
SOFTmax[®] PRO for Lmax User's Manual

Version 1.0L Software

for Macintosh[®] and Windows[®] Operating Systems



Molecular Devices Corporation
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SOFTmax[®] PRO for Lmax User's Manual

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Introduction

The Lmax™ Microplate Luminometer uses SOFTmax PRO® for Lmax software. This software allows you to create files containing all of the parameters required to acquire and analyze data from the Lmax. SOFTmax PRO for Lmax (version 1.0L) is available in either Macintosh or Windows format. The Macintosh version of the software is a fat binary application that includes native code for both 68K and Power Macintosh computers. The Windows version is a 32-bit application that is Windows 95/98/2000 and Windows NT 4.0 compliant.

SOFTmax PRO for Lmax is powerful, yet easy to use. It performs three major functions:

- **Instrument Control:** You can set up and run a complete assay. Instrument settings can be saved as a protocol file and used repeatedly for reading different microplates.
- **Data Collection:** SOFTmax PRO for Lmax collects and stores all raw data received from the Lmax. Data is displayed on the computer screen in a grid format that corresponds to the wells in a microplate. How the data is shown depends on other settings made using the software.
- **Data Reduction and Plotting:** You can reduce the data using built-in formulas or you can define custom reduction formulas. More than one reduction can be shown and results from different microplates can be compared within the same experiment.

SOFTmax PRO for Lmax can collect data from one or more microplates and store it in a single data file, using the same or different instrument settings for different microplates. Four read modes are possible using SOFTmax PRO for Lmax,:

- **Endpoint:** Allows a single integration of samples in 96- or 384-well plates with two injections possible, if desired, in any or all wells of a 96-well plate (no injection possible in 384-well plates). A pre-read of the microplate may be made before injection of buffer or reagent and /or reading of the samples. The signal is integrated for each well over a user-selectable reading time (maximum 5000 seconds). You can program a delay after each injection, if desired (no minimum delay for the M injector; the P injector has a minimum delay of 1.6 seconds; both have a maximum setting of 1000 seconds), and you can read all or only some of the wells in the plate. Default values reported are relative luminescence units (RLU).
- **Dual Read:** Also an endpoint reading, this mode is designed to perform two separate integrations, one after each of two possible injections in any or all wells of a 96-well microplate (no injection possible in a 384-well plate). Integration times for both readings must be the same (minimum integration time is 0.1 second; maximum is 1000 seconds). You may program a delay after each injection, if desired (no minimum delay for the M injector; the P injector has a minimum delay of 1.6 seconds; both have a maximum setting of 1000 seconds). You may read only some or all of the wells in the plate; pre-reading the microplate is not possible with this mode. Default values reported are relative luminescence units (RLU).



- **Long Kinetics:** Allows repeated measurements on one or more wells of a 96- or 384-well microplate. Pre-reading the microplate is not possible with this mode. One or two injections can be made in any or all wells of a 96-well plate at the beginning of the reading (no injection possible in 384-well plates). An important application for this mode is cellular luminescence (see page B-5). Default data reductions for kinetic data are Vmax per Sec, Vmax, Time to Vmax, or Onset Time.
- **FastKinetics:** Allows repeated measurements of one or more wells of a 96- or 384-well microplate up to a 100-point maximum integration. Pre-reading the microplate is not possible with this mode. One or two injections can be made in each well of a 96-well plate at the start of the reading (no injection possible in 384-well plates). Integration time can be set from 1 to 100 seconds. Default data reductions for kinetic data are Vmax per Sec, Vmax, Time to Vmax, or Onset Time.

About the Documentation

The SOFTmax PRO for Lmax documentation set consists of this manual, an on-line help system, and a formula reference manual. Additional documents on disk may also be provided to cover the latest information regarding the software. An outline of the contents of this manual follows, along with introductory information that should help you get started using SOFTmax PRO for Lmax,.

NOTE: Screens and parts of screens represented in this manual may depict either the Macintosh or the Windows version of the software. The two interfaces are quite similar, but bear subtle differences such as where the Help menu is located, etc. Depending upon which computer platform you are using, which operating system you are using, and the type of screen that is depicted, your screen may look different from the one shown. Note that Macintosh screens are depicted using OS versions 7 through 9.

- The rest of this chapter, "Welcome to SOFTmax PRO for Lmax," provides introductory information regarding the program and its features. A glossary of terms is included at the end of this chapter.
- Chapter 2, "Installation," contains basic information about installing the program on your computer and making sure the correct connections are made between the computer and the instrument.
- Chapter 3, "SOFTmax PRO for Lmax Overview," explains how SOFTmax PRO for Lmax is organized and how it is used with the Lmax.
- Chapter 4, "Reading Microplates," contains instructions regarding how to prepare for and conduct a reading using SOFTmax PRO for Lmax,.
- Chapter 5, "Displaying and Analyzing Data," describes how data is displayed during and after a reading and how this display can be customized. Additionally it describes data reduction and the different means for analyzing the raw data received from the Lmax.
- Chapter 6, "File Management and Printing," explains the printing options available within SOFTmax PRO for Lmax along with how to customize reports. Other topics include managing files and importing, exporting,



copying, pasting, and duplicating data, both within SOFTmax PRO for Lmax as well as to and from other external programs.

- If you are new to SOFTmax PRO for Lmax, Chapter 7, "Tutorial," provides a written series of steps that will help you learn the basics of working with the software.
- Chapter 8, "Troubleshooting," describes alert messages that may appear while using SOFTmax PRO for Lmax and what to do if they occur. Other information in this chapter pertains to possible communication problems with the Lmax and other computer-related issues.

This manual assumes that you are familiar with the basic operating environment of your computer. We recommend that you become familiar with your computer's operating environment prior to installing and using SOFTmax PRO.

Conventions Used in This Manual

This manual uses visual cues and standard text formats as follows.

Visual Cues

The following typographic conventions are used in this manual.

Format	Meaning
Menu Item	Menu titles and menu items.
<i>Text Entry/Key Name</i>	Entries that you type from the keyboard or keys that you press on the keyboard. Information shown in this font should be entered exactly as it appears.
<i>Text Entry</i>	Placeholders for information you must provide. For example, if you are asked to type <i>filename</i> , you should type the actual name for the file instead of the word shown in this other font.
Button	Buttons shown on the screen. Note that sometimes an actual representation of the button may be shown.



The accompanying text applies only to SOFTmax PRO for Lmax for Macintosh.






The accompanying text applies only to SOFTmax PRO for Lmax for Windows.



Keyboard Formats

Key designations, combinations, and sequences appear in the following formats.

Format	Meaning
 ⌘Key	Selections on the Macintosh can be made using the mouse, but often shortcuts are available using the keyboard. Shortcuts commonly use the command (⌘) key in conjunction with another key and are shown with the command key symbol (⌘) followed by the accompanying key. To use a shortcut, you must <i>press and hold</i> the command key while pressing the second key.
 Key1+Key2	A plus sign (+) between key names means to <i>press and hold down the first key while you press the second key</i> (within Windows). For example, “press ALT+ESC” means to press and hold down the ALT key and press the ESC key. Then release both keys.
 Key1, Key2	A comma (,) between key names means to <i>press and release the keys one after the other</i> . For example, “press ALT, F” means to press and release the ALT key, and then press and release the F key.

NOTE: The names of keys found on different Windows-compatible PC computer keyboards may vary somewhat from machine to machine. The key names shown in this manual reflect typically used key names. Yours should be similar but may not be exactly like those shown. For example, the “print screen” key may be shown on some keyboards as “Prt Scrn”; other keyboards may spell out the complete key name.

Messages

The following types of messages may appear in this manual:

NOTE: Calls attention to information that should be followed during installation, use, and/or servicing of the instrument or software.

CAUTION: Indicates a potentially hazardous situation which, if not avoided, might result in minor or moderate injury, loss of data, or mechanical damage. It is also used to alert against unsafe practices.

WARNING: Used whenever an action or condition might potentially cause serious injury to yourself or to others who might use the instrument or computer.



Getting Help



Macintosh On-Line Help

On-line help is available while working with SOFTmax PRO for Lmax for the Macintosh by clicking the Help menu at the far right of the menu bar.

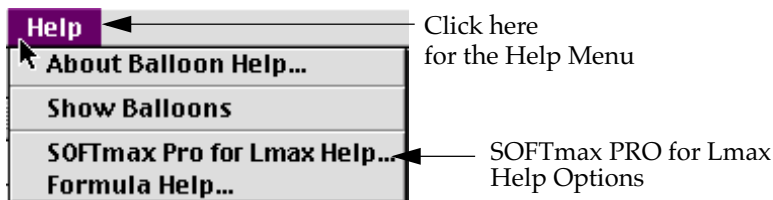


Figure 1.1: Help Menu for SOFTmax PRO for Lmax for the Macintosh

Choosing either of the two help options at the bottom of this menu (**SOFTmax PRO for Lmax Help** or **Formula Help**) causes a Help window to appear. This window provides a list of topics on the left and information about the selected topic on the right. Figure 1.2 shows the window for Formula Help. In this figure, the topic “Math Functions” has been selected (on the left) and the corresponding information about that topic appears on the right.

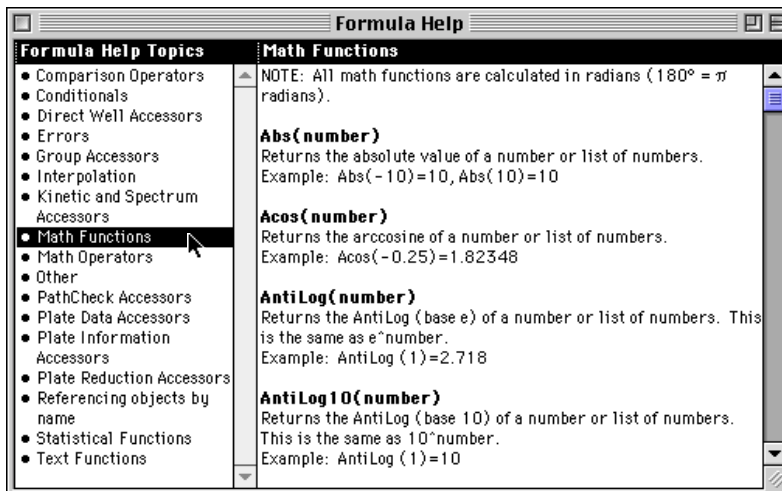


Figure 1.2: Formula Help Window



Windows On-Line Help

You can access on-line help within SOFTmax PRO for Lmax for Windows by selecting the **Help** menu in the menu bar. Three types of help are immediately available: you can view the contents of the help file, seek specific help about formulas, or get general information about how to use the help function.

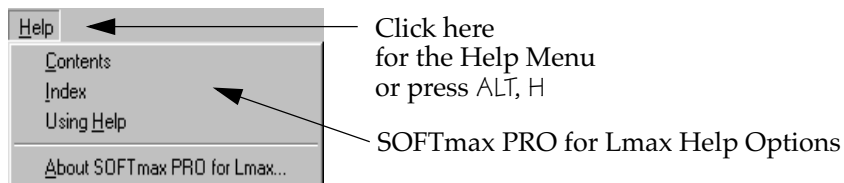


Figure 1.3: Help Menu for SOFTmax PRO for Lmax for Windows

Choosing Contents (by clicking on the menu item with the mouse or pressing ALT, H, C) brings up the SOFTmax PRO for Lmax help Contents window, showing the general organization of the help that is available.

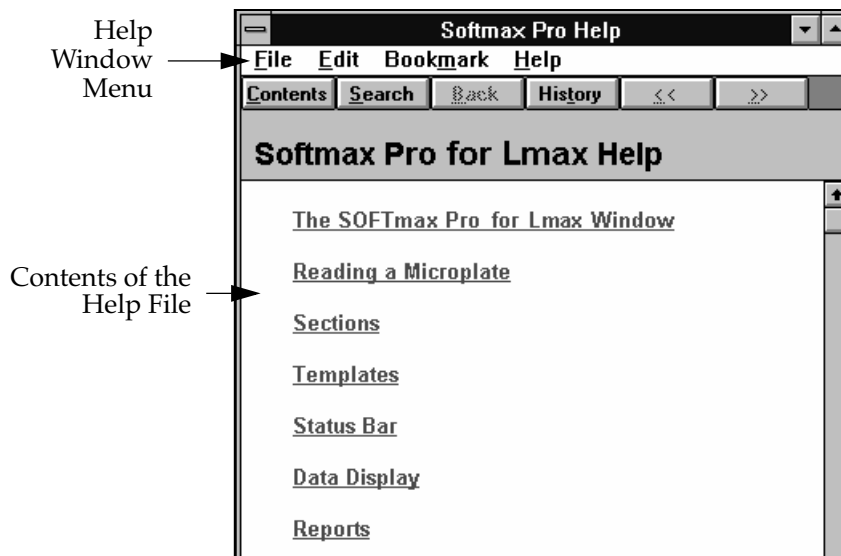


Figure 1.4: Help Contents for SOFTmax PRO for Lmax for Windows



Help Options from the Contents Window

- You can search for a particular item or subject by clicking the **Search** button (located in the tool bar at the top of the Contents window).

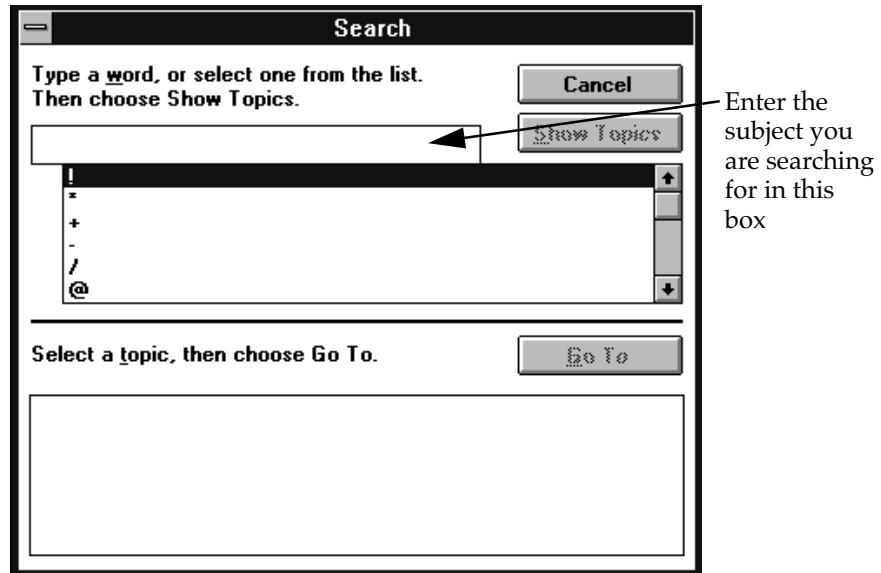


Figure 1.5: Using the Search Function within Help in SOFTmax PRO for Lmax for Windows

After entering a search subject, clicking the **Show Topics** button will cause a list of related topics to appear in the box below.

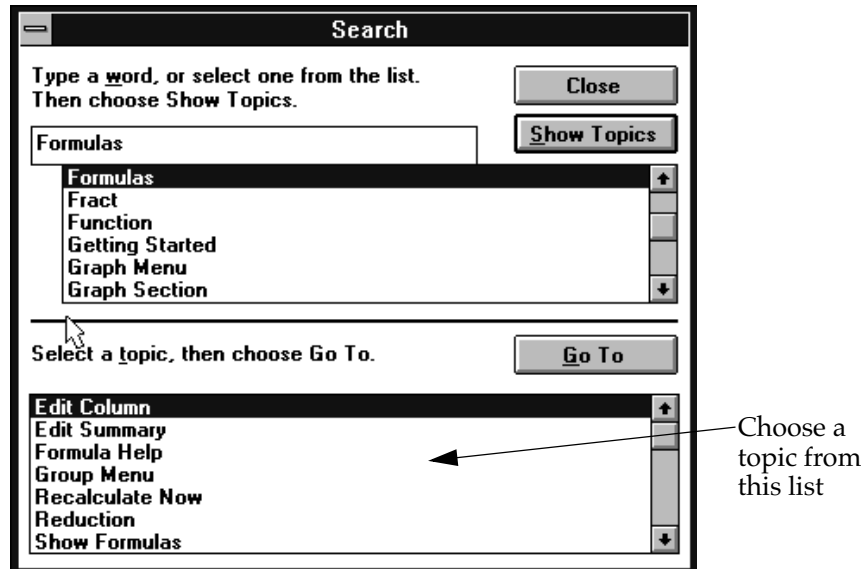
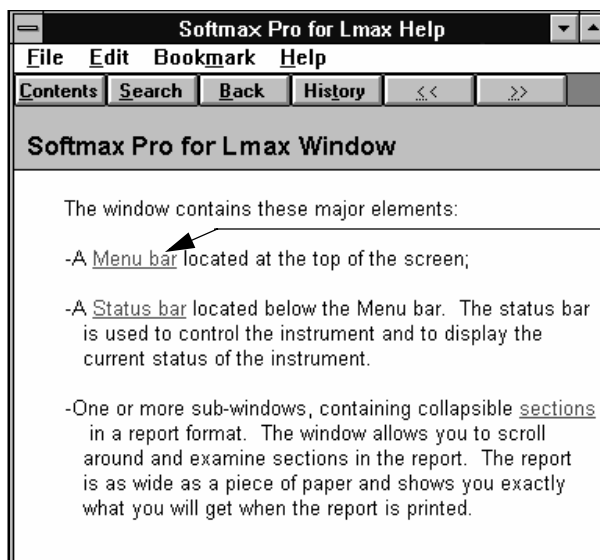


Figure 1.6: Choose a Topic within Help in SOFTmax PRO for Lmax for Windows

- You can return to the window viewed previously by clicking the **Back** button.



- Clicking the **History** button will provide a list of all areas you have viewed within the Help function.
- Text that appears in green with an underline contains a hypertext link to more detailed information about that item. Figure 1.7 below shows some examples of hypertext links.



Hypertext linked item (clicking here will give more information about this item)

Figure 1.7: Hypertext Links within SOFTmax PRO for Lmax for Windows

Additional options are available, depending upon where you are within the Help function. More information about Windows Help can be found in the *Microsoft Windows User's Guide* that accompanies your Windows software.

Troubleshooting and Technical Support

If you encounter a problem while using SOFTmax PRO for Lmax, refer first to Chapter 8, "Troubleshooting," and try to locate a remedy for the problem. For problems with SOFTmax PRO for Lmax that are not listed there, or if you need additional assistance, in the U.S., call Molecular Devices Technical Services group at 800-635-5577 or 408-747-1700; elsewhere, call your local representative.



Glossary of Terms

Active

The window or icon that you are currently using or that is currently selected. The active section is the section in which you are currently working. The title bar for the active section or window will be highlighted (gray); inactive sections are not highlighted (white). Only one section or window can be active at a time. To make a section or window active, click anywhere within it. If you scroll past the bottom or top of the active section, the tool bar for that section “sticks” to the top of the window (you can always tell which section is active in this way and can always access the items in its tool bar).

ANSI Character Set

The American National Standards Institute 8-bit character set. It contains 256 characters.

Area Under Curve

Reduction formula for a kinetic reading that determines the area under the kinetic plot, or under a plot in a Graph section. First the area under the curve is divided into a series of trapezoids; then the area defined by each trapezoid is computed and all areas are summed.

ASCII Character Set

The American Standard Code for Information Interchange 7-bit character set. It consists of the first 128 (0–127) characters of the ANSI character set (and most other 8-bit character sets). The ASCII character set is the most universal character-coding set.

Autosave

Enabling the Autosave feature in the Preferences dialog box will save a data file automatically each time a plate is read. You can Autosave to one of two different file formats: SOFTmax PRO for Lmax (used to save your data automatically in SOFTmax PRO for Lmax format) or Text File (used when you want to have the data available for use with a spreadsheet or other program for data analysis external to SOFTmax PRO for Lmax).

Blanking

Pre-Read Blanking subtracts background signal caused by the microplate itself, as well as by solvents, reagents, or samples. This feature is available only for endpoint mode. The individual blank reading from each well can be subtracted from the final reading from the same individual wells (choose “Pre-read plate” from within the Instrument Settings dialog box).

Plate Blanks are created by entering a Blank group in the template. After a reading, the average of the blank values obtained from the Blank group is subtracted from all wells in the plate. Plate blanks can be used with all read modes.

Group-Associated Blanks are created by placing blank wells within a group in the template. The values of group blanks are averaged and subtracted from the values of the wells within that group only.



Checkbox

A small, square box that appears in a dialog box and that can be selected or cleared. When the checkbox is selected, an X appears in the box. A checkbox represents an option that you can turn on or off.

Clear

Within the Template Editor, **Clear** removes information that was designated previously for selected wells. By highlighting wells and then choosing **Clear**, any information regarding those wells will be erased (the wells will return to their default status of empty). Note that if you clear the entire template, the groups themselves will not be deleted and are still selectable. Keeping the groups allows you to reassign wells without having to recreate groups.

Click

You click an object on the screen by pressing and quickly releasing the mouse button while the pointer is on that object. When you click once on an object, such as an icon or file name, it becomes highlighted, which means it is selected. Whatever action you choose next will affect a selected object.

Clipboard

A temporary storage location within the operating system used to transfer data within and between files and applications.

Concentration

The amount of a substance present per unit volume. Used as the default sample descriptor for a series of samples.

Control

A sample that is run as an internal check of the assay. The control has a predictable or known assay result against which other results can be compared.



Control Menu Box (Windows)

The icon that opens the Control menu for the window. It is located at the left of the SOFTmax PRO for Lmax title bar. The Control menu can be activated by clicking in this box with the mouse or by pressing ALT, SPACEBAR.

Copy

A function common to most software applications that duplicates the selected text or item and places this duplicate on the clipboard. The copy function is enabled by pressing \mathbb{C} on the Macintosh and CTRL+C in Windows.

Copy-and-Paste

A method of copying selected text or graphics from one location and pasting them to a different location in the same file or into another file (which could be in an application other than SOFTmax PRO for Lmax).

Cubic Spline (Curve Fit)

This curve fitting option generates a fit to a cubic equation between each pair of data points. The cubic equations are computed such that these equations and their first two derivatives are continuous everywhere.

More information regarding curve fits can be found in Chapter 5, "Displaying and Analyzing Data."



Curve Fit

Specifies the type of curve (linear, logarithmic, quadratic, and so on) that is applied to the data points in a plot. The curve is a graphic representation of the mathematical relationship between the data points in the plot. More information regarding curve fits can be found in Chapter 5, "Displaying and Analyzing Data."

Cut

A function common to most software applications that removes a selection of text or graphics and places it on the clipboard. The cut function is enabled by pressing \mathbb{X} on the Macintosh and CTRL+X in Windows.

Cut-and-Paste

A method of moving selected text or graphics from one location to a different location in the same file or into another file (which could be in an application other than SOFTmax PRO for Lmax).

Data Display

Located in the Plate section, the data display is a grid that corresponds to the layout of the wells in the microplate. This display can show the results of the reading in various formats [for example: raw signal data (RLU), reduced data (number or plot), Threshold, Ranged, and Gray Scale].

Data File

Contains the raw data collected from the instrument, as well as any associated sections or settings (Notes, Graphs, Groups, Instrument Setup, Display Options, and Data Reductions).

Default Protocol

A protocol file, included with the SOFTmax PRO for Lmax program or created by a user. The Default Protocol file defines the initial information that will appear when you open SOFTmax PRO for Lmax or when you create a new file from within the program.

Desktop

The background on which windows and icons appear.

Dialog Box

A rectangular window that appears temporarily to request information. Some dialog boxes have options you must choose before SOFTmax PRO for Lmax can carry out a command and must be closed before you can continue in the software.

Dilution Factor

Specifies the amount of dilution for a given sample. This factor can be multiplied against interpolated values to determine concentration of undiluted sample.

Dimmed

Unavailable or disabled. A dimmed button or command is displayed in light gray instead of black and it cannot be chosen.

**Double-Click**

To rapidly press and release the mouse button twice while the pointer is on an object. Double-clicking carries out an action, such as opening an icon.

Drag

To move an object on the screen by clicking it, holding down the mouse button, and then moving the mouse. Also to select an item in a list by dragging through the list or to select multiple items by dragging across them.

Dual Read

An endpoint reading, this mode is designed to perform two separate integrations, one after each of two possible injections in any or all wells of a 96-well microplate (no injection is possible in a 384-well plate).

Duplicate

A command available in the **Edit** menu that allows you to create identical copies (except data) of all sections and experiments. The name of the duplicated item will be the same as the previously active item with the word "Copy" appended.

Endpoint

A single integration of samples in 96- or 384-well plates with two injections possible, if desired, in any or all wells of a 96-well plate (no injection possible in 384-well plates).

End Time

Reduction parameter used to omit the end of a kinetic run from data reduction.

Export

Data from SOFTmax PRO for Lmax is copied to a new file that can be opened by another program such as a spreadsheet or word processing application. Data from some or all Plate or Group sections may be exported as tab-delimited ASCII files.

Exponential (Curve Fit)

The exponential function used to generate this curve fit is:

$$y = A + B * (1 - \exp^{(-x / C)})$$

More information regarding curve fits can be found in Chapter 5, "Displaying and Analyzing Data."

Fast Kinetics

During fast kinetics readings, data is collected over time, with multiple readings made at regular intervals up to a specified number of points. The values calculated based on raw kinetic data are Vmax, Vmax per Sec, Time to Vmax, and Onset Time.

Fluorophore

A material which absorbs light energy of a characteristic wavelength, undergoes an electronic state change, and instantaneously emits light of a longer wavelength.



Four Parameter Logistic (Curve Fit)

The equation used to generate this curve fit is:

$$y = ((A - D) / (1 + (x / C)^B)) + D$$

More information regarding curve fits can be found in Chapter 5, "Displaying and Analyzing Data."

Gain

The amount of increase in signal power expressed as the ratio of output to input for a photomultiplier tube.

Gray Scale Data Display

Raw or reduced data is displayed proportionally in a plate format using seven shades of gray, ranging from light shading at the low end to dark shading at the high end, based on user-defined high and low limits.

Group

Wells can be assigned to group types using the Template Editor. Depending on the default protocol used, certain group types (such as Standard or Unknown) may be created automatically; you can create others as required.

Highlighted

Indicates that an object or piece of text is selected and will be affected by your next action. Highlighted text appears in reverse video or in color. Highlighted objects may change color or be surrounded by a selection cursor.

Icon

A graphical representation of an element of the computer or software such as a disk drive, application, or file.

Import

Bringing information or data from another application into SOFTmax PRO for Lmax. Information can be brought into SOFTmax PRO for Lmax in two ways: by copying tab-delimited text to the clipboard and then pasting this information into the Plate section within SOFTmax PRO for Lmax; or by using the **Import** command under the **File** menu to import ASCII text files.

Incubator

Choosing **Incubator** from the **Control** menu or clicking the incubator button opens a dialog box allowing you to start or stop temperature regulation and to select an elevated temperature for the microplate chamber on instruments that have temperature control capability.

Injection

The process of adding reagent and/or buffer to a microplate well. Lmax has two injectors that may be used with the 96-well plate format (injection not available with 384-well plates).

Instrument Icon

Located in the status bar, the instrument icon shows the status of the connection between the computer and the instrument. If the icon has an "X" through



it, the connection is not functioning or no instrument is connected; if the instrument icon appears without an “X,” the connection is working properly.

Instrument Setup

Defines the parameters (read mode, run time, read interval, etc.) used to integrate data from the microplate.

Interleaved Display

A display choice available with 384-well plates is useful when four 96-well daughter plates are combined into one 384-well plate.

Interprocess Messaging

Messages that can be sent to and received from other applications. This feature is very useful for interfacing Lmax with robotic systems and for sending and receiving data from LIMS systems.

Lag Time

Lag time is the period of time in a kinetic assay of very slow growth (microorganisms) or rate of reaction that may precede the rapid (or linear) phase of reaction. When a lag time is specified in SOFTmax PRO for Lmax, data collected prior to the lag time is not included in data reduction.

Large Display

A display choice available with 384-well plates. This setting is useful when looking at large numbers if you have a larger (17-inch or greater) monitor.

Linear (Curve Fit)

The linear function fits the best straight line to the data. The equation for this fit has the form of:

$$y = A + Bx$$

where A is the y-intercept of the line and B is the slope. A linear fit should be used whenever the values appear to lie on or scattered around a straight line.

More information regarding curve fits can be found in Chapter 5, “Displaying and Analyzing Data.”

Log-Logit (Curve Fit)

The Log-Logit is also called a two-parameter curve fit. The equation used to generate this curve fit is:

$$y = ((A - D) / (1 + (X / C)^B)) + D$$

More information regarding curve fits can be found in Chapter 5, “Displaying and Analyzing Data.”

Long Kinetics

During long kinetics readings, data is collected over time, with multiple readings made at regular intervals. The values calculated based on raw kinetic data are Vmax, Vmax per Sec, Time to Vmax, and Onset Time.



Luminescence

The emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation.

Mask

Lets you “hide” the data in selected wells of the Plate display so that the data stored there will not be used for calculations and will not be reported. The Mask function is commonly used to suppress outliers. Masked wells are shown with diagonal lines across them and the word “Masked” is shown over each masked well.

MaxRLU

The limit for the maximum value you wish to use for displaying and analyzing kinetic data. The default is 20,000 RLU. Any values that are above this limit will not be shown and will be excluded from data reduction.



Maximize Button (Windows)

The small box containing an up arrow at the right of the title bar. Mouse users can click the Maximize button to enlarge a window to its maximum size. Keyboard users can use the **Maximize** command on the **Control** menu.

Menu

A list of items, most of which are commands. Menu item names appear in the menu bar at the top of the window.

To choose an item in a menu, place the pointer on the menu heading and drag to the selection you desire—then release the mouse button.

Menu Bar

A list of items, most of which are commands. Menu item names appear in the menu bar (the horizontal bar that appears below the title bar at the top of the window).

MinRLU

The limit for the minimum value you wish to use for display and analysis of kinetic data. Any values that are under this limit will not be shown and will be excluded from data reduction. The default is 0. To display negative Kinetics, the value should be set below 0 (zero).




Minimize Button (Windows)

The small box containing a down arrow at the right of the title bar. Mouse users can click the Minimize button to reduce a window to its icon. Keyboard users can use the **Minimize** command on the **Control** menu. A minimized application continues running and you can select the icon to make it the active application.



Modem Port (Macintosh)

A serial communication port on the back panel of the Macintosh marked with the icon of a telephone handset ().

Normal Display

The default display for 384-well plates.



NVRAM

Refers to the Non-Volatile Random Access Memory of the instrument. Information stored in the NVRAM is not lost when power to the instrument is turned off.

Onset RLU

The change in signal required to compute the onset time (for kinetic readings).

Onset Time

The time it takes for a given increase in signal, called the onset RLU, to occur.

Open/Close Triangle

A small triangle located on the left-hand side of section and experiment tool bars. Used to open/close sections and experiments within a SOFTmax PRO for Lmax file.

Option Button (Radio Button)

A small, round button that appears in a dialog box or window. Within a group of related option buttons, you can select only one.



Parallel Port (Windows)

A connection on a computer, usually LPT1, to plug in the cable for a parallel printer. Windows supports parallel ports LPT1 through LPT3.

Paste

A function that copies the contents of the clipboard and inserts this copied information at the current selection or cursor position. The paste function is enabled by pressing $\mathfrak{A}V$ on the Macintosh and CTRL+V in Windows.



Path (Windows)

Specifies the location of a file within the directory tree. For example, to specify the path of a file named ASSAY.BAT located in the SOFTmaxPRO directory on drive C, you would type `c:\softmaxpro\assay.bat`.

Photomultiplier Tube (PMT)

A vacuum tube that detects light especially from dim sources through the use of photoemission and successive instances of secondary emission to produce enough electrons to generate a useful current.

Plate Section

The Plate section is used to display data, to specify how microplates will be read, and to define how the data received from the instrument should be reduced. The data display in this section is a matrix that corresponds to the well format of the microplate in use.

Plate Type

A choice in the Instrument Settings dialog box to distinguish between 96- and 384-well microplates. The specification of the plate type depends on the well format and manufacturer of the microplate.



Point (Using the Mouse)

You point to an object on the screen by moving the mouse so that the pointer is positioned on that object. When the pointer is an arrow, the tip of the arrow must be on the object.

Point-to-Point

This curve-fitting option fits a linear equation to each pair of data points.

Port

A connection or socket on the computer. Ports are common used for connecting devices, such as printers or monitors, to your computer, and for sending information to and/or from your computer to such devices.

Protocol File

Contains the template(s), instrument settings, and reduction parameters. Protocol files are useful if you repeat a particular type of assay frequently.

Quadratic (Curve Fit)

A mathematical description of a parabola:

$$y = A + Bx + Cx^2$$

RAM (Random Access Memory)

The memory that can be used by applications to perform necessary tasks while the computer is on. When you turn the computer off, all information in RAM is lost.

Ranged Data Display

Raw or reduced data is assigned proportionally to integer values from 0 through 9 based on user-defined limits. Values above the high limit are displayed as plus (+) and values below the low limit are displayed as minus (-).

Raw Data

Signal reported as RLU from the Lmax with no alteration.

Read Interval

The interval of time between the start of one reading and the start of the next reading during a kinetic run.

Read Button or Command

Starts the reading process and shows the endpoint, dual read, or kinetic data in the data display of the Plate section.

Read Mode

The method used to read the microplate: endpoint, dual read, or kinetic.

Reduced Data

Data reduction causes the raw signal values reported by the instrument to be calculated and displayed according to user-defined formula settings.

Reduction Limits

Sets limits on data to be included in data reduction for kinetic readings.



Restore Box (Windows)

The small box containing both an up and a down arrow at the right of the title bar. The Restore box appears only after you have enlarged a window to its maximum size. Mouse users can click the Restore box to return the window to its previous size. Keyboard users can use the **Restore** command on the **Control** menu.

Rotated Display

A display option for 384-well plates that shows the plate rotated 90 degrees.

Scroll

To move up, down, right, or left through text or graphics in order to see parts of the file or list that cannot fit on the screen. A scroll bar appears at the bottom and/or right edge of a window whose contents are not entirely visible. Each scroll bar contains a scroll box and two scroll arrows. A scroll box is a small box that shows the position of information currently in the window or list box relative to the contents of the entire window. A scroll arrow is an arrow on either end of a scroll bar that you can use to scroll through the contents of the window or list box.

Semi-Log (Curve Fit)

The semi-log function fits the best straight line to a set of data for $\log(X)$ plotted against Y . The resulting curve displayed will be a straight line with the X -axis drawn in logarithmic scale.



Serial Port (Windows)

A connection on a computer, usually COM1, where you plug in the cable for a serial device such as a plate reader, a printer, or a modem. Serial, asynchronous, and RS232 interfaces are all the same type by which the computer sends single bits of information to the connected device, one after the other. Windows supports COM1 through COM4. The Lmax requires COM1 or COM2.

Series

Lets you assign a set of wells within groups in the Template so that the standard value or dilution factor increases or decreases in specified steps and the name increments as well.

Slope

A reduction option for a kinetic run that determines the slope of the combined plot (i.e., the slope of the line using linear regression). This reduction uses all timepoints visible in the reduction window.

Standard Curve

A mathematically idealized representation of the relationship between the concentration of a standard calibrator (X -value) and the optical density (Y -value). The standard curve may be set to a linear, semi-log, log-log, quadratic, 4-parameter logistic, log-logit (2-parameter logistic), exponential, cubic spline, or point-to-point curve fits.



Status Bar

Shown above the SOFTmax PRO for Lmax window, the Status Bar contains the Lmax icon, the current temperature within the microplate chamber, and buttons allowing you to activate the Read function, the incubator, etc.

Template Editor

A representation of the microplate shown as a grid of wells that can be used to designate the location of blanks, standards, controls, unknowns, empty wells, or to assign wells to other groups you create. The template is a map of the microplate. It tells the software what is in the microplate and where it is located.

Threshold Data Display

Data is displayed as plus (+) for values above, asterisk (*) for values within, and minus (-) for values below user-defined limits.

Time at Half Maximum

Reduction for a kinetic run that reports the time at half the maximum signal within the reduction limits as follows: SOFTmax PRO for Lmax first determines the kinetic point (within the reduction limits) that has the maximum signal level. It then scans the kinetic plot from left to right until it finds two points that have signals bracketing one-half of that signal value. A linear interpolation between these two points is used to estimate the Time at Half Maximum.

Time at Maximum

Reduction for a kinetic run that reports the time at the maximum signal within the reduction limits.

Time at Minimum

Reduction for a kinetic run that reports the time at the minimum signal within the reduction limits.

Time to Vmax

The elapsed time until the maximum reaction rate of a kinetic reading is reached, reported in seconds.

Title Bar

The horizontal bar (at the top of a window) that contains the title of the window or dialog box. On many windows within the Windows environment, the title bar also contains the Control-menu box and Maximize and Minimize buttons. On the Macintosh, the title bar also contains the Close box and Zoom box.

Undo

Cancels the previous operation (if possible). The paste function is enabled by choosing **Undo** from the **File** menu, pressing \mathbb{V} on the Macintosh, or pressing CTRL+V in Windows.

Vertical Display

A display option for 384-well plates which shows the plate as two 8 × 12 arrays stacked vertically.



Vmax Points

The number of points in the kinetic curve included in each of a series of regressions to calculate the maximum rate, Vmax, or the time segment used to calculate the Time to Vmax.

Vmax (units per sec)

The kinetic rate reported as signal/sec (RLU units per second). It is calculated using a linear curve fit, $y = Ax + B$. A creeping iteration is performed using Vmax Points and the slope of the steepest line segment is reported as Vmax Rate.



Zoom Box (Macintosh)

A small box containing a smaller box at the right of the title bar. You can click the Zoom box to return the window to its previous size.

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Connect the Hardware

⚠ CAUTION: Turn OFF the power to both the Lmax and the computer before connecting or disconnecting any components or cables. Not doing so could potentially result in damage to components in either or both machines.



Macintosh

NOTE: If your Macintosh has a single serial port, refer to Chapter 8, “Troubleshooting,” for information regarding connection to the Lmax.

Serial Port Connection

Use an 8-pin DIN to DB-25 Macintosh modem cable to connect the Macintosh to the Lmax. Connect the 8-pin, round DIN connector to the Macintosh (to the printer or modem port) and the 25-pin connector to the Lmax. Connecting to the modem port of the Macintosh is preferred, allowing the printer port to remain available. If you *do* connect the Lmax to the Macintosh printer port, first inactivate AppleTalk using the Chooser desk accessory in the Apple () menu.

NOTE: If you are running other applications that use AppleTalk, it may be necessary to quit them before making AppleTalk inactive.

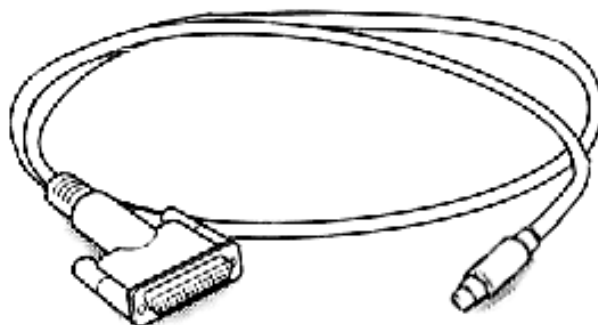


Figure 2.1: 8-Pin DIN to DB-25 Macintosh Straight-through Serial Cable

USB Connection

Although the Lmax requires a serial connection, through the use of a USB-to-serial adapter you can connect a serial cable between the Macintosh and the Lmax instrument. Molecular Devices has tested and can recommend the Keyspan USB Twin Serial Adapter (not the PDA adapter). Connect the USB connector from the adapter to an open USB connection and choose the “printer” port from the Preferences in SOFTmax PRO for Lmax (the Keyspan software only supports direct access to the emulated printer port and may cause the Mac to freeze if the modem port is chosen). Connect the serial cable to *port 1 of the adapter (not port 2)* and plug the other end of the serial cable into the receptacle on the back of the Lmax instrument.



Windows-Compatible Computer

The type of cable required to connect your computer and the Lmax will depend upon the type of serial port(s) available on your computer. The end of the cable that will attach to the Lmax must be a 25-pin connector. The cable itself should be a straight-through serial (null modem) cable. The connector for your computer may be a 9-pin or 25-pin connector (or you can use an adapter that converts from 9-pins to 25-pins—see Figure 2.2).

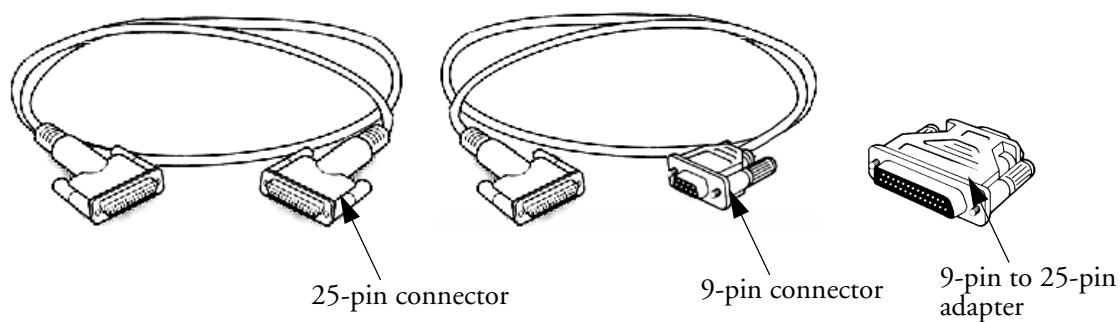


Figure 2.2: 25-Pin DIN to 25-Pin or 9-Pin Serial Cable



Computer System Requirements

The following computer hardware and operating system software specifications are recommended to ensure proper operation of SOFTmax PRO for Lmax. The minimum configuration required is also given.



Macintosh Computer¹

Recommended System Configuration
<ul style="list-style-type: none">• Apple Power Macintosh G3• Operating software System 9.0• Sony 17-inch Multisync Color Monitor• 64 MB RAM, 6 GB hard disk drive• CD-ROM drive• Extended keyboard• Keyspan USB Twin Serial Adapter• Epson 740I color printer
Minimum System Configuration
<ul style="list-style-type: none">• Apple Macintosh PowerPC• Operating software System 7.5.5• Apple 14-inch color monitor• 24 MB RAM, 270 MB hard disk drive• 1.44 MB HD (high-density) 3.5-inch floppy disk drive• Access to a CD-ROM drive• Available serial communications port

¹. Macintosh hardware tested for the SOFTmax PRO for Lmax release: iMac with OS 8.1; Macintosh G4; PowerMac 7600/120 with OS 8.1; PowerMac 7100/80AV with OS 7.5.5. Printer tested: Apple Laserwriter Select 360.



Windows-Compatible Computer²

Windows 95/98 Recommended System Configuration	Windows NT/2000 Recommended System Configuration
<ul style="list-style-type: none"> • Pentium III, 450 MHz computer • Microsoft Windows 95 or 98 • 17-inch color SVGA monitor • Video adapter and software driver capable of displaying 800 x 600 minimum resolution set to 16-bit high color and small fonts or better • 64 MB RAM or greater • 6 GB hard disk drive • 1.44 MB HD (high-density) 3.5-inch floppy disk drive • CD-ROM drive • Bus mouse • Serial port (COM1 or COM2) 	<ul style="list-style-type: none"> • Pentium III, 450 MHz computer • Microsoft Windows NT 4.0 or 2000 • 17-inch color SVGA monitor • Video adapter and software driver capable of displaying 800 x 600 minimum resolution set to 16-bit high color and small fonts or better • 128 MB RAM or greater • 6 GB hard disk drive • 1.44 MB HD (high-density) 3.5-inch floppy disk drive • CD-ROM drive • Bus mouse • Serial port (COM1 or COM2)

Windows 95/98 Minimum System Configuration	Windows NT/2000 Minimum System Configuration
<ul style="list-style-type: none"> • 486 33 MHz computer • Microsoft Windows 95 or 98 • Color monitor • SVGA graphics adapter (set to 256 colors and small fonts or better) • 24 MB RAM or greater • 1 GB hard disk drive • 1.44 MB HD (high-density) 3.5-inch floppy disk drive • Access to a CD-ROM drive • Bus mouse • Serial port (COM1 or COM2) 	<ul style="list-style-type: none"> • 486 33 MHz computer • Microsoft Windows NT 4.0 or 2000 • Color monitor • SVGA graphics adapter (set to 256 colors and small fonts or better) • 32 MB RAM or greater • 1 GB hard disk drive • 1.44 MB HD (high-density) 3.5-inch floppy disk drive • Access to a CD-ROM drive • Bus mouse • Serial port (COM1 or COM2)


² PC hardware tested for the SOFTmax PRO for Lmax release: Dell Dimension XPS R350 with Windows 98; Dell Dimension XPS H266 with Windows 2000; HP Vectra XU 3/200 with Windows NT; Compaq Presario 9234 with Windows 95. Printers tested: HP DeskJet 812C and HP LaserJet 4000N.



Install SOFTmax PRO for Lmax

Follow these instructions to install SOFTmax PRO for Lmax from the CD to your computer's hard disk.

- 1) Insert the SOFTmax PRO for Lmax CD into the CD-ROM drive.
- 2) **Macintosh:** Double-click the CD-ROM drive icon to open it.
Windows: Open "My Computer" and then double-click the CD-ROM drive icon to open it.
- 3) Double-click the Installer icon (Macintosh) or Setup icon (Windows) and follow the screen prompts.
- 4) The "Welcome" screen suggests that you close all other programs before beginning installation of SOFTmax PRO for Lmax software. It is also recommended that you disable any virus protection software you may have running before installing SOFTmax PRO for Lmax. Click **[Cancel]** to exit the installer and close any programs and/or disable virus protection and then start the installer again from step 3 above. To continue with installation, click **[Next]**.
- 5) The screen named "Choose Destination Location" asks you to select the drive where the software will be installed. A default location will be shown on the screen. If you have more than one hard drive in your computer, you may change the destination to the disk drive where you wish SOFTmax PRO for Lmax to reside or accept the default location.
- 6) The "Setup Type" screen asks you to choose between "Complete" (the default) or "Basic." Basic installation installs all but the tutorial files. If you wish to omit the tutorials, choose Basic. Otherwise, just click **[Next]** to continue.
NOTE: The **[Back]** button is available on all but the first screen, allowing you to go back to previous screens and review or change information that you have entered.
- 7) Click **[Next]** to start the actual installation of SOFTmax PRO for Lmax files.
- 8) When installation is complete, the "Finished" screen will appear. Click **[Close]** to end the installer/setup program. On the Macintosh, remove the CD by dragging its icon to the Trash. On the PC, close the window for the CD and "My Computer" and then press the CD eject button on the CD-ROM drive. Replace the CD in its holder and store it in a safe place.

An open folder named "SOFTmax PRO for Lmax" will be visible on your screen. It will contain the SOFTmax PRO program. If you want SOFTmax PRO for Lmax to be able to communicate with your Lmax, be sure to turn the Lmax on prior to starting the SOFTmax PRO for Lmax program. After the instrument is powered on and has completed its start-up sequence, double-click the "lpf" icon () to start SOFTmax PRO for Lmax.

NOTE: In Windows, a shortcut for the program will be available in the Start menu under "Programs\SOFTmax PRO for Lmax\lpf."




What Is Installed

A folder called “SOFTmax PRO for Lmax” will be installed in the area you specified on your computer’s hard drive. Within this folder are a number of different files and subfolders (folder and filenames are the same for Windows and Macintosh except that Windows files have the extension “.lpr” appended to the file name).

NOTE: An uninstall program is available for Windows computers. Should you need to remove an older version of SOFTmax PRO for Lmax from your hard drive, we recommend using the uninstall program since it also removes information regarding SOFTmax PRO for Lmax from the Windows Registry.

To remove any version of SOFTmax PRO for Lmax from a Macintosh computer, simply drag the SOFTmax PRO for Lmax folder and any other SOFTmax PRO for Lmax files you may have on your hard drive to the trash.

Enter Registration Information

Turn on the Lmax and allow it to complete its start-up sequence. Then double-click the program icon ( inside the SOFTmax PRO for Lmax folder—Mac—or on the desktop—Windows) or choose SOFTmax PRO for Lmax from the Windows Start menu to begin the software program.

The first time you open SOFTmax PRO for Lmax, a registration dialog box will appear, asking that you enter your name, company, and the serial numbers of the software program and Lmax. The software serial number can be found on the original SOFTmax PRO for Lmax CD, on the inside cover of this manual, and on the software Warranty Registration Card (please remember to fill out this card and return it to Molecular Devices Corporation to validate your warranty). The instrument serial number (S/N) is located on a label affixed to the back of the Lmax. Type the information into the appropriate boxes and then click . Note: the option to click will not be available until all information has been entered. If you have difficulty entering the registration information, refer to Chapter 8, “Troubleshooting,” for assistance.



The registration dialog box features the Molecular Devices logo at the top left. Below the logo, the text reads: "Please register this copy of SOFTmax PRO for Lmax by providing the following information." There are four input fields: "Name:", "Company:", "Software Serial Number:", and "Instrument Serial Number (S/N):". At the bottom right, there are two buttons: "Quit" and "OK".

Figure 2.3: Registration Dialog Box

After you click [OK], a title screen will appear (see Figure 2.4) showing the registration information you entered.

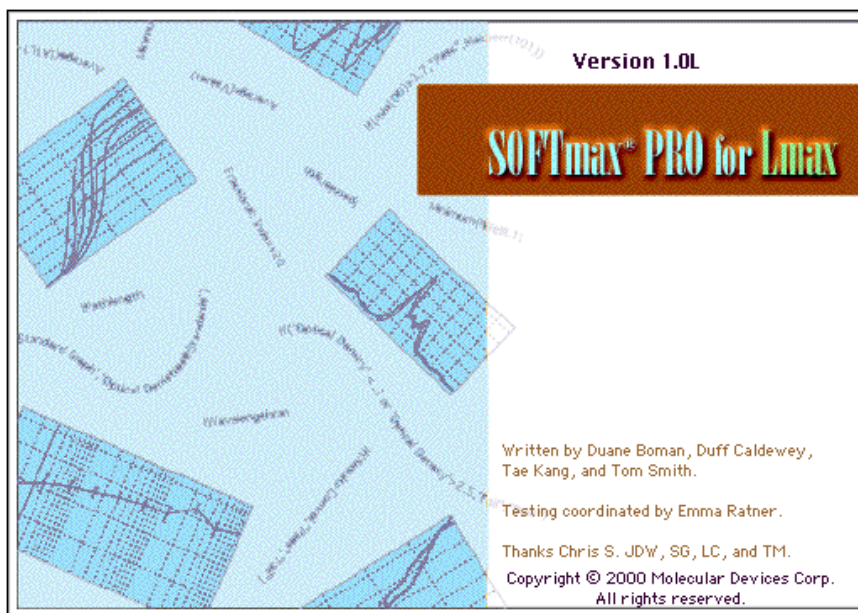


Figure 2.4: SOFTmax PRO for Lmax Title Screen

After a few seconds, the title screen will be replaced by an untitled SOFTmax PRO for Lmax window (see Figure 2.5).

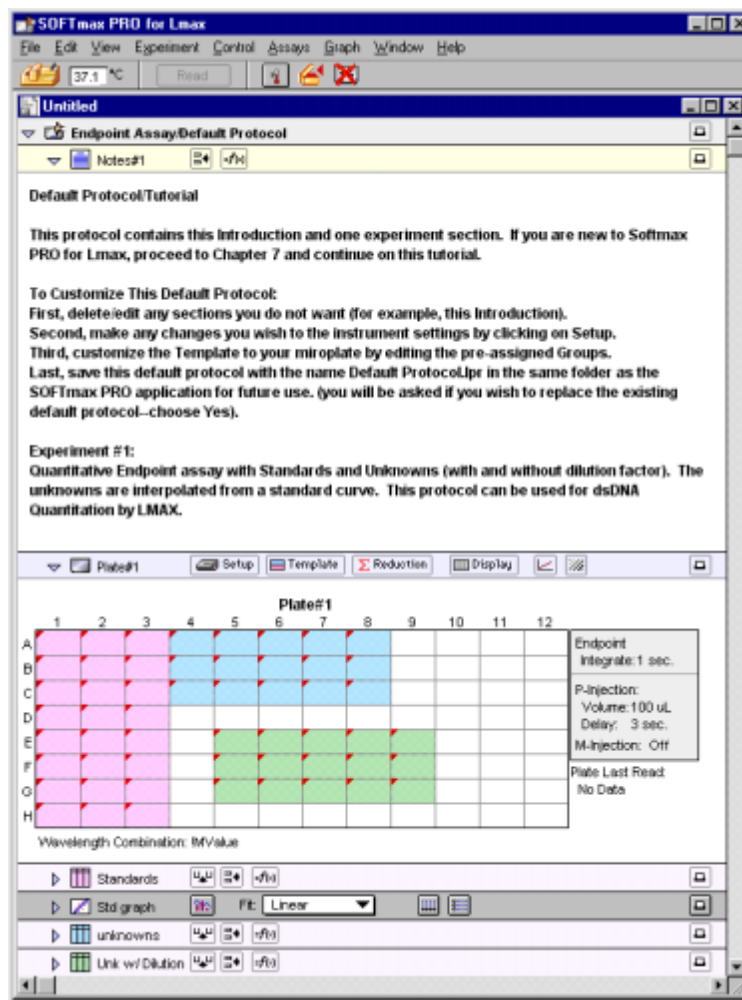


Figure 2.5: SOFTmax PRO for Lmax Default Protocol Untitled Window

Communicating with the Lmax

After installation of SOFTmax PRO for Lmax, you will note that the instrument icon in the upper left-hand corner of the window appears with an “X” and the words “No port selected” through it. You must access the Preferences settings (from the **Edit** menu, choose the **Preferences** command) to select the proper communications port (modem or printer for the Macintosh, COM1 or COM2 for the PC). An additional setting, which is the factory default, is “Neither” (used when you do not have an instrument connected to the computer and wish to use SOFTmax PRO for Lmax or if the port connection must be disabled for other reasons). Choose the appropriate setting for your computer’s physical serial port connection and click **[OK]**.

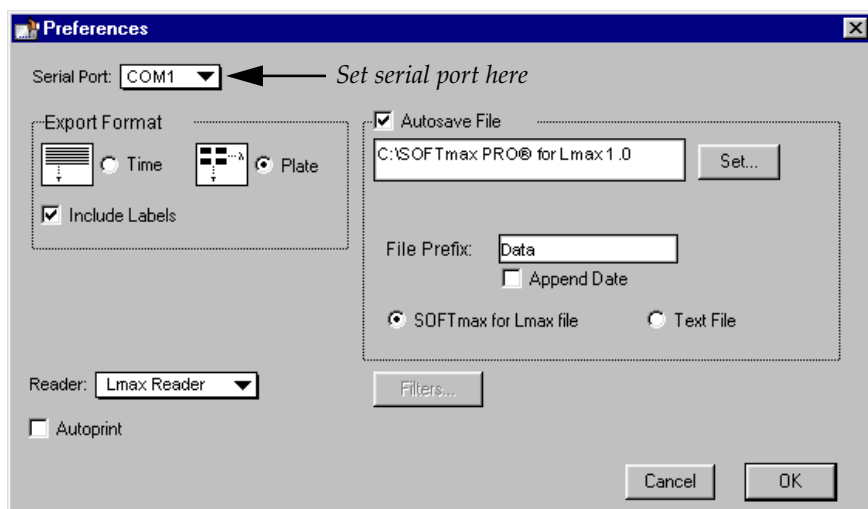


Figure 2.6: Preferences Dialog Box (Windows)

The instrument icon should now appear normally (without an X through it).

If the port setting is correct and the Lmax is on, yet communication is still *not* functioning properly, quit SOFTmax PRO for Lmax, turn off the power to both the computer and the Lmax, and check that the cable connections between the instrument and computer are secure. Turn both machines on again and restart SOFTmax PRO for Lmax. If an “X” still appears over the icon, refer to Chapter 8, “Troubleshooting,” for more information.

Set Preferences

It is a good idea to set the SOFTmax PRO for Lmax preferences before proceeding to use the program. The preferences include certain configuration settings, such as which port is used for the connection to the instrument, as well as settings for automatic printing and saving of files, and the format for exported data. To make these selections, choose **Preferences** from the **Edit** menu to bring up the Preferences dialog box (see Figure 2.6).

Serial Port

Check that the setting for the serial port matches the physical port used to connect the computer and the instrument.

NOTE: A selection of “Neither” can be made for the serial port which can be selected if communication with an instrument is not required or if no instrument is connected to the computer. When this option is selected, the Status Bar in SOFTmax PRO for Lmax will reflect it by showing an instrument icon with an “X” through it, along with the words “No port selected.” This selection allows you to use the serial ports for other purposes (modem connection, network connection, or printing, for example), but does not allow you to communicate with a Lmax.



Export Format

The preferences settings regarding the Export Format allow you to choose whether to export the data as it appears in plate format or linearly (over time). The option to include text labels along with numerical data can be activated by clicking “Include Labels.” For more information on the **Export...** function, refer to “File Creation and Management” on page 6-3.

Autosave

Enabling the Autosave feature in the Preferences dialog box will save a data file automatically each time a plate is read.

Autosave saves a copy of the file that is currently open. If the file that is open is a protocol file, note that the file will still be “Untitled” (i.e., not named) even after an autosave has occurred. This allows you to autosave to a text file format and then save manually as a SOFTmax PRO for Lmax file.

You can Autosave to one of two different file formats:

- SOFTmax PRO for Lmax—used to save your data automatically in SOFTmax PRO for Lmax format.
- Text File—used when you want to have the data available for use with a spreadsheet or other program for data analysis external to SOFTmax PRO for Lmax.

