SoftMax® Pro Microplate Data Acquisition and Analysis Software

Version 6

User Guide

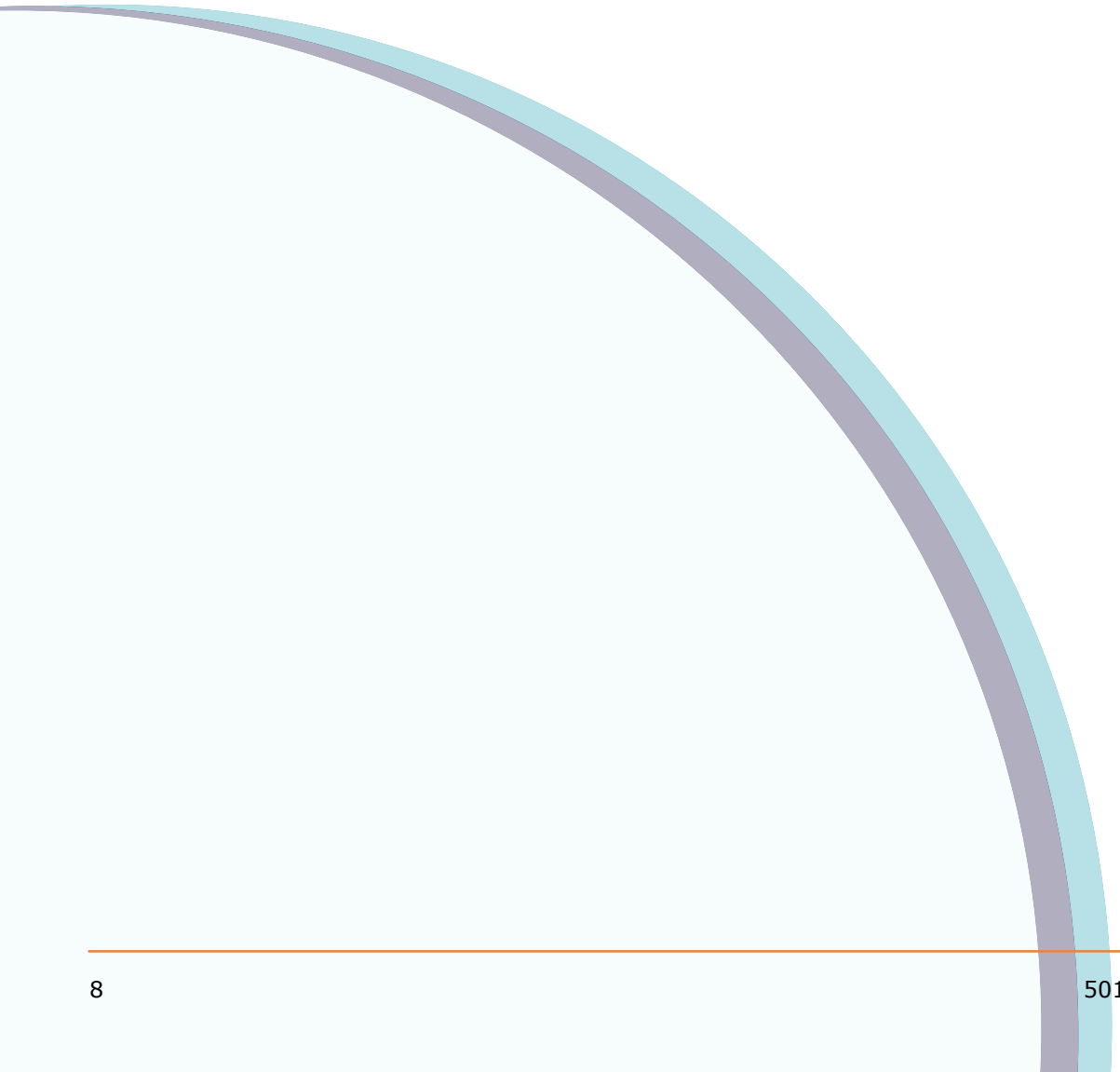
5014177 C
October 2011

Molecular Devices

For research use only. Not for use in diagnostic procedures.

Product manufactured by Molecular Devices, LLC.
1311 Orleans Drive, Sunnyvale, California, United States of America 94089.
Molecular Devices, LLC is ISO 9001 registered.

Contents



1 Installation and Setup

SoftMax® Pro Microplate Data Acquisition and Analysis Software controls Molecular Devices spectrophotometers, absorbance, luminescence, and fluorescence microplate readers, and the SpectraMax® Paradigm® Multi-Mode Detection Platform.

For a complete list of the instruments supported by this release of the SoftMax Pro Software, see Supported Instruments on page 111.

This section contains the following topics:

Computer System Requirements

SoftMax Pro Software version 6 can be installed on a computer with the following system specifications.

Minimum Requirements

Software

- Windows XP, 32-bit (x86), with Service Pack 3
- Windows 7, 32-bit or 64-bit (x86 or x64)
- .NET Framework 4.0[1]

Hardware

- Computer that has a 2 GHz or faster processor
- 2 GB RAM
- 100 MB of available hard disk space
- 1024 x 768 or higher-resolution display
- CD-ROM Drive

Recommended Requirements

Software

- Windows 7, 32-bit or 64-bit (x86 or x64)
- .NET Framework 4.0[1]
- Internet Connection

Hardware

- Computer that has a 2 GHz or faster processor with quad core or higher CPU[2]
- 4 GB RAM[2]
- 500 MB of available hard disk space
- 1280 x 1024 or higher-resolution display
- DVD-ROM Drive

[1] .NET Framework 4.0 is installed automatically by the SoftMax Pro Software installer if necessary.

[2] If running in a virtual machine, Molecular Devices recommends a quad-core or faster processor with at least 6 GB RAM.

Installing SoftMax Pro Software

Note: Molecular Devices recommends that you disable your anti-virus program before installing SoftMax Pro Software, as it might interfere with the installation process.

To install SoftMax Pro Software:

1. Insert the SoftMax Pro Software CD into the CD-ROM drive. The installation program runs automatically.
2. If the installation program does not start, navigate to your CD-ROM Drive and double-click **SoftMaxPro6.1Setup.exe**.
3. Follow the on-screen instructions.
4. Optionally, re-enable anti-virus programs.

Note: If you have anti-virus programs installed, Molecular Devices recommends that you add the SafeNet HASP License Manager Service (hasplms.exe) to the list of trusted applications in your anti-virus programs.

Uninstalling SoftMax Pro Software

Before uninstalling the program, make sure to backup your data and any saved files to a folder outside of the SoftMax Pro Software folder.

1. Click **Start > Control Panel**.
2. Click **Programs and Features**.

   In Windows XP, double-click **Add or Remove Programs**.
3. In the list that appears, click **SoftMax Pro 6.1** and then click **Uninstall** or **Remove**.

Note: This is the recommended method of removing SoftMax Pro Software from a Windows-based computer since it also removes related information from the Windows Registry.

Registering the Software

The software product key can be found on an insert in the SoftMax Pro Software CD package. The instrument serial number is located on a label affixed to the back of the instrument.

Activating Your Software License

To active your SoftMax Pro Software license:

1. Start the SoftMax Pro Software application.
2. Click the **Help** tab in the ribbon.
3. Click **Software License**.
4. If you have internet connectivity, type the Product Key in the field and click **Activate Online**, and then follow the on-screen instructions.
5. If you do not have internet connectivity, click **Activate Offline** and follow the on-screen instructions.

   To activate offline, you need your Product Key, a computer with internet connectivity, and a USB drive for transferring files between the computers.

Starting the Software

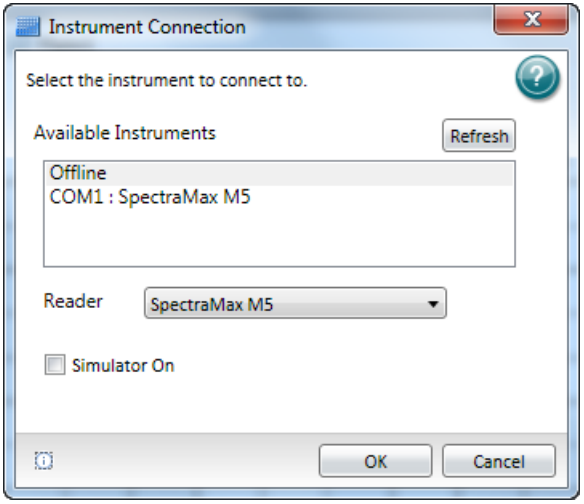
To start the software under normal conditions, wait for the connected instrument to complete its start-up sequence, then double-click the **SoftMax Pro** icon on your desktop to start the program. To start the program from the Windows Start menu, click **Start > All Programs > Molecular Devices > SoftMax Pro 6.1 > SoftMax Pro 6.1**.

Note: You can start the SoftMax Pro Software with or without an attached instrument. When no instrument is attached you cannot acquire data. To perform operations that require data you must be able to open an existing data file.

Selecting and Connecting to an Instrument

To select a reader instrument:

1. Click on the **Instrument** icon on the **Home** tab. The **Instrument Connection** dialog opens.



2. In the **Available Instruments** list, select the communication port to which the instrument is connected or select **Offline**.

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument, select **Offline** and you can select a reader to work with.

3. When you are working offline, select **Offline** and then select the reader you want to connect to from the **Reader** menu.
4. Optionally, to work in simulation mode, select **Offline**, select the reader from the **Reader** menu, and then click **Simulator On**.
5. Click **OK**. An icon for the selected instrument appears on the **Home** tab.

Note: The **Information** button on the lower-left of the dialog is provided for troubleshooting purposes only.

SoftMax Pro Software supports the following instruments:

- VersaMax ELISA Microplate Reader on page 112
- SpectraMax Plus 384 Absorbance Microplate Reader on page 113
- SpectraMax M2 and M2e Multi-Mode Microplate Readers on page 114
- SpectraMax M3 Multi-Mode Microplate Reader on page 115
- SpectraMax M4 Multi-Mode Microplate Reader on page 116
- SpectraMax M5 and M5e Multi-Mode Microplate Readers on page 117
- SpectraMax 340PC 384 Absorbance Microplate Reader on page 118
- SpectraMax 190 Absorbance Microplate Reader on page 118
- Gemini XPS Fluorescence Microplate Reader on page 119
- Gemini EM Fluorescence Microplate Reader on page 119
- FilterMax F5 Multi-Mode Microplate Reader on page 120
- FilterMax F3 Multi-Mode Microplate Reader on page 121
- DTX 800 and DTX 880 Multi-Mode Microplate Readers on page 121
- Vmax Kinetic ELISA Microplate Reader on page 122
- Emax Endpoint ELISA Microplate Reader on page 122
- SpectraMax Paradigm Multi-Mode Detection Platform on page 123
- StakMax Microplate Handling System on page 132

When connected to a SpectraMax Paradigm instrument, see Selecting the SpectraMax Paradigm Detection Cartridge on page 149.

Setting Instrument Calibration Options

To calibrate the reader:

1. Click **Calibration** on the **Operations** tab in the ribbon.
   The **Instrument Control** dialog opens.
2. To calibrate a plate, select **Calibrate Plate** and click **Calibrate Now**.
3. To calibrate a cuvette, select **Calibrate Cuvette** and click **Calibrate Now**.
4. When the calibration indicator shows that the calibration is complete, click **Close**.

The calibration settings are saved in the firmware of the instrument.

Note: If the **Calibration** button is not active in the **Operations** tab, then either the instrument is not connected to the computer or the connected instrument does not support the calibration process.

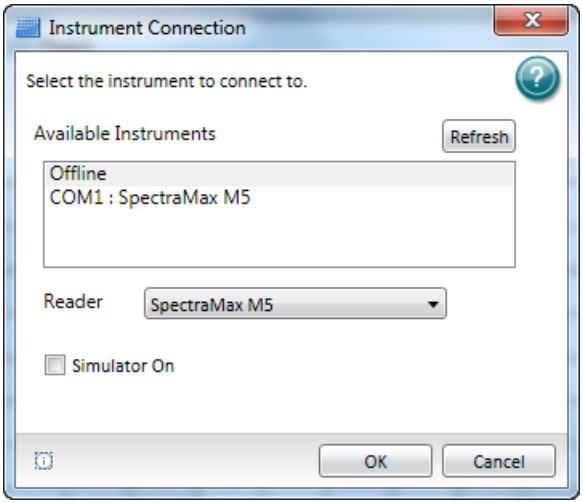
Troubleshooting Instrument Connections

If the instrument is properly connected and turned on, the icon for the instrument appears in the **Home** tab of SoftMax Pro Software main window, and the correct instrument is shown as selected.

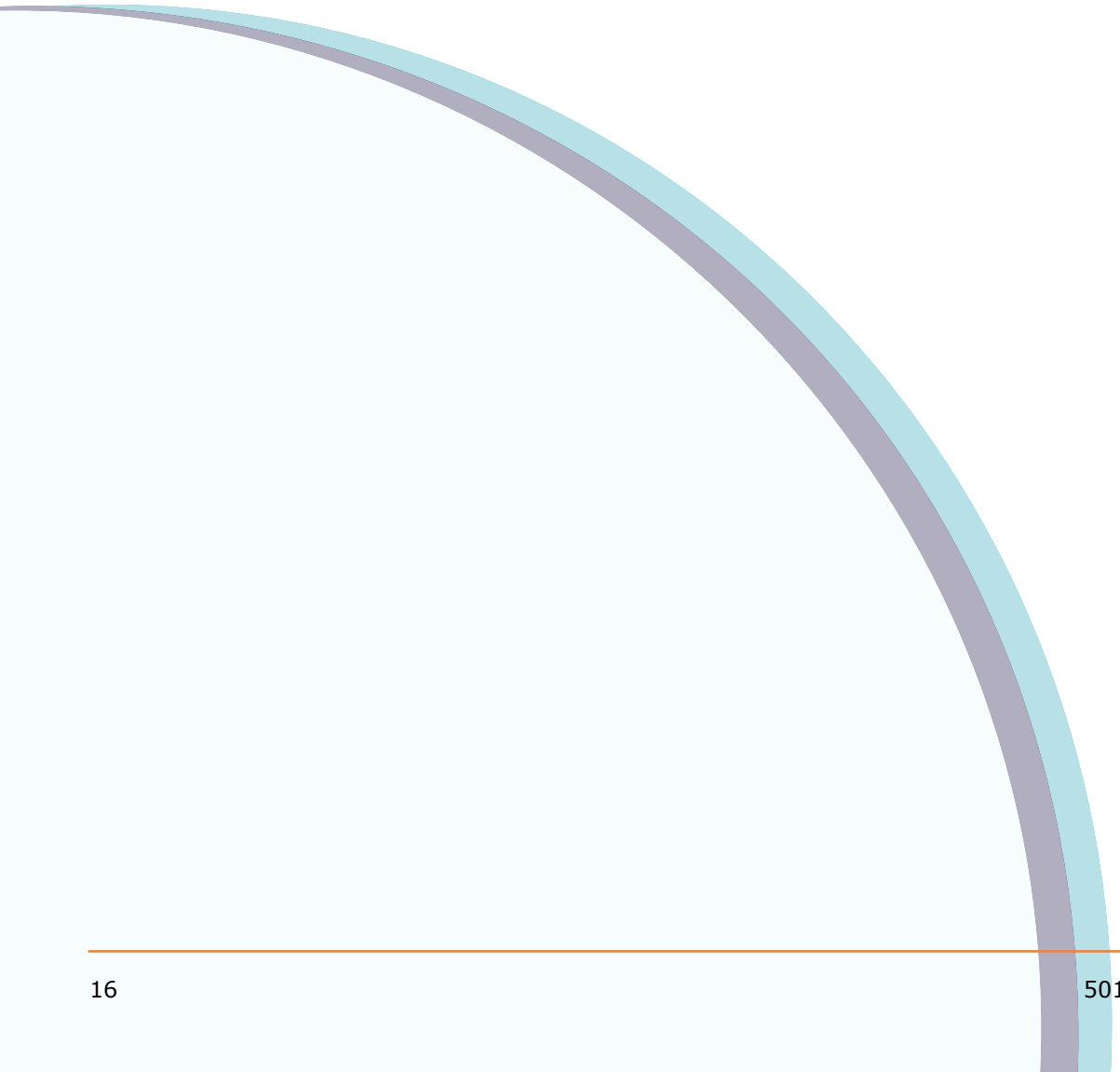
If the instrument is not properly connected or is turned off, the instrument icon in the upper-left corner of the window appears with the  reader disconnected icon. Check that the connection is secure and that the instrument is turned on.

Maintaining the Instrument

Instrument maintenance is detailed in the user guide for instrument. Please refer to the user guide that came with your instrument for instructions.



Note: The **Information** button on the lower-left of the **Instrument Connection** dialog is provided for troubleshooting purposes. Click this button to open a list of plug-ins currently installed in the SoftMax Pro Software.



User Interface Overview

2

SoftMax® Pro Software allows you to set up and run a complete protocol for the supported instruments. Instrument settings can be saved as a protocol (template) file and used repeatedly for reading different microplates or cuvettes. All standalone instrument functions can be controlled using the software.

Workflow Overview

1. Select an instrument. See Selecting and Connecting to an Instrument on page 12.

   The default protocol opens in the workspace with sample sections.

   From here you can add as many plate or cuvette sections as your experiment needs.

2. Click a plate or cuvette section to make it active in the workspace.

   The **Home** tab changes to reflect the tools available for the active plate or cuvette section.

3. Create a protocol. Click the **Settings**  button to set the instrument options. See Selecting Instrument Settings on page 148.

   As you create the protocol, you might need to click the **Template**  button to add a plate template which can include defining groups, blank cells, and a sample series. See Configuring a Microplate Template on page 150.

   Click **OK** to save the settings.

4. Click the **Read**  button to run the protocol and have SoftMax Pro software collects and stores all raw data received from the instrument. See Collecting Data from a Microplate on page 194 or Collecting Data From a Cuvette on page 203.

As information is read, the SoftMax Pro Software display updates showing the new data. During the read, the read button changes to  . You can click this button to stop the read operation. Data is displayed in a grid format that corresponds to the wells in a microplate or individual cuvettes.

SoftMax Pro Software can collect data from one or more microplates or cuvettes and store it in a single data file, using the same or different instrument settings for different microplates or cuvettes. For example, microplates containing different samples can be read using the same or different modes, all within the same experiment.

5. View and analyze the data. See Viewing the Data Display on page 206, Performing Data Reduction on page 208 and Graphing Data on page 230.

   You can manipulate or reduce the raw data using dozens of built-in formulas or define your own analysis structure to quickly and easily summarize the raw data. More than one reduction can be shown, and results from different microplates and cuvettes can be compared within the same experiment.

Note: After you have defined instrument settings, and have customized a SoftMax Pro Software data file with assay information, reduction settings, custom columns in Group sections, and summary objects, you can Save a Protocol file type to create an assay template. This template can then be used and distributed throughout a department or company for highly repeatable data collection and analysis.

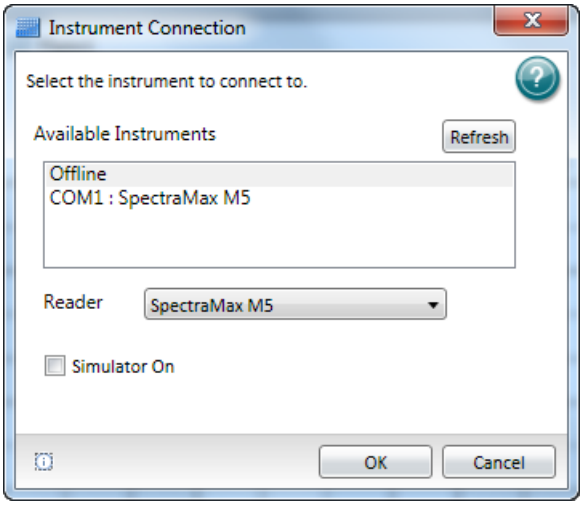
Using SoftMax Pro Software

For those familiar with working with earlier versions of SoftMax Pro Software, adapting to the new SoftMax Pro Software 6 user interface will be quick and easy. The SoftMax Pro Software 6 user interface provides not only advanced features and state-of-the-art navigation, analysis, and data display tools, but it also supports a version of the classic SoftMax Pro Software interface.

SoftMax Pro Software 6 not only improves the basic functionality of earlier versions of the software, but it also adds new features that further contribute to the usability of SoftMax Pro Software's powerful computing engine.

Connecting to an Instrument

1. Click on the Instrument icon on the Home tab. The Instrument Connection dialog opens.



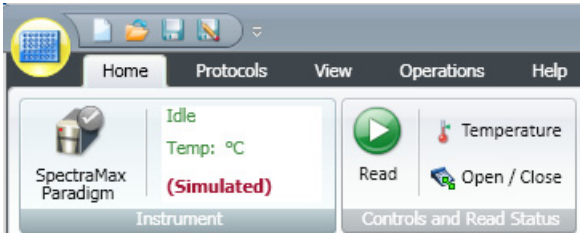
Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument, select Offline and enable simulation mode.

2. Select the reader you want to connect to from the **Reader** menu.
3. To work in simulation mode, select **Offline** and click **Simulator On**.
4. Click **OK**.

An icon for the selected instrument appears in the Home tab.

Reading a Plate

1. Click on the target plate to make it active in the workspace. When enabled, the Read button turns green.
2. Click the **Read** button.



Using the Main Window

The SoftMax Pro Software application window consists of three main areas: the ribbon, the navigator, and the workspace area.

Navigation Tree

The **Navigation Tree** shows the contents of the data file that is currently open and available for display in your Workspace.

When you save a data file, the name that you save the file to is shown on the tab in the Navigation Tree.You can have more than one data file open at a time. Each data file can contain an unlimited number of experiments. Each experiment can contain an unlimited number sections.Section types include: Notes, Plate, Cuvette Set, Group, and Graph.

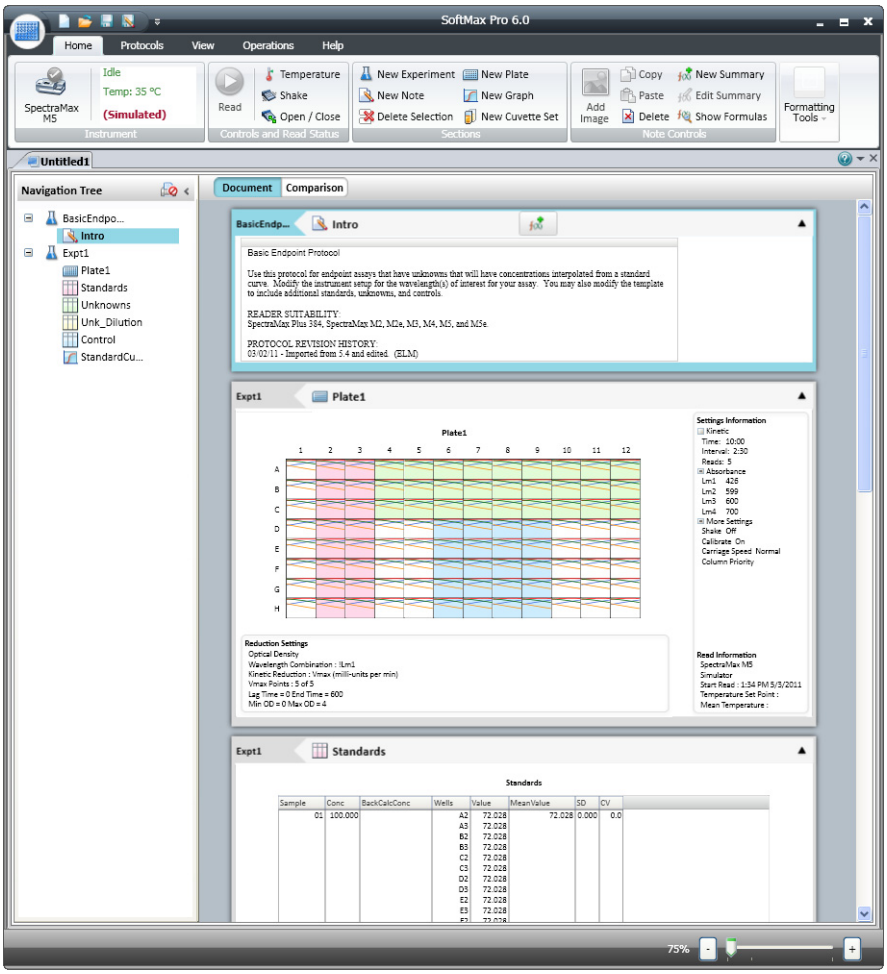
The order of the sections can be moved within an experiment only and not between experiments.

Workspace Views

The SoftMax Pro Software workspace has two main views: Document view and Comparison view.

Document View

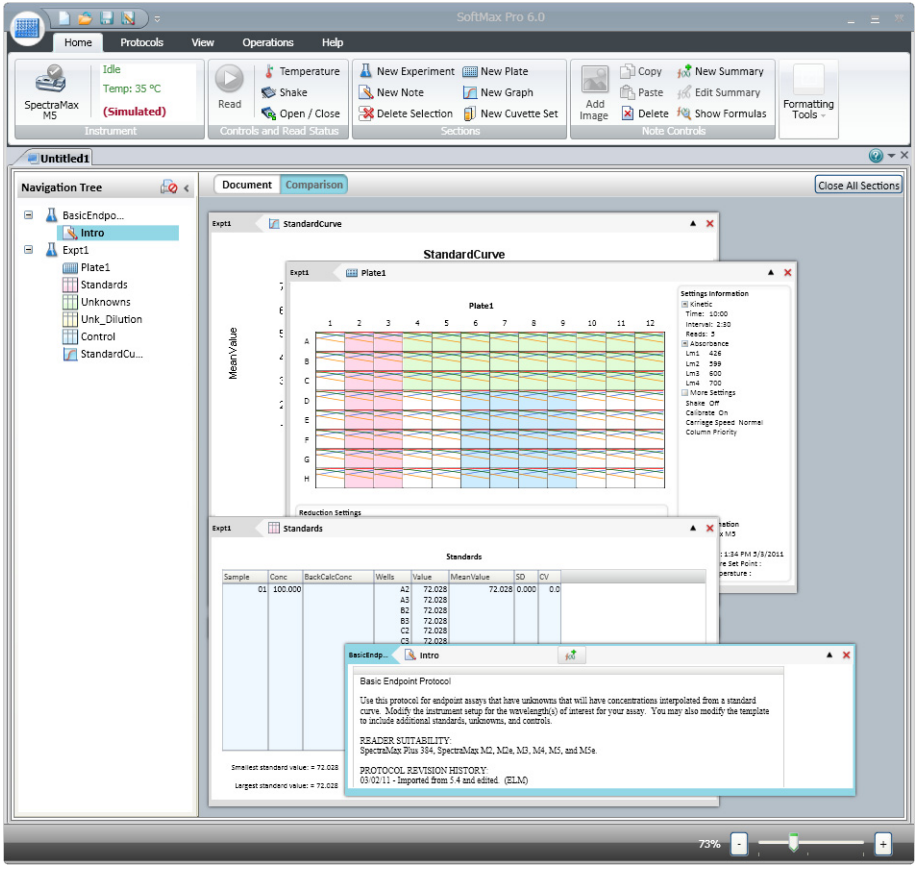
Document view displays information in a manner that is similar to previous SoftMax Pro Software releases. The Document view provides you with basic experiment sections viewing capabilities. To view a section either click on it in the navigation tree or use the scroll bar until the desired section comes into view.



Comparison View

To change to the Comparison view, click the **Comparison View** button.

The **Comparison** view provides you with advanced display options. In Comparison view you can move the sections around in the workspace to position them where they are most useful.



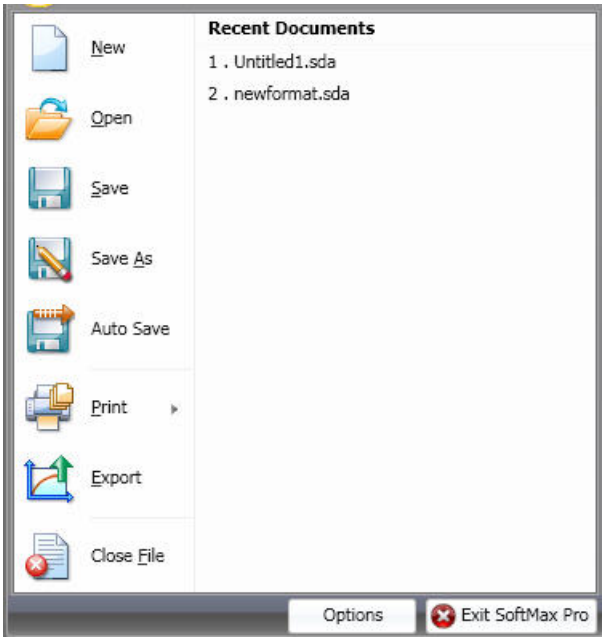
- You can drag sections from the navigation tree and position them in the workspace.
- Sections can overlap.
- Double clicking a section in the navigation tree adds that section to the bottom of the workspace.
- The workspace automatically resizes to display the entire currently active section.

Tools Bar Ribbon

The application ribbon toolbar contains buttons and tabs with the tools needed to work with SoftMax Pro Software.

Application Menu

Click the **Application** button to open the Application menu that contains the options needed for file management and printing. For more information, see Using the Application Menu on page 26.

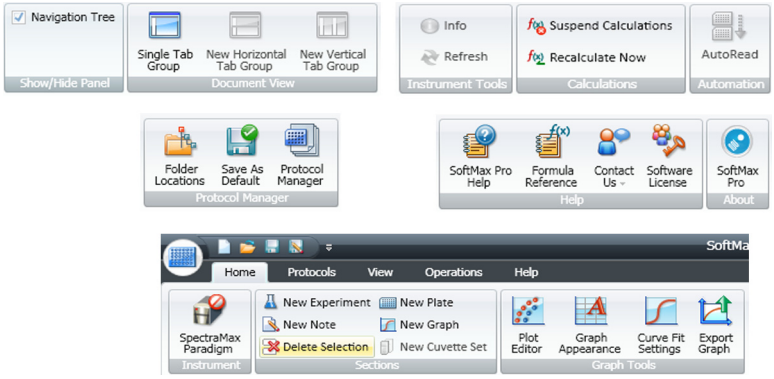


Quick Access Toolbar

The **Quick-Access** toolbar is available for you to customize access to the workspace tools. For more information, see Using the Quick-Access Toolbar on page 38.