NOTE: If you autosave as a text file, the SOFTmax PRO for Lmax version of the data will not be saved automatically and you will not be able to automatically re-open the data file in SOFTmax PRO for Lmax. If you choose to save to this format, you will need to save your data manually, using the **Save** or **Save As...** commands in the **File** menu if you also want to save a SOFTmax PRO for Lmax file. If you attempt to quit SOFTmax PRO for Lmax before saving a file containing data that has not yet been saved, a message box will appear asking if you want to save the file. You can assign any name to a file you save manually.

NOTE: If your protocol contains more than one experiment section when you Autosave to SOFTmax PRO for Lmax file format, each experiment will be saved to a separate data file.

When Autosave is enabled, the box below the Autosave option becomes active, allowing you to choose the folder within which data files will be saved and the type of file to be saved. You may choose to autosave as a SOFTmax PRO for Lmax file (default) or as a text file and, when saving as a text file, you may choose to append to the same file.

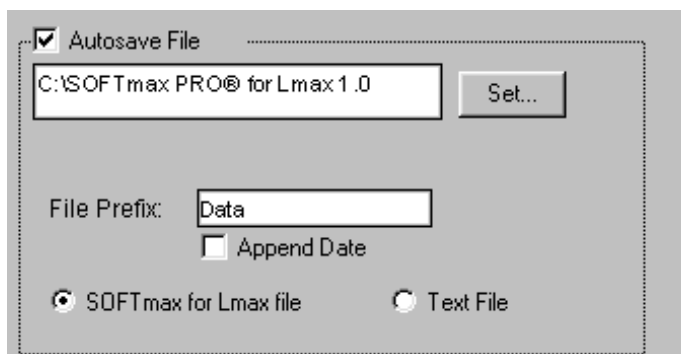


Figure 2.7: Autosave Information (Windows)

The default folder for saving files is the SOFTmax PRO for Lmax folder. To save data files in a different folder, click the [Set] button. A dialog box will open, allowing you to choose a new location for saved files.

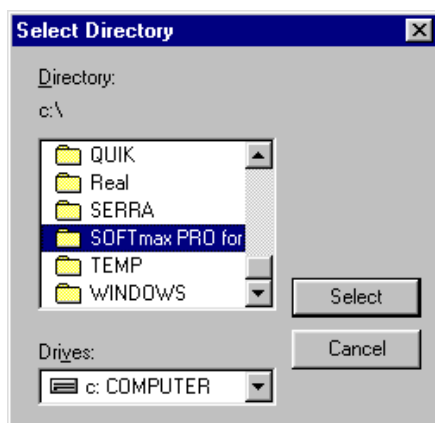


Figure 2.8: Autosave Location Dialog Box (Windows)



Highlight the folder where you want files to be saved. The **Select** button at the bottom of the dialog box will reflect this choice; clicking the **Select** button will set this folder as the default location for saved data files. You can change the location at any time by repeating this process.

To change the file format from SOFTmax PRO for Lmax to ASCII text, select the Text File format from the list.

NOTE: If the Autosave function is reporting an error, or if files are saved in the wrong location, it may be that a previous version of SOFTmax PRO was not uninstalled prior to installing the newest version. Follow the instructions given above to choose the correct folder for saved files.

Create New File (Text only)

After each Plate section is read, the data will be saved in a new file. This file will contain *all* the data from *the current and previous readings* (for example, if you read a Plate section and then another Plate section, File1 will contain data for Plate1; File2 will contain data for Plate1 *and* Plate2. This option is available for both file formats (SOFTmax PRO for Lmax and ASCII text).

CAUTION: If you Autosave to ASCII text format, you will **not** have a SOFTmax PRO for Lmax version of the file unless you also manually save the file using the **Save as...** command.

Append to File (Text only)

If you are saving data to an ASCII text file, you may choose to save all data to a single file for all plates by choosing "Append to File." This option is dimmed (not available) if you are saving to a SOFTmax PRO for Lmax file.

File Prefix

The information entered into the "File Prefix" field is automatically given to new files that are saved automatically. The default prefix is "Data." Accepting this default saves files starting with the name "Data" followed by a sequential number beginning with "1." For example, the first microplate read would be saved under the file name "Data 1."

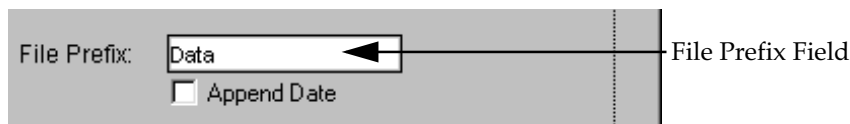


Figure 2.9: File Prefix Field (Windows)

You can change this default prefix to any other prefix, provided the entire file name will not exceed 31 characters. To change the prefix, click within the File Prefix field, highlight the existing information, and type over the word to enter a new prefix.

If the File Prefix field is left blank, the date of the run will be used as the file prefix.



Append Date

Enabling the checkbox for Append Date will cause the date to be included in the automatic file name for the saved data file. For example, using the file prefix “Data,” the first file saved on January 31, 2000, would have the name “Data 01/31/2000 1.”

Autoprint

Checking this box will enable automatic printing of a report after each plate is read. This report will contain information from each section that is designated to be included in the report (the default is for all sections to be included). For more information on reports, and including or excluding sections, see Chapter 3, “SOFTmax PRO for Lmax Overview.”

Assays Folder

The **Assays** menu is used to choose the folder that will contain protocol files you wish to access most often. After installation of SOFTmax PRO for Lmax, the protocol files shown under the **Assays** menu will be the basic protocols shipped with the software. Other folders may be installed with SOFTmax PRO for Lmax that contain different protocol files for other applications—you may select one of these folders instead, if desired, or create a new one of your own.

To change the default folder, choose **Assays** and then choose **Set Folder...** . A dialog box will appear, asking you to choose the folder or directory containing the protocol files you wish to be shown in the **Assays** menu. Select one of the other folders within the SOFTmax PRO for Lmax directory / folder or, if you have other protocol files in a different location, choose that location and then click **Select**.

NOTE: If you do not see any files in the Assays menu or if the files shown are not correct, it may be that another or previous version of SOFTmax PRO was not uninstalled prior to installation of the current version. This can cause SOFTmax PRO for Lmax to default to using the old directory for assay files rather than the directory associated with the most current program version. Follow the instructions given above to choose the correct folder for the default assays folder.



Chapter 3: SOFTmax PRO for Lmax Overview

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Introduction

This chapter presents a general description of the features and use of SOFTmax PRO for Lmax. It describes:

- the parts of the SOFTmax PRO for Lmax window, including experiments and sections;
- the menus and tool bars found within the SOFTmax PRO for Lmax program;
- the basic steps required to perform a reading (complete information regarding reading using SOFTmax PRO for Lmax can be found in Chapter 4, “Reading Microplates”); and
- the basic types of data analysis (detailed information regarding data analysis can be found in Chapter 5, “Displaying and Analyzing Data”).

SOFTmax PRO for Lmax employs standard Macintosh and Windows conventions for using menus, dialog boxes, windows, and the mouse. This manual assumes that you are familiar with the basic operation of your computer. While some general instructions are given here, and many of the terms used in SOFTmax PRO for Lmax are defined in Chapter 1, “Glossary of Terms,” if you need additional assistance regarding the basic use of your computer, please review the documentation accompanying it.

The SOFTmax PRO for Lmax Window

When you start SOFTmax PRO for Lmax, a title screen will appear, showing the information entered during registration, along with the SOFTmax PRO for Lmax version, the instrument type (if connected), the ROM version installed in the instrument (if available), and copyright information.

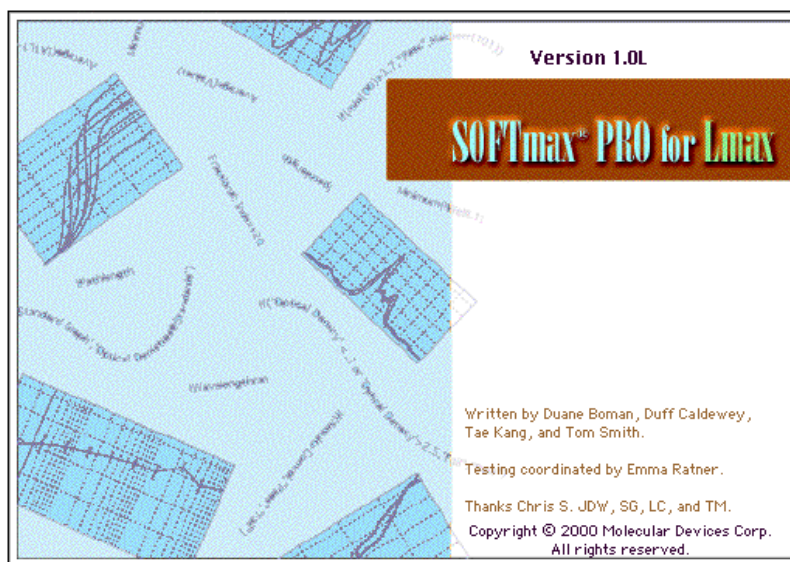


Figure 3.1: SOFTmax PRO for Lmax Title Screen.



After a few seconds, the title screen will be replaced by an untitled SOFTmax PRO for Lmax window. Figure 3.2 below shows an example default protocol screen for the PC.

The SOFTmax PRO for Lmax screen contains these major elements:

- A menu and status bar located at the top of the screen;
- An untitled window showing an experiment containing various sections, depending upon the configuration of the default protocol used and the type of instrument chosen in the Preferences or connected to the computer.

The menu and status bars are always visible while you are using SOFTmax PRO for Lmax, no matter where you are within the program.

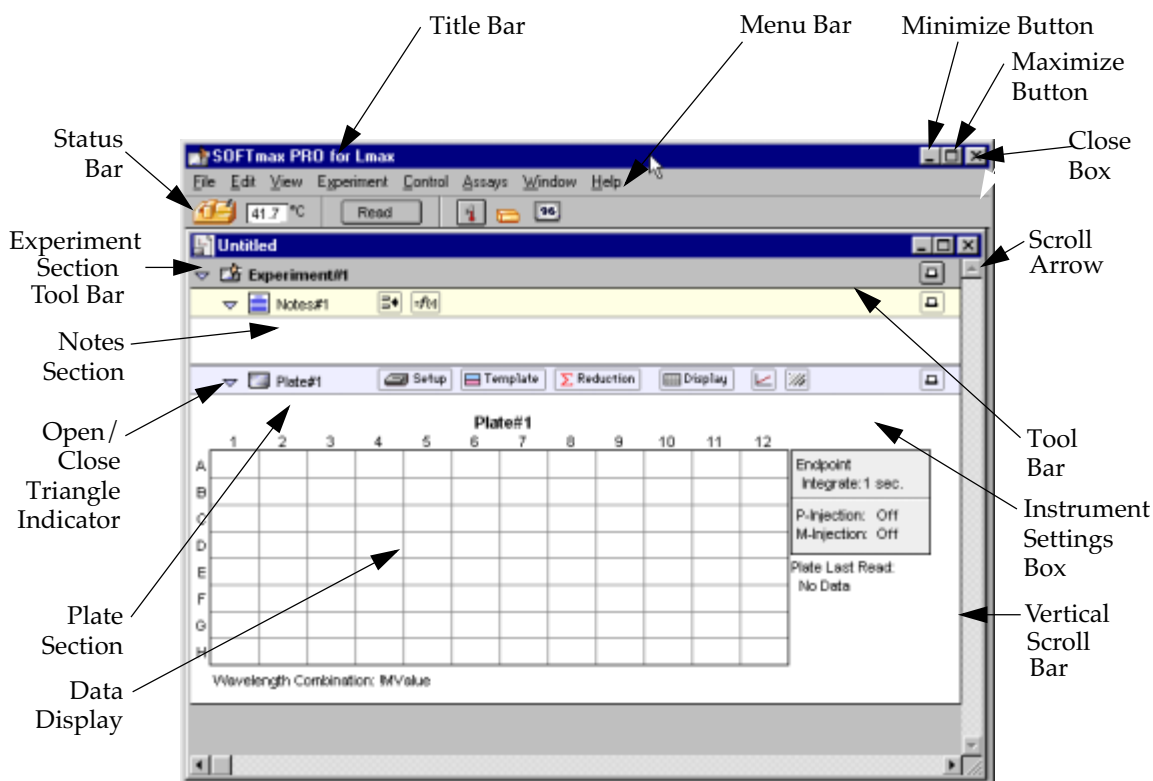


Figure 3.2: SOFTmax PRO for Lmax Screen and Window for the PC (Windows).



Window Size



You may change the size of the window shown on the Macintosh by dragging the size box (at the bottom right corner of the window) in any direction. You can expand the window to the full size of the screen by clicking the zoom box (in the upper right corner of the window). If you extend the bottom edge of the window beyond the last section or the right edge beyond the edge of the sections, the intervening space will be gray. To resize the window again, drag the size box to a different position or click the zoom box again. Clicking the zoom box will restore the previous window size.

NOTE: If you move the SOFTmax PRO for Lmax window such that the title bar is under the status bar, you will have to hide the status bar before you can move the window to see the title bar again.



You may change the size of the window shown in Windows by dragging the side, bottom, or corner of the window (when the cursor changes into a double-headed arrow). You can expand the window to the full size of the screen by clicking the Maximize button in the upper right corner of the window. If you extend the bottom edge of the window beyond the last section or the right edge beyond the edge of the sections, the intervening space will be gray. You can also reduce the window to an icon by clicking the Minimize button in the upper right corner of the window. When the window is shown as an icon, it is still functional; double clicking the icon will restore the window to its previous size. Other options are available for resizing windows—refer to the documentation that came with your Windows software for more information.



NOTE: The width of the experiment and sections shown in the window is constrained by the width set for the printed page size. The dimensions and orientation of the printed page (and, consequently, the display size) may be changed by selecting either **Page Setup...** (Macintosh) or **Print Setup...** (Windows) from the **File** menu.



Status Bar

Near the top of the SOFTmax PRO for Lmax window is a status bar that starts with an icon representing the Lmax instrument (see Figure 3.3).

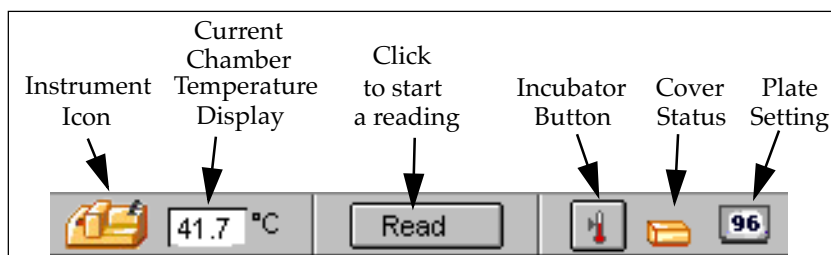


Figure 3.3: Status Bar

NOTE: The status bar can be hidden from view by selecting **Hide Status** from the **View** menu. Selecting **Show Status** from the view menu will restore it to view.

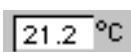
The status bar shows the following items (from left to right):

Instrument icon. An icon such as that shown at the left indicates that the Lmax is communicating properly with the computer. If an “X” appears on top of the icon, the computer is not making proper contact with the Lmax.



NOTE: Double-clicking the Lmax icon will open the Preferences dialog box.

Temperature display. A small window after the Lmax icon shows the current temperature within the microplate chamber in degrees Celsius.



The [Read] button. If a single Plate section has been created in the experiment window, clicking the [Read] button starts the reading. This reading is based on the settings chosen in the Instrument Settings dialog box (refer to “Instrument Setup”) for the plate.

If more than one Plate section has been created in the experiment window and no Plate section is currently active, clicking the [Read] button opens a dialog box asking you to select which plate to read. If a Plate section is active when you click the [Read] button, that section is read automatically.

The Incubator button. Clicking this button causes the “Incubator” dialog box to appear, allowing you to set and regulate the temperature of the microplate chamber. The temperature setting can be left at the default or can be specified by typing a different value in the box.

NOTE: The incubator setting is *independent* of the protocol being run. Running an assay does not automatically set the temperature set point. After a reading, however, the temperature set point, range, and average temperature are recorded in the saved file.



Cover status. This icon displays the status of the cover over the microplate tray of the Lmax (closed or open). You must close the lid prior to reading a microplate.



96- or 384-well plate setting display. This icon shows whether or not a microplate is present in the reading chamber and also if the Lmax is currently set to read 96-well or 384-well plates (when no plate is present, an “X” appears over the icon as shown). The Lmax requires that two changes be made when switching from reading one plate size to the other: you must physically reconfigure the position of the Lmax read head and you must change the software setting for the plate size in the SOFTmax PRO for Lmax **Control** menu. Selecting the 384-well plate size in the Instrument Settings without configuring that setting in the **Control** menu will produce an error.

Experiments and Sections

Experiments

Each SOFTmax PRO for Lmax file contains at least one *experiment* (and can contain more than one). Within an experiment are one or more *sections* (discussed below). Experiments define a naming scope for the sections within them and provide you with quick access to different types of information without opening or referring to multiple files.

An *experiment* can be *selected* by clicking its tool bar (the area that shows the experiment name) with the mouse. Selecting an experiment causes its tool bar to change from light gray to darker gray and the experiment title to be shown in boldface type. *Selecting* is different from *activating* an experiment. *Selecting* an experiment allows you to perform actions on the entire experiment (such as deleting or duplicating it). Multiple experiments can be selected by holding down the *Shift* key while clicking the individual tool bars of the experiments.

A single click anywhere within an inactive experiment *activates* it. *Activating* an experiment allows you to add new sections to it or to perform other operations within that experiment. (With *sections*, however, *selecting* and *activating* are identical functions. Clicking anywhere within an inactive section activates and selects it.)

Creating a new experiment by choosing either **New** or **Duplicate** from the **Edit** menu creates an identical copy of the experiment (excluding data). The new experiment retains the same name as the original, with the word “Copy” appended when you choose to **Duplicate** (e.g., the duplication of “Expt1” produces “Expt1 Copy”) and an increment of number when you choose **New** (e.g., “Expt1” becomes “Expt2”).

An experiment can be deleted if it is no longer needed. The **Delete** command in the **Edit** menu changes depending upon which experiment is active. For example, if the experiment titled “Experiment #1” is active, the **Edit** menu will show **Delete Experiment #1**. Using the Macintosh, you can also delete the selected experiment by pressing \mathfrak{K} -; within Windows, you can press CTRL+-. Take care when deleting experiments that they do not contain information you wish to save.

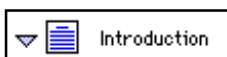
You may also choose to delete multiple experiments simultaneously by holding down the *Shift* key and activating the tool bars of the experiments you wish to delete. Having done this, the **Edit** menu will now show **Delete Selection** instead of **Delete Experiment**.



You can add new sections to the active experiment using the **New Section** command under the **Edit** menu.

Sections

SOFTmax PRO for Lmax contains four types of sections: Notes, Plate, Group, and Graph. A brief synopsis of these sections is given below.



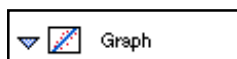
Notes sections are used to record information pertaining to the experiment. You can enter text in this section as you desire; in addition, you can create summaries containing formulas for displaying reduced data. Text in Notes sections can be formatted (font, size, and style any way you choose).



Each **Plate section** shows a grid of cells which corresponds to the wells of the microplate. To the right of or underneath this grid is a box showing the current instrument settings (except incubator settings, which are always shown at the bottom of the Plate section after data is acquired). In the Plate section, you define a template to be used for analysis, determine the instrument settings, choose how the data will be displayed, and define the type of reduction (if any) that will be performed on the raw data received from the instrument.



Group sections are created automatically when groups are defined in the Template Editor. The example shown at left is for a group named "Standard." Protocol files generally contain default groups; you can create others as needed. Each Group section contains default columns of data which can also be changed, if desired. Within a Group section, you can resize columns, modify existing columns to use different analysis formulas, add columns containing new formulas, and/or add one or more summaries at the bottom of the section.



Graph sections are used to plot information from groups (defined in the Template Editor). Values associated with the X- or Y-axes depend on the type of group selected for graphing. More about Graph sections can be found in Chapter 5, "Displaying and Analyzing Data."

General Information about Sections

Sections are either active or inactive. Multiple sections can be active at the same time although it is typical to have only one active section. The tool bar of an active section is dark gray. Inactive sections are color coded to make it easier to discern a section's type:

Inactive Experiment or Section Color

Experiment	Gray
Plate	Purple
Notes	Yellow
Group	Pink
Graph	Blue

Clicking anywhere in an inactive section makes it active. Selecting an experiment deactivates any individual section within it that may have been active and vice versa. Activating a section also activates the experiment containing that section. To activate multiple sections at the same time, hold down the Shift key and click in the tool bars of the sections you wish to activate. Doing this allows you to move, copy, duplicate, print, and delete multiple sections simultaneously.



The last item (farthest right) in the SOFTmax PRO for Lmax menu bar changes to reflect the section type that is currently active. If a Plate section is active, for example, the last menu item will be **Plate**. If an experiment is selected, this menu will not be shown.

New Plate, Notes, or Graph sections can be created whenever you need them and more than one of each type of section can exist in a single SOFTmax PRO for Lmax experiment. (Group sections are created automatically when you define new groups in the Template Editor or may be created with the **Duplicate** command.)

A section can be deleted if it is no longer needed. The **Delete** command in the **Edit** menu changes depending upon which section is active. For example, if the section titled "Plate #1" is active, the **Edit** menu will show **Delete Plate #1**. Using the Macintosh, you can also delete the active section by pressing \mathbb{C} -; within Windows, you can press $\text{CTRL}+-$. Take care when deleting sections that they do not contain information you wish to save. You may also delete multiple sections simultaneously by making them active—the **Delete** command in the **Edit** menu will change to show **Delete Selection**.

NOTE: Deleting a Group section automatically deletes references to that group from the Template. See Chapter 4, "Reading Microplates," for more information.

Section tool bars contain buttons and pull-down menus. Buttons in the tool bar are only enabled when that section is *active* (the section does *not* need to be *open*). Menu equivalents exist for most tool bar functions. Figure 3.4 shows the active tool bar from a Graph section.



Figure 3.4: Tool Bar from an Active Graph Section

If you scroll the window vertically so that part of an open/active section is hidden, its tool bar "sticks" to the top of the window (See Figure 3.5). This feature prevents the active tool bar from becoming unavailable if you are viewing the bottom of a section that is longer than the size of the window. When the next section scrolls to the top of the window, its tool bar replaces the previous one *but is not active*, even if the section is open. If you scroll back so that even a small part of the previous section is visible, its tool bar returns to the top of the window.

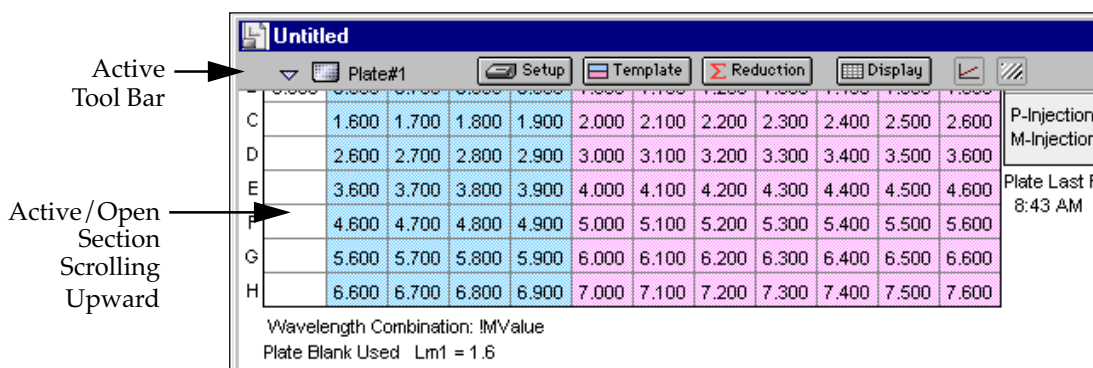


Figure 3.5: Example of a Tool Bar “Sticking” to the Top of the Window.

Opening and Closing Sections

Newly created experiments and sections are initially shown in their full-size (open) form. You can close them, however, to show just their tool bars, if desired. Sections that are created automatically by SOFTmax PRO for Lmax may be shown initially either open or closed, depending on the default protocol file in use.

To open or close a single section within SOFTmax PRO for Lmax, click its indicator located in the top left corner of the tool bar. When an experiment or section is closed, the indicator points to the right (▶); when open, the indicator points down (▼). Figure 3.6 shows an open experiment containing three sections—the first two are closed, the last is open.

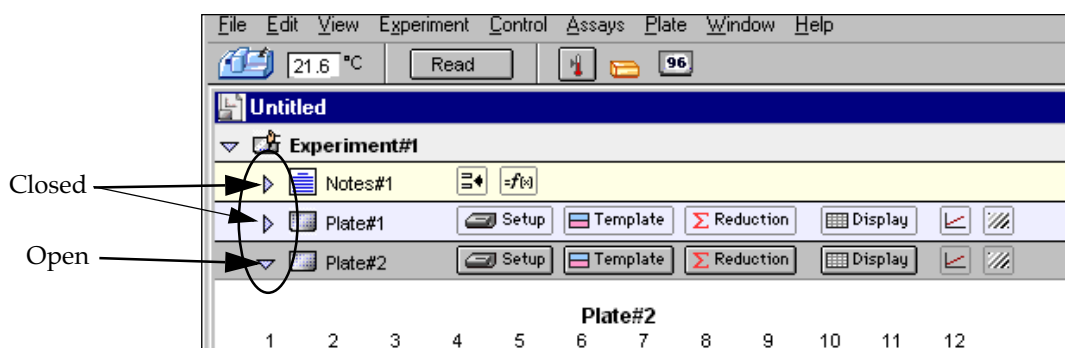


Figure 3.6: Closed and Open Sections. The Notes#1 and Plate#1 sections are closed; the Plate#2 section is open.

To open or close multiple sections within an experiment (or multiple experiments in a file) simultaneously, use the **Expand** and **Minimize** commands in the **View** menu. Selecting **Minimize** (⌘M for Macintosh; CTRL+M for Windows) will close *all* sections (leaving experiments open) to show only section title bars. Selecting **Expand** (⌘E; CTRL+E) will open all sections.

NOTE: If you close or minimize an experiment, the tool bars in that experiment will be hidden (see Figure 3.7). If all sections are presently expanded, the **Expand** command is dimmed (unavailable).

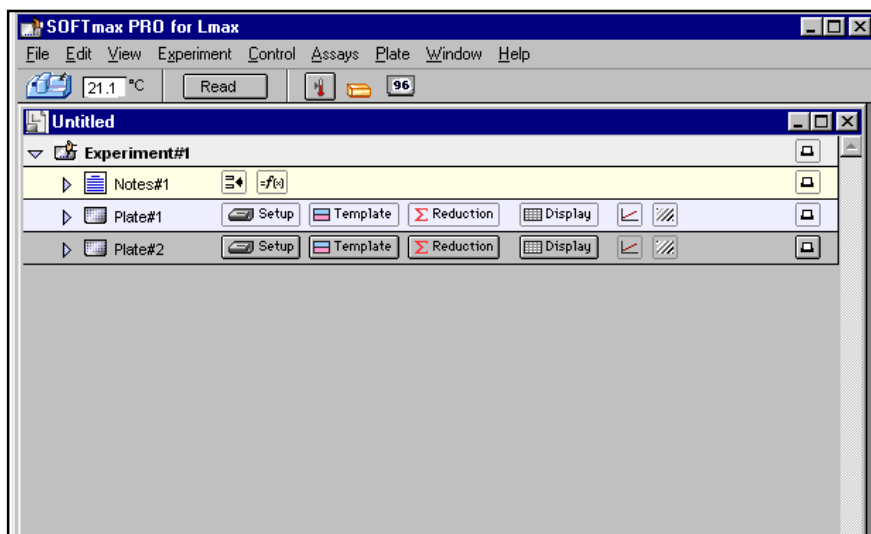


Figure 3.7: Result of Minimizing an Experiment (Tool Bars Hidden)

You may also open a section in a separate window. Double-clicking the section icon (in the tool bar) or selecting **New Window** from the active section menu (either **Plate**, **Graph**, **Notes**, or **Group**) causes the currently active section to open as a separate window (a copy of the tool bar still remains in the original experiment, but the section is closed; clicking the closed section places the new window behind the main window (the original section remains closed). Clicking on the triangle in the section tool bar closes the new window and causes the section in the experiment to open (and closing the separate window opens the section within the experiment again). All of the tool bar options applicable to the section are available in the separate window (except the triangle indicator).

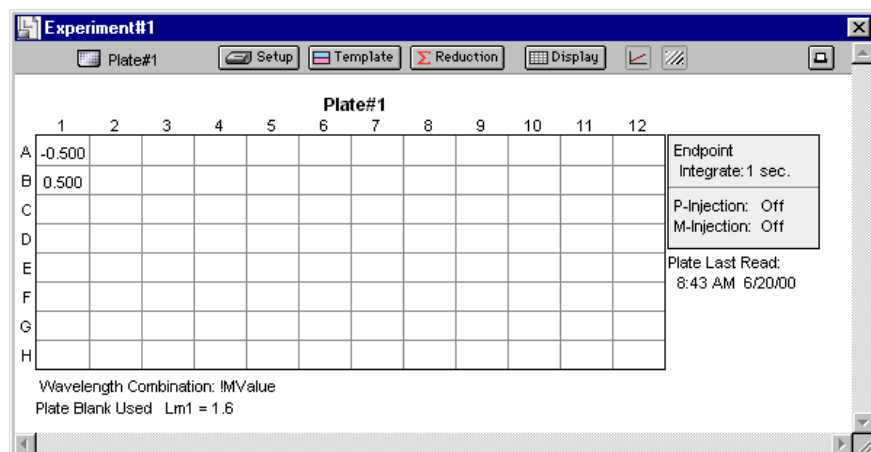


Figure 3.8: Separate Section Window



Notes Section

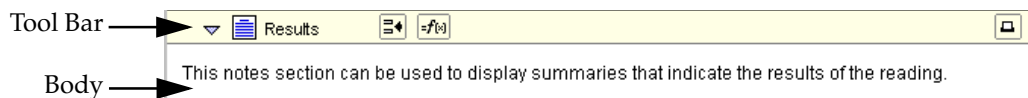


Figure 3.9: Example Notes Section, Named “Results.”

The Notes section is used to record information that pertains to the experiment. You can enter text of any kind and add summaries containing reduction formulas.

The Notes section is divided into two areas:

- The tool bar
- The body

You may create multiple Notes sections within the same experiment.

The tool bar contains the following items (shown with their tool bar or mouse equivalents). These menu items and buttons are active only when a Notes section is active.

Table 3.1: Notes Section Menu Commands and Tool Bar Buttons





Menu Command/ Mouse Equivalent	Description	Tool Bar Button
Notes section icon	Opens the Notes section in a new window	 (double-click)
Name of Notes section	Opens the Section dialog box	Introduction (double-click)
New Window in the Notes menu	Opens the section in a new window	
Section Name in the Notes menu	Opens the Section Name dialog box	
Create Summary in the Notes menu	Opens the Calculation dialog box, allowing you to create a summary	
Edit Summary in the Notes menu or double-click the summary (pointer changes to double-headed arrow)	Opens the Calculation dialog box, allowing you to edit the summary	
Show/Hide Formulas in the Notes menu	Shows/hides the calculations used to derive summaries	
Printer icon	Includes/excludes section from printed report	



Plate Section

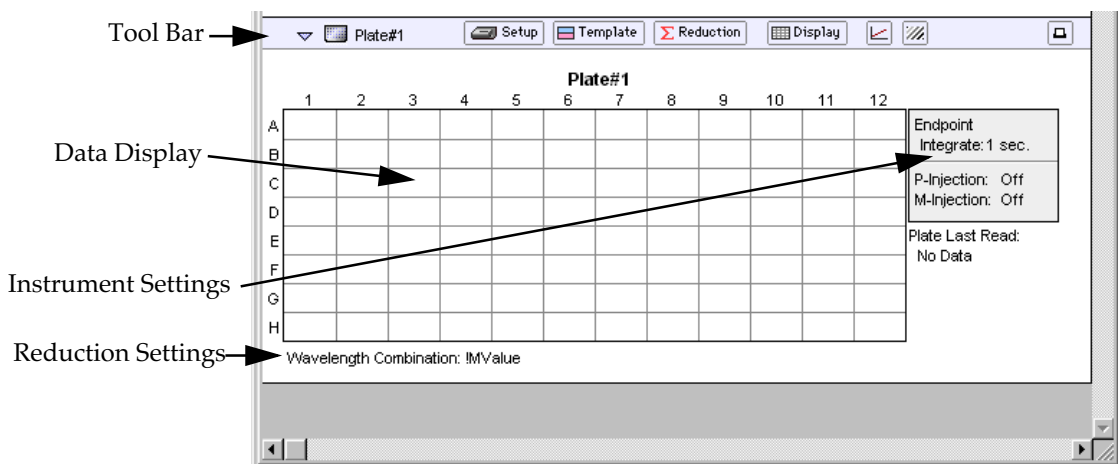


Figure 3.10: Plate Section.

The Plate section is used to collect data from the instrument, define how the data is displayed, and what the initial reduction of the data will be. In addition, the Template Editor in the Plate section is used to create a “map” of the contents of the microplate.

Choosing a particular function or command in the Plate section can be done in several ways. You can use the mouse to click on a button, choose a command from one of the drop-down menus in the menu bar, or press a key-stroke combination to activate a command or function.

The Plate section is divided into four areas:

- Tool bar
- Data display (shown in a microplate grid format)
- Instrument settings, shown to the right of or below the data display, depending on the plate type and printer settings
- Reduction settings, shown to the right of or below the data display, depending on the plate type and printer settings


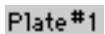




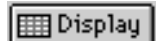


When the grid in the Plate section shows colors, this means that a template has been defined for this Plate section. Each group defined in the template has a different color (the icon of the corresponding group table will have the same color).

If your experiment requires multiple plates, you can create as many Plate sections as needed.

The **Plate** menu contains the items shown in Table 3.2 (shown with their tool bar or mouse equivalents). These menu items and buttons are active only when a Plate section is active.



Table 3.2: Plate Section Menu Commands and Tool Bar Buttons

Menu Command/ Mouse Equivalent	Description	Tool Bar Button
Plate section icon	Opens the Plate section in a new window	 (double-click)
Name of Plate section	Opens the Section dialog box	 (double-click)
Instrument Setup... in the Control menu	Opens the Instrument Settings dialog box for this plate	
Template... in the Plate menu	Opens the Template dialog box allowing you to create or edit the template	
Reduction... in the Plate menu	Opens the Reduction dialog box containing reduction settings	
Double-click in a single well or when several wells are selected; Graph in the Plate menu	Enlarges selected well(s) to a Well Graph display (kinetic mode only); choosing Graph again returns to regular display (toggle)	
Display... in the Plate menu	Opens the Display dialog box containing display choices	
Mask in the Plate menu	Masks the selected wells	
Printer icon	Includes/excludes section from printed report	
Section Name... in the Plate menu	Opens the Section dialog box, allowing you to rename the section	
New Window in the Plate menu	Opens the section in a new window	
Copy Template in the Plate menu	Copies the template, allowing it to be pasted to another Plate section	
Paste Template in the Plate menu	Pastes a previously copied template to a new Plate section	
Export Template in the Plate menu	Opens the Export Template to... dialog, allowing you to export a template to an ASCII text file	
Import Template in the Plate menu	Opens the Import Template to... dialog, allowing you to import a template from an ASCII text file	



Group Section

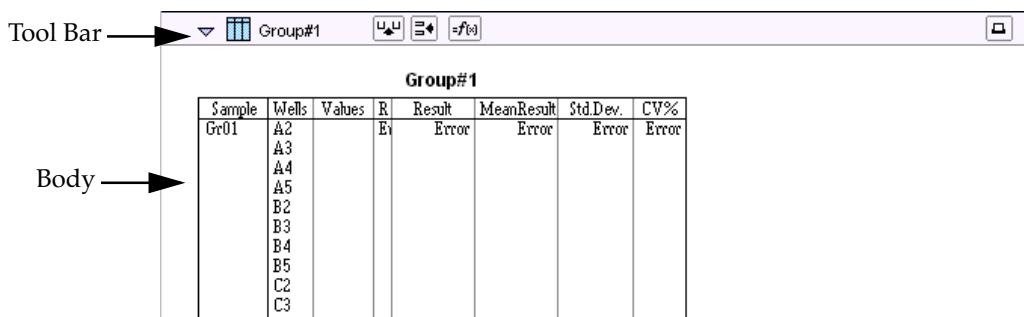


Figure 3.11: Group Section (example shown prior to collecting data)

Group sections are created automatically when you create or select groups for the experiment in the Template Editor. A Group section is a table which shows all of the information for a particular group. A group is a set of associated samples which have been created in the Template. Depending on the default assay chosen, certain group types (such as Standard or Unknown) may have been created; you can create others as required. Group sections may also be created by duplicating existing Group sections.

The Group section is divided into three areas:

- The tool bar
- The body of the Group table
- The summaries

The **Group** menu contains the following items (shown with their tool bar or mouse equivalents). These menu items and buttons are active only when a Group section is active.

Table 3.3: Group Section Menu Commands and Tool Bar Buttons






Menu Command/ Mouse Equivalent	Description	Tool Bar Button
New Window in the Group menu	Opens the Group section in a new window	 (double-click)
Name of Group section	Opens the Group settings dialog box	Group (double-click)
Group Settings in the Group menu	Opens the Group Settings dialog box	
Create Summary in the Group menu	Opens the Calculation dialog box, allowing you to create a summary	
Create Column in the Group menu	Opens the Calculation dialog box, allowing you to create a column	



Table 3.3: Group Section Menu Commands and Tool Bar Buttons

Menu Command/ Mouse Equivalent	Description	Tool Bar Button
Edit Summary in the Group menu or double-click the summary (pointer changes to double-headed arrow)	Opens the Calculation dialog box, allowing you to edit the summary	
Edit Column in the Group menu or double-click the column	Opens the Calculation dialog box, allowing you to edit the column	
Show/Hide Formulas in the Group menu	Shows/hides the formulas used in columns and summaries	
Autosize in the Group menu	Enlarges or reduces column size to fit the largest piece of text	
Hide/Show All in the Group menu	Hides one or more selected columns or shows all columns that were hidden	
Hide Replicates in the Group menu	Hides data from all replicates	
Printer icon	Includes/excludes section from printed report	



Graph Section

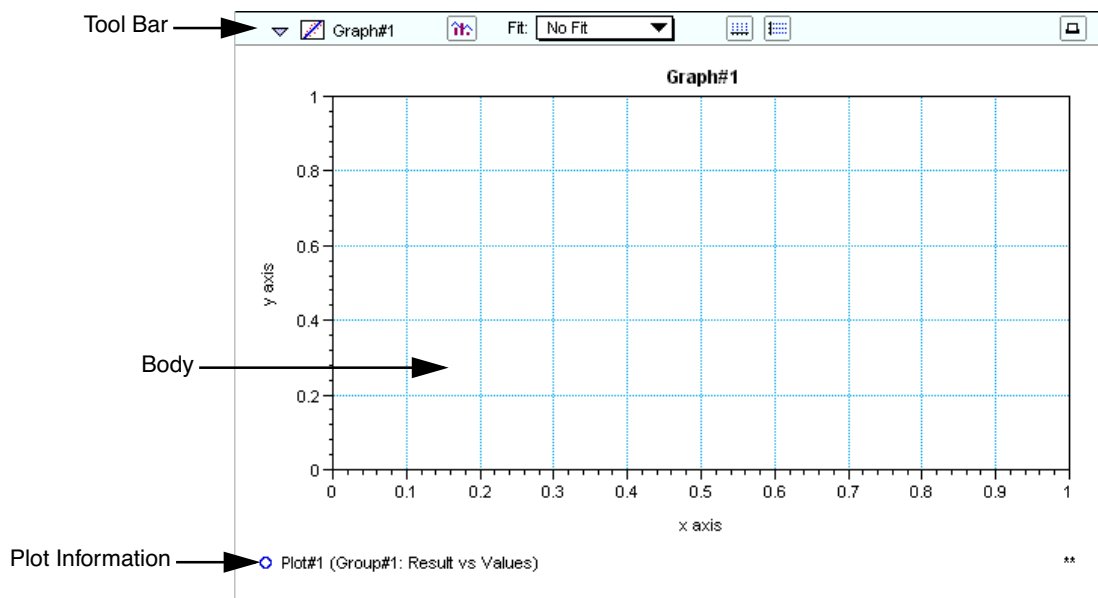


Figure 3.12: Graph Section (example shown before data collection)

To create a graph section, choose **New Graph** from the **Experiment** menu. The Plots dialog box opens automatically and a default plot called “Plot#1” is selected. The plot will default to the first group available in the experiment.

Once a graph has been created, new plots can be added and deleted, the axes can be customized, and the size (displayed area) of the graph can be changed. Grid lines can be enabled or disabled (default is enabled). Graphs may be displayed as bar graphs or scatter plots.

The Graph section is divided into three areas:




- The tool bar
- The body of the Graph section
- Plots information below the graph

You may create more than one Graph section within an experiment. Plots in the Graph section must be defined from groups within the same experiment section as the graph, with one exception: if you *duplicate* an experiment that contains data in the Plate section and has a plot defined in a Graph section, that plot will remain in the duplicated Graph section (and new plots may be added).



The **Graph** menu contains the following items (shown with their tool bar or mouse equivalents). These menu items and buttons are active only when a Graph section is active.

Table 3.4: Graph Section Menu Commands and Tool Bar Buttons

Menu Command/ Mouse Equivalent	Description	Tool Bar Button
New Window in the Graph menu	Opens the Graph section in a new window	(double-click)
Name of Graph section	Opens the Section dialog box	Graph (double-click)
Graph Settings in the Graph menu	Opens the Graph Settings dialog box	
	Drop-down menu allows you to choose a curve fit	
X Axis... in the Graph menu	Opens the X Axis Settings dialog box	
Y Axis... in the Graph menu	Opens the Y Axis Settings dialog box	
Printer icon	Includes/excludes section from printed report	
Graph Options in the Graph menu	Opens the Graph Options dialog box	
Export Graph in the Graph menu	Opens the Export Graph As... dialog box	



Instrument Settings

You must specify the type of reading to be performed prior to actually reading a microplate.

Instrument settings allow you to choose a read mode (endpoint, dual read, long kinetics, or fast kinetics), timing for a kinetic run, and so on.

Complete information regarding instrument settings can be found in Chapter 4, “Reading Microplates.”

Menus and Commands

The menu bar for SOFTmax PRO for Lmax is located at the top of the window (just beneath the title bar).

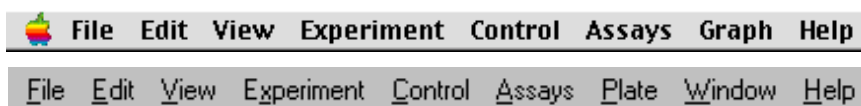


Figure 3.13: Menu Bar (Macintosh menu top, Windows menu bottom)

You may access SOFTmax PRO tools and features in several ways: menus, tool bars, dialog boxes, and keystrokes. Figure 3.14 shows all of the menu items that are available in SOFTmax PRO for Lmax.

When menu items are unavailable, they are dimmed (shown as gray rather than black)—available choices depend on the action you are performing with the software. An ellipsis (three dots) following a menu item indicates that choosing this item will bring up a dialog box. Menu items followed by a triangle are hierarchical menus: choosing such an item causes a submenu to appear to the right of the original item. Certain menu items can also be chosen by pressing keys on the keyboard.



Within Windows, menu items contain an underlined letter in their names. Pressing the ALT key will activate the menu bar; pressing a letter key that corresponds to the underlined letter of a menu name will then activate that menu. Subsequent menu items can be activated (chosen) by simply pressing the underlined letter in their names (you do not need to press ALT again). For example, to open a file from within SOFTmax PRO for Lmax using the keyboard shortcut, you would press ALT, F, O which will first activate the menu bar, then select the **F**ile menu, then choose **O**pen from the **F**ile menu.



When available, a shortcut key combination will be shown to the right of the command in the menu. On the Macintosh, begin by pressing and holding down the command (⌘) key followed by the key shown in the shortcut. Windows users should press and hold the control (CTRL) key followed by the key shown in the shortcut.

Some menus are divided into groups of items that perform related functions. Dividers are shown as thin, horizontal lines between menu items.

File. Items in this menu relate to entire SOFTmax PRO for Lmax files. Commands to open, print, apply password protection, and save files are located here.



Edit. This menu contains features for changing items within SOFTmax PRO for Lmax. Commands to select, copy/paste, delete and duplicate items are located here. This menu also contains the **Preferences** command, which allows you to set options regarding printing, communication with the Lmax, and saving files.

View. Choices regarding what is shown and how it is shown are available here. The last section in this menu provides a navigational tool, allowing you to choose the particular section you would like to view (a section chosen in this manner will open if it was closed and will be brought into view within the window).

Experiment. Choices in this menu allow you to create new sections within the current experiment, as well as to create a completely new experiment.

Control. Items in this menu allow you to set up and activate Lmax functions.

Assays. This menu allows you to choose the folder containing the protocols you have saved and shows those protocols below the divider, making it easy to open one or more of them. A second level of folders can be stored within the Assays folder and will appear on the Assays menu, allowing you to separate protocols further, as desired.

Notes/Plate/Group/Graph. The name of this menu item changes to reflect the *section type* that is currently active. If a Plate section is active, for example, the last menu item will be **Plate**. An active Graph section causes this menu to show **Graph**. If an experiment is selected, this menu will not be shown.



Window. Selections in this menu allow you to position the windows or icons on your desktop in different ways.



Apple. Displays the SOFTmax PRO for Lmax version, registration information, ROM version (if available from the instrument), and copyright information.

Additionally, a number of buttons are available in different sections of the SOFTmax PRO for Lmax experiment and section tool bars. Clicking these buttons accomplishes many of the same actions as choosing menu items.

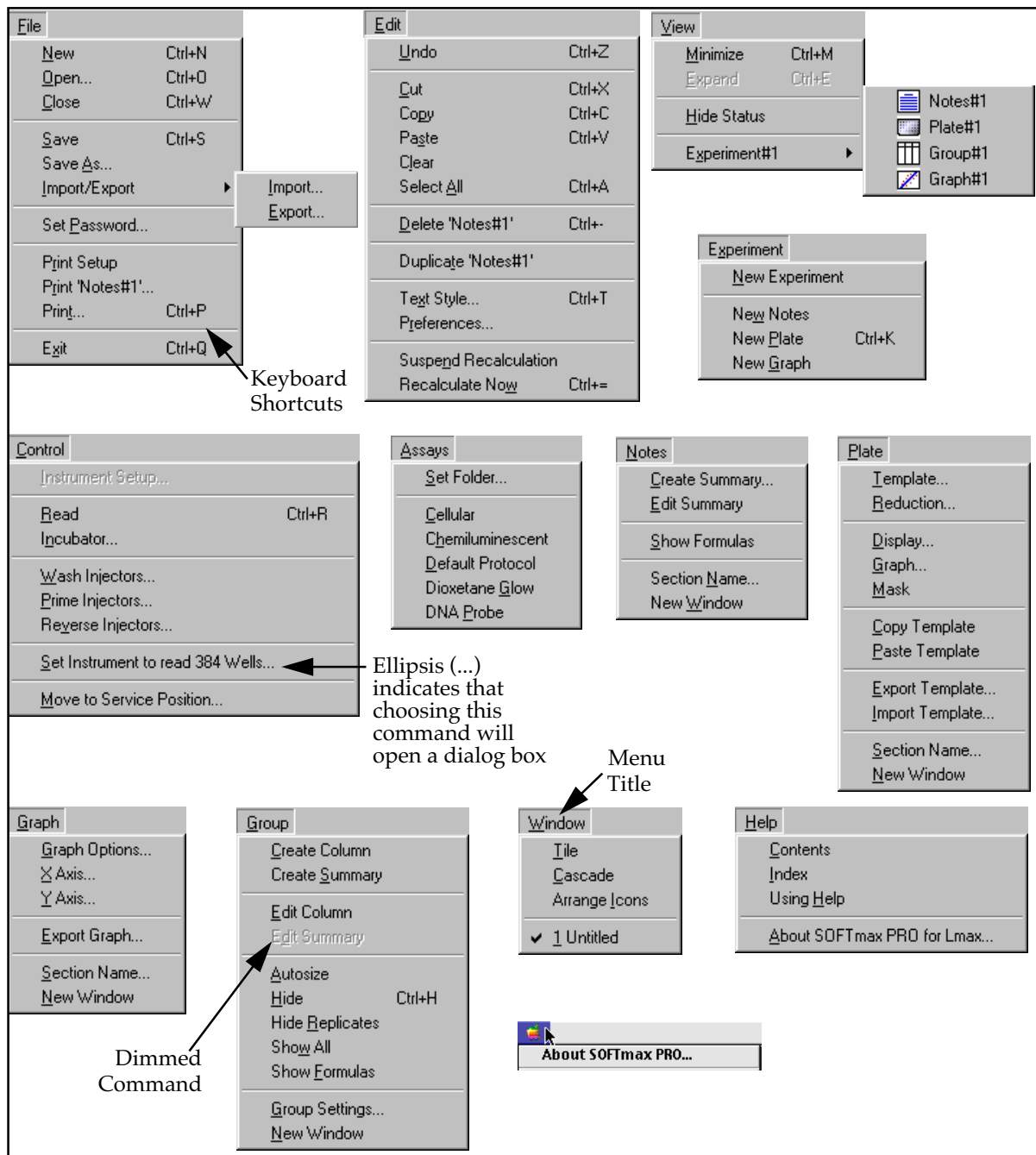


Figure 3.14: SOFTmax PRO for Lmax Menus (Windows version)



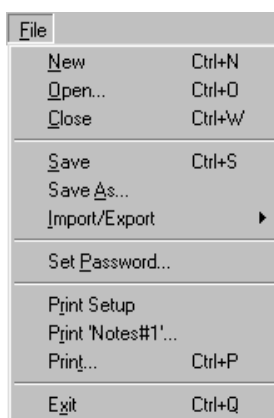
Apple Menu



About SOFTmax PRO for Lmax

(*Macintosh only*) Displays the SOFTmax PRO for Lmax version, registration information, ROM version (if available from the instrument), and copyright information. If an instrument is connected to the Macintosh running SOFTmax PRO for Lmax, the instrument type will also be displayed. You will need to know this information if you call for technical support. Other items in the Apple menu depend on the settings of your Macintosh.

File Menu



New... (⌘N; CTRL+N)—Opens a new file window, allowing you to begin a new or different data file. Information from each SOFTmax PRO for Lmax window (which can consist of one or more experiments with their sub-sections) is saved to a separate file.

Open... (⌘O; CTRL+O)—Opens an existing SOFTmax PRO for Lmax file (or an older SOFTmax file). When you choose **Open** from the **File** menu, a dialog box appears. To open a file from the list of available files, double-click the filename to be opened, or click once on the filename and then click the **Open** button.

Close (⌘W; CTRL+W)—Closes the front-most (active) window. If you have only one file open, this file will be closed. If you have changed the file in any way since it was opened, a dialog box will open asking if you want to save the file before closing. (This menu item is dimmed if no window is open.)

Save (⌘S; CTRL+S)—Saves the active window to a file. The active file name is shown at the top of the status window (protocol files are always shown as “Untitled”). To save the file in a different folder/subdirectory or under a different name, use **Save As...** If the file has not yet been saved and given a name (is “Untitled”), the **Save As...** dialog box will appear and you will be asked to name the file. (This menu item is dimmed if the file has already been saved and no changes have been made.)

Save As...—Saves a file with a new file name or into a new folder or subdirectory. See Chapter 6, “Saving Files Manually,” for more information about using the **Save As...** command for saving files.

Import/Export...—**Import** brings tab-delimited ASCII text file information into SOFTmax PRO for Lmax and **Export** creates a file of Plate section and/or Group table section data. All information from SOFTmax PRO for Lmax can be saved as tab-delimited ASCII (or text) format, which is suitable for importing into word processing or spreadsheet programs.

After choosing **Import**, a dialog box will appear asking you to choose the file you wish to import. Files to be imported must contain specific header information. For more information on importing files into SOFTmax PRO for Lmax, see “Exporting/ Importing Information” on page 6-16.



After choosing **Export**, a dialog box will appear, asking you to name the file and choose a location where the file should reside. In addition, the dialog box will ask you to choose which sections should be exported (you can choose to export all or some Plate or Group table sections).

The data that is exported out of the Plate section will be the same data currently displayed in that Plate section: either raw or reduced data.

One additional parameter that affects export function is located under **Preferences** in the **Edit** menu. You may choose to export the data in plate format (8 × 12 array of numbers for a 96-well plate, for example) or in time format (a single line or row). You may also choose for the exported file to be created with or without labels.

Set Password—Allows you to enable password protection for the file to protect it from unauthorized or unintentional changes.

Page Setup/Print Setup—You should access this command after you select a different printer in the Chooser (Macintosh), if you choose a different printer using Windows, or if you want to use a different paper size or printing option. Choosing **Page Setup/Print Setup** opens a dialog box with various options for printing. More about this function can be found in the documentation that accompanies your computer and/or printer.

NOTE: The **Page Setup/Print Setup** command also controls how SOFTmax PRO for Lmax sections are displayed on your monitor (the width of the display corresponds to the width of the page size).

Print (Section name/Selection)—Allows you to print just the active section(s).

Print... (⌘P; CTRL+P)—With the Macintosh, choose this command and then click either “Print all” or “Print report.” Print all sends all sections to the printer in the order they appear in the window. Print report sends those sections selected to be in the report to the printer in the order they appear in the window. From Windows, choose **Print** and then specify a page range or leave the default to print all pages.

NOTE: Make sure you have selected the correct printer prior to choosing one of the Print options in the **File** menu. If you start to print and discover the wrong printer is selected, choose **Cancel** to exit from the Print dialog box, select the correct printer (using the Chooser or the **Print Setup** command), and then repeat the printing process.

Quit (⌘Q; CTRL+Q)—Closes SOFTmax PRO for Lmax as well as any SOFTmax PRO for Lmax files you may have open. If you have open files that have changed and have not yet been saved, a dialog box will ask if you want to save them before quitting.



Edit Menu

Edit	
Undo	Ctrl+Z
Cut	Ctrl+X
Copy	Ctrl+C
Paste	Ctrl+V
Clear	
Select All	Ctrl+A
Delete 'Notes#1'	
Duplicate 'Notes#1'	
Text Style...	Ctrl+T
Preferences...	
Suspend Recalculation	
Recalculate Now	Ctrl+=

Undo (⌘Z; CTRL+Z)—Restores the last text operation to the condition that existed before the editing was done (used primarily when entering text in Notes sections). This menu item is dimmed if no actions have been taken or if undoing the last action is not possible.

Cut (⌘X; CTRL+X)—Removes highlighted information (text or data) from the document and stores it on the clipboard so it can be pasted elsewhere. (This menu item is dimmed if no selection has been made or if the selection cannot be cut.)

Copy (⌘C; CTRL+C)—Copies selected (highlighted) text or data and stores it on the clipboard so it can be pasted elsewhere. (This menu item is dimmed if no selection has been made or if the selection cannot be copied.)

Parts of an experiment can be copied within the same experiment or to another application using the Copy and Paste commands. You may also use this command to copy information from SOFTmax PRO for Lmax into other programs.

Paste (⌘V; CTRL+V)—Pastes information from the clipboard just to the right of the insertion point in the document. Can also be used to import tab-delimited data into SOFTmax PRO for Lmax. (This menu item is dimmed if nothing has been copied to the clipboard.)

Clear—Clears or erases highlighted text (or data) without storing it on the clipboard. (This menu item is dimmed if nothing has been selected.) You can remove one or more columns from a Group table, or text or formulas from a Notes section, by highlighting the column or text/formula and then choosing **Clear** from the **Edit** menu. Columns and text or formulas can also be removed by highlighting them and pressing the Delete key.

Select All (⌘A; CTRL+A)—The **Select All** command can be used in Notes, Plate, and Group sections when a section is active (it is not available in Graph sections).

In a Notes section, if no summaries are present, it will select all text. If summaries are present at the top or bottom of the Notes section, this command will select all text but not the summaries (which must be selected individually). If the text is broken up by summaries, this command will select the largest block of text that it can (bounded by summaries).

In a Plate section, this command will select all wells, if one or more wells in the grid array has already been selected. If no wells have been selected, this command will be dimmed.

In a Group section, this command will select all columns in the group table but will not include any summaries at the bottom of the group table (summaries must be selected individually).

Delete Section/Selection (⌘ - ; CTRL+ -)—Deletes the currently active section(s) or experiment(s). A warning dialog box will appear asking if you really want to delete the section(s) or experiment(s).

Duplicate Section/Experiment—Creates new section(s) or experiment(s) that are exactly like the active section(s) or experiment(s) and positions the duplicated selection(s) after the currently active section(s). The names



of the new section(s) or experiment(s) will have the word “Copy” appended to their name(s). For example, if you duplicate a section named “Plate#1,” the duplicate section will be called “Plate#1Copy.” Subsequent duplications will append an incremented number after the name (e.g., “Plate#1Copy#2”). For more information about this command and what is included when a section is duplicated, see “Duplicate and New” on page 6-24.

Text Style... (⌘T; CTRL+T)—Allows you to change the attributes of selected text. (This command is dimmed if no text has been selected.)

Preferences... (double-click the instrument icon)—Through the Preferences dialog box, you can choose settings for the following options:

- Serial port connection (modem port or printer port for the Macintosh; COM1 or COM2 for the PC) that will be used to connect to the Lmax. A third option, “Neither,” should be used when no instrument is connected to the computer.
- Export format (Time versus Plate, and whether or not to include labels).
- Whether or not files will be saved automatically and the location where saved files will be stored (Autosave).
- Whether or not reports should be printed automatically (Auto-print).

Suspend Recalculation—Stops SOFTmax PRO for Lmax from performing calculations based on changes you make to the settings or other program information. This command is used most often when you want to make a number of changes to the settings without having to wait while the results of each change are recalculated.

Recalculate Now (⌘=; CTRL+=)—Causes SOFTmax PRO for Lmax to recalculate data based on the current settings. This command is used most often when you have suspended automatic recalculation.



View Menu



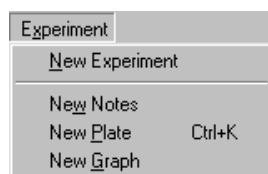
The View menu provides three commands: **Minimize** (⌘M; CTRL+M), **Expand** (⌘E; CTRL+E), and **Hide Status**. In addition to these choices, the names of the experiments appear at the bottom of the menu (sections within experiments are shown as submenus), allowing you to go to a specific experiment or section within an experiment by choosing the item. If the section was closed, selecting it from the **View** menu will open and activate it. The currently active section is shown in the menu with a checkmark next to it.

Minimize (⌘M; CTRL+M)—If you select **Minimize** while a section within an experiment is active, the sections within that experiment are minimized. If you choose **Minimize** while an experiment tool bar is active, all experiments will be minimized (sections within are hidden and only the experiment tool bars are visible).

Expand (⌘E; CTRL+E)—If you select **Expand** while a section within an experiment is active, the sections within that experiment are expanded. If you choose **Expand** while an experiment tool bar is active, all sections within all experiments become visible (the sections may be minimized or expanded).

Hide Status/Show Status—Hides or displays the status bar.

Experiment Menu



The Experiment menu provides five commands: **New**, **New Notes**, **New Plate**, and **New Graph**.

New—Creates a new experiment directly following the active experiment (the new experiment then becomes the active experiment). All sections shown in the experiment that was active when this command is chosen are duplicated in the new experiment.

New Notes—Creates a new Notes section below the active section.

New Plate—Creates a new Plate section below the active section. The new Plate section will be a copy of the active Plate section or, if no Plate section currently exists, the new Plate section will use SOFTmax PRO for Lmax default settings.


New Graph—Creates a new Graph section below the active section and brings up the Plots dialog box, allowing you to select the graph options.



Control Menu



Instrument Setup...—This command is available only if a Plate section is active. Choosing **Instrument Setup** opens the Instrument Settings dialog box which allows you to enter the mode for the run (endpoint, dual read, or kinetic) and the settings for pre-read blanking, strips, timing for kinetic runs, and plate type.

Button Equivalent:  in the Plate section tool bar.

Read—In a single plate experiment, the **Read** command reads the plate immediately. If no Plate section is active in an experiment containing multiple Plate sections, the **Read** command displays a dialog box asking you to select which Plate section to read. The default Plate section, selected by pressing the Return key, is chosen on these criteria:

- The selected Plate section, if one is selected.
- The next unread Plate section.
- The first plate, if all have been read.

Once a reading is in progress, menu items are no longer available until the reading is finished.

Button Equivalent:  in the Status bar.

Incubator—Allows you to turn the incubator on or off. When on, you can enter a setting for regulation of the temperature in the microplate chamber.

Button Equivalent:  in the Status bar.

Wash Injectors—Performs a manual wash of either the P or M injector, or both, for a specified number of injections (default is 30). Choosing this option causes a dialog box to appear which contains a note that reminds you to change the reagent bottles, if necessary. Choose the settings you desire and click the **Wash** button to start the wash process.

Prime Injectors—After changing reagents, you should prime the lines to remove any air that may be present and to ensure that the lines are filled with the new reagent. Choosing this menu option opens a dialog box that allows you to prime the P or M injector line, or both. You can choose the number of injections to perform to prime the line (the default is 7). The dialog box reminds you to change reagent bottles, if necessary, prior to starting the priming procedure.

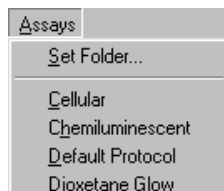
Reverse Injectors—Choosing this menu option allows you to send fluid from the lines back into the reagent bottle (for example, to preserve costly reagents). You can set this option for as many “injections” as desired (default is 7) through either the P or M injector, or both.

Set Instrument to Read 384 (96) wells ...—One of two settings that need to be changed in the SOFTmax PRO for Lmax software in order to switch from 96- to 384-well capability and vice versa (the other setting is found in the Instrument Settings parameters). Additionally, a hardware reconfiguration must be made to change from one plate size to the other. See the *Lmax User's Manual* for more information.



Move to Service Position ...—Moves the read head to the service position, allowing you to perform service-related activities such as changing the injector tips. More about such functions is included in the *Lmax User's Manual*.

Assays Menu

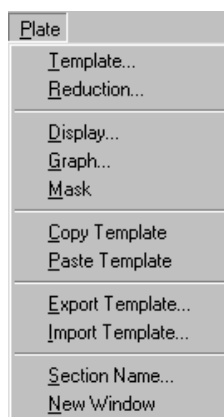


Set Folder...—Allows you to select a folder or subdirectory containing commonly used protocol files. In addition to any protocol files residing within this folder (which will be listed below the dividing line and may be opened by clicking them), an additional level of subfolders (if any) will be shown below a center division. Protocol files can be used repeatedly (for specific types of assays, for example). These files can be named in any way you choose.

Folder name(s) after dividing line—Choosing any of these folders will open a submenu showing protocol files residing in this subfolder. Select one of the protocol files to open it.

File name(s) after dividing line—The files that appear here are protocol files that reside within the folder determined using the **Set Folder...** command. You can quickly open and use one of these protocol files by selecting it from the **Assays** menu.

Plate Menu



This menu item changes to show Plate, Notes, Graph, or Group, depending on the type of section that is currently active. If no section is active, this menu item does not appear.


Template...—Opens the Template Editor.

The Template Editor allows you to describe the samples that are in the wells, thus providing the link between the raw data and the analysis groups. You can create new groups in the template or assign wells to already established groups.

If your experiment contains multiple Plate sections, the template for each Plate section must be defined using the Template Editor in that section. You can create a new template or modify an existing one using the Template Editor.

NOTE: Each Plate section requires that a template be created in order to identify groups within that plate. However, two or more Plate sections may use the same template, or the same groups may be used in different templates.

After groups are defined in the template and you return to the Plate section, the data display will show the group areas in different colors.

Button Equivalent:  **Template** in the Plate section tool bar.

Reduction...—Opens the Reduction dialog box. Options available in the Reduction dialog box depend on the type of reading performed (end-point, dual read, or kinetic). The Reduction dialog box is used for initial reduction of the data collected from the microplate reader. The luminescence information collected is reduced to a single number per




well. More information regarding reduction options can be found in Chapter 5, “Displaying and Analyzing Data.”

Button Equivalent:  in the Plate section tool bar.


Display...—Opens the Display dialog box. Allows you to decide how the information in the Plate section will be displayed. More information regarding display options can be found in Chapter 5, “Displaying and Analyzing Data.”

Button Equivalent:  in the Plate section tool bar.

Graph...—The Well Graph command can be activated both during and after data acquisition with a kinetic reading. You can get a “close up” view of the plot shown in one or more wells by first selecting the well(s) and then choosing the **Graph...** command. (This command is dimmed if the instrument settings are for an endpoint reading or if no well in the Plate display has been selected.)

Button Equivalent:  in the Plate section tool bar.

Mask—Allows you to “hide” certain wells so that they will not be taken into account when performing analyses. (This command is dimmed if no data has been acquired or if no wells in the plate have been selected.)

Button Equivalent:  in the Plate section tool bar.

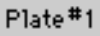
Copy Template—While a Plate section is open and active, choosing this command allows you to copy the template from this Plate section to the Clipboard, allowing you to paste it into a different Plate section (in the same or in a different experiment or into a Plate section in another SOFTmax PRO for Lmax file).

Paste Template—If you have copied a template from a Plate section, you can then create or activate/open another Plate section and choose **Paste Template**. In this way, you can use the same template for more than one Plate section or you can modify the pasted template as desired. If you are pasting a template into a new experiment and groups from the source template do not exist in the destination experiment, pasting the template will create them. Existing groups of the same name will not be changed by the pasting process. For example, if the template being copied contains the groups “Standards” and “Unknown,” and the group “Standards” already exists in the destination experiment, the group “Unknown” will be created but all information regarding the group “Standards” in the destination experiment will remain unchanged. If you copy and paste the template with the same experiment, no new groups are created. Group names can be edited in the pasted template (these changes will be reflected in the original template also).

Export Template—Opens the Export Template to... dialog, allowing you to export a template to an ASCII text file.


Import Template—Opens the Import Template to... dialog, allowing you to import a template from an ASCII text file.

Section Name...—Allows you to change the name of the Plate section.

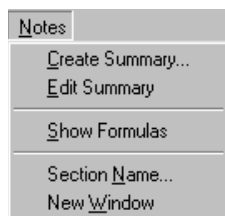
Mouse Equivalent: double-click the name of the Plate () in the Plate section tool bar (to the right of the Plate section icon).



New Window—Opens the active section in a separate window.

Mouse Equivalent: double-click the  icon in the Plate section tool bar.

Notes Menu




This menu item changes to show Plate, Notes, Graph, or Group, depending on the type of section that is currently active. If no section is active, this menu item does not appear.

Create Summary...—When the Notes section is active, choosing **Create Summary** opens a dialog box allowing you to give a title to a new summary and then enter a formula to be used for the summary.

Button Equivalent:  in the Notes section.

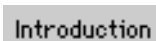
Edit Summary—If you have created a summary and need to change it, you can do so by first highlighting the summary you wish to edit and then choosing **Edit Summary**. (This command is dimmed if you have not yet created a summary or if the summary has not been selected.) The dialog box then appears showing the name and formula.

Mouse Equivalent: Double-clicking the summary performs the same function.


Button Equivalent:  in the Notes section.

Show Formulas—Choosing this command shows the formulas associated with the summaries you have created in the Notes section. After choosing **Show Formulas**, the menu name changes to **Hide Formulas**. (This command is dimmed if you have not created any summaries.)

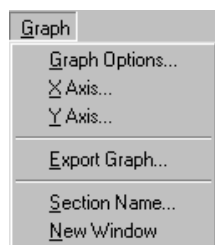
Section Name...—Allows you to change the name of the Notes section.

Mouse Equivalent: double-click the name of the Notes section () to the right of the Notes section icon.

New Window—Opens the active section in a separate window.

Mouse Equivalent: double-click the  icon in the Notes section.

Graph Menu



This menu item changes to show Plate, Notes, Graph, or Group, depending on the type of section that is currently active. If no section is active, this menu item does not appear.

Graph Options...—You can select from three types of graphs: Scatter (with symbols plotted and/or the points connected), Cluster Bar, or Stack Bar and you can set the height of the graph to be something other than the default percentage (100%). The font used for the title, legend, and/or axes can be changed and/or modified in size and style. Plots can be added, deleted, or edited. Editing plots allows you to change the plot name, its source (from which experiment and group), as well



as the items to be plotted (for example, concentration vs. values).

Button Equivalent:  in the Graph section.

X-axis...—Sets the X-axis parameters (tick marks, scale, label, gridlines, and autorange).

Button Equivalent:  in the Graph section.

Y-axis...—Sets the Y-axis parameters (tick marks, scale, label, gridlines, and autorange).


Button Equivalent:  in the Graph section.

Section Name...—Allows you to change the name of the Graph section.

Mouse Equivalent: double-click the name of the Graph section (**Standard Curve**) to the right of the Graph section icon.

Export Graph—Creates a file containing a picture of the graph with a filename of your choice. On the Macintosh, the graphic is in PICT format; in Windows, the graphic is in EMF (Enhanced MetaFile) format.

New Window—Opens the active section in a separate window.

Mouse Equivalent: double-click the  icon in the Graph section.

Group Menu



This menu item changes to show Plate, Notes, Graph, or Group, depending on the type of section that is currently active. If no section is active, this menu item does not appear.

Create Column—If a Group section is currently active, you can add a column to the display by choosing this command. If no columns are highlighted, the column will be added at the end of the group. If you select a column first, the new column will be added to the right of the highlighted column.

Button Equivalent:  in the Group section.

Create Summary—Allows you to create a summary and enter a formula for this summary for the Group. Summaries appear at the bottom of the group table.

Button Equivalent:  in the Group section.

Edit Column—Selecting a column makes this command active. Choosing **Edit Column** allows you to change the formula associated with the column and to change the column name.

Mouse Equivalent: Double-clicking the column performs the same function.

Button Equivalent:  in the Group section.

Edit Summary—Allows you to edit the formula associated with the summary, as well as to change the summary name. (This command is dimmed if a summary has not yet been created.) First highlight the summary and then select this command.

Mouse Equivalent: Double-clicking the summary performs the same function.

Button Equivalent:  in the Group section.



Autosize—Choosing this command expands or shrinks the width of selected columns to accommodate the largest piece of text in the column.
 Mouse Equivalent: Double-clicking the line to the right of the column heading performs the same function.

Hide—Hides the selected column from view. (This command is dimmed if a column has not been selected.)
 Mouse Equivalent: Dragging the line to the right of the column heading until it is on top of the line to the left of the column performs the same function.

Hide Replicates—Hides data for all replicates (if any) from view.


Show All—Shows all columns or replicates that were hidden previously.

Show Formulas—Causes a new row to appear beneath the headings of each column showing the formulas used to determine the data in the columns. After choosing this command, the menu name changes to **Hide Formulas**.

Group Settings—Opens the Group Settings dialog box, allowing you to rename a group and /or change the sample or group descriptors used, as well as to modify the units used with each descriptor.

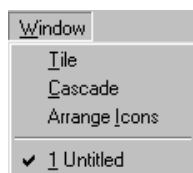
Mouse Equivalent: double-click the name of the Group section (**Standards**) to the right of the Group section icon.

New Window—Opens the active section in a separate window.

Mouse Equivalent: double-click the  icon in the Graph section.



Window Menu



Tile—If you have several windows open, choosing the **Tile** command will resize and arrange all open windows so that they are all visible at the same time (generally side by side).

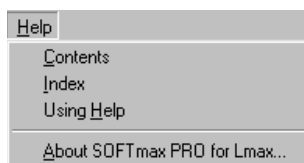
Cascade—If you have several windows open, choosing the **Cascade** command will resize and layer the open windows so that each title bar will be visible.

Arrange Icons—If you have several files open but reduced to icons, choosing the **Arrange Icons** command will move all icons to the bottom of the SOFTmax PRO for Lmax window and put them in order.

Beneath the line will appear the names of all open files within SOFTmax PRO for Lmax. The active file will have a checkmark next to it. Choosing one of the file names shown here will cause that file to come to the forefront of the screen (and will restore the file to its previous size if it had been minimized).



Help Menu—Windows



Contents—Displays the contents of the Help screens, allowing you to choose from the listed topics. Clicking a topic opens the help screen associated with it.

Index—Shows the alphabetical listing of all items that can be viewed within the Help function. Clicking a topic opens the help screen associated with it.

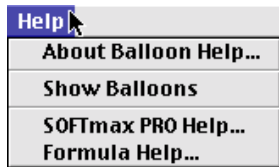


Using Help—Allows you to choose from an indexed list of topics or to search for the type of help you're looking for.

About SOFTmax PRO for Lmax—Brings up the About screen (splash screen) which gives the serial number, ROM version, and the instrument type connected to the computer, as well as the software version for SOFTmax PRO for Lmax.



Help Menu—Macintosh



About Help...—Brings up a dialog box explaining how to use the Apple Guide which gives detailed information about the help function on the Macintosh.

Show/Hide Balloons—Depending upon whether or not you have balloon help turned on or off, this menu presents the choice to reverse the setting.

SOFTmax PRO Help...—Opens the help section devoted to SOFTmax PRO for Lmax. Items are presented in alphabetical order.

Formula Help...—Opens the formula help section which provides information about how to use formulas, including formula examples.

Interprocess Messaging



(This feature is available on the Windows Operating System only)

Many operations found in SOFTmax PRO for Lmax can be controlled by other Windows applications by means of an ASCII string-based messaging system. Commands are available for opening and saving files, controlling instruments, selecting sections, exporting data from Plate and Group sections, and importing Templates. The commands for interprocess messaging are described in Appendix A, "SOFTmax PRO for Lmax Remote Command Language," along with examples for programming these commands.



Chapter 4: Reading Microplates

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Introduction

Reading a microplate using SOFTmax PRO for Lmax is a three-part process:

- Prepare the software to collect data;
- Prepare the microplate(s) to be read; and
- Read the microplate(s).

Before reading a microplate, you must create the Plate section(s) and define the instrument settings for the reading. *Instrument Settings must be defined prior to collecting data and may NOT be changed after data is collected.* In addition, it is recommended that you define a template, the reduction parameters, and display parameters prior to reading the microplate, as these parameters determine how data is displayed and analyzed, but these last parameters may be set up and/or modified after data collection.

A typical process for preparing, collecting, and analyzing data is as follows:

- 1) Start SOFTmax PRO for Lmax.
- 2) Create Plate section(s) as needed.
- 3) Define instrument settings (use the button in the Plate section tool bar or the command in the **Control** menu).
- 4) Define the template, reduction parameters, and display parameters.
- 5) Prepare the microplate to be read and place it in the Lmax.
- 6) Initiate the reading.
- 7) Save the data file.
- 8) Modify the template, reduction parameters, or display parameters as you wish them to be (or, if you didn't set them up in Step 4, do so now).
- 9) Save the data file.
- 10) Analyze the data using the Group section tables and Graph sections.
- 11) Save the data file when you are finished.

This chapter discusses how to define instrument settings, create a template, and initiate a reading. Data analysis is covered in Chapter 5, "Displaying and Analyzing Data."



Instrument Settings

You must specify the instrument settings prior to actually reading a microplate. These settings include:

- The read mode—endpoint, dual read, long kinetics, or fast kinetics.
- Timing for kinetic runs
- Plate type: number of wells
- Blanking (pre-read plate)
- Wells to be read
- Integration time

Settings for the reading are made in the Instrument Settings dialog box, which can be accessed three ways:

- 1) Choose **Instrument Setup...** from the **Control** menu;
- 2) Click the **[Setup]** button in the tool bar of the active, expanded Plate section; or
- 3) Double-click anywhere in the settings box (located to the right of the well grid) in the active, expanded Plate section.

NOTE: You may create more than one Plate section in a single experiment, and the instrument settings associated with each Plate section can be different. **Instrument Setup...** can only be chosen while a Plate section is active, and the settings you choose will affect only that section. A new Plate section that is created in an experiment that contains an active Plate section will contain the same instrument settings found in the previously active section. The settings for the new section can be modified as desired.

Table 4.1 lists all possible instrument settings choices based on instrument type. When you have defined the instrument settings, click the **[OK]** button to close the Instrument Settings dialog box. After closing, a summary of the instrument settings will appear in the box to the right of the data display in the Plate section. The Lmax will use these settings to read the plate.

Table 4.1: Features Available in the Instrument Settings Dialog Box

	Integration Time	Pre-Read Plate	Injection: P and/or M Injection	Injector Volume	Post-Injection Delay	Injection Wells (P & M Injector)	Wells to Read	Run Time	Number of Reads per Well	Plate Type
Endpoint	◆	◆	◆	◆	◆	◆	◆			◆
Dual Read	◆		◆	◆	◆	◆	◆			◆
Fast Kinetics	◆		◆	◆	◆	◆	◆		◆	◆
Long Kinetic	◆		◆	◆	◆	◆	◆	◆	◆	◆

NOTE: Incubator control is an instrument setup parameter that is *not* found in the Instrument Settings dialog box.

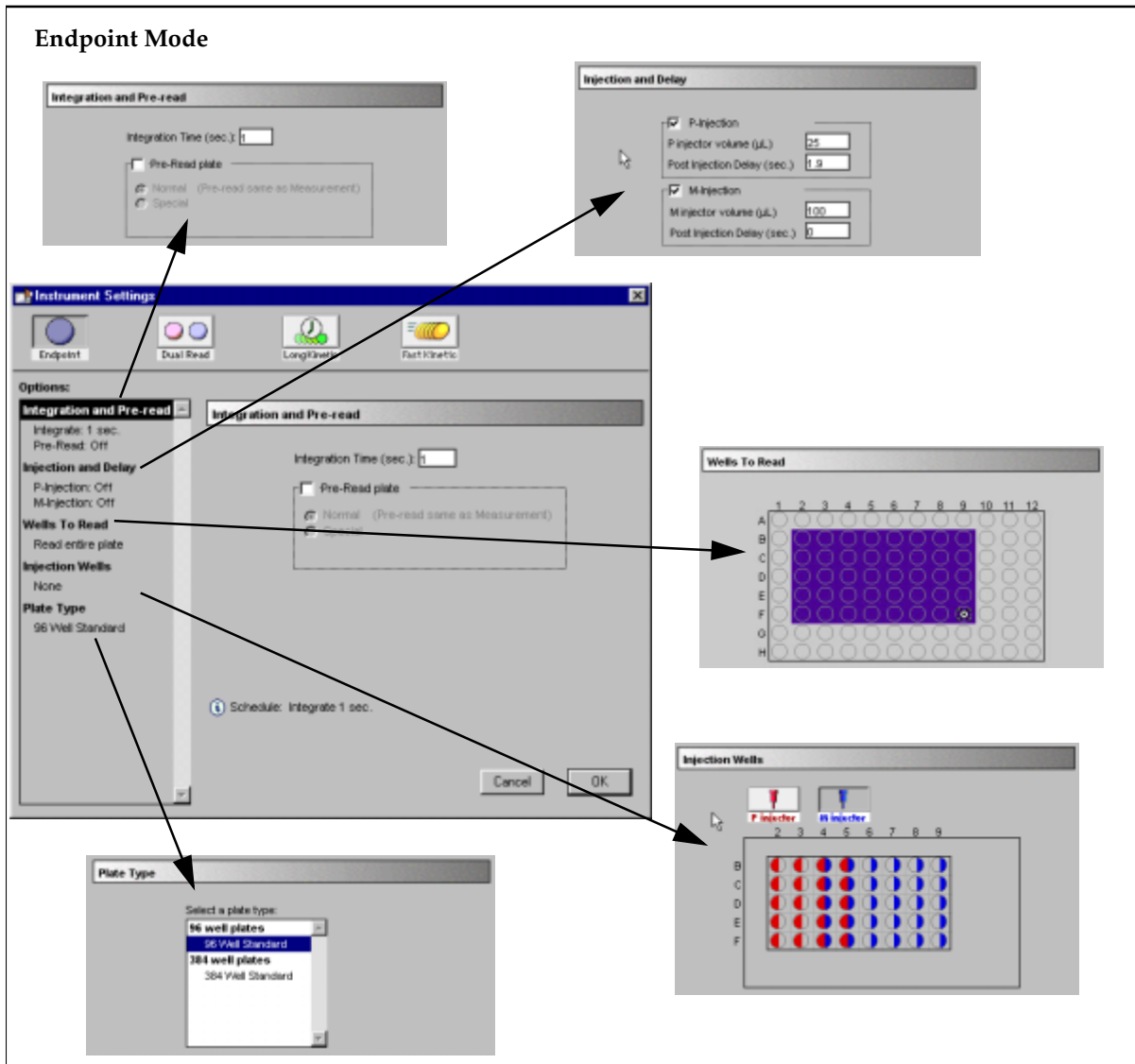


Figure 4.1: Instrument Settings Dialog Boxes for an Endpoint Reading

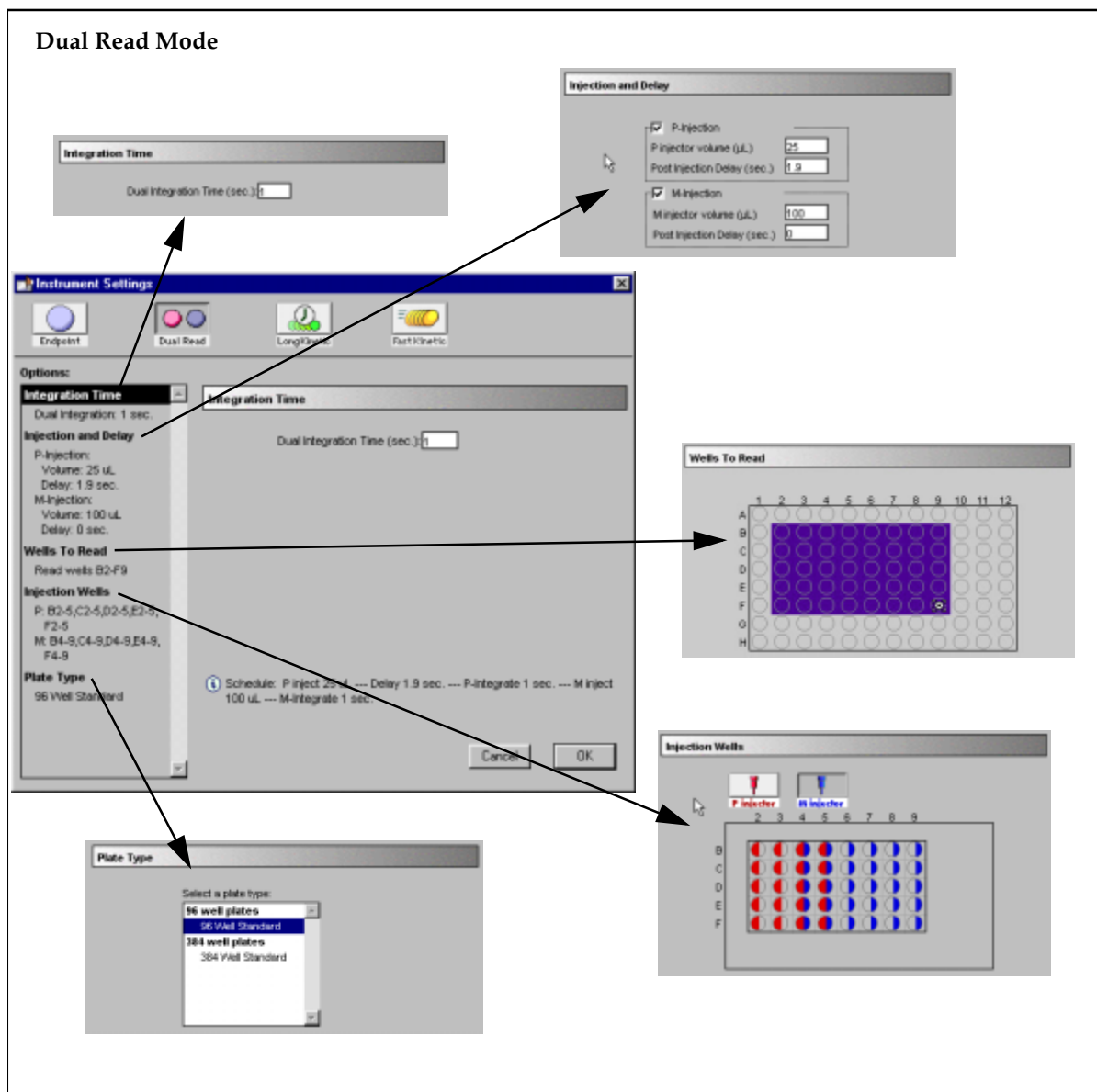


Figure 4.2: Instrument Settings Dialog Boxes for a Dual Read

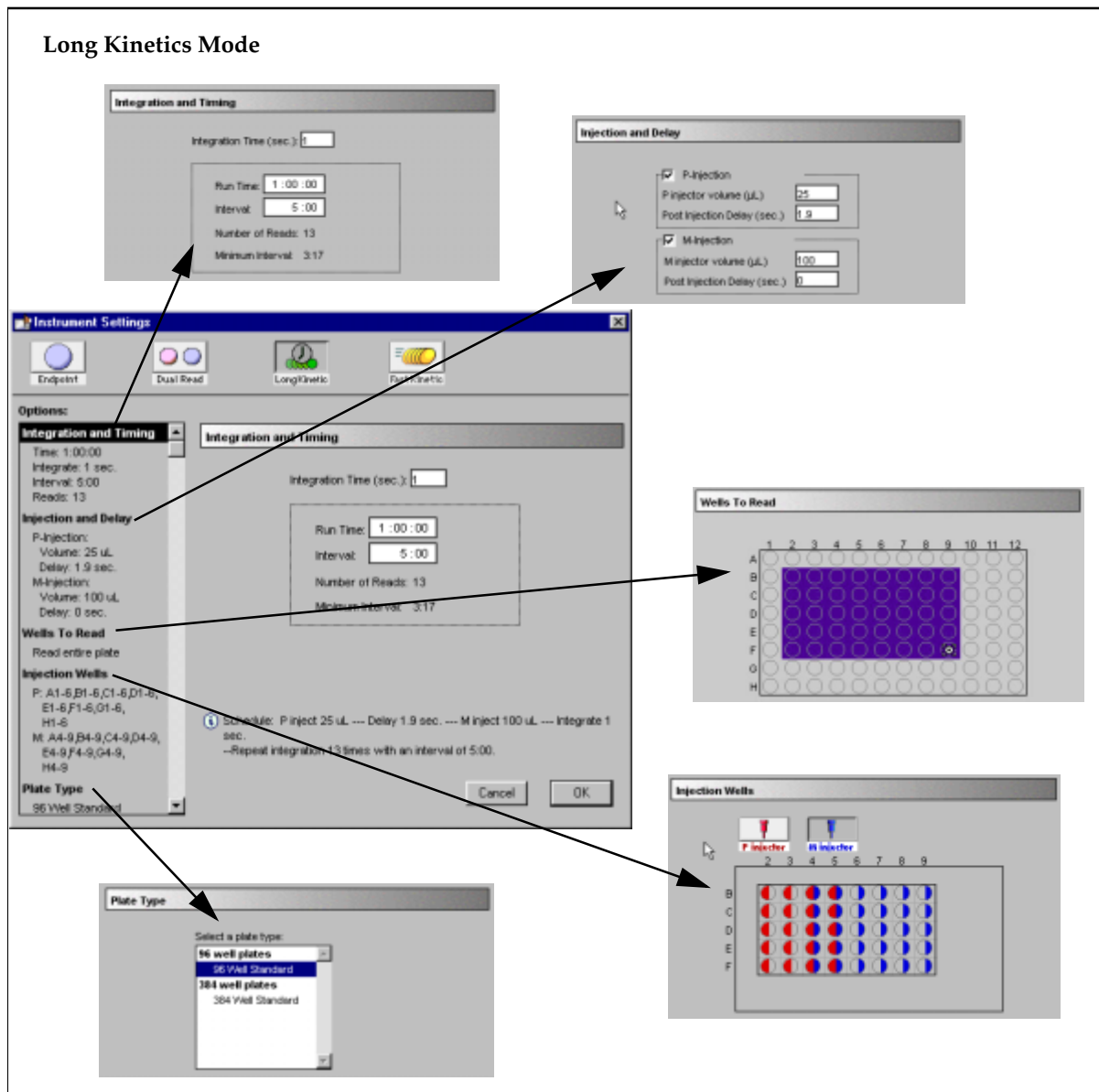


Figure 4.3: Instrument Settings Dialog Boxes for a Long Kinetics Reading

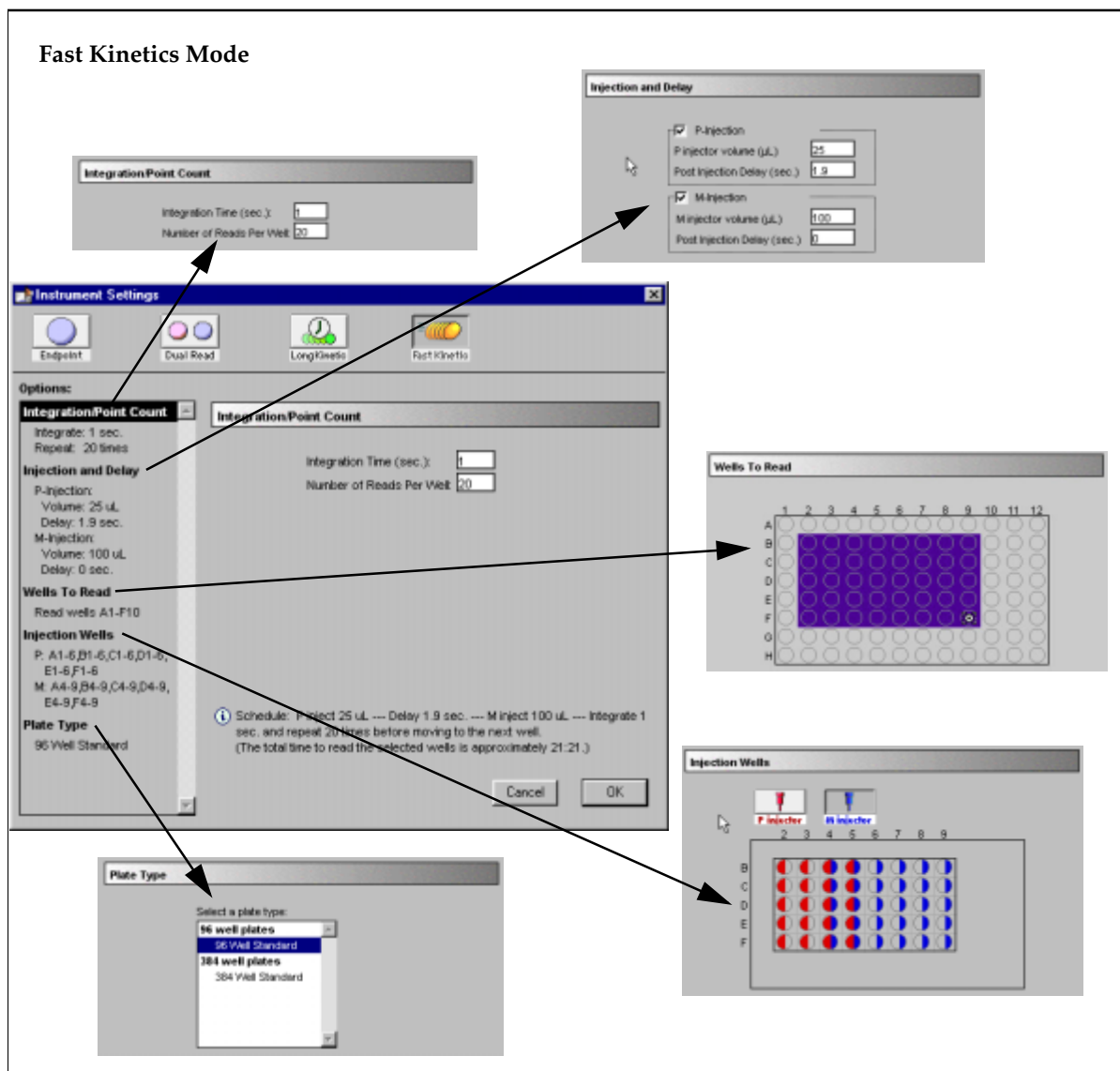


Figure 4.4: Instrument Settings Dialog Boxes for a Fast Kinetics Reading



Instrument Settings Options

Read Mode

Choices for the read mode are endpoint, dual read, long kinetics, or fast kinetics. To select a read mode, click the appropriate icon in the Instrument Settings dialog box.

Endpoint

A single integration of samples in 96- or 384-well plates with two injections possible, if desired, in any or all wells of a 96-well plate (no injection possible in 384-well plates). The minimum integration time is 0.1 second. A pre-read of the microplate may be made before injection of buffer or reagent and/or reading of the samples. The signal is integrated for each well over a user-selectable reading time (maximum 5000 seconds). You can program a delay after each injection, if desired (up to 1000 seconds with a minimum of 1.6 seconds for the P injection), and you can read all or only some of the wells in the plate. Values are reported as relative luminescence units (RLU); the default reduced value is !MValue.

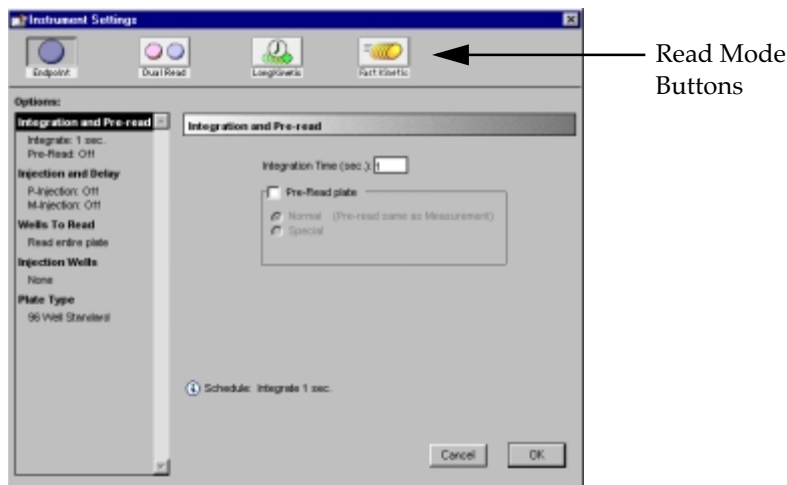


Figure 4.5: Instrument Settings for an Endpoint Reading.

Dual Read

Also an endpoint reading, this mode is designed to perform two separate integrations, one after each of two possible injections in any or all wells of a 96-well microplate (no injection possible in a 384-well plate). Integration times for both readings must be the same (minimum integration time is 0.1 second; maximum is 1000 seconds). You may program a delay after each injection of up to 1000 seconds (a minimum of 1.6 seconds for the P injector), and you can read all or only some of the wells in the plate. Pre-reading of the microplate is not possible with this mode. Values are reported as relative luminescence units (RLU); the default reduced value is !MValue.

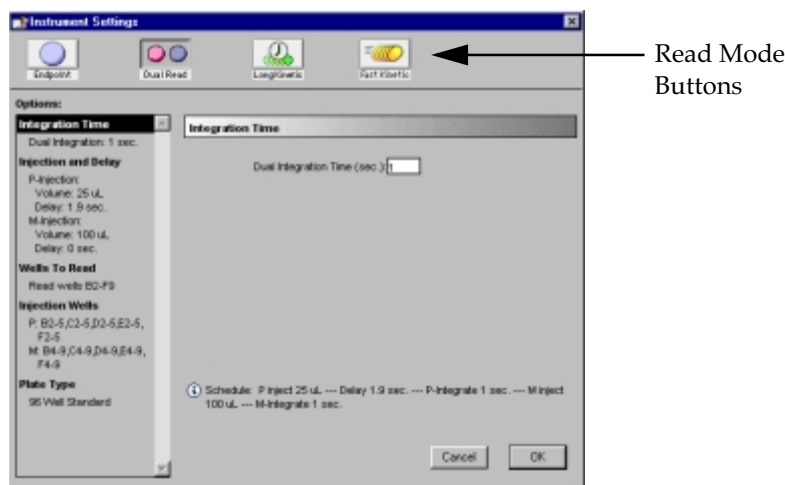


Figure 4.6: Instrument Settings for a Dual Reading.

Long Kinetics

Reads all of the selected wells of a 96- or 384-well microplate and then repeats this process for as many readings as are specified in the Instrument Settings. One or two injections can be made in any or all wells of a 96-well plate at the beginning of the reading (no injection possible in 384-well plates). The default reduced values calculated for long kinetics data are Vmax per Sec (RLU/Sec), Vmax, Time to Vmax (seconds), Onset Time (seconds), Time at Minimum, Time at Maximum, Time at 1/2 Maximum, Slope, and Area Under Curve. An important application for this mode is cellular luminescence.

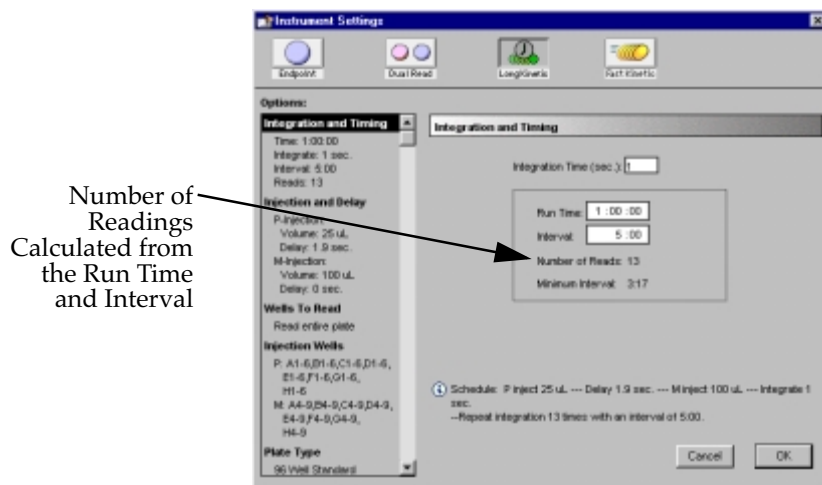


Figure 4.7: Instrument Settings for a Long Kinetics Reading.

Timing—Enter the total kinetic run time and the time interval between readings. A default value will be shown for the run time; to change this value, click in the box. Up and down arrows will appear, enabling you to increase or decrease the value shown. Highlight the hours, minutes, or seconds shown



and then click the up or down arrow until the desired value is reached. Alternatively, you can type over an existing value using the keyboard.

After choosing a total run time and an interval between readings, the total number of readings that will result is shown below the run time. If you set the interval time to be shorter than the instrument's minimum read interval, the program will alert you with a warning message.

Fast Kinetics

Repeated integrations are made for a specified number of data points, up to a 100-point maximum integration, in a single well of a 96- or 384-well microplate. This process is then repeated for each of the rest of the selected wells. One or two injections can be made in each well of a 96-well plate at the start of the integration (no injection possible in 384-well plates). Integration time can be set from 1 to 100 seconds. Data is collected over time with readings taken at regular intervals. The default reduced values calculated for kinetic data are Vmax per Sec (RLU/Sec), Time to Vmax (seconds), Onset Time (seconds), Time at Minimum, Time at Maximum, Time at 1/2 Maximum, Slope, and Area Under Curve.

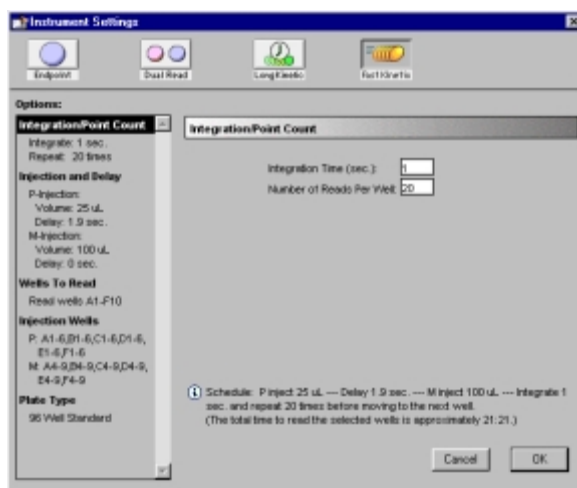


Figure 4.8: Instrument Settings for a Fast Kinetics Reading.

Plate Type

This setting determines the display of wells in the microplate—it should be set to match the type and number of wells in the actual microplate that will be read.

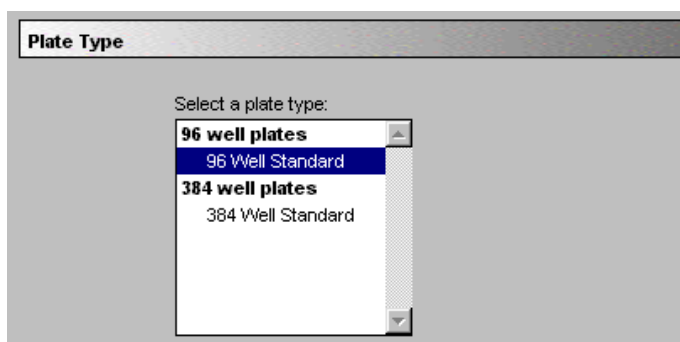


Figure 4.9: Plate Type Setting. Choose either 96-well or 384-well standard plates.

NOTE: Changing the Plate Type within a Plate section causes well assignments stored in the previous template to be discarded. The groups that were created previously remain, however, so you can select new wells and simply apply existing groups to these wells.

NOTE: Switching between 96- and 384-well plates requires a hardware reconfiguration on the Lmax as well as the designation of Plate Type in the software. Choosing a different plate type will cause a message to appear on the screen reminding you that the physical setup must be altered and that you must also choose an additional setting in the Control menu to ensure that the proper hardware for that plate type has been configured.

Wells to Read Selection

You may choose to read only certain wells in the microplate or to read the entire plate. Partial-plate reading is available for all read modes; the time required for readings may be significantly reduced using this setting if the instrument does not have to read the entire plate.

To configure the settings for a partial-plate read, choose the Wells to Read setting.

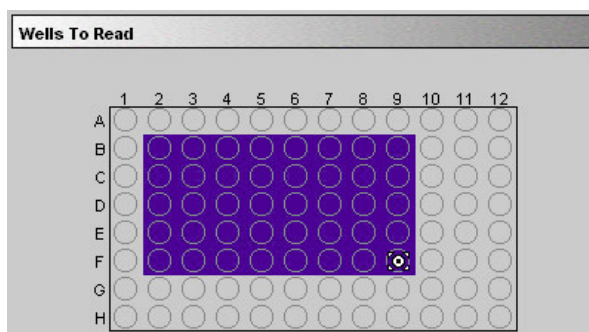


Figure 4.10: Partial Plate Read (Wells to Read) Dialog Box with wells B2 through F9 Selected

Highlight the wells to be read. Wells must be contiguous but do not need to start in any particular place. If you enable a partial-plate reading, only the



wells to be read will be visible in the data display, indicating that no data will be collected for the other wells (all wells will be present in the Template Editor, however).

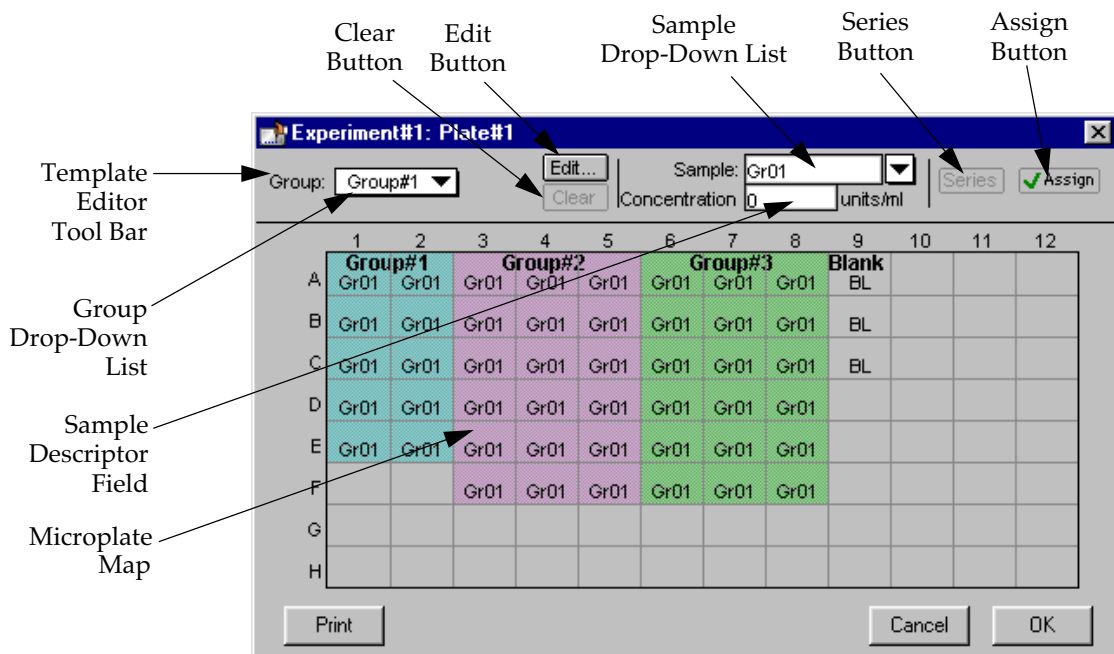
Template Editor

The Template Editor is used to describe the location of samples in the wells of the microplate, providing the link between the raw data and the analysis groups. The Template Editor is used to create a template which provides SOFTmax PRO for Lmax with a map of what is in the microplate.

When you first open the Template Editor for a new Plate section, the grid or display is empty. The number of wells shown depends upon the type of plate selected in the Instrument Settings. After selecting wells, you can assign groups and indicate the types of samples they will contain.

Depending upon the default protocol you are using, certain group names (and associated sample descriptions) may have been created. You can use these predefined groups and/or create new groups to suit your needs. When creating new groups, you may enter any name you wish. Once entered, this name will be available for use in that experiment thereafter. New groups can be created independently from selecting or assigning any wells in the Template Editor.

Each well designated as being part of a group has associated with it a group name, a sample name (or replicate ID), a sample descriptor (optional), and a default column format (group type, such as Standards, for example). When a group is applied to selected wells, the group name is displayed in bold type over the wells and the wells bear the same color.





Groups are always visible, both in the Template and in the data display of the Plate section. Even though they are grouped, single wells are still selectable in either the Template Editor or the data display.

Samples and groups may exist across multiple Plate sections as well as within a single Plate section.

Each template is built upon a hierarchy as follows: the template is composed of samples and groups. A set of one or more replicate wells makes up a *sample*. A set of related samples forms a *group*. For example, you may have a group named Standards that consists of seven samples named STD01, STD02 ... STD07. Group names may be up to 32 characters long, while sample names may be 300+ characters long. Samples may be assigned one at a time using the **[Assign]** button or several at the same time using the **[Series]** button.

If you create a group in the Template Editor but do not assign any wells to the group, a *section* for that group will still be created automatically when you close the template. You can remove the group (both its section in the window and its group name in the template) by making that Group section active and choosing **Delete Groupname** (where “Groupname” is the name assigned to that group in the template) from the **Edit** menu. Using the Macintosh, you can also delete the selected Group by pressing **⌘ -**; within Windows, you can press **CTRL+-**. Note that deleting the wells assigned to a group from the template *does not delete the corresponding Group name* from the drop-down list. The only way to remove a group name from this list in the Template Editor is to delete the corresponding Group section from the experiment.

Selecting Wells in the Template Editor

In the Template Editor for a Plate section, you can select one or more wells by clicking or dragging on the grid. Multiple (contiguous) wells can be selected by dragging across them. Non-contiguous selections can be made by holding down the Shift key and clicking individual wells. Clicking a row label will select the entire row; clicking a column label will select the entire column. Contiguous columns or rows (1-12 or A-H, for a 96-well plate, for example) can also be selected by dragging. Non-contiguous columns or rows can be selected by holding the **⌘** (*command*) key on the Macintosh or the **CTRL** key in Windows and clicking in labels.

- Double-clicking anywhere within a group will select the entire group.
- To deselect all wells quickly, click in the empty border around the Template.
- When you leave the Template Editor (by clicking the **[OK]** button), the Plate section will show the boundaries of the groups created in the template. Group labels and sample descriptors will not be visible—you can see them by holding down the **Ctrl+Shift** keys. When you release the key(s), the original view of the Plate section will return.



The Template Editor Tool Bar

The tool bar will appear differently depending upon what type of group is selected in the Group drop-down list. The following illustration shows how the tool bar will appear under various circumstances.

Group: Unknowns Edit... Clear Sample: Un01

A group is selected that does not contain sample descriptor information (such as Unknowns column type).

Group: Standards Edit... Clear Sample: Sta01 Concentration 0 µg/ml

A group is selected for which a concentration has been defined (such as Standards column type).

Group: Unknow...ution Edit... Clear Sample: Un01 Dilution Factor 0

A group is selected for which a dilution factor is defined (such as UnknownsDilution column type).

Figure 4.12: The Appearance of the Template Editor Tool Bar under Different Circumstances

The tool bar is divided into three areas: the Group area, the Sample area, and the Assign area.

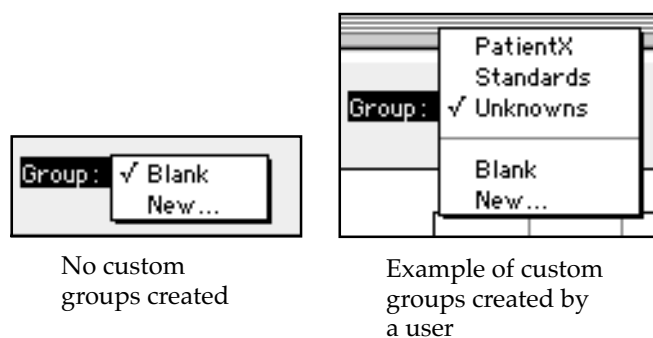
The Group Area

The Group area contains the Group drop-down list which allows you to assign wells to existing groups, create new groups, or modify (using the **[Edit]** button) existing groups. In addition, it contains the **[Clear]** button which allows you to clear well assignments in the template.

Group Drop-Down List

This drop-down list consists of two areas, divided by a horizontal gray line. The two items below the gray line (**Blank** and **New**) are always available. Items above the gray line are custom groups that may be created as you wish.

The Group drop-down list will appear differently depending upon the custom groups that have been created (by default or by the user). The figure below shows two different instances of the appearance of the Group list.



No custom groups created

Example of custom groups created by a user

Figure 4.13: The Group Drop-Down List

NOTE: Depending on the default template used when opening SOFTmax PRO for Lmax version 1.0, initially no groups may appear above the gray line. As you create new groups, they will be positioned above the line.

- **Blank**—Creates a plate blank in the Plate section. Note: the name “blank” is reserved for use by the SOFTmax PRO for Lmax program and should not be used when creating custom groups.
- **New**—Creates a new group. The Group Settings dialog box will appear (see Figure 4.14) showing default settings. This dialog box allows you to define the name for a group of related samples, what type of descriptor will be associated with these samples, and the initial column format for the Group section. Whenever a new group is created (whether or not wells are selected in the template), a Group section (table) will also be created. To delete a group, you must delete the Group section. Simply clearing a group from the Template Editor will remove the *assignment* of wells to that group name, but the name will still show in the group pull-down menu and the Group section will still exist.

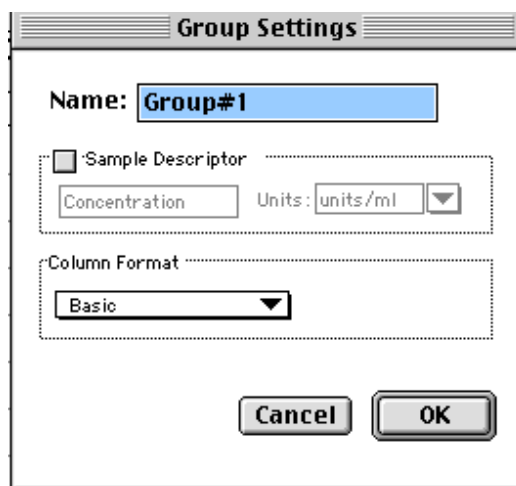


Figure 4.14: Group Settings Dialog Box Using Default Settings



Name: Use either the default name, "Group#X," where "X" is an integer that is initially set to "1" and increments (i.e., "1," "2," "3," etc.) as other groups are created, or enter a custom name (up to 32 characters in length).

In the **Sample Descriptor** area of the Group Settings dialog box, you can enable the application of the sample descriptor, if appropriate by clicking the checkbox. You may also choose the units used for the sample descriptor by selecting an option from the **Units** drop-down list or by typing the units you wish to use into the list box.

The Sample Descriptor is automatically enabled when either the Standards or Unknowns(Dilution) column format (see below) is selected. The descriptor defaults to Concentration with the Standards column format and to Dilution with the Unknowns(Dilution) column format. The Sample Descriptor may also be used for time or fraction number information.

NOTE: No sample descriptor is assigned automatically for the Basic or Unknowns column format. You may assign a sample descriptor to these column formats manually but, if you choose to do this, the information will not appear automatically in the Group section. To see it, you will need to create a new column containing the formula `!Sampledescriptor`. For more information about creating columns, see "Adding Columns" on page 5-26.

Column Format defines the default column types that will be created for a new group or shows the original column format setting that was used to create the group you are editing.

Four selections are offered: Basic, Standard, Unknowns, and Unknowns (Dilution). Different types of columns will be created for a *new* group, or changed in an *existing* group, depending on which of these column format settings you select. Table 4.2 shows the default columns created with each selection type.

NOTE: If you are creating a new group for unknowns that have a dilution factor, use the Unknowns(dilution) column format as a base rather than the Unknowns group included in the default protocol.



Table 4.2: Columns Created and Their Formulas

	Basic	Standard	Unknown	Unknown(Dilution)
Name	Sample	Sample	Sample	Sample
Formula	!SampleNames	!SampleNames	!SampleNames	!SampleNames
Name	Wells	Wells	Wells	Wells
Formula	!WellIDs	!WellIDs	!WellIDs	!WellIDs
Name	Sample#	Concentration	Values	Values
Formula	Index	!Concentration*	!WellValues	!WellValues
Name	Values	Values	R (Outside Standard Range)	R (Outside Standard Range)
Formula	!WellValues	!WellValues	If(Values)>='MinStd@Standards' and Values<='MaxStd@Standards', "", "R"	If(Values)>='MinStd@Standards' and Values<='MaxStd@Standards', "", "R"
Name	Mean Value	MeanValue	Result	Result
Formula	Average(Values)	Average(Values)	InterpX(STD@StandardCurve,Values)	InterpX(STD@StandardCurve,Values)
Name		Std.Dev.	MeanResult	MeanResult
Formula		Stdev(Values)	Average(Result)	Average(Result)
Name		CV%	Std.Dev.	Std.Dev.
Formula		Cv(Values)	Stdev(Result)	Stdev(Result)
Name			CV%	CV%
Formula			Cv(Result)	Cv(Result)
Name				Dilution
Formula				!Factor*
Name				Adj.Result
Formula				MeanResult*!Factor

* !Concentration, !Factor, and !SampleDescriptor return identical information.