Ribbon Tabs

The ribbon has five tabs containing the tools needed to use SoftMax Pro Software.



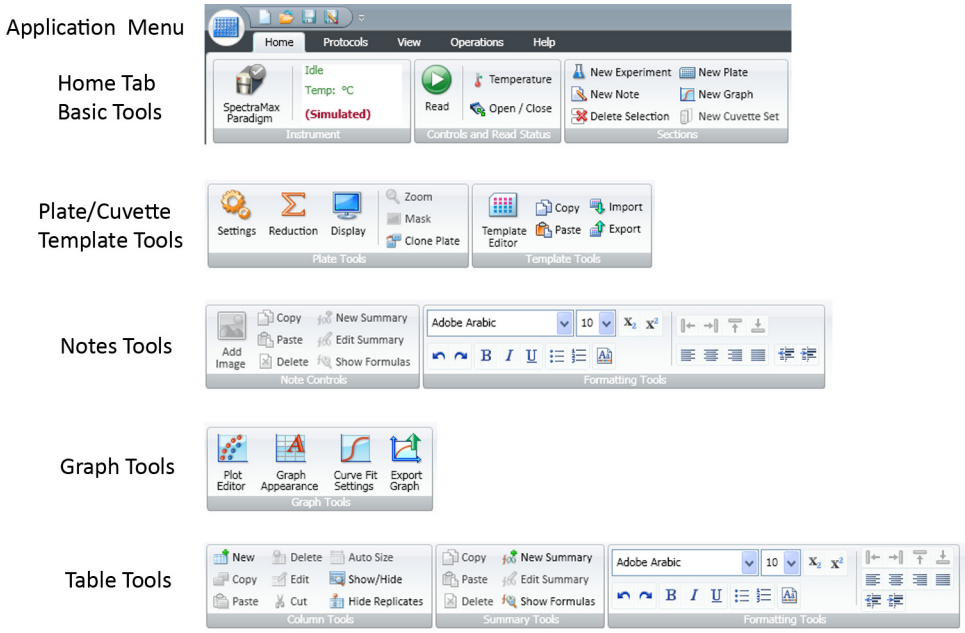
The **Operations** tab contains the tools for maintaining the instrument and controlling programmatic calculations.

The **Protocols** tab provides easy access to protocol files that are stored in the file system.

In the **View** tab has the **Show/Hide Panel** where you can select to display sections, protocols, both sections and protocols, or deselect everything and remove the navigation tree from the application window.

The **Help** tab provides access to the SoftMax Pro Software help documents.

The contents of the **Home** tab changes depending on the section type that is active in the workspace.



For more information about using of the ribbon tabs, see Using the Ribbon on page 39

Workspace Sections

The workspace can contain five different types of sections.

- **Notes** sections are used to record text or to report summary data pertaining to the experiment. For more information, see Using the Notes Section on page 81.
- **Plate** sections are used to collect data from the instrument, and to define data display and data reduction. For more information, see Using the Plate Section on page 72.
- **Cuvette Set** sections are used to collect data from a cuvette port. For more information see Using the Cuvette Set Section on page 92.
- Unlike other section types, **Group** sections are created automatically when you create and assign a Group to a Plate section within the **Template Editor** dialog (after clicking the Template button in a Plate section). For more information, see Using the Group Section on page 97.
- **Graph** sections are used to plot information from groups as scatter plots or bar graphs. For more information, see Using the Graph Section on page 104.

Using the Application Menu

Click the **Application**  button to open the **Application** menu.
This menu contains options for working with files including:

Creating a New Data File

You can create a new empty data file and save it into a folder.

The **Application** button **New File** command opens a new data file named "Untitled", based on the default protocol.

To create a new data file follow these steps:

1. Click the **Application**  button.
2. Select **New**. An experiment file opens and a new tab appears on the navigation tree.

Note: It is recommended that you name the file before you configure settings and collect data.

3. Click the **Application** button.
4. Select **Save as**.
5. The **Save as** dialog opens.
6. Navigate to where you want to save the file.
7. Type the file name in the **File name** field.
8. Select **Data Files (*.sda)** file type.
9. Click **Save**.

   The name of the data file appears on the tab in the navigation tree.

Opening a Data File

To open a supported protocol or data file, use the **Application** menu. SoftMax Pro Software 6 can open files created by version 4.0 or later. However, after you save a file, it is readable only by the current version.

Note: Before you open a file you must make sure that your currently selected instrument is the same model as the one used to create the file, or a model with instrument settings that are compatible with the model used to create the file.

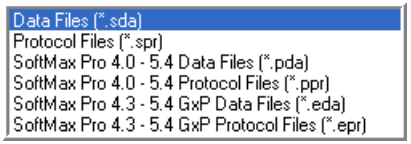
SoftMax Pro Software 6 can open files created by version 4.0 or later. If you want to open a file created in a version earlier than version 4.0, you must first open the file in SoftMax Pro Software 4.0 or later, and then save it as a version 4.0 or later file. Instrument compatibility requirements also must be met.

If you need to continue using a data file with an older version of SoftMax Pro Software, then save the file with a different name or in a different location after you open the file with the current version.

To open a data file:

1. Click the **Application** button.
2. Select **Open**. The **Data Files (*.sda)** file type is selected by default.

   SoftMax Pro Software 6 can open files created by version 4.0 or later. When you open the file, it is converted to SoftMax Pro Software version 6 format.

3. Navigate to the data file you want to open.
4. Select the file.
5. Click **Open**.

   A tab with the name of the file opens in the navigation tree, and the file opens in the workspace.

Saving Data Files

Use the File menu **Save** or **Save As** commands to save data files.

1. Click the **Application** button.
2. Select **Save** (for first time file saving) or **Save As**.
3. Navigate to where you want the file to be saved.
4. Type the file name in the **File name** field.
5. Select the file type from the **Save as type** field. For a data file, select **Data Files (*.sda)**.
6. Click **Save**.

Copying and Pasting Data

Endpoint **Plate** section data can be copied from external programs and pasted into a **Plate** section.

For **Plate** section data, the format of the copied Endpoint data must match the Instrument Settings of the target **Plate** section.

Note: After data is pasted into a **Plate** section, the information to the right of the display shows imported data.

Managing Protocol Files

The **Protocols** tab provides easy access to protocol files that are stored in the file system.

Protocol files are Experiment template files that contain microplate well layout assignments and all other reader configuration data, but no data. A large number of predefined protocols are installed with SoftMax Pro Software.A group pre-defined protocols are provided in the SoftMax Pro Software Protocols folder. During software installation, the Basic Endpoint protocol is set as the default protocol.

Saving a data file as a protocol file removes any data in the file, leaving only the configuration information.

You can use the functions provided on the **Protocol** tab on the ribbon to open and manage protocol files.

Note: When you add a new plate or cuvette section to an experiment, the settings in the default protocol are automatically applied to that section. You can change these settings individually using the **Settings** dialog or apply a pre-defined or saved protocol to the new section.

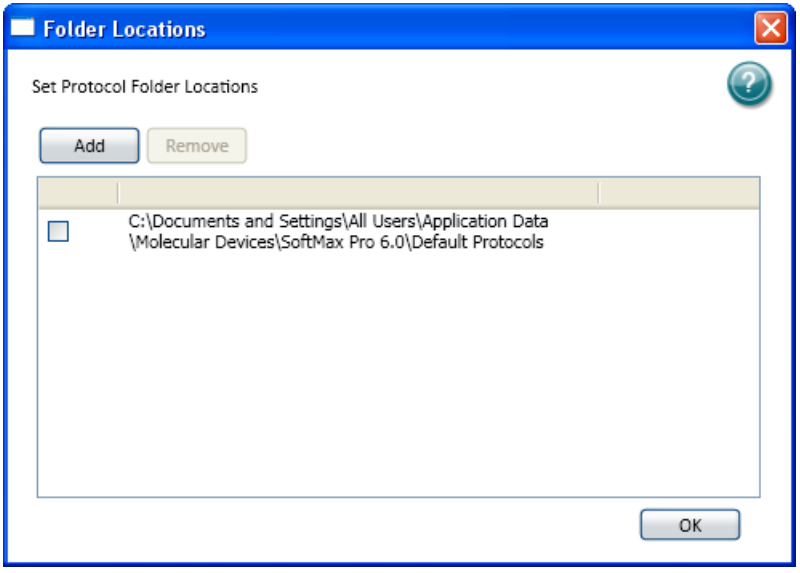
Using the Folder Locations Button

Use the Protocol Manager shortcut to add folders in your file system containing saved protocols.

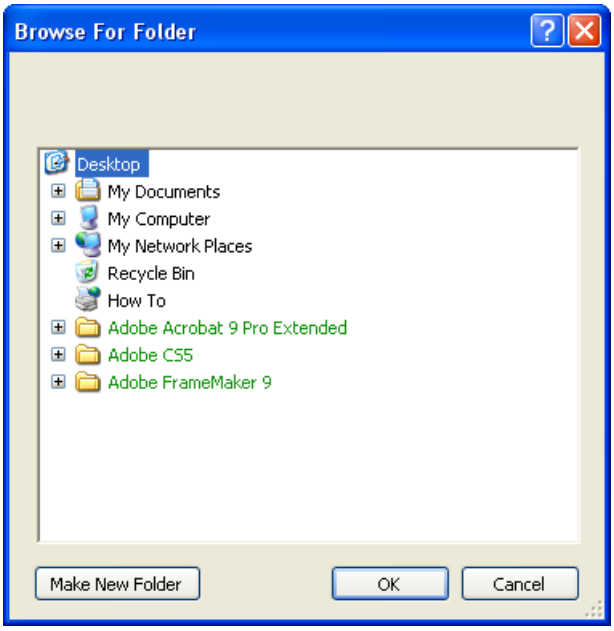
Adding a Protocol Folder Location

To add a protocol folder location:

1. Click **Folder Locations** to open the **Folder Locations** dialog.



2. Click **Add** to open browse the file system to a folder to be added.

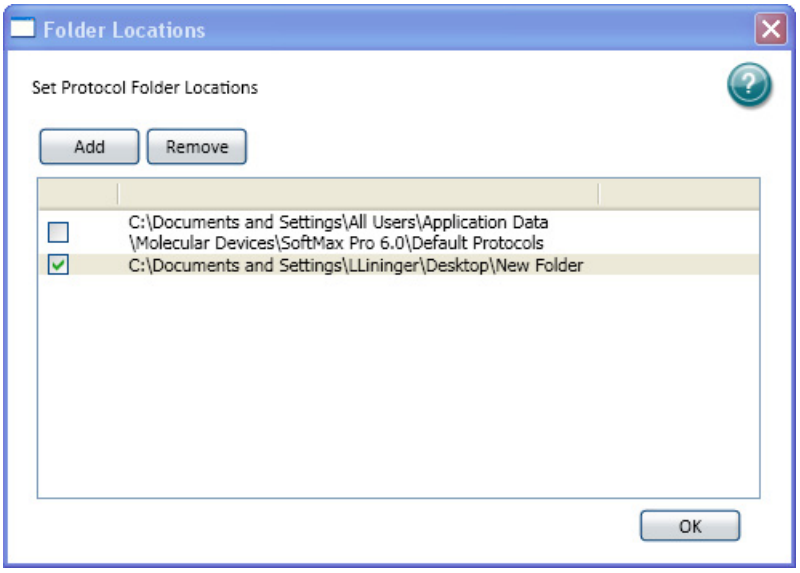


3. Select the folder where you want to store protocols.
4. Optionally, click **Make a New Folder** to add a folder inside the selected folder and type the folder name to replace New Folder.
5. Click **OK**. The selected folder is added to the folder list in the Folder Locations dialog.
6. Click **OK** to close the Folder Locations dialog.

Deleting a Protocol Folder Location

To remove a protocol folder location:

1. Click **Folder Locations** to open the **Folder Locations** dialog.
2. Click the check box to select a folder location.



3. Click **Remove**. The selected folder is removed to the folder list in the **Folder Locations** dialog.
4. Click **OK** to close the **Folder Locations** dialog.

Creating a Default Protocol

You can save the settings of the section currently active in the workspace (minus the data) as the default protocol.

Saving a Default Protocol

1. Click **Save as Default**  to activate the protocol folder locations list.
2. Click an entry in the list to display the entire list.
3. Select a folder from the list.
4. Select a file name from the list.
5. The settings from the currently active section are saved as the default protocol and the file is renamed to **default.spr**.
6. Click **OK** to overwrite the default protocol file with this file. With the exception of acquired data, all settings and sections (with their contents) are saved as part of the new default protocol. Any new file you create will initially be identical to this current data file (minus existing data).

Creating a New Protocol File

You can create a new empty protocol file and save it into a folder.

To create a new protocol file follow these steps:

1. Click the **Application** button.
2. Click **New**.
3. Configure the protocol settings, see Creating a Protocol on page 147.
4. Click the **Application** button.
5. Select **Save as**.
6. The **Save as** dialog opens.
7. Navigate to where you want to save the protocol file.
8. Type the file name in the **File name** field.
9. Select **Protocol file (*.spr)** file type.
10. Click **Save**.

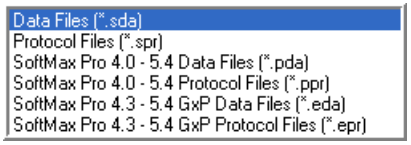
Opening a Protocol File

To open a protocol file:

1. Click the **Application** button.
2. Click **Open**.

   The **Open** dialog appears.
3. In the **Files of Type** field, select the **Protocol Files (*.spr)**.

   You can open protocol files from pervious releases of SoftMax Pro software as shown below. When you open the file it is converted to SoftMax Pro Software version 6 format.



4. Navigate the protocol file you want to open.
5. Select the protocol file.
6. Click **Open**.

   An untitled file opens in the workspace with the settings of the selected protocol applied to it.

Saving Protocol Files

Use the File menu **Save** or **Save As** commands to save data or protocol files or projects.

1. Click the **Application** button.
2. Select **Save** (for first time file saving) or **Save As**.

   The **Save As** dialog opens.
3. Navigate to where you want the protocol file to be saved.
4. Type the file name in the **File name:** field.
5. Select the file type from the **Save as type:** field. For a Protocol file, select **Protocol files (*.spr)**.
6. Click **Save**.

Exporting Plate Data

The Application menu **Export** command creates a data file from the data in one or more Plate sections, or Group table sections within a SoftMax Pro Software file. Please contact Molecular Devices Technical Support for a copy of the SoftMax Pro XML schema.

1. Click the **Application** button.
2. Select **Export** to open the **Export** dialog.
3. Select the Section(s) to export:
   - Select the Experiments.
   - Select the Notes, Plates and Groups individually.

   or
   - Select **All Plates** to export all plate data.
   - Select **All Group Sections** to export all group data.
   - Select **All Notes** to export all notes data.
4. Choose from the data options:
   - **Raw**
   - **Reduced**
   - **Both**

Note: These options apply only if one or more plate sections are selected.

5. Choose one of the following three output formats:
   - **Columns** exports data in single column text for each well.
   - **Plate** exports data in a text matrix corresponding to a microplate grid.
   - **XML** exports data in an XML file format.

Note: These options apply only if one or more plate sections are selected.

Selecting an Export Format

There are three export format options: **Columns**, **Plate**, and **XML**.

- **Columns** exports data in single column text for each well.
- **Plate** exports data in a text matrix corresponding to a microplate grid.
- **XML** exports data in an XML file format.

CuvetteSet sections are automatically exported in the **List** format.

Printing

You can print the contents of the currently selected project.

1. Click the **Application** button.
2. Click **Print** and select one of the following:
   - **Print All** to print the all the sections in an experiment or those selected in the **Print Selected** dialog.
   - **Print Preview** to display a preview of the sections selected for printing.
   - **Print Selected** to select the sections you want to print.

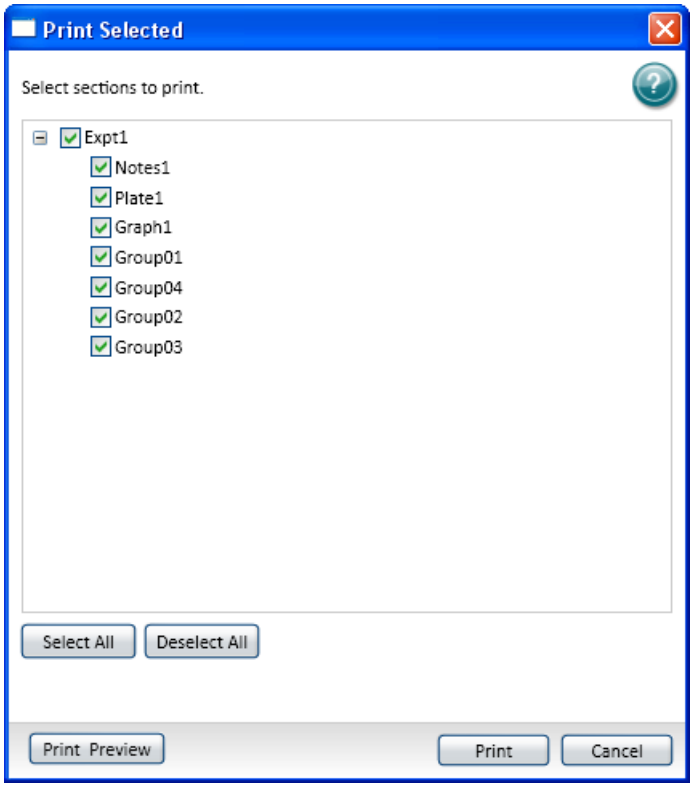
Note: There is a print exclusion icon  on the navigation tree at the far right top of the tree area. Click a section or sections in the navigation tree that you do not want to print and then click the print exclusion icon to remove the items from the print selection list.

   - **Printing Options** to specify and edit the header and footer text of the printed output. For more information, see Setting Printing Options on page 37.

Printing Selected Sections

You select which section you want to print out.

1. Click the **Application** button.
2. Select the **Print** command.
3. Select **Print Selected**.



Note: There is a print exclusion icon on the navigation view and at the far right top of the sections. Click the print exclusion icon to remove the item from the print selection.

- Click **Select All** to select all sections for printing.
- Click **Deselect All** to remove selection of all sections.
- You can also use the check boxes to select section for printing individually.

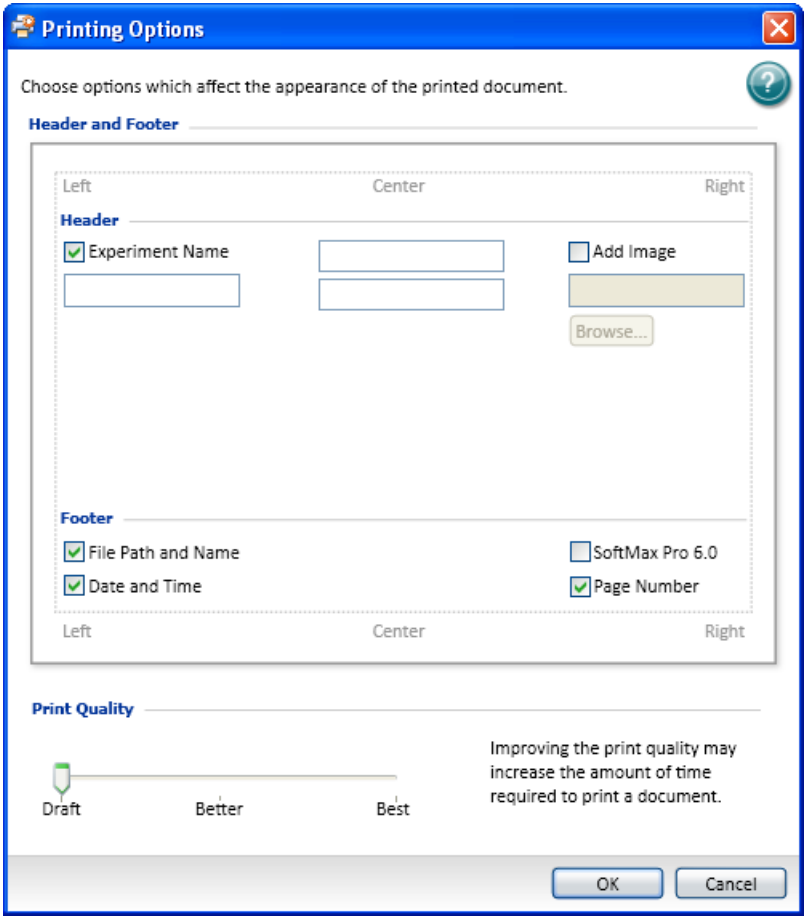
4. Click **Print Preview** to view the selected sections in preview format.

5. Click **Print** to print the selected sections and close the dialog.
6. Click **Cancel** to discard print selections and close the dialog.

Setting Printing Options

You can define the contents of the header and footer of the printed output.

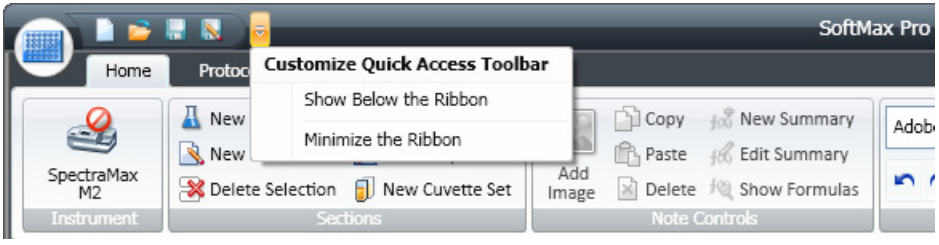
1. Click the **Application** button.
2. Select the **Print** command.
3. Select **Printing Options** to specify and edit the header and footer of the printed output.



4. In the **Print Options** dialog use the check boxes to select to print information from the data file. Text entry fields are available for you to type information you want to include.
5. You can add an image, such as a logo, on the right side of the header. Use the check box to enable the field and click the **Browse** button to locate and select the image file.
6. You can use the **Print Quality** slide bar to select the output quality.
7. When all contents are defined, click **OK** to save the information and close the dialog.

Using the Quick-Access Toolbar

The Quick-Access toolbar is available for your customization. The Quick-Access toolbar initially opens directly to the right of the File menu button in the upper left corner of the SoftMax Pro Software application window.



You can move the location to above or below the ribbon and add any function buttons you want to have quick-access to.

The Quick Access Toolbar has buttons to perform the following actions:

- Create a new data file.
- Open a data file.
- Save a new data file or a new protocol file.
- Save (Save as) a data file or protocol file with a new name.
- The actions button.

Reposition the Quick-Access Toolbar

The Quick-Access toolbar can appear above or below the application ribbon.

1. To reposition the Quick-Access Toolbar, place the cursor over the toolbar and right-click.
2. Choose either **Show Quick-Access Toolbar above the ribbon** or **Show Quick-Access Toolbar below the ribbon**.

Add a Button

1. To add a function button to the Quick-Access toolbar, place the cursor over the button you want to add and right-click.
2. Choose **Add to the Quick-Access toolbar** from the pop-up menu.

Remove a Button

1. To remove a function button from the Quick-Access toolbar, place the cursor over the button you want to remove and right-click.
2. Choose **Remove from the Quick-Access toolbar** from the pop-up menu.

Minimize the Ribbon

You can choose to minimize the ribbon display.

1. Place the cursor over the Quick-Access toolbar and right-click.
2. Choose Minimize the Ribbon from the pop-up menu.

To return the minimized ribbon to the display.

1. Place the cursor over the Quick-Access toolbar and right-click.
2. Click to deselect Minimize the Ribbon.

Using the Ribbon

The application ribbon contains tabs with the tools needed to work with SoftMax Pro Software.

Application Menu

See Using the Application Menu on page 26 for more information.

Home Tab

The Home tab always contains the following sections:

- Instrument
- Instrument Control and Read Status
- Section Tools

The contents of the **Home** tab changes depending on the section type that is active in the workspace.

Plate Section Tools

When a **Plate** section is active, the Home tab contains:

- Plate Tools
- Template Tools

See Using Plate Tools on page 72.

Graph Section Tools

When a **Graph** section is active, the Home tab contains:

- Graph Tools

See Working with Graphs on page 231

Group Section Tools

When a **Group** section is active, the Home tab contains:

- Formatting Tools
- Column Tools
- Summary Tools

See Using Column Tools on page 97.

Notes Section Tools

When a **Notes** section is active the Home tab contains:

- Notes Controls
- Formatting Tools

See Using the Notes Section on page 81.

Cuvette Section Tools

When a Cuvette Set section is active, the Home tab contains:

- Cuvette Tools
- Template Tools

See Using Cuvette Tools on page 93.

Protocols Tab

The **Protocols** tab provides easy access to protocol files that are stored in the file system.

See Using the Protocols Tab to Manage Protocols on page 50.

View Tab

The **View** tab contains the following:

- Show/Hide Panel
- Document View

See Using the View Tab on page 56.

Operations Tab

The **Operations** tab contains the following:

- Instrument Tools
- Calculations
- Automation

See Using the Operations Tab on page 56.

Help Tab

The **Help** tab contains the following:

- SoftMax Pro Help
- Formula Reference
- Contact Us
- Software License
- About SoftMax Pro

See Using the Help Tab on page 69

Using the Home Tab

The tools and controls displayed on the **Home** tab change depending on the type of section that is active in the workspace.

- Using Instrument Controls on page 42
- Using Section Controls on page 48
- Using Plate Tools on page 72
- Using Template Tools on page 80
- Using Cuvette Tools on page 93
- Using Column Tools on page 97

Using Instrument Controls

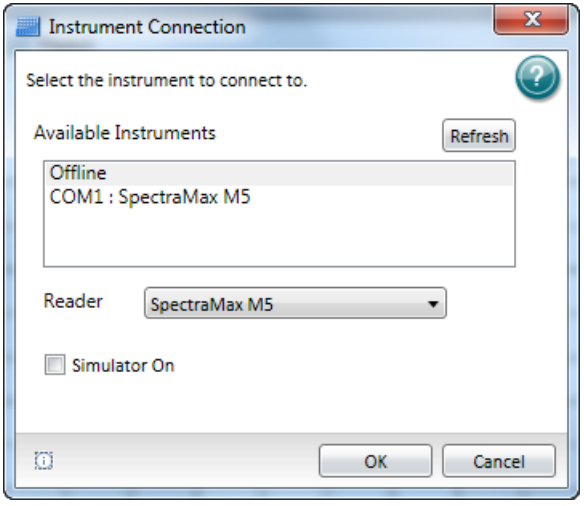
Instrument Controls and **Read Status** options on the **Home** tab enable:

- Selecting an Instrument on page 42
- Enabling Simulation on page 45
- Reading Data on page 48
- Setting the Temperature on page 47
- Shaking the Plate on page 47
- Opening/Closing the Drawer on page 48

Selecting an Instrument

To select a connected reader instrument:

1. Click **Instrument** in the **Home** tab. The **Instrument Connection** dialog opens.



2. Select the reader you want to connect to from the **Reader** menu.
3. Optionally, select **Offline** and **Simulator On** if you want to work in simulation mode.
4. Click **OK**. An icon for the selected instrument now appears on the **Home** tab.

Note: It is not necessary to be physically connected to an instrument in order to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline in simulation mode.

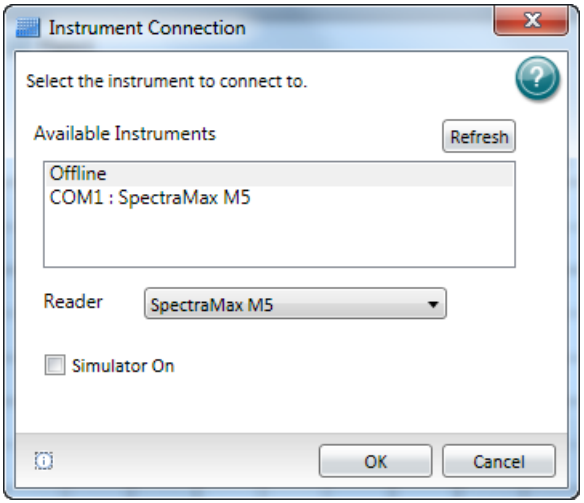
SoftMax Pro Software supports the following instruments:

- VersaMax ELISA Microplate Reader, see page 112
- SpectraMax Plus 384 Absorbance Microplate Reader, see page 113
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 114
- SpectraMax M3 Multi-Mode Microplate Reader, see page 115
- SpectraMax M4 Multi-Mode Microplate Reader, see page 116
- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 117
- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 118
- SpectraMax 190 Absorbance Microplate Reader, see page 118
- Gemini XPS Fluorescence Microplate Reader, see page 119
- Gemini EM Fluorescence Microplate Reader, see page 119
- FilterMax F5 Multi-Mode Microplate Reader, see page 120
- FilterMax F3 Multi-Mode Microplate Reader, see page 121
- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 121
- Vmax Kinetic ELISA Microplate Reader, see page 122
- Emax Endpoint ELISA Microplate Reader, see page 122
- SpectraMax Paradigm Multi-Mode Detection Platform, see page 123
- StakMax Microplate Handling System, see page 132

Enabling Simulation

To enable simulation mode, connected to an instrument:

1. Click **Instrument** on the **Home** tab. The **Instrument Connection** dialog opens.



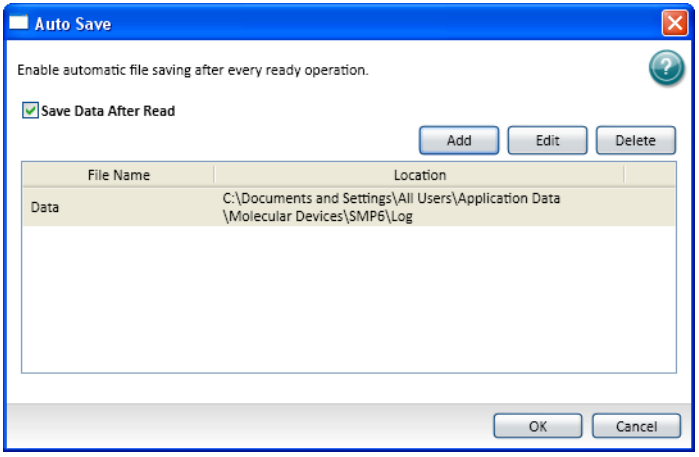
2. Select the reader you want to connect to from the **Reader** menu.
3. Select **Offline** and **Simulator On** if you want to work in simulation mode.
4. Click **OK**. An icon for the selected instrument now appears on the **Home** tab.

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline or in simulation mode.

Enabling the Auto Save Function

To enable Auto Save:

1. Click the **Application** icon to open the menu.
2. Click **Auto Save**.



3. Select **Save Data After Read**.
4. Click **OK**.

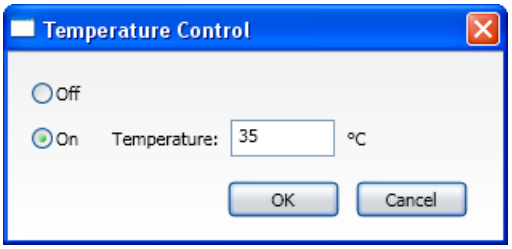
Note: Auto Save settings are saved with each document.

Setting the Temperature

When connected to an instrument with temperature control, you can set the temperature in the microplate or cuvette chamber in degrees Celsius.

To set the temperature:

1. Connect to an instrument that is capable of temperature control.
2. Click **Temperature** on the **Home** tab.
   The **Temperature Control** dialog opens.



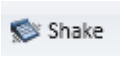
3. Click **On**.
4. Type the desired temperature in degrees Celsius.
5. Click **OK**.

With the SpectraMax M2, M2e, M5, M5e, and Plus 384, the **Instrument Controls** section of the **Home** tab shows the temperature within the microplate chamber. This can be different from the front panel of the instrument, which displays the temperature within the cuvette chamber.

The readings should be very similar to one another after both chambers have reached equilibrium. During warm-up, however, you might notice a discrepancy in temperatures, which is normal.

Shaking the Plate

To shake the plate follow these steps:

1. Insert the prepared plate into the instrument.
2. Connect to the instrument.
3. Click and hold **Shake** 

on the **Home** tab
4. Release the mouse button to stop the shaking operation.

Opening/Closing the Drawer

To open the drawer on the connected instrument:

1. Click **Open/Close** 

on the **Home** tab.
2. Insert the prepared plate into the instrument.
3. With the drawer open on the connected instrument, click **Open/Close** on the **Home** tab to close it.

Reading Data

To read data:

1. Select an instrument.
2. Select or define a data acquisition protocol.
3. Click **Read**  on the **Home** tab.

Using Section Controls

The Section controls appear on the Home tab in the ribbon. Section controls enable:

- Creating an Experiment on page 48
- Creating a Notes Section on page 48
- Deleting a Section on page 49
- Creating a Plate Section on page 49
- Creating a New Graph Section on page 49
- Creating a Cuvette Set Section on page 49

Creating an Experiment

To create a new experiment:

1. Open a data file in the workspace.
2. Click **New Experiment** in **Sections** on the **Home** tab.

This action causes a new experiment that is duplicate of the currently selected experiment to be added to the navigation tree.

Creating a Notes Section

To create a **Notes** section:

1. Select the experiment where you want to add a **Notes** section in either the **Navigation Tree** or in the **Workspace**.
2. Click **New Note** in **Sections** on the **Home** tab.

Deleting a Section

To delete a section:

1. Select a section to or multiple sections to be deleted in either the **Navigation Tree** or in the **Workspace**.

Note: To select multiple sections, press and hold the Control or Shift key and click on the sections to be selected.

2. Click **Delete Selection** in **Sections** on the **Home** tab.

Creating a Plate Section

To create a new **Plate** section:

1. Select the experiment where you want to add a plate section in either the **Navigation Tree** or in the **Workspace**.
2. Click **New Plate** in **Sections** on the **Home** tab.

Creating a New Graph Section

To create a new graph:

1. Select the plate section to relate to the graph in either the **Navigation Tree** or the **Workspace** and click **New Graph** in **Sections** on the **Home** tab.
2. This action opens the **New Graph** dialog.
3. See Graphing Data on page 230 for more information about configuring graphs.
4. When the graph options have been set, click **OK**.

The new graph section is added to the selected experiment.

Creating a Cuvette Set Section

To create a new cuvette set section:

1. Select the experiment you want to add the cuvette set to in either the **Navigation Tree** or in the **Workspace**.
2. Then click **New Cuvette Set** in **Sections** on the **Home** tab.

Using the Protocols Tab to Manage Protocols

The **Protocols** tab in the ribbon provides easy access to protocol files that are stored in the file system. It also gives you access to the SoftMax Pro Software user's community for protocol sharing, exchanging tips and tricks, and networking with other experienced users.

During software installation, the Basic Endpoint protocol is set as the default protocol with the filename **default.spr**. Whenever you click the **New Experiment** button on the **Home** tab, the contents of the default.spr protocol file is loaded into the new experiment. In addition, a group of pre-defined protocols are provided in the SoftMax Pro Software Protocols folder.

You can use the predefined protocols and you can create your own protocols. For more information on protocols, see Creating a Protocol on page 147. When you create protocols, save them into folders on your file system. Name the folders appropriately so that you will be able to find the protocols.

Three buttons are provided on the **Protocol Manager** area of the **Protocols** tab:

- Click **Folder Locations** to add the folders containing the protocols you want to use to the short cut menu you can access by clicking **Protocol Manager**. See Adding a Protocol Folder to the Protocol Manager Shortcut on page 51.
- Click **Save As Default** to overwrite the default.spr file with the protocol setting of the experiment that is active in your workspace. After you save the active protocol file as the default, whenever you create a new experiment, the settings in the default.spr that you have saved are loaded into the new protocol. See Saving a Protocol as the Default Protocol on page 54.

Note: When you save the file as the default protocol, any data you have collected in the current experiment remains unaltered. If you want to keep the data, you need to save the data into a data file.

- Click **Protocol Manager** to open the Protocol Manager shortcut and select a protocol to be assigned to the open experiment. See Using the Protocol Manager to Open a File on page 55.

Note: When you add a new plate or cuvette section to an experiment, you can configure settings using the **Settings** dialog or apply a pre-defined or saved protocol to the new section.

Two buttons are provided in the **Community** area of the **Protocols** tab:

- Click **Protocol Home Page** to access the SoftMax Pro Software community web site for protocol sharing, exchanging tips and tricks, and networking with other experienced users. To access this site, you must have internet access on your computer.
- Click **Export for Sharing** to export the active protocol to an encrypted file format for sharing with the online community. No data is exported with the protocol. Before exporting the protocol, make sure that the first **Notes** section has a clear description of the protocol, since the first **Notes** section will be used as the description of the protocol on the web site.

  After exporting a protocol for sharing, you can upload the exported protocol to the community web site. Protocols exported for sharing have a **.spz** extension. Do not rename the file. The file is encrypted and will not upload properly if it is renamed. To upload your exported protocol, follow the instructions on the community web site.

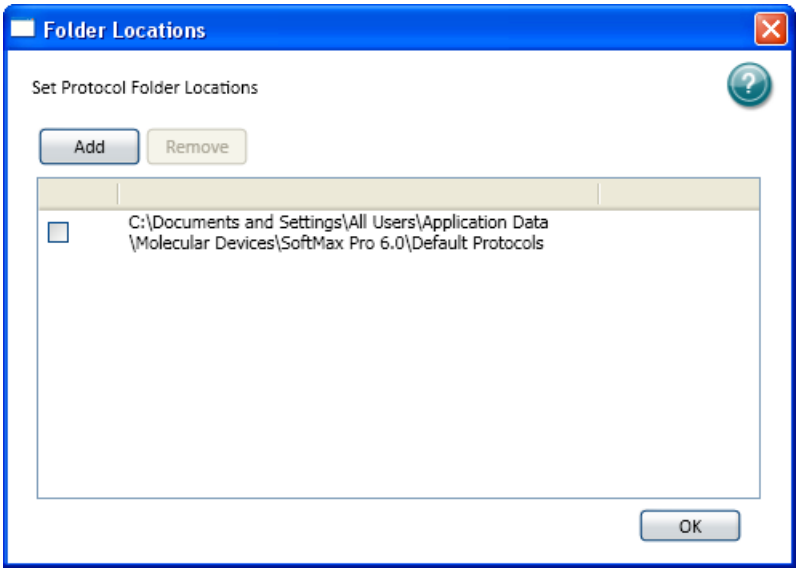
Adding a Protocol Folder to the Protocol Manager Shortcut

You can add folders in your file system to those folder where protocols can be stored. Whenever you store protocol files in the added folder, those protocols are available in the **Protocol Manager** shortcut. See Using the Protocol Manager to Open a File on page 55.

Adding a Protocol Folder Location

To add a protocol folder location:

1. Click **Folder Locations**  to open the **Folder Locations** dialog.



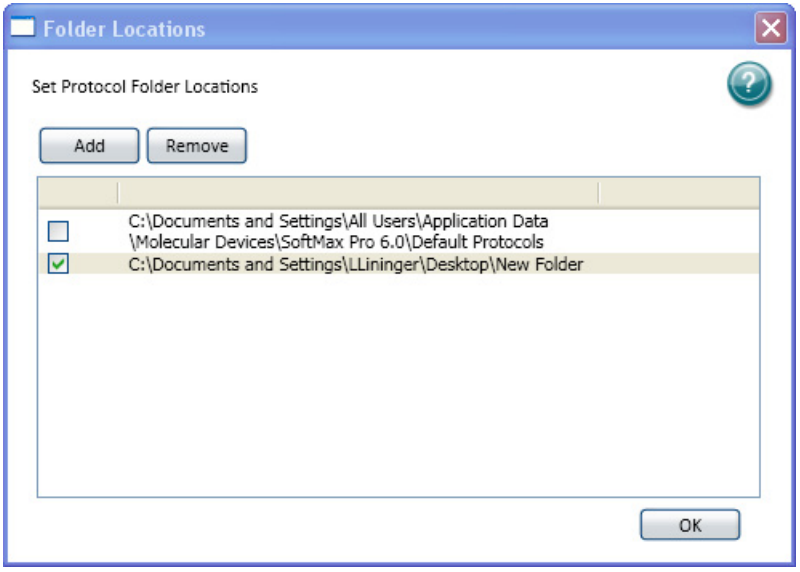
2. Click **Add** to browse the file system to the folder to be added.

3. Select the folder where you want to store protocols.
4. Optionally, click **Make a New Folder** to add a folder inside the selected folder and type the folder name to replace New Folder.
5. Click **OK**. The selected folder is added to the folder list in the Folder Locations dialog.
6. Click **OK** to close the Folder Locations dialog.

Deleting a Protocol Folder Location

To remove a protocol folder location:

1. Click **Folder Locations** to open the Folder Location dialog.
2. Click the check box to select the folder location you want to remove.



3. Click **Remove**. The selected folder is removed from the folder list in the Folder Locations dialog.
4. Click **OK** to close the **Folder Locations** dialog.

Saving a Protocol as the Default Protocol

During software installation, the Basic Endpoint protocol is set as the default protocol with the filename **default.spr**. Whenever you click the **New Experiment** button on the **Home** tab, the contents of the default.spr protocol file is loaded into the new experiment. In addition, a group of pre-defined protocols are provided in the SoftMax Pro Software Protocols folder.

You can save the settings of the file currently active in the workspace as the default protocol. After you save the active protocol file as the default, whenever you create a new experiment, the settings in the default.spr that you have saved are loaded into the new protocol.

Saving a Default Protocol

1. Click **Save as Default**  to overwrite the default.spr file with the protocol setting of the experiment that is active in your workspace.
2. Click **OK** to overwrite the default protocol file with this file. With the exception of acquired data, all settings and sections (with their contents) are saved as part of the new default protocol. Any new file you create will initially be identical to this current data file (minus existing data).

Note: When you save the file as the default protocol, any data you have collected in the current experiment remains unaltered. If you want to keep the data, you need to save the data into a data file. See Saving Data Files on page 28.

Using the Protocol Manager to Open a File

A group of pre-defined protocols are provided in the SoftMax Pro Software Protocols folder. You can use the predefined protocols and you can create your own protocols. For more information on protocols, see Creating a Protocol on page 147.

To open a file available in the Protocol Manager shortcut:

1. Click **Protocol Manager**  on the **Protocols** tab to open the Protocol Manager shortcut and select a protocol to be assigned to the open experiment.
2. Select **Default Protocols >** *Yourfoldername* or **Default**.
3. Select the protocol you want to open.
4. When you save the section as a data file the settings from the protocol are saved along with the data.

Note: When you add a new plate or cuvette section to an experiment, you can apply a pre-defined or saved protocol to the new section or configure settings using the **Settings** dialog. See Instrument Settings on page 133.

Using the View Tab

In the **Show/Hide Panel** you can select to display sections, deselect everything, and remove the navigation tree from the application window.

Table 2-1 Navigation Tree Document Views

Document Views	
	**Single Tab Group** combines all tab groups into one.
	Moves the active document to a horizontal tab group.
	Moves the active document to a vertical tab group.

Using the Operations Tab

Instrument Tools

The buttons that appear in the **Instrument Tools** group depend on the currently selected instrument.

- **Calibration**: The instrument calibration values are stored in the firmware of the instrument. For information about updating the instrument calibration, see Calibrating the Reader on page 58.
- **Filters**: The positions of the filters in the Emax and Vmax ELISA Microplate Readers can be defined using the **Filter Configuration** dialog. For instructions, see Configuring the Filters for Emax and Vmax ELISA Microplate Readers on page 58.
- **Info**: In the **Instrument Information** dialog, you can view a list of the installed detection cartridges in a SpectraMax Paradigm Multi-Mode Detection Platform or the installed filters in FilterMax F3 and F5 Multi-Mode Microplate Readers. This dialog also lists other instrument information like the serial number and firmware version. For FilterMax F3 and F5 Multi-Mode Microplate Readers, you can configure the excitation and emission filter slides. See Using the Instrument Information Dialog on page 59.

- **Refresh**: To update the software with the currently installed detection cartridges in a SpectraMax Paradigm Multi-Mode Detection Platform or the currently installed filter slides in FilterMax F3 and F5 Multi-Mode Microplate Readers, click the **Refresh** button.

Calculations

By default, SoftMax Pro Software performs continuous recalculation of the data when you read plates, create or change formulas, or change settings affecting data. At certain times, it is useful to disable recalculations so that you can edit the Experiment without waiting for recalculations to be completed.

- **Suspend Calculations** disables automatic recalculation. While suspended, no recalculation occurs regardless of what you change, add, or delete from the Experiment. This is useful when creating or changing column formulas within Group sections.
- **Resume Calculations** re-enables automatic recalculation.
- When recalculation has been suspended, you might want to see the results of the changes you have made but might still not want to enable continuous recalculation. Click **Recalculate Now** to recalculate all data once without enabling continuous recalculation.

Under certain circumstances, a calculation status message appears in the workspace footer.

- **Calculating** appears when SoftMax Pro Software is calculating an entire experiment automatically or when **Recalculate Now** is selected.
- **Calculating Suspended** appears when the **Suspend Calculation** is selected.
- Under all other conditions, no message is displayed.

Automation

Auto Read: You can automatically read plate sections as they appear within an experiment. See Enabling Auto Read on page 68.

Plate Stacker: The StakMax Microplate Handling System runs using the StakMax Software that is integrated with the SoftMax Pro Software. See StakMax Microplate Handling System on page 132.

Calibrating the Reader

To calibrate the reader:

1. Click **Calibration** on the **Operations** tab.

   The **Calibration** dialog opens.

2. To calibrate a plate, select **Plate** and click **Calibrate Now**.
3. To calibrate a cuvette, select **Cuvette** and click **Calibrate Now**.
4. When the calibration indicator shows that the calibration is complete, click **Close**.

The calibration values determined using this operation are stored in firmware.

Configuring the Filters for Emax and Vmax ELISA Microplate Readers

The Emax and Vmax ELISA Microplate Readers have configurable filters. If you change the order of the filters, or add or remove filters, you need to use the **Filter Configuration** dialog to define the positions of the filters. If the filter settings in the **Filter Configuration** dialog are incorrect, the reader will generate incorrect data. For information about changing the filters, see the user guide for the Emax and Vmax ELISA Microplate Readers.

To define the filter positions:

1. Click **Filters** on the **Operations** tab.
2. In the **Filter Configuration** dialog, type the wavelength values in the correct positions for each installed filter.

   You can define up to six filters. The allowed wavelength range is 400 nm to 750 nm.

3. After all the installed filters have been defined, click **OK**.

The defined filters are available for selection in the **Settings** dialog. See Creating an Absorbance Mode Protocol on page 167.

Using the Instrument Information Dialog

To view the **Instrument Information** dialog, go to the **Operations** tab in the ribbon and click **Info**.

The Instrument Information dialog lists the PIC firmware version, the instrument firmware version, the instrument serial number, and the automation device number for the selected SpectraMax Paradigm Multi-Mode Detection Platform, FilterMax F3 Multi-Mode Microplate Reader, or FilterMax F5 Multi-Mode Microplate Reader. To update the PIC or instrument firmware, click the corresponding **Update** button. For more information about updating firmware, contact Molecular Devices support.

The lower portion of the dialog shows instrument-specific information for either the SpectraMax Paradigm Multi-Mode Detection Platform or FilterMax F3 and F5 Multi-Mode Microplate Readers.

SpectraMax Paradigm Multi-Mode Detection Platform Instrument Information

The software detects the installed detection cartridges and displays them in the **Instrument Information** dialog. The name and serial number for the detection cartridge appears under its position in the drawer.

To open or close a detection cartridge drawer, click  to the left of the detection cartridge definitions for that drawer.

You can lock or unlock the cartridge drawers when you are using the StakMax Microplate Handling System:

- When the stacker is installed, the detection cartridge drawers must be locked. To lock the cartridge drawers, click **Lock Drawers for StakMax**.
- When you need to open a detection cartridge drawer, remove the stacker from the detection platform and then unlock the drawers. To unlock the cartridge drawers, click **Unlock Drawers for StakMax**.

For more information, see the user guide for the StakMax Microplate Handling System.

To lock all the drawers closed, click **Lock Instrument for Shipment**. For more information about preparing an instrument for shipment, contact Molecular Devices support.

To align the transfer position of the StakMax Microplate Handling System with the plate drawer of the SpectraMax Paradigm Multi-Mode Detection Platform, click **StakMax Alignment Wizard**. For information about using the StakMax Alignment Wizard, see the user guide for the StakMax Microplate Handling System.

FilterMax F3 and F5 Multi-Mode Microplate Readers Instrument Information

The software detects the installed excitation and emission filter slides and displays their saved configurations in the **Instrument Information** dialog. The identification number for the slide appears on the right. Each filter has its defined wavelength, bandwidth, and read mode displayed under its position in the slide.

To eject or retract a filter slide, click  to the left of the slide definition.

To edit the filters in a slide, or to define additional slides, click **Edit Slide Configuration**. See Configuring Filter Slides on page 60.

Configuring Filter Slides

The FilterMax F3 and F5 Multi-Mode Microplate Readers have configurable filter slides. If you add or remove filter slides, or change the order of the filters in a slide, you need to use the **Filter Settings** dialog to define the filters in the slide. If the defined filter settings in the **Instrument Information** dialog are incorrect, the reader will generate incorrect data. For information about changing the filter slides, see the user guide for the FilterMax F3 and F5 Multi-Mode Microplate Readers.

To view the **Filter Settings** dialog, go to the **Operations** tab in the ribbon and click **Info**. Then, in the **Instrument Information** dialog, click **Edit Slide Configuration**.

The filters used to perform measurements are mounted on two types of interchangeable slides. One slide is reserved for excitation filters used in absorbance and fluorescence measurements; the other is used for emission filters used in fluorescence and some luminescence measurements. Each slide can hold up to six filters.

Note: Excitation and emission filter slides are different sizes to prevent them from being installed in the incorrect position.

When exchanging slides, an identification code built into the slide allows the SoftMax Pro Software to recognize the new slide and filter configuration. When a slide with a new configuration is inserted, or the filters on a slide change, the slide must be configured in the **Filter Settings** dialog. Up to 31 excitation filter slides and 31 emission filter slides can be stored in the software at one time.

- For quantitation protocols, a genomic filter slide, which contains narrow bandwidth 260 nm and 280 nm filters must be installed and configured.
- For protocols that use PathCheck Pathlength Measurement Technology, a filter slide designed for PathCheck technology must be used.

To work with a filter slide, click **Excitation** or **Emission** to display a list of the selected type of filter slides. To add, remove, export, or import filter slides, click a button on the left side of the dialog.

- Click **Add Slide** to add a new filter slide to the list. See Adding a Filter Slide on page 62.
- Click **Remove Slide** to delete the selected filter slide. See Removing a Filter Slide on page 63.
- Click **Export selected Slide** to export the filter definitions for the selected filter slide. See Exporting a Filter Slide on page 64.
- Click **Import Slides** to import a filter slide definition file that defines one or more filter slides. See Importing Filter Slides on page 65.
- Click **Load Slides** to replace all the current filter slide definitions with the imported definitions. See Replacing the Filter Slide Definitions on page 66.
- Click **Export Slides** to export all the defined filter slides. See Exporting the Filter Slide Definitions on page 67.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Adding a Filter Slide

The filters used to perform measurements are mounted on two types of interchangeable slides. One slide is reserved for excitation filters used in absorbance and fluorescence measurements; the other is used for emission filters used in fluorescence and some luminescence measurements. Each slide can hold up to six filters.

To add a filter slide and configure the filters:

1. Go to the **Operations** tab in the ribbon and click **Info**.
2. In the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. In the **Filter Settings** dialog, click **Excitation** or **Emission** to display a list of the selected type of filter slides.
4. Click **Add Slide** to add a new slide to the list.
5. The **Filter Slide Properties** pane displays information about the selected slide.
6. In the **Slide ID** field, type the identification number printed on the slide.
7. In the list of filter slides, click the **+** to the left of the filter slide name to display the list of filters installed on the slide.
8. Click a filter in the list to display the **Filter Properties** for the selected filter.
9. In the **Wavelength** field, type the wavelength of the filter.
10. In the **Technique(s)** field, select the read mode for which the filter applies. The filter can be used only for measurements of the selected read mode. For the FilterMax F5 instrument only, you can select **Polarization** for filter positions that have a polarization filter installed.
    - After a read mode is selected, the **Installed** field displays **Yes**.
    - If no read mode is selected, the **Installed** field displays **No**, and the filter position cannot be used for measurements.

Note: The **Installed** and **Position** fields cannot be edited.

11. In the **Bandwidth** field, type the bandwidth of the filter.
12. In the **Order#** field, type the manufacturer's part number for the filter for your reference.
13. Continue configuring the rest of filters on the slide.

14. After the filters on the slide have been properly configured, click **OK**.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Removing a Filter Slide

If a filter slide is no longer used with an instrument, it can be removed from the list.

CAUTION: After you remove a filter slide from the list, it cannot be recovered. If you think you might want to use this filter slide definition again, export the slide definition before removing it from the list. See Exporting a Filter Slide on page 64.

To remove a filter slide from the list:

1. Go to the **Operations** tab in the ribbon and click **Info**.
2. In the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. In the **Filter Settings** dialog, click **Excitation** or **Emission** to display a list of the selected type of filter slides.
4. In the slide list, click the slide that you want to remove.
5. Click **Remove Slide** to remove slide from the list.
6. Click **OK**.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Exporting a Filter Slide

Exported slide definitions are saved in XML files that can be imported again later or imported into the software on another computer.

Using the **Export selected Slide** feature exports the definition for a single filter slide. To export all the current filter slide definitions, see Exporting the Filter Slide Definitions on page 67.

To export a filter slide in the list:

1. Go to the **Operations** tab in the ribbon and click **Info**.
2. In the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. In the **Filter Settings** dialog, click **Excitation** or **Emission** to display a list of the selected type of filter slides.
4. In the slide list, click the slide that you want to export.
5. Click **Export selected Slide**.
6. In the **Save As** dialog, navigate to the folder where you want to save the exported XML file and type a name for the file in the **File name** field.

   The default location for filter slide definitions is:
   C:\ProgramData\Molecular Devices\SMP61\MultiMode\Detection Software\Filters
7. Click **Save**.
8. In the **Filter Settings** dialog, click **OK**.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Importing Filter Slides

Filter slide definitions can be imported from XML files that were previously exported or provided by Molecular Devices. Slide definition files can contain definitions of one or more filter slides.

Each filter slide definition must have a unique **Slide ID**. If the **Slide ID** of a filter slide that you want to import already exists in the list, rename the **Slide ID** or remove the slide from the list before importing.

To import filter slide definitions into the list:

1. Go to the **Operations** tab in the ribbon and click **Info**.
2. In the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. In the **Filter Settings** dialog, click **Excitation** or **Emission** to display a list of the selected type of filter slides.
4. Click **Import Slides**.
5. In the **Open** dialog, navigate to the folder where XML file is saved and select the file.

   The default location for filter slide definitions is:
   C:\ProgramData\Molecular Devices\SMP61\MultiMode\Detection Software\Filters
6. Click **Open**.
7. In the **Filter Settings** dialog, click **OK**.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Replacing the Filter Slide Definitions

Filter slide definitions can be imported from XML files that were previously exported or provided by Molecular Devices. Slide definition files can contain definitions of one or more filter slides for both excitation and emission filters.

CAUTION! When you use the **Load Slides** feature, all the current definitions are deleted, and are replaced by the imported definitions for both excitation and emission filters. If you think you might want to use any of the current filter slide definitions again, export the slide definitions before replacing them in the list. See Exporting a Filter Slide on page 64 or Exporting the Filter Slide Definitions on page 67.

To replace the current filter slide definitions:

1. Go to the **Operations** tab in the ribbon and click **Info**.
2. In the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. In the **Filter Settings** dialog, click **Load Slides**.
4. In the message that appears, click **Yes**.
5. In the **Open** dialog, navigate to the folder where XML file is saved and select the file.

   The default location for filter slide definitions is: C:\ProgramData\Molecular Devices\SMP61\MultiMode\Detection Software\Filters
6. Click **Open**.
7. In the **Filter Settings** dialog, click **OK**.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Exporting the Filter Slide Definitions

Exported filter slide definitions are saved in XML files that can be imported again later or imported into the software on another computer.

Using the **Export Slides** feature exports all the filter slide definitions in both the excitation and emission lists. If you want to export a single filter slide definition, see Exporting a Filter Slide on page 64.

To export all the filter slide definitions in the lists:

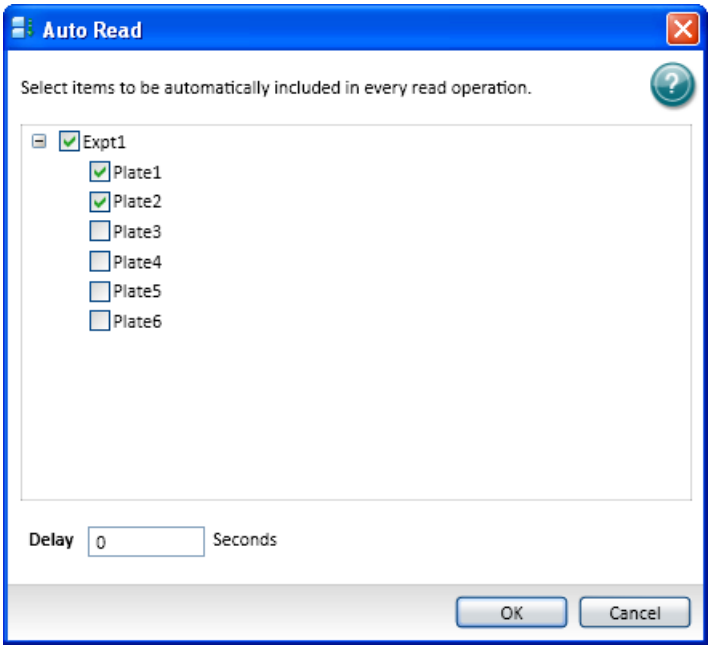
1. Go to the **Operations** tab in the ribbon and click **Info**.
2. In the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. In the **Filter Settings** dialog, click **Export Slides**.
4. In the **Save As** dialog, navigate to the folder where you want to save the exported XML file and type a name for the file in the **File name** field.

   The default location for filter slide definitions is: C:\ProgramData\Molecular Devices\SMP61\MultiMode\Detection Software\Filters
5. Click **Save**.
6. In the **Filter Settings** dialog, click **OK**.

Note: Molecular Devices recommends that you do not reconfigure standard filter slides.

Enabling Auto Read

1. Click **Auto Read** to enable automatic reading of plate sections as they appear within an experiment. When the data is acquired for any plate in the experiment that is enabled for automatic reading, all enabled plates are read.



2. Select the plates you want to automatically read.
3. Optionally, type the number of seconds to delay (the interval) between plate readings.
4. Click **OK** to close the dialog.

Note: When a plate is selected to be automatically read, the following empty plate is also read. For example, if you have plates 1 through 6 and you select Plate 1 and Plate 2 for AutoRead, when the Read operation is executed, Plate 1, Plate 2, and Plate 3 are read in sequence.

Using the Help Tab

SoftMax Pro Software has a comprehensive help library.

You can open the application help documents from the **Help** tab in the ribbon.