The **Edit** Button

When you click the **Edit** button, the Group Settings dialog box will appear, reflecting the settings for the *active* group (currently visible in the group drop-down list). If you wish to edit a group that is not currently active, first choose the group from the drop-down list and then click the **Edit** button. The figure below shows the Group Settings dialog box for the active group called "Patient#1."

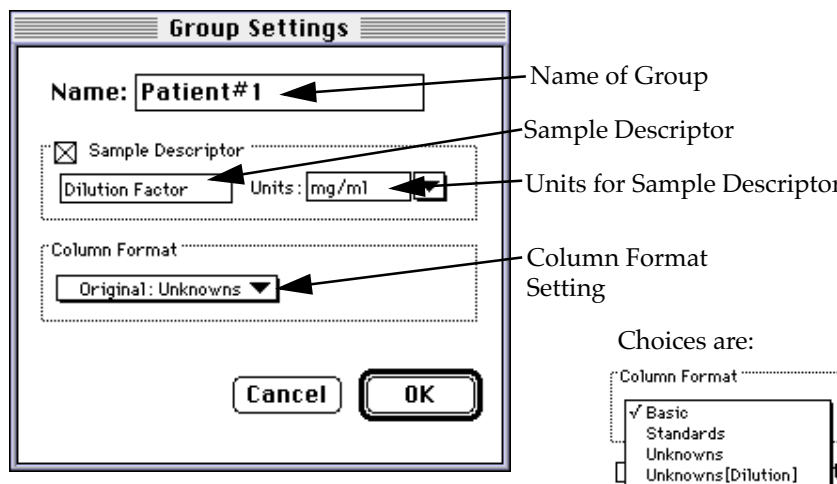


Figure 4.15: Group Settings Dialog Box for the Group Named "Patient#1"

The name, sample descriptors, and column format can be changed for the group that you are editing.

When you *edit* a group, you will notice that the information shown for the Column Format in the Group Settings dialog box changes to show the name of the *original* group used to define the group columns (see Figure 4.16).

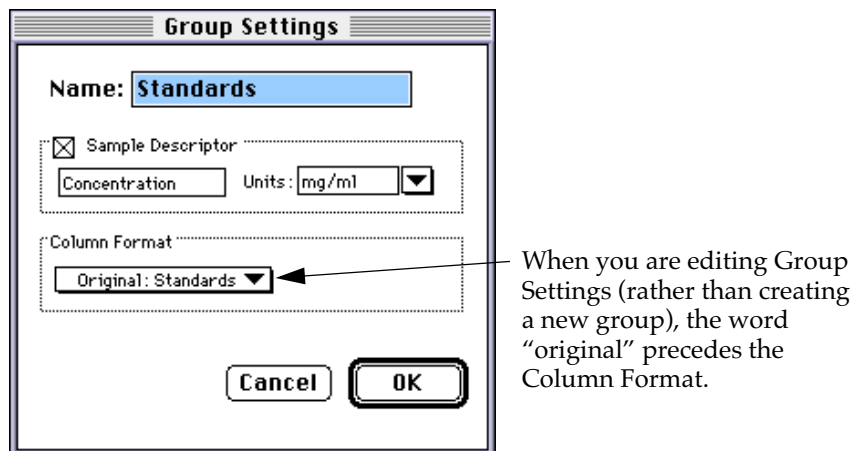


Figure 4.16: Groups Settings Dialog Box Showing Original Column Format



⚠ CAUTION: Changing the column format setting will cause the columns in the group to revert to their original defaults as shown in Table 4.2 on page 4-18. If you have added columns or changed column information within the existing section for the group you are editing, these changes will be removed.

The **Clear** Button

Allows you to deselect, or clear, wells in the map of the microplate. Highlight the wells you wish to clear and then click the **Clear** button. Data for cleared wells will no longer be shown in the data display.

The Sample Area

The Sample area contains the Sample drop-down list and sample descriptor values (active if defined in the Group Settings dialog box or if assigned automatically by SOFTmax PRO for Lmax).

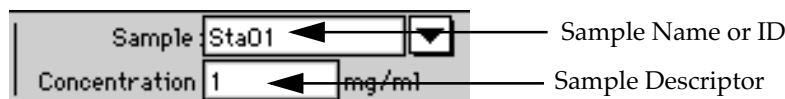


Figure 4.17: Modifying Sample Names and Descriptor Values in the Template Editor

⚠ CAUTION: When selecting wells to change sample names or descriptors, be sure all wells are replicates of the same sample and group or that you want them to become replicates of the same sample and group. SOFTmax PRO for Lmax does not prohibit selecting individual wells from *different* samples and/or groups, for example, and changing their descriptions—and they will change to become the same.

Sample Drop-Down List

The sample drop-down list consists of two areas divided by a horizontal gray line. Before other sample names have been defined, a single, pre-defined name ("BL") appears below the gray line (the sample name "BL" is the name reserved by SOFTmax PRO for Lmax for group-associated blanks). The item above the gray line is the sample ready to be or currently being assigned. After being assigned, it will be shown below "BL" below the gray line.

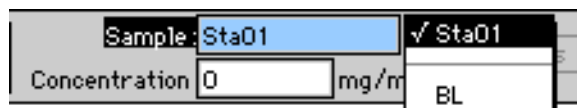


Figure 4.18: The Sample Drop-Down List. When this list first appears, the only selection shown below the dividing line is "BL"; the active sample name that is ready to be assigned appears above the line.

When samples are assigned to a group, the default name is the first 3 letters of the group name followed by a number. For a group called "Standard," for example, the default sample names would be Sta01, Sta02, Sta03, and so on.



Sample names can be 300+ characters long; however, only the first five or six characters will be displayed in the template and in the Plate section.

NOTE: You can type a sample name directly into the box next to the arrow for the drop-down list by highlighting and typing over the existing sample name. If one or more wells were highlighted, typing a different name will replace the sample name shown in the template with the name you have typed and, if the name is new, will add this name to those that can be chosen in the drop-down list.

When wells have been selected in the template and sample names have been defined, the sample menu will look like the one below. The checkmark indicates the sample that is currently being named.

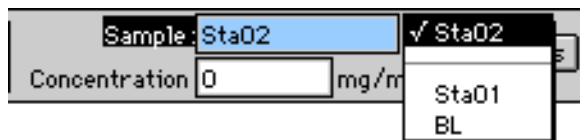


Figure 4.19: Sample Drop-Down List—Sample Names Defined and Wells Selected

Sample Descriptor Field

The field associated with the sample descriptor only appears in the Template Editor tool bar if the group column type that has been selected requires this type of information. Generally the information entered in this field is a dilution or concentration. Position the cursor in this field and enter a number. The label and units assigned to the sample descriptor in the Group Settings dialog box are shown on either side of the field.

Assign Area

The Assign area contains the **Series** and **Assign** buttons.

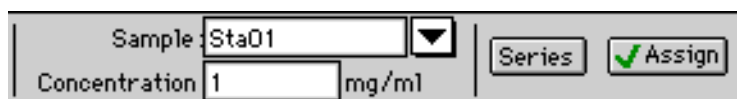


Figure 4.20: Assign Area of the Template Editor Tool Bar

Assign Button and Function

Click this button to apply a sample name to one or more wells that have been selected in the template. You may also perform this operation by using the Enter key on your keyboard.

Series Button and Function

This button is used to define several samples as a *series*, allowing you to easily enter incremental sample descriptors (for example, dilutions or concentrations) and sample names to the template as long as the increment can be expressed as a mathematical operation.

Replicate Samples and Samples in a Series

You can assign the same group or sample definition (same sample name and description within a group) to multiple wells to create *replicates*. For example, you might want to read standards in replicate to ensure that anomalies can be excluded prior to generating a standard curve. Replicates can be created using the **Assign** button or with the *series* function.



Creating a series is faster than assigning replicates individually. The series option can be used as long as the replicate values conform to a pattern that can be described by a mathematical operator (multiply by 2 or add 5, for example).

A series is defined in one direction (starting from left, right, top, or bottom) and therefore samples must be arranged sequentially in either ascending or descending order.

To create a series, do the following:

- 1) In the Template Editor, highlight the wells to be part of the series. The wells must be in the shape of a rectangle.

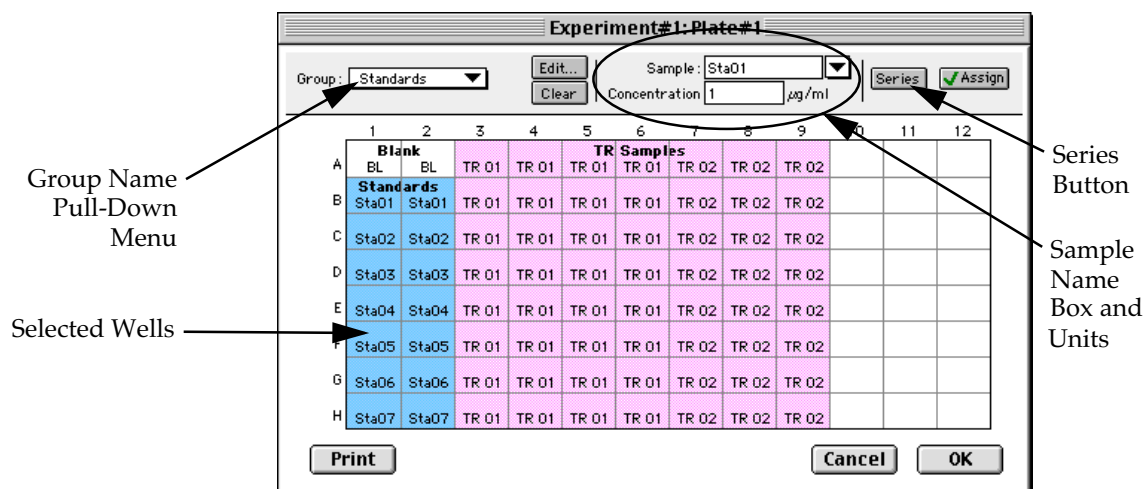


Figure 4.21: Template with Wells Selected for a Series

- 2) Click the **Series** button to open the Series dialog box.

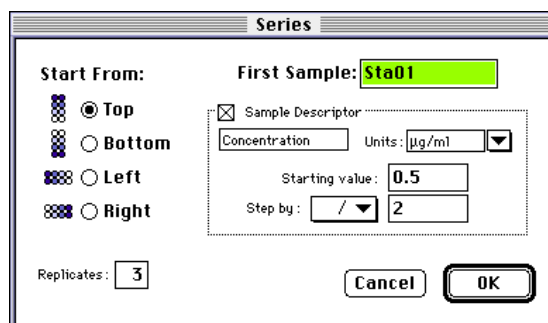


Figure 4.22: Series Dialog Box

- 3) Specify the name of the first sample in the series in the box (or accept the default provided by SOFTmax PRO for Lmax). Subsequent replicates use this name as a base and either increment the number within the name or append a number to it. In the example above, the sample name provided by SOFTmax PRO for Lmax describes the first sample ("01" in a group named "Standard," here shortened to "Sta.")
- 4) The information shown in the box labeled Sample Descriptor is derived from the information entered in the Group Settings dialog box. If the information for



sample descriptor or units is not correct, you may change it here. If you did not already do so, specify the starting value for the first sample in the series. Values for subsequent samples are calculated from this starting value.

- 5) Choose the operator for the series (+, -, *, or /) from the Step by: drop-down list. Enter the starting value for the series in the Starting value: box. The increment is entered in the box to the right of the Step by: box and below the Starting value: box. Figure 4.22 shows a starting value of “.5” with the operator being division (“/”) and the increment being “.2.” Thus the series will start with a value of “.5” and divide each subsequent sample by 2 to produce the following series values: .5, .25, .125, etc.
- 6) You must assign a fill direction (from the top, bottom, left, or right—to describe how the series will fill the wells) and a number of replicates. Various options are diagrammed in Figure 4.23 using a 4 x 4 block of wells (for more information about replicates, see “Replicate Samples and Samples in a Series” on page 4-21). The maximum number of replicates depends on the selection of wells: if you are filling down from the top, for example, and you have chosen a block of wells that is 4 wells wide by 8 wells high, the maximum number of replicates will be four; with the same block of wells selected, filling from the left would allow eight replicates. If the number of replicates you choose does not divide evenly into the number of rows or columns you select, depending on the filling direction, the remaining wells that cannot contain replicates will be labeled as additional individual wells in the series. For example, if you fill a block of 4 x 4 wells from the top, and choose three replicates, then the first three columns will contain the replicates and the last column will contain the additional wells in the series (see the last diagram in Figure 4.23). This replicate feature allows you to quickly define the number of replicates and, in cases where the series has more samples than a single row or column, to name them simultaneously. To create a series with all 96 samples, highlight all the wells in the plate, select the fill direction, and set the replicates to 1.

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Figure 4.23: Fill Options and Replicate Settings



- 7) Click **OK** to save the series and close the dialog box. While still in the Template Editor, you can view the value assigned to any well by clicking that well. The value will be displayed at the top of the Template Editor. Holding down the **Ctrl+Shift** keys (both Mac and PC) will display the values for *all* wells.

NOTE: Sometimes you may wish the *names* of a series of samples to increment but may not have a concentration or dilution factor that you wish to enter, or you want the concentration or dilution factor to remain constant. This can be done using the **Series** button, setting the starting value to 1 or another constant value, and either multiplying by 1 or dividing by 1. In this way, the names will increment but the value of concentration or dilution will not change. If you don't have a concentration or dilution factor, simply deselect the sample descriptor checkbox if it is selected.

NOTE: If sample names are set to increment automatically using the Series function, be aware that SOFTmax PRO for Lmax will automatically truncate the sample name to three or four characters, including the incrementing number if the sample name starts with letters. If the sample name is entirely numeric, it will not be truncated. If sample names are very long, and they must be kept long for reference, you will have to set up your replicates manually and will not be able to use the series function.

Blanking

Blanking (Pre-Read Plate)

The blanking selection found in the Instrument Settings dialog box for endpoint mode (only) is known as *pre-read plate blanking*, but pre-reading the plate is only one of five types of blanking available in SOFTmax PRO. Understanding the differences between the types of blanking functions will enable you to choose the type of blanking that is appropriate for your assay. Note that all types of blanks can be used in combination and have *cumulative* effects. Pre-read plate blanking for endpoint mode is discussed here; for a description of the other types of blanking that are available and how these blanks work in combination, see "Blanking" on page 4-24.

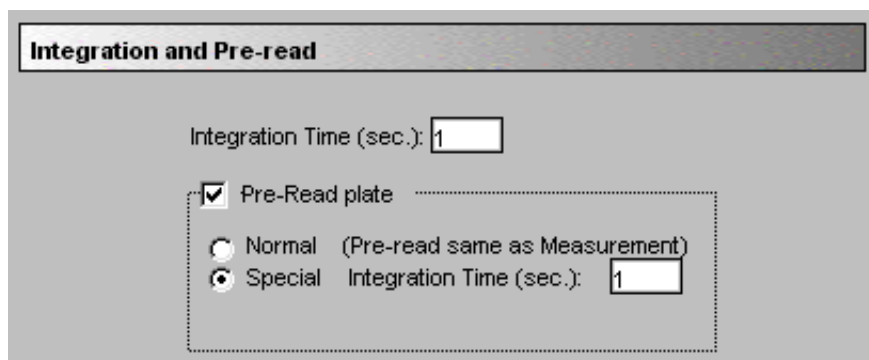


Figure 4.24: Pre-Read Plate Blanking Section of the Instrument Settings Dialog Box (Endpoint Mode Only)



The purpose of pre-read plate blanking is to correct for well-to-well variability. The pre-read values will be stored in the data file and *the individual well values collected during the pre-reading of the plate will be subtracted from the individual well values* obtained during the actual reading of samples. Pre-read blanking may be turned off in the Reduction dialog box (click on the **Reduction** button) so the data may be seen with and without pre-read blanking subtracted. This option is only available within the Reduction dialog box if pre-read blanking was chosen in the Instrument Settings for endpoint mode.

NOTE: Molecular Devices recommends that you fill the wells of the pre-read plate with appropriate buffer.

Other Types of Blanks

In addition to the pre-read plate blanking selection found in the Instrument Settings dialog box, other types of blanking are available in SOFTmax PRO for Lmax and these others can be used with other read modes. Understanding the differences between the types of blanking functions will enable you to choose the type of blanking that is appropriate for your reading. Note that blanks can be used in combination and have *cumulative* effects.

The other types of blanking besides pre-read plate blanks that are defined in SOFTmax PRO for Lmax are:

- Plate blanks
- Group-associated blanks

Plate Blanks

Wells can be assigned to a “Blank” group in the Template Editor. Unlike pre-read plate blanks, *the average value of all the wells in a plate blank group is subtracted from all individual well values* obtained during the reading of microplates. Plate Blank subtraction may be turned off in the Reduction dialog box so that the data may be seen with or without plate blank subtraction.

NOTE: Plate blanks are only subtracted from wells on the same plate to which they are assigned. The checkbox for Plate Blank subtraction only appears in the Reduction dialog box after data has been collected.

For kinetic data, the blank value is averaged and subtracted on a point-by-point basis. The example below shows a graphic representation of original and average plate blank data, followed by the resulting data with average subtracted.

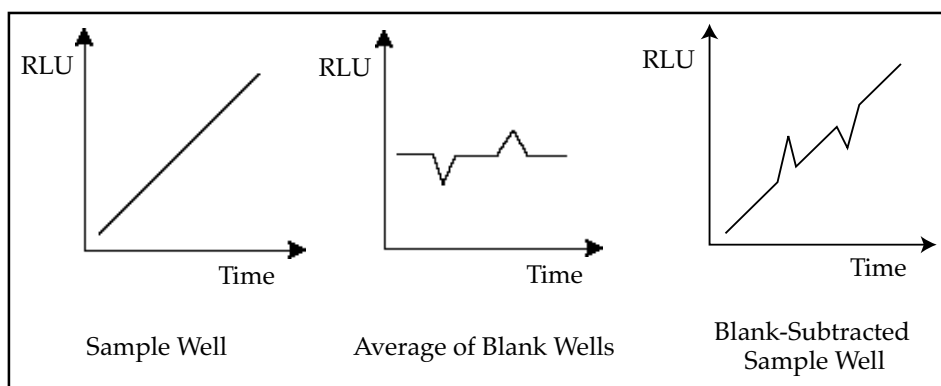


Figure 4.25: Graphic Representation of Plate Blank Subtraction

Group-Associated Blanks

Blank wells can be assigned within any group (other than the “Blank” group) in the Template Editor. *The average reduced value of the blank wells within that group will be subtracted from individual reduced values within that group only.* This blanking function uses the reduced number (example: !Lm1-!Lm2, Vmax, etc.) only—it does not subtract on a point-by-point basis. When a group blank has been assigned, it is automatically subtracted. To see the reduced number without the group blank subtracted, mask the group blank wells.

Group blanks apply to all wells in a group and thus may be subtracted from wells in more than one Plate section.

Blanks in Combination

It is possible to use the blanking functions in combination. For example, you could perform plate blanking and also include blank wells within certain groups. The effects of the different blanking functions are *cumulative*, so it is important to understand the function of each before using both types of blanking in a single assay. Plate blanking is applied to the raw data acquired from the instrument whereas group-associated blanks are applied to the reduced number. Group-associated blank subtraction is only visible in the Plate section when the reduced number is displayed. Figure 4.26 shows plate blanks and group-associated blanks in the Template Editor.

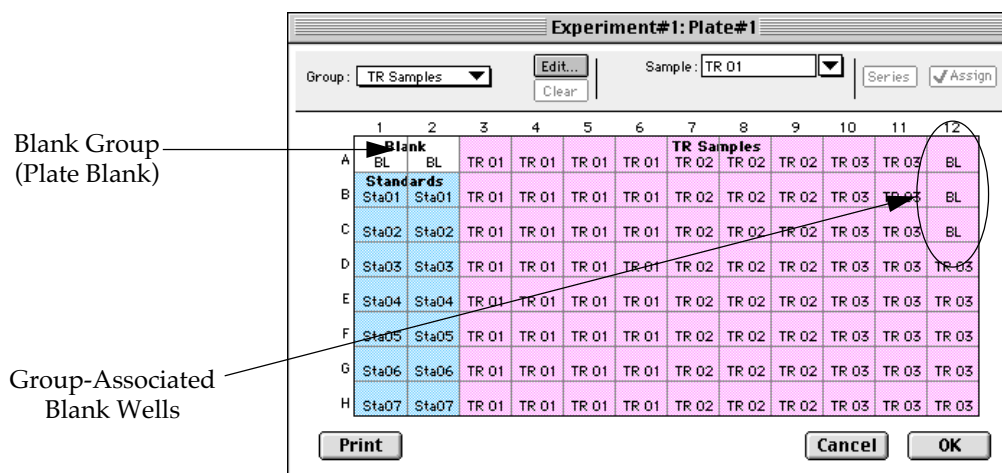


Figure 4.26: Template Editor Showing Plate Blank and Group-Associated Blank Wells

In the figure above, note that the plate blank in the upper left appears as a separate group named “Blank” and individual samples within this group are named “BL.” The group-associated blank wells, shown in the upper right, are not a separate group—the samples, also named “BL,” are contained within the boundaries of the large group called “Unknowns” and are the same color as that group.



NOTE: In order to save changes you have made to the template in the Windows version of the Template Editor, be sure to click the **OK** button to close the window rather than clicking the Control Menu box. Clicking the Control Menu box (located in the upper left of the window) performs the same function as the **Cancel** button: the window will close but any changes you have made to the template will not be saved.

Copying and Pasting Templates

Copying Complete Template Information

Two template actions—Copy and Paste—can only be activated from the **Plate** menu (when a Plate section is active) rather than from the Template Editor. If you have created a template in a Plate section and wish to use it in another Plate section, you may copy that template and paste it into the new section. The results of copying and pasting a template depend upon whether the action is performed within the same experiment or between experiment sections.

Copying and Pasting within the Same Experiment Section

To copy a template, make the section containing that template active and choose **Copy Template** from the **Plate** menu (the menu name changes to reflect the type of active section). Then activate the destination Plate section and choose **Paste Template** from the **Plate** menu.

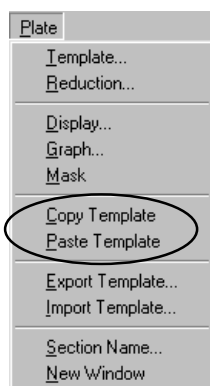


Figure 4.27: Copy Template and Paste Template Commands in the Plate Menu

The group names and sample names of the pasted template are identical to those of the source template. In effect, the destination template is considered an extension of the original template and all wells on the destination plate are considered replicates of the wells on the source plate (same group and sample names). Any changes made to the template on the destination plate will also be made on the source plate. *Data from the two Plate sections will be analyzed together.*

Copying and Pasting between Different Experiment Sections

To copy a template, make the section containing that template active and choose **Copy Template** from the **Plate** menu (the menu name changes to reflect the type of active section). Then activate the destination Plate section and choose **Paste Template** from the **Plate** menu.

The group names and sample names of the pasted template will be identical to those of the source template but, since they are in different experiments, the full name will be different (for example: group@experiment#2 instead of group@experiment#1). Any changes made to the template of the destination Plate section will not affect the source section. *Data from the two Plate sections will **not** be analyzed together.*

Copying and Pasting Template Regions

Contiguous regions of templates can be copied and pasted to other regions of templates (within the same or to a different experiment). All template settings are pasted into the new template except the group name: the new group is given the name "Group#X" where X is the next greater number than that used already for group names.

To copy a region of a template, highlight the region and then select **Copy** from the **Edit** menu or press **⌘C** (Macintosh) or **CTRL-C** (Windows). Only the highlighted wells will be copied.

To paste the region into another area of a template, highlight the region and select **Paste** from the **Edit** menu or press **⌘V** (Macintosh) or **CTRL-V** (Windows).

Only the highlighted wells will have template information pasted into them. Data is pasted using row priority: all wells in a row of highlighted wells have information pasted into them before proceeding to the next row, from left to right. If fewer wells are highlighted for pasting than were copied, the data



will be pasted until all paste-highlighted wells are filled. If more wells are highlighted for pasting than were highlighted for copying, the extra wells are left empty.

NOTE: If you try to paste template information between Plate sections having different plate format settings, an error will result. Plates must be of the same type to copy and paste template information.

Reading a Microplate

When you read a microplate using an Lmax, the information sent by the instrument to SOFTmax PRO for Lmax is luminescence data which is the measurement of light given off by a chemical or biochemical reaction.

You can start a reading at any time after defining instrument settings. It is not necessary to define groups and assign wells within the Template Editor first. The values received from the Lmax are raw luminescence readings and are not affected by settings in the Template Editor. Only instrument settings must be defined prior to reading a microplate.

To start a reading, insert the prepared microplate in the drawer and then click the **Read** button in the status bar or choose **Read** from the **Command** menu.

If you have created more than one Plate section, and no Plate section is active, choosing the **Read** command will cause a dialog box to appear asking you to choose which section to read (see Figure 4.28). Select the correct section and then click **OK** to close the dialog box. If a Plate section is active, choosing the **Read** command will read that plate.

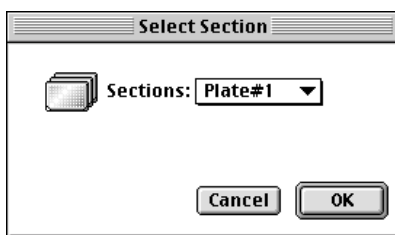


Figure 4.28: Select Section Dialog Box

If you select a Plate section that already contains data and choose to **Read** again, an alert appears, asking you to confirm replacing data in the destination plate (see Figure 4.29). If you select **Replace**, the reading will proceed and the previous data will be replaced with new data.

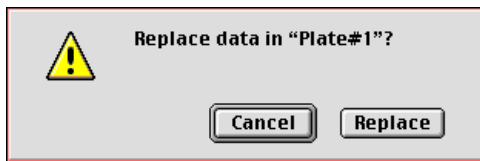


Figure 4.29: Alert Regarding Replacing Data for Plate Section

After beginning a reading, the status bar will show the actions that are occurring in the Lmax, along with a box showing the elapsed time for each action



in minutes and seconds. The **Read** button will change to **Stop**, allowing you to terminate a reading, if necessary. An example of the appearance of the status bar at the start of a kinetic reading is shown below.



Figure 4.30: Status Bar during a Long Kinetics Reading

The values read by the Lmax will appear in the data display of the Plate section as they are received (in real time). Depending upon the read mode (end-point, dual read, or kinetic), the plate type, and the display options selected, the data display will show one or more numbers or plots.

You can enlarge the display of one or more wells while a kinetic reading is in progress. Double-click a single well to enlarge its display to a Well Graph or hold the *Shift* key while selecting more than one well, and then choose **Graph...** from the **Plate** menu or click the graph button on the tool bar to display the data from multiple wells in one enlarged Well Graph. The data in the enlarged Well Graph will update to show new data points as they are received from the Lmax.

Other choices and settings within SOFTmax PRO for Lmax affect the way in which data is displayed—the type of reduction, specific time settings, and more. These settings are discussed in Chapter 5, “Displaying and Analyzing Data.”

Chapter 5: Displaying and Analyzing Data

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Introduction

Initial display and reduction of the data received from the Lmax is performed in the Plate section in which the data has been collected. Display and reduction parameters can be set up either prior to collecting data or after data has been collected. The display and reduction parameters may be modified at any time.

Data analysis and the display of analyzed data is also performed in the Group sections (created when wells are assigned to groups in the Template Editor) and the Graph section(s).

Notes sections may be used in conjunction with any other section to provide text commentary and/or a summation of data through the use of summaries. Notes sections are used most commonly to provide an introduction to or summary of results for a data file.

All data files have a minimum of one Plate section containing data. Multiple Plate sections may be present. Most files will have a template and Group, Graph, and Notes sections. However, depending upon the assay and how you wish to report the data, only some of these sections may need to be present in a file. Some simple assays allow you to calculate concentration in the Plate section. In such an example, Group and Graph sections would be necessary only if you wished to display the data graphically.

Standards and unknowns are defined in a template. Unknowns are then interpolated from a standard curve. The following diagram shows the relationship between the different sections in SOFTmax PRO for Lmax:

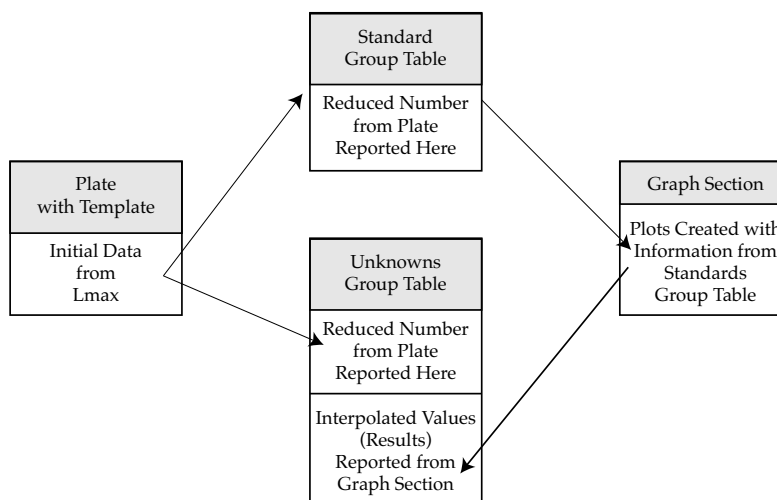


Figure 5.1: Relationship of Sections

This chapter discusses the initial display and reduction of data in the Plate section first, followed by display and data analysis in the Group, Graph, and Notes sections.



Initial Data Display and Reduction

The manner in which data is displayed in a Plate section depends on the type of reading that was performed (endpoint, dual read, long kinetics or fast kinetics), but can be customized by choices made in the data display and reduction settings. Depending on the type of reading performed, display options include:

- Number or plot displays for RLU (relative luminescence units), with or without reduced number.

NOTE: The number format (use of commas or periods in numeric notation) chosen for the computer operating system in general is reflected in the way that SOFTmax PRO for Lmax displays data.

- Number, Plot, Threshold, Ranged, or Grayscale (with or without reduced number)
- If you have chosen 384-well plates from the Instrument Settings, you can display the wells in different ways: Normal, Vertical, Rotated, Large, or Interleaved.

Default displays are shown automatically unless you choose a different display option.

Reduction options allow you to display the raw data received from the Lmax in a reduced (analyzed) form. Which reduction options are available depend on the read mode; custom settings, using formulas you create, are available for all read modes and read types.

Reduction settings do not affect the raw data but simply cause it to be displayed differently, allowing you to change reduction settings without affecting the raw data from the plate reading in any way.

Custom reduction formulas require the use of accessors and operators that are understood by SOFTmax PRO for Lmax. Information regarding custom formulas can be found in the *Formula Reference Guide*.

The Data Display

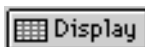
After a plate has been read, the information sent from the Lmax is shown in the data display in the active Plate section. The default display for data from endpoint readings for the Lmax is raw RLU numeric values. Data from kinetics readings is displayed in plots format as raw RLU values with respect to time. While a kinetic reading is in progress, the display updates dynamically to show the data collected so far.

All choices except those defined in the Instrument Settings affect only the manner in which the data is displayed—the *raw* data is not changed—permitting you to optimize the display *after* data is collected.

NOTE: Data *reduction* changes the way in which data is displayed and also affects the values used in calculations within SOFTmax PRO for Lmax. While the raw values remain unchanged, calculations will use reduced values, if any, and are, therefore, affected by settings chosen in the Reduction dialog box. For more information, see “Data Reduction” on page 5-16.



The Display Dialog Box



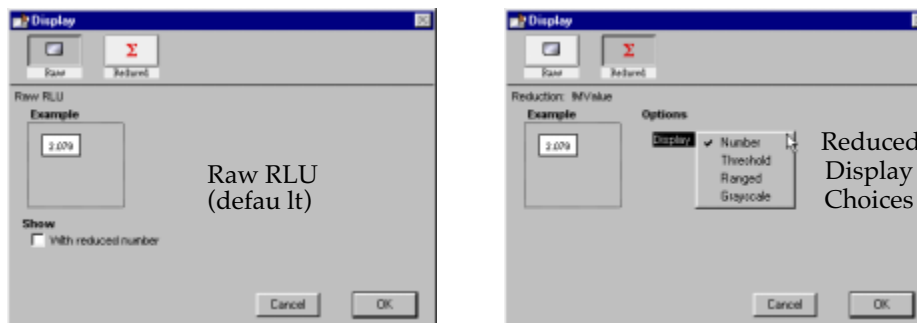
Before or after a plate has been read, you can click the **Display** button to open the Display dialog box which allows you to change the way the data is presented. Choices available in this dialog box depend on whether a Plate section is currently active and which read mode you have chosen (for example, the choice for "Plots" is not shown if endpoint mode is selected). Figure 5.2 shows the default Display dialog boxes for the different read modes.

The Display dialog box is divided into two parts (left and right): the default display, "Raw RLU," is shown to the left of a dividing line; the choices for reduced data displays are shown to the right of the dividing line. Note: data reduction itself is performed separately; settings here simply determine how the reduced data will be *displayed* in the Plate section.

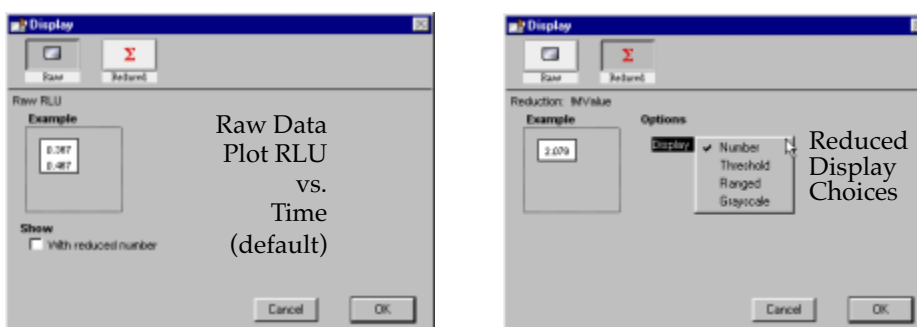
The dialog boxes below show the *default* display choices for the three modes: raw RLU values (numbers) for endpoint; raw RLU values (numbers) for dual read; the change in raw RLU values over time displayed as a plot for both long and fast kinetics. Other choices available in this dialog box include displaying the data as a single reduced value (number), in threshold, ranged, or gray scale formats, or any combination of display choices with the reduced number (the reduced number display choice cannot be used with the reduced number checkbox).



Endpoint



Dual Read



Long and Fast Kinetics

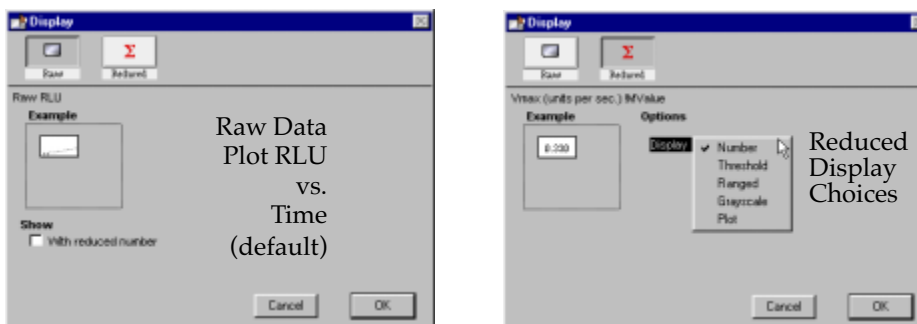
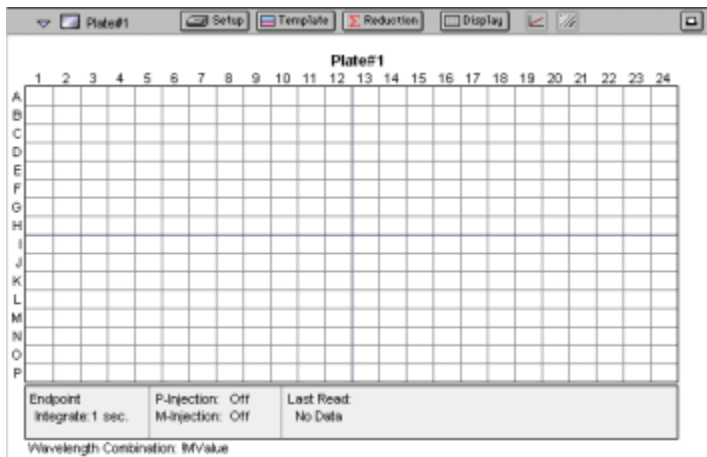


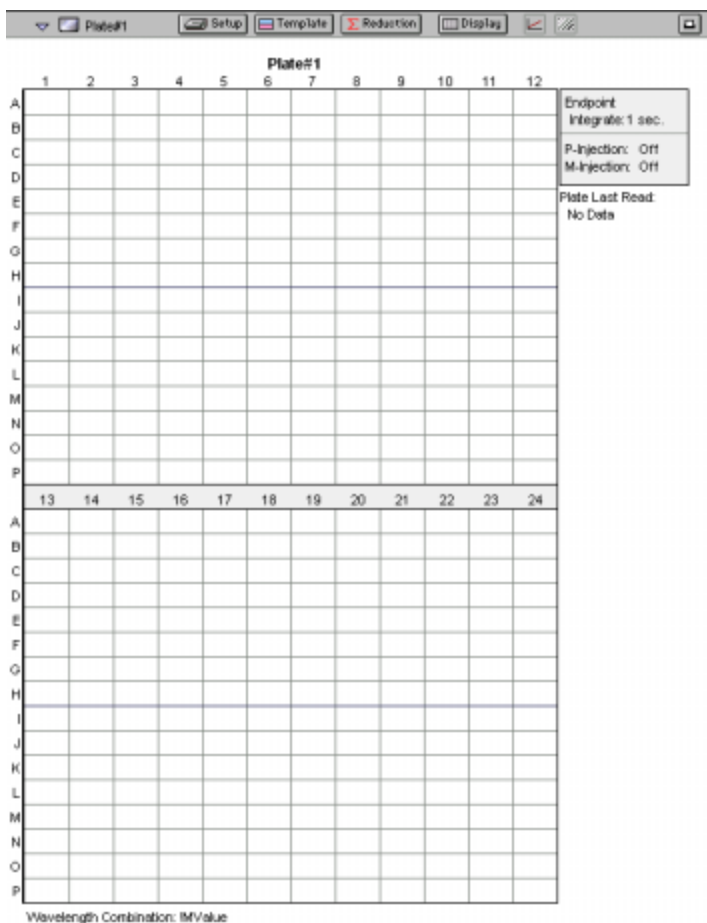
Figure 5.2: Endpoint, Dual Read, and Long and Fast Kinetics Display Dialog Boxes.

Data in the Plate section is displayed in a grid array (microplate format). With all plates other than 384-well plates, the display is always shown as a standard microplate, with well A1 in the top left corner. If you are reading 384-well plates, additional display options can be chosen to make the display of data clearer:

- Normal: shows the data in standard left-to-right format, with well A1 in the upper left corner. Columns 1 through 24 are along the top and rows A through P are shown from top to bottom. The wells are smaller and data is shown with fewer digits (less precision).

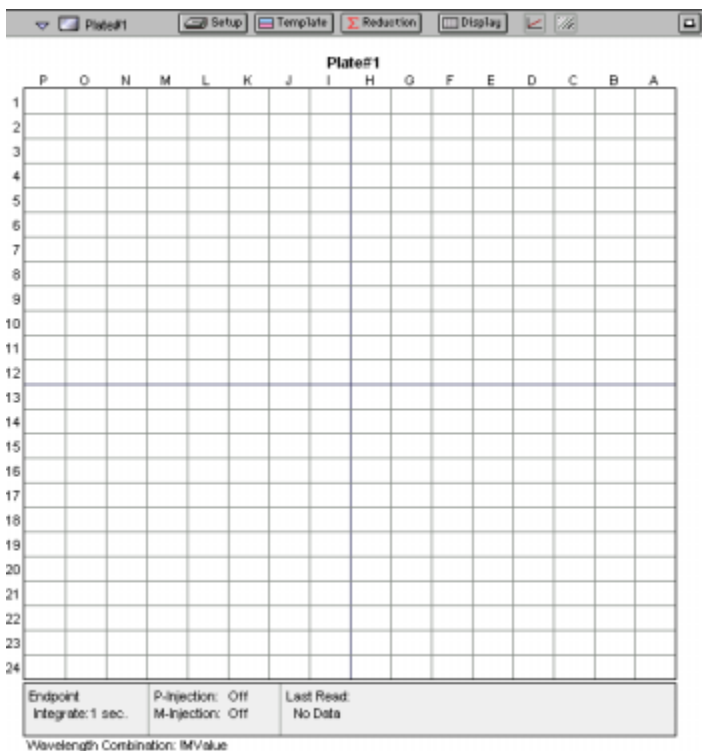


- Vertical: the plate is shown in the same orientation as Normal except that columns 1 through 12 (all rows) are shown above columns 13 through 24 (all rows).

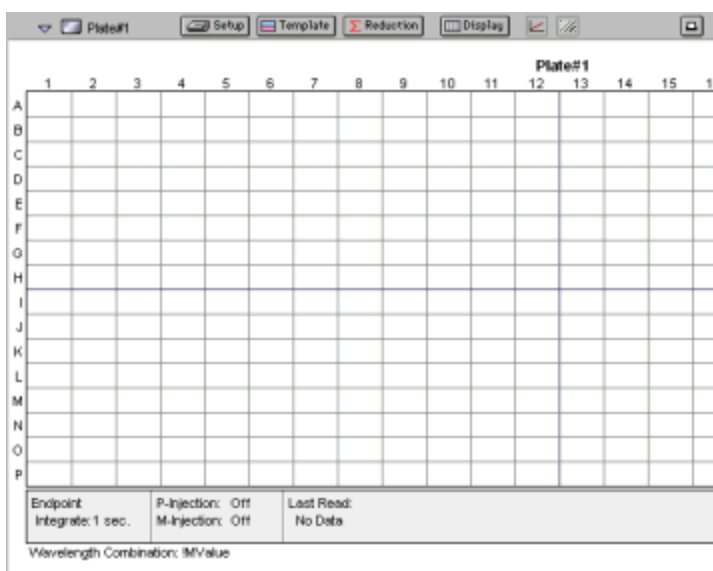




- Rotated: Same as the Normal view except that it is rotated 90 degrees clockwise (well A1 is in the upper right corner).

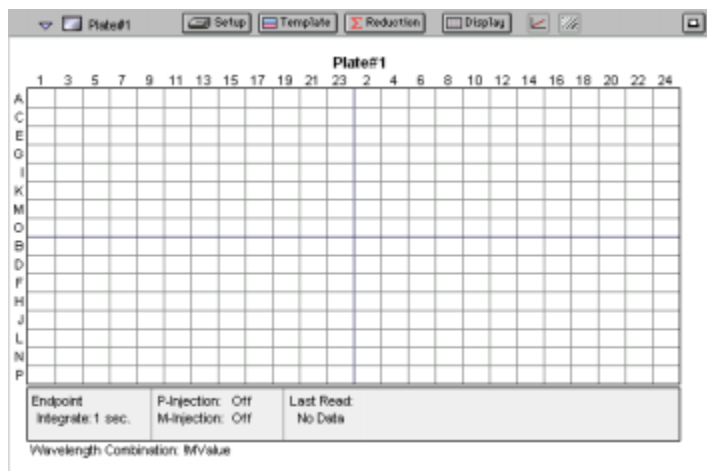


- Large: Same as the Normal view except larger. This display can be seen in its entirety only if you have the page size set for *legal paper* (8.5 x 14 inches) in *landscape* orientation (set this in **Page Setup** or **Preferences** under the **File** menu). In the example below, the display is shown as it appears if you do *not* change the page size from letter-size to legal.





- **Interleaved:** In this display, the wells are shown in a format that “skips” every other well as follows: all odd columns and rows begin in the upper left corner of the plate display and are followed by all even columns and rows. So well A1 is still in the upper left corner, but it is followed horizontally by well A3, A5, ... A23, A2, A4, etc., and vertically by well C1, E1, ... O1, B1, D1, etc. This display is most useful when the 384-well plate is composed of 4 daughter plates of 96 wells each.



Reduced Number

The reduced number is based on the settings defined in the Reduction dialog box. It is a combination of plate blank subtraction and/or endpoint reduction or kinetic reduction. The information acquired from the microplate reader is reduced to a single number or value. The reduced number will be reported in the Group section when a template has been defined.

To view by reduced number alone, select the Number option (the first choice to the right of the default, just after the dividing line); to see a reduced number as well as another type of display, click the box next to the option that says “With reduced number” in the lower left of the dialog box to enable it. Changing the reduction parameters after choosing a reduced data display mode will cause the display to update accordingly. More information about reduction formulas and how to customize them follows later in this chapter.

Threshold, Ranged, and Gray Scale

To display **Threshold, Ranged, or Gray Scale** data, high and low values must be entered. When you choose one of these display options, SOFTmax PRO for Lmax provides default values for the high and low limits by means of an autoranging function (encompassing all the data from lowest to highest value). You can change the high and low limits by highlighting the existing value and entering a new value. An example of the Display dialog box for a kinetic reading with the Threshold display option chosen is shown in Figure 5.3.

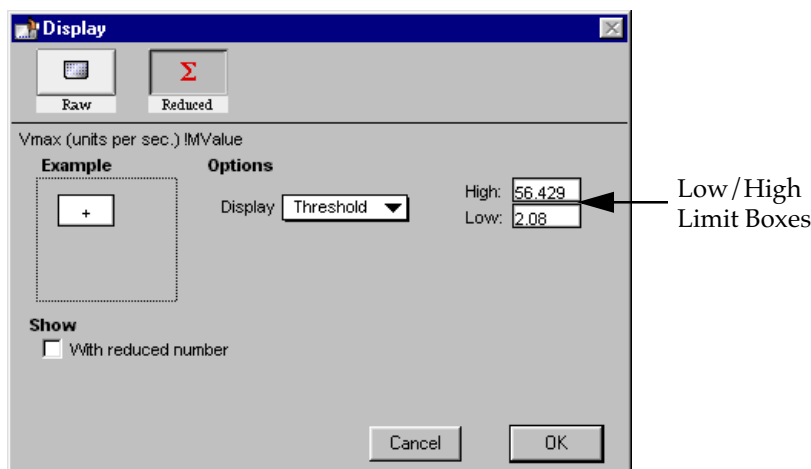


Figure 5.3: Display Dialog Box for Long Kinetics Threshold Display

Threshold

The **Threshold** display shows the raw data as a plus (+) for values above the high limit, an asterisk (*) for values within the limits, and a minus (-) for values below the low limit. Figure 5.4 shows an endpoint reading displayed using the Threshold option along with the reduced number.

Plate#1													Endpoint Integrate: 1 sec.
1	2	3	4	5	6	7	8	9	10	11	12		
A	-	*	*	*	*	*	*	*	*	*	*	*	P-Injection: Off M-Injection: Off
	1.100	1.200	1.300	1.400	1.500	1.600	1.700	1.800	1.900	2.000	2.100	2.200	
B	*	*	*	*	*	*	*	*	*	*	*	*	Plate Last Read: 10:01 AM 6/21/00
	2.100	2.200	2.300	2.400	2.500	2.600	2.700	2.800	2.900	3.000	3.100	3.200	
C	*	*	*	*	*	*	*	*	*	*	*	*	
	3.100	3.200	3.300	3.400	3.500	3.600	3.700	3.800	3.900	4.000	4.100	4.200	
D	*	*	*	*	*	*	*	*	*	*	*	*	
	4.100	4.200	4.300	4.400	4.500	4.600	4.700	4.800	4.900	5.000	5.100	5.200	
E	*	*	*	*	*	*	*	*	*	*	*	*	
	5.100	5.200	5.300	5.400	5.500	5.600	5.700	5.800	5.900	6.000	6.100	6.200	
F	*	*	*	*	*	*	*	*	*	*	*	*	
	6.100	6.200	6.300	6.400	6.500	6.600	6.700	6.800	6.900	7.000	7.100	7.200	
G	*	*	*	*	*	*	*	*	*	*	*	*	
	7.100	7.200	7.300	7.400	7.500	7.600	7.700	7.800	7.900	8.000	8.100	8.200	
H	*	*	*	*	*	*	*	*	*	*	*	*	
	8.100	8.200	8.300	8.400	8.500	8.600	8.700	8.800	8.900	9.000	9.100	9.200	

Wavelength Combination: IMValue

- < 1.1 * 1.1 to 9.2 + > 9.2

Figure 5.4: Endpoint Data Displayed as Threshold with Reduced Number

Ranged

When the **Ranged** display is chosen, raw data that falls between the high and low limits is assigned proportionally to integer values from 0 through 9. Values above the high limit are displayed as a plus (+) and values below the low limit are displayed as a minus (-). Figure 5.5 shows reduced kinetic data displayed in Ranged format.

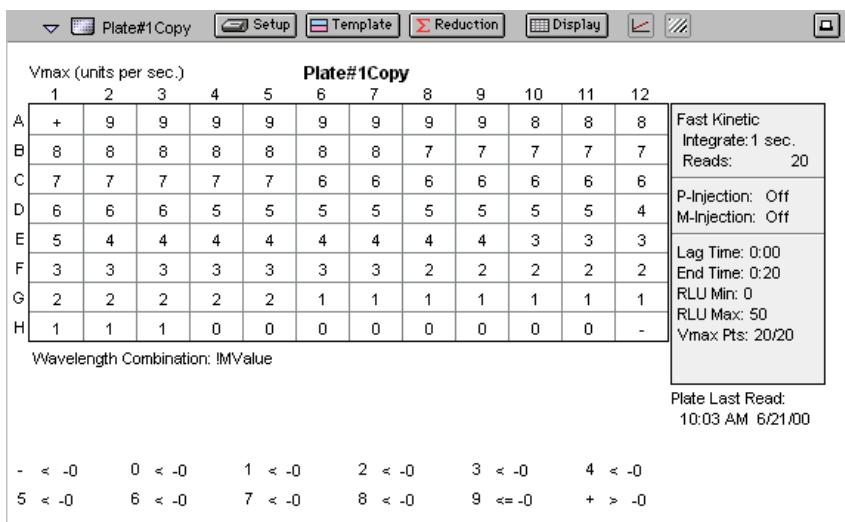


Figure 5.5: Fast Kinetics Data (Reduced) Displayed as Ranged

Gray Scale

The **Gray Scale** option presents the raw data in eight shades of gray, changing from light (for values less than or equal to the low limit) to dark (for values greater than or equal to the high limit). Figure 5.6 shows reduced endpoint data displayed as Gray Scale.

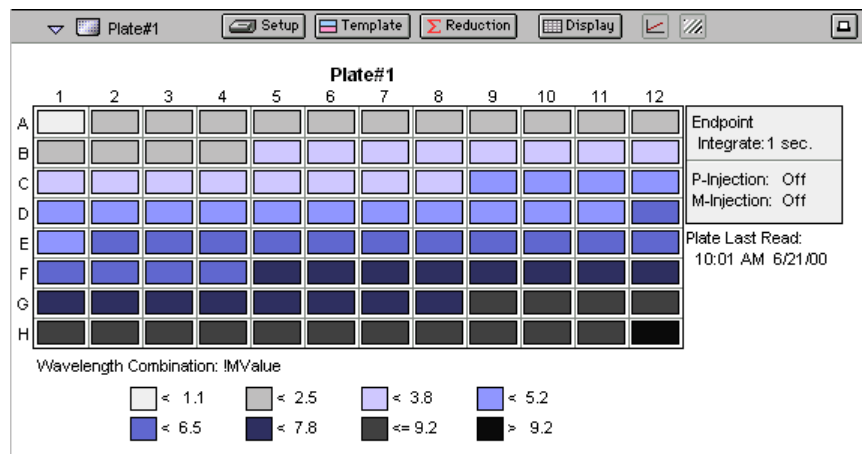



Figure 5.6: Endpoint Data (Reduced) Displayed as Gray Scale



Well Graphs of Kinetic Wells


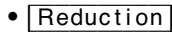
 You can enlarge the display of the wells shown with either long or fast kinetics mode to get a close-up view of the data. This is done by either double-clicking the particular well in the data display or by selecting the well and then clicking the Well Graph button in the Plate section tool bar.

Well Graphs plot the data for the individual wells in a microplate and show the well ID and reduction information, including the goodness of fit (R^2 value), if appropriate. The default display for the X-axis shows time; RLU is displayed on the Y-axis.

Positioning the cursor within a Well Graph shows X and Y values for the cursor location, allowing you to determine more precisely the location of data points.

Well Graph Options



Along the bottom of each Well Graph dialog box are eight buttons that perform the following functions:

-  **Print**
Opens the Print dialog box.
-  **Reduction**
Opens the Reduction dialog box.
By default, the ranges on the enlarged plot are set to the limits defined in the Reduction dialog box, and the information included near the bottom of the window is based on the reduction settings currently in use. You may change these settings (type of reduction used, minimum and maximum RLU limits, lag time and end time, and number of Vmax Points). More information about the reduction options can be found later in this chapter.

NOTE: Changes to the reduction settings made within well graphs will be applied to *all* wells in the Plate section. When viewing Raw data, you can scale the graph to see data outside the reduction limits [line(s) are included on the graph indicating the limits and the area outside of them will be shaded slightly]. This view should assist you in determining the best limits for the reduced data.

-   **Show Raw/Show Reduced button**

This button toggles the Well Graph between views of raw and reduced data. When the Well Graph is opened, the initial view depends on the Display settings for the Plate section. If raw data is being displayed in the Plate, the initial view in the Well Graph will be raw data and the button will be set to "Show Reduced." If reduced data is being displayed in the Plate section, the initial view in the Well Graph will be reduced data and the button will be set to "Show Raw."


-   **Scale to Data/Scale to Limits button**

This button toggles between a view of the data within the reduction limits and a view of the entire data set. Switching between these views helps to



visualize how the limit settings affect data reduction. If raw data is being viewed in the Plate section, the display in the Well Graph is set initially to show the plot scaled to the reduction limits. If reduced data is being viewed in the Plate section, the Well Graph display is set initially to show the plot scaled to the data.

NOTE: If you are viewing raw data and click the [Scale to Limits] or [Scale to Data] button, only the display of raw data in the Well Graph will be affected (the button will not affect the view of reduced data). The same is true if you click this button while viewing reduced data. To produce comparable scaling of both raw and reduced displays, this button must be set to either Scale to Limits or Scale to Data for both raw and reduced views of the Well Graph.

-  Graph Options button
Opens the Well Graph Options dialog box which allows you enable or disable connected points or plotted symbols the graph.

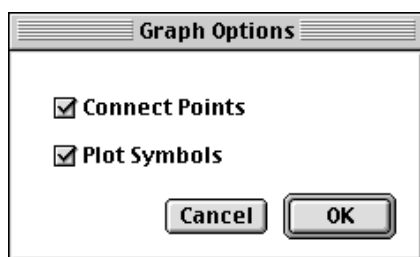




Figure 5.7: Graph Options Dialog

-  X-Axis Settings button
-  Y-Axis Settings button

Opens the Well Graph Axis Settings dialog box for the particular axis, allowing you to set Auto Range parameters, choose the minimum and maximum values for the selected axis, cause the axis to be auto-ranged, add or remove gridlines, and add or remove axis tick marks. When a Well Graph is opened, the default settings are to show the reduction limits for that axis with Auto Ranging off and gridlines and tick marks selected. Only the Well Graph being viewed is affected by changes in these dialog boxes.

- **Done**
Closes the Well Graph dialog box.

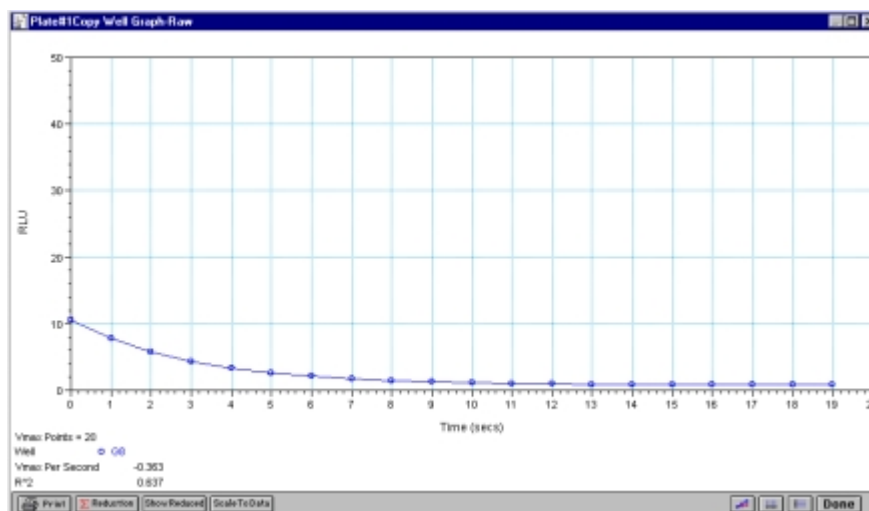


Figure 5.8: Well Graph for a Single Fast Kinetics Microplate Well (Raw Data)

Multiple wells from the same Plate section can be graphed together in one enlarged window. Select the well in the data display (hold the Shift key if you are selecting non-contiguous wells) and then click the Graph button or double-click in one of the selected wells.