- Click **SoftMax Pro Help**  to view the *SoftMax Pro Application Help*. This help contains task-oriented information about how to use SoftMax Pro Software. You can find topics by reviewing the table of contents and by using the search function. This document also features a linked index and glossary.
- Click **Formula Reference**  to view the *SoftMax Pro Formula Reference Guide*. This document is broken into four main sections:
  - The first section provides a general introduction to SoftMax Pro Software formulas.
  - **Operators** describes the different types of operators that can be used to build formulas.
  - **Functions** describes the different types of built-in functions that can be used to build formulas.
  - **Accessors** describes the special functions that provide access to other specific information.

Note: In these help files, you can find topics through the table of contents and by using the search function. These documents also feature a linked index.

- Click **Contact Us**  to view the Molecular Devices customer home page, request technical support, or open the knowledge base which contains documentation supporting Molecular Devices products. You can use the customer login tools and featured links to help you get the information you need.
- To see release information about the SoftMax Pro Software application, click **SoftMax Pro**  in the **About** section of the **Help** tab.
- To activate your SoftMax Pro Software license, click **Software License**  and follow the instruction in the dialog.

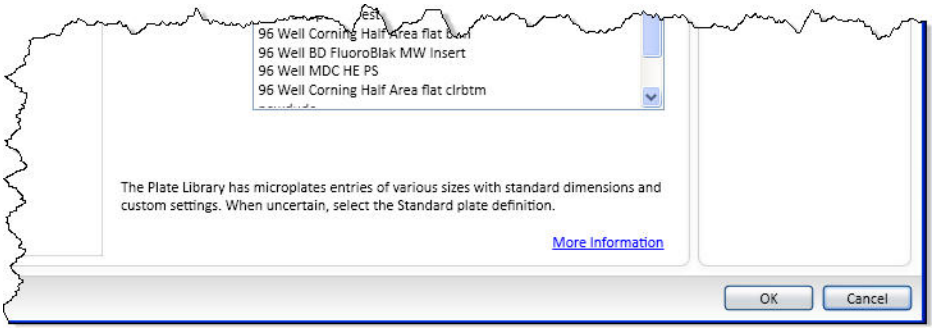
Context-sensitive help is available for the dialogs that have a help button.

You can view the user guides using Adobe Reader or other PDF viewer.

- The *SoftMax Pro Software User Guide* and the *SoftMax Pro Software Formula Reference Guide* can be found in the Windows Start menu at **Start > Programs > Molecular Devices > SoftMax Pro 6.1**.
- The user guides for the supported instruments can also be found in the Windows Start menu at **Start > Programs > Molecular Devices > SoftMax Pro 6.1 > Hardware User Guides**.

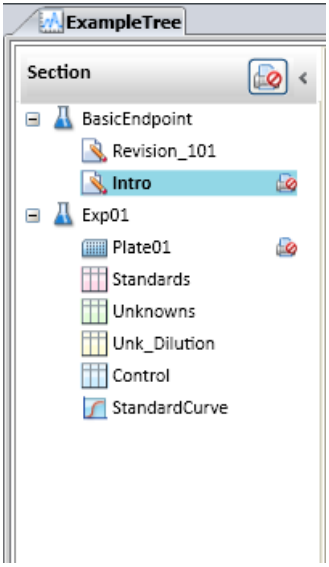
Viewing On-Screen Help

On-screen help is displayed when the dialog requires specific user interaction. When more information about the topic is available the **More Information** link opens help that is specific to the option being set.



Using the Navigation Section

The **Navigation** section contains the **Navigation Tree**.



The **Navigation Tree** shows the sections that are available for display in your **Workspace**.

- When you save a data file, the name that you save the file to is shown on the tab in the **Navigation Tree**.
- You can have more than one data file open at a time.
- Each data file can contain an unlimited number of experiments.
- Each experiment can contain an unlimited number sections.

Section types include:

- Notes
- Plate
- Cuvette Set
- Group
- Graph

Note: The order of the sections can be moved within an experiment only, and cannot be moved between experiments.

Using the Plate Section

A Plate section is a part of the SoftMax Pro program Experiment workspace.

Plate sections are used to collect data from the instrument, and to define data display and data reduction. If you read the same physical plate twice with different instrument settings, you would have two **Plate** sections.

The **Template Editor** in the **Plate** section is used to create a map of the contents of the microplate.

Plate sections are divided into:

- Tool bar
- Data display (shown in a microplate grid format)
- Instrument settings (shown to the right of the data display)
- Reduction settings (shown below the data display)

If the plate grid in the **Plate** section is colored, then a template has been defined for the **Plate** section. Each group defined in the template has a different color and the corresponding group table has the same color.

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

Adding a Plate Section

To add a plate section:

1. With a data file open in the workspace, click **New Plate** .
2. A blank **Plate** section opens in the **Workspace**. You can add as many **Plate** sections as needed to your data file.

Return to Using the Plate Section on page 72.

Using Plate Tools

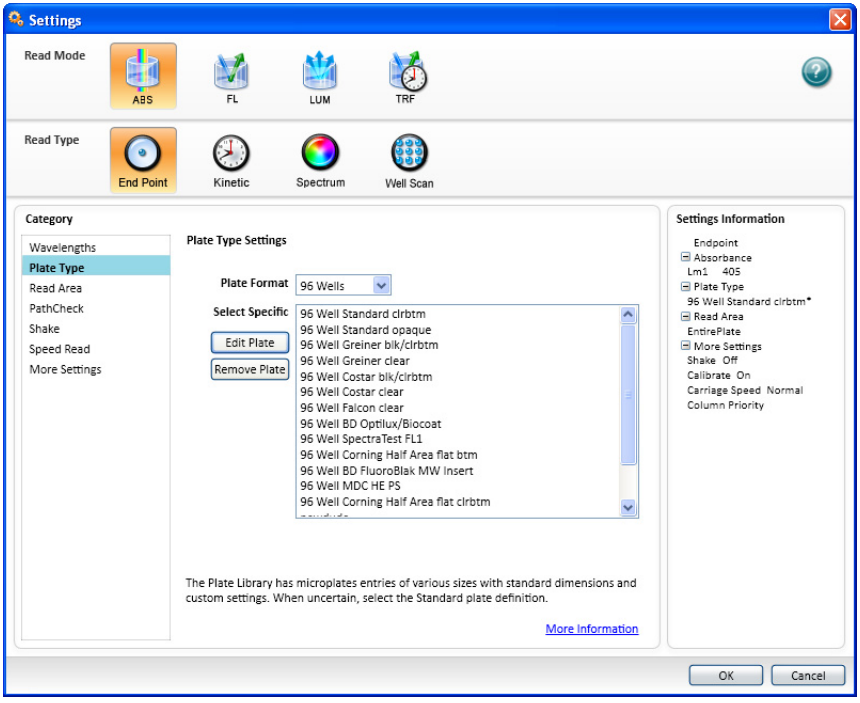
Plate tools are located on the Home tab when a plate section is active in the workspace.

Plate tools are available for:

- Modifying Instrument Settings on page 73
- Modifying Data Reduction Settings on page 75
- Modifying the Display on page 76
- Masking Wells on page 77
- Zooming the Well Display on page 78

Modifying Instrument Settings

Instrument settings are part of the protocol used to collect data. To modify settings for the selected instrument, select a plate or cuvette section in the workspace and then click **Settings** on the **Home** tab. The **Settings** dialog opens.



For more information, see Selecting Instrument Settings on page 148.

Using the Plate Editor

To view the Plate Editor, in the Settings dialog in the Plate Type Settings category, click **Edit Plate** on the left side of the plate list.

The Plate Editor has five fields and one check box that you can use to define plate dimensions, in millimeters.

Modifying the Dimension of an Existing Plate

To modify the dimension of an existing plate when you do not want to change the plate name:

1. Select the plate from the list and click **Edit Plate**.
2. Enter the custom dimensions as needed.
3. Click **Save**.

The changed plate has an asterisk at the end of the plate name.

Creating a New Plate

1. Select any plate from the plate list and click **Edit Plate**.
2. In the **Plate Name** entry field, type the name of the new plate.
3. Enter the dimensions of the new plate as needed.
4. Click **Save**.

The new plate name appears in the plate list.

Table 2-2 Plate Editor Tools

Dimension Field	Icon	Description
Left Edge to Left Well Center		The distance from the left edge of the plate to the center of well A1.
Top Edge to Top Left Well Center		The distance from the top edge of the plate to the center of well A1.
Horizontal Center to Center		The horizontal distance between well centers.
Vertical Center to Center		The vertical distance between well centers.
Well Diameter		The diameter of the well.

Table 2-2 Plate Editor Tools (cont'd)

Dimension Field	Icon	Description
Well Bottom Type	Check box	Clear well bottom.

The distance from the left edge of the plate to the center of well A1 must also include an offset to accommodate the angle of the optics.

Appropriate measurements should be taken in the lab to determine the correct settings for these fields.

Plate information is saved to the file Custom Plates in the SoftMax Pro folder. Because this is specific to the computer on which it is saved, when a file with a custom plate is opened on another computer, the plate type is changed to the standard plate type.

Importing a Plate Definition

For the SpectraMax Paradigm instrument, you can import a previously saved microplate definition.

1. Click **Import Plate**.
2. In the **Open** dialog, navigate to the location where the microplate definition is saved.
3. Select the **Microplate File (*.plt)** or **Multimode File (*.xml)**.
4. Click **Open** to add the plate definition to the list.

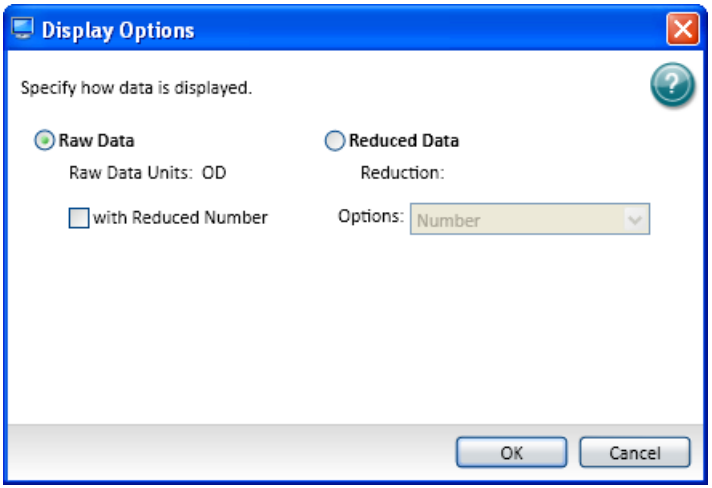
Modifying Data Reduction Settings

To modify the data reduction setting for a plate or cuvette:

1. Click **Reduction** ∑ on the **Home** tab or the Reduction shortcut button on the window bar. The **Data Reduction** dialog opens.
2. For more information about data reduction, see Performing Data Reduction on page 208.

Modifying the Display

At any time in an active **Plate** or **Cuvette** section you can click the **Display** button on the Home tab to change how the data is presented.



Choices available in the **Display Options** dialog include the selection between **Raw** and **Reduced Data**.

Raw Data

Selecting Raw displays the default data type for the selected read type:

- **Endpoint**: Raw absorbance, fluorescence, or luminescence values.
- **Kinetic**: The change in raw OD/RFU/RLU values over time, displayed as a plot.
- **Spectrum**: Raw OD/RFU/RLU values for the range of wavelengths, displayed as a plot.
- **Well Scan**: Raw OD/RFU/RLU values as shades of blue to red.

To see a reduced number, click **with Reduced Number**.

Reduced Data

The reduced data number is a combination of plate blank subtraction in the Plate section, wavelength reduction, and if applicable, a Spectrum, Well Scan, or Kinetic reduction.

The reduced number is reported in the Group section when a template has been defined.

- **Number**
  To view by reduced number alone, select **Number**.
- **Plot**
  For Kinetic reads, the reduced number and a plot of the data can be selected for display.
- **Grayscale**
  Grayscale 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).
- **Color Map**
  Color Map presents the raw data in eight colors, changing from blue (for values less than or equal to the low limit) to red (for values greater than or equal to the high limit).

Masking Wells

Masking is the process of excluding outliers in certain wells from data reduction calculations.

To mask wells:

1. Click to select a well or click and drag to select multiple wells.
2. Click **Mask**  .

Masking can be used as a "what if?" tool. 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 suppresses the blanking function, while unmasking them enables it again.

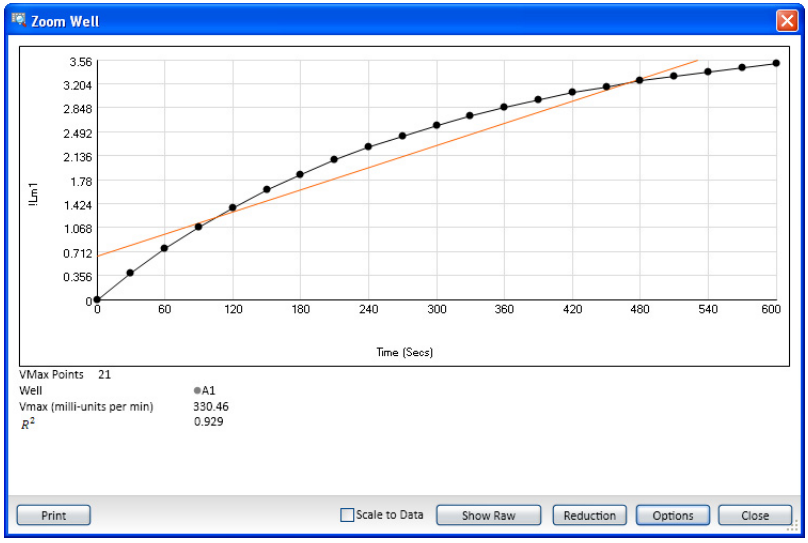
Zooming the Well Display

The **Zoom** icon becomes active when the data in a well is available for a zoomed display.

To display the zoom window:

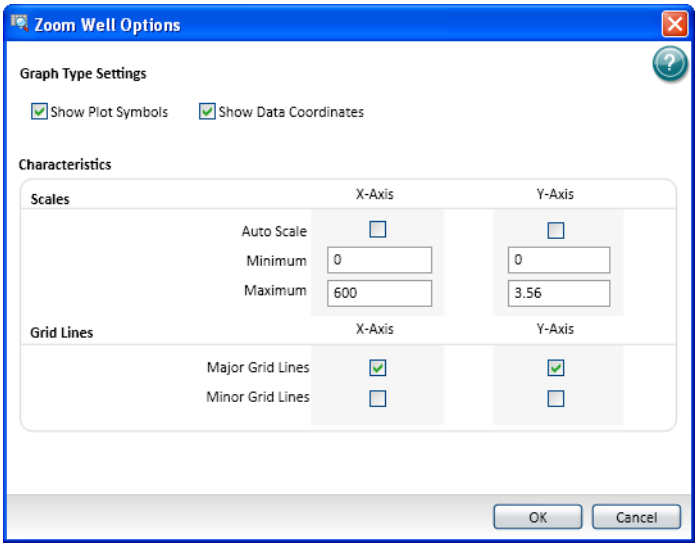
1. Click the well you want to zoom.
2. Click **Zoom** .

   The data from the selected well appears in a zoomed graph window.



3. Click **Print** to print the graph window.
4. To scale to the data, select **Scale to Data**.
5. To toggle between reduced and raw data, click **Show Reduced** or **Show Raw**.
6. To modify the reduction settings, click **Reduction**. The **Data Reduction** dialog opens. See Modifying Data Reduction Settings on page 75 for instructions.

7. To modify the zoom well display settings, click **Options**. The **Zoom Well Options** dialog opens.



- **Graph Type Settings** allows you to enable or disable connected points or plotted symbols on the graph.
- **Characteristics** allows you to set Auto Scale parameters, choose the minimum and maximum values for the selected axis, and you can add or remove grid lines.

Only the well graph being viewed is affected by these changes.

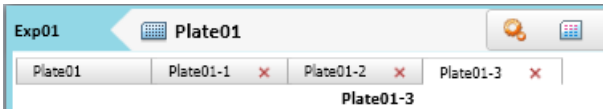
8. To close the window, click **Close**.

Cloning a Plate Section

Cloning a plate causes a duplicate copy of the active plate to be added to the plate section allowing multiple templates and reductions to be applied to the same set of raw data.

To clone a plate:

1. With the plate section to be cloned active in the workspace, click **Clone Plate** on the **Home** tab.
2. The cloned plate is added to the active plate section.



You can add as many plate clones as needed for your experiment. The cloned plates are shown in a horizontal list at the top of the plate section. The first plate on the list is the original plate. Clones are listed with the original plate name and a number corresponding to the number of the clone.

3. Click the cloned plate on the list to make it the active plate in the section.
4. Use the left and right arrows to scroll through the list of cloned plates.

Using Template Tools

A Template is used to describe the location of samples in a microplate or cuvette, and thus provides the link between raw data and analysis groups.

Each 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 can have a group named “Standards” that consists of seven samples named STD01, STD02, …, STD07, and a group named “Unknowns” that consists of five samples names UNK01, UNK02, …, UNK05.

Each sample would be generally applied to a column or some other subset of wells on a microplate, or to one or more cuvettes. 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 column format for the calculations and data reported in the associated Group section.

Samples and groups can exist across multiple Plate sections as well as within a single Plate section. Deleting the wells assigned to a group from the template does not delete the group. To delete a group name in the Template Editor you must delete the corresponding Group section from the Experiment.

The template tools are located in the Template tools section of the Home tab when a plate or cuvette section is open in the workspace.

You use template tools for:

- Modifying a Template on page 152
- Copying and Pasting Template Contents on page 155
- Importing a Template on page 160
- Exporting a Template on page 160

Using the Notes Section

Notes sections are used to record text or to report summary data pertaining to the experiment.

You can type text in a **Notes** section, add images, and create summaries containing formulas for displaying reduced data.

- A **Summary** is part of a **Notes** section or **Group** section that is generated by a user-entered formula applied to data in the file. It can also be text or a user-entered constant, such as certificate value.
- Text in **Notes** sections can be formatted (font face, font size, and font style) using the **Notes Controls** formatting tools on the **Home** tab.
- Images can be inserted into the **Notes** section including, images from other programs and section images from the current document.

Note: Each image must be placed in its own frame, and the frame cannot contain any text.

You can create multiple **Notes** sections within the same experiment.

To work in a **Notes** section, see the following topics:

Adding a Notes Section

To add a **Notes** section:

1. Make any section in an experiment active in the workspace.
2. Click the **New Notes** button in **Sections** on the **Home** tab.
3. An empty **Notes** section opens in the workspace.

Inserting an Image

To insert an image into a **Notes** section:

1. With a **Notes** section active in the workspace, click in a blank area of the **Notes** section to place the cursor in a new frame where you want to insert the image.

Note: Each image must be placed in its own frame, and the frame cannot contain any text.

2. Click the **Add Image** button in the **Notes Controls** on the **Home** tab, or right-click and then click **Add image**.
   The **Open** dialog appears.
3. Browse to locate the image to be inserted.
4. Select the image and click **Open**.
   The image appears in the **Notes** section.

Deleting an Image

To delete an image from a **Notes** section:

1. Click to select the image to be deleted.
2. Right-click the image and then click **Delete**.
   The image is deleted from the **Notes** section.

Inserting an Image of a Section

To insert an image of a section from the current data file into a **Notes** section:

1. With a **Notes** section active in the workspace, click in a blank area of the **Notes** section to place the cursor in a new frame where you want to insert the section image.

Note: Each image must be placed in its own frame, and the frame cannot contain any text.

2. Click the **Add Section Image** button in **Note Controls** on the **Home** tab or in the toolbar at the top of the **Notes** section.
3. In the **Add Section Image** dialog, locate the section to be inserted.
4. Select the section and then click **Add Section Image**.

   The section image appears in the **Notes** section.

Note: You can also right-click in the **Note** section and then click **Add Section Image** to select the section from the cascading menus.

Refreshing Section Images

To refresh all the section images in a **Notes** section, click **Refresh Section Images** in the toolbar at the top of the **Notes** section.

All the section images in the **Notes** section are updated to their current state. For example, if you inserted an image of a **Graph** section and then made changes to the graph section, you can refresh the **Graph** section image in the **Notes** section.

Note: The section images are automatically refreshed periodically, such as when you open the data file, scroll up and down, switch between Document and Comparison views, or start to print or print preview the file.

Deleting a Section Image

To delete a section image from a **Notes** section:

1. Click to select the section image to be deleted.
2. Right-click the section image and then click **Delete**.

   The section image is deleted from the **Notes** section.

Note: When you delete a section from the data file, any images of that section are also deleted from the **Notes** sections. When you delete an experiment from the data file, any images of the sections in the experiment are also deleted from the remaining **Notes** sections.

Adding Text to a Notes Section

To add text to a **Notes** section:

1. Click to place the cursor in the **Notes** section where you want to add the text.
2. Use the keyboard and type the text you want to appear.
3. A text frame appears as you type.
4. To exit from the text frame, click somewhere else in the **Notes** section.

Note: You can add additional text to any text frame by clicking where you want to add text within the frame.

Aligning Text

Text in a text frame is aligned on a line by line basis. Each line in the text frame can be aligned separately.

To adjust the alignment of text within a text frame:

1. Click to select the lines of text to be aligned.
2. To align the line to the left edge, click the (left-alignment) button in **Notes Controls** on the **Home** tab.
3. To align the line to the right edge, click the (right-alignment) button in the **Notes Controls** on the Home tab.
4. To center the line, click the (center-alignment) button in the **Notes Controls** on the **Home** tab.
5. To left and right justify the line, click the (left-right justification) button in the **Notes Controls** on the **Home** tab.
6. To indent the selected line, click the (indent) button in the **Notes Controls** on the **Home** tab.
7. To outdent an indented line, click the (outdent) button in the **Notes Controls** on the **Home** tab.

Editing Text in a Text Frame

To edit text in a text frame:

1. Click on the word or click and drag on a group of words to highlight the text to be edited.
2. Type the revised text.
3. The highlighted text is replaced with the text being typed.

Copying Text

To copy text in a text frame:

1. Click on the word or click and drag on a group of words to highlight the text to be copied.
2. Right-click to display the pop-up menu.
3. Click **Copy.**
4. Or use the **Control+C** keyboard shortcut.

   The highlighted text is placed in the clipboard.

Pasting Text

To paste text from the clipboard into a text frame:

1. Copy text into the clipboard.
2. Click to place the cursor in the text frame where you want to insert the text.
3. Right-click to display the pop-up menu and click **Paste**.
4. Or use the **Control-V** keyboard shortcut.

   The text in the clipboard is pasted into the text frame.

Cutting Text

To cut text out of a text frame:

1. Click on the word or click and drag on a group of words to highlight the text to be cut.
2. Right-click to display the pop-up menu.
3. Click **Cut.**
4. Or use the **Control+X** keyboard shortcut.

   The highlighted text cut from the text frame and not placed in the clipboard.

Formatting Text in a Text Frame

You can format text in a text frame using the formatting tools located in **Notes Controls** on the **Home** tab.

Click and drag or double-click text in a text box to highlight it and then click the formatting tools to perform the following functions:

- Select a font style
- Select a font size
- Format a bulleted list 
- Format a numbered list 
- Superscript  or subscript characters
- Bold , italic , or underline characters
- Select a font color 

You can also click the  (undo) and  (redo) arrows for quick formatting changes.

Positioning Text Within the Notes Section

To auto-position a text frame within a Notes section:

1. Click on a text frame border to select a text.
2. Hover the cursor over the border until the four-arrowhead cursor appears.
3. Right-click to display the pop-up menu.
4. To align the text to the right edge of the **Notes** section, click **Right Align** .
5. To align the text to the left edge of the **Notes** section, click **Left Align** .
6. To align the text to the top of the **Notes** section, click **Top Align** .
7. To align the text to the bottom of the **Notes** section, click **Bottom Align** .

You can manually position the text within the **Notes** section:

1. Click and hold down the mouse button on the border of a text frame.

   The four-arrowhead cursor appears.
2. Drag the text to the desired location and release the mouse button.

You can also select multiple text frames, summaries, and images by selecting using **Ctrl-Click**, and then align them with each other using the alignment buttons described above.

Resizing a Text Box

To resize a text box:

1. Click on text to select it.
2. Click and hold the text box on the right or left side border.
3. The double-ended arrow appears.
4. Drag the box to the desired size and release the mouse button.

Deleting Text

To delete text:

1. Click on a text frame boarder to select a text frame.
2. Right-click to display the pop-up menu.
3. Click **Delete**.

   The text is deleted from the **Notes** section.

Adding a New Summary Formula

To add a new New Summary to a Notes section:

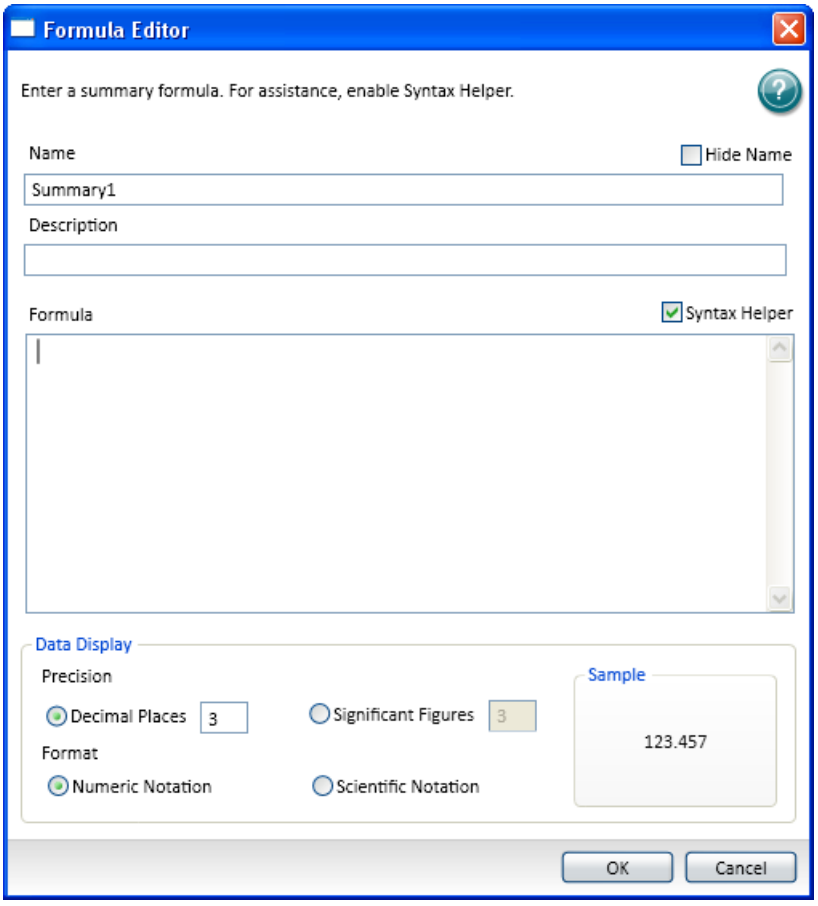
1. Click on a **Notes** section to make it active in the **Workspace**
2. Click **New Summary**  in the **Note Controls** on the **Home** tab.
   This opens the **Formula Editor**.
3. See Using the Formula Editor Dialog on page 90 for details about entering the formula.

Click **Formula Reference Guide**  on the **Help** tab for a complete guide to formulas.

Using the Formula Editor Dialog

To enter a new formula:

Click New Summary in the **Note Controls** on the **Home** tab. This opens the **Formula Editor**.



To edit and existing summary formula, double click on the formula text box. This opens the **Formula Editor** with the existing formula in the editor window.

To edit the formula:

1. For a new formula, type the name of the summary in the **Name** field.
2. Optionally, click **Hide Name**.

   This hides the name of the summary in the **Notes** section.

3. Enter a brief text description of the summary.
4. Specify the data display characteristics including:
   - **Significant Figures** and type the number of figures to display
   - **Decimal Places** and type the number of places to display
   - **Scientific Notation**
   - **Numeric Notation**
5. Type in a formula to be used for the Summary.
6. Optionally, click **Syntax Helper** to turn on the formula completion tool. See Enabling Syntax Helper on page 92.
7. Click **OK**.

For a complete guide to formulas, see the **SoftMax Pro Formula Reference Guide**  help on the **Help** tab in the ribbon.

Editing a Summary Formula

1. To edit an existing summary formula, click on the formula text box in the **Notes** section. This opens the **Formula Editor** with the existing formula in the editor window.
2. Optionally, change the name of the formula in the **Name:** field.
3. Optionally, click **Hide Name**.
   This hides the name of the summary in the **Notes** section.
4. Enter a brief text description of the summary.
5. Specify the data display characteristics including:
   - **Significant Figures** and type the number of figures to display
   - **Decimal Places** and type the number of places to display
   - **Scientific Notation**
   - **Numeric Notation**
6. Optionally, click **Syntax Helper** to turn on the formula completion tool. See Enabling Syntax Helper on page 92.
7. Revise the formula to be used by the **Summary**.
8. Click **OK**.

For a complete guide to formulas, see the **SoftMax Pro Formula Reference Guide** help on the **Help** tab in the ribbon.

Deleting a Summary Formula

To delete and existing summary formula:

1. Click the formula text box.
2. Click **Delete**  in the **Notes Controls** section of the **Home** tab.

Enabling Syntax Helper

For assistance creating formulas, click **Syntax Helper**. When the Syntax Helper is enabled, click **Syntax Helper** to disable it.

When you first start typing a formula, a popup displays below your text as you type. This is **Syntax Helper**, a technology that analyzes text as you type and compares it to formulas that are available for use in the SoftMax Pro Software.

Syntax Helper allows you to quickly see what formulas are available and what parameters these formulas expect to receive.

By default, **Syntax Helper** is enabled when you first launch the SoftMax Pro Software. You can enable and disable this feature whenever you are working in the **Formula Editor** dialog.

Using the Cuvette Set Section

Cuvette Set sections are used to collect data from the cuvette port of the SpectraMax M2, M2e, M5, M5e, or Plus 384, to define an analysis template, and to define data display and data reduction. The Template Editor in the Cuvette Set section is used to describe the contents of each cuvette within the Cuvette Set.

Cuvette Set sections show:

- Cuvette tools in the Home tab, see Using Cuvette Tools on page 93.
- Cuvette data display(s)
- Instrument settings, shown to the right of the data display
- Reduction settings, shown at the bottom of the data display

When colors appear in the name labels above individual cuvettes this means a template has been defined for the Cuvette Set section.

Each group defined in the template has a different color (the icon of the corresponding group table has the same color).

If your experiment requires multiple cuvettes, you can add up to 96 cuvettes in a single Cuvette Set section or create more than one Cuvette Set section. Cuvettes are read one at a time.

Using Cuvette Tools

Cuvette Tools appear on the **Home** tab when a cuvette set section is active in the workspace. **Cuvette Tools** include the following:

Creating a New Cuvette Set Section

To add a new Cuvette Set section:

1. Click **New Cuvette Set** in **Sections** on the **Home** tab.
2. A new **Cuvette Set** section is added to the Experiment and appears on the navigation tree.

Note: When you create a new Cuvette Set and an existing Cuvette Set is active in the workspace, SoftMax Pro Software copies the active Cuvette Set section and the new Cuvette Set section is a duplicate of the active Cuvette Set section.

Adding a Cuvette to a Cuvette Set

To add a cuvette to a Cuvette Set:

1. Click on a Cuvette Set section to make it active in the workspace.
2. Click **New** in the **Cuvette Tools** on the **Home** tab.

Copying a Cuvette Set Section

To duplicate a new Cuvette Set section:

1. Make the **Cuvette Set** section you want to duplicate active in the workspace.
2. Click **New Cuvette Set** in **Sections** on the **Home** tab.
3. A new **Cuvette Set** section that is a duplicate of the active Cuvette Set section is added to the Experiment and appears on the navigator tree.

Deleting a Cuvette

To delete a cuvette from a Cuvette Set:

1. Select the cuvette to be deleted.
2. Click **Delete** in **Cuvette Tools** on the **Home** tab.

Modifying Instrument Settings

Changing the instrument setting for a cuvette changes the setting for all the cuvettes in the Cuvette Set.

To change the instrument settings:

1. Make a **Cuvette Set** section active in the workspace.
2. Click **Settings** in the **Cuvette Tools** on the **Home** Tab or click the **Settings** shortcut in the Cuvette Set Section header. The **Settings** dialog opens.
3. Set the instrument settings options as needed.
4. **Click** OK.

Modifying Data Reduction Settings

To change the data reduction settings:

1. Click **Reduction** in the **Cuvette Tools** on the **Home** tab or the **Reduction** shortcut on the Cuvette Set section header bar.
2. Change the reduction settings as needed.The Data Reduction dialog opens. For information about changing data reduction settings, see Modifying Data Reduction Settings.
3. Click **OK**.

Note: Changing the data reduction setting for one cuvette, changes the settings for all the cuvettes in the active Cuvette Set section.

Modifying the Display

To change the display settings:

1. Click **Display** in the **Cuvette Tools** on the **Home** tab or the **Display** shortcut on the **Cuvette Set** section header bar. The **Data Display** dialog opens.
2. Change the display settings as needed. For information about changing the display settings, see Modifying the Display on page 76.
3. Click **OK**.

Note: Changing the display setting for one cuvette, changes the display settings for all the cuvettes in the active Cuvette Set section.

Masking Cuvettes

Masking is the process of excluding outliers in certain cuvettes from data reduction calculations.

To mask cuvettes:

1. Select the cuvettes to be masked.
2. Click **Mask** in Cuvette Tools on the Home tab or the Mask shortcut on the Cuvette Set Section header bar.

   You can also right-click and select **Mask** from the pop-up menu.

Masking can be used as a "what if?" tool. 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 cuvettes suppresses the blanking function, while unmasking them enables it again.

Zooming the Display

Zoom becomes active when the data in a cuvette is available for a zoomed display.

To display the zoom window:

1. Click the cuvette you want to zoom in on.
2. Click **Zoom** in the **Cuvette Tools** on the **Home** tab or the **Zoom** shortcut on the Cuvette Set section header bar.

Copying and Pasting Cuvette Data

To copy the cuvette data.

1. Select the cuvette with the data to be copied.
2. Right-click and select **Copy Cuvette Data** from the pop-up menu.
3. The data is copied into the clipboard.

To paste cuvette data that is stored in the clipboard.

1. Select the cuvette that the data should be pasted into.
2. Right-click and select **Paste Cuvette Data** from the pop-up menu.

Using the Template Tools With a Cuvette Set

When working within a Cuvette Set section, adding template information in the form of groups automatically adds cuvettes to the active cuvette set.

Up to 96 cuvettes can be added to one Cuvette Set section. When you open the template editor with a Cuvette Set section active in the workspace, the template editor window contains 96 wells in the column and row format of a 96 well plate.

Cuvette in the template are numbered as if they were wells in a microplate.

This representation provides easy access to the data analysis capabilities of the formula engine.

Use the Cuvette Set Template tools for:

- Adding a New Cuvette Set Template on page 96
- Modifying a Cuvette Set Template on page 96

Adding a New Cuvette Set Template

To add a template to a **Cuvette Set** section:

1. Click **Template** in either the **Template Tools** of the **Home** tab
2. Or click the **Template** shortcut on the **Cuvette Set** section header bar.

Modifying a Cuvette Set Template

To change the template settings in a Cuvette Set section:

1. Click **Template** in Template Tools on the Home tab or the Template short cut on the Cuvette Set section header to open the **Template Editor** for the Cuvette Set section.
2. Change the template settings as needed.
3. Click **OK**.

Using the Group Section

Unlike other section types, Group sections are created automatically when you create and assign a Group to a Plate section within the **Template Editor** dialog (after clicking the Template button in a Plate section).

Reduced numbers from the Plate section are displayed in the Values column of the Group section tables by default.

Custom reduction formulas require the use of accessors and operators that are understood by SoftMax Pro Software. For a list of these accessors and operators see the *SoftMax Pro Formula Reference Guide* help.

Reusing Formulas

Formulas are used in Group columns and custom reductions. Sometimes it is useful to copy an existing formula, column from one area of the program to another, or from one Experiment to another, rather than recreating it. After pasting, formulas can be edited.

Contiguous column formulas can be copied and pasted together.

When a Group section is active in the workspace Group Tools appear on the Home tab.

For information about group tasks, see Using Column Tools on page 97.

Using Column Tools

When an column or data is selected in the group table, the buttons in the Column tools that can be used on the selected items are active. Actions not appropriate for a selected item are disabled.

After selecting a column or data in a group table, functions appropriate to the selected item are available by right-clicking to open the Group tools pop-up menu.

Using the Column tools on the Home tab you can perform the following operations:

- Adding a New Column Formula on page 100
- Modify a Column on page 101
- Deleting a Column on page 101
- Autosizing a Column on page 101
- Hiding a Column on page 101
- Hiding Replicates on page 101
- Showing Hidden Columns on page 102
- Cutting Column Content on page 102
- Pasting Column Contents on page 102
- Copying Column Contents on page 102

When you have finished configuring groups, return to Using the Group Section on page 97.

Using the Group Settings dialog

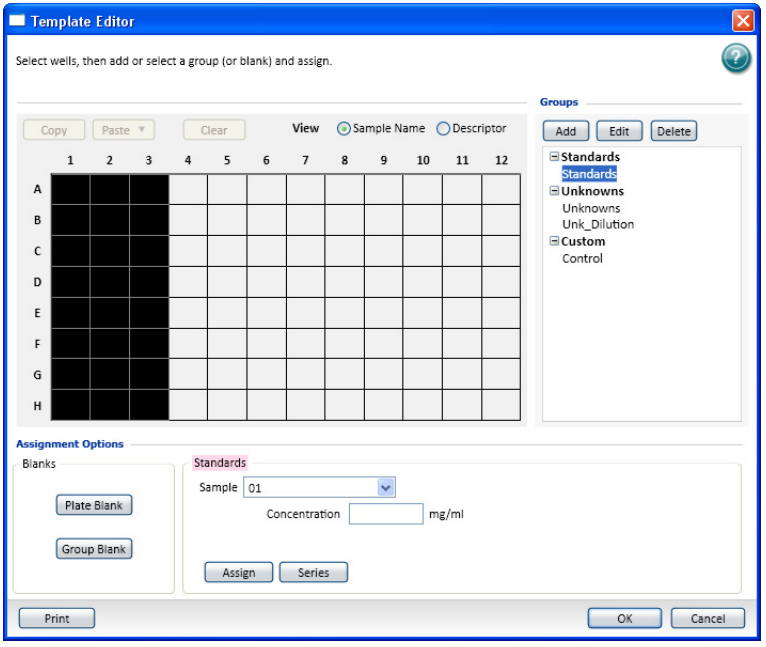
The **Group Settings** dialog allows you to define the name for a group of related samples, a descriptor associated with the samples, and the initial column format for the data calculated and reported in the associated **Group** section.

Whenever a new group is created (whether or not wells are selected in the template), a Group section is created in your Experiment. To delete a group, you must delete the Group section. Clearing a group from the Template Editor removes only the assignment of wells to that group name; it does not delete the group.

See Group Column Format on page 163 for information about the column formats.

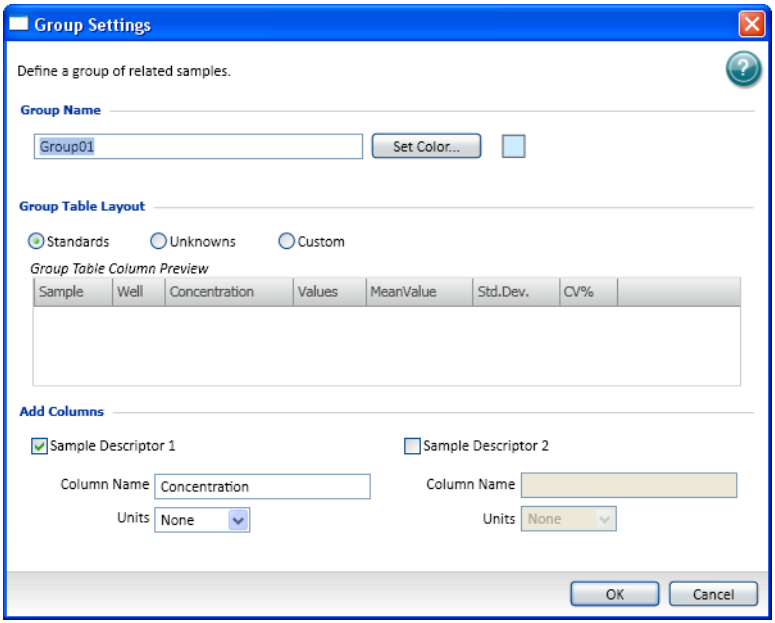
Defining a Group

1. Click the **Template** button in the **Home** tab to open the **Template Editor** dialog.



2. Click and drag the mouse to select one or more wells to be defined as a group.

3. Click **Add** in the **Group Type** to open the **Group Settings** dialog.



4. Type the name to assign to the group in the **Group Name** field.
5. If you want to set a color for the group, click **Set Color** and select the color to be displayed for the group.
6. In the **Group Table Layout** area, click **Standards**, **Unknowns**, or **Custom** to specify the column format.

   For information about the column formats, see Group Column Format on page 163.

   When the Standards group type is selected, Sample Descriptor1 is automatically activated and the assumption is that it will be used for concentration information. However, it could be used for any numerical data, that is, dilution factor or fraction number. The other two group types do not have sample descriptors by default.
7. In the **Add Columns** area, if it is not already selected, click **Sample Descriptor1** to apply a descriptor and its units to the group.
8. Type a description of the sample descriptor in the **Column Name** field.
9. Select a set of units from the list or type them directly in the **Units** field.

10. Optionally, define a second descriptor.

    The **Group Table Column Preview** shows an example of how the columns will appear in the group table.

11. When all groups have been defined, click **OK** to close the **Group Settings** dialog.

    The highlighted wells are placed in the newly defined group.

Note: No sample descriptor is assigned automatically for the Unknowns column format. You can assign a sample descriptor to these column formats manually but the information does not appear automatically in the Group section. To see it, create a new column containing the formula !Sampledescriptor, !factor, or !concentration. For more information about creating columns, see Adding a New Column Formula on page 100.

Adding a New Column Formula

To add a new column to a group:

1. Click on a **Group** section to make it active in the workspace.
2. Click the **New** button in the **Column Tools** to add a column formula to the active Group table.

   If no columns are highlighted, the column is added at the end of the group.

   If you select a column first, the new column is added to the right of the highlighted column.

New columns can contain references to other columns, either in the current section or in a different one. 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)

Many possibilities exist for creating and editing column formulas: see the **SoftMax Pro Formula Reference Guide** help for a complete discussion.

Modify a Column

1. Click on a **Group** section to make it active in the workspace.
2. To modify a column formula, double click on the column to open the **Formula Editor** dialog.
3. To resize the column width, click and hold the column border and drag it to the desired column width.

Deleting a Column

To delete a column:

1. Click on a **Group** section to make it active in the workspace.
2. Select a column by clicking on the column title box.
3. Click **Delete** in the **Group Tools** or right-click and select **Delete Column** from the pop-up menu.

Autosizing a Column

You can autosize a column to set the width of the selected column(s) to accommodate the longest text string:

1. Click on a **Group** section to make it active in the workspace.
2. Select a column or multiple columns.
3. Click **Auto Size** in the **Group** tools or right-click on a column and select **Auto Size**.

Hiding a Column

To hide one or more columns:

1. Click on a **Group** section to make it active in the workspace.
2. Select a column or columns.
3. Click **Hide** in the Group tools or right-click and select **Hide Column** to hide the selected column(s) from display.
4. You can show or hide columns by clicking **Show/Hide** in the **Group** tools and checking/unchecking columns to show or hide them.

Hiding Replicates

To hide data for all replicates

1. Click on a **Group** section to make it active in the workspace.
2. Click **Hide Replicates** in the Group tools.

Showing Hidden Columns

To show all columns:

1. Click on a **Group** section to make it active in the workspace.
2. Click **Show/Hide** in the Column tools to open the **Show/Hide Group Table columns** selection box.
3. Select the hidden columns to return the hidden column(s) to the display.

Cutting Column Content

To cut content out of a column:

1. Click on a **Group** section to make it active in the workspace.
2. Select the content to be removed.
3. Click **Cut** in the Group tools.

Note: Content that is cut from a column is not placed in the clipboard. To place content in the clipboard, the content must be copied.

Pasting Column Contents

The content to be pasted into a column must be copied from another column location and be present in the clipboard.

To paste content into a column:

1. Click on a **Group** section to make it active in the workspace.
2. Click to select the target column location.
3. Click **Paste** in the Column Tools.

Note: If the Paste button is grayed out when you attempt this operation, there is no appropriate content present in the clipboard. You must first load the clipboard with content.

Copying Column Contents

To paste content into a column you must first copy appropriate content into the clipboard using the copy operation provided in the **Group tools**.

To copy content to the clipboard:

1. Click on a **Group** section to make it active in the workspace.
2. Select the content to be copied.
3. Click **Copy** in the Column tools.

Using Summary Tools

When a Group section is active in the workspace, the Home tab displays the Summary tools.

Using the Summary tools on the Home tab you can perform the following operations:

- Adding a New Formula on page 103
- Editing a Formula on page 103
- Deleting a Formula on page 103
- Copying/Pasting a Formula on page 104

Adding a New Formula

When a Group section is active in the workspace Column Tools appear on the Home tab.

To add a new formula to a column:

1. Click on a **Group** section to make it active in the workspace.
2. Select the column where the new formula will be added.
3. Click **New Summary** in Summary tools to open the **Formula Editor** dialog. For more information, see Using the Formula Editor Dialog on page 90.
4. Type in the formula to be added and click **OK**.

Editing a Formula

When a Group section is active in the workspace Summary Tools appear on the Home tab.

To edit a formula in a column:

1. Click on a **Group** section to make it active in the workspace.
2. Select the formula to be changed.
3. Click **Edit Summary** in Summary tools.
4. Type in the change to formula and click **OK**. For more information, see Using the Formula Editor Dialog on page 90.

Deleting a Formula

To delete a formula:

1. Click on a **Group** section to make it active in the workspace.
2. Click a formula to select the formula to be deleted.
3. Click **Delete** in **Summary** tools.

Copying/Pasting a Formula

When a **Group** section is active in the workspace **Summary Tools** appear on the **Home** tab.

To copy formula and paste it into a column:

1. Click on a **Group** section to make it active in the workspace.
2. Select the formula to be copied.
3. Click **Copy** in Summary tools.
4. Click to select the location where you want to paste the Summary.
5. Click **Paste** in Summary tools. The copied formula is pasted into the Group section and labeled as a copy of the selected formula.

Using the Graph Section

Graph sections are used to plot information from groups as scatter plot graphs.

Once a graph has been created, new plots can be added and deleted, the axes can be customized, and the size of the graph can be changed. Grid lines can be enabled or disabled (default is enabled).

Graph sections are divided into:

- The body of the **Graph** section
- The Legend

You can create more than one **Graph** section within an Experiment data file, and plots in the **Graph** section can be created from any Experiment in the file.

For more information see Working with Graphs on page 231.

File Management

SoftMax Pro Software uses two basic file types: data files and protocol files.

Data files

Data files contain the raw data collected by your reader along with any data analysis you might have completed.

SoftMax Pro Software 6 can open files created by version 4.0 or later. Before you open a file you must make sure that your currently selected instrument is the same model as the one used to create the file, or a model with instrument settings that are compatible with the model used to create the file.

SoftMax Pro Software automatically creates data files based on the capabilities of the connected reader, or if a reader is not present, based on the reader type specified in the **Instrument Status** section of the **Home** tab. Data file options include:

- .sda
- .xml
- .txt (list)
- .txt (plate)
- .xls (list)
- .xls (plate)

Note: All data file options except ".sda" are for export file types.

Export Data Formats Options

The Auto Save function using the supported export data formats can be used to prevent data loss. For more information and a description of the data file types, see Setting Auto Save Options on page 190.

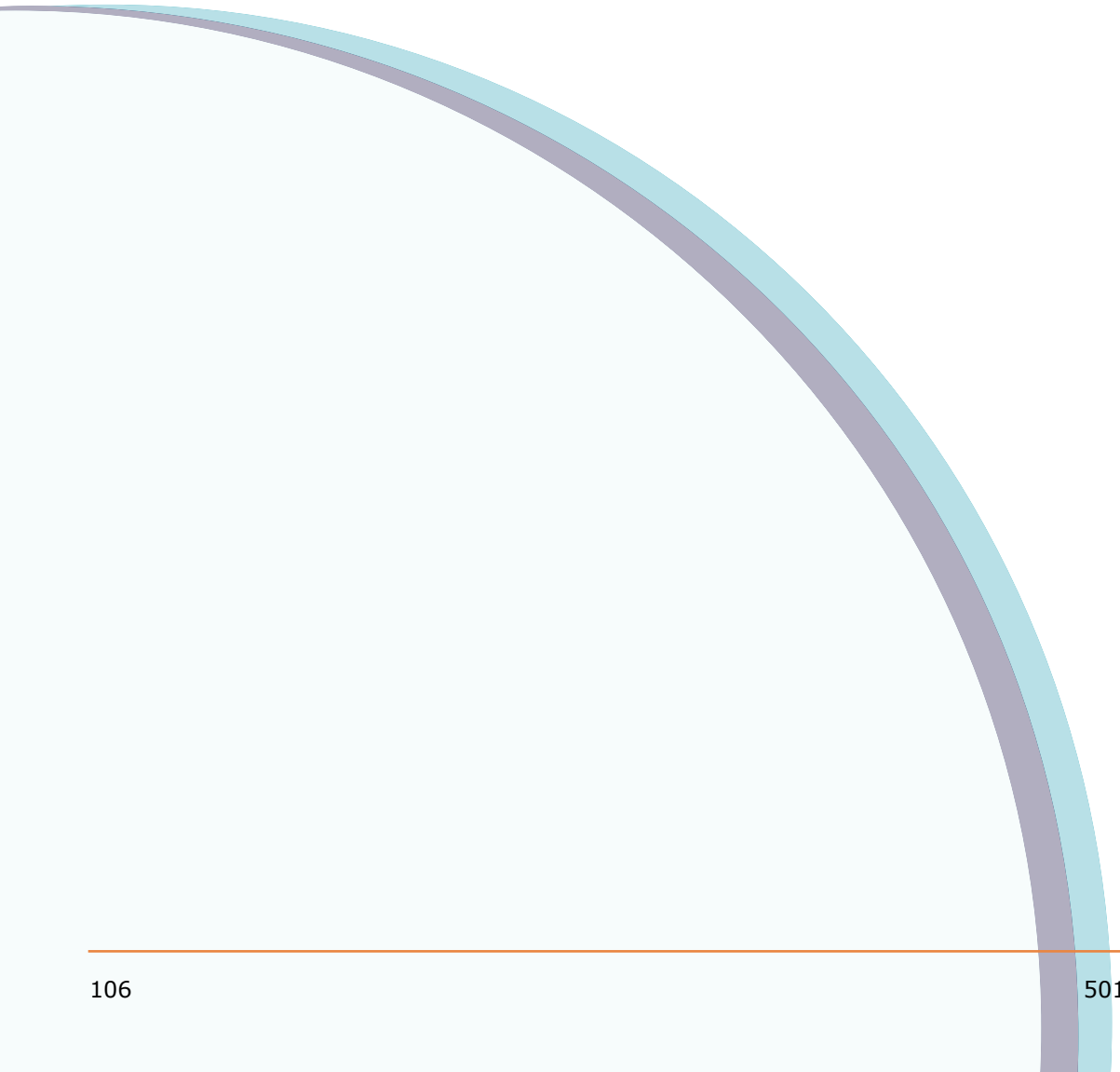
Protocol Files

Protocol files are Experiment template files that contain microplate well layout assignments and all other reader configuration data, but no data. A large number of predefined protocols are installed with SoftMax Pro Software. SoftMax Pro Software protocol files have the file extension **.spr**.A large number of predefined protocols are installed with SoftMax Pro Software and are placed in the default protocols folder. During software installation, the Basic Endpoint protocol is set as the default protocol.

Saving a data file as a protocol file removes any data in the file, leaving only the configuration information.

You can use the functions provided on the **Protocol** tab on the application ribbon to open, create and save protocol files.

See Using the Application Menu on page 26.



Instrument and Detection Settings

3

SoftMax® Pro Microplate Acquisition and Analysis Software controls Molecular Devices spectrophotometers, absorbance, luminescence, and fluorescence microplate readers, providing extensive data calculation and analysis capabilities under a Good Manufacturing Practices (GMP), Good Laboratory Practices (GLP) work environment for pharmaceutical, biotechnology, academic, hospital, and government customers.

Over 120 assay protocols are included in the software to speed life science research and drug discovery assay development and screening. Researchers can customize experiment protocols, analyze and display data, and create meaningful reports. The straightforward yet powerful programming capabilities of SoftMax Pro Software can further enhance any specialized data collection and analysis needs through custom assay development.

This software package is widely integrated with industry-leading robotics systems.

This guide describes features available in SoftMax Pro Software version 6 and later. SoftMax Pro Software 6 is available for PC-compatible computers using Windows 7.

Supported Read Types

SoftMax Pro Software supports the following Read Types:

Endpoint Read Type

In an Endpoint read, a reading of each microplate well or cuvette is taken once, in the center of each well, at a single wavelength or at multiple wavelengths. Depending on the read mode, raw data values are reported as optical density, %Transmittance, RFUs, or RLUs.

Kinetic Read Type

In a Kinetic read, the data are collected over time with multiple readings taken at regular intervals. To achieve the shortest possible interval for Kinetic readings, choose wavelengths in ascending order.

Spectrum Read Type

Depending on the read mode selected, a Spectrum read measures optical density, %Transmittance, relative fluorescence units (RFUs), or relative luminescence units (RLUs) across a spectrum of wavelengths.

Well Scan Read Type

Well Scan allows readings to be taken at more than one location within a well. A Well Scan read takes one or more readings of a single well of a microplate on an evenly spaced grid inside of each well at single or multiple wavelengths. Every option available for Endpoint reads is available for Well Scans.

Supported Read Modes

SoftMax Pro Software supports the following Read Modes:

- ABS Read Mode on page 108
- FL Read Mode on page 109
- LUM Read Mode on page 109
- TRF Read Mode on page 109
- FP Read Mode on page 110
- FRET Mode on page 110
- AlphaScreen Mode on page 111

ABS Read Mode

In the Absorbance (ABS) read mode, the instrument measures the Optical Density (OD) of the sample solutions. Absorbance is the amount of light absorbed by a solution.

Absorbance detection measures how much light is absorbed by a sample. Absorbance-based detection has been commonly used to assess changes in color or turbidity, allowing for widespread use including ELISAs, protein quantitation, endotoxin assays, and cytotoxicity assays. With absorbance readers that are capable of measuring in the ultraviolet (UV) range, the concentration of nucleic acids (DNA and RNA) can be found using their molar extinction coefficients.

FL Read Mode

In the Fluorescence Intensity (FL) read mode, the instrument measures Fluorescence Intensity (in Relative Fluorescence Units, RFUs). Fluorescence occurs when absorbed light is re-radiated at a longer wavelength.

Fluorescence intensity detection uses an external light source to excite molecules (fluorophores), which then emit light of a longer wavelength. The emitted light is then captured and analyzed to quantitate changes in the fluorophores. Fluorescence intensity is used widely in applications not limited to fluorescent ELISAs, protein assays, nucleic acid quantitation, reporter gene assays, cell viability, cell proliferation, and cytotoxicity. Another major application of this mode is to study the kinetics of ion release.

LUM Read Mode

In the Luminescence read mode, Relative Luminescence Units, RLU(RLUs) is 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.

Luminescence detection relies on the production of light from a chemical reaction in a sample. The highly efficient conversion of chemical energy to light has been used in reporter gene assays, aequorin GPCR, and secreted luciferase assays, to name a few.

TRF Read Mode

In Time-Resolved Fluorescence (TRF) read mode, the instrument detects the extremely long emission half-lives of rare earth elements called lanthanides. The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes interference from fluorescence excitation light.

Time-resolved fluorescence (TRF) detection is similar to fluorescence intensity (FL) detection. The difference is that the fluorophores are a special type called lanthanides that have a longer lifetime than standard fluorophores used with FI detection. Assays with these long-lifetime fluorophores have the advantage of very low background fluorescence. TRF detection is widely used in high throughput screening applications such as kinase assays.

The Cisbio Bioassays HTRF (Homogeneous Time-Resolved Fluorescence) technology is a proprietary time-resolved fluorescence technology that overcomes many of the drawbacks of standard Fluorescence Resonance Energy Transfer (FRET) techniques, such as the requirements to correct for autofluorescence and the fluorescent contributions of unbound fluorophores. The HTRF technology is available for the SpectraMax M5e reader and the SpectraMax Paradigm Cisbio HTRF Detection Cartridge.

FP Read Mode

In Fluorescence Polarization (FP) mode, the relative change of polarization of emitted fluorescent versus excitation light is measured. The change in polarization angle between the excitation and the emitted light is higher, the lower the molecular weight and thus the higher the speed of rotation.

Fluorescence polarization detection is also similar to fluorescence intensity, with the important difference that it uses plane polarized light, versus non-polarized light. Plate readers measure FP of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation. Depending on the size of the molecule bound to the fluorophore, you will get a corresponding decrease in rotation; the larger the molecule, the greater the degree of polarization (mP). For this reason FP is typically used for molecular binding assays in HTS.

FRET Mode

Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule *without emission of a photon*.

FRET relies on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. Due to its sensitivity to distance, FRET has been used to investigate molecular interactions. FRET is the radiationless transmission of energy from a donor molecule to an acceptor molecule. The donor molecule is the dye or chromophore that initially absorbs the energy and the acceptor is the chromophore to which the energy is subsequently transferred. This resonance interaction occurs over greater than interatomic distances, without conversion to thermal energy, and without any molecular collision. The transfer of energy leads to a reduction in the donor's fluorescence intensity and excited state lifetime, and an increase in the acceptor's emission intensity. A pair of molecules that interact in such a manner that FRET occurs is often referred to as a donor/acceptor pair.

While there are many factors that influence FRET, the primary conditions that need to be met in order for FRET to occur are relatively few. The donor and acceptor molecules must be in close proximity to one another. The absorption or excitation spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor. The degree to which they overlap is referred to as the spectral overlap integral (J).

AlphaScreen Mode

ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. AlphaScreen is a bead-based chemistry used to study molecular interactions between moieties A and B, for example. When a biological interaction between A and B brings beads—coated with A and B, respectively—together, a cascade of chemical reactions acts to produce a greatly amplified signal.

The cascade finally resulting in signal is triggered by laser excitation (680 nm), making a photosensitizer on the A-beads convert oxygen to an excited (singlet) state. That energized oxygen diffuses away from the A-bead. When reaching the B-bead in close proximity, it reacts with a thioxene derivative on the B-bead generating chemiluminescence at 370 nm. Energy transfer to a fluorescent dye on the same bead shifts the emission wavelength into the 520 nm to 620 nm range. The limited lifetime of singlet oxygen in solvent (~4 microseconds) allows diffusion reach only up to about 200 nm distance. Thus, only B-beads in the proximity of A-beads yield signal, which indicates binding between moieties A and B.

An AlphaScreen measurement includes a light pulse, by turning on the laser diode for a specified time, turning off the laser diode, followed by the measurement of the AlphaScreen signal, as specified in the measurement protocol timing parameters.

Note: AlphaScreen mode is available with a SpectraMax Paradigm instrument that has an AlphaScreen detection cartridge installed.

Supported Instruments

User guides for each of the supported instruments are installed during the SoftMax Pro Software installation. You can view these user guides from the Windows Start menu at **Start > All Programs > Molecular Devices > SoftMax Pro 6.1 > Hardware User Guides**.