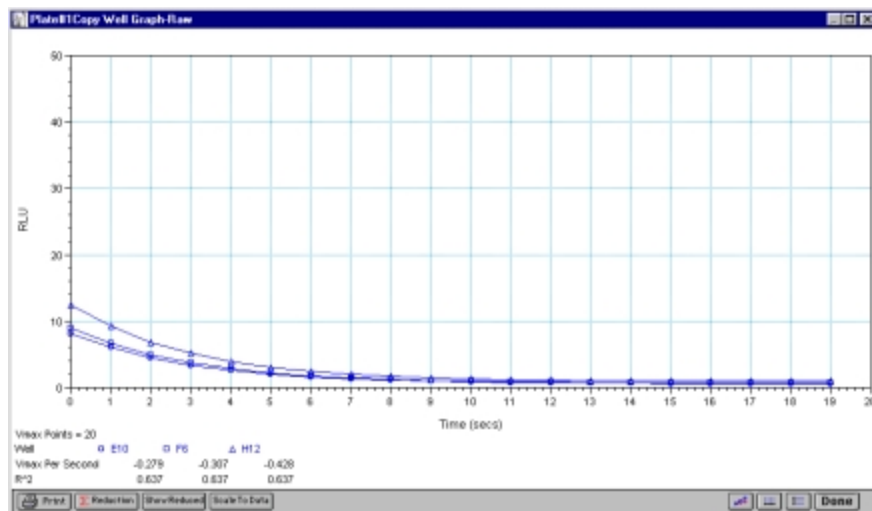


Figure 5.9: Well Graph for Three Fast Kinetic Wells of a Microplate (Raw Data)

The choices you make for viewing the data in the Plate section also affect the way the Well Graph appears. Figures 5.8 and 5.9 show Well Graphs for *raw* data displays. If you display one of the *reduced* options (reduced number, Threshold, Ranged, or Gray Scale), the Well Graph will show the plot with an indicator of the reduced value and will include the word “Reduced” in the window title.



Zoom Box

You can enlarge the Well Graph even further by clicking the zoom box in the upper right-hand corner of the window (the center box of three in the upper right corner in Windows). Clicking the zoom box once will cause the window to fill the total display on your monitor; clicking the zoom box again will return the window to its previous size.

Size Box

Clicking and dragging the size box located in the lower-right corner (Macintosh) or clicking and dragging the lower right corner of the window (Windows) allows you to make the Well Graph any size desired.

Printing the Well Graph

You can print the Well Graph window, if desired, by clicking the **Print** button at the bottom left corner of the dialog box.

NOTE: If you enlarge the Well Graph to greater than 12 inches, Molecular Devices does not recommend printing it since the graph will not fit on an 8.5- by 11-inch page and the printout will be truncated.

Masking Wells

If you find data in certain wells (outliers) that should be excluded from the calculations to be performed by data reduction, you can mask these wells. Select the well(s) to be masked and then click the Mask button in the Plate section tool bar or choose **Mask...** from the **Plate** menu. Masked wells are shown with an overlay of diagonal lines and are marked with the word "Masked."

The masking function can be used as a "what if?" tool. For example, you could suppress or enable certain functional groups within the experiment. Suppose you have included a group blank in the template and want to see the data with and without the blank. Masking the group blank wells would suppress the blanking function; unmasking them would enable it again.

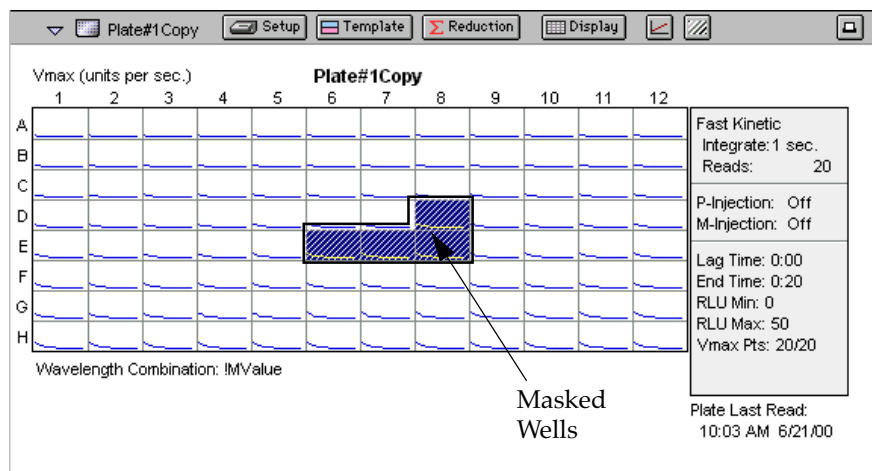
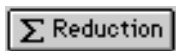


Figure 5.10: Masked Wells



To remove masking from one or more wells, highlight the masked well(s) and click the Mask button in the Plate section tool bar (toggles masking on and off) or choose **Mask...** from the **Plate** menu again. The diagonal lines covering those wells (and masking function) will be removed.

Data Reduction



Raw data received from the Lmax can be displayed in an analyzed (or reduced) form. The reduction process within SOFTmax PRO for Lmax is based on formulas that reduce the raw data to show a single number for each well. Further analysis of this reduced number then takes place in the Group and Graph sections.

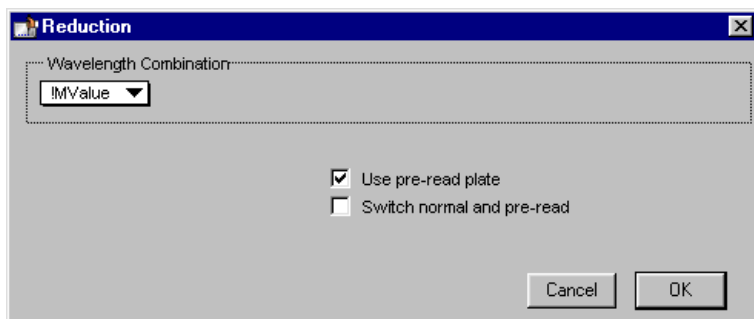
SOFTmax PRO for Lmax performs calculations hierarchically when reducing the luminescence information collected from the instrument. Calculations in the Plate section are performed in the order shown below. If an option has either not been selected in the Instrument Settings dialog box or has not been defined in the template and/or Reduction dialog boxes, SOFTmax PRO for Lmax starts/continues with the next listed calculation.

- 1) Plate blank subtraction
- 2) Endpoint or kinetic reduction
- 3) Group blank subtraction

You can access the Reduction dialog box in several ways: by clicking the **Reduction** button in the tool bar of the active Plate section, by choosing **Reduction** from the **Plate** menu, or by clicking the **Reduction** button in the Well Graph display. The appearance of the Reduction dialog box will be different depending on the type of read mode you have chosen, and the options you have chosen in the Instrument Settings dialog box. Figure 5.11 shows the Reduction dialog box for endpoint, dual read, and kinetic modes, along with the default reductions for each of those modes.

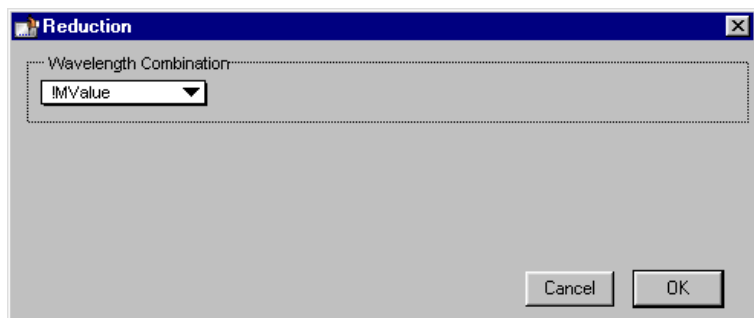
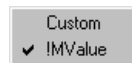


Reduction Dialog Boxes

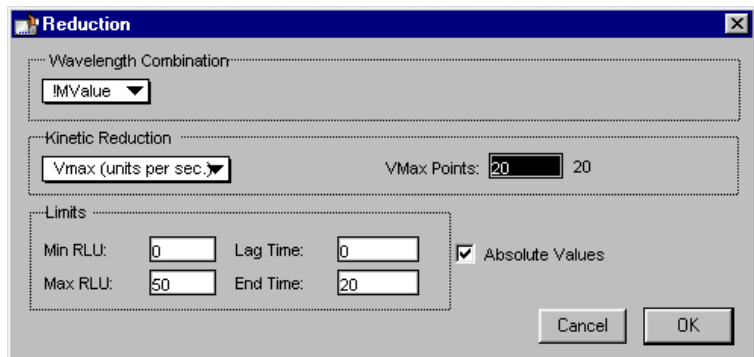
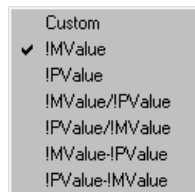


Endpoint

Default Reductions



Dual Read



Long Kinetics and Fast Kinetics

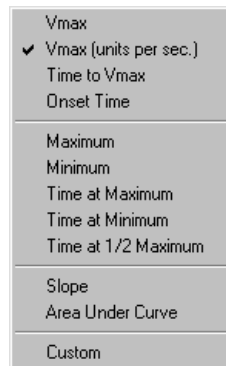
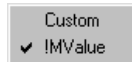


Figure 5.11: Reduction Dialog Boxes for All Read Modes.



All of the read modes offer one or more predefined choices for reduction. The predefined reductions are described below under the appropriate read mode heading. In addition, with any read mode, you can create a custom reduction formula to better suit your needs. Since all custom reduction formulas are defined the same way, regardless of read mode, they are described together after the individual read mode sections.

Endpoint

The default reduction for endpoint readings is !MValue. You can also choose Custom in order to create a different reduction formula.

Dual Read

The default reduction for dual readings is !MValue. Other reductions available from the pop-up menu are !PValue, !MValue!/PValue, !PValue!/MValue, !MValue-!PValue, and !PValue-!MValue. You can also choose Custom in order to create a different reduction formula.

Long and Fast Kinetics

The default reduction for kinetic readings is !MValue. If you do not wish to use the default reduction, you can choose Custom and create a different reduction formula.

Kinetic Limits

The Limits settings for the display of kinetic data are MaxRLU, MinRLU, Lag Time, and End Time.

NOTE: The display of RLU values is shown relative to the first point measured for each well. Negative kinetic values decrease with time, and limits should be set accordingly (below 0) to view negative kinetic data.

Limits define the data that will be *viewed and included in data reduction*, but do not affect data collection. If you alter a limit to show less data, you can always display the “hidden” data again by changing the limit.

MaxRLU

The limit for the maximum value you wish to report. Any values from the reading that are above this limit will not be shown and will be excluded from data reduction. The default is 20,000 for RLU.

The MaxRLU reduction parameter can be used to exclude the non-linear portion of the reaction from data analysis. This type of data reduction is most useful in reactions where the initial portion of the data is linear. You might also adjust the End Time setting to remove a non-linear portion after a certain time point in the reaction. Using MaxRLU allows the use of the maximum number of linear points to calculate the slope of the line and, thus, to determine the rate for each well.



MinRLU

The limit for the minimum value you wish to report. Any values from the reading that are under this limit will not be shown and will be excluded from data reduction. The default is 0 RLU. To display negative kinetics, the value should be set below 0 (zero).

Lag Time

Specifies how many initial data points are excluded from the calculation of Vmax Rate. Lag Time truncates the data used in the calculation. It does not prevent data from being collected. The kinetic plots will not display the data collected prior to the set Lag Time. The value for Lag Time can be changed after the plate is read. The default is 0.

End Time

This setting specifies the time at which you wish to stop showing data in the display. Any values occurring after this limit will not be reported in the display. The default setting is the total assay time. The value for end time may not be greater than the total assay time.

Absolute Values

This setting causes the plots shown in the data display to be drawn at their absolute RLU as opposed to the default (with the box unchecked) which offsets the first point to the (0,0) coordinate. Enabling Absolute Values may cause some data points that do not fall within the default limits for the plots to “disappear.” To see more data, yet still display absolute values, increase the limits for MinRLU and /or MaxRLU found in the Reduction dialog box (Figure 5.12).

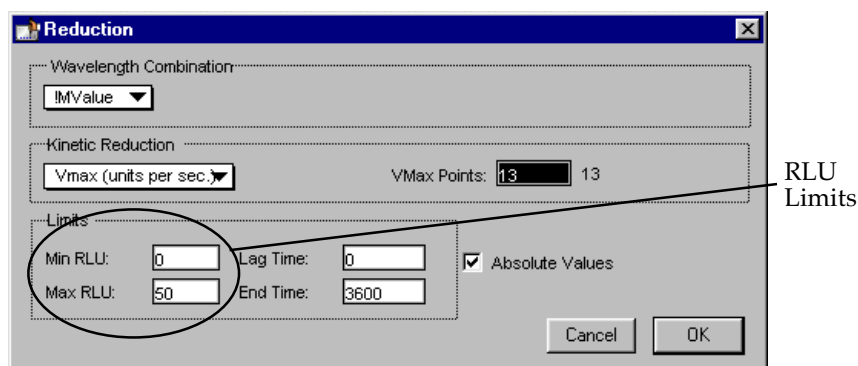


Figure 5.12: Limits in Reduction Dialog Box

If you increase the MaxRLU setting to be at least as large as the largest luminescence value expected, most or all of the data plots should appear in the data display.

Kinetics Reduction

The default kinetics reduction is Vmax (units per sec). Other reductions available from the pop-up menu are Vmax, Time to Vmax, Onset Time, Time at Minimum, Time at Maximum, Time at 1/2 Maximum, Slope, Area Under Curve, or you can choose Custom and create a different reduction formula.



Vmax and Vmax(units per sec)

The maximum slope of the kinetic display of relative luminescence units versus time. The default value displayed is in units/second, where units are relative luminescence units (RLU). The rate is calculated with the use of Vmax Points (see below) which determines the number of contiguous points over which Vmax is calculated. The number of Vmax Points defaults to the maximum taken during the reading; you can change this number in the Reduction dialog box if desired.

NOTE: $V_{max} = V_{max}/sec \cdot 60,000$

A linear regression is performed to determine the slope of the line using the equation:

$$y = m \cdot X + B$$

where m is the slope of the line.

The reaction rate is reported in units/sec.

Time to Vmax

This is an alternative method for analyzing non-linear kinetic reactions which reports the elapsed time until the maximum reaction rate is reached rather than reporting the maximum rate itself. Used in conjunction with Vmax Points, Time to Vmax is the time to the midpoint of the line defined by Vmax Points and used to calculate Vmax. This elapsed time data is useful for applications including coagulation chemistry where the changing concentration of the reagents does not change Vmax, but rather will change the time at which the reaction reaches the maximum rate.

Onset Time

This is another method for analyzing non-linear kinetic reactions. Onset Time reports the time required for a kinetic reaction to change by a specified amount of luminescence (onset RLU above the initial RLU reading for the well).

Time at Minimum

This setting reports the time at the minimum RLU that falls within the reduction limits.

Time at Maximum

This setting reports the time at the maximum RLU that falls within the reduction limits.

Time at 1/2 Maximum

This setting reports the time at the half of the maximum RLU that falls within the reduction limits. To calculate this reduction, SOFTmax PRO for Lmax first determines the kinetic point (within the reduction limits) that has the maximum signal level. It then scans the kinetic plot from left to right until it finds two points having signals that bracket half of that value. A linear interpolation between these two points is used to estimate the Time at 1/2 Maximum.



Slope

This setting determines the slope of the combined plot (i.e., the slope of the line using linear regression). This setting uses all visible timepoints in the reduction window.

Area Under Curve

This reduction estimates the area under the curve as defined by the data plots in wells (within the reduction limits). The data plots are treated as a series of trapezoids with vertices at successive data points and at the X-axis coordinates of the data points. The areas defined by each of the trapezoids are then computed and summed.

Custom Reduction Formulas

If the predefined reduction formulas do not meet your needs, you can create one or more different reduction formulas for any or all read modes (endpoint, dual read, or kinetic). Choosing “Custom” from any of the menus or dialog boxes causes a Formula button to appear (an example of this button in an endpoint Reduction dialog box is shown in Figure 5.13).

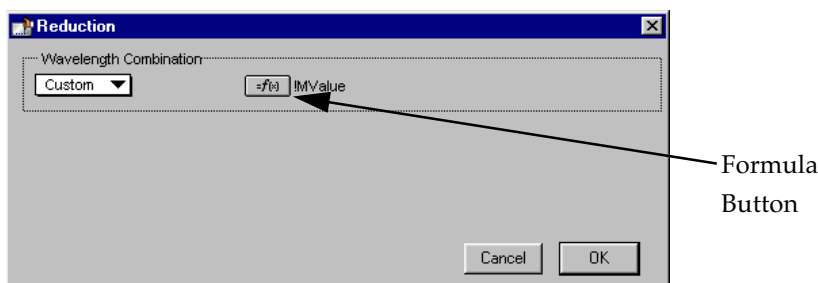


Figure 5.13: “Custom” Selected Showing Formula Button (Endpoint)

Clicking the Formula button opens the Calculation dialog box in which you can name and define a new reduction formula.

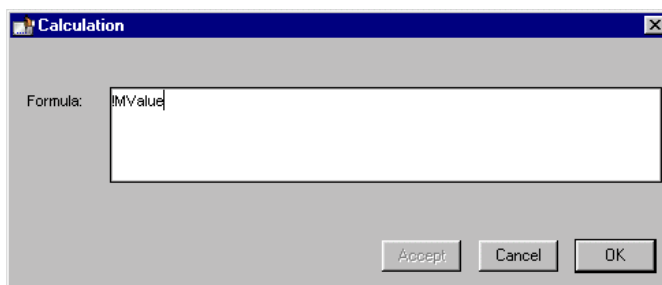


Figure 5.14: Calculation Dialog Box (Endpoint)

When you finish your entry in the Calculation dialog box and click **OK**, the formula you have entered then appears to the right of the word “Custom” and becomes the default selection for the Custom option. You may subsequently edit this formula or enter a new one by clicking the Formula button again. For a full discussion of custom formulas, see the *Formula Reference Guide*.



Recalculation Options

By default, SOFTmax PRO for Lmax performs continuous recalculation of the data when you read plates, create or change formulas, and so on. At certain times, it would be useful to disable the recalculation feature so that you can make alterations to the experiment without waiting for individual recalculations to be completed. Two commands are available in the **Edit** menu that allow you to modify the way in which SOFTmax PRO for Lmax performs recalculation: **Suspend Recalculation** and **Recalculate Now** (⌘ =; CTRL+=).

Choosing **Suspend Recalculation** disables the automatic recalculation feature of the program and places a checkmark to the left of the command in the menu to indicate that it has been selected. While this function is enabled, no recalculation will occur, regardless of what you may change, add, or delete from the experiment. This is especially useful when creating or changing column formulas within Group sections, for example, as it can speed up the process greatly.

When recalculation has been suspended, you may wish to see the results of the changes you have made but may still not wish to enable continuous recalculation. Choosing **Recalculate Now** (⌘ =; CTRL+=) from the **Edit** menu will cause SOFTmax PRO for Lmax to examine all elements of the experiment and perform complete recalculation of the data. Choosing this command does not enable continuous recalculation but simply causes the program to perform recalculation once.

To return to continuous recalculation, choose **Suspend Recalculation** again. The checkmark next to the command will disappear, indicating that the function is no longer in use.

NOTE: **Recalculate Now** (⌘ =; CTRL+=) can be used to see intermediate results during long kinetic readings. At any time, you can choose **Suspend Recalculation** to delay calculations until a later time.

Displaying Data in Group, Graph, and Notes Sections

Group Sections

The manner in which data is displayed in a Group section depends on the type of reading that was performed (endpoint, dual read, or kinetic), but can be customized by choices made in the data display, reduction settings, and the **Edit** menu.

- Resizing/hiding columns
- Adding/deleting columns
- Showing/editing column formulas
- Adding/editing summaries
- Changing text style

Default displays for Group sections are shown automatically unless you choose a different display option.

The particular font to be used in a Group section table, as well as its size and style, can be changed by first selecting one or more columns in the Group sec-



tion table and then choosing the **Text** command from the **Edit** menu and making the desired choices from the dialog box that appears.

Settings from the Plate Section Affect Data Display in Group Section Tables

Reduction options in the Plate section allow you to display the raw data received from the Lmax in a reduced (analyzed) form. Which reduction options are available depend on the read mode used and reduction settings chosen. Reduced number from the Plate section is displayed in the Values column of the Group section tables by default.

Reduction settings do not affect the raw data but simply cause it to be displayed differently. You can change reduction settings without affecting the raw data from the plate reading in any way.

Custom reduction formulas require the use of accessors and operators that are understood by SOFTmax PRO for Lmax. A list of these accessors and operators is given at the end of this chapter.

Resizing and Hiding Columns

The columns in a Group section can be resized in several ways. One of the simplest is to drag the line dividing the columns to another position (moving it horizontally). When you point to a line between columns, the cursor changes to a double arrow with a vertical line through it, showing that it is ready to move left or right (see Figure 5.15).

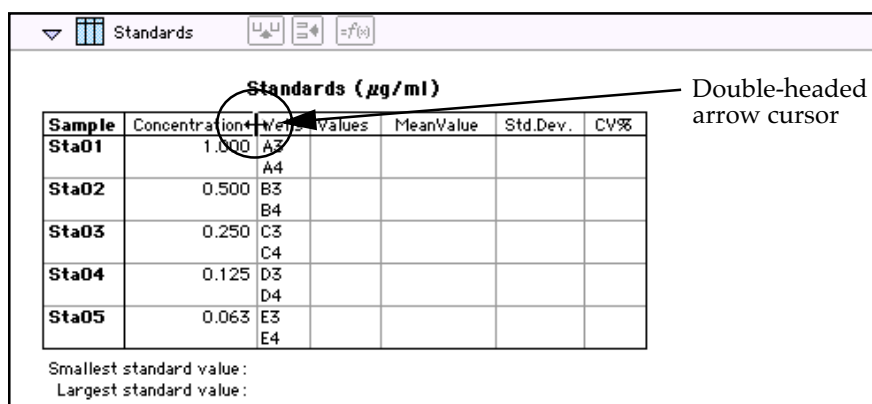


Figure 5.15: Cursor Positioned on a Line between Columns

Reducing Column Size and Hiding Columns

You can reduce a column to a size smaller than the width of its title, in which case the title will not be completely visible. You can also hide a column by reducing its size completely (drag one edge of the column on top of the other) or by selecting that column and choosing **Hide** from the **Group** menu.

NOTE: If you hide a column in this way and then wish to view it again, use the **Show All** command in the **Group** menu. All columns that were hidden will again be visible. There is no way to show only *one* hidden column.



Enlarging Column Size

You can enlarge the width of columns to any size. Increasing the size of columns may make the width of the Group section increase beyond the page width boundary. If this occurs, the columns will “disappear” off the edge of the screen but are really still there. To bring these columns back into view, reduce the width of the columns that are still visible until the others reappear.

Divider being dragged to the left

Standards ($\mu\text{g/ml}$)						
Sample	Concentration	Wells	Values	MeanValue	Std.Dev.	
Sta01	0.00	A3				
		A4				
Sta02	0.500	B3				
		B4				
Sta03	0.250	C3				
		C4				
Sta04	0.125	D3				

Figure 5.16: Dragging a Column Divider Line

NOTE: If the width of a section extends beyond the page width boundary, the portion that is not visible will not be printed.

To size two or more columns to the same width, highlight those columns and then drag one of the dividing lines to the desired width. The width of all highlighted columns will be sized the same as the column to the left of the dividing line you are moving.

Columns can also be resized to the minimum amount of space required by choosing **Autosize** from the **Group** menu. By highlighting the entire table or individual columns and then choosing **Autosize**, the amount of space each column requires will be reduced to the minimum size required by the largest piece of text in that column.

Double-clicking the line dividing two columns causes the single column to the left of the divider to be sized according to the largest amount of text in that column.

Showing and Editing Existing Column Formulas

When group sections are first created, the formulas used to calculate the data are not displayed.

These formulas can be shown (until you choose to hide them again) by making a Group section active and then choosing **Show Formulas** from the **Group** menu. (Note that the command in the menu changes to **Hide Formulas** after they are shown.)

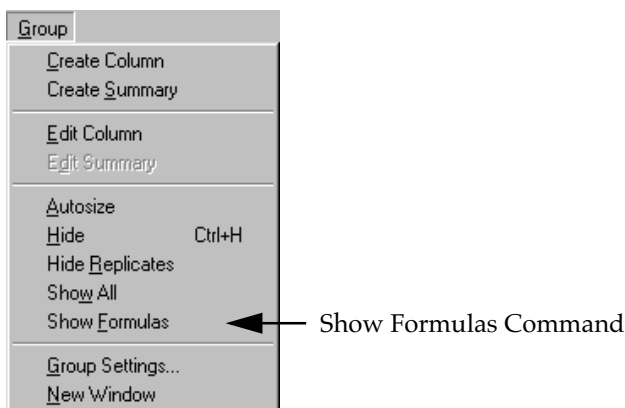


Figure 5.17: The "Show Formulas" Command in the Group Menu

Choosing this option causes an additional row to appear (beneath the heading for each column) which shows the formula used to calculate the data in each column. To see formulas briefly, hold down the shift and control (Ctrl) keys.

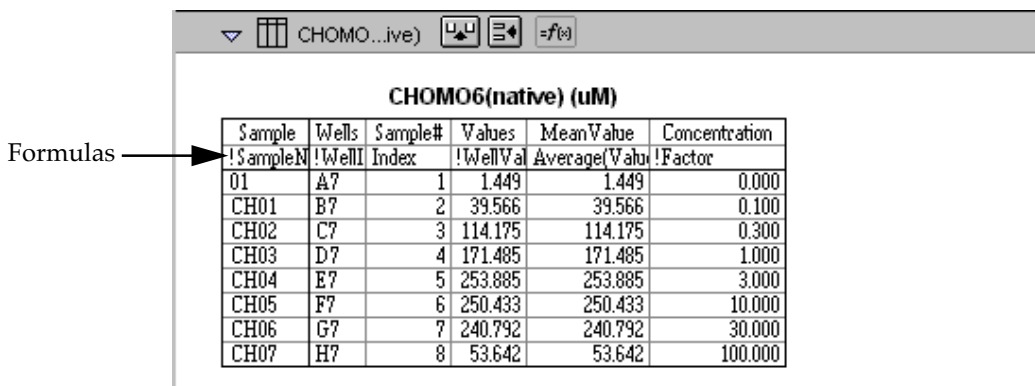


Figure 5.18: Formulas Shown in Group Columns

Modifying Column Formulas

To modify the formula for an existing column, double-click the column or click the column once and then click the formula button in the section tool bar. The dialog box associated with the column formula will open, allowing you to edit the formula (and the name) associated with the column.

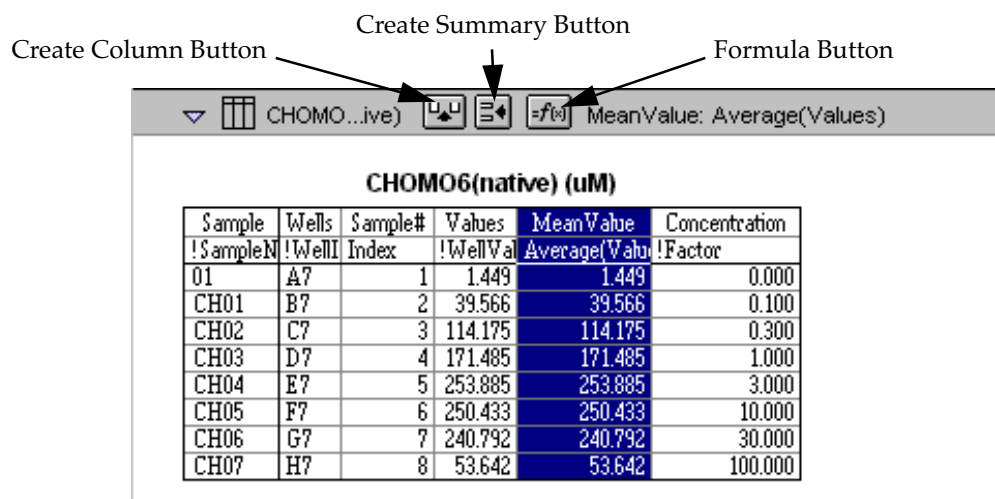


Figure 5.19: Formula Button Activated and Column Formulas Shown

For a complete discussion of custom formulas, see the accompanying *Formula Reference Guide*.

Adding Columns

You can add a new column to a Group section by clicking the Create Column button in the Group tool bar or by choosing **Create Column** from the **Group** menu which will open the Column Formula dialog box.

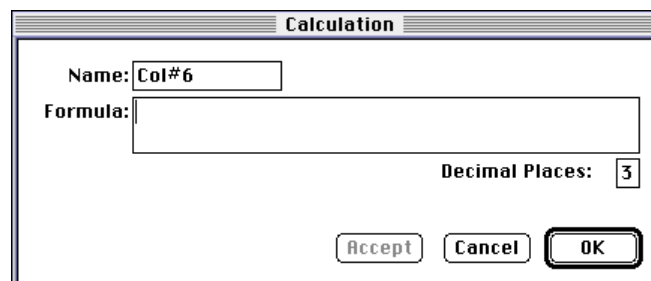


Figure 5.20: Column Formula Dialog Box

In the Column Formula dialog box, you can change the name of the new column (from the default which simply lists the column by number) to reflect the data that it will contain.

If no existing column is selected when you click the Create Column button, the new column will be created to the right of all existing ones. If you select (highlight) a column and then add a new column, it will be positioned to the right of the one you selected. If the table is currently as wide as the sheet of paper specified in **Page Setup** when a new column is added, the columns will “disappear” off the right side of the window but are still there. To bring these columns back into view, resize visible columns smaller.

New columns can contain references to other columns, both in the current section or in a different one within the same experiment. For example, if you wanted to subtract the mean values in one group from those in another, you



could create a new column (in either group) to do this. If the two groups were named "Group 1" and "Group 2," for example, and both groups contained a column entitled "Mean," you could create a column within "Group 1" that would subtract the mean values in "Group 2" from those in "Group 1." The column formula needed to do this would be:

Mean-Mean@Group2

Numerous possibilities exist for creating and editing column formulas to display the data you desire. For a complete discussion of formulas and operators, see the accompanying *Formula Reference Guide*.

NOTE: To delete a column, highlight it and press the Delete key or choose **Cut** from the **Edit** menu.

Adding or Editing a Summary in a Group Section

To add a summary at the bottom of a Group section, click the Create Summary button in the tool bar or choose **Create Summary** from the **Group** menu. The Calculation dialog box will appear, allowing you to change the name of the summary or hide the name, if desired, as well as to enter a description of the summary and to create a formula for it (including the number of decimal places for the result, if appropriate). Double-clicking an existing summary causes the same dialog box to appear, allowing you to modify summary information.

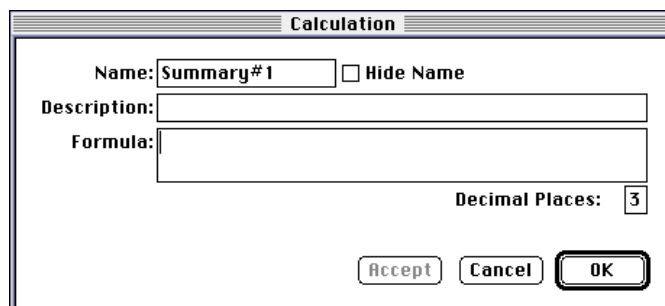


Figure 5.21: Calculation Dialog Box

Information entered in this dialog box will be placed at the bottom of the Group section as follows:

Name = Description Formula Result

You can click the checkbox next to Hide Name which will change the way the summary appears to show:

Description Formula Result

NOTE: The name assigned to a summary is important since you can reference the summary by name in other formulas (building one upon another). The summary name can be referenced even if it is hidden.

If you wish to see the formulas associated with summaries without calling up the Calculation dialog box for each one, you can choose **Show Formulas** from the **Group** menu. To see these formulas only briefly, hold down the shift and control (Ctrl) keys while that Group section is active. The summaries will "expand" to show the complete formula along with the title. They will resume their previous appearance when you release the option key.



Summaries in Group sections can be moved horizontally, if desired, by dragging them left or right. When you position the pointer over a Group summary, the cursor changes to show a double-headed horizontal arrow. Clicking once highlights the summary; dragging and then releasing the summary leaves it in the position where the mouse button was released.

To have summaries line up with particular columns in the Group, hold down the control (Ctrl) key while dragging the summary. When you release the control (Ctrl) key, the summary will “snap back” so that it begins flush to the left side of the column under which it was released. Each summary can be positioned in a different place in the Group section, if desired.

NOTE: To delete a summary, highlight it and press the Delete key or choose **Cut** from the **Edit** menu. A summary can also be copied or cut and then pasted in a different location.

You can also change the format of the text in Group section columns and/or summaries. By selecting the column or summary and then choosing **Edit** and then **Text Style** (Ctrl+T), you can change the font, size, and/or style of the text.

Notes Sections

The Notes section allows you to enter text describing the experiment and one or more summary lines that contain formulas. To create a summary, click the Create Summary button in the tool bar or choose **Create Summary...** from the **Notes** menu.

The Calculation dialog box that appears (see Figure 5.21) allows you to enter a description of the summary, accept the default title for the summary, change it, or hide it, and to enter a formula (including the number of decimal places the reduction should show, if appropriate). Detailed information regarding operators and accessors can be found at the end of this chapter.

If you wish to see the formulas associated with the summaries without calling up the Calculation dialog box for each one, you can choose **Show Formulas** from the **Notes** menu. To see these formulas only briefly, hold down the shift and control (Ctrl) keys while that Notes section is active. The summaries will “expand” to show the complete formula along with the other summary information. They will resume their previous appearance when you release the option key.

Graph Section


The Plot#X Dialog Box

Whenever you create a new Graph section or add a plot to an existing graph, the Plot#X dialog box opens automatically (“X” starts with “1” and increments each time a new plot is added to a Graph section). In this dialog box, you can rename the plot, assign specific information to be plotted on the X- and Y- axes, and choose which icon and color will be used. If you rename the plot, the title of the dialog box will change to show the new plot name.

All groups that have been created or assigned within the file are listed on the left side of the “Source:” field in the Plot#X dialog box. The first group in the file is automatically selected. Other groups can be selected by highlighting them. When a group is selected, the pull-down menus in the X-axis and Y-axis




subfields change to show the group columns that are available for graphing. Group columns can be assigned for both the values to be plotted and any error bars to be included for both axes.

Summaries and user-defined formulas can also be graphed. Pushing the  button for either the X-axis or Y-axis opens a dialog box which allows you to enter formulas. To plot a summary, enter the summary name in this dialog box. Formulas can be plotted for comparison with other data. For example, if the data is assumed to follow a specific model, the model formula can be entered as a plot and then the data can be chosen for another plot within the same graph. You can also use this feature to plot kinetic data in Graph sections. For example, to plot kinetic data from well A1 in a Graph section, you can enter the formula !A1Lm1 for the Y-Axis and the !TimeRun accessor for the X-axis. The entered formulas are also shown in the "Source:" field of this dialog box.

The size, color, and style of the icon used for a plot can be selected on the right side of this dialog box. Four sizes, nine colors, and nine styles are available from drop-down menus.

The Graph Options Dialog Box

The Graph Options dialog box allows you to change the name of the Graph section, change the height of a graph, select the type of graph to be displayed, change the fonts used on the title, legend, and axes, access the Plot#X dialog box for editing plots, entering new plots, and/or to delete plots. This dialog box is opened after pressing in the Plot#X dialog box when a new Graph section is being created. This dialog can also be accessed by pressing the Graph Options button  on the Graph section toolbar or by choosing **Graph Options** from the **Graph** menu.

Scatter plots, cluster bar charts and stack bar charts can be created. Error bars can be used with all graph types. You can also choose whether to connect the points and/or plot the symbols in the graph.

In the "Plots:" field of this dialog box, each of the plots that have been created for this Graph section are listed. Choosing any of these plots provides an indication of the data to be used for the X- and Y- axis data points and error bars on the right side of this field. Plots can be deleted by highlighting the plot name and clicking the button. Clicking on the button opens the Plot#X dialog box, allowing you to create a new plot for the graph. Existing plots can be edited by highlighting the plot name and clicking on the button which opens the Plot#X dialog box containing all the information for the selected plot.

When creating a new Graph section, the Graph Options dialog box opens after pressing either or in the Plot#X dialog. The new Graph section is not created, however, until you press in the Graph Options dialog box.

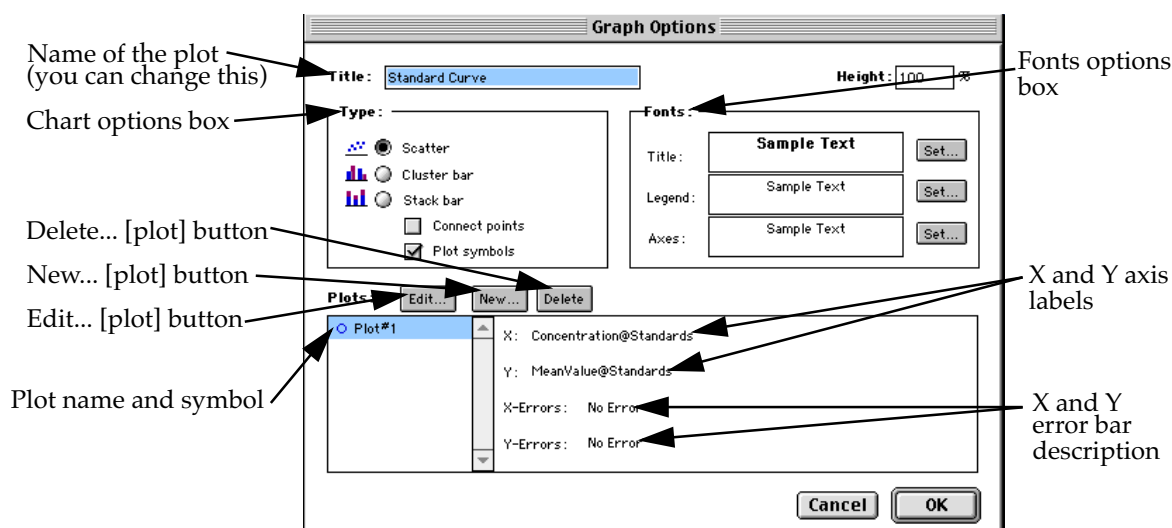


Figure 5.22: Graph Options Dialog Box

Creating Multiple Plots

SOFTmax PRO for Lmax makes it possible to create multiple plots within a simple graph. Each plot will represent data from a group that has been created in the Template Editor or data from a summary or custom formula. You may plot data from any group or from several groups on a graph, even if the groups are in separate experiments. It is possible, for example, to plot multiple dilution series of an unknown, to graph together a series of patient samples, or to plot several controls that are run over time against one another.

Following is a summary of the steps required to create multiple plots within a single graph, after which is an example that provides more detail.

Multiple Plots Step Summary

- Create all required groups in the Template Editor.
- Read the microplate to obtain data.
- Create a graph section.
- Add plots to the graph and choose the group and the X and Y values to be shown for each plot.

Example of Creating Multiple Plots

This example shows how six groups, all of which are Standards, were plotted on the same graph.

- 1) Groups are created in the Template Editor. For this example, eight groups are created: GA(h), GA(native), G5A(h), G5A(native), plec2(h), plec2(native), CHOMO6(h), and CHOMO6(native).

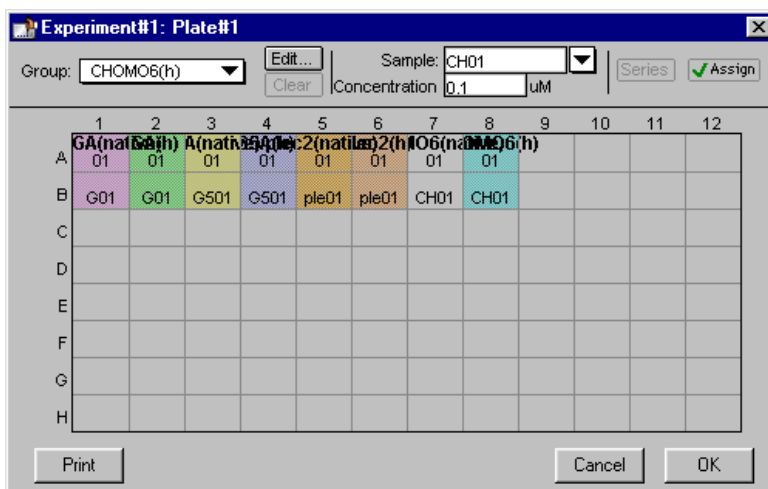


Figure 5.23: Standards and Unknowns Groups Created

- 2) These Group sections are created using the Basic column format which, by default, generates columns for values, concentration, and mean values. The example below shows the Group section for GA(native).

GA(native)

GA(native) (uM)					
Sample	Wells	Sample#	Values	MeanValue	Concentration
01	A1	1	1.664	1.664	0.000
G01	B1	2	13.678	13.678	0.100
G02	C1	3	39.515	39.515	0.300
G03	D1	4	42.618	42.618	1.000
G05	E1	5	63.994	63.994	3.000
G06	F1	6	64.058	64.058	10.000
G07	G1	7	76.077	76.077	30.000
G08	H1	8	40.684	40.684	100.000

Figure 5.24: GA(native) Group Section

- 3) A new Graph section is created by selecting **New Graph** from the **Experiment** menu. This causes the Plots dialog box to open automatically. Clicking **OK** opens the Graph Options dialog box which allows you to choose the graph type, modify the fonts used for the graph, and so on. Choices should be made as follows: GA(native) for the group to be plotted, the concentration of GA(native) for the X-axis, and values of GA(native) for the Y-axis. No error bars are added.

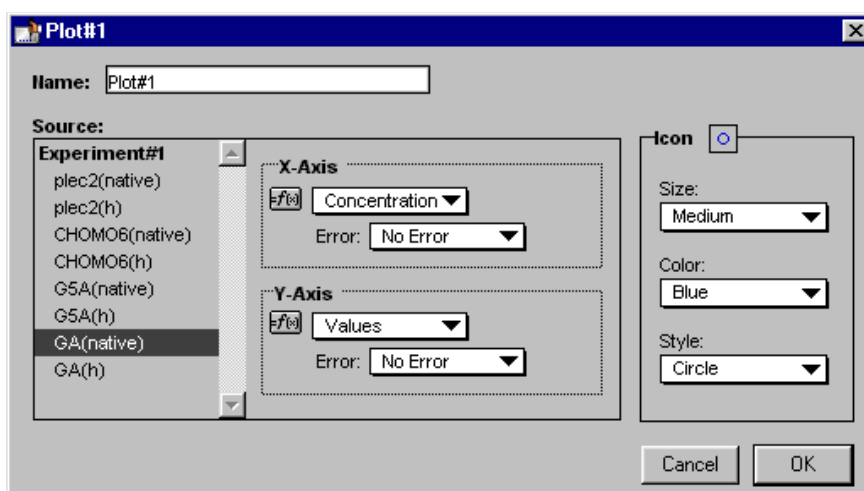


Figure 5.25: Graph Options Dialog Box

- 4) A second plot is added to this graph by clicking the **New** button in the Graph Options dialog box. After clicking **New**, a dialog labeled Plot #2 is opened. Choose GA(h) for the group, and change the X- and Y-axes to show concentration and values, respectively, for GA(h).

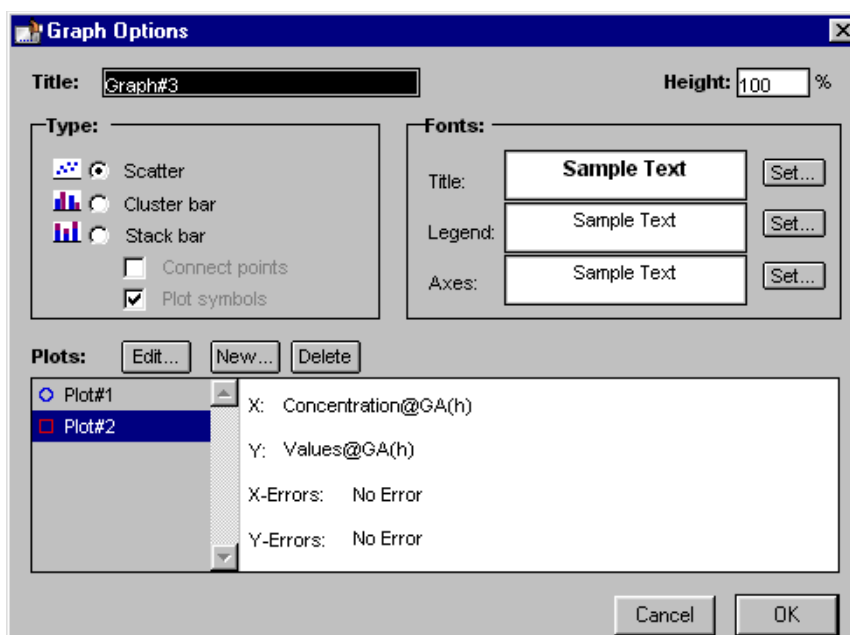


Figure 5.26: Graph Options Dialog Box with Second Plot Added for GA(h)

- 5) This process is repeated for the rest of the Group plots, in this order:
 Plot#3 (G5A(native): Concentration vs Values)
 Plot#4 (G5A(h): Concentration vs Values)
 Plot#5 (plec2(native): Concentration vs Values)
 Plot#6 (plec2(h): Concentration vs Values)
 Plot#7 (CHOMO6(native): Concentration vs Values)
 Plot#8 (CHOMO6(h): Concentration vs Values)



- 6) Now that all plots information is complete, clicking **[OK]** in the Graph Option dialog box will close it, and the resulting graph will show the eight plots created.

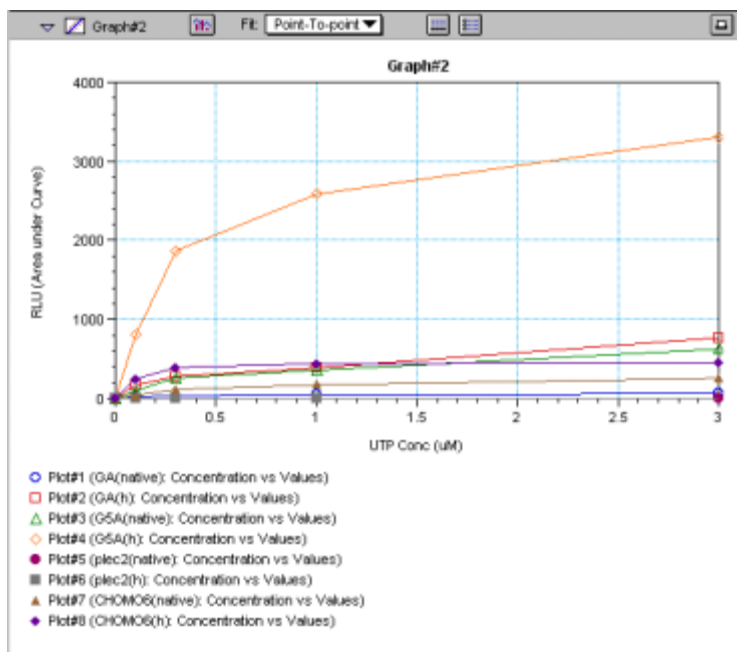



Figure 5.27: Graph Displaying Eight Plots

You can add as many plots to a graph as you wish, and these can be of the same or different types of groups. If you create a graph and later wish to add one or more plots to it, click the Graph Options button () on the Graph section tool bar and then click the **[New]** button. This will cause the Plots dialog box to reappear, allowing you to add or modify the plot information.



Error Bars

When data is displayed as a scatter or bar graph, you can choose to display error bars for the plot of the data for the X- and/or Y-axis. Error bars are lines that extend beyond a plotted value in either or both directions and graphically represent some amount of error in plotted data.

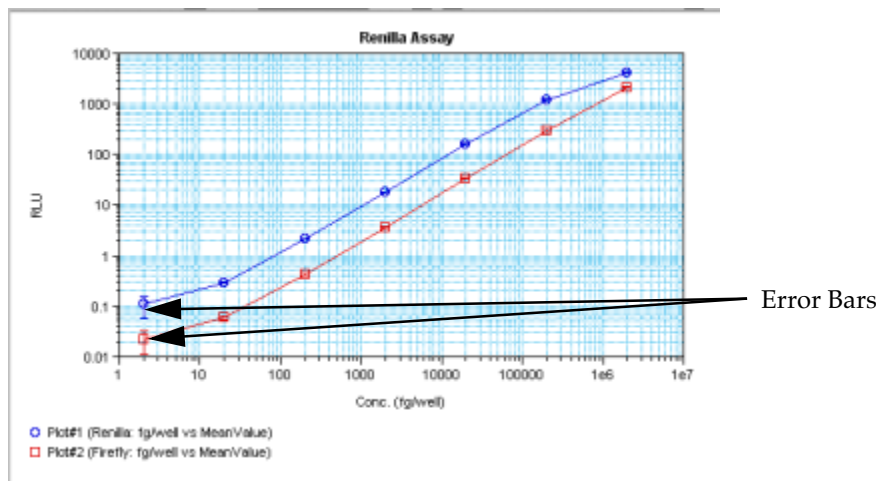


Figure 5.28: Plot Showing Error Bars

Choices for displaying error bars relative to a particular axis are the same as those available for displaying data on that axis: any column from that group that contains numerical information (not columns with text). To display error bars, click and hold the box next to "Error Bars" in the Plot#X dialog and drag to select one of the choices in the menu. To disable error bars, choose "No Error" for the X- and/or Y-axis.

Error bars show both positive and negative error. Depending on the range settings for the graph, portions of the error bars may not be visible.

Curve Fitting

When you first create a graph of the data, it does not have a fit associated with it. The graph might appear something like this:

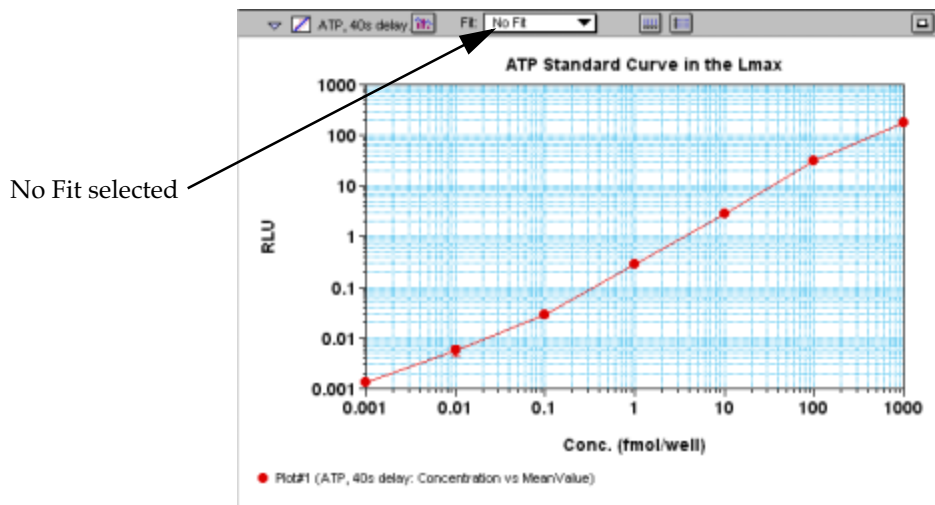


Figure 5.29: Graph with No Fit

You can fit any plot to one of nine curve-fitting algorithms: Linear, Semi-Log, Log-Log, Quadratic, 4-Parameter (logistic), Log-Logit, Point-to-Point, Exponential, and Cubic Spline. These selections are shown in the Fits pop-up menu in the Graph section toolbar (click on the box next to “Fit”).

Typically, a standard curve refers to the curve fitted to the plot of concentration versus mean value for the Standard group.

Once you have selected a Fit type, SOFTmax PRO for Lmax determines the parameter values that best fit the data. The function with these parameters is then drawn on the graph. Ideally, the type of fit used should be determined by the underlying chemistry of the assay and could be set before data is read.

NOTE: A good discussion of curve fitting appears in “Data Analysis and Quality Control of Assays: A Practical Primer” by R. P. Channing Rogers in *Practical Immuno Assay*, edited by Wilfrid R. Butt (published by Marcel Dekker, Inc., New York, 1984).

When a fit is chosen, the Graph section is updated to show the coefficients describing the fit when applicable. These values are displayed in the legend at the bottom of the graph. Figures 5.30 through 5.37 show the same graph with the different fit algorithms applied. Note that the legend information changes (along with the formula for the fit) at the bottom of each Graph section for each different fit.

NOTE: All plots on a graph must have the same type of fit.

When a curve fit is chosen, the values for the coefficients of the equation (A, B, C, etc.) will be shown as will the square of the correlation coefficient (R^2). The correlation coefficient describes how well a change in x values correlates with a change in the y values. Please see “Judging a Good Curve Fit” on page 5-43 for more information. The R^2 value should only be used for curve fits having linear axes.

Linear

The linear function fits the best straight line to the data. The equation for this fit has the form of:

$$Y = A + (B * X)$$



where A is the y-intercept of the line and B is the slope. A linear fit should be used whenever the values appear to lie on or scattered around a straight line.

This equation is shown on the screen as:

$$y = A + Bx$$

A linear fit should be used whenever the standard values appear to lie on or are scattered around a straight line. Figure Figure 5.30 shows an example of a linear fit.

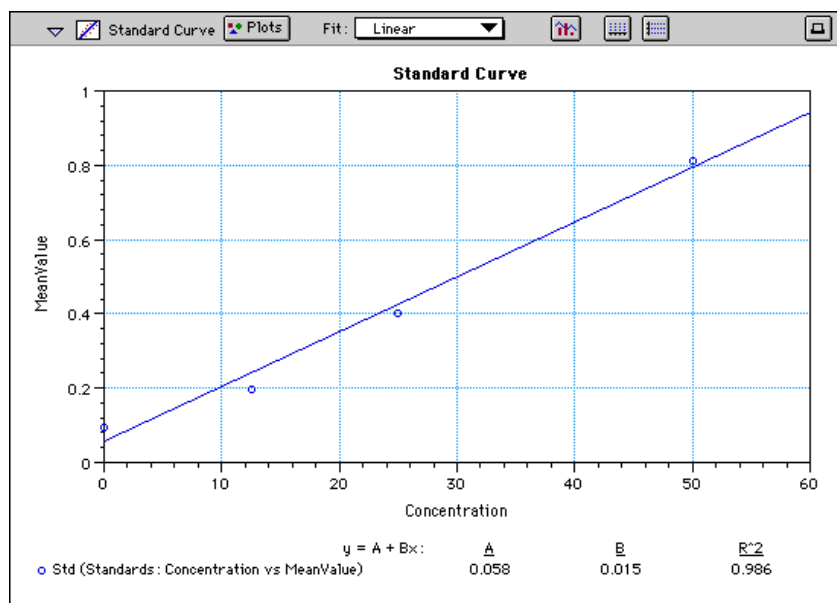


Figure 5.30: Graph with Linear Fit (simulated data)

Semi-Log

The semi-log function fits the best straight line to a set of data for $\log(X)$ plotted against Y. The resulting curve displayed will be a straight line with the X-axis drawn in logarithmic scale. The equation for a semi-log fit is:

$$Y = A + [B * \log_{10}(X)]$$

and is shown on the screen as:

$$y = A + B * \log(x)$$

where A is the y-intercept of the line and B is the slope (note: log in this equation is the common or base-10 logarithm).

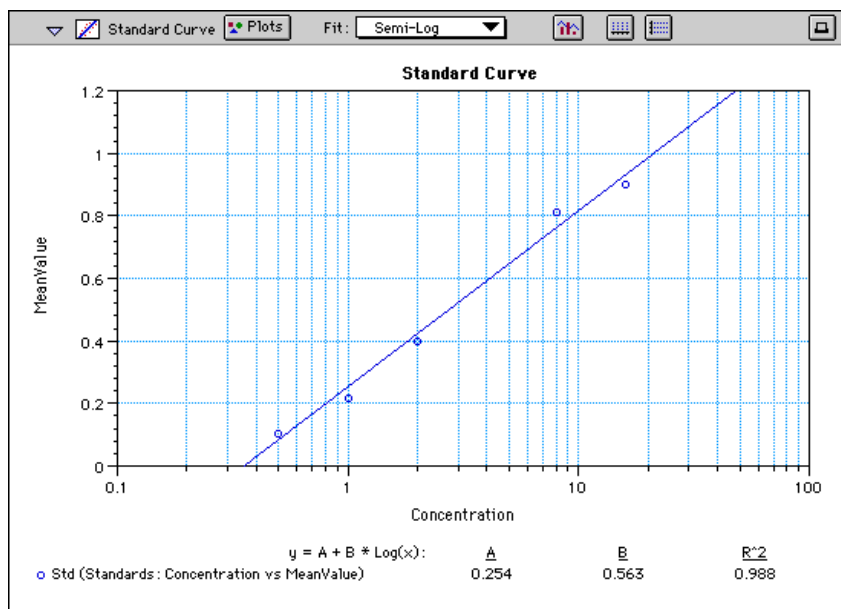


Figure 5.31: Graph with Semi-Log Fit (simulated data)

Log-Log

The log-log function fits the best straight line to the set of data which consists of the logarithm of the readings on the Y-axis (the response) and the logarithm of the dose on the X-axis. The resulting display will be a straight line with both axes drawn in logarithmic scale. The equation for the log-log fit is:

$$\log_{10}(Y) = A + [B * \log_{10}(X)]$$

and is shown on the screen as:

$$\log(y) = A + B * \log(x)$$

where A is the $\log_{10}Y$ -intercept of the line for $\log_{10}(X) = 0$ and B is the slope (note: log in this equation is the common or base-10 logarithm).

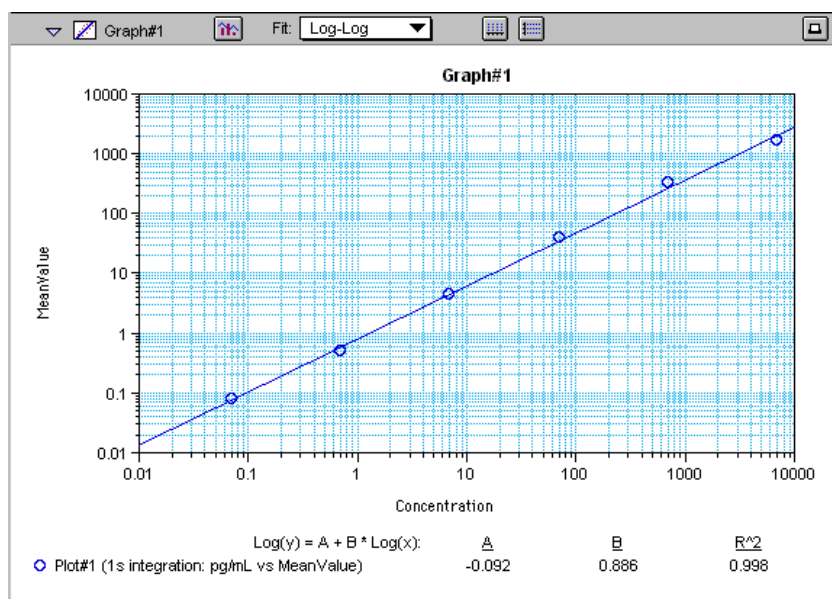


Figure 5.32: Graph with Log-Log Fit (simulated data)

Quadratic

The quadratic function fits the best parabola to the data. The parabola is a curved line based on the equation:

$$y = A + (B * x) + (C * x^2)$$

which is shown on the screen as:

$$y = A + B*x + C*x^2$$

where A is the intercept, B is the slope of the curve at the intercept, and C is the measure of the curvature of the parabola.

The quadratic fit is most appropriate when the standard curve has a tendency to curve up or down. An example of a quadratic fit is shown in Figure 5.33.

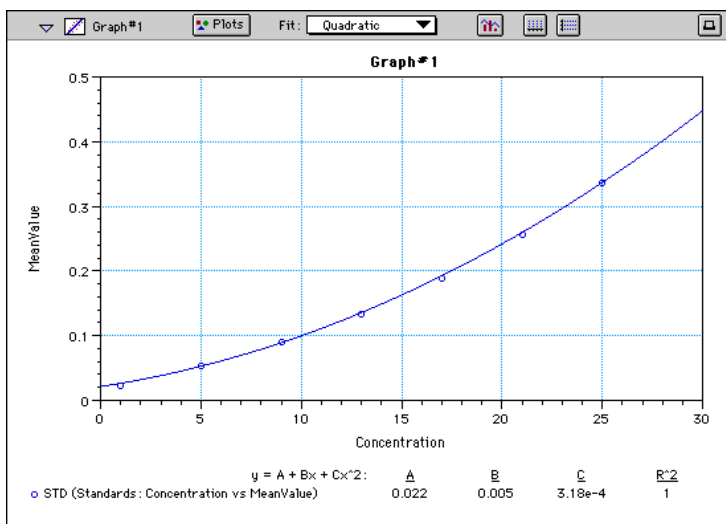


Figure 5.33: Graph with Quadratic Fit (simulated data)

4-Parameter Logistic and Log-Logit

If the standard curve has a sigmoidal shape when plotted on the semi-log axes, it may be appropriate to use either the log-logit or 4-parameter fit. Both fits are based on the equation:

$$y = \frac{A - D}{1 + \left(\frac{x}{C}\right)^B} + D$$

where D is the Y-value corresponding to the asymptote (i.e., the flat part of the curve) at high values of the X-axis and A is the Y-value corresponding to the asymptote at low values of the X-axis. The coefficient C is the X-value corresponding to the midpoint between A and D. The coefficient B describes how rapidly the curve makes its transition from the asymptotes in the center of the curve. A large value of B describes a sharper transition. Typically, B has a magnitude of about 1.

Both the log-logit and 4-parameter equations are shown on the screen as:

$$y = (A - D) / (1 + (x / C)^B) + D$$

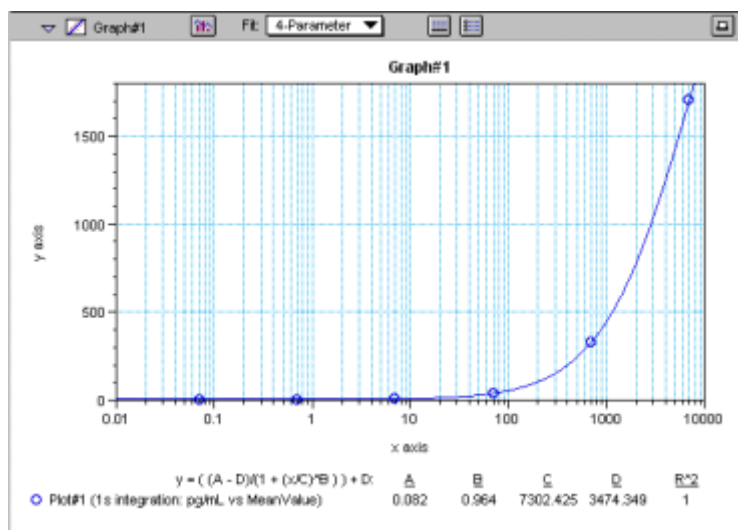


Figure 5.34: Graph with 4-Parameter Fit (simulated data)

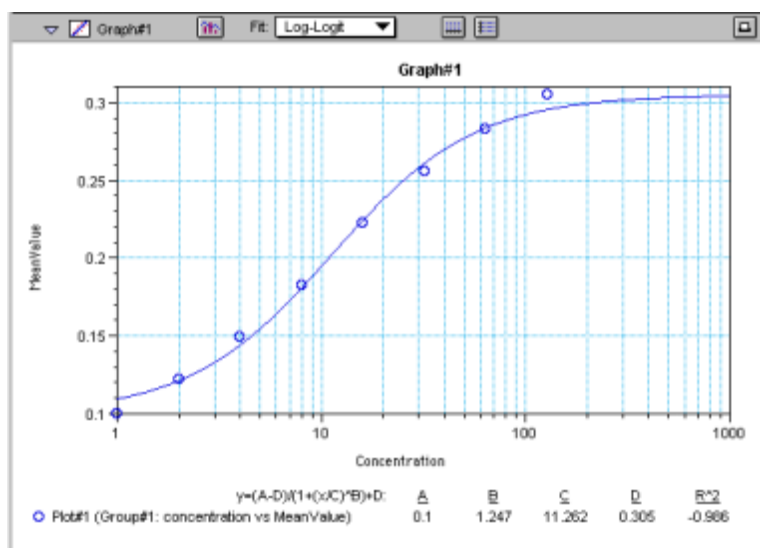


Figure 5.35: Graph with Log-Logit Fit (simulated data)

Some publications show the log-logit equation in another form:

$$\log_{10} \frac{y}{1-y} = bx - b \log_{10} c$$

where

$$y = \frac{y-A}{D-A} \quad x = \log_{10} x$$

A different algebraic expression of these is:

$$y = \frac{A-D}{1-(x/C)^B} + D$$



The difference between the log-logit fit and the 4-parameter fit are in the way the coefficients A and D are calculated. For the log-logit method, the standard values for the lowest and highest values of x are used. The corresponding y values are assigned to A and D, respectively. Based on these fixed values for A and D, the algorithm then computes values for B and C. This technique works well if there are standard points along the upper and lower asymptotes. If this is not true, the log-logit fit should be avoided in favor of the 4-parameter logistic.

In SOFTmax PRO for Lmax 2.x, the 4-parameter logistic curve fit uses 2000 iterations. If the fit does not converge in this number of iterations, the program will report a fit error.

NOTE: The curve-fitting algorithm for the 4-parameter equation was based on the Levenberg-Marquardt Method. Discussion of this method can be found in *Numerical Recipes in C: The Art of Scientific Computing* by William H. Press, Brian P. Flannery, Saul A Teukolski, and William T. Vetterling, published by Cambridge University Press, New York, 1988.

Point-to-Point

A linear equation is fit to each pair of data points. Fit parameters are not given. The point-to-point curve fit is a linear fit composed of pieces that assume a linear relationship between each pair of data points. The line segment defined by each pair is used to interpolate data between those points. Since there are multiple line segments, fit parameters are not shown on the graph.

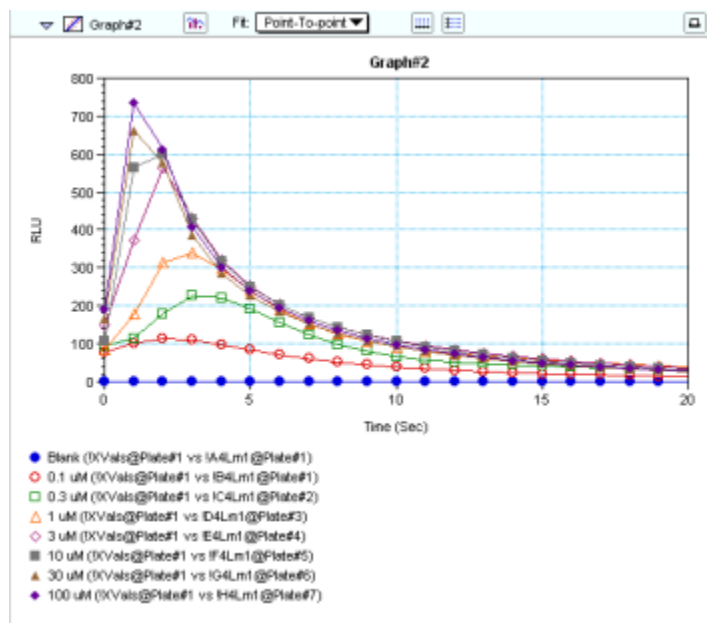


Figure 5.36: Graph with Point-to-Point Fit



Cubic Spline

This curve fit generates a fit to a cubic equation between each pair of data points. The general form of a cubic equation is:

$$y = A + Bx + Cx^2 + Dx^3$$

The equations are computed with the requirement that the first and second derivatives of the equations are continuous throughout the range of the data. As multiple curve fits are performed with this routine, fit parameters are not shown with the graph.

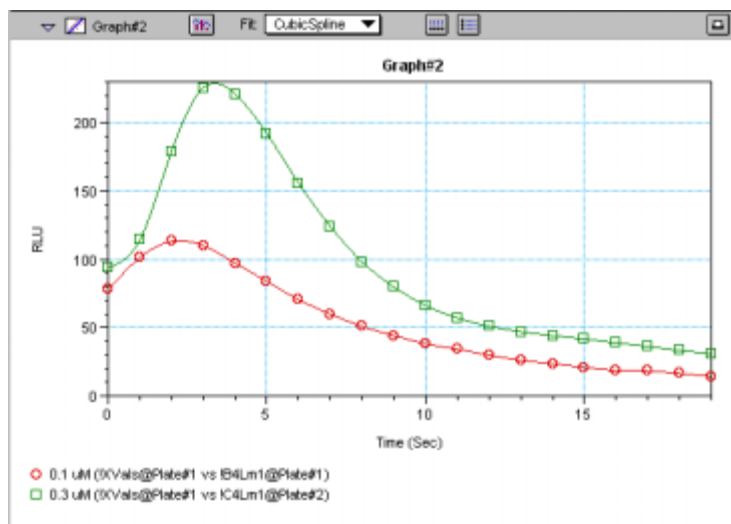


Figure 5.37: Graph with Cubic Spline Fit

Exponential

The exponential function used to generate this curve fit is:

$$y = A + B * (1 - \exp^{-x / C})$$

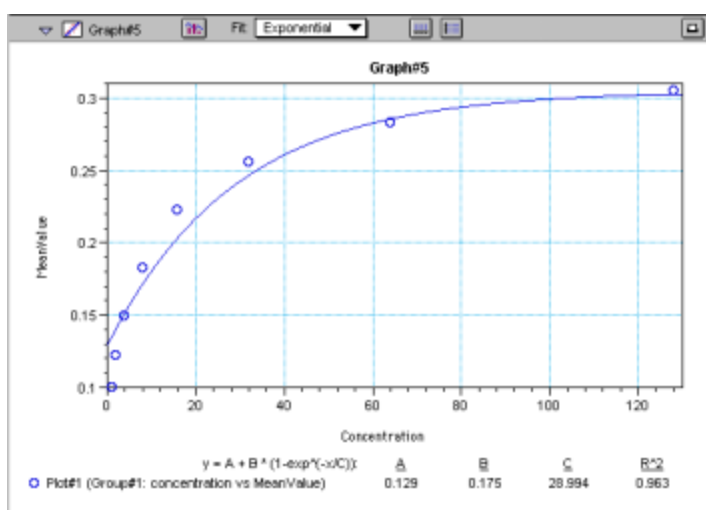


Figure 5.38: Graph with Exponential Fit (simulated data)



NOTE: SOFTmax PRO for Lmax does not plot samples with descriptor values of zero (0) on a log axis. Curve fits affected by this are the semi-log, log-log, log-logit, and 4-parameter curve fits.

Minimum Number of Standards

The number of standards used in a standard curve depends on the particular curve fit selected and on the judgment of the operator. The table below gives the minimum number of standards required mathematically by each of the curve fitting algorithms.

Table 5.1: Minimum Number of Standards Required for Curve Fits

Curve	Minimum Number of Standards
Linear	2
Quadratic	3
Semi-Log	2
Log-Log	2
Log-Logit	4
4-Parameter	4
Point-to-Point	2
Exponential	3
Cubic Spline	4

For optimal results, you should always exceed the minimum number of standards required for any given fit.

There are other requirements on the standards for the log-logit and 4-parameter logistic curves. The fits for the 4-parameter logistic curve are based on the assumption that the curve has a high and a low asymptote and a certain "steepness" for the linear portion around the inflection point between the asymptotes. If a sufficient number of points do not define the inflection point and the asymptotes, you will receive a "no fit" error message or an inappropriate fit. For best results, make sure that the 4-parameter fit is appropriate for your assay and that the entire range of the assay is represented in your standards.

If your standards do not clearly define both a high and low asymptote, the spline fit may be more appropriate for your assay.

Judging a Good Curve Fit


The correlation coefficient is also known as the goodness of fit value because it shows how good a fit exists between the computer curve and the standard data points. It measures the degree to which the points fall on the computer curve and may give an indication as to what residuals are to be expected. The correlation coefficient is popularly known as R and is between -1 and 1, inclusive. When the data points lie on a perfectly straight line with negative slope, then $R = -1$. If the correlation coefficient is not suitably close to 1 or -1 (a per-



fect fit), you can simply apply one of the other types of curves without having to reread the plate.

For nonlinear curve fits, SOFTmax PRO for Lmax performs transformations that cause the curve to become more linear. The correlation coefficients are derived from that transformed data. Because of this, you should be cautious about using correlation coefficients as an indicator of goodness of fit. Do not let this single value for goodness of fit sway your intuition about which fit is best. Some curve fits may seemingly give a good fit (e.g., fit values close to 1) but, when inspected by eye, show a poor fit along a major portion of the plot.

Graph Options

Graphs can be customized further through the Graph Options dialog box which is accessed by choosing **Graph Type...** from the **Graph** menu or by clicking the Graph button () in the section tool bar.

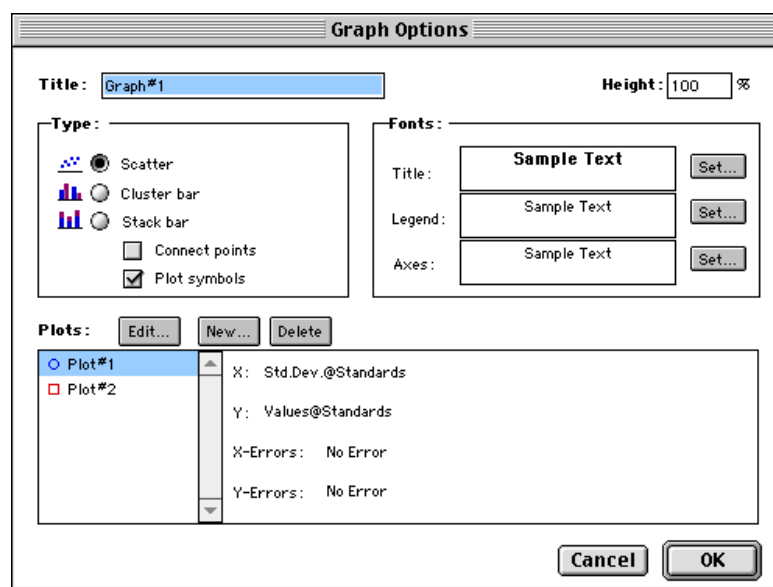


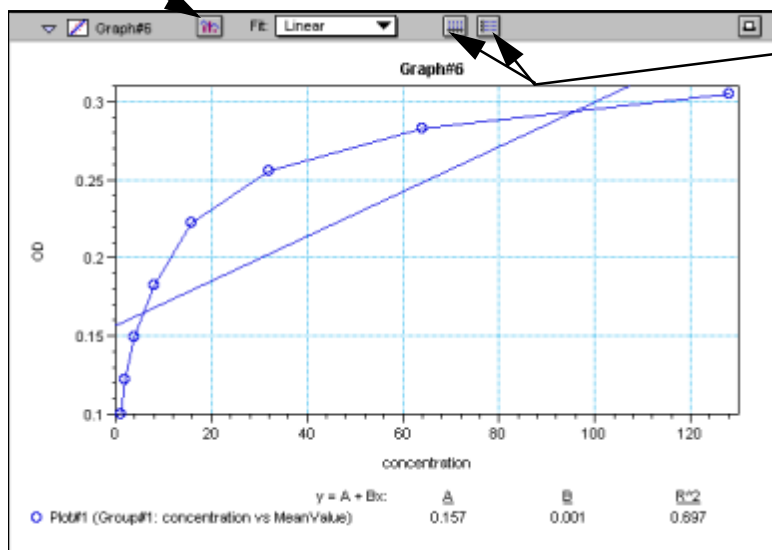
Figure 5.39: Graph Options Dialog Box

The default options are shown in Figure 5.39, but you can alter these as desired: you may change the font and type size used for the title, legend, and/or axes of the graph, and you can choose the color and type of symbol used on a scatter graph to show the plot. The graphs shown in preceding Figures reflect the default graph options. Connecting the points of a scatter graph can be useful in determining the closeness of the fit. Figure 5.40, below, shows a scatter graph with points connected compared to a linear fit.

NOTE: The Connect Points option connects the points in order of appearance by sample name—not the appearance of points from left to right on the graph.



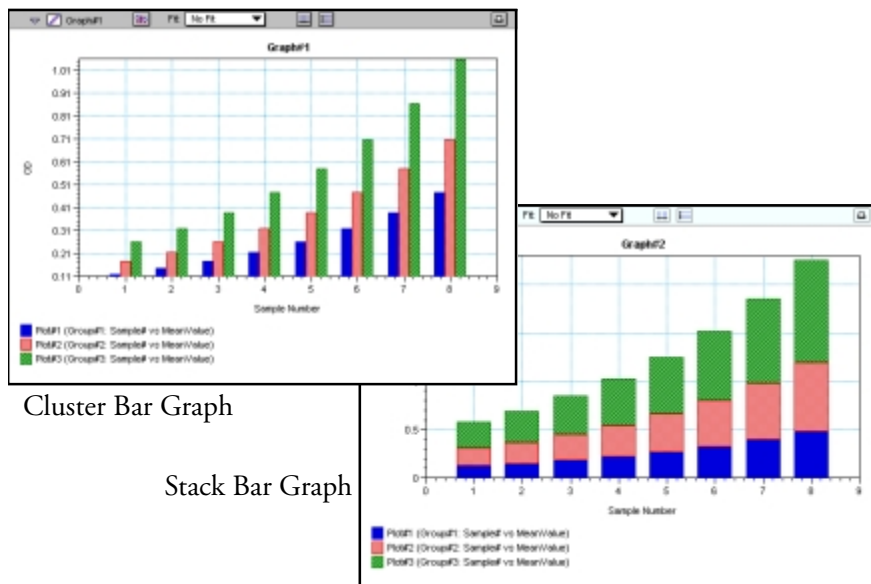
Graph Options Button



X- and Y-Axis Buttons

Figure 5.40: Scatter Graph with Points Connected (Linear Fit Applied)

Other options in this dialog box allow you to plot the data as a bar graph with data shown in clusters or stacked. Figure 5.41 shows both of these bar graph options.



Cluster Bar Graph

Stack Bar Graph

Figure 5.41: Bar Graphs—Cluster and Stack

NOTE: Bar graph options for multiple plot graphs are only available when each plot contains the same number of data points and has matching X-axis coordinates.

NOTE: When choosing the Connect Points option, the order in which points are connected is sample name dependent.



Axes Options

Double-clicking either graph axis, clicking the X-axis or Y-axis button in the Graph section toolbar, or selecting either **X-axis...** or **Y-axis...** from the **Graph** menu opens a dialog box concerning the configuration of that axis. Figure 5.42 shows the dialog box for the X-axis.

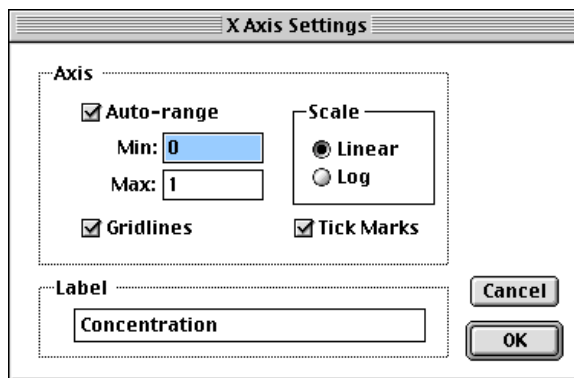


Figure 5.42: X-Axis Dialog Box

The default settings are shown in the Figure above. You can change these as desired for either or both axes.

Axis Settings

Enabling the Auto-range option causes the graph to be drawn such that all data points are shown (by automatically adjusting the minimum and maximum range settings to encompass the data). You can disable the Auto-range function by clicking the checkbox or by changing either or both values for the Min and/or Max settings.

Formulas in SOFTmax PRO for Lmax

You can enter custom calculations in several places within SOFTmax PRO for Lmax to provide the information you desire:

- In Plate sections, you can specify formulas to be used for reduction.
- In the Group table sections, you can view and edit the formulas associated with the various columns or create new columns containing new formulas. The data shown in columns from one group can relate to or contain information derived from columns in another group. You can also create summaries which consist of formulas and text.
- Notes sections allow you to enter summaries which consist of formulas and related text.

Use of formulas is described in detail in a separate manual, *The SOFTmax PRO for Lmax Formula Guide*.

Chapter 6: File Management and Printing

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File Creation and Management

During routine use of SOFTmax PRO for Lmax, you will create, open, and save numerous files. You may wish to create file folders that will make it easy to locate particular files. It is also important to understand the way in which SOFTmax PRO for Lmax finds and uses default protocol files to enable certain assay options automatically.

When you install SOFTmax PRO for Lmax, certain files and folders/directories are created on your computer's hard disk. One of these files is known as the "Default Protocol" (so named on the Macintosh; called "DEFAULT.LPR" in Windows). The Default Protocol file is automatically used as a "template" for new files you create. You can, however, create one or more different "template" protocol files.

Default Protocol

When first installed, SOFTmax PRO for Lmax looks for the Default Protocol file in the same folder or subdirectory as the SOFTmax PRO for Lmax program file. You can instruct SOFTmax PRO for Lmax to look in a different location, however, through the **Assays** menu. Suppose, for example, that you create a folder/subdirectory called "MYASSAYS" and that you create a new protocol file (called "Default Protocol" or "DEFAULT.LPR") within this folder. The **Assays** menu shows two sections: at the top, a command entitled **Set Folder...**, which allows you to choose the folder within which SOFTmax PRO for Lmax will look to find the Default Protocol file when creating a new experiment. At the bottom of this menu, beneath the dividing line, is a list of all protocol files contained in the currently chosen folder.

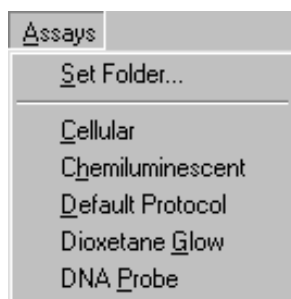


Figure 6.1: Example Assays Menu

To have SOFTmax PRO for Lmax look in a different folder for the Default Protocol file, choose **Set Folder...**. A dialog box will open, allowing you to locate and set a different default folder.

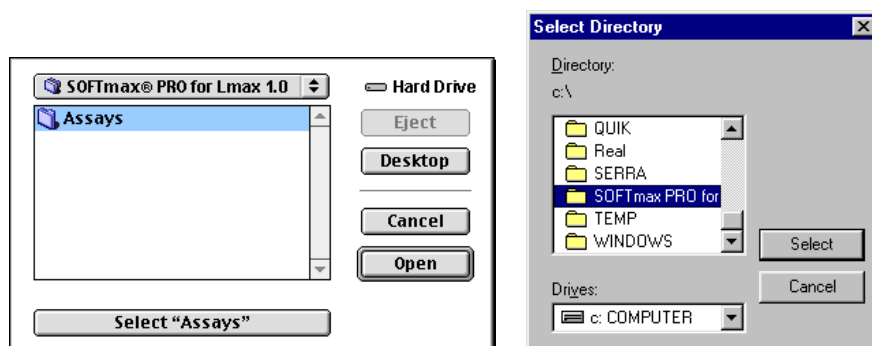


Figure 6.2: Set Folder Dialog Box—Macintosh and PC

Select the folder you wish to use and then click the large button at the bottom (Mac) or right (Windows) of the dialog box to set this folder as the default.

NOTE: Molecular Devices recommends that you have only one default protocol.

Opening Other Protocol Files

The bottom half of the **Assays** menu provides a “shortcut” for opening other protocol files contained in the set folder. These usually consist of protocols you or others create for repeated use. To choose a different protocol file, click the protocol file you wish to use. An untitled copy of that protocol file will open in a new window.

Locating Protocol and Data Files

You may find it useful to create separate folders/subdirectories within which to store the data files that SOFTmax PRO for Lmax creates. In addition, you may want to create and save various protocol files for use as “templates” for later readings. In a multi-user environment, it might be helpful to create a folder/subdirectory for each SOFTmax PRO for Lmax user or to create separate folders/subdirectories for different types of experiments. We recommend that you consider the way in which you will organize your files before creating too many of them—otherwise you may find that locating a particular file among the many that fill your hard disk difficult and/or time consuming.

NOTE: Creating new folders or subdirectories must be done outside of SOFTmax PRO for Lmax (using the Finder for Macintosh or the File Manager or Windows Explorer within Windows, for example).

Creating New Files

Selecting **New** (⌘N; CTRL+N) from the **File** menu causes a new, untitled file to open, creating a new window on the screen. The initial contents of this window are based on the default protocol file chosen in the Preferences dialog box.

NOTE: You can create and/or open more than one SOFTmax PRO for Lmax file at a time. Subsequently opened files will appear in separate windows in a cascading, diagonal order.



New files can be saved as protocol files at any time, either before or after data is acquired. To create a new protocol file, enter all the instrument settings for the reading and then choose **Save** or **Save as...** from the **File** menu. The dialog box that appears presents the option of saving a file as either a protocol or a data file (the default). On the Macintosh, click the option button next to “Protocol” and enter a name for the file in the text box (type over “Untitled”). In Windows, choose the appropriate format from the list in the box under the heading, “Save File as Type:.” Also remember to choose the folder or subdirectory in which to save this file. When you have entered all the information, click **Save** or **OK**.

NOTE: If you are creating a protocol file from a file containing data, first save the file as a data file (if you wish to save the data) and then perform a second save as a protocol file. Data will not be saved with a protocol file.

Protocol files are stationery pad files—when you choose to open a protocol file, you are actually presented with a *copy* of the file, which appears as an untitled file.

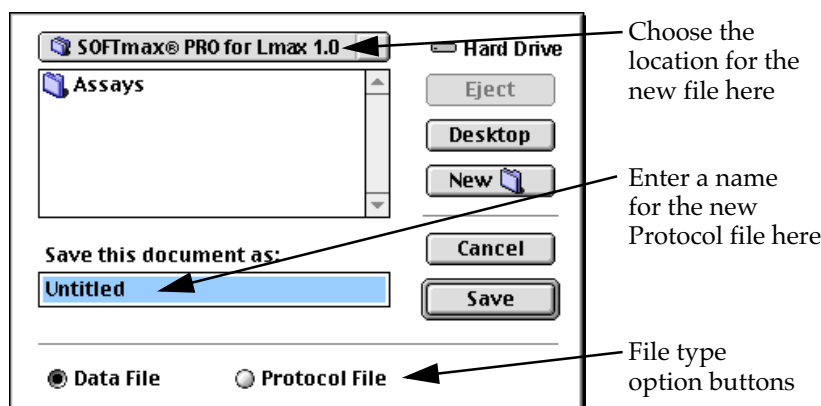


Figure 6.3: Save as... Dialog Box—Macintosh

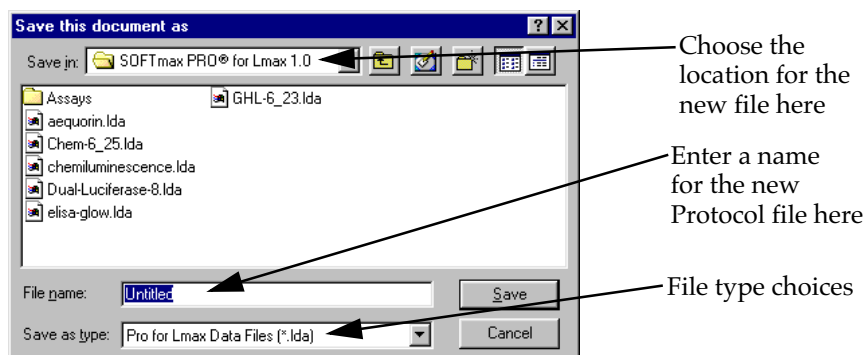


Figure 6.4: Save as... Dialog Box—Windows



Opening Files

You can open a SOFTmax PRO for Lmax file by selecting **Open...** (⌘O; CTRL+O) from the **File** menu within SOFTmax PRO for Lmax or by double-clicking on the file name or icon when you are in the Finder (Macintosh) or in the File Manager (Windows). Note that you can open SOFTmax PRO for Lmax files using either platform regardless of whether they were created using Macintosh or Windows (see “Cross-Platform Files,” below, for more information).

Opening SOFTmax/SOFTmax PRO for Lmax Files (Earlier Versions)

SOFTmax PRO for Lmax allows you to open files from previous versions of SOFTmax PRO for Lmax as well as from SOFTmax. From within SOFTmax PRO for Lmax, select the **Open** (⌘O; CTRL+O) command from the **File** menu. The file will open and will function in the same way as a SOFTmax PRO for Lmax file. If you wish, you can save the file in SOFTmax PRO for Lmax format by selecting **Save As...** from the **File** menu. If you do not change the name of the file and save it to the same location, a message box will appear, asking whether or not you want to replace the existing SOFTmax file with the new SOFTmax PRO for Lmax version. If you do not, you can either choose a different location or rename the file.

NOTE: While SOFTmax files can be opened using SOFTmax PRO for Lmax, two items do not translate completely across the programs: sample blanks and auto range limits (in graph sections). The data for sample blanks obtained using SOFTmax will be available when these files are opened in SOFTmax PRO for Lmax, but no calculations are performed automatically using this data. Auto range limits are not available when SOFTmax files are opened using SOFTmax PRO for Lmax, so limits for the display of information in graphs must be entered manually.

Cross-Platform Files

If you have SOFTmax or SOFTmax PRO for Lmax files that were created on a platform other than the one you are currently using, you can transfer these files to your computer and use them, provided you have the required software and/or hardware to do so.

Transferring Macintosh Files to the PC



Third-party software is available for the Windows-compatible PC that allows the PC to read Macintosh-formatteed 3-1/2-inch disks. However, if you need to transfer files between Macintosh and PC platforms and do not have such software, you can use PC disks with both platforms since the Macintosh will be able to read them. Macintosh computers are equipped with software that allows you to “read” and transfer files from PC disks. If you are using System 7.5 or later, you will need to make sure that File Exchange is available in the Control Panels. The process of using File Exchange is described below.

- 1) If you have not yet enabled File Exchange on your Macintosh, select Control Panels from the Apple (⌘) menu. From within the Control Panels, choose the Extensions Manager and turn File Exchange on by placing a checkmark next to it. Restart your computer—File Exchange will now be enabled.
- 2) Rename the Macintosh files you wish to transfer to PC disks so that their names have appended the proper 3-letter extension preceded by a period.



Table 6.1, found later in this chapter, lists the three-letter extensions that are associated with data and protocol files from SOFTmax and SOFTmax PRO.

- 3) Insert a PC-formatted disk into the Macintosh floppy drive. It will appear as an icon on the Macintosh desktop.
- 4) Drag the files (renamed with 3-letter file extensions) onto the IBM disk icon to copy the files to the disk. Drag the disk to the trash to eject it.

NOTE: Certain special characters will not translate accurately across platforms. Characters that are created on the Macintosh using the option key, such as ®, ©, °C, ™, or • (bullet character) for example, will be shown on the PC as other letters or characters. Also, if you align items using spaces, these may or may not align when viewed on a different platform since fonts on the different platforms have different character widths.

Transferring PC Files to a Macintosh



Third-party software is available for the Windows-compatible PC that allows the PC to read Macintosh 3-1/2-inch disks. However, if you need to transfer files between Macintosh and PC platforms and do not have such software for the Windows-compatible PC, you can use PC disks for both platforms since the Macintosh will be able to read them. Macintosh computers are equipped with software that allows you to “read” and transfer files from PC disks. If you are using System 7.5 or later, you will need to make sure that File Exchange is available in the Control Panels.

- 1) If you have not yet enabled File Exchange on your Macintosh, select Control Panels from the Apple () menu. From within the Control Panels, choose the Extensions Manager and turn File Exchange on by placing a checkmark next to it. Restart your computer—File Exchange will be enabled.
- 2) Choose File Exchange from the Control Panels. A dialog box like the one below will appear.



Figure 6.5: PC Exchange Dialog Box

- 3) In this dialog box, click **Add...**. This will cause a second dialog box to appear.

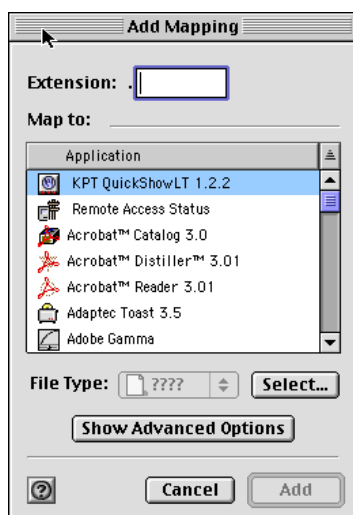


Figure 6.6: Add Mapping Dialog Box

- 4) In order to allow the Macintosh to automatically recognize protocol and data files of PC origin as SOFTmax PRO for Lmax files, you need to enter the associated three-letter extensions that correspond to these files in the File Exchange Add Mapping dialog box. The table below lists the type of file and its corresponding extension that should be entered in the dialog box.

Table 6.1: PC File Name Extensions and Macintosh Document Types for Use with File Exchange

Type of File	3-Letter Extension	Macintosh Document Type
SOFTmax PRO for Lmax Protocol File	.LPR	sPFd
SOFTmax PRO for Lmax Data File	.LDA	SPFd

For example, if you will be working with SOFTmax PRO for Lmax *protocol files* created on the PC, enter .LPR in the box under “Extension . [_____]”

- 5) Then click the application icon (currently shown as “File Type ???” in the center of the box under “Application Program.” From the menu choices, navigate to the folder where the SOFTmax PRO for Lmax application resides. Highlighting this application will enable the Macintosh to start SOFTmax PRO for Lmax and read the corresponding protocol file whenever you double-click a PC file bearing the “.LPR” extension. Also select the appropriate Macintosh document type (shown in the table above). Clicking [OK] causes this dialog box to close and updates the earlier dialog box to show the extension and associated program you just entered.
- 6) Repeat steps 3 through 5 for other PC file name extensions with which you will be working.



Saving Files Manually

SOFTmax PRO for Lmax provides several ways to save files manually. If the current file has not been saved (is “Untitled”), it can be saved under any name. If you have opened and modified an existing file, you can save it under the same name (overwriting the old information) or under a different name. You can also save the file in the same or in a different location on your hard disk (in a different folder or subdirectory).

Saving Files under the Same Name

If you have made changes to a file and want to save these changes, thereby updating the same file, select **Save** (⌘S; CTRL+S) or **Save As...** from the **File** menu. If you select **Save** (⌘S; CTRL+S), the current version of the file will *replace the old one (without notification)*. If you select **Save As...**, an alert box will appear, asking if you want to replace the previous file by that name. If you are intentionally replacing the file, click or . The new information will be written over the old information under the same file name.

Saving Files under a Different Name or in a Different Folder

If you have made changes to a file and want to save these changes as a new file, or if you wish to save the file to a different location, select **Save As...** from the **File** menu. A dialog box will open allowing you to enter a new name and/or a new location for the file. To choose a different location for the file, use the pull-down menu at the top of the dialog box to select a different folder. To change the name, overtype the existing file name. Click when you have entered all the necessary information and you are ready to save the file.

Autosave

SOFTmax PRO for Lmax can be configured to save a copy of your data file automatically (Autosave) after a reading. This function is enabled in the Preferences dialog box (choose **Preferences...** from the **Edit** menu).

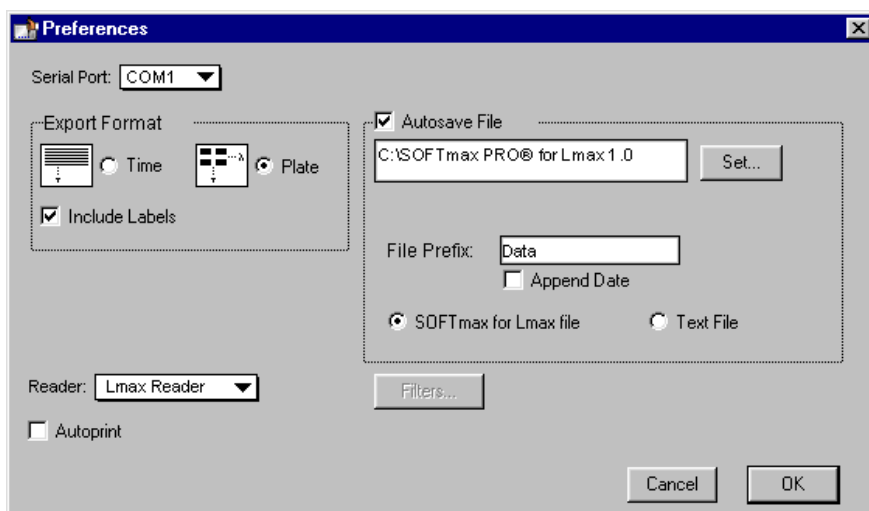


Figure 6.7: Preferences Dialog Box

When you enable the Autosave function (by clicking its checkbox in the Preferences dialog box), the textbox below it becomes active, allowing you to accept or change the default location for saving data files. The default location is the folder containing the SOFTmax PRO for Lmax program. You may accept the default file prefix or change it to another prefix of your choice. For example, you may wish to use a prefix that is specific for a certain group of experiments or one of several users. You may also choose the file type for the Autosaved file (SOFTmax PRO for Lmax file or ASCII text file), choose to append the date to the autosaved file name, and, if you select the Text file option, you can then choose either the “Create a new file” or “Append to file” option.

NOTE: When Autosave is enabled in SOFTmax PRO for Lmax file mode within files containing multiple experiment sections, each experiment section will be autosaved to a separate file.



Printing

Five printing options are available for an experiment:

- 1) You can print a report at any time by choosing **Print...** (⌘ P; CTRL+P) from the **File** menu. You can determine which sections (or whether all sections) will be printed in this report (see "The Printed Report," below).
- 2) A copy of the template can be printed while you are in the Template Editor by clicking the **Print** button in the bottom left corner.
- 3) A report can be printed automatically at the end of a reading. To enable this function, choose **Preferences...** from the **Edit** menu and select "Autoprint."
- 4) You can print the active section or selection. This option is available within the **File** menu. If only one section is selected, the name shown in the menu will reflect the name of the active section. For example, if the active section is named "Plate #1," the topmost print command in the **File** menu would be **Print 'Plate #1'...** If more than one section is selected, the menu will show **Print Selection**. You can select multiple sections to be printed by holding down the Shift key and clicking on the section tool bars to make them active.
- 5) Enlarged displays (well graphs) of kinetic data can be printed by clicking the **Print** button in the Well Graph window. The size of the printout is dependent on the size of the well graph.

NOTE: If you enlarge the well graph to greater than 12 inches, the printout will be truncated.

Regardless of which print option you select, make sure the printer is connected properly to the computer and that it is turned on prior to sending the print request.

If Autoprint is enabled, make sure the printer is connected and that it is turned on prior to starting a reading.



If you are using a Macintosh, you can check the Chooser for the currently selected (connected) printer.





If you are using Windows, you can select a printer by choosing **Print Setup** from the **File** menu. In the Print Setup dialog box, choose the default printer or select from any other printers have been configured for use with Windows. See your Windows documentation for further information.




The Printed Report

You can create customized reports by including or excluding experiments and individual sections, changing the order in which sections will print, and adding text describing each experiment in Notes sections. You can also create summaries (consisting of reduction formulas and associated text) in Notes and Group sections. The size and content of Group sections can also be changed.

Including or Excluding Sections

When sections are created, they default to being included in the printed report. You can exclude sections from printing by clicking the print setting button on the tool bar of that section (it is a toggle, so it will change from enabled  to disabled  or vice versa).

Changing the print setting option for the experiment will change the state of all sections within that experiment. Changing the print setting option for one or more sections within an experiment will cause the print setting button for the experiment to change to reflect an “undecided” status with regard to the sections within it (). Toggling the experiment’s print setting button will override any changes made to individual sections and set all of them to printing.

When **Print** is chosen from the **File** menu, selecting **Print All** will override the print settings made to sections or experiments.

Customizing the Report

In addition to choosing which sections will print in the report by toggling their print status, you can choose to print all items in a file or to print only the report from the dialog box that appears when you choose **Print** from the **File** menu.

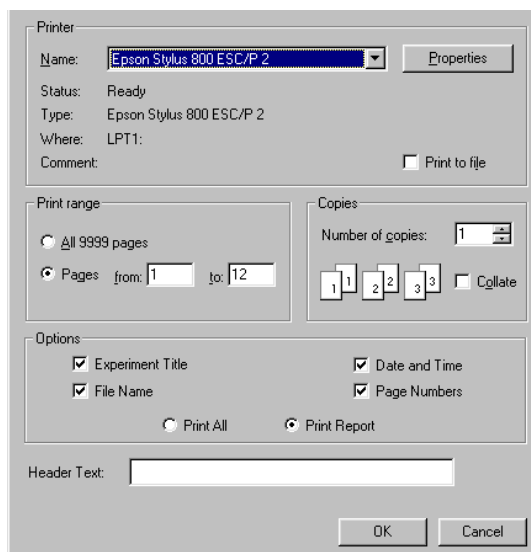


Figure 6.8: Print Dialog Box



Other options regarding the appearance of the printed report can be chosen in this dialog box: you can add text to be included in the header on every page of the report (maximum of 100 characters in a single row), and you can include or exclude the experiment title (the name of the experiment in the file) as well as the name(s) of sections, and you can include the date and time of printing, as well as add page numbers to the report which are printed at the bottom right corner of each page. These are all enabled, by default, until you disable them by clicking in the box with the checkmark next to each option (which will deselect that option).

NOTE: If a Notes section is longer than can be printed on one page, a line may be cut off (not printed) at the bottom of the first page. To fix this problem, insert a carriage return in the appropriate place (above the page break) in the Notes section and print again.

Changing the Section Order

When sections are created (either automatically by SOFTmax PRO for Lmax or by you), their order may not be optimal for either viewing or printing. The order of sections in the window determines how they will print in the report, and you may find that changing the order of sections makes it easier to view the data.

When you create a new section, it is positioned directly after the currently active section. To change the order of a section, first make it active by clicking on it, then click in its tool bar and drag it to a new position (on top of the section or section tool bar you want it to precede). Sections may be open or closed during this procedure.

Changing the Text Format

The text in Notes and Group sections can be formatted to change its appearance. The Format Text dialog box, accessible by choosing **Text Style...** (⌘T; CTRL+T) from the **Edit** menu, provides a number of different options (see Figure 6.9).

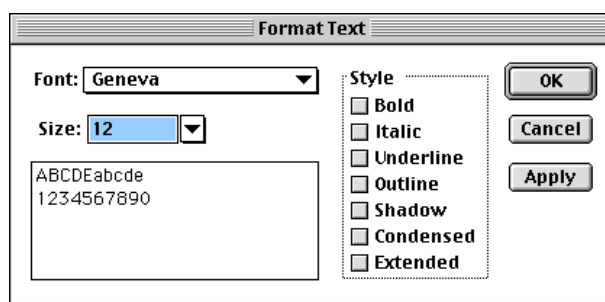


Figure 6.9: Format Text Dialog Box (Macintosh)

First highlight the text you wish to format. Then choose **Text Style...** (⌘T; CTRL+T) and select the options you wish to apply to the selected text. You can click on the **Apply** button to see the result of the selections you have made, allowing you to select more or different options before finally setting the options by clicking on **OK**.



The Print Dialog Box

Choosing any of the **Print...** commands brings up a dialog box. The contents of this box will vary depending upon the type of printer you have selected or the particular printing option you have chosen, but the basic elements are the same. The dialog boxes shown below represent those displayed when you choose the **Print...** command from the Macintosh and from Windows.

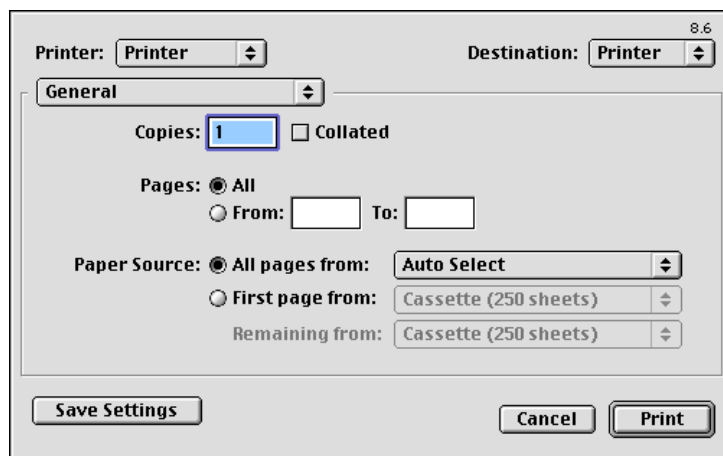


Figure 6.10: Print Dialog Box for the Macintosh

You can choose to print all of the sections, regardless of whether they are included in the report, by clicking the option button next to “Print all” at the bottom left of the Print dialog box.

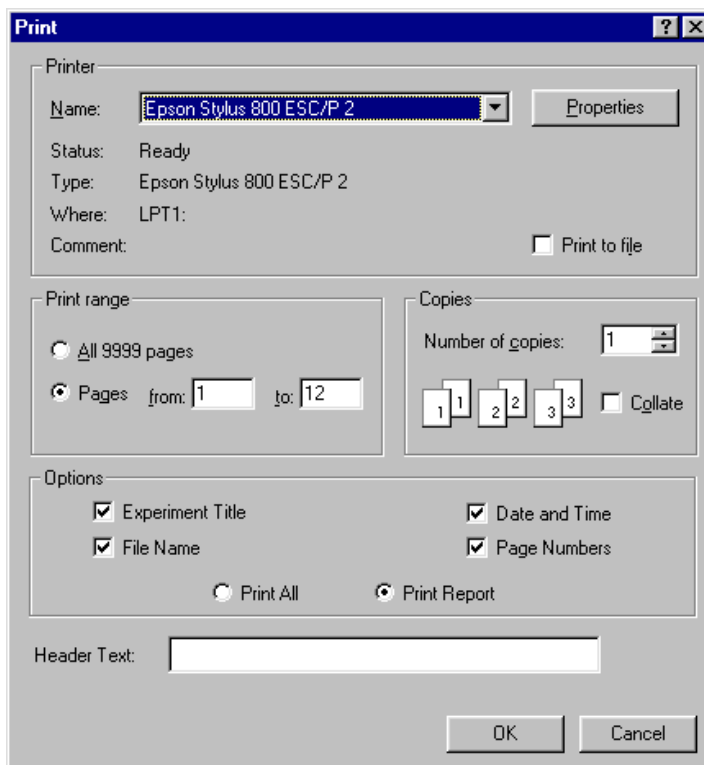


Figure 6.11: Print Dialog Box for Windows (Epson Stylus 800 Shown)

NOTE: If more than one printer is available to your computer, make sure you select the desired printer prior to selecting one of the **Print** options in the **File** menu. If you start to print and discover the wrong printer is selected, choose **Cancel** to exit from the Print dialog box. Select the correct printer (using the Chooser for the Macintosh or the Print Setup dialog box for Windows) and then repeat the print process.

Additional information regarding the general print functions available in these dialog boxes can be found in the documentation that accompanies your computer and/or printer.



Exporting/ Importing Information

Exporting Data for Later Reimport

If you are exporting data so that you can manipulate it using another program and then reimport it into SOFTmax PRO for Lmax, *the data must be displayed as raw data (without a reduced number)* prior to being exported. Follow the steps below to ensure that the exported data will be able to be reimported properly into SOFTmax PRO for Lmax. Complete instructions regarding how to perform each of these steps can be found in the following section.

- 1) Check that the correct plate format and export options are specified in the Preferences dialog box.
- 2) Make the Plate section from which you wish to export data the active section (click somewhere in it or on its toolbar).
- 3) Check that the reduction settings are as you wish them to be by clicking the **Reduction** button on the Plate section toolbar.
- 4) Click on the **Display** button and verify that *the display settings are set to show raw data without a reduced number*.
- 5) Choose **Export** from the **File** menu and save the exported data to a file on your computer.

Exporting Data—General Information

The **Export...** command (a sub-menu of **Import/Export** located in the **File** menu) creates a file of data from one or more Plate sections and/or Group table sections within a SOFTmax PRO for Lmax file. The type of data that is exported depends upon three things:

- 1) Selections made in the Preferences dialog box regarding the export format. Choose **Preferences** from the **Edit** menu. Within the Preferences dialog box that appears, select the format for data export that you prefer: for Plate sections, you can choose either time-based, which produces a linear format for the data, or plate-based, which exports data in a matrix format corresponding to a microplate grid. You also must choose whether or not to include labels with the exported data.¹ Click **OK** to close the dialog box.



Figure 6.12: Choosing an Export Format from the Preferences Dialog Box

NOTE: The preference settings chosen above also affect the copying and pasting of data and Autosaving to ASCII text.

¹ Including labels will affect all data that is exported, but doing so is most noticeable with Group section data, since the column headings in the Group table will be shown, as will any other calculated data in summaries, etc. Labels included with Plate sections show “1-12” headers for 96-well Plate section data represented as an 8 x 12 matrix; 384-well plate data will show “1-24” headers.



- 2) Display options chosen for the Plate section section.
Whether *raw* or *reduced* data will be exported depends upon the display options. If raw data is displayed, raw data will be exported; if reduced data is displayed, this reduced data will be exported.
Open the Plate section section from which you will be exporting data. Click the **[Display]** button in the tool bar of the Plate section to access the Display dialog box. Choose the elements of the display settings that match the data you would like to export: raw or reduced data, with or without number, and so on. When finished selecting display preferences, click **[OK]** to close the dialog box.

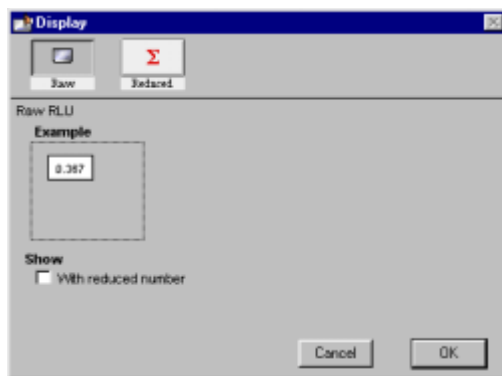


Figure 6.13: Display Dialog Box

NOTE: When exporting data as tab-delimited ASCII text, all *reduced* displays will be exported as numbers, regardless of how the data is shown in SOFTmax PRO for Lmax. Choosing Grayscale, Ranged, or Threshold will export the same information as the reduced number display. Choosing “with number” will also not affect the exported data, since only the raw number is exported when raw numbers are displayed and only the reduced number will be exported when reduced data is displayed.

- 3) Choices made in the Export dialog box.
After choosing **Export...** from the **File** menu, a dialog box will appear, asking you to choose which sections you wish to export. You can choose to export Plate sections (all or selected) and/or Group sections (all or selected). After choosing a name for the exported data file, click **[OK]** to save the exported data.

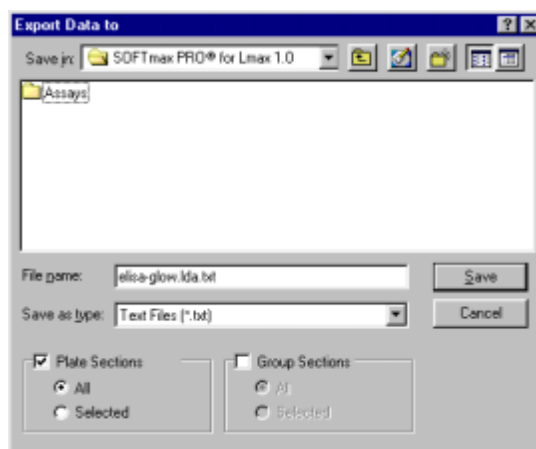


Figure 6.14: Export Dialog Box

Exported tab-delimited ASCII data files have a minimum of five lines and, in most cases, will be considerably longer. The first line of an exported ASCII data file includes one field indicating the number of data sets in the file. This single field includes the text "##BLOCKS=" followed by a number that equals the number of data sets.

The second line of a file for a Plate section includes 19 fields that provide all of the information needed to determine the type of test that was run to measure the first data set. The table following describes each of these fields.

**Table 6.2: ASCII Data File Fields and Descriptors**

Field	Field Entry	Description
1	Section kind	Plate:
2	Section name	Defined in Section dialog
3	Export version	1 (2 if there are headers)
4	Export format	PlateFormat or TimeFormat, set in Preferences
5	Read mode	Endpoint, dual read, long kinetics, or fast kinetics
6	Data mode	Luminescence
7	Data type	Raw or Reduced
8	Pre-read included	TRUE or FALSE
9	Kinetic points	Reduced plates and endpoint plates = 1 Kinetic plates: number of reads
10	Kinetic read time	Kinetic read time in seconds; otherwise blank field
11	Kinetic interval	Kinetic read interval in seconds; otherwise blank field
12		Blank field
13		Blank field
14		Blank field
15	Number of wavelengths read	1 for endpoint and kinetics; 2 for dual read
16	Wavelengths used	Always All
17	First column read	First column read (1 to max)
18	Number of columns	Last column read (1 to max)
19	Number of wells	Number of wells in a plate (96 or 384)

The information for the first plate section is provided in the lines following the descriptors. The number of lines of data depend on the export format and the type of test that is run. A minimum of one line of data is exported when the Time format is used with an endpoint read.

NOTE: The temperature at the time of the reading will appear before the first data point in the reading.

The second line of a file for a Group table section includes fields that correspond to the number and types of columns found in that section (they are labeled as the table is labeled and look very much like the table itself.

Following the data, a blank line is included and then a line with a single termination field: ~End.

Each subsequent data set in the file includes the lines described above, a blank line, and a line with the termination field.



Importing Data from Outside of SOFTmax PRO for Lmax

SOFTmax PRO for Lmax imports data in tab-delimited ASCII text format, allowing you to import data collected by a non-Molecular Devices instrument into SOFTmax PRO for Lmax. Note that only plate data can be imported.

Selecting **Import...** from the **Import/Export** menu under **File** causes the Import dialog box to appear.

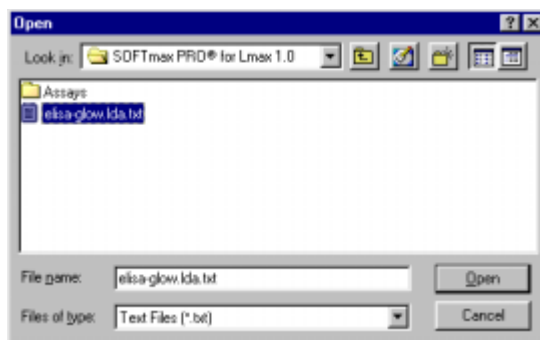


Figure 6.15: Import Dialog Box

Choose the file you wish to import and click the **Import** button. *Note that data to be imported must contain the appropriate header information.* A new experiment is created in the open SOFTmax PRO for Lmax file for the imported data. New Plate sections are created for each data set within the file. Plate pre-read data can also be imported. Note that the time/date stamp for a section containing imported data shows “imported data” rather than a date or time.

Importing and Exporting Templates

Template information, saved as an ASCII text file, can be exported from Plate sections and can also be imported into such sections. A template file consists of seven columns of data that are separated by tabs. Each line of the file provides information for one well in a plate.

When template information is exported, the information for well A1 is written into the first line of the file, information for well A2 is written to the second line of the file, and so on, until a line has been created in the export file for each well in a plate. If no template information exists for a well in a plate, that line is left blank. The ASCII file terminates with the string “~End”.

The order for importing template information must match the export order. Similarly, blank lines in the file are assigned to a well, providing a well with no template setting.

Table 6.3 describes the data that is included in each column of the ASCII template file.