SoftMax Pro Software supports the following instruments:

- VersaMax ELISA Microplate Reader on page 112
- SpectraMax Plus 384 Absorbance Microplate Reader on page 113
- SpectraMax M2 and M2e Multi-Mode Microplate Readers on page 114
- SpectraMax M3 Multi-Mode Microplate Reader on page 115
- SpectraMax M4 Multi-Mode Microplate Reader on page 116
- SpectraMax M5 and M5e Multi-Mode Microplate Readers on page 117
- SpectraMax 340PC 384 Absorbance Microplate Reader on page 118

VersaMax ELISA Microplate Reader

The VersaMax™ ELISA Microplate Reader provides rapid and sensitive measurements of a variety of analytes across a wide range of concentrations. You can run both standard spectrophotometer and microplate reader applications on the same instrument. Read one sample or up to 96 at a time. Use any standard 96-well microplate.

Detection mode includes:

- Visible Absorbance (Abs), see ABS Read Mode on page 108.

VersaMax ELISA Microplate Readerfeatures include:

- Full spectral range: 340 nm to 850 nm, tunable in 1 nm increments.
- Superior performance: 2 nm bandwidth provides exceptional accuracy and linearity for the widest range of assays.
- Endpoint and Kinetic reads.

SpectraMax Plus 384 Absorbance Microplate Reader

The SpectraMax® Plus 384 Absorbance Microplate Reader has a built-in cuvette port and microplate drawer. You can run both standard spectrophotometer and microplate reader applications on the same instrument. Read one sample or up to 384 at a time. Use any standard cuvette, 12 x 75 mm test tube, or 96-well or 384-well microplate. For more sample throughput, the SpectraMax Plus 384 Absorbance Microplate Reader can be integrated into a full robotic system.

Detection mode includes:

- UV and Visible Absorbance (Abs), see ABS Read Mode on page 108.

SpectraMax Plus 384 Absorbance Microplate Reader features include:

- Full spectral range: 190 nm to 1000 nm, tunable in 1 nm increments.
- Superior performance: 2 nm bandwidth provides exceptional accuracy and linearity for the widest range of assays.
- Speed read mode: Reads 96-well microplates in just 5 seconds and 384-well microplates in 16 seconds for ultra-fast sample throughput.
- PathCheck® Pathlength Measurement Technology reports the microplate data as standard 1 cm cuvette values.

SpectraMax M2 and M2e Multi-Mode Microplate Readers

The SpectraMax® M2 and M2e Multi-Mode Microplate Readers are instruments with two monochromators, dual-mode cuvette ports, and top-plate-and bottom-plate reading capability (top-reading only on the SpectraMax M2 Multi-Mode Microplate Reader). Area-well scanning read types and the Molecular Devices PathCheck® Pathlength Measurement Technology allow homogeneous and heterogeneous microplate assays to be performed in one flexible system.

Detection modes include:

- UV and Visible Absorbance (ABS), see ABS Read Mode on page 108.
- Fluorescence Intensity (FI), see FL Read Mode on page 109.

Features of the SpectraMax M2 and M2e Multi-Mode Microplate Readers include:

- Multi-detection capability
- Top-read and bottom-read (M2e only)
- Dual-mode cuvette port
- Two monochromators
- PathCheck® Pathlength Measurement Technology and well volume sensors
- Instrument and software validation
- Automation compatible

SpectraMax M3 Multi-Mode Microplate Reader

The SpectraMax® M3 Multi-Mode Microplate Reader is a modular, dual-monochromator microplate reader platform offering a wide range of high performance multi-mode capabilities ideal for life science research and drug discovery screening. The SpectraMax M3 Multi-Mode Microplate Reader contains a triple-mode cuvette port, accurate temperature control, and microplate shaking capability.

Detection modes include:

- UV and Visible Absorbance (Abs), see ABS Read Mode on page 108.
- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.

Features of the SpectraMax M3 include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- Automated Absorbance Pathlength Correction
- Endpoint, kinetic, spectral and well-scanning read types
- Comprehensive Data Analysis with SoftMax Pro Software
- Validation and compliance
- Robotics compatibility

SpectraMax M4 Multi-Mode Microplate Reader

The SpectraMax® M4 Multi-Mode Microplate Reader is a modular, dual-monochromator microplate reader platform offering a wide range of high performance multi-mode capabilities ideal for life science research and drug discovery screening.

The SpectraMax M4 Multi-Mode Microplate Reader offers a triple-mode cuvette port, accurate temperature control, and microplate shaking capability.

Detection modes include:

- UV and Visible Absorbance (Abs), see ABS Read Mode on page 108.
- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.
- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 109.

Features of the SpectraMax M4 Multi-Mode Microplate Reader include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- PathCheck® Pathlength Measurement Technology
- Endpoint, kinetic, spectral, and well-scanning read types
- Comprehensive Data Analysis with SoftMax Pro Software
- Validation and compliance
- Automation compatibility

SpectraMax M5 and M5e Multi-Mode Microplate Readers

The SpectraMax® M5 and M5e Multi-Mode Microplate Readers are modular, upgradable, dual-monochromator microplate reader platforms offering a wide range of high performance multi-mode capabilities ideal for life science research and drug discovery screening.

The SpectraMax M5 and M5e Multi-Mode Microplate Readers offer triple-mode cuvette port, accurate temperature control, and microplate shaking capability.

Detection modes include:

- UV and Visible Absorbance (Abs), see ABS Read Mode on page 108.
- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.
- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 109.
- Fluorescence Polarization (FP), see FP Read Mode on page 110.

Features of the SpectraMax M5 and M5e Multi-Mode Microplate Readers include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- PathCheck® Pathlength Measurement Technology
- Endpoint, kinetic, spectral, and well-scanning read types
- Comprehensive Data Analysis with SoftMax Pro Software
- Validation and compliance
- Automation compatibility

SpectraMax 340PC 384 Absorbance Microplate Reader

The SpectraMax® 340PC 384 Absorbance Microplate Reader provides rapid and sensitive measurements of a variety of analytes across a wide range of concentrations. You can run both standard spectrophotometer and microplate reader applications on the same instrument. Read one sample or up to 384 at a time. Use any standard 96-well or 384-well microplate.

Detection mode includes:

- Visible Absorbance (Abs), see ABS Read Mode on page 108.

SpectraMax 340PC 384 Absorbance Microplate Reader features include:

- Full spectral range: 340 nm to 850 nm, tunable in 1 nm increments.
- Superior performance: 2 nm bandwidth provides exceptional accuracy and linearity for the widest range of assays.
- PathCheck® Pathlength Measurement Technology reports the microplate data as standard 1 cm cuvette values.

SpectraMax 190 Absorbance Microplate Reader

The SpectraMax® 190 Absorbance Microplate Reader provides rapid and sensitive measurements of a variety of analytes across a wide range of concentrations. Read one sample or up to 96 at a time. Use any standard 96-well microplate.

Detection mode includes:

- UV and Visible Absorbance (Abs), see ABS Read Mode on page 108.

SpectraMax 190 Absorbance Microplate Reader features include:

- Full spectral range: 190 nm to 850 nm, tunable in 1 nm increments.
- Superior performance: 2 nm bandwidth provides exceptional accuracy and linearity for the widest range of assays.
- PathCheck® Pathlength Measurement Technology reports the microplate data as standard 1 cm cuvette values.

Gemini XPS Fluorescence Microplate Reader

The Gemini™ XPS Fluorescence Microplate Reader can perform a variety of fluorescent applications. The extreme flexibility and high sensitivity makes the Gemini XPS Fluorescence Microplate Reader appropriate for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology.

Detection modes include:

- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.
- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 109.

Gemini XPS Fluorescence Microplate Reader features include:

- Dual monochromators allow the selection of any wavelength in 1 nm increments.
- Cutoff filters reduce stray light and minimize background interference.
- Wavelength scanning ensures that the most sensitive assay conditions are used.

Gemini EM Fluorescence Microplate Reader

The Gemini™ EM Fluorescence Microplate Reader can perform a variety of fluorescent applications. The extreme flexibility and high sensitivity makes the Gemini EM Fluorescence Microplate Reader appropriate for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology. The top-read and bottom-read optical design allows for measurements for both solution and cell-based assays.

Detection modes include:

- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.
- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 109.

Gemini EM Fluorescence Microplate Reader features include:

- Dual monochromators allow the selection of any wavelength in 1 nm increments.
- Cutoff filters reduce stray light and minimize background interference.
- Wavelength scanning ensures that the most sensitive assay conditions are used.

FilterMax F5 Multi-Mode Microplate Reader

The FilterMax™ F5 Multi-Mode Microplate Reader is ideal for a broad range of applications, including drug discovery, genomics, proteomics, and cell-based research. The unique patented design ensures precise performance and sensitivity across all detection modes.

Detection modes include:

- UV and Visible Absorbance (Abs), see ABS Read Mode on page 108.
- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.
- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 109.
- Fluorescence Polarization (FP), see FP Read Mode on page 110.
- Fluorescence Resonance Energy Transfer (FRET), see FRET Mode on page 110.

Features of the FilterMax F5 Multi-Mode Microplate Reader include:

- Absorbance detection in the UV and visible range (230 nm to 650 nm)
- Fluorescence Intensity (FL) top read and bottom read
- Endpoint, kinetic, multiple wavelength, linear scan, and area scan read types
- PathCheck® Pathlength Measurement Technology
- Automatic z-height optimization (top read)
- Temperature Control
- Comprehensive Data Analysis with SoftMax Pro Software

The filters used to perform measurements are mounted on two types of interchangeable slides. One slide is reserved for excitation filters used in absorbance and fluorescence measurements; the other is used for emission filters used in fluorescence and some luminescence measurements. Each slide can hold up to six filters. For more information, see Configuring Filter Slides on page 60.

FilterMax F3 Multi-Mode Microplate Reader

The FilterMax™ F3 Multi-Mode Microplate Reader is ideal for a broad range of applications, including drug discovery, genomics, proteomics, and cell-based research. The unique patented design ensures precise performance and sensitivity across all detection modes.

Detection modes include:

- Visible Absorbance (Abs), see ABS Read Mode on page 108.
- Fluorescence Intensity (FL), see FL Read Mode on page 109.
- Luminescence (LUM), see LUM Read Mode on page 109.
- Fluorescence Resonance Energy Transfer (FRET), see FRET Mode on page 110.

Features of the FilterMax F3 Multi-Mode Microplate Reader include:

- Absorbance detection in the visible range (340 nm to 650 nm)
- Endpoint, kinetic, multiple wavelength, linear scan, and area scan read types
- Comprehensive Data Analysis with SoftMax Pro Software

The filters used to perform measurements are mounted on two types of interchangeable slides. One slide is reserved for excitation filters used in absorbance and fluorescence measurements; the other is used for emission filters used in fluorescence and some luminescence measurements. Each slide can hold up to six filters. For more information, see Configuring Filter Slides on page 60.

DTX 800 and DTX 880 Multi-Mode Microplate Readers

The DTX 800 and DTX 880 Multi-Mode Microplate Readers are earlier models of the FilterMax F3 and F5 Multi-Mode Microplate Readers. For information about the DTX 800 and DTX 880 Multi-Mode Microplate Readers, refer to information in this documentation regarding the FilterMax F3 and F5 Multi-Mode Microplate Readers.

- For information about the DTX 800 Multi-Mode Microplate Reader, see FilterMax F3 Multi-Mode Microplate Reader on page 121.
- For information about the DTX 880 Multi-Mode Microplate Reader, see FilterMax F5 Multi-Mode Microplate Reader on page 120.

Vmax Kinetic ELISA Microplate Reader

The Vmax® Kinetic ELISA Microplate Reader measures optical density (OD) and can conduct a kinetic analysis, the rate of optical density change per minute (milliOD/min). The optical array is designed to analyze the absorbance of multiple sample sites with 9 mm centers. Read one sample or up to 96 at a time. Use any standard 96-well microplate.

Detection mode includes:

- Visible Absorbance (Abs), see ABS Read Mode on page 108.

Vmax Kinetic ELISA Microplate Reader features include:

- Filter range: 400 nm to 750 nm
- Filter capacity: 6
- Optical density: single wavelength and dual wavelength

Emax Endpoint ELISA Microplate Reader

The Emax® Endpoint ELISA Microplate Reader measures optical density (OD). The optical array is designed to analyze the absorbance of multiple sample sites with 9 mm centers. Read one sample or up to 96 at a time. Use any standard 96-well microplate.

Detection mode includes:

- Visible Absorbance (Abs), see ABS Read Mode on page 108.

Emax Endpoint ELISA Microplate Reader features include:

- Filter range: 400 nm to 750 nm
- Filter capacity: 6
- Optical density: single wavelength and dual wavelength

SpectraMax Paradigm Multi-Mode Detection Platform

The SpectraMax® Paradigm® Multi-Mode Detection Platform is a modular multi-mode microplate reader. User-installable and removable detection cartridges allow the reader to be configured for specific applications and easily expand the capabilities of the reader at any time. The measurement capabilities of the reader are defined by the installed detection cartridges.

Up to six detection cartridges can be installed in each of the two detection cartridge drawers of the SpectraMax Paradigm Multi-Mode Detection Platform.

Every detection cartridge starts with an excitation energy source either in the form of long-life, high-powered LED, a xenon flash lamp, or a laser diode. By concentrating the excitation energy on the sample through label-specific optics, high levels of sensitivity and performance are reached.

For information about the different detection cartridges, see SpectraMax Paradigm Detection Cartridges on page 123.

Features of the SpectraMax® Paradigm® Multi-Mode Detection Platform include:

- Array of detection cartridge capabilities
- Automatic Z-height optimization (for both top and bottom read)
- Patent-pending LED intensity adjustment
- Up to 1536-well format
- Temperature control
- Monochromator and filter based options available
- PathCheck® Pathlength Measurement Technology
- AlphaScreen

SpectraMax Paradigm Detection Cartridges

The SpectraMax Paradigm Multi-Mode Detection Platform supports the following detection cartridges:

- Absorbance Detection Cartridge on page 124
- Tunable Wavelength (TUNE) Detection Cartridge on page 125
- Multi-Mode (MULTI) Detection Cartridge on page 126
- AlphaScreen Detection Cartridges on page 127
- Cisbio HTRF Detection Cartridge on page 127
- Time Resolved Fluorescence (TRF) Detection Cartridge on page 128
- Fluorescence Intensity (FI) Detection Cartridges on page 128
- Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge on page 129

Absorbance Detection Cartridge

The SpectraMax® Paradigm® Absorbance Detection Cartridge combines wavelength scanning and a broad spectrum wavelength range necessary to address a variety of nucleic acids, proteins, ELISAs and immunoassays found in the laboratory. The Absorbance Detection Cartridge uses a monochromator to perform absorbance endpoint, kinetic, well scan, and spectrum read types (wavelength scanning) measurements.

The Absorbance Detection Cartridge consists of two components: a detection component which measures the absorbance, and an excitation component which sets the absorbance wavelengths. The detection component is installed in the top read detection cartridge drawer and occupies one (1) detection cartridge slot. The excitation component is installed in the bottom read detection cartridge drawer and occupies two (2) detection cartridge slots. Both components must be installed to perform absorbance measurements with the SpectraMax Paradigm Multi-Mode Detection Platform.

Typical Applications

- Nucleic Acid Quantitation
- Protein Quantitation
- ELISA
- Immunoassay
- Proliferation/Viability

Tunable Wavelength (TUNE) Detection Cartridge

The SpectraMax® Paradigm® Tunable Wavelength (TUNE) Detection Cartridge is a detection cartridge for use with the SpectraMax Paradigm Multi-Mode Detection Platform. Installing the Tunable Wavelength (TUNE) Detection Cartridge enables several detection modes, including:

- Fluorescence Intensity (FL)
- Time-Resolved Fluorescence (TRF)
- Glow Luminescence (LUM)

The spectral optimization feature of the Tunable Wavelength (TUNE) Detection Cartridge can help to get the maximum signal to background ratio for virtually any fluorophore or luminescence label compatible with the wavelength ranges.

In addition, the Tunable Wavelength (TUNE) Detection Cartridge can perform a Spectrum read to apply either excitation or emission scanning.

The Tunable Wavelength (TUNE) Detection Cartridge occupies three (3) slots. It can be installed in either the top read detection cartridge drawer for top reading, or in the bottom read detection cartridge drawer for bottom reading.

Multi-Mode (MULTI) Detection Cartridge

Optimized for 96-well and 384-well plates, the SpectraMax® Paradigm® Multi-Mode (MULTI) Detection Cartridge provides a broad array of measurement capabilities:

- Fluorescence Intensity (FL)
- Time-Resolved Fluorescence (TRF)
- Luminescence (LUM)

Typical Applications

- Proliferation/Viability
- Nucleic Acid Quantitation
- cAMP Quantitation
- GPCR
- Immunoassay
- Ion Channel
- Reporter

Typical Fluorophores

- Coumarin
- Fluorescein
- Rhodamine
- Texas Red
- Europium (TRF only)

Note: This list does not represent a complete list of applications or labels which can be performed by the Multi-Mode (MULTI) Detection Cartridge.

AlphaScreen Detection Cartridges

SpectraMax® Paradigm® AlphaScreen® Detection Cartridges utilize a 680 nm laser diode to provide a sensitive reading system for AlphaScreen assays. In addition, a patent pending design that isolates each well enables optimal performance for AlphaScreen assays.

Typical Applications

The AlphaScreen Detection Cartridge provides optimal performance for key applications in drug discovery:

- GPCR Assays
- Cytokine Assays
- cAMP Quantitation
- Kinase Assays

ALPHASCREEN is a registered trademark of PerkinElmer, Inc.

Cisbio HTRF Detection Cartridge

The SpectraMax® Paradigm® Cisbio HTRF® Detection Cartridge uses a high-energy Xenon flash lamp for sensitive reading of HTRF reagents. In addition, the standard dual emission design gives the most accurate results in short overall read time.

Typical Applications

The Cisbio HTRF Detection Cartridge provides optimal performance for key applications for drug discovery, bioprocess development, and others.

- GPCR Assays
- Cytokine Assays
- cAMP Quantitation
- Human mAb Screening
- Kinase Assays

HTRF is a registered trademark of Cisbio Bioassays.

The fluorescence ratio associated with the HTRF readout is a correction method developed by CisBio and covered by the US patent 5,527,684 and its foreign equivalents, for which CisBio has granted a license to Molecular Devices. Its application is strictly limited to the use of HTRF reagents and technology, excluding any other TR-FRET technologies such as IMAP TR-FRET calculations of acceptor to donor ratios.

Time Resolved Fluorescence (TRF) Detection Cartridge

The SpectraMax® Paradigm® Time Resolved Fluorescence (TRF) Detection Cartridge is the benchmark against which all other readers will be measured. Built to measure Europium and Samarium, it is ideal for your protein interaction, GPCR, and enzyme activity applications.

Typical Applications

- cAMP Quantitation
- Immunoassays
- GTP Binding
- Apoptosis
- GPCR Ligand Binding

Typical Fluorophores

- Europium Chelates
- Samarium Chelates

Note: These lists do not represent a complete list of applications or labels that can be performed by the Time Resolved Fluorescence (TRF) Detection Cartridge.

Fluorescence Intensity (FI) Detection Cartridges

The SpectraMax® Paradigm® Fluorescence Intensity (FI) Detection Cartridges provide speed, sensitivity, and flexibility. The cartridge design provides uncompromised performance for both top and bottom reads. In addition, the standard dual-emission design enables simple, straightforward FRET (fluorescence resonance energy transfer) measurement.

Typical Applications

- Nucleic Acid Quantitation
- Molecular Interaction
- Ion Channel
- Protein Quantitation
- Reporter Gene Assays
- Apoptosis
- Proliferation/Viability

Note: This list does not represent a complete list of applications or labels which can be performed by the Fluorescence Intensity (FI) Detection Cartridges.

Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge

The SpectraMax® Paradigm® Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge is designed for use with Invitrogen GeneBLAzer assays. The cartridge design provides uncompromised performance for both top and bottom reads. In addition, the standard dual-emission design enables simple, straightforward FRET (fluorescence resonance energy transfer) measurement.

Typical Applications

Cell Based assays:

- GPCR Assays
- Ion Channel
- Nuclear & Cytokine Receptor Assays
- Signal Transduction Pathways
- Transcriptional Regulators (Kinases)

Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge

The innovative SpectraMax® Paradigm® Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge is designed for use with Promega's Multi-Tox Fluor assays. It uses two high-powered LEDs to provide fast, sensitive, simultaneous reads of two distinct labels.

In a dual color method, dual labels (such as gene reporters) causing the emission of different wavelength bands at the same time are measured.

Typical Applications

The Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge provides optimal performance for key applications for Drug Discovery, Cancer Research, ADME-Tox studies, and others.

Fluorescence Polarization (FP) Detection Cartridge

The SpectraMax® Paradigm® Fluorescence Polarization (FP) Detection Cartridge takes full advantage of the standard dual photomultiplier tube design of the SpectraMax Paradigm System to collect all the necessary data in a single read of a microplate. The resulting speed and performance are exceptional.

Typical Applications

- cAMP Quantitation
- Genotyping (AcycloPrime)
- Immunoassay
- Molecular Interaction
- Nuclear Receptor

Typical Fluorophores

- Fluorescein
- FP - green
- Rhodamine
- FP - red-shifted dye

Note: This list does not represent a complete list of applications or labels that can be performed by the Fluorescence Polarization (FP) Detection Cartridge.

Glow Luminescence (LUM) Detection Cartridges

The SpectraMax® Paradigm® Glow Luminescence (LUM) Detection Cartridges use a patent-pending design that isolates each well to provide optimal performance for glow-luminescence applications.

The three Glow Luminescence (LUM) Detection Cartridges are each optimized for a popular plate format.

Typical Applications

- Apoptosis
- cAMP Quantitation
- GPCR Ligand Binding
- GTP Binding
- Immunoassay

Note: This list does not represent a complete list of applications or labels that can be performed by the Glow Luminescence (LUM) Detection Cartridges.

Dual Color Luminescence (LUM) (BRET2) Detection Cartridge

The SpectraMax® Paradigm® Dual Color Luminescence (LUM) (BRET2™) Detection Cartridge uses a patent-pending design that isolates each well to provide optimal performance for chemiluminescence applications. In addition, the standard dual emission design gives most accurate results in short overall read time.

In a dual color method, dual labels (such as gene reporters) causing the emission of different wavelength bands at the same time are measured.

The Dual Color Luminescence (LUM) (BRET2) Detection Cartridge is designed to provide optimal performance when used with PerkinElmer's BRET2 reagents. When inserted, the template protocols are enabled with predefined data reduction such as blank subtraction, green to blue ratio, and determination of the Z' factor.

BRET2 is a trademark of PerkinElmer, Inc.

Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge

The SpectraMax® Paradigm® Dual Color Luminescence (LUM) (Chroma-Glo™) Detection Cartridge uses a patent-pending design that isolates each well to provide optimal performance for chemiluminescence applications. In addition, the standard dual emission design gives most accurate results in short overall read time.

In a dual color method, dual labels (such as gene reporters) causing the emission of different wavelength bands at the same time are measured.

Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge contains emission filters designed for Promega Chroma-Glo reagents.

Chroma-Glo is a trademark of Promega Corporation, Madison, WI, USA.

StakMax Microplate Handling System

The StakMax® Microplate Handling System is an integrated microplate stacker for use with Molecular Devices microplate readers, providing simple, powerful, walk-away benchtop automation.

The StakMax Software is accessed from the **Operations** tab in the ribbon. When you click the **Plate Stacker** button, the StakMax Software starts and automatically connects to the StakMax Microplate Handling System. The current status of the instrument appears in the status bar of the StakMax Software window.

CAUTION: To prevent data loss, make sure that **Auto Save** is enabled for each protocol you want to run before you start the StakMax Software to run automated reads. See Setting Auto Save Options on page 190.

The StakMax Microplate Handling System is compatible with the following microplate readers:

- SpectraMax M2 and M2e Multi-Mode Microplate Readers
- SpectraMax M3 Multi-Mode Microplate Reader
- SpectraMax M4 Multi-Mode Microplate Reader
- SpectraMax M5 and M5e Multi-Mode Microplate Readers
- Gemini™ EM and XPS Fluorescence Microplate Readers
- SpectraMax Paradigm Multi-Mode Detection Platform

In addition, newer models of the following readers are compatible with the StakMax Microplate Handling System:

- SpectraMax Plus 384 Absorbance Microplate Reader
- SpectraMax 340PC 384 Absorbance Microplate Reader
- SpectraMax 190 Absorbance Microplate Reader

- VersaMax ELISA Microplate Reader

For more information about using the StakMax Microplate Handling System, see the StakMax *Microplate Handling System User Guide* or the help available in the StakMax Software.

Instrument Settings

Use the **Settings** dialog to configure all parameters for a plate or cuvette read, such as the read type (End-point, Kinetic, Fast Kinetic, Spectrum, Well Scan), the read mode (Absorbance, Fluorescence, Luminescence, Fluorescence Polarization, Time Resolved Fluorescence), wavelength settings, Shake parameters, PathCheck, Auto-Read, timing for Kinetic runs, Speed Read for Spectrum scans, and plate type.

Each Plate or Cuvette Set section can have a different instrument setup.

The available options in the **Settings** dialog depend on the type of instrument that is selected.

Settings include the following:

- Wavelength Settings on page 134
- Plate Type Library on page 135
- Read Area on page 136
- Shake Settings on page 136
- Sensitivity Settings on page 137
- Timing on page 137
- PathCheck Pathlength Measurement Technology on page 138
- More Settings on page 143
- Well Scan Settings on page 144
- Cuvette Reference on page 142
- TRF Settings on page 144
- Background Constant Subtraction on page 141
- Interfering Substances on page 140
- Settling Time on page 144
- Speed Read on page 144
- Column Wavelength Priority on page 145
- Paradigm Detection Cartridge Settings on page 145

Wavelength Settings

Wavelength settings vary depending on the selected instrument, read mode, and read type.

Procedures to optimize excitation and emission wavelengths for a given protocol are described in the instrument user guides for the readers that support this function. For more information, see those user guides.

For Endpoint or Kinetic Readings

Choose the number of wavelengths to read.

Specify the number of wavelengths and type any wavelength within the available range of the instrument in the wavelength settings fields.

For filter-based instruments, select from the available filters.

For Spectrum Readings

Choose a Start and a Stop wavelength, and a wavelength step for each increment between reads.

The minimum selectable increment is 1 nm.

Cutoffs

The choices in this portion of the Wavelengths setting depend upon the selections for read type (Endpoint, Kinetic, Spectrum, or Well Scan) and read mode (Fluorescence, Fluorescence Polarization, or Time Resolved Fluorescence). The term cutoff refers to filters used to block unwanted residual excitation light and minimize background interference.

No emission cutoff filter is used when Luminescence is selected. With other read types, choices are to enable or disable Auto Cutoff with specific filter settings available if it is disabled (manual cutoff selection). When Auto Cutoff is enabled, the instrument sets the cutoffs based upon the wavelengths chosen for reading; when Spectrum is selected as the read type, however, a manual setting for the emission monochromator is required, with the default being no cutoff filter.

Determining a manual setting for a cutoff filter is based upon the known value of the Stokes shift, which is the difference between the wavelengths of the excitation and emission maxima. If the Stokes shift is small, it is advisable to choose an excitation wavelength that is as far as possible from the emission maximum while still being capable of exciting the fluorophore. Doing this causes less of the excited light to overlap the emission spectrum, allowing better selection and quantitation of the emitted light. See the individual instrument user guides for more information regarding cutoff filter settings.

Plate Type Library

The Plate Library includes entries for microplates of various sizes with standard dimensions.

If you are uncertain which plate to select, then select the Standard plate definition.

You can choose to edit the dimensions of a plate or add a custom plate to the plate library.

- To edit the dimensions of a plate in the library, select the plate from the list and click **Edit Plate**.
- To add a new plate to the library, select any plate from the list and click **Edit Plate**. Type a name for the new plate in the **Plate Name** field of the **Plate Editor** dialog.
- To include a plate from a predefined plate description, click **Import Plate** and use the file system browser to locate the plate description file.

Note: The **Import Plate** option is available only for the SpectraMax Paradigm Multi-Mode Detection Platform and the FilterMax F3 and F5 Multi-Mode Microplate Readers.

- To delete a plate from the plate library, select the plate to be removed and click **Remove**.

For information about entering custom plate dimensions, see Using the Plate Editor on page 73.

When using a SpectraMax Paradigm Multi-Mode Microplate Detection Platform you have plate orientation options.

Choose the plate orientation to match the orientation of the plate in the plate drawer.

- **Landscape** puts the A1 location in the upper-left corner closest to the instrument.
- **Portrait** puts the A1 location in the upper-right corner closest to the instrument.
- **Opposite Landscape** puts the A1 location in the lower-right corner farthest from the instrument.
- **Opposite Portrait** puts the A1 location in the lower-left corner farthest from the instrument.

Plate Type setting determines the display of wells in the microplate—it should be set to match the type and number of wells in the microplate to be read.

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

Read Area

You can choose to read a subset of wells or columns of wells in the microplate. Partial-plate reading is available for all read types, although it is most useful for fast Kinetic analysis. The time required for Kinetic readings can be significantly reduced using this setting since the instrument does not have to read the entire plate.

To enable a partial-plate read, highlight the columns to be read. Columns do not need to start with "1" but must be contiguous.

If you enable a partial-plate reading, only the wells to be read are visible in the data display, indicating that no data will be collected for the other wells (all wells are present in the Template Editor, however).

Shake Settings

Shake is a feature that allows you to set automated shaking of the microplate. The options available for Shake depend on the read type chosen and the type of instrument.

Before First Read

This setting can be used with any read type. Whether you read at a single wavelength or at multiple wavelengths, checking Before First Read shakes the plate for the set amount of time before the first wavelength reading only.

Between Reads

This option is available only when Kinetic read is selected.