**Table 6.3: ASCII Template File Columns and Descriptions**

Column	Column Entry	Description
1	Group name	Assigned in the Group Settings dialog box. If this field is missing, the rest of the line is ignored. Any text string can be entered in this field.
2	Column format	Assigned in the Group Settings dialog box. Four text strings are supported in SOFTmax PRO for Lmax: <i>Basic</i> , <i>Standards</i> , <i>Unknowns</i> , and <i>Unknowns[Dilution]</i> . Blank groups have a Basic column format. If this field is empty, the Basic column format is assigned.
3	Sample name	The sample name is set in the "Sample:" field of the Template Editor dialog box. If this field is left blank, no sample name will be assigned to the well, although the sample name will be included in the group.
4	Number of descriptors	0 for Unknown column formats; 1 for other column formats.
5	Descriptor units	Assigned in the Group Settings dialog box. Supported strings are <i>unit/ml</i> , <i>mg/ml</i> , <i>ug/ml</i> , <i>ng/ml</i> , <i>mg</i> , <i>ng</i> , and <i>ml</i> . If this column is left blank, <i>unit/ml</i> is assigned.
6	Sample descriptor	Assigned in the Group Settings dialog box. Any text string can be entered in this field or it may be left blank.
7	Descriptor value	Assigned in the Series dialog box. Can be set to any number or left blank.

Exporting Graphs

Graphs from Graph sections and Well Graphs can be exported in PICT format (Macintosh) and EMF format (Enhanced MetaFile in Windows). Graph sections can be exported by choosing **Export Graph** from the **Graph** menu. Well Graphs can be exported by choosing **Export Graph** from the **Plots** menu that appears when a Well Graph is opened.

Copying and Pasting

Parts of an experiment can be copied within a SOFTmax PRO for Lmax file, to or from another SOFTmax PRO for Lmax file, or to or from another program using the **Copy** and **Paste** commands. Plate section data and text and summaries in Notes sections can be copied and pasted to other sections within the same or a different experiment within SOFTmax PRO for Lmax or to other programs. Virtually any ASCII-formatted text can be copied and pasted into a Notes section. A graph can be copied and the resulting picture pasted into another application. Template information can be copied and pasted from one part of a template to another (within the same or to different templates and from one experiment to another).

Plate Section Data and Templates

Copying and Pasting within SOFTmax PRO for Lmax

Data can be copied from one Plate section to another within SOFTmax PRO for Lmax. Make the source section active by clicking on it and then choose **Copy** (⌘C; CTRL+C) from the **Edit** menu. Create a new section, if necessary, and make the destination section active. Choose **Paste** (⌘V; CTRL+V) from the **Edit** menu to paste the data into this section.



NOTE: When copying Plate sections, all well data is copied. It is not possible to copy and paste the data from only certain wells. Even if you select only one well prior to choosing the **Copy** (⌘C; CTRL+C) command, all plate data will be copied and pasting will replace all data in the destination plate.

If the Instrument Settings of the target section do not match the settings from the section being copied, the Instrument Settings for the target section will be updated appropriately to match.

Templates can also be copied from one Plate section to another within SOFTmax PRO for Lmax. However, the effects of this action depend on whether you are pasting the template to another section in the same experiment from which it was copied or to a section in a different experiment. Pasting a template to a section in the same experiment from which it was copied is useful for cross-plate analyses since corresponding wells in each plate will be assigned to the same group and sample.

Pasting a template to a section in a different experiment will create new groups in that experiment if those groups have not been created previously in that experiment. If you are pasting templates between experiments, and groups from the source template do not exist in the destination experiment, pasting a template will create them. Existing groups of the same name will not be changed by the pasting process. For example, if the template being copied contains the groups “Standard” and “Unknown,” and the group “Standard” already exists in the destination experiment, the group “Unknown” will be created but all information regarding the group “Standard” in the destination experiment will remain unchanged.

Experiments define a naming scope for the sections within them, which is especially important for Group sections: it allows you to have identical groups (name and column format) within two or more different experiments in the same file. Having multiple experiments in the same file also provides you with quick access to different types of information without having to open or refer to multiple files.

Copying from Other Programs

Plate section data and Notes section text can be copied from programs other than SOFTmax PRO for Lmax and pasted into a Plate section or Notes section. For Plate section data, the format of the copied information must match the Instrument Settings of the target Plate section and the settings chosen in the Export Format in the Preferences. Pre-read data cannot be pasted into a Plate section. If there is a mismatch between the data to be pasted and the target sec-



tion, SOFTmax PRO for Lmax will attempt to paste the data, but if it cannot do so, an error message will result (an example of such an error message for a Plate section is shown in Figure 6.16). After data is pasted into a Plate section, the information to the right of the display will show “imported data.”

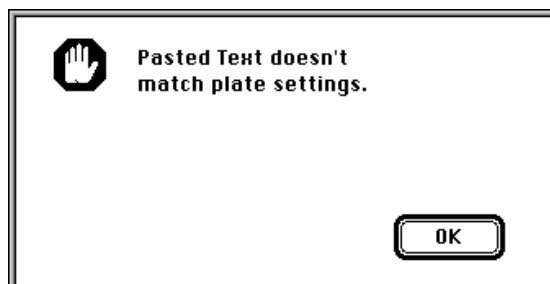


Figure 6.16: Error Message Generated When Pasting Mismatched Data

NOTE: If you have created a template for a Plate section prior to pasting data, numbers will be shown only in the group(s) you have created in the template. Data in other wells will not be reported (but will be available if you alter the template). Clearing the template or assigning groups to all wells in the plate will show the complete data set.

Copying to Other Programs

Plate section, Group table section, and Notes section data can be copied from SOFTmax PRO for Lmax and pasted into another program that will accept the data (such as a spreadsheet or word processing program). No copy or paste features are available for Graph sections or Well Graphs, but these graphs can be exported as either PICT or EMF files (see “Exporting Graphs” on page 6-21 for more information).

Group Columns

You can copy a Group column and paste it within another Group in the same experiment or into a Group of another experiment. The data included in the pasted columns will be appropriate for the target group.

Highlight the column, select **Copy** (⌘C; CTRL+C) from the **Edit** menu; then activate the section into which you want to paste the column and select **Paste** (⌘V; CTRL+V) from the **Edit** menu. If you highlight a column in the destination section, the column you are pasting will appear to the right of the highlighted column. Otherwise it will be appended to the right of all existing columns.

Columns and summaries in Group sections can also be copied and pasted to other programs. Data from other programs cannot be pasted into Group sections within SOFTmax PRO for Lmax, however.

Copying Formulas and Summaries

Formulas are used in Group columns, custom reductions, and summaries. Sometimes it is useful to copy an existing formula, column, or summary from one area of the program to another, or from one experiment to another, rather than taking time to recreate it. Summaries can also be copied and pasted within SOFTmax PRO for Lmax. After pasting, both formulas and summaries can be edited.



NOTE: Contiguous *column formulas* may be copied and pasted together. *Summaries* must be copied and pasted individually.

To copy a formula or summary, highlight it and then select **Copy** (⌘C; CTRL+C) from the **Edit** menu. Activate the area or section in which you would like to paste it; if necessary, with a formula, access the particular dialog box into which it will be pasted. After placing the cursor in the correct location and highlighting any existing information to overwrite it, select **Paste** (⌘V; CTRL+V) from the **Edit** menu. The pasted formula or summary will appear in the new location.

Formulas and summaries can be deleted by highlighting them and pressing the Delete key or choosing **Cut** from the **Edit** menu.

Duplicate and New

Duplicate

All sections and even complete experiments can be *duplicated* by making the section or experiment active and then selecting **Duplicate** from the **Edit** menu. All of the information in the section or experiment (including data) is copied to the newly created section or experiment. The name of the new section or experiment is the same as that of the original with the addition of the word “Copy” (e.g., “OldName Copy”). Duplicated sections are created at the end of the experiment. Duplicated experiments are created beneath the other experiments.

Group section tables can also be duplicated. When a Group table is duplicated, its name is added to the Template Editor’s list of group names (but the group is not assigned to any wells).

Graph sections that are duplicated include all settings for plots, fit, etc.

Notes sections are duplicated exactly, including any summaries and all text.

New

New sections and new experiments can be created by selecting **New** from the **Section** or **Experiment** menu. If an existing section or experiment is active when this command is chosen, all of the information in the section or experiment (*except data*) is copied to the newly created section or experiment. The name of the new section or experiment is the same as that of the original with the addition of an incrementing number (e.g., “OldName#2”). A new section is created immediately below the active section. A new experiment is created immediately below the active experiment.

New Group section tables can also be created but are devoid of any data.

New Graph sections include all settings for plots, fit, etc., but contain no data.

New Notes sections are exact copies of the active Notes section, including any summaries and all text (same as duplicated Notes sections except for the name).

**Table 6.4: New vs. Duplicate within an Experiment**

	New	Duplicate
Notes Section	Empty Notes section.	Replicates text and summaries from source Notes section.
Plate Section	Replicates Instrument Setup, Reduction, and Display parameters from last Plate section created (if no Plate section exists, default values are used).	Replicates Instrument Setup, Reduction, and Display parameters from last Plate section created.
Graph Section	Creates a new Graph section with default settings and asks for plot information.	Replicates all Graph settings, including any plots that were defined in the source Graph section.
Group Section	Not available	Creates a replicate of the source Group section table. The new group will be in the template Group drop-down menu but no wells will be assigned to this group.

Password Protection

Individual protocol and/or data files can be protected by adding a password to them. When a protocol file is password protected, you may read data into the file but you may not change the file in any other way. When a data file is password protected, you may view the file but are unable to change it. The correct password must be entered to make changes to the files.

The table below shows the various actions that are protected when a password is assigned to a SOFTmax PRO for Lmax file.



Table 6.5: Actions Protected by Password

Menus	Tool Bar Buttons	Dialog Boxes	Others
File>Page Setup	Read*	Summary	Moving a formula in a Notes section
File>Import		Instrument Setup	Resizing columns in a Group
File>Save As...		Template Editor	Moving sections and experiments
Edit>Undo		Reduction	Typing in sections (Notes and Plate)
Edit>Cut		Display	
Edit>Paste		Mask Well	
Edit>Clear		New Column	
Edit>Delete Section		Edit Formula	
Edit>Duplicate Section		Plots	
Edit>New Experiment		Graph Options	
Edit>New Section		X-Axis/Y-Axis	
Edit>Text Style		All Section Settings	
Control>Instrument Setup			
Notes (all items)			
Plate (all items)			
Group (all items)			
Graph (all items)			

* Only if data exists in the section.

Setting and Changing a Password

Choosing **Set Password...** from the **File** menu causes the Password dialog to appear. If the active document is already password protected, the menu command changes to **Change Password...** .

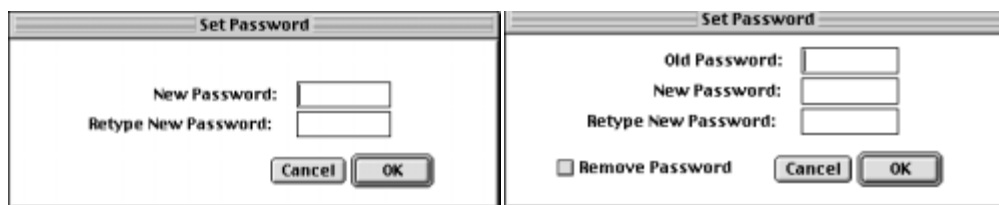



Figure 6.17: Set Password Dialog Box. Left, no password set previously; right, with previously set password.

This dialog appears differently depending upon whether or not you are setting a password for the first time or if you are changing/removing an existing password. If you have not previously set a password, the dialog box will appear like that on the left of Figure 6.17. To set a password, type it in both



boxes (first to set it and then to confirm it). After password protection is enabled, a “locked” icon will appear on the right side of the status bar (.

When changing or removing a password, the dialog box will appear as shown on the right of Figure 6.17. Before you can change or remove the password, you are required to enter the old password in the top text box. After entering the old password, checking the box next to “Remove Password” and typing nothing in the rest of the boxes will clear the password from the file when you click [OK].

As with all password protection, be sure to choose a password that you will be able to remember, since the file will be locked to changes of nearly any kind should you forget it. If you try to perform an unauthorized action on a protected file, a dialog box will appear asking you to enter the password.



Figure 6.18: Password Required Dialog Box

When this dialog box appears, you must enter the password in order to continue with the action you have chosen.

If you do not remove password protection prior to trying to change the file, every attempt to change the file will result in this dialog box appearing. This repeat prompting can be stopped by enabling the Suspend Password option (click the box to check it).

⚠ CAUTION: SOFTmax PRO for Lmax provides no hidden commands that will override password protection. If you are concerned that you might forget your password, write it down and store it in a safe place for future reference.



Chapter 7 Tutorial

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Introduction

The “hands-on” written tutorial included in this chapter is designed to take you through the basic features of the SOFTmax PRO for Lmax program while creating and running a simulated quantitative endpoint assay. We suggest that you read and follow the steps in this “hands-on” tutorial before using SOFTmax PRO for Lmax for the first time. Going through this tutorial will help you learn to use the basic features quickly. To learn more about SOFTmax PRO for Lmax, you will want to read through Chapter 3, “SOFTmax PRO for Lmax Overview,” Chapter 4, “Reading Microplates,” and the introduction to the *Formula Reference Guide*.

NOTE: If you have not yet installed SOFTmax PRO for Lmax, refer to Chapter 2, “Installation,” and follow the steps to install the software before continuing with this tutorial.

Hard and Soft Parameters

The only settings that must be defined before reading a microplate are the instrument settings (found in the Instrument Setup dialog box). These settings tell the instrument what conditions should be in place in order to collect data. They include the read mode (endpoint, dual read, long kinetics, and fast kinetics), the wells to read, any injections required, and so on. These settings, called “hard” parameters, define the conditions under which the data is obtained. Once a microplate has been read, the instrument settings cannot be changed without deleting the data.

“Soft” parameters include the template definition, data reduction, and data display settings. These parameters can be defined prior to reading a microplate, but they are applied only after the plate is read and can be changed at any time.

In this tutorial, you will build a complete protocol that will include both hard and soft parameters. You will save the completed protocol file prior to collecting data.

NOTE: This tutorial has been written specifically for use with the Lmax Microplate Luminometer.

A synopsis of the steps you will take during this tutorial is given below.

1. Create a new file
2. Instrument setup
3. Template setup
4. Set reduction parameters
5. Set display parameters
6. Save the protocol
7. Read the plate (start the simulator and then open the Tutorial file)
8. Analyze data—Standard Curve
9. Analyze data—Standard section
10. Print the report



Description of the Assay Example

Following are the details about the quantitative assay settings we will use to create a protocol file using SOFTmax PRO for Lmax.

Type of Assay:	Endpoint, quantitative assay with Standards and Unknowns (with and without dilution factor)
Standards in pg/ μ L:	500.0, 250.0, 125.0, 62.5, 31.3, 15.6, and 7.8 (each standard run in triplicate)
Blanks:	Group-associated blanks (with Standards), in triplicate
Integration:	1 second
Injection:	P injector; volume: 100 μ L; delay: 3 seconds
Standard Curve Fit:	Linear
Reduction:	Default (!MValue)

Define the Protocol

The following steps will guide you through the process of creating a protocol file for a quantitative assay. Reading an actual plate will not be required—data will be acquired using a simulator program that is part of SOFTmax PRO for Lmax.



Step 1: Start SOFTmax PRO for Lmax and Open the Tutorial File

Double-click the SOFTmax PRO for Lmax icon to start the program. Choose **Tutorial.lpr** from the **Assays** menu. You should see a new file labeled “Untitled” which should resemble the one shown below.

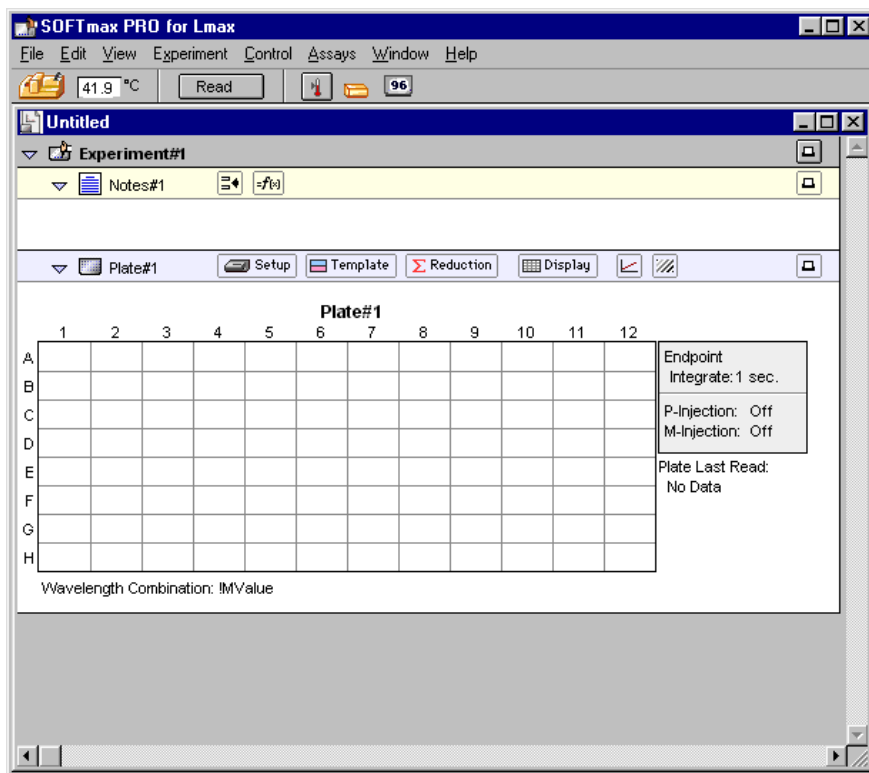


Figure 7.1: Tutorial File (Blank) with Notes # 1 and Plate#1 Sections Open



Step 2: Define the Instrument Settings

Click on the Plate#1 section to make it active and then click the **[Setup]** button to open the Instrument Settings dialog box. The option button next to “Endpoint” at the top is automatically selected; no change is required. On the left side of this dialog box are main section headings, followed by descriptions of the current settings for those headings. The right side of the dialog box shows the options available for the selected heading on the left. The first section, “Integration and Pre-read” is selected automatically, showing that the Integration Time is set to the default of 1 second and Pre-Read plate is disabled. These settings are fine for this assay.

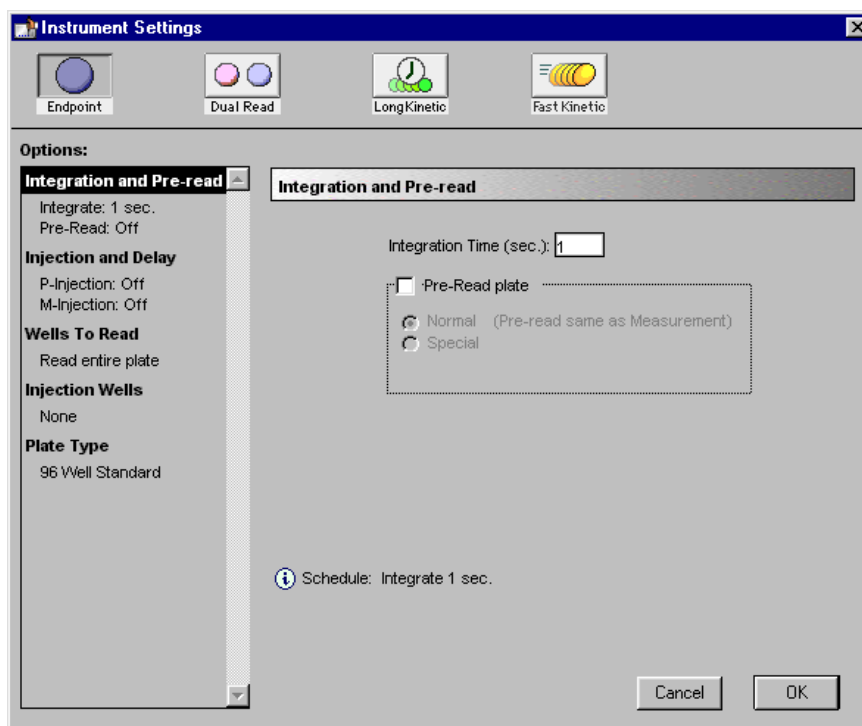


Figure 7.2: Instrument Settings Dialog Box—Integration and Pre-read Section Open



Click the next heading on the left side, "Injection and Delay." The Instrument Settings window should look like the following.

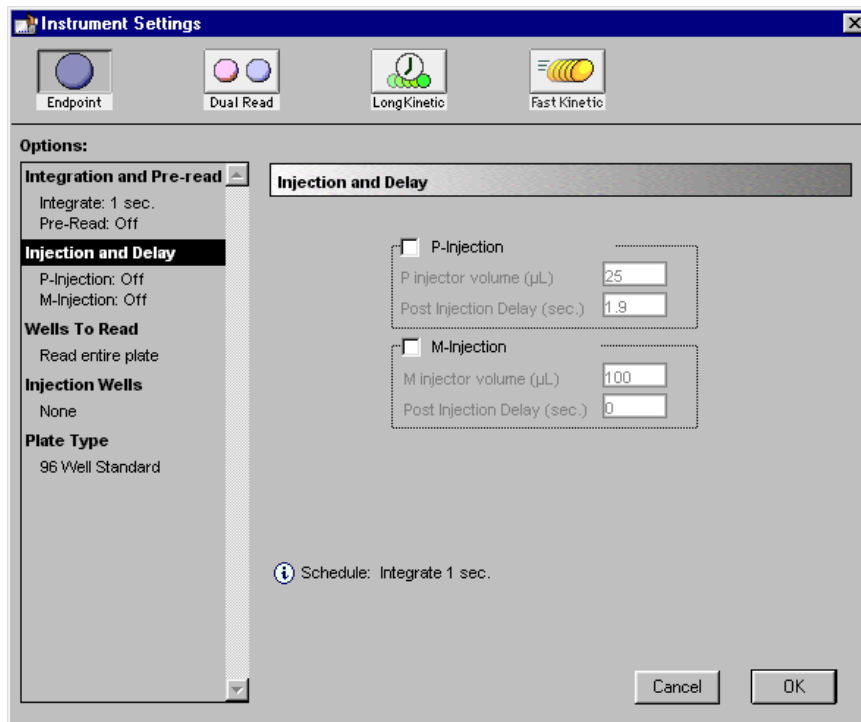


Figure 7.3: Instrument Settings Dialog Box – Injection and Delay Section Open



Click in the checkbox next to P-Injection to enable it. Drag over the default volume (25 μL) to highlight this value and overtype it with the new volume of 100 μL . Highlight the default of 1.9 seconds for the delay and enter the new value of 3 seconds. Leave M-injection disabled.

The Instrument Settings dialog box should now look like this:

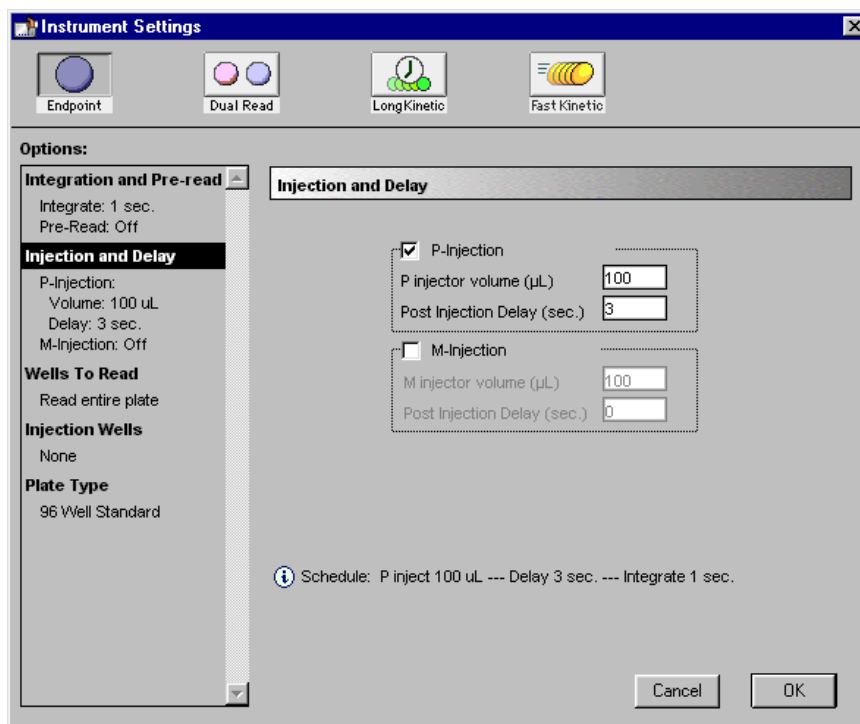


Figure 7.4: Instrument Settings Dialog Box—Injection and Delay Section Revised



Since the entire plate will be read and that is the default setting, you do not need to do anything to the “Wells To Read” section.

You do need to define the wells that will be injected by the P-injector, however. Click on the “Injection Wells” heading on the left side of the dialog box to open that section on the right. The default shows no wells selected and the P-injector enabled as shown below.

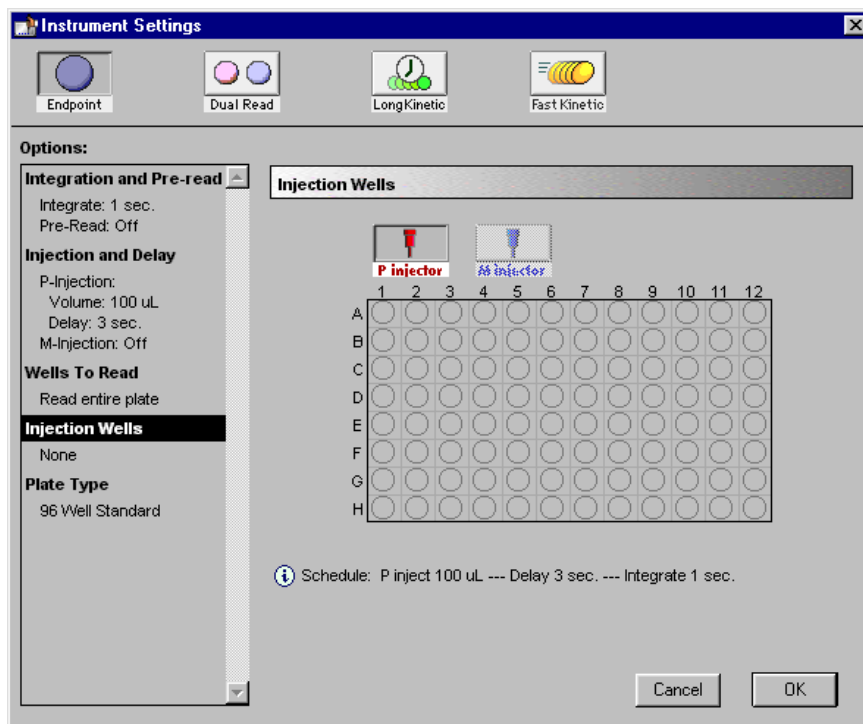


Figure 7.5: Instrument Settings Dialog Box—Injection Wells Section Open with P Injector Enabled



You can click individual wells or click and drag across groups of contiguous wells to enable injection within them. Note that the wells you select for the P-injector will be shown with a red half-circle—if the M injector were enabled, its wells would be shown with blue half-circles on the other side of the wells. Wells can be selected in any order; if you select an incorrect well by mistake, click it again to deselect it. Select wells as shown in the following figure:

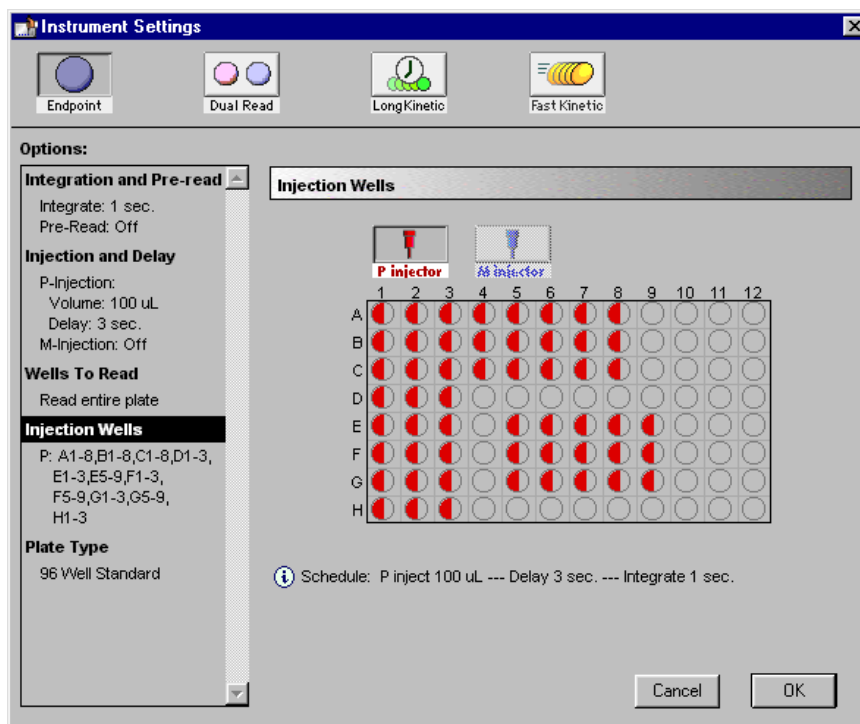


Figure 7.6: Instrument Settings Dialog Box—Injection Wells Selected

The wells you have selected are now also shown on the left side of the dialog box beneath the “Injection Wells” heading.

You do not need to change the default setting of 96-Well Standard for the Plate Type.

The settings are now complete. Click **OK** to accept the settings as shown and close the dialog box.



Step 3: Define the Template

- A) Click the **Template** button in the Plate section tool bar. This will open the Template Editor, showing all wells empty. Drag the mouse (or shift-click) to select the first three columns of wells, 1 through 3. These wells will be defined as Standards for the assay. Click the **Group** pull-down menu. Because no groups have been created yet (Blank is all that is available), you will have to create a new one. Choose New... .

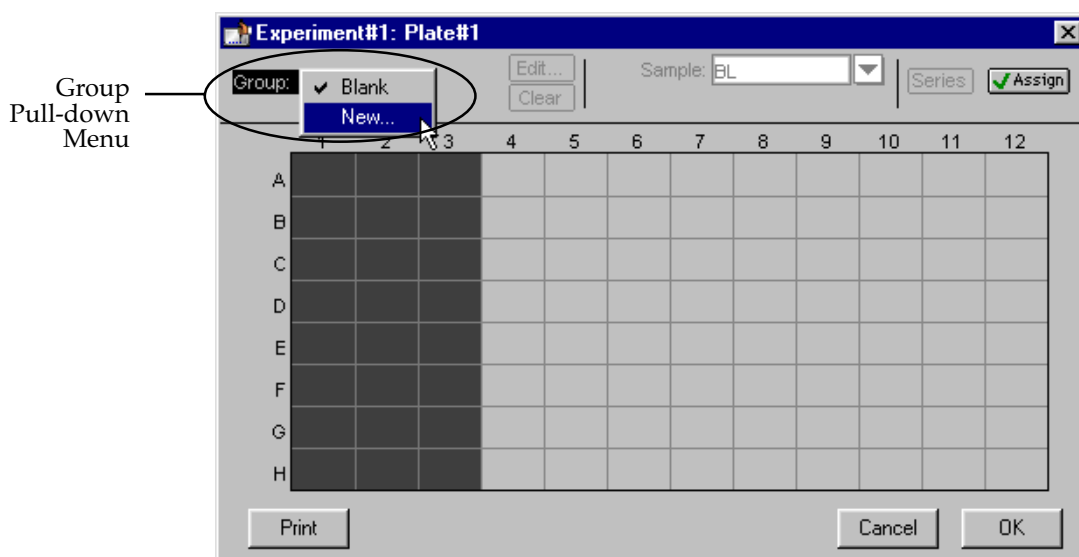


Figure 7.7: Template with Three Columns of Wells Selected and New Group Being Created

- B) The Group Settings dialog box will appear, allowing you to name the new group and select the appropriate information for it. Name the new group "Standards" and choose the "Standards" Column Format. Doing this automatically enables the units, "pg/ μ L." The tutorial assay uses "pg/ μ L," however, so you will need to change this. Clicking on the arrow next to units shows all possible options—but "pg/ μ L" is not among them. You must enter it manually by overtyping the value in the box. When you are done entering information, the Group Settings dialog box should appear like the one on the right, below.

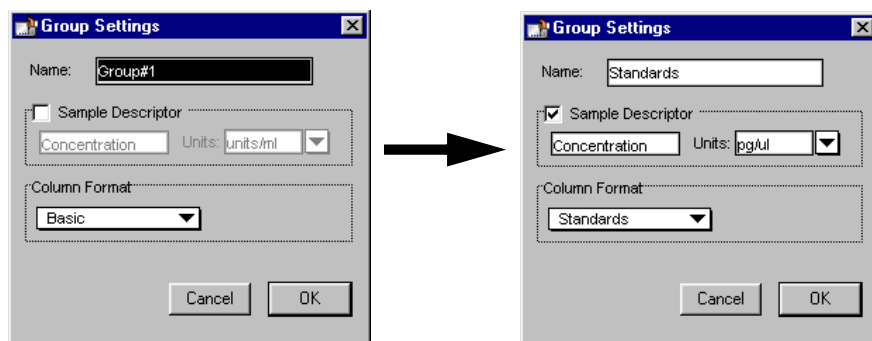


Figure 7.8: Instrument Settings Dialog Box. Left shows initial settings; right shows settings after entering information for the new group, Standards.



Click **OK** to close the dialog box and apply the Standards group to the wells you had selected in the template. It should now look like this:

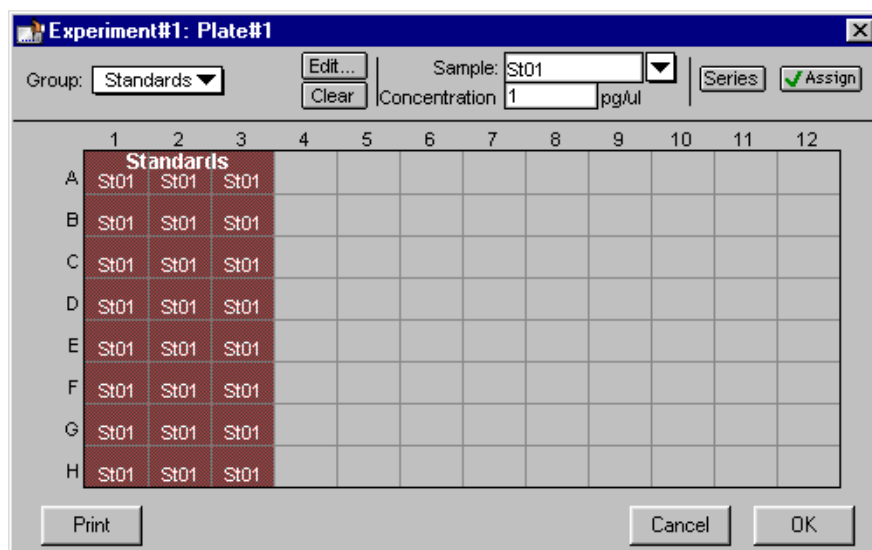


Figure 7.9: Template with Standards Assigned.

- C) The first three wells in row A are to be designated as a group-associated blank within the Standards group. To do this, first click on the template somewhere other than on the Standards group and then highlight wells A1 through A3. Click the arrow to the right of the Sample descriptor in the upper middle of the Template. From the drop-down menu, choose "BL." The template should look like this:

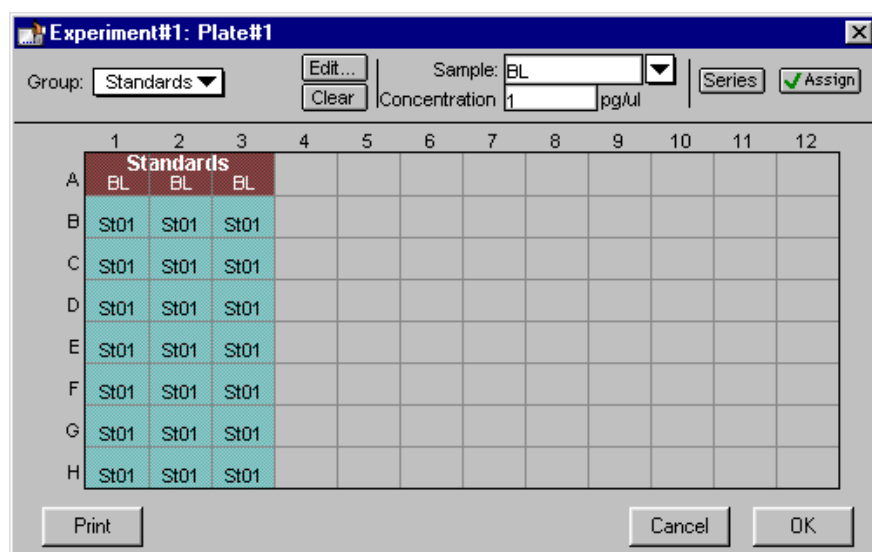


Figure 7.10: Template with Group-Associated Blank Included with Standards.

- D) The Standards entered into the template are currently all the same. However, this assay requires the use of standards with different concentrations, run in



triplicate. The easiest way to start setting this up is to create a “series” by first highlighting the remaining Standards wells and then clicking the **Series** button in the upper right of the Template Editor. In the dialog box that appears, leave the default values for the First Sample name (Std01) and Sample Descriptor (Concentration in pg/ μ L). Choose to have this series start from the bottom and enter 500 for the starting value, choose the function “/” and enter 2 for the increment of the series. These choices will cause the series of wells you have selected to be assigned concentrations of 500, 250, 125, 62.5, 31.25, 15.625, and 7.813 pg/ μ L, respectively, in triplicate from the bottom up. The choices you have made should look like this:

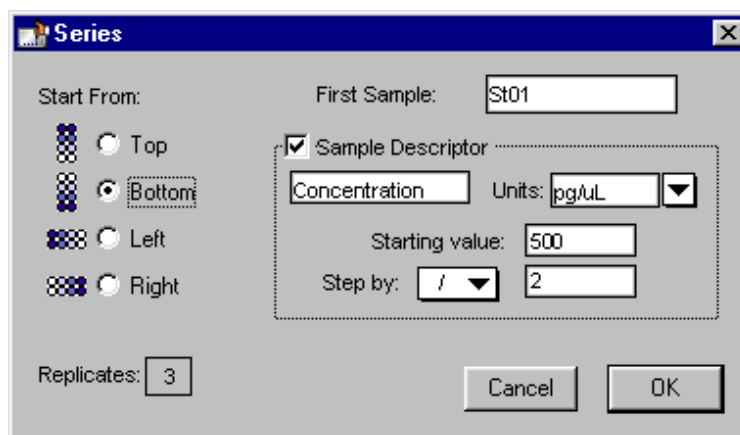


Figure 7.11: Series Dialog Box

Click the **OK** button to close the dialog box and return to the Template Editor. The wells you selected should now be assigned the values entered for the series. You can check the concentrations assigned to these wells by pressing and holding the CTRL+Shift keys.

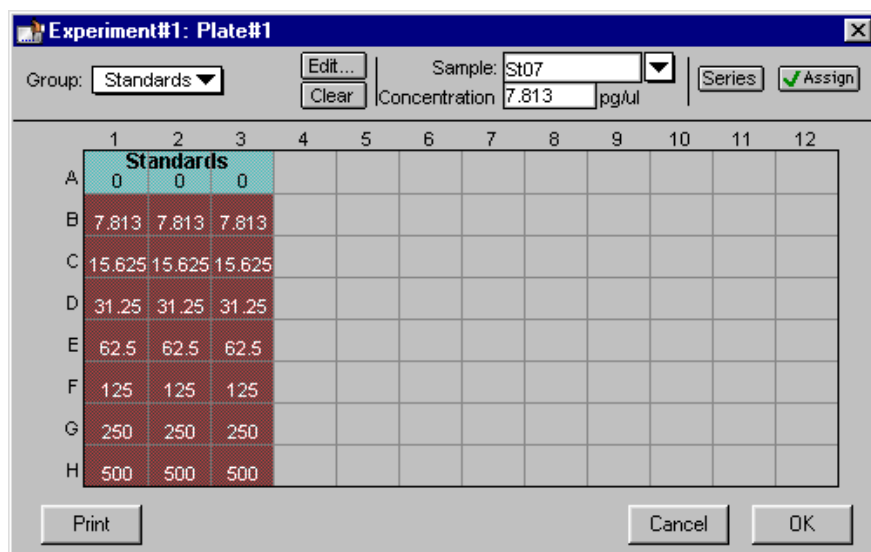


Figure 7.12: Template with CTRL+Shift Keys Depressed



- E) This tutorial assay uses two groups of unknown samples, one with and one without dilution. Each unknown is run in triplicate.

Start by highlighting wells A4 through C5. Select “New” from the Group menu and enter “Unknowns” for the name, Unknowns for the Column Format, and then click [OK]. The selected wells will now show the default first sample name for the Unknowns group, “Un01.” The template should look like the one below.

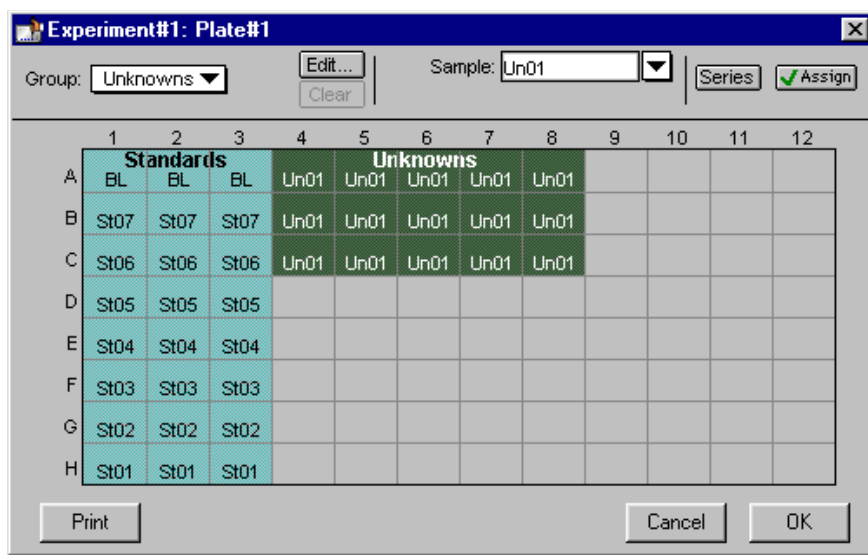


Figure 7.13: First Unknowns Added to the Template

- F) These unknowns need to be identified with different sample names. The shortcut to doing this is to make them a series but change only the name. To do this, click the [Series] button and, in the dialog that results, leave all settings as they are but enable the Sample Descriptor section by clicking in the checkbox. Leave the information in this section as it is (starting with 1 and dividing by 1). This will not change the group in any way except to increment the name of each sample from left to right. When finished, click [OK] to close the dialog box. The template should now look like this:

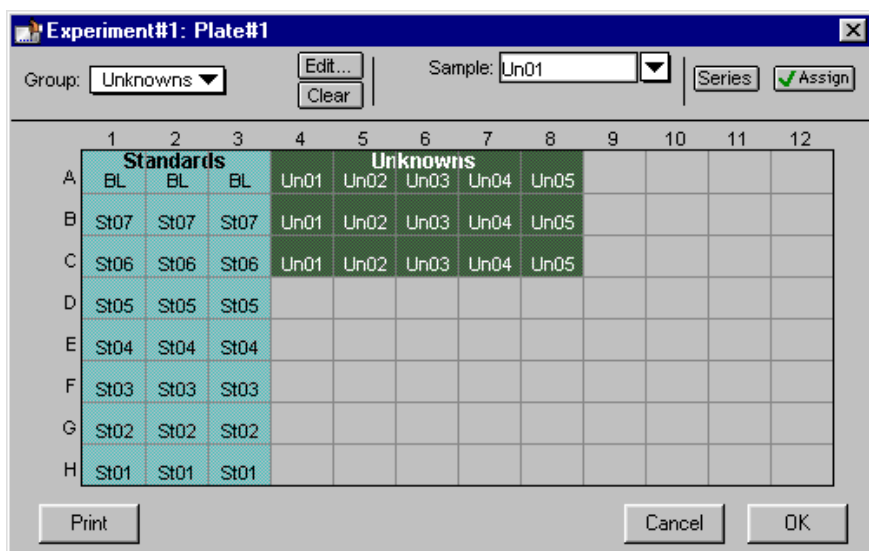


Figure 7.14: Unknowns Changed to a Series

- G) The next group to add is the Unknowns with Dilution. Highlight wells E5 through G9. Choose “New” from the Group menu and, in the Group Settings dialog box, enter the name “Unk w/Dilution,” choose the Unknowns(dilution) Column Format, and enter $pg/\mu L$ for the units. The Group Settings dialog box should look like the one below. When all settings have been entered, click [OK] to close the dialog box.

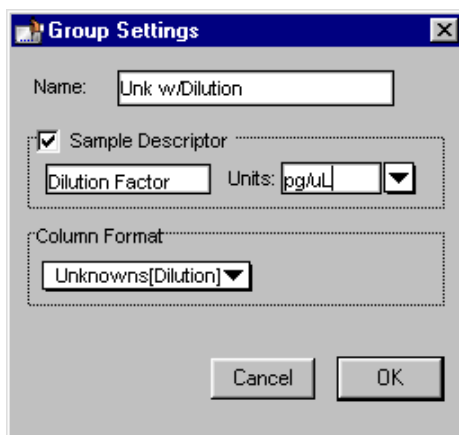


Figure 7.15: Group Settings Dialog Box for Unknowns with Dilution

- H) These Unknowns with Dilution need to have names that reflect distinct samples run in triplicate and they also need to all show a dilution factor of 10. Choose the **Ser ies** button again and, in the dialog box that appears, enter “10” for the starting dilution. Leave the rest of the settings in this dialog box as they are (the series will progress from left to right and will divide the starting concentration by 1). Clicking [OK] will update the template so that all wells in the Unknowns with Dilution now show a dilution factor of 10 and sample names increment from left to right in replicates of 3 as shown below.

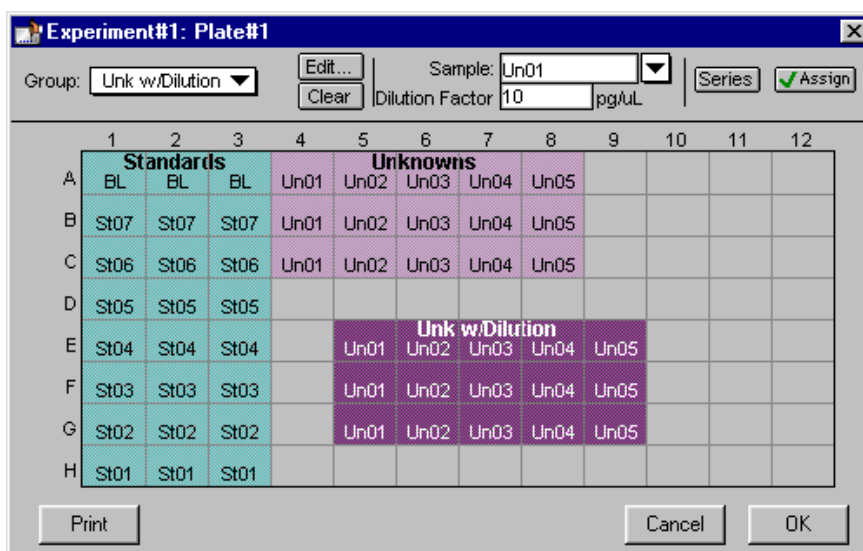


Figure 7.16: Template Complete

- I) The template is now complete. Click the **OK** button to accept the entries and close the Template Editor. The outlines of the groups you have defined in the Template Editor will now be shown in the Plate section.

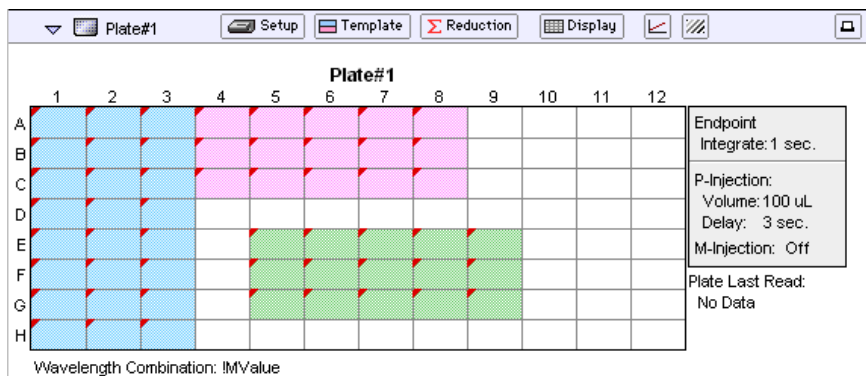


Figure 7.17: The Plate Section Showing Template Group Boundaries



Step 4: Set the Display Parameters

Click the **Display** button to open the Display dialog box.

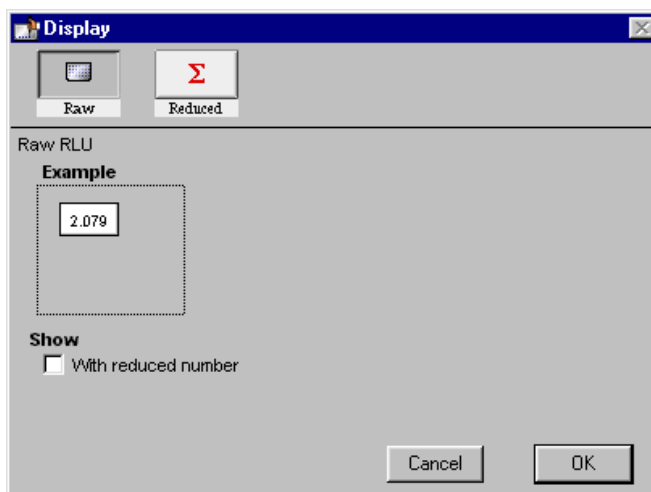


Figure 7.18: Display Dialog Box (Endpoint)

The default display for Endpoint readings is raw RLU. This tutorial example displays reduced data as a number. To do this, click the **[Reduced]** button at the top of the dialog box and then choose “Number” from the choices that appear:

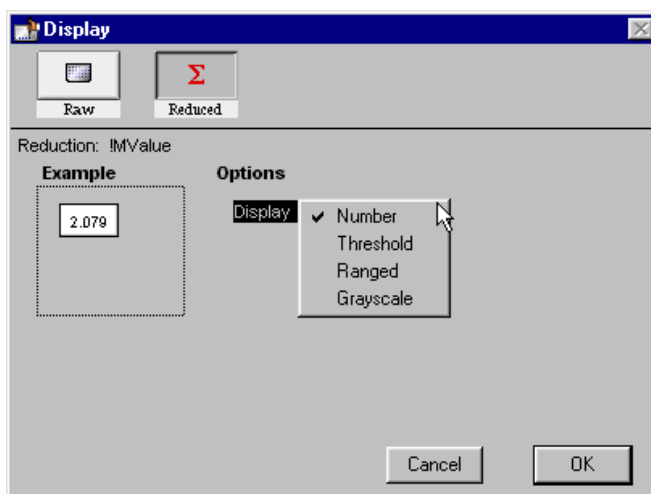


Figure 7.19: Display Dialog Box after Choosing Reduced and Clicking the Display Choices Drop Down Menu

Click **OK** to close the dialog box.



Step 5: Save the Protocol

Choose **Save** from the **File** menu. Because the file has not been saved before and is not named, the Save As... dialog box will open.

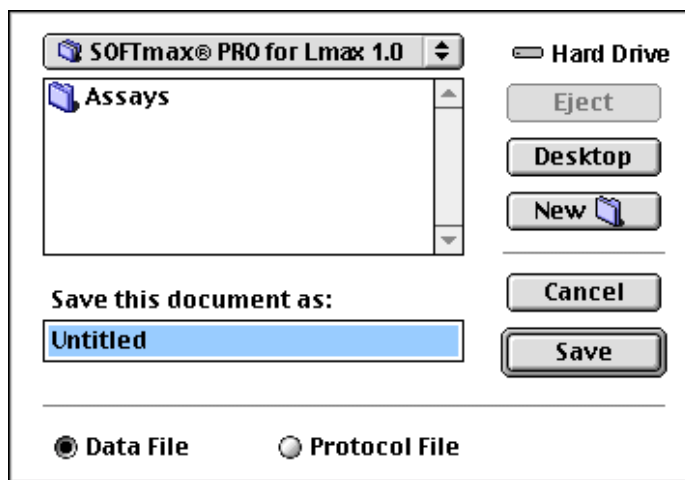


Figure 7.20: Save As... Dialog Box – Macintosh

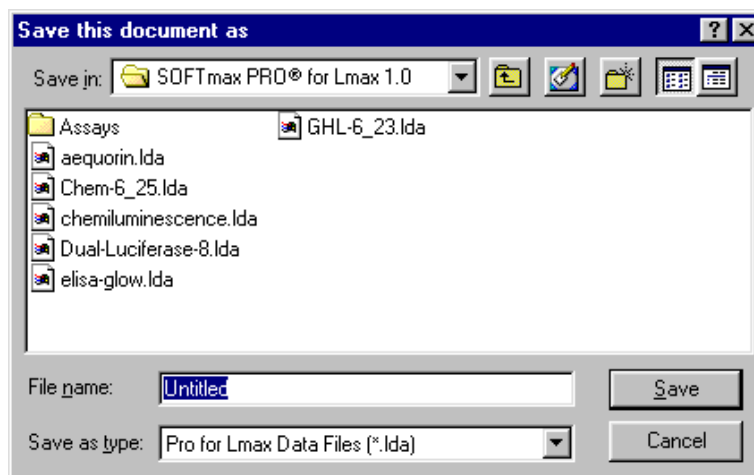


Figure 7.21: Save As... Dialog Box – Windows

On the Macintosh, click the option button next to “Protocol File.” In Windows, click in the box under “Save File as Type:” and choose “Pro for Lmax Protocols (*.lpr).” Type *Tutorial* (the name for the file) in the text box (highlighted over “Untitled”). Then click **Save** to place a copy of the protocol file to the hard disk.

NOTE: The title bar of the window will still show “Untitled” rather than “Tutorial” after saving. This does not mean the protocol has not been saved. When you create a *protocol* file, SOFTmax PRO for Lmax saves a *copy* of the current file under that file name but does not display the name on the screen. Protocol files are saved as *template* files (on the Macintosh, these are known as *stationery* files). When you open a pro-



toocol file, an “Untitled” window appears, containing all the protocol information saved previously, so that you can collect data and save the resulting *data* file under a different name.

Congratulations! You have just created your first SOFTmax PRO for Lmax protocol file. Now would be the time to read the plate and then save the data file. In this tutorial example, however, you will not perform an actual plate reading but will use a simulator program built into SOFTmax PRO for Lmax to generate “data” that you will manipulate as if it had been obtained from an actual reading.

Note that the Default Protocol which ships with SOFTmax PRO for Lmax contains a template and display parameters that are equivalent to the protocol file you just created in this tutorial example.

Step 6: “Read” the Plate

If you were reading an actual microplate at this time, you would prepare the microplate as it is defined in the Template Editor. (Before an actual reading, you might find it helpful to print a copy of the template to assist in properly filling the plate: from the Template Editor, click the **[Print]** button in the lower left corner.) You would then place the filled microplate in the drawer of the MAXline instrument. Since we are using a simulator, you don’t need to load anything in the instrument. To enable the simulator, open the Preferences (choose **Edit > Preferences**) and then click on the instrument type shown in the lower left corner of the dialog box. A menu will appear, offering a choice of instrument or simulator. Choose **Simulator** (see the figure below).

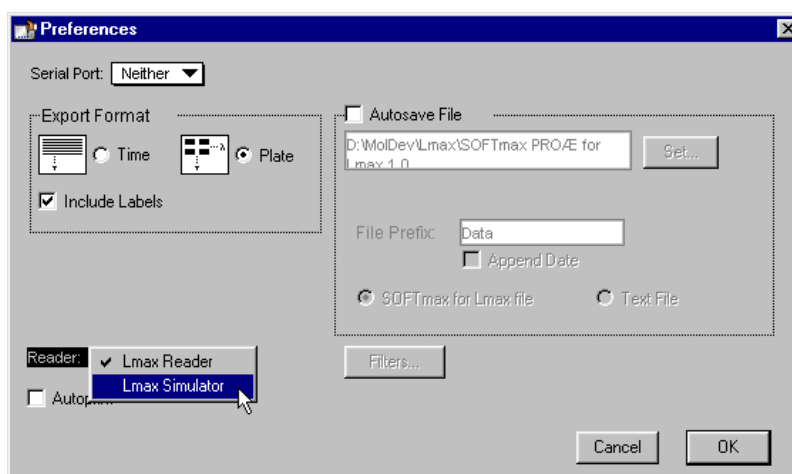


Figure 7.22: Simulator Choice in the Preferences Dialog Box

When the simulator is active, the instrument icon in the upper left corner of the window will be blue instead of orange. With the simulator active, click the **[Read]** button in the status bar at the top of the screen to initiate the simulated reading.

Values will appear in the data display in the Plate section as if they were being read by the Lmax. When the simulated reading is complete, the plate section should look similar to the one below.

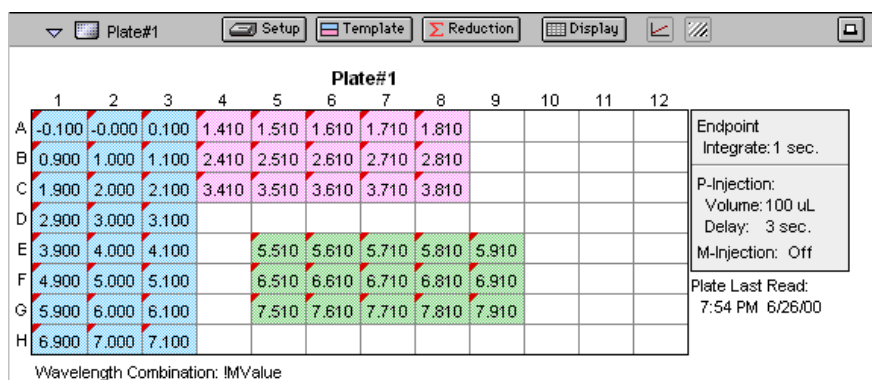


Figure 7.23: Plate Section with Simulated Data

The display for each well that is defined by a group boundary shows a raw RLU value displayed in a reduced form as a number. The reduction formula is shown at the bottom of the display: “Wavelength Combination: !MValue.”

In this part of the tutorial, you will display the data in various other *reduced* formats.

- A) Click the **Display** button again to open the Display dialog box. Earlier in this tutorial, we chose to display the data as a reduced number. The other display choices for endpoint data are Threshold, Ranged, and Grayscale. You can experiment more with the various display options, if desired, at a later point. For now, choose “Grayscale” from the drop-down menu. The dialog box will update to show the low and high limits for grayscale display values (which are automatically set to the minimum and maximum data values).

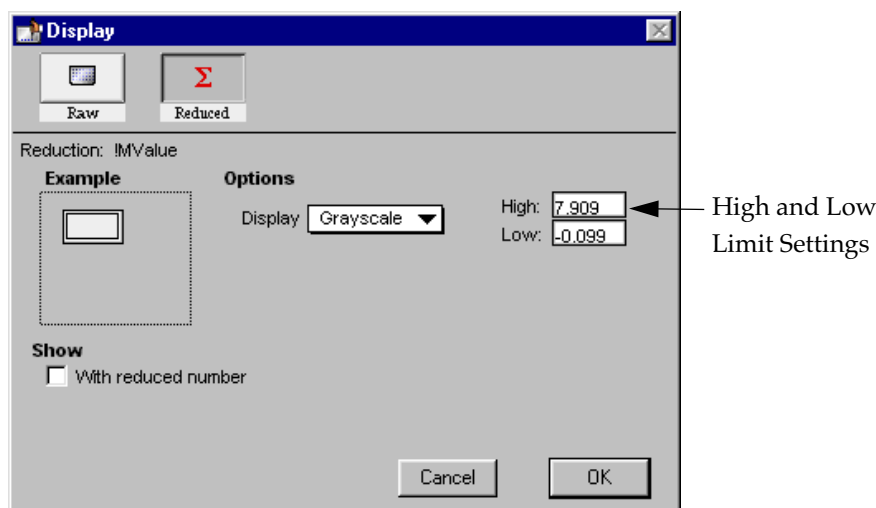


Figure 7.24: Display Dialog Box Updated with Low and High Limit Settings



Click **OK**. The dialog box will close, and the data display in the Plate section will update to show the data represented by shades of gray as shown in Figure 7.25.

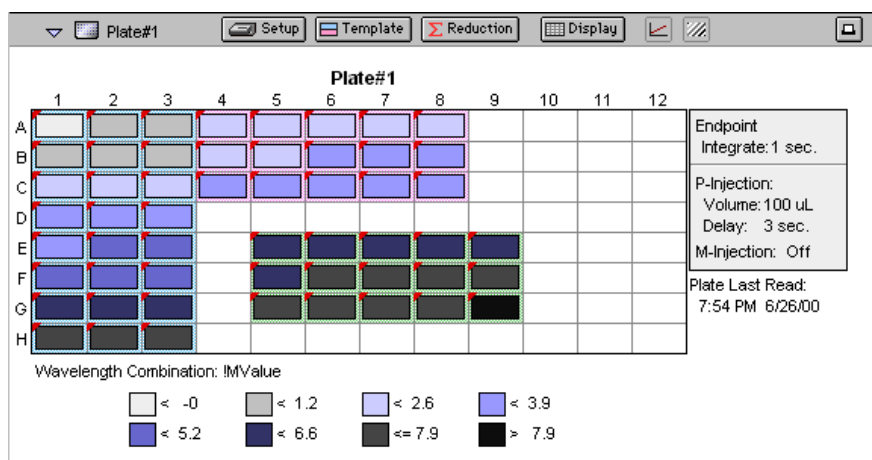


Figure 7.25: Plate Display with Data Displayed as Gray Scale

Step 7: Data Analysis—Group Sections

Group sections are created automatically by SOFTmax PRO for Lmax when you define groups in the Template Editor. The content of Group sections changes depending on the wells that are assigned to these groups in the Template Editor. Before a plate is read, these sections show no data for the wells. After a reading, Group sections contain information about the values for the wells contained within the groups.



Scroll down until the Standards Group is visible. It should look like this:

Standards (pg/μl), group blank = 1.210						
Sample	Concentration	Wells	Values	Mean Value	Std.Dev.	CV%
St01	500.000	H1	6.900	7.000	0.100	1.4
		H2	7.000			
		H3	7.100			
St02	250.000	G1	5.900	6.000	0.100	1.7
		G2	6.000			
		G3	6.100			
St03	125.000	F1	4.900	5.000	0.100	2.0
		F2	5.000			
		F3	5.100			
St04	62.500	E1	3.900	4.000	0.100	2.5
		E2	4.000			
		E3	4.100			
St05	31.250	D1	2.900	3.000	0.100	3.3
		D2	3.000			
		D3	3.100			
St06	15.625	C1	1.900	2.000	0.100	5.0
		C2	2.000			
		C3	2.100			
St07	7.000	B1	0.900	1.000	0.100	10.0
		B2	1.000			
		B3	1.100			

Smallest standard value: 1.000
Largest standard value: 7.000

Figure 7.26: The Group Section for Standards

The columns in this group are set up as follows, from left to right:

- **Sample:** the seven sample names assigned for the Standards group.
- **Concentration:** the concentrations assigned to each sample in the template.
- **Wells:** The wells that are assigned for each sample in triplicate in the template.
- **Values:** raw RLU values obtained for each sample.
- **Mean Value:** the mean of data from the Value column for each sample.
- **StdDev:** standard deviation for each sample value.
- **CV%:** coefficient variation percent for each sample value.

In the following part of this tutorial, you will view formulas associated with columns and summaries, resize individual columns, autosize all the columns, add a column, and hide replicates.



- A) **Viewing Formulas.** To view the formulas associated with *all columns* in the Standards Group section, hold down the CTRL+Shift keys while the Standards group is active. Another row will appear beneath the column titles, showing the formula for each column. Depending on the size of the column, all or only part of each formula may be shown.

Standards (pg/μl), group blank = 1.210

Sample	Concentration	Wells	Values	MeanValue	Std.Dev.	CV%
!SampleN	!Concentration	!WellI	!WellVal	Average(Valu	Stdev(Valu	Cv(Valu
St01	500.000	H1	6.900	7.000	0.100	1.4
		H2	7.000			
		H3	7.100			
St02	250.000	G1	5.900	6.000	0.100	1.7
		G2	6.000			

Figure 7.27: A Portion of the Standard Group Section with Formulas Shown (by Pressing the CTRL+Shift Keys)

If column formulas are long, as in this tutorial example, they may be seen in their entirety in the default column size. To see the complete formula for a *particular column*, click once on a column to highlight it. The formula for that column will be shown in the tool bar.

Standards (pg/μl), group blank = 1.210

Sample	Concentration	Wells	Values	MeanValue	Std.Dev.	CV%
St01	500.000	H1	6.900	7.000	0.100	1.4
		H2	7.000			
		H3	7.100			
St02	250.000	G1	5.900	6.000	0.100	1.7
		G2	6.000			

Figure 7.28: Standards Group Section with Formula Shown for Highlighted Column (MeanValue). Click the button to modify or change a formula.



- B) **Resize and then Autosize a Column.** Position the pointer on the line dividing the columns named “Concentration” and “Wells.” The pointer will change to a double-headed arrow. Click and drag the line dividing these columns to the right. Figure 7.29 shows these actions and the resulting wider Concentration column.

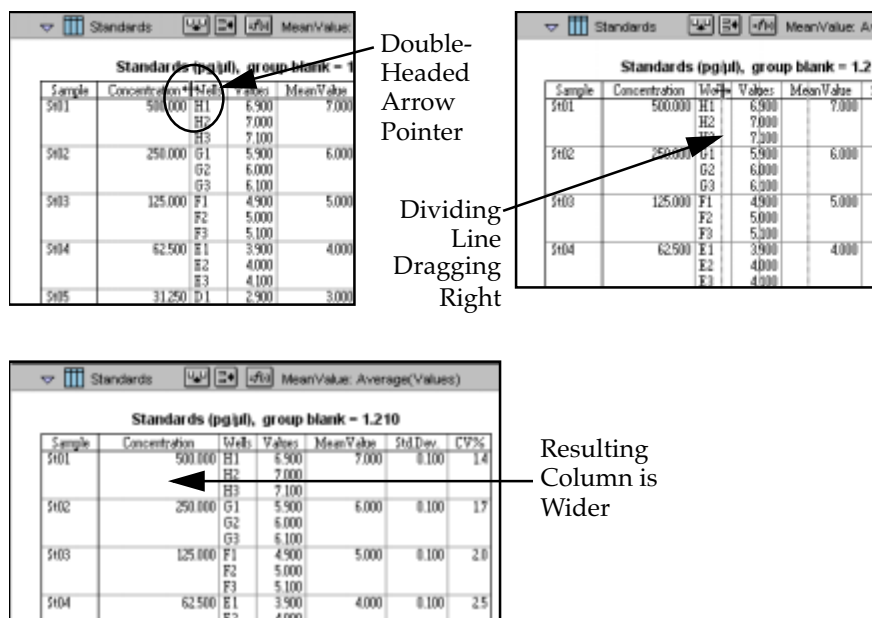


Figure 7.29: Sizing the Concentration Column Wider

To shrink this column to the minimum size needed to display the complete title, click once in the Concentration column to highlight it. Then choose **Autosize** from the **Group** menu. The column will be reduced in width as shown in Figure 7.30.

NOTE: Several columns can be Autosized at one time by highlighting (dragging across) multiple columns in the Group and then choosing **Autosize**.



Sample	Concentration	Wells	Values	Mean Value	Std.Dev.	CV%
St01	500.000	H1	6.900	7.000	0.100	1.4
		H2	7.000			
		H3	7.100			
St02	250.000	G1	5.900	6.000	0.100	1.7
		G2	6.000			
		G3	6.100			
St03	125.000	F1	4.900	5.000	0.100	2.0
		F2	5.000			
		F3	5.100			
St04	62.500	E1	3.900	4.000	0.100	2.5
		E2	4.000			


Before

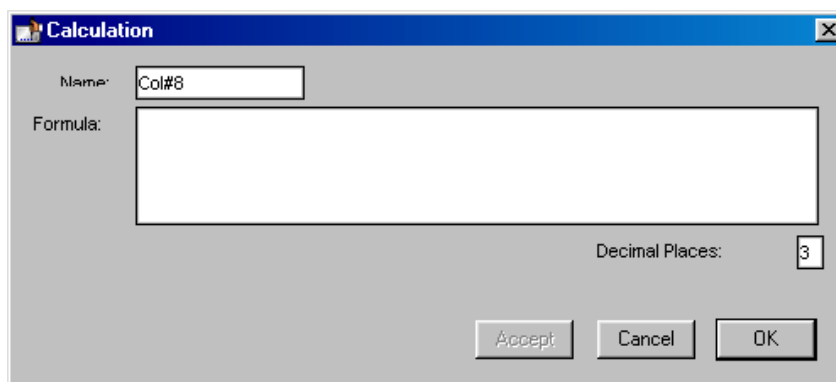
Sample	Concentration	Wells	Values	Mean Value	Std.Dev.	CV%
St01	500.000	H1	6.900	7.000	0.100	1.4
		H2	7.000			
		H3	7.100			
St02	250.000	G1	5.900	6.000	0.100	1.7
		G2	6.000			
		G3	6.100			
St03	125.000	F1	4.900	5.000	0.100	2.0
		F2	5.000			
		F3	5.100			
St04	62.500	E1	3.900	4.000	0.100	2.5
		E2	4.000			

After

Figure 7.30: Columns Before and After Autosize

- C) **Add a Column.** The columns that are created by default with various Group sections may not always meet all the needs of a particular experiment. You can add a column to any Group section and define the purpose of this column in your data analysis. By default, new columns are added to the right of any selected (highlighted) column or, if no columns are selected, after the last column in the Group.

To add a new column to the right side of the Standards Group, make sure this section is active. Then deselect any highlighted columns by clicking outside the column area. Click the Create Column button () or choose **Create Column** from the **Group** menu. A dialog box will open, asking you to enter information for the new column.



The Calculation dialog box has a title bar with a close button. It contains a 'Name' field with the text 'Col#8', a large 'Formula' text area, and a 'Decimal Places' field with the value '3'. At the bottom, there are three buttons: 'Accept', 'Cancel', and 'OK'.

Figure 7.31: Calculation Dialog Box



In this tutorial example, the new column will show the maximum value obtained for each sample. Type over the default column name, entering **MaxValue** in the box next to "Name." Now enter **Max(Values)** in the box next to "Formula" and click . (Be sure to enter the formula *exactly* as shown.) The dialog box will close and a new column will be shown at the right of the previous columns, showing the maximum value obtained for each sample.

Standards (pg/ul), group blank = 1.210							
Sample	Concentration	Wells	Values	MeanValue	Std.Dev.	CV%	MaxValue
St01	500.000	H1	6.900	7.000	0.100	1.4	7.100
		H2	7.000				
		H3	7.100				
St02	250.000	G1	5.900	6.000	0.100	1.7	6.100
		G2	6.000				
		G3	6.100				
St03	125.000	F1	4.900	5.000	0.100	2.0	5.100
		F2	5.000				
		F3	5.100				
St04	62.500	E1	3.900	4.000	0.100	2.5	4.100
		E2	4.000				
		E3	4.100				
St05	31.250	D1	2.900	3.000	0.100	3.3	3.100
		D2	3.000				
		D3	3.100				
St06	15.625	C1	1.900	2.000	0.100	5.0	2.100
		C2	2.000				
		C3	2.100				
St07	7.000	B1	0.900	1.000	0.100	10.0	1.100
		B2	1.000				
		B3	1.100				

Smallest standard value: 1.000
Largest standard value: 7.000

Figure 7.32: New MaxValue Column Appended to Standards Section

- D) **Create an "If" Statement in a Column Formula.** An "If" statement in a formula defines a condition that, if found to be true, causes a certain result and, if not true, causes a different result. The basic structure of an If statement is:

An "If" statement in a formula defines a condition that, if found to be true, causes a certain result and, if not true, causes a different result. The basic structure of an If statement is:

If (condition,result1,result2)

We are going to create an If statement (formula) within the Unknowns section that will compare data from the Values column in that section against calculations found in the Standards section. Here's the formula we will create:

If(Values>=MinStd@Standards and Values<=MaxStd@Standards,"","Outlier")

This statement says that, if the data shown in the Values column (of the Unknowns section) is greater than or equal to the calculation of "MinStd" found in the Standards group section and less than or equal to the calculation of "MaxStd" found in the Standards group section, then show nothing (signified by two quotation marks with nothing between them); otherwise show the word "Outlier."

Looking at the Standards section, you will not immediately see the MinStd or MaxStd calculations used by this If statement. They are there, but their names



are hidden. Look at the bottom of the Standards section and you will see two summaries as shown in the following figure.

		F2	5.000					
		F3	5.100					
\$t04	62.500	E1	3.900	4.000	0.100	2.5	4.100	
		E2	4.000					
		E3	4.100					
\$t05	31.250	D1	2.900	3.000	0.100	3.3	3.100	
		D2	3.000					
		D3	3.100					
\$t06	15.625	C1	1.900	2.000	0.100	5.0	2.100	
		C2	2.000					
		C3	2.100					
\$t07	7.000	B1	0.900	1.000	0.100	10.0	1.100	
		B2	1.000					
		B3	1.100					

Smallest standard value: 1.000
Largest standard value: 7.000

Figure 7.33: Summaries in the Standards Section

Double-click the first summary, “Smallest standard value”. A Calculation dialog box will appear:

Calculation

Name: Hide Name

Description:

Formula:

Decimal Places:

Figure 7.34: Calculation Dialog Box from Summary in Standards Section

This dialog box contains a checkbox for “Hide Name” which is currently enabled. Unchecking the box will allow you to view the name of the summary to the left of its text in the Standards section.

The formulas for MinStd and MaxStd are:

Min(MeanValue) Max(MeanValue)

The MeanValue column is derived from the average of the well values found in the Values column (the Standard replicates). The minimum and maximum values from the MeanValue column in the Standards group are used to create the summaries, and the summary formulas are used to determine our “Outliers.” Such a formula might be useful in finding points that might be better masked (not used in the calculations), for example.

Basic information regarding formulas and the information they can contain can be found in Chapter 5, “Displaying and Analyzing Data.” For a complete discussion of writing formulas in SOFTmax PRO for Lmax, as well as detailed



examples of formulas and customized protocols, refer to the *Formula Reference Guide*.

- E) **Hide Replicates.** While the Standards section is active, choose **Hide Replicates** from the **Group** menu. The display before and after hiding replicates is shown in Figure 7.35.

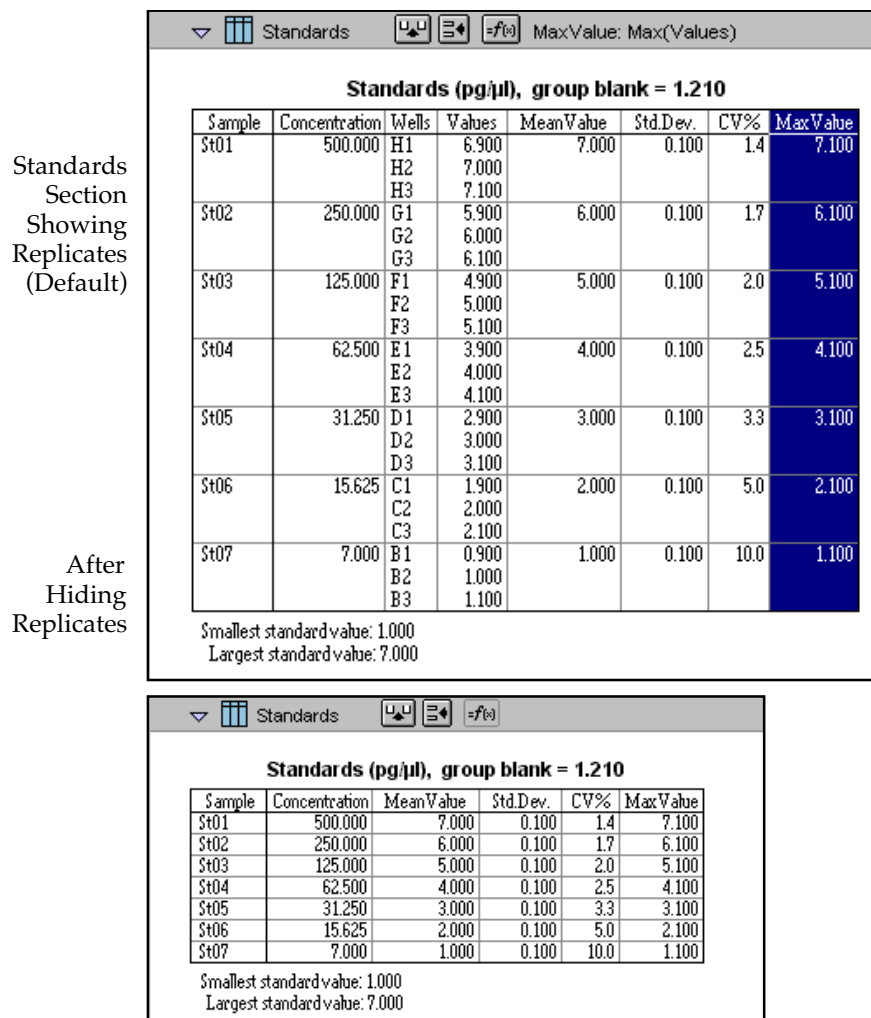


Figure 7.35: Standard Section Before and After Hide Replicates

More information about modifying group sections can be found in Chapter 5, "Displaying and Analyzing Data."



Step 8: Data Analysis—Standard Curve

The Default Protocol (which is the same as the protocol file created in this tutorial example) contains the predefined groups Standards, Unknowns, and Unk w/ dilution. In addition, it contains a graph section which has been created to display a standard curve. If you have not already done so, open the Default Protocol (to create a new “Unknown” file) and, with the simulator still running, click the **[Read]** button to gather simulated data.

Scroll down within the SOFTmax PRO for Lmax window until you see the section tool bar for “Std graph.” This section is closed; click the indicator on the left side of the tool bar to open it. This graph contains a single plot as shown in Figure 7.36.

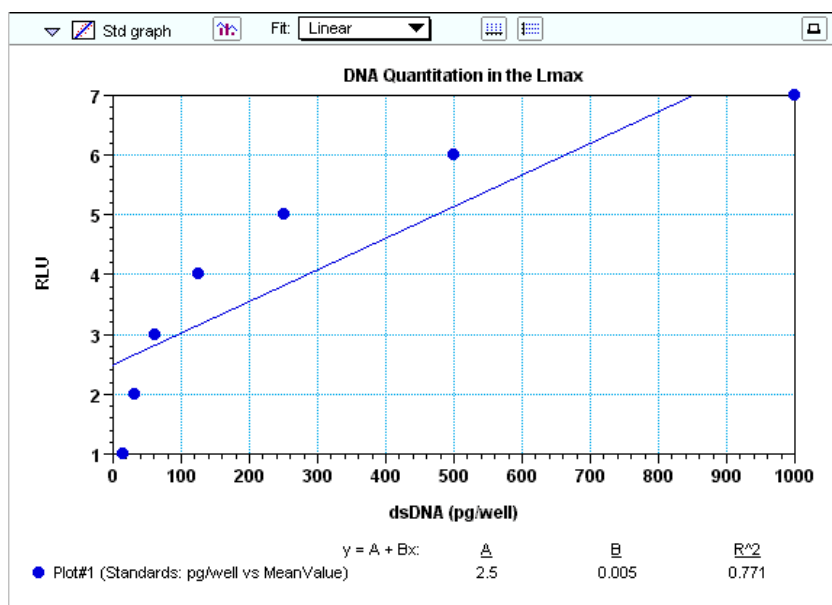


Figure 7.36: Standard Curve Graph Section—Linear Fit Applied. Note that this simulated data does not work well with this linear curve fit.

This graph is set to show pg/well on the X-axis and mean value (RLU) on the Y-axis. A linear fit has been applied to this data but, because the simulated data is not accurate, the fit is not good. If you were to try to find a good fit for this data, you could apply other curve fits by clicking in the box next to “Fit:” in the Graph section toolbar and making a different fit selection. Try some of them, as you wish. The figure below shows the result of applying a 4-parameter curve to this simulated data for a much closer fit.

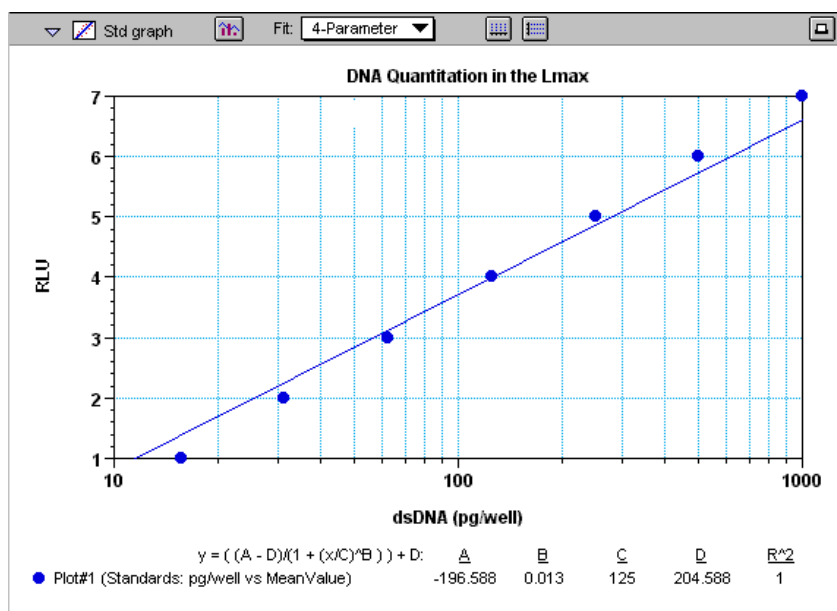


Figure 7.37: Standard Curve Graph Section with 4-Parameter Fit Applied

You can also display the data in a bar-graph or another format by clicking the graph options button  which opens a dialog box like that shown in the following figure.

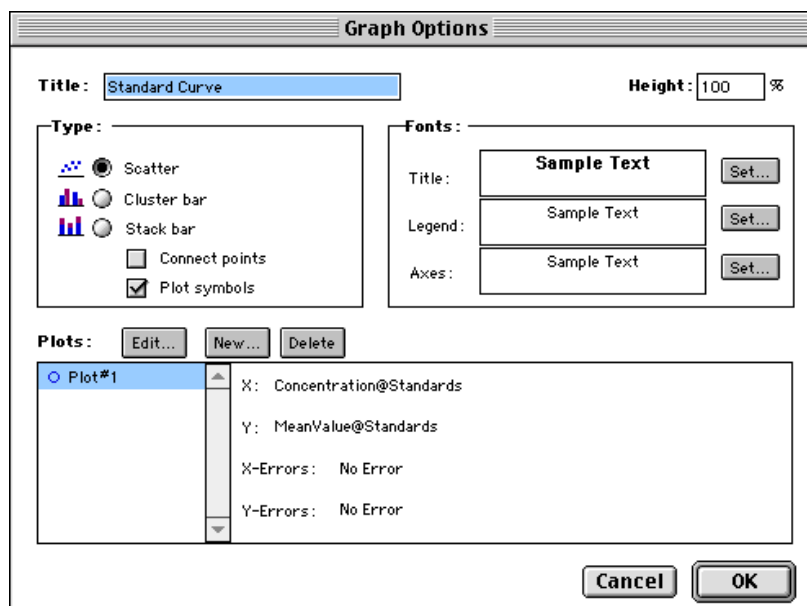


Figure 7.38: Graph Options Dialog Box

This dialog box allows you to rename the graph, choose the type of graph that will be shown, and to format the text used in the graph. You can add or remove plots (click the or buttons) and you can also edit existing plots. See Chapter 5, "Graph Section," for more information regarding the graphing options that are available.



Step 9: Print a Report

SOFTmax PRO for Lmax allows you to print several types of information. In addition to printing a copy of the template, you can print the active section, the sections that have been designated to be included in the report, or all sections.

The default setting in SOFTmax PRO for Lmax is for all sections to be included in the report. You can exclude sections from printing as described in Chapter 6, "File Management and Printing." Otherwise, choosing **Print...** from the **File** menu and choosing "Print report" will print all of the sections that are designated to be included in the report, regardless of whether or not they are open.

A standard Print dialog box will appear (the appearance of this box depends upon the type of computer and printer you are using). Select the appropriate settings for your printer and then click **Print** or **OK**.

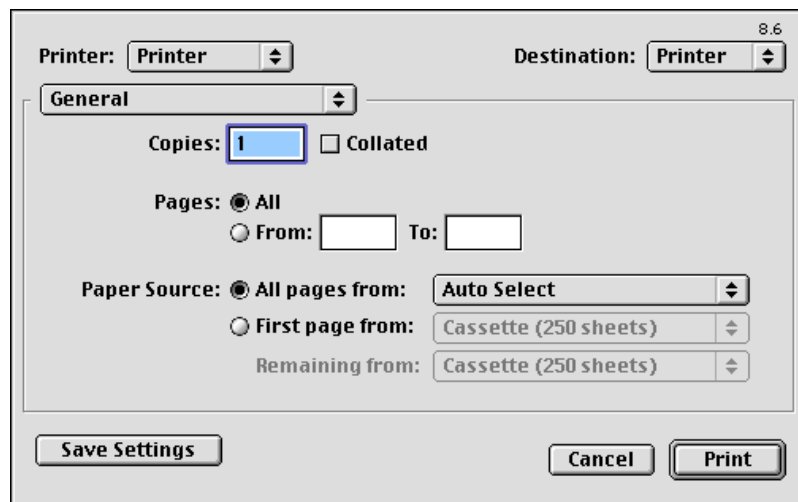


Figure 7.39: Print Dialog Box for Macintosh

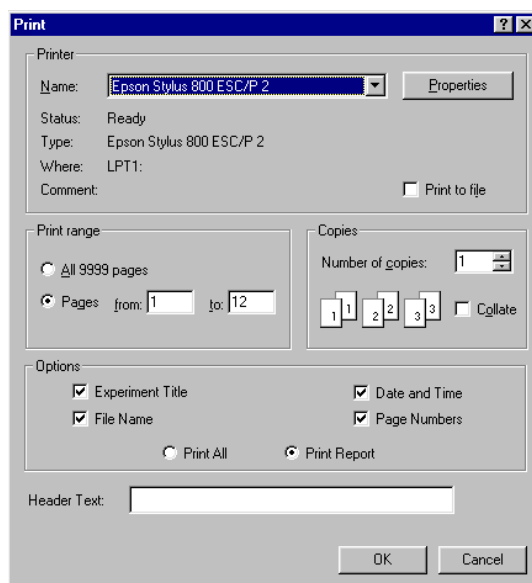


Figure 7.40: Print Dialog Box for Windows (Epson Stylus 800 Shown)

You have completed this tutorial!—but SOFTmax PRO for Lmax has many other features that you will find useful. The next chapter describes in detail items only briefly discussed up to this point and will provide information regarding customizing and using the rest of the features of SOFTmax PRO for Lmax.

Chapter 8: Troubleshooting

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Introduction


When alerts, message boxes, or error boxes appear while you are using SOFTmax PRO for Lmax, they usually contain information describing the situation. In some circumstances, however, the information may not be complete enough to indicate what you should do to remedy the problem. At other times, errors may occur that are not directly associated with SOFTmax PRO for Lmax but affect its operation. The information in this chapter is provided to assist you if an error occurs for which you do not have a solution or if you don't know the source of a problem.

This chapter presents general information regarding problems you may encounter first, followed by specific types of problems grouped by kind: display issues, printing errors, etc. Within each group is general information regarding such problems, sometimes followed by a table containing specific problems and their suggested remedies. If you encounter a problem during operation of SOFTmax PRO for Lmax, first check to see if a table is associated with that type of problem and see if a remedy is listed there. When remedies require more space than the table can allow, a reference to a numbered text section will be listed in the table. Refer to that text section (by number) beneath the table to learn more about solving the problem.

If you need additional assistance or if you encounter a problem with SOFTmax PRO for Lmax that is not listed here, in the U.S., call Molecular Devices Technical Services group at 1-800-635-5577; elsewhere, call your local representative. For problems with an instrument that do not relate to the use of SOFTmax PRO for Lmax, please see the instrument manual for more information.

Message Boxes and Alerts

Message boxes typically appear to inform you that the action you are attempting needs adjustment in some way. Message boxes contain only an **[OK]** button that you can click to remove the message after you have read the information.

Alerts inform you that the action you are about to take will have consequences that may not be reversible or may make a significant change within the program. Alerts are indicated by an alert symbol (); typically, alerts do not prohibit an action but warn you about a condition that may cause a problem.

One common example of an alert occurs when you are defining instrument settings for a kinetic assay and the read interval is too short. The message might look like this:

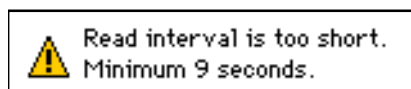
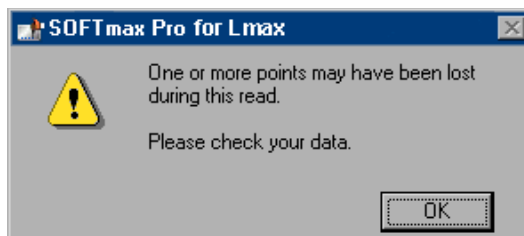


Figure 8.1: Example of an Alert Message



One or More Points Lost Message



A problem may exist with the instrument or the computer, or a communication problem may exist between the instrument and the computer. This error message means that no signal was recorded in a given well (for an endpoint reading) or at one or more timepoints for a given well (for a kinetic run). The instrument sent a placeholder (“NAN” — stands for “Not A Number”) in lieu of a number and continued to collect data.

Depending on the type of assay(s) conducted, it may be very easy or difficult to see which wells were not read. During an endpoint reading, it might be quite easy to spot a missed data point; finding one during a long kinetics reading might be quite difficult, however. Follow the instructions below for the type of reading that was performed when the error occurred in order to assist you in finding lost data points.


Lost Data with an Endpoint Reading

If you get such an error message during an endpoint reading, try displaying raw signal and visibly inspect the plate data for missing read points.

Lost Data with a Kinetics Reading

If the error occurs during a kinetics run, access the Reduction dialog box (click the **[Reduction]** button) for the plate section that was read. Choose “Custom” for the reduction and then click the formula button that appears (**[=f(x)]**) to bring up the Calculation dialog box. For the kinetic reduction, enter the formula $\ln(\text{numpointsread})$ and then close the dialog box. In the Plate section, set the display (click the **[Display]** button) to raw data plot with reduced number. Compare the number shown in the Plate display with the number of Vmax Points listed at the right-hand side of the Plate section. The numbers should be the same. If a time point was not collected in a well, the Plate display number will be lower.

Error Message Boxes

Error boxes contain a stop graphic () and text explaining the error, if the software can report it. Some errors are not recoverable and require that you perform the action or assay again. Others, such as an obstruction of the drawer of the microplate reader, report the problem and allow you to correct it and then proceed with the assay.



You have attempted to paste values from an external file containing ASCII tab-delimited data into a Plate section, but the number of fields of data that you have copied does not match those required by the setup of that Plate section.

The format of the copied information must match the Instrument Settings of the target Plate section and the settings chosen in the Export Format in the Preferences. Check that all the required fields are present and that you have highlighted all of these fields prior to copying the data and try pasting again.

Communication Problems

Symptom	Possible Cause/Solution
The instrument icon shown in the upper left corner of the SOFTmax PRO for Lmax window has an "X" through it	<ol style="list-style-type: none"> 1) Is the Lmax turned on? 2) If the icon also has the words "No port selected" through it along with an "X," open the Preferences and select the proper communications port (setting should agree with the physical port to which the cable from the instrument is connected to the computer). 3) Quit SOFTmax PRO for Lmax, turn off power to both the computer and the instrument. Check that cable connections between the instrument and computer are secure. Turn both machines on again and restart SOFTmax PRO for Lmax. If an "X" still appears over the icon, it may be that you are using the wrong type of cable. See "Communication #1" (following) for more information.
The Macintosh computer has only one serial port and can't connect to an Lmax and printer at the same time.	You can purchase a serial switch that will allow you to choose between communication with the Lmax or the printer. See "Communication #2" (following) for more information.

Communication #1

Instrument Icon Shown with an "X"

If the instrument icon shown in the upper left corner of the SOFTmax PRO for Lmax window has an "X" through it, check whether the Lmax is turned on. If it is, then check the SOFTmax PRO for Lmax Preferences settings (see Setting Preferences below) to ensure that the proper communications port (modem or printer for the Macintosh; COM1 or COM 2 for the PC) is selected. This setting should agree with the physical port to which the cable from the instrument is connected to the computer. If the port setting is correct, the instrument is on, and communication is still not functioning properly, quit SOFTmax PRO for Lmax, turn off power to both the computer and the Lmax, and check that the cable connections between the instrument and computer



are secure. Turn both machines on again and restart SOFTmax PRO for Lmax. If an "X" still appears over the icon, it may be that you are using the wrong type of cable.

Communication #2

Macintosh Computers Having a Single Serial Port

Some Macintosh computers have only a single 8-pin DIN serial port which serves as both the printer and modem port. Please check to see if your model is listed as compatible hardware on the *SOFTmax[®] PRO for Macintosh Version System Requirements and Compatible Hardware* sheet. If your computer is not listed, the performance of SOFTmax PRO for Lmax version 3.0 for Macintosh cannot be guaranteed when using this model.

When running SOFTmax PRO for Lmax, the single serial port Macintosh can either communicate with the Lmax or print through the single serial port, but both printer and instrument cannot remain connected to the serial port at the same time. To avoid uncabling the instrument in order to attach the printer, you must either print across a network using the ethernet port of the computer, or the instrument and printer can be connected to an A/B switch box (one 8-pin DIN input port with two 8-pin DIN output ports) available from MacWarehouse (1/800/255-6227, part number BND0199, Din-8 A/B switch box). This switch box can then be attached to the single serial port of the computer. The A/B switch of the switch box is used to physically switch the connection between the instrument and the printer. Because of the limitations of the single serial port Macintosh, the Autoprint feature of SOFTmax PRO for Lmax cannot be used unless you use the ethernet port of the computer for printing.

To print from SOFTmax PRO for Lmax for Macintosh using the single serial port Macintosh, connect to an AppleTalk-compatible printer such as the Hewlett-Packard DeskWriter C or 550C using an AppleTalk cable connection and the AppleTalk printer driver. Serial printers such as the Apple StyleWriter which use an Apple

8-pin DIN to 8-pin DIN peripheral cable and a serial printer driver cannot be used with SOFTmax PRO for Lmax for Macintosh on a Macintosh with a single serial port.

Configuration for Reading

The computer, SOFTmax PRO for Lmax for Macintosh, and switch box must be configured as follows in order to establish communication with the Lmax: AppleTalk Inactive in the Chooser dialog, serial port set to modem in the SOFTmax PRO for Lmax Preferences dialog, and the switch box set to communicate with the Lmax (to access the Chooser or Preferences dialogs, refer to "To Switch from Printing to Reading," below).

Configuration for Printing

The computer, SOFTmax PRO for Lmax for Macintosh, and switch box must be configured as follows in order to print from SOFTmax PRO for Lmax: AppleTalk active in the Chooser dialog, serial port set to modem in the SOFTmax PRO for Lmax Preferences dialog, and the switch box set to communicate with the printer (to access the Chooser or Preferences dialogs, refer to "To Switch from Reading to Printing," below).



Connecting to the Switch Box

These instructions are for connecting to the A/B switch box available from MacWarehouse (800-255-6227, part number BND0199, DIN-8 A/B switch box).

- 1) Attach one end of the 8-pin DIN to 8-pin DIN serial cable provided with the switch box to the single 8-pin DIN serial port of the computer.
- 2) Attach the other end of the cable to the input port (I/O) of the A/B switch box.
- 3) Attach one end of the 8-pin DIN to 8-pin DIN serial cable provided in the SOFTmax PRO for Lmax software package to one of the two output ports (A or B) of the A/B switch box.
- 4) Attach the other end of the cable to the Lmax.
- 5) Attach one end of the 8-pin DIN to 8-pin DIN AppleTalk cable to the remaining output port of the switch box.
- 6) Attach the other end of the cable to the AppleTalk printer.
- 7) Place a note on the top of the switch box indicating the identity of each output port.

To Switch from Reading to Printing

- 1) Configure the computer, SOFTmax PRO for Lmax, and the switch box as described above for reading a microplate.
- 2) Read the microplate.
- 3) Set the switch box to communicate with the printer.
- 4) Quit SOFTmax PRO for Lmax.
- 5) In the Finder, select **Chooser** from the Apple menu to bring up the Chooser dialog.
- 6) Set AppleTalk to active.
- 7) In the box on the left side of the Chooser dialog, choose the AppleTalk driver for the printer. The printer name should appear in the box on the right side of Chooser dialog.
- 8) Close the Chooser dialog.
- 9) Start SOFTmax PRO for Lmax.
- 10) Print the desired sections.



To Switch from Printing to Reading

- 1) Configure the computer, SOFTmax PRO for Lmax, and the switch box as described above for printing.
- 2) Print the desired sections.
- 3) Select **Chooser** from the Apple menu to bring up the Chooser dialog.
- 4) Set AppleTalk to Inactive.
- 5) Set the switch box to communicate with the instrument.
- 6) Select **Preferences** from the **Edit** menu.
- 7) Without changing any settings, click OK.
- 8) Read the microplate.



Macintosh Cable Connections

For an Lmax connected to a Macintosh, one end of the cable should end with an 8-pin DIN connector. This connector is small and round and should be connected to either the modem or printer port of the Macintosh.

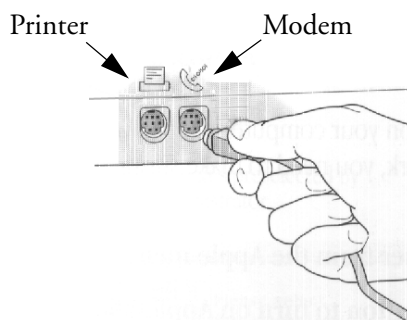


Figure 8.2: 8-Pin Din Connection to Macintosh Computer

If you are connecting the Macintosh to an Lmax, the other end of the cable should also be an 8-pin DIN connector.

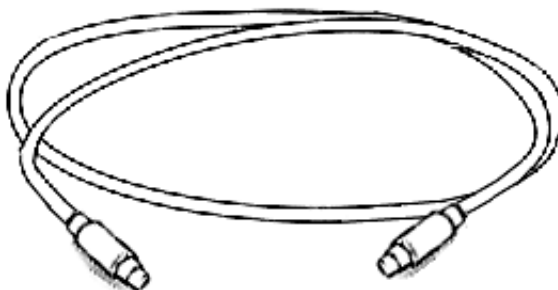


Figure 8.3: 8-Pin DIN to 8-Pin DIN Serial Cable

You should use a double-shielded, straight-through serial cable. If you are unsure of the quality of the cable you are using, Molecular Devices suggests that you purchase a cable of known quality and try the connection again.



PC Cable Connections

The connector required to attach to your PC will depend upon the type of serial port(s) available on your computer. Typically, at least one 9-pin or 25-pin serial port is available on a PC; most have two, which are known as COM1 and COM2, respectively. The connector for your computer may be a 9-pin or 25-pin connector (or you can use an adapter that converts from 9-pins to 25-pins—see Figure 8.4).

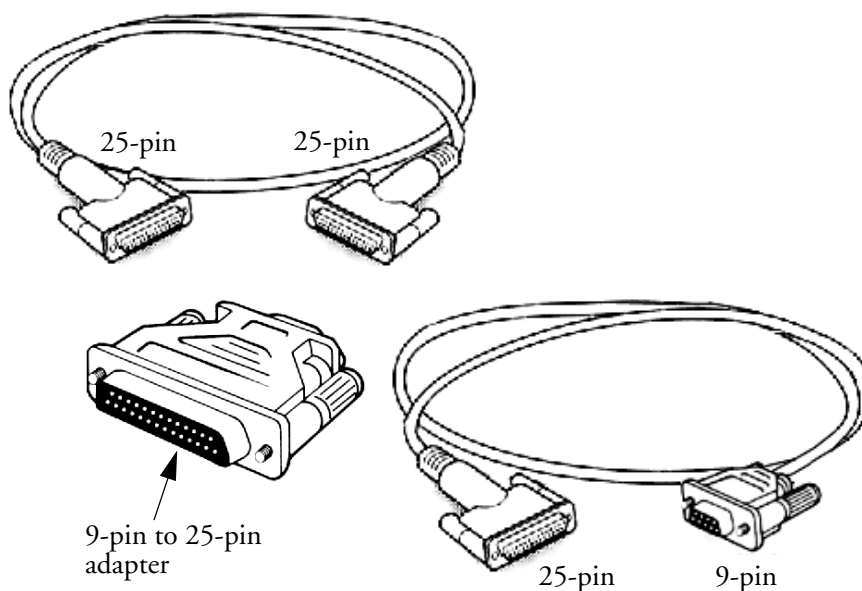


Figure 8.4: 25-Pin DIN to 25-Pin or 9-Pin Serial Cable

The cable itself should be a double-shielded, straight-through serial cable. If you are unsure of the quality of the cable you are using, Molecular Devices suggests that you purchase a cable of known quality and try the connection again.



Display Problems

Symptom	Possible Cause/Solution
A large Group section table becomes gray or disappears.	You are attempting to display more than 2500 data points in a group. 1) From the View menu, choose Minimize. Delete the large Group table; then create a new, smaller table. 2) If this does not work, from the View menu, select the large group; then choose Delete groupname from the Edit menu. Save the file; close the file; reopen the file.
Checkbox for Plate blank subtraction in the reduction dialog box is not visible.	This option is not visible until after data has been collected.
Y-axis units on a graph (either Well Graph or Graph section) are repeated.	This can occur when the range on the Y-axis is very small. Increase the height of the Y-axis.
Text appears "too large" for wells in plate on Windows platform.	In the Windows Control Panel , check the settings for Display . Ensure that you are using "small fonts."
General display problems in Windows	Change the video display settings to 16-bit and high color, or better.

Printing Problems

Symptom	Possible Cause/Solution
Text in the header field of the print dialog box "disappears" if you press the return or enter key.	The text is still there but is not visible. It will print. If you click in the box and drag the mouse to the left, it will reappear.
The Notes section is longer than one page. The bottom line of the first page and the top line of the second page are partially printed.	Insert a linefeed into the Notes section at the appropriate place.



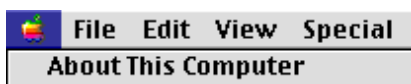
General Problems



Insufficient Memory in a Macintosh

If errors occur while using SOFTmax PRO for Lmax for the Macintosh, it may be that the amount of memory allocated for the program is insufficient. Two items need to be checked: the amount of memory your Macintosh has available for use, and the amount of memory allocated to SOFTmax PRO for Lmax.

To see the amount of memory available within the Macintosh, first quit all open applications, then go to the Finder, and choose **About This Macintosh...** from the Apple () menu.



The information box that opens will list the amount of memory used by the System file and the amount of memory remaining (under the heading, "Largest unused block") that can be assigned to applications (such as SOFTmax PRO for Lmax). The amount of memory available should be equal to or greater than minimum amount required by SOFTmax PRO for Lmax.



Figure 8.5: About This Macintosh Dialog Box

If you open many files simultaneously, or if you open SOFTmax PRO for Lmax while other applications are open in the background, memory errors can occur. Also, if you make a kinetic run with a large number of data points, you may not have enough memory with a typical allocation. SOFTmax PRO for Lmax requires a minimum of 7223K (over 7 megabytes) of memory to run, but this is not optimal. The preferred size is 8223K (over 8 megabytes) or larger. Try increasing the amount of memory allocated to the program. To do this, quit SOFTmax PRO for Lmax (if it is running). Then highlight the SOFTmax PRO for Lmax program icon (click it once), and then choose **Get Info** (⌘I) from the **File** menu. The dialog box that opens lists suggested and minimum memory allocations (see Figure 8.6).

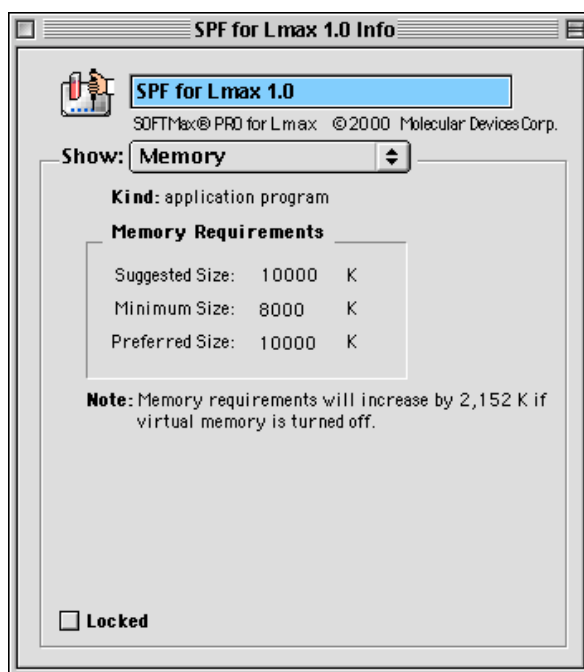


Figure 8.6: SOFTmax PRO for Lmax Info Dialog Box

Enter a larger amount of memory in the box next to “Suggested size”—the amount you enter may be limited by the total amount of memory available. The next time you start SOFTmax PRO for Lmax, it will use the larger memory size you have allocated. You may also enter a different number into either the minimum or preferred size boxes. It is suggested that you do not *reduce* the number in the “Minimum size” box (you may increase it, however). The “Preferred size” can be as large as you wish, depending on the amount of memory available.

Appendix A SOFTmax PRO for Lmax Remote Command Language

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Introduction

SOFTmax PRO for Lmax can be controlled by other Windows applications by means of a simple ASCII string-based messaging system. A command string such as "Read" or "SaveAs:myFile" is sent to SOFTmax PRO for Lmax which then parses and executes the command.

Interprocess communication is implemented by using the Windows Send-Message/PostMessage system. When SOFTmax PRO for Lmax starts up, it uses `RegisterWindowMessage` to register a unique message tag. Other applications can retrieve this message tag by using the same mechanism:

```
smaxMsg=RegisterWindowMessage("SOFTMaxPROMsg");
```

ASCII commands are sent to SOFTmax PRO for Lmax using the `WM_SETTEXT` message, a special message used for interprocess Windows messages because it allows character strings from the address space of one process to be accessed (read-only) by a process running in another address space.

All messages must be sent to the SOFTmax PRO for Lmax main window. To get the proper `HWND`:

```
hwnd = FindWindow("SOFTMaxPROMainWnd", "SOFTmax PRO for Lmax");
```

Finally to send the message:

```
SendMessage(hwnd,WM_SETTEXT,smaxMsg,(LPARAM)myCmd);
```

Notes:

- The `smaxMsg` tag MUST be sent as the `wParam` so that SOFTmax PRO for Lmax knows this is an external command and not a Windows generated message.
- The tag and `hwnd` should be checked for non-zero values before sending the message.
- Opening and/or closing the Lmax chamber cover must be done manually and so cannot be automated by means of interprocess messaging.

SOFTmax PRO for Lmax Responses

Most commands that are sent to SOFTmax PRO for Lmax are just that: commands that tell SOFTmax PRO for Lmax to do something. However, a few SOFTmax PRO for Lmax commands respond with ASCII results. Examples are "ReturnStatus" and "ReturnData".

Two response modes are available. In the first mode, SOFTmax PRO for Lmax responds by putting the requested information on the clipboard. The data can then be pasted into other applications, such as into Excel spreadsheets. For many other applications, however, the clipboard mechanism is not appropriate for retrieving data from SOFTmax PRO for Lmax. Because of this, SOFTmax PRO for Lmax can also send the response strings back in a separate message, using the MFC C++ `WinProc`. This mechanism is discussed in detail later in the Appendix.



SOFTmax PRO for Lmax Commands

SOFTmax PRO for Lmax commands are single words. If parameters are required with the command, a colon is used between the command and the parameters.

Close

Close the current document.

⚠ CAUTION: If the data has not been saved, the document is closed anyway with no user warning.

Copy

This is equivalent to pressing CTRL-C or selecting **Copy** from the **Edit** menu.

Notes:

- If a plate section has just been read or is the only selected section, the plate data will be copied to the clipboard.
- The data copied will follow the display settings for the current plate (normally Raw Data).
- Other sections such as Groups can also be copied. The *SelectAll* command should be used first.

ExportAs:XXX

Tell SOFTmax PRO for Lmax to export data in the standard SOFTmax PRO for Lmax format.

ImportTemplate:XXX

Have SOFTmax PRO for Lmax import a template file. The file should comply with SOFTmax PRO for Lmax's template file format. A plate section should be selected prior to importing a template.

NameCurSection

This (re)names the current selected section. If no section is selected, nothing happens.

Notes:

- This command is used typically to identify different plates in an automated environment.
- The data copied will follow the display settings for the current plate (normally Raw Data).

Example:

"NameCurSection:Plate#002345"



New

Have SOFTmax PRO for Lmax send a *New* command. This creates a new document and reads in the values contained in the “Default Protocol.lpr” protocol file.

OpenAssay:XXX

Have SOFTmax PRO for Lmax open a protocol file. The file should be in SOFTmax PRO for Lmax’s Assays folder which is set using the Assays menu. Protocols can also be stored in subdirectories of the Assays folder.

Notes:

- Only protocol files can be opened.
- SOFTmax PRO for Lmax will append .lpr to any filename passed in.
- If the file is not found, SOFTmax PRO for Lmax does nothing.

Examples:

“OpenAssay:myFile”

“OpenAssay:\mySubDirectory\myFile”

Read

Select a plate section and send SOFTmax PRO for Lmax a read command.

Notes:

- If more than one plate section exists in the current document, the first selected plate will be read. If no plate is selected, the first plate after the currently selected section will be read. If nothing is selected, the first plate in the document will be read (typical case).
- If the plate already has data, the data will be overwritten without confirmation.
- If Autosave is turned on, the document will be saved normally after the read is complete.
- Pre-reads of plates should not be performed.

ReturnData

This command is used to ask SOFTmax PRO for Lmax to send back the data from the currently selected plate. Generally this is used to send the data directly to the caller instead of using the clipboard.

In the Visual Basic or Excel environments, this command functions like the SOFTmax PRO for Lmax “Copy” command if a plate is the currently selected section.

ReturnStatus

This command sends back the current instrument status. The status is an ASCII string that has information about the instrument type and version, whether the instrument is busy or idle, its current temperature, and the state of its drawer. The exact format of this response depends on the type of instrument in use.



ReturnTiming

This command sends back the number of seconds that it would take to read the current plate. For multi-plate documents, the plate used is chosen in the same way as described in the “Read” command above.

SaveAs:XXX

Have SOFTmax PRO for Lmax save the current document file. The file will be saved in the current SOFTmax PRO for Lmax Autosave directory which is set in the Preferences dialog accessed through the **Edit** menu. Data files can also be saved to subdirectories of the Autosave directory.

Notes:

- SOFTmax PRO for Lmax will append *.lda* to any filename passed in to it.
- If a file with the same name already exists, SOFTmax PRO for Lmax will automatically overwrite the file with NO warning.
- If the subdirectory does not exist, the file will not be saved.

Examples:

“SaveAs:myFile”

“SaveAs:\mySubDirectory\myFile”

SelectAll

This is equivalent to pressing CTRL-A or choosing **Select All** from the **Edit** menu. This can be used to select data in group tables for copying (not needed for copying plate data).

SelectSect

Select a section by name. This can be useful for multi-plate protocols or for selecting Group tables for copying.

Examples:

“SelectSect:Plate#0023499”

“SelectSect:Group#1”

SelectSectNum

Select a section by its order in the document. This can be useful for multi-plate protocols or for selecting Group tables for copying.

Example:

“SelectSectNum:3 ”

SetTemp:XX.X

Send a command to set the current instrument incubator temperature.

Stop

Send SOFTmax PRO for Lmax a command to stop reading. This is equivalent to pressing the Stop button on the SOFTmax PRO for Lmax tool bar. This should be sent before any Close or Quit command if there is a possibility that the instrument is still reading.



Quit

Tell SOFTmax PRO for Lmax to close all documents and Quit.

⚠ CAUTION: If the data has not been saved, the application quits anyway with no user warning.

Visual Basic (Excel Macro) Example

```
' First some housekeeping code:
Private Declare Function FindWindow Lib "user32" Alias _
"FindWindowA" (ByVal lpClassName As String, _
    ByVal lpWindowName As String) As Long
Private Declare Function SendMessage Lib "user32" Alias _
"SendMessageA" (ByVal hWnd As Long, ByVal wParam As Long, _
    ByVal lParam As String) As Long

Private Declare Function RegisterWindowMessage Lib "user32" Alias _
"RegisterWindowMessageA" (ByVal msgName As String) As Long
'-----
Const WM_SETTEXT = 12 ' define a windows message value
Function SendMsgToPRO(ByVal msgStr As String) As Long
    hWnd = FindWindow("SOFTMaxPROMainWnd", "SOFTmax PRO for Lmax")
    If hWnd = 0 Then ' one beep means Pro is not running.
        Beep
    Else
        ' Pro is running so use the SendMessage API
        softmaxMsg = RegisterWindowMessage("SOFTMaxPROMsg")
        If softmaxMsg = 0 Then
            ' This version of PRO does not understand external msgs
            Beep
            Beep
        Else
            SendMessage(hWnd, WM_SETTEXT, softmaxMsg, msgStr)
        End If
    End If
End Function
'-----
' This is an example of how to use the above routine
```



```
Sub SendNewMsgToPRO()
    SendMsgToPRO ("New")
End Sub
```

MFC C++ Interface to SOFTmax PRO for Lmax Remote Commands that Return Values

Some SOFTmax PRO for Lmax IPC commands return values. By default they put the data in the clipboard, but optionally they will use the WM_COPYDATA mechanism to return data directly to the caller.

In order to have the SOFTmax PRO for Lmax response returned in this fashion, the commands must be sent using WM_COPYDATA so that SOFTmax PRO for Lmax knows what to send the response to.

SOFTmax PRO for Lmax will then reply with another WM_COPYDATA message that contains the response.

MFC Example Code:

```
long cmdStr;
CString cmdStr;
smaxMsg=RegisterWindowMessage("SOFTMaxPROMsg");
cd.dwData=(DWORD)GetSafeHwnd(); // pass our hwnd for return messages
cd.cbData=cmdStr.GetLength()+1; // add one for /0
cd.lpData=cmdStr.GetBuffer(0);
::SendMessage(hwnd,WM_COPYDATA,smaxMsg,(LPARAM)&cd);
```

Later, SOFTmax PRO for Lmax will reply with a WM_COPYDATA message. To get it in MFC, create a routine like this:

```
BOOL XXXXXX::OnCopyData(CWPropWnd, COPYDATASTRUCT* pCopyDataStruct){
    UINTmsgTag;
    CString aStr;
    msgTag=RegisterWindowMessage("SOFTMaxPROReplyMsg"); // get tag
    if (pCopyDataStruct->dwData==msgTag) {
        aStr= pCopyDataStruct->lpData,pCopyDataStruct->cbData);
        // do whatever
        return(TRUE);
    } else {
        return(FALSE); // message is for someone else
    }
}
```




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