For readings at a single wavelength, Between Reads shakes the plate for the specified amount of time prior to each reading at that wavelength.

For readings at multiple wavelengths, enabling Between Reads shakes the plate for the specified amount of time before each reading at the first wavelength only.

SpectraMax Paradigm Microplate Shaking Options

The SpectraMax Paradigm Multi-Mode Detection Platform can perform microplate shaking operations inside the microplate chamber as part of a measurement protocol.

Microplate shaking options include:

- Intensity: Low, Medium, or High. Actual shake speed is based on the microplate format.
- Direction: Linear or Orbital patterns.
- Duration: Length of time in seconds (1 to 60) to shake.

Sensitivity Settings

PMT sensitivity can be set to **Low**, **Medium**, or **High**. To get optimal PMT response without saturation, set the PMT gain to **High** for samples having lower concentration (dim samples), or use a lower PMT gain for samples having a higher concentration (bright samples).

To define the exact voltage to use for the **PMT Gain**, select **Manual** and then type a value in the field between 200 volts and 1000 volts.

In Endpoint reads, an **Automatic** setting is available allowing the instrument to adjust the PMT voltage automatically for varying concentrations of sample in the plate.

Automatic is not available for Fluorescence Polarization reads.

For the SpectraMax M5 and M5e readers, the PMT Sensitivity is always set to Automatic for Luminescence and Time Resolved Fluorescence read modes.

Timing

For Kinetic reads you can configure the total Kinetic run time and the time interval between readings.

After entering a total run time and an interval between readings, the total number of readings is calculated and reported. The minimum read interval is determined by the instrument, and depends on many factors including the number of wavelengths, the number of wells being read, and the distance the instrument's filter wheel must move.

To achieve the shortest possible interval for Kinetic readings, choose wavelengths in ascending order.

PathCheck Pathlength Measurement Technology

The (missing or bad snippet) normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

Microplate readers use a vertical light path so the distance of the light through the sample depends on the volume. This variable pathlength makes it difficult to perform extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a cuvette is the conventional basis for quantifying the unique absorbtivity properties of compounds in solution. Quantitative analysis can be performed on the basis of extinction coefficients, without standard curves (for example, NADH-based enzyme assays). When using a cuvette, the pathlength is known and is independent of sample volume, so absorbance is proportional to concentration.

In a microplate, pathlength is dependent on the liquid volume, so absorbance is proportional to both the concentration and the pathlength of the sample. Standard curves are often used to determine analyte concentrations in vertical-beam photometry of unknowns, yet errors can still arise from pipetting the samples and standards. The PathCheck technology automatically determines the pathlength of aqueous samples in the microplate and normalizes the absorbance in each well to a pathlength of 1 cm. This approach to correcting the microwell absorbance values is accurate to within 2.5% of the values obtained directly in a 1 cm cuvette.

The 1 cm values can be obtained by using the factory installed *Water Constant* or by reading a cuvette containing deionized water, or buffer, at the time that the microplate is read, creating a *Cuvette Reference* for the read. PathCheck technology is used to normalize the data acquired from absorbance endpoint microplate readings to a 1 cm pathlength, correcting the OD for each well to the value expected if the sample were read in a 1 cm cuvette.

The Water Constant correction method is supported by all readers that have PathCheck technology enabled.

The PathCheck technology is based on the absorbance of water in the near infrared spectral region (between 900 nm to 1000 nm). If the sample is completely aqueous, has no turbidity and has a low salt concentration (less than 0.5 M), the Water Constant is adequate. The Water Constant is determined during manufacture and is stored in the instrument.

The Cuvette Reference correction method is supported by the SpectraMax M2, M2e, M3, M4, M5, M5e, and Plus 384 readers.

If the sample contains an organic solvent such as ethanol or methanol, we recommend using the Cuvette Reference. It is important that the solvent does not absorb in the 900 nm to 1000 nm range. To determine whether or not a given solvent would interfere, see the discussion of interfering substances in Interfering Substances on page 140. When a non-interfering solvent is added to the aqueous sample, the water absorbance decreases proportionally to the percentage of organic solvent present. For example, 5% ethanol decreases the water absorbance by 5% and results in a 5% underestimation of the pathlength. You can minimize the error by putting the same water/solvent mixture in a cuvette and using the Cuvette Reference.

To use the Cuvette Reference, place into the cuvette port a standard 1 cm cuvette containing the aqueous/solvent mixture that is used for the samples in the microplate. The cuvette must be in place when you read the microplate. When you click the Read button, the instrument first makes the 900 nm and 1000 nm measurements in the cuvette, and then makes the designated measurements in the microplate. The cuvette values are stored temporarily and used in the PathCheck calculations for the microplate samples.

Use of Cuvette Reference with the PathCheck Pathlength Measurement Technology is different from a reference reading of a cuvette in a CuvetteSet section (by clicking the Ref button in the CuvetteSet section tool bar). The cuvette reference used for PathCheck calculations (measurements at 900 nm and 1000 nm) does not produce data that can be directly viewed in a CuvetteSet section and is used only with data in microplates, not cuvettes. However, you can obtain these values using accessors in the formula editor. See the !PathCheckLm1000 and !PathCheckLm900 accessor described in the *Formula Reference Guide* help.

Note: After you have read a plate with PathCheck technology turned on, PathCheck information is stored permanently in the data file. You have the option of applying, or not applying, PathCheck technology to the absorbance values. If you do not have PathCheck technology turned on during the plate read, you cannot apply the PathCheck Pathlength Measurement Technology feature after the read.

Interfering Substances

Any material that absorbs in the 900 nm to 1000 nm spectral region could interfere with PathCheck program measurements. Fortunately, there are few materials that do interfere at the concentrations typically used.

Turbidity is the most common interference: if you can detect any turbidity in your sample, you should not use the PathCheck Pathlength Measurement Technology. Turbidity elevates the 900 nm measurement more than the 1000 nm measurement and causes an erroneously low estimate of pathlength. Using Cuvette Reference does not reliably correct for turbidity.

Samples that are highly colored in the upper visible spectrum might have absorbance extending into the near infrared (NIR) and can interfere with the PathCheck Pathlength Measurement Technology. Examples include Lowry assays, molybdate-based assays and samples containing hemoglobins or porphyrins. In general, if the sample is distinctly red or purple, you should check for interference before using the PathCheck program.

To determine possible color interference, do the following:

- Measure the OD at 900 nm and 1000 nm (both measured with air reference).
- Subtract the 900 nm value from the 1000 nm value.

Do the same for pure water.

If the delta OD for the sample differs significantly from the delta OD for water, then it is advisable not to use the PathCheck Pathlength Measurement Technology. Use of Cuvette Reference (on SpectraMax Plus, Plus 384, M2, M2e, M5, M5e readers) does not correct for the interference with the current calculation scheme in SoftMax Pro Software. Currently, Cuvette Reference involves a single (automated) read at 900 nm and 1000 nm and the automated calculations in SoftMax Pro Software do not compensate for color or solvent interference. However, you could correct for such interference by taking two cuvette measurements and using a different set of calculations. For further information, contact Molecular Devices Technical Support.

Organic solvents could interfere with the PathCheck program if they have absorbance in the region of the NIR water peak. Solvents such as ethanol and methanol do not absorb in the NIR region, so they do not interfere, except for causing a decrease in the water absorbance to the extent of their presence in the solution. Their passive interference can be avoided by using the Cuvette Reference. If, however, the solvent absorbs between 900 and 1000 nm, the interference would be similar to the interference of highly colored samples described above. If you are considering adding an organic solvent other than ethanol or methanol, you are advised to run a Spectrum scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck program.

Background Constant Subtraction

Raw OD measurements of microplate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of microplate material). The latter must be eliminated from the PathCheck program calculation to get valid results that have been normalized by the PathCheck program. There are two ways to accomplish this, described below, beginning with the easiest.

Use a Plate Blank

This method can only be used if all samples in the microplate are the same volume and you are not depending on the PathCheck program to correct for variability in volumes.

To use this method:

1. Designate at least one well (preferably several) as Plate Blank. See Blanking on page 157.
2. Pipette buffer (for example, your sample matrix) into those wells and read along with your samples. SoftMax Pro Software software automatically subtracts the average of the blank wells from each of the samples. The OD of the microplate material is subtracted as part of the blank.
3. Make sure that **Use Plate Blank** is checked under **Other Options** in the **Data Reduction** dialog.

Use Plate Background Constant

If your sample volumes are not identical or if you choose not to use a Plate Blank, then you can use the Plate Background Constants.

To determine Plate Background Constants:

1. Fill a clean microplate with water.
2. Read at the wavelengths you will be reading your samples.
3. The average OD value is the Plate Background Constant.
4. If you intend to read your samples at more than one wavelength, there should be a corresponding number of Background Constants.

Note: It is important that you put water in the wells and not read a dry plate for the Background Constant. Dry plates have a slightly higher OD value than a water-filled plate because of differences in refractive indices. Using a dry plate results in PathCheck normalized values that are lower than 1 cm cuvette values. Omitting the Background Constant results in values that have been normalized by the PathCheck program and are higher than 1 cm cuvette values.

Cuvette Reference

PathCheck Pathlength Measurement Technology is based on the absorbance of water in the near infrared region (between 900 nm and 1000 nm). If the sample is completely aqueous, has no turbidity and has a low salt concentration (less than 0.5 M), the Water Constant is adequate. The Water Constant is determined during manufacture and is stored in the instrument.

If the sample contains an organic solvent such as ethanol or methanol, we recommend using the cuvette reference. It is important that the solvent does not absorb in the 900 nm to 1000 nm range (to determine whether or not a given solvent would interfere, see the discussion of interfering substances below). When a non-interfering solvent is added to the aqueous sample, the water absorbance decreases proportionally to the percentage of organic solvent present. For example, 5% ethanol decreases the water absorbance by 5% and results in a 5% underestimation of the pathlength. You can avoid the error by putting the same water/solvent mixture in a cuvette and using the Cuvette Reference.

To use the Cuvette Reference, place into the cuvette port a standard 1 cm cuvette containing the aqueous/solvent mixture that is used for the samples in the microplate. The cuvette must be in place when you read the microplate. When you click the Read button, the instrument first makes the 900 nm and 1000 nm measurements in the cuvette, and then makes the designated measurements in the microplate. The cuvette values are stored temporarily and used in the PathCheck program calculations for the microplate samples.

Use of Cuvette Reference with PathCheck software is different from a reference reading of a cuvette in a Cuvette Set section (by clicking the Ref button in the Cuvette Set section tool bar). The cuvette reference used for PathCheck calculations (measurements at 900 nm and 1000 nm) does not produce data that can be viewed in a Cuvette Set section and is used only with data in microplates, not cuvettes.

After you have read a plate with the PathCheck program turned on, PathCheck application information is stored permanently in the data file. You have the option of applying, or not applying, the PathCheck application to the absorbance values as you choose. If you did not have the PathCheck program turned on during the plate read, you cannot apply the PathCheck program after the read.

More Settings

The **More Settings** tab in the **Settings** dialog provides settings options that are not otherwise related to the other settings tabs.

The content of this tab varies depending on the selected instrument, read mode, and read type.

Calibrate

For absorbance reads, calibrate makes an air calibration measurement at each wavelength before reading the microplate. If you turn calibration off, SoftMax Pro uses the calibration values stored in the firmware of the instrument.

If **Calibrate** is set to **On** in the **More Settings** tab, the wavelengths specified in the protocol are calibrated before each read.

Carriage Speed

Carriage Speed can be slowed down to accommodate samples (such as, fragile clots) that could be disrupted by rapid carriage movement.

Read Order

Read Order determines the order that wavelengths are read:

- Wavelength Priority reads the entire plate at the first wavelength, and then reads the entire plate at the second wavelength, and so on.
- Column Priority reads column 1 at all wavelengths, then reads column 2 at all wavelengths, and so on.

Settling Time

The selected delay is added between the reading of each column. There is no delay between each well in a column.

Well Scan Settings

A Well Scan read takes one or more readings of a single well of a microplate at single or multiple wavelengths. Every option available for Endpoint reads is available for Well Scans.

Values reported are optical density, %Transmittance, relative fluorescence units (RFU), or relative luminescence units (RLU).

You can specify the scanning pattern, and the scanning density. Only odd numbers of points are included in density settings.

Four scanning patterns are available:

- a horizontal line
- a vertical line
- a cross (which is a combination of a horizontal and vertical line)
- a fill pattern

The density setting determines:

- The number of points read in the line patterns.
- The maximum number of horizontal and vertical points included in the grid pattern.
- With a grid pattern, all points that land within 2.8 mm of an edge are excluded.
- The minimum number of points is 3 for most plate sizes, while the maximum number of points depends on the well diameter; for 384-well plates, only one read per well is allowed.

TRF Settings

TRF measures fluorescence as a function of time after excitation. You can set a time delay before collecting data and the integration time of the fluorescence signal.

Settling Time

Settling Time specifies a well-to-well read delay. After the read head moves to the next well, the read is delayed by the settling time to allow the fluid in the microplate wells to settle, thereby minimizing sloshing.

Speed Read

Enable Speed Read for an Absorbance read to read the plate faster by substituting firmware values for calibration values and by decreasing the number of lamp flashes.

Speed Read turns off Calibration and uses the air calibration values stored in the instrument. As a result, Speed Read might not provide as accurate an absorbance measurement at each wavelength of a scan as with the normal read.

Column Wavelength Priority

Choosing column or wavelength priority determines the order in which a plate is read in multi-wavelength readings:

- In column priority, the instrument reads all wavelengths for the first column of wells in the microplate first, and then reads all wavelengths for the second column, third column, etc.
- In wavelength priority, an instrument reads the entire microplate (or chosen number of strips) at the first wavelength, and then goes back and reads the microplate at the second and any additionally selected wavelengths.

Paradigm Detection Cartridge Settings

On the Fly Detection

On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to **Off-stop and go** mode where the plate stops moving for each read.If using on-the-fly detection, specify whether the detection method should be optimized for **Performance** or **Speed** using the On the Fly Detection menu.Selecting **Performance** results in a faster read time than not using On the Fly Detection, but not as fast as the Speed mode. Performance provides considerably better results than Speed for demanding assays. Selecting **Speed** results in the fastest possible read time per plate. However there is a trade-off between the data quality and read speed because each well is sampled for shorter integration times.

Integration Time

Type a value in the **Integration Time** field in milliseconds, to specify the measurement time per well to be validated.

Attenuation

Very bright samples can exceed the linear dynamic range of the detection system. If such is the case, reading can be performed using an attenuation filter.

Microplate Read Order

- **Row** reads plates row-by-row.
- **Column** reads plates column-by-column.
- **Well** reads each well individually before reading the next well, which is useful for short-interval kinetic, area scan, and wavelength scan measurements.

Show Pre-Read Optimization Options

Selecting **Show Pre-Read Optimization Options** causes the **Pre-Read Optimization Options** dialog to open before the microplate is read. The available optimization options are dependant on the read mode.

Microplate Optimization

Microplate dimensions can vary slightly between production lots, which potentially affects measurement accuracy. SoftMax Pro Software allows labware dimensions to be optimized by determining the centers of the four corner wells on the plate. Each time a labware type is optimized before a read, a new plate definition is added to the Plate Library with dimensions from the labware optimization.

If a microplate type is to be used in different plate orientations for measurements, labware optimization must be done for each plate orientation separately.

Read Height Optimization

The SpectraMax Paradigm Multi-Mode Microplate reader features an objective lens that can be moved up and down to optimize the read height used in luminescence, fluorescence intensity top, fluorescence polarization, and time-resolved fluorescence protocols. Read height is the distance between the top (using top reading) or bottom (using bottom reading) surface of the microplate being read and the surface of the objective lens. Optimizing read height matches the focus of the optics with the sample volume. This maximizes the raw signal, which yields the highest precision and maximum sensitivity.

Collecting Data

4

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

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.
3. Choose a data acquisition protocol. You can use an existing protocol or create a new one.
   - To use an existing protocol, see Opening a Protocol File on page 33.
   - To set up a new protocol, see Creating a Protocol on page 147.
4. Prepare the microplate or the cuvettes and insert it into the instrument.
5. Click the **Read** button to read the microplate or the cuvette.
6. Analyze the data. See Analyzing Data on page 205.
7. Save the acquired data, see Saving Data Files on page 28.

Creating a Protocol

Protocols contain instrument settings, template maps, Data Reduction and Display choices. Templates are maps to the contents of a plate or cuvettes, and how the data will be collected and displayed.

You manage protocols using the **Protocol Manager** tab on the application ribbon. See Using the Protocols Tab to Manage Protocols on page 50.

You can create as many different protocols as you need. And, you can create a new protocol by modifying an existing one and saving it under a new name.

To create a protocol, follow these steps:

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select the instrument to be used with the protocol. See Selecting and Connecting to an Instrument on page 12.
3. Define the instrument settings to use with the protocol. See Selecting Instrument Settings on page 148.
4. Create a template, if applicable. See Configuring a Microplate Template on page 150.
5. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
6. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.
7. Set Display options, if applicable. See Viewing the Data Display on page 206.

Selecting Instrument Settings

To select instrument settings:

1. Click on a plate or cuvette section to make it active in the workspace.
2. Click the **Settings**  button on the **Home** tab.
3. When working with a SpectraMax Paradigm instrument, select a detection cartridge. See Selecting the SpectraMax Paradigm Detection Cartridge on page 149.
4. Select a **Read Mode**. See Selecting the Read Mode on page 149.
5. Select a **Read Type**. See Selecting the Read Type on page 149.
6. Click each of the category tabs and change the default settings as needed.
7. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.

To continue creating the protocol see the following:

- Create a template, if applicable. See Configuring a Microplate Template on page 150.
- Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
- Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

Connecting to an Instrument

To select and connect to an instrument see, Selecting and Connecting to an Instrument on page 12.

Selecting the SpectraMax Paradigm Detection Cartridge

1. Select the SpectraMax Paradigm instrument using the **Instrument Connection** dialog. See Selecting and Connecting to an Instrument on page 12.
2. Click on a plate section to make it active in the workspace.
3. Click the **Settings** button.
4. Select a **Cartridge** from those shown at the top of the **Settings** dialog.

Note: When you select to work **Offline** in the Instrument Connection dialog, you can see all the cartridges that the SpectraMax Paradigm Multi-Mode Detection Platform can support.

When you are actually connected to the SpectraMax Paradigm instrument, you see only the cartridges that are installed in the instrument.

Selecting the Read Mode

1. Click on a plate section to make it active in the workspace.
2. Click the **Settings** button in the **Plate Tools** on the **Home** tab or click the **Settings** shortcut button on the section header.

   The **Settings** dialog opens.
3. Click a **Read Mode** button.

Selecting the Read Type

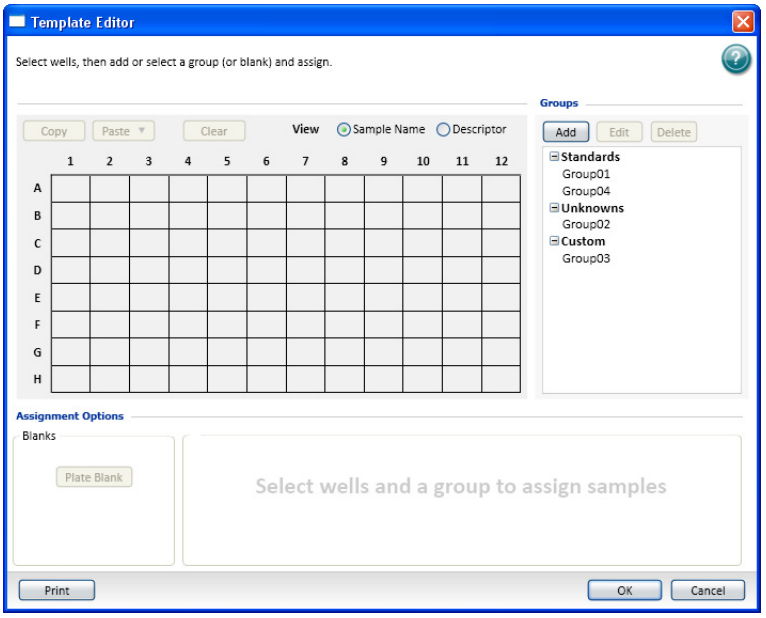
1. Click on a plate section to make it active in the workspace.
2. Click the **Settings** button in the **Plate Tools** on the **Home** tab or click the **Settings** shortcut button on the section header.

   The **Settings** dialog opens.
3. Click a **Read Type** button.

Configuring a Microplate Template

The **Template Editor** is used to create a map of the contents of the microplate and save it into a template.

1. Click on a plate section to make it active in the workspace.
2. Click the **Template** button in the **Template Tools** section of the **Home** Tab. This opens the **Template Editor**.



3. Define a group of wells, see Using the Group Settings dialog on page 98.
   - To assign well(s) to an existing group, select the wells to add to the group, click the group name, and click the **Assign** button.
   - To specify well(s) as Plate Blank, select the well(s) and click the **Plate Blank** button.
   - To specify well(s) as Group Blank, select the well(s) and click **Group Blank**, then click **Assign**.

Note: For more information about using blanks, see Blanking on page 157.

   - To remove wells from a group, select the wells to be removed and click **Clear**.
   - To print the **Template Editor** dialog, click the **Print** button.
4. To assign wells to a series, select the wells (must be a contiguous rectangle) and click the **Series** button. See Creating a Series on page 164.
5. To save the template for use on future experiment into a protocol file, select **File> Save**. See Saving Protocol Files on page 33.

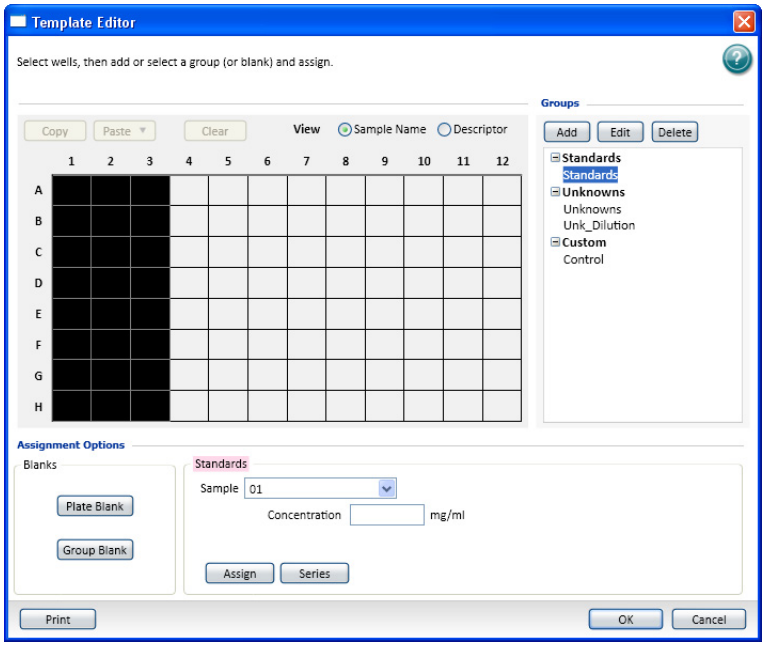
Working with Templates

Template tasks include the following:

Modifying a Template

To modify a template:

1. Make the **Plate** section with the template to be modified active in the workspace.
2. Click the **Template** button in **Template Tools** on the **Home** tab.



3. Click **OK** to close the Template Editor.

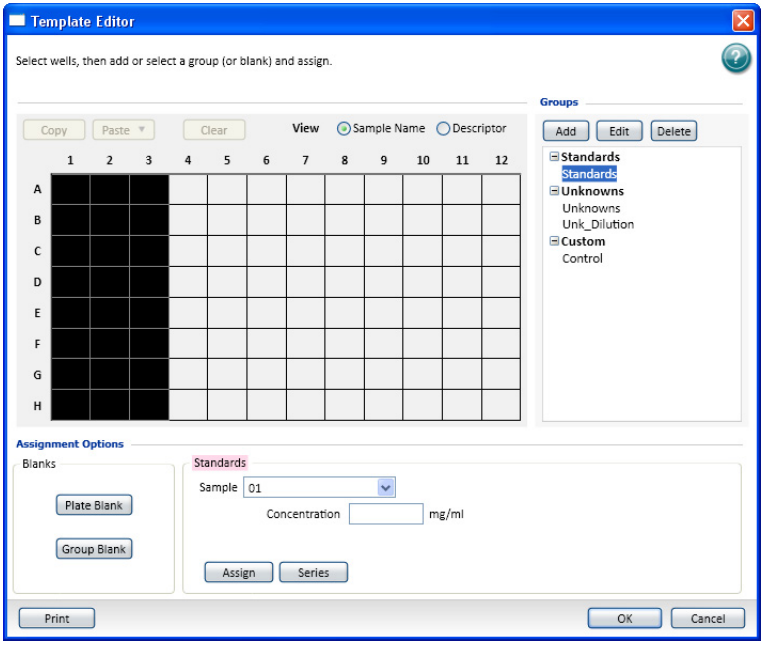
For additional template modification options, see:

- Defining a Group on page 153
- Copying and Pasting Template Contents on page 155

Defining a Group

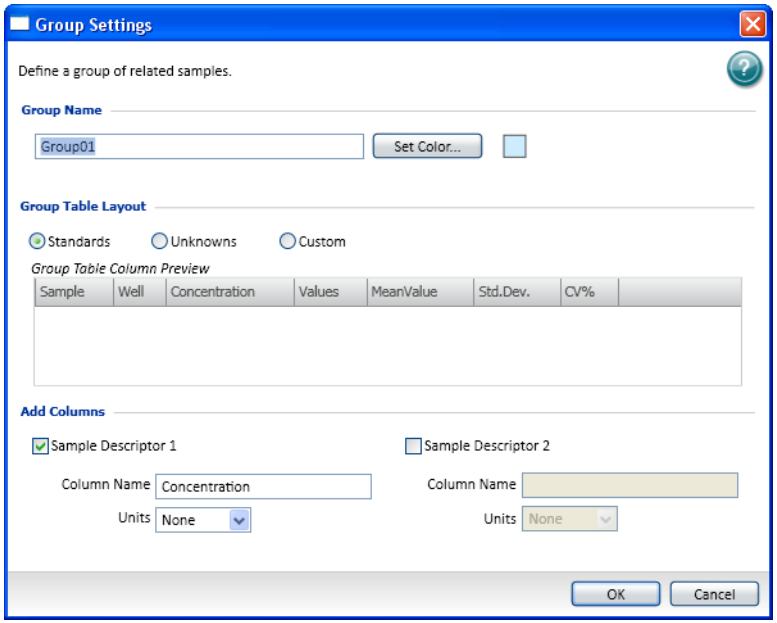
Creating a Group and Assigning Wells to It

1. Click the **Template** button in the **Home** tab to open the **Template Editor** dialog.



2. Click and drag the mouse to select one or more wells to be defined as a group.

3. Click **Add** in the **Group Type** to open the **Group Settings** dialog.



4. Type the name to assign to the group in the **Group Name** field.
5. If you want to set a color for the group, click **Set Color** and select the color to be displayed for the group.
6. In the **Group Table Layout** area, click **Standards**, **Unknowns**, or **Custom** to specify the column format.

   For information about the column formats, see Group Column Format on page 163.

   When the Standards group type is selected, Sample Descriptor1 is automatically activated and the assumption is that it will be used for concentration information. However, it could be used for any numerical data, that is, dilution factor or fraction number. The other two group types do not have sample descriptors by default.
7. In the **Add Columns** area, if it is not already selected, click **Sample Descriptor1** to apply a descriptor and its units to the group.
8. Type a description of the sample descriptor in the **Column Name** field.
9. Select a set of units from the list or type them directly in the **Units** field.

10. Optionally, define a second descriptor.

    The **Group Table Column Preview** shows an example of how the columns will appear in the group table.

11. When all groups have been defined, click **OK** to close the **Group Settings** dialog.

    The highlighted wells are placed in the newly defined group.

Copying and Pasting Template Contents

The content of a template can be copied and pasted to another section within the same experiment, between different experiments. Contiguous subsets can be copied within the template only.

Within the Same Experiment Section

The destination template is 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).

- Changes made to the template on the destination plate are also made on the source plate.
- Data from the two Plate sections are analyzed together.

Between Different Experiment Sections

When copying and pasting between different Experiment sections, make sure the group names and sample names of the pasted template are identical to those from the source template but, since they are in different Experiments, the full name is different (for example: group@experiment#2 instead of group@experiment#1).

- Changes made to the template of the destination Plate section do not affect the source section.
- Data from the two Plate sections are not analyzed together.

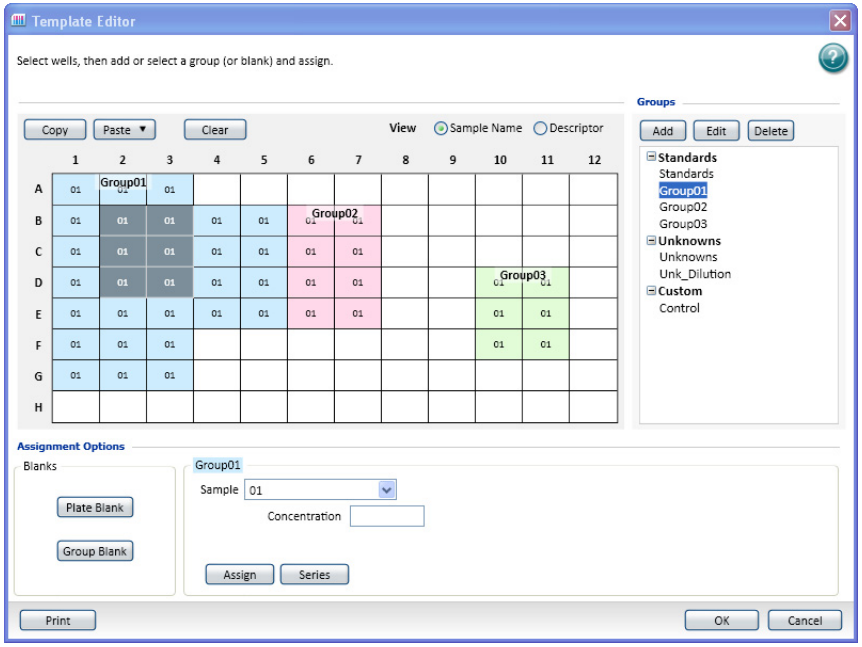
Regions within a Template

Contiguous subsets can only be copy and pasted within a template.

To copy and paste a region of a template:

1. Click on the source **Plate** section to make it active in the workspace.
2. Click **Template Editor** in the **Template Tools** on the **Home** tab.

3. Click and drag to select the wells to copy.
4. Click the **Copy** button (on the left side of the **Template Editor** dialog).



5. Click elsewhere within the same Template and click the **Paste** button

Note: You can highlight the same footprint, or simply click on the top-left well of the desired target.

6. A menu appears asking whether to **Paste** or **Paste-with-New-Groups**.

- **Paste** adds the copied wells to the current group.
- **Paste with a new Group** pastes a the copied cells into a new group.

If more wells are selected for pasting than were selected for copying, the extra wells are left empty.

The real value in this method is the ability to copy a complicated template and then to be able to analyze the data separately from the original Plate.

Blanking

In the Template Editor you can configure Plate Blanks and Group Blanks.

The different blank types have these features:

- Plate blanks are used to correct readings when all samples on a plate have been prepared in the same buffer or matrix, and therefore can use the same correction value. Plate blanks are subtracted from the raw data (or after pathlength correction if PathCheck is selected).
- Group blanks are used to correct readings when samples on a plate have been prepared in a different buffer or matrix, and therefore have to be individually corrected. Group blanks can be subtracted from raw data, like plate blank, or after post-reduction calculations, for example, wavelength, kinetic reduction, and so on.

Blanks can be used in combination and have cumulative effects.

Plate Blanks

Plate blanks can be assigned in Plate sections and in Cuvette Set sections.

Plate Sections

Wells can be assigned to a Plate Blank:

1. Click **Template** on the Home tab to open the **Template Editor** for the **Plate** section.
2. Select the wells to be used for the Plate Blank.
3. Click **Plate Blank**.

Use Plate Blanks when all samples on the plate have been prepared in the same way, and therefore can be corrected from a single blank reading. Plate Blank subtraction can be turned off in the Reduction dialog so that the data can be reviewed with or without plate blank subtraction.

For Kinetic and Spectrum data, the blank value is averaged and subtracted from each point in the read.

Cuvette Set Sections

To perform a similar function to Plate Blanking for Cuvette Set sections, you can assign cuvettes to a Blank group in the Template Editor of the Cuvette Set section and create a Template Blank:

1. Click the **Template** button in the Home tab to open the **Template Editor** for the CuvetteSet section.
2. Select the cuvettes to be used for the Template Blank.
3. Click **Blank**.

The average value of all the cuvettes in a Template Blank group is subtracted from individual cuvette readings in the same CuvetteSet section.

For Kinetic and Spectrum data, the blank value is averaged and subtracted from each point in the read.

Template blanks are subtracted after the cuvette reference reading is subtracted.

Group Blanks (Plates and Cuvettes)

Blank wells can be assigned within any group (other than the "Blank" group) in the Template Editor:

1. Click the **Template** button to open the Template Editor for the plate.
2. Highlight the wells or cuvettes to be used for the group blank. These should be wells or cuvettes that are already assigned to a Group (not the "Blank" group).
3. Select a group from the Group Types list.
4. Select the **Group Blank** button and then click **Assign**.

In a plate blank group the average value of all the wells in the Blank group is subtracted from each individual well value on the microplate. If you have an instrument that supports PathCheck, the Plate Blank is subtracted after pathlength correction. The average reduced value of the blank wells or cuvettes within that group is subtracted from individual reduced values within that group only.

Use Group Blanks when samples on the plate have been prepared differently, and therefore require individual blank correction.

The Group Blank function offers the options of subtraction before or after data reduction. When a group blank has been assigned, it is automatically subtracted and you do not have the option of reviewing the uncorrected data. To see the reduced number without the group blank subtracted, you must mask the group blank wells or cuvettes. See Masking Wells or Cuvettes on page 208.

Group blanks apply to all wells or cuvettes in a group and thus can be subtracted from wells or cuvettes in more than one Plate or Cuvette Set section.

Exporting a Template

Template information can be exported from a Plate section.

1. Click on the **Plate** section with the template to be exported to make it active in the workspace.
2. Click **Export** in the **Template Tools** section on the **Home** tab.

Note: You might need to scroll the **Home** tab ribbon to show the entire **Template Tools** section.

3. Navigate to the location in the file system where you want to save the template file.
4. Type the name the file should be saved to in the **Name** field with the .txt (tab-delimited) or .xls (Excel) file type.
5. Click **OK**.

   The template information is exported and saved.

For basic information about the format of an imported or exported template file, see Template File Formats on page 161.

Importing a Template

Template information can be imported into a Plate section. Template files can be prepared outside SoftMax Pro Software and then imported as needed when ready to run samples.

For basic information about the format of an imported or exported template file, see Template File Formats on page 161.

Note: Perhaps the quickest way to determine the correct format for importing a template is to export a template from SoftMax Pro Software and examine the contents of the resulting file.

1. Click on the **Plate** section where you want to import template information to make it active in the workspace.
2. Click **Import** in the **Template Tools** section on the **Home** tab.
3. Navigate to and select the file to be imported.
4. Click **OK**.

Note: You might need to scroll the **Home** tab ribbon to show the entire **Home Tools** section.

Template File Formats

Template information saved as a tab-delimited ASCII text file or in XML format, can be imported to or exported from Plate or Cuvette sections. A template file consists of twelve columns of data separated by tabs. Each line of the file provides information for one well in a plate or one cuvette.

When template information is exported, the information for well/cuvette A1 is written into the first line of the file, information for well/cuvette 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 or all cuvettes. If no template information exists for a well in a plate or for a cuvette, that line is left blank.

The order for importing template information must match the export order. Similarly, blank lines in the file are assigned to a well or cuvette, providing a well/cuvette with no template setting.

Table 4-1 ASCII Template File Columns and Descriptions

Column	Column Entry	Description
A	Well Location	In this column, the wells do not need to be in order.
B	Group Name	Assigned in the Group Settings dialog. If this field is missing, the rest of the line is ignored. Any text string can be entered in this field.
C	Group type	Assigned in the Template Editor dialog. three text strings are supported in SoftMax® Pro Software: Standards, Unknowns, Custom. Blank groups have an Empty column format. 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.
D	Sample name	The sample name is set in the Sample field of the Template Editor dialog. 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.
E	Descriptor1 Name	Assigned in the Group column name assigned to the descriptor 1 is shown here.
F	Descriptor1 Value	Assigned in the Group Settings dialog. A numeric value can be entered in this field or it can be left blank.

Table 4-1 ASCII Template File Columns and Descriptions (cont'd)

Column	Column Entry	Description
G	Descriptor1 Units	Assigned in the Group Settings dialog. Supported strings are unit/ml, mg/ml, µg/ml, ng/ml, mg, ng, and ml. If this column is left blank, unit/ml is assigned.
H	Descriptor2 Name	Assigned in the Group column name assigned to the descriptor 2 is shown here.
I	Descriptor2 Value	Assigned in the Group Settings dialog. A numeric value can be entered in this field or it can be left blank.
J	Descriptor2 Units	Assigned in the Group Settings dialog. Supported strings are unit/ml, mg/ml, µg/ml, ng/ml, mg, ng, and ml. If this column is left blank, unit/ml is assigned.

The tab-delimited ASCII files can be opened with spreadsheet programs, such as Microsoft Excel, but if edited in Microsoft Excel they cannot be imported back into SoftMax Pro Software.

Group Column Format

The Group Type setting defines the default columns for the data calculated and reported in the associated Group section of the current sample group.

Each of the three column formats, Standard, Unknowns and Custom creates a different set of columns in a new or existing group. The table below shows the default columns created with each selection type.

Table 4-2 Group Type Column Format

Name	Formula	Standard	Unknown	Custom
Sample	!SampleNames	X	X	X
Wells	!WelllDs	X	X	X
Sample#	Index			
Concentration	!Concentration	X		
Mean Value	Average(Values)	X		
Values	!WellValues	X		
R (Outside Standard Range)	If(Values)>=‘MinStd @Standards’ and Values<=‘MaxStd@ Standards’,“”,“R”		X	X
Result	InterpX(STD@ StandardCurve, Values)		X	X
MeanResult	Average(Result)		X	X
Std.Dev.	Stdev(Result)	X	X	X
CV%	Cv(Result)	X	X	X
Dilution	!Factor			X
Adj.Result	MeanResult*!Factor			X

Creating a Series

You can 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.

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 dialog.

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:

1. Make a plate or cuvette section active in the workspace, click **Template Editor** on the **Home** tab or click the **Template Editor** shortcut button on the section header bar.
2. In the **Template Editor** dialog, highlight a rectangular selection of wells to be part of the series.

Note: A series is associated with a group. If no groups have been defined, you will need to define a group and then define the series. If you select a group of wells that are not currently in a group, when you define a series these wells will be associated with whatever group has already been defined.

3. Click the **Series** button.
4. Select options in **Series Layout**.
   - Choose a fill direction to describe how the series fills the wells from the **Start From** options. Select one of Top to Bottom, Bottom to Top, Left to Right, or Right to Left.
   - In **Patterns of Replicates**, type the number of replicates in the Y-Direction and type the number of replicates in the X-Direction.

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. 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.

- Optionally, click **Reverse Series Order** to reverse the order of the series.

5. In **Sample Information** type the name of the first sample in the series in the **Starting Sample Name** field. Subsequent replicates use this name as a base and either increment the number within the name or append a number to it.
6. Click **Concentration** and type the starting value for the first sample in the series in the **Starting Value** field.

Note: "Concentration" is the default sample descriptor name. You can change this name in the Group Settings dialog by giving the column a different name.

7. Choose the operator for the series (+, -, *, or /) from the **Step by** list and enter a value for the increment. For example, with a starting value of 0.5, the division operator (/) and the increment 2, the series will start with 0.5 and divide each subsequent sample by 2 to produce the series 0.5, 0.25, and 0.125.
8. Click **OK** to save the series and close the dialog.

Incrementing the Names Only

To increment the names of a series of samples without applying a concentration or dilution:

1. Create a series in the **Series** dialog.
2. Uncheck **Concentration**.

Note: "Concentration" is the default sample descriptor name. You can change this name in the Group Settings dialog by giving the column a different name.

Incrementing with a Constant

To increment the names of a series of samples with a constant concentration or dilution:

1. Create a series in the **Series** dialog.
2. Check the **Concentration** field.

Note: "Concentration" is the default sample descriptor name. You can change this name in the Group Settings dialog by giving the column a different name.

3. Enter the concentration or dilution, and select the multiply operator with a factor of 1.

   If sample names are set to increment automatically using the Series function, be aware that SoftMax Pro Software 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 is not truncated. If sample names are very long, and they must be kept long for reference, you will have to set up your replicates manually.

Creating an Absorbance Mode Protocol

To create an Absorbance Mode protocol, follow these steps:

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

3. With the plate or cuvette section active, click the **Settings** button on the **Home** tab.
4. When working with a SpectraMax Paradigm instrument, click the Absorbance Detection Cartridge from the **Cartridges** list.
5. Click **ABS** to select the Absorbance read mode.
6. Click a Read Type.
7. Define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
8. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
9. Create a template, if applicable. See Configuring a Microplate Template on page 150.
10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
11. Set Display options, if applicable. See Viewing the Data Display on page 206.
12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

For more information, see Absorbance Mode Protocol Overview on page 168 and Optimizing Absorbance Assays on page 168.

Absorbance Mode Protocol Overview

Absorbance is the amount of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. In the absence of turbidity, absorbance = optical density.

$$A=\log(I_0/I)$$

where I_0 is incident light before it enters the sample, I is the intensity of light after it passes through the sample, and A is the measured absorbance.

Applications of Absorbance

Absorbance measurements are used to quantify the concentration of an absorbing species. It must be known at what wavelength a species absorbs light. For example, the concentration of nucleic acids can be quantified by measuring the absorbance at 260 nm.

Optimizing Absorbance Assays

The wavelength of the incident light can be adjusted in 1 nm increments. SoftMax Pro Software also allows reading up to six wavelengths per plate or cuvette for **Endpoint** and **Kinetic** read types, and four wavelengths for **Well Scans**. It is always good scientific practice to designate wells in the microplate to serve as plate blanks or group blanks. This can be done easily in the **Template** dialog of the Plate section in the SoftMax Pro Software program.

If desired, the PathCheck feature can be used to normalize the data to a 1 cm pathlength. For more information, see PathCheck Pathlength Measurement Technology on page 138.

Note: Assay optimization requires the use of a computer and SoftMax Pro Software.

Creating a Fluorescence Intensity Mode Protocol

To create a Fluorescence Intensity protocol, follow these steps:

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

3. With the plate or cuvette section active, click the **Settings** button on the Home tab ribbon.
4. When working with a SpectraMax Paradigm instrument, click one of the Fluorescence Intensity cartridges from the **Cartridges** list.
5. Click **FL** to select the Fluorescence Intensity read mode.
6. Click a Read Type.
7. Define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
8. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
9. Create a template, if applicable. See Configuring a Microplate Template on page 150.
10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
11. Set Display options, if applicable. See Viewing the Data Display on page 206.
12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

For more information about Fluorescence Intensity mode protocols, see Fluorescence Intensity Mode Overview on page 170 and Optimizing Fluorescence Intensity Assays on page 172.

Fluorescence Intensity Mode Overview

Fluorescence is light emitted by a substance resulting from the absorption of incident radiation. The governing equation for fluorescence is:

> Fluorescence = (extinction coefficient) × (concentration) × (quantum yield) × (excitation intensity) × (pathlength) × (emission collection efficiency)

Fluorescent materials absorb light energy of a characteristic wavelength (excitation), undergo an electronic state change, and instantaneously emit light of a longer wavelength (emission). Most common fluorescent materials have well-characterized excitation and emission spectra. The following figure shows an example of excitation and emission spectra for a fluorophore. The excitation and emission bands are each fairly broad, with half-bandwidths of approximately 40 nm, and the wavelength difference between the excitation and emission maxima (the Stokes shift) is typically fairly small, about 30 nm. There is considerable overlap between the excitation and emission spectra (gray area) when a small Stokes shift is present.

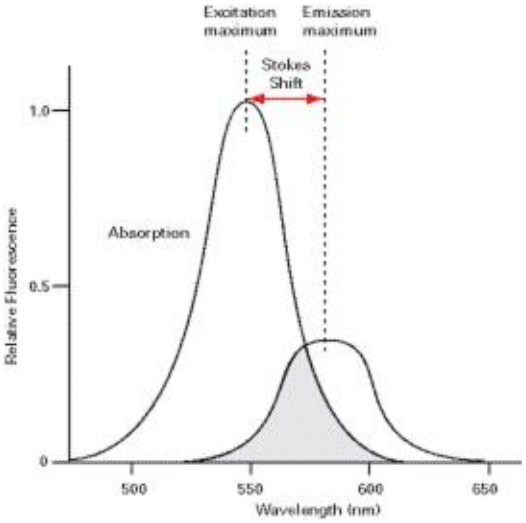


Figure 4-1 Excitation and emission spectra.

Because the intensity of the excitation light is usually many tens of thousands of times greater than that of the emitted light, some type of spectral separation is necessary to reduce the interference of the excitation light with detection of the emitted light. The SpectraMax reader incorporates many features designed to restrict interference from reflected excitation light. Among these features is a set of long-pass emission cutoff filters that can be set automatically by the instrument or manually by the user. If the Stokes shift is small, it is advisable to choose an excitation wavelength that is as far away from the emission maximum as possible while still being capable of stimulating the fluorophore so that less of the excited light overlaps the emission spectrum, allowing better selection and quantitation of the emitted light.

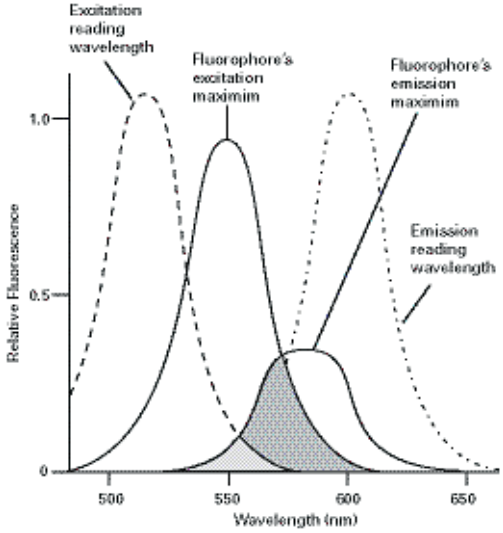


Figure 4-2 Optimized excitation and emission reading wavelengths.

The previous figure shows that the best results are often obtained when the excitation and emission wavelengths used for reading are not the same as the peak wavelengths or maxima of the excitation and emission spectra of the fluorophore. When the reading wavelengths for excitation and emission are separated, a smaller amount of excitation light passes through to the emission monochromator (gray area) and on to the PMT, resulting in a purer emission signal and more accurate data.

The SpectraMax readers allows scanning of both excitation and emission wavelengths, using separate tunable monochromators. One benefit of being able to scan emission spectra is that you can assess more accurately whether the emission is, in fact, the expected fluorophore, or multiple fluorophores, and not one generated by a variety of background sources or by contaminants. Another benefit is that you are able to find excitation and emission wavelengths that avoid interference when interfering fluorescent species are present.

For this reason, it is desirable to scan emission for both an intermediate concentration of labeled sample, as well as the background of unlabeled sample. The optimum setting is where the ratio of the sample emission to background emission is at the maximum.

Optimizing Fluorescence Intensity Assays

The optimum instrument settings for detection of a particular fluorophore depend on a number of different factors. Settings that can be adjusted for assay optimization include the excitation and emission wavelengths, emission cutoff filter, readings per well, the PMT voltage, and the temperature of the reading chamber.

Another important factor that is independent of the instrument but which affects assay optimization is the Stokes' shift. When the Stokes' shift is very small, optimizing the excitation and emission wavelengths and correct cutoff filter choices are very important.

- Excitation and Emission Wavelengths
  The excitation and emission wavelengths can be set in 1-nm increments between 250 nm and 850 nm. The available wavelengths are dependent on the instrument. For more information about the available wavelengths, see the documentation for the instrument you are using.
  A procedure to optimize excitation and emission wavelengths for a given assay is outlined in Spectral Scanning on page 174.
- Emission Cutoff Filter
  The emission cutoff filters assist in reducing background. Sources of background include stray excitation light and native fluorescence of plate materials, sample constituents, and solvents (including water). The default setting allows the instrument and SoftMax Pro Software to determine which cutoff filter should be used in endpoint and kinetic modes. The spectral scan mode default uses no cutoff filter.

- Readings Per Well
  The number of readings per well can vary between 1 (used for a quick estimate) and 100 (for very precise measurements). The default number of readings per well varies with the read mode: for fluorescence, the default is 6, and for luminescence the display shows 1 read per well.
- PMT Voltage
  The voltage of the photomultiplier tube can be set to low (for higher concentration samples), medium, or high (for lower concentration samples) in all read modes. In endpoint and spectrum mode, there is an additional setting, automatic, in which the instrument automatically adjusts the PMT voltage for varying concentrations of sample in the plate.
- Temperature Control
  The chamber of the SpectraMax reader is isothermal at ambient as well as at elevated temperatures. The temperature in the reading chamber can be adjusted from 2° C above ambient to 60° C.

For information about using spectral scanning to optimize excitation and emission wavelengths for Fluorescence Assays, see Spectral Scanning on page 174.

Spectral Scanning

This procedure optimizes excitation and emission wavelengths for fluorescence assays using spectral scanning.

If you are using the SpectraMax Paradigm Tunable Wavelength (TUNE) Detection Cartridge, see Spectral Optimization on page 201.

Pipette 200 µL of sample that includes the fluorophore and 200 µL of a buffer control into separate wells of a microplate.

1. Perform the excitation scan:
   - Using SoftMax Pro Software, set up a Plate section for a fluorescence read, spectrum mode, Em Fixed/Ex Scan, with no cutoff filter (default), and medium PMT.
   - Set the emission wavelength based on the tentative value from the literature (or from a customary filter set used to measure your fluorophore). If the emission wavelength is not known, select a tentative emission wavelength about 50 nanometers greater than the absorbance maximum of the fluorophore. If necessary, the absorbance maximum can be determined by performing an optical density spectral scan first.
   - Set the excitation scan to start/stop approximately 50 nm below/above the tentative excitation value obtained from the literature (or the customary excitation filter).
   - Set the step increment to 2 nm or 3 nm. You can choose to do a preliminary scan with a 10 nm increment to determine the approximate peak location, and then repeat the scan over a narrower wavelength range with a 2 nm or 3 nm increment.
   - Perform the scan and view the results as a plot of emission fluorescence vs. excitation wavelength. Note the excitation wavelength at the emission peak and the maximum RFU value.

   If an error message reporting missing data points occurs, it can be due to possible saturation reported by the SoftMax Pro Software program at the end of the spectral scan. Reset the PMT to "low" and rescan the sample (scan the buffer blank with the PMT set to "medium" or "high"). If the error occurs after scanning with the PMT set to "low," it can be necessary to dilute the sample.

If the excitation scan shows no apparent peak, change the PMT setting to "high" and rescan the sample. If the spectral scan still shows no apparent peak, adjust the Y-scale of the zoom plot so that the plot fills the graph.

- Select the optimal excitation wavelength. If the excitation peak wavelength and emission wavelength are separated by more than 80 nm, use the excitation peak wavelength value. If the excitation and emission wavelengths are less than 80 nm apart, use the shortest excitation wavelength that gives 90% maximal emission. Follow the plot to the left of the peak until the RFU value falls to approximately 90% of the maximum, and then drop a line from the 90% point on the plot to the x-axis as shown in the following figure.

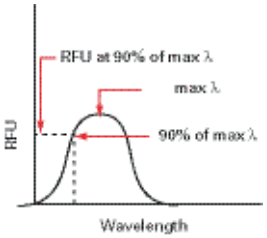


Figure 4-3 Plot of RFU vs. wavelength

2. Perform emission scan #1:
   - In the SoftMax Pro program, set up a second plate section for a fluorescence read, spectrum mode, Ex Fixed/Em Scan, with no cutoff filter (default), and medium PMT.
   - Set the excitation wavelength to the value determined in Step 1 above.
   - Set the emission scan to start/stop approximately 50 nm below or above the tentative emission value obtained from the literature (or existing filter pair).

Note: If the Stokes shift is less than 50 nm, then start the emission scan above the excitation wavelength.

   - Set the step increment to 2 to 3 nm (or do a preliminary scan with a 10 nm increment to determine the approximate peak location and then repeat the scan over a narrower wavelength range using a 2 to 3 nm increment.)
   - Perform the scan and view the results as a plot of fluorescence vs. emission wavelength.

3. Choose the emission filter:
   - Select an emission cutoff filter that blocks as much of the residual excitation light as possible without unduly reducing the fluorescence signal. The cutoff wavelength choices are 325, 420, 435, 475, 495, 515, 530, 550, 570, 590, 610, 630, 665, or 695 nm. The cutoff value should be near the maximum emission wavelength (preferably between the excitation wavelength and the maximal emission wavelength) but at least 10 nm less than the emission wavelength. If you have questions about this procedure please contact Molecular Devices Technical Support and ask to speak to an applications scientist.
4. Perform emission scan #2:
   - In the SoftMax Pro Software, set up a third plate section for an emission scan as specified in Step 2 above, except selecting Manual Cutoff Filter and setting the wavelength to that determined in Step 3.
   - Perform the scan and view the results as a plot of fluorescence vs. emission wavelength. Note the wavelength giving the maximum emission (the optimal emission wavelength).
   - Compare the spectra of the sample containing the fluorophore to the spectra of the buffer blank to get an estimate of the signal-to-noise ratio. If there is significant background interference, repeat Steps 3 and 4 with another choice of cutoff filter.
5. Results

   The optimal excitation and emission wavelengths are those determined in Steps 1 and 4, above.
6. Comments
   - In endpoint or kinetic fluorescence modes, the “AutoCutoff” feature generally selects the same cutoff filter wavelength as the previously described optimization method. If desired, however, you can specify the cutoff filters manually.
   - For emission wavelengths less than 325 nanometers, experimental iteration is usually the best method of determining the optimal emission and excitation wavelengths. Begin optimization by performing Steps 1 through 4 above. Try emission and excitation wavelength combinations with the 325 cutoff or with no cutoff filter. Similarly, for excitation wavelengths greater than 660 nanometers, try emission and excitation wavelength combinations with the 695 cutoff or with no cutoff filter.

The following table lists default settings for the emission cutoff filters.

Table 4-3 Emission cutoff filter default settings for SpectraMax M2, M2e, M3, M4, M5, and M5e readers

	Automatic Cutoff Selection	Endpoint and Kinetic Modes
#	Wavelength (nm)	Emission Wavelength (nm)
1	None	< 415
2	420	415–434
3	435	435–454
4	455	455–474
5	475	475–494
6	495	495–514
7	515	515–529
8	530	530–549
9	550	550–569
10	570	570–589
11	590	590–609
12	610	610–629
13	630	630–664
14	665	665–694
15	695	695–850

Creating a Luminescence Mode Protocol

To create a Luminescence Mode protocol, follow these steps:

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.
3. When working with a SpectraMax Paradigm instrument, click a Luminescence detection cartridge from the **Cartridges** list.
4. With the plate or cuvette section active, click the **Settings** button on the **Home** tab.
5. Click **LUM** to select the Luminescence read mode.
6. Click a read type.
7. Define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
8. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
9. Create a template, if applicable. See Configuring a Microplate Template on page 150.
10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
11. Set Display options, if applicable. See Viewing the Data Display on page 206.
12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

Luminescence Mode Protocol Overview

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

Optimizing Luminescence Assays

Luminescence can be read from the top or the bottom of a microplate. Solid white plates or white plates with clear bottoms are recommended for luminescence reads.

For standard luminescence a separate light path without monochromators carries the emitted light to a dedicated PMT. The optimum emission wavelength is between 360 nm and 630 nm. Under reader set-up the emission says **All**.

For wavelength-selectable luminescence, the emission monochromator is used to differentiate the wavelengths being emitted from the well. Up to four emission wavelengths within the specified reader range can be selected. If reading only one luminescent event in the well, best sensitivity should be achieved using the standard luminescence measurement, without a wavelength selected.

Luminescence read times are not designated by multiple reads per well, but rather by choosing the total integration time desired between 1 ms and 1,500 ms. Typical luminescence assays require between 500 ms and 1000 ms integration.

If wells have been incubating for a long period of time, it is a good idea to mix the plate before reading. This can be done using the Shake function.

If it appears that the signal is always higher in the first wells read (for example, column A), the plate might need to be "dark adapted" to reduce the auto-luminescence of the white plastic. The auto-luminescence decreases quickly, so manually load the plate using the hardware controls and wait for 1 to 2 minutes before initiating the read and determine if the read-out is more consistent across the plate.

Creating a Time-Resolved Fluorescence Mode Protocol

To create a Time-Resolved Fluorescence Mode protocol, follow these steps:

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.
3. With the plate or cuvette section active, click the **Settings** button on the Home tab ribbon.
4. When working with a SpectraMax Paradigm instrument, click an appropriate detection cartridge from the **Cartridges** list.
5. Click **TRF** to select the **Time-Resolved Fluorescence** read mode.
6. Click a read type.
7. Define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
8. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
9. Create a template, if applicable. See Configuring a Microplate Template on page 150.
10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
11. Set Display options, if applicable. See Viewing the Data Display on page 206.
12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

For more information about the Time-Resolved Fluorescence mode, see Time-Resolved Fluorescence Mode Overview on page 181 and Optimizing Time-Resolved Fluorescence Assays on page 181.

Time-Resolved Fluorescence Mode Overview

Time-Resolved Fluorescence (TRF) is a method of detection, which uses a delay between the excitation, and emission detection. This delay allows for the reduction of background fluorescence. Most fluorescence substances are not suitable for this type of reading. However, the fluorescence emitted by the lanthanide dyes are delayed long enough to measure fluorescence to use for this detection mode. This is different from Fluorescence Intensity mode reads, which do not have a delay between the excitation and emission.

TRF is used to reduce the amount of background noise that interferes with fluorescence. The excitation lamp flashes, and after it is off, the delayed emission is collected for a set period of time before the lamp is flashed again.

In normal Fluorescence Intensity mode, the SpectraMax readings are taken while the lamp is on. The most common limitation to sensitivity in normal fluorescence is excitation energy or background fluorescence that cannot be eliminated from the emission signal. Since the lamp is the source of excitation energy, turning it off provides the best means of eliminating background excitation. The elimination of background excitation is the critical difference between fluorescence intensity measurements and TRF measurements.

Optimizing Time-Resolved Fluorescence Assays

Time-resolved fluorescence is performed by flashing the excitation lamp and, after it is off, collecting the delayed emission for a period of time before the lamp is flashed again. Long-lifetime rare-earth lanthanide dyes are typically used to provide a long-lived fluorescent signal that persists after the lamp is turned off. Background fluorescence usually fades, while lanthanide chelates and cryptates have fluorescent lifetimes between 100 µs and 2 ms.

To optimize data collection for a particular assay, you can select when to start and end data acquisition-the minimum is 50 µs after the lamp has been turned off, and the maximum is 1450 µs, in 50 µs or 200 µs steps.

Some examples of TRF assays are:

- IMAP® TR-FRET
- Cisbio HTRF
- LanthaScreen TR-FRET
- LANCE TR-FRET
- DELFIA TRF

Creating a Fluorescence Polarization Mode Protocol

To create a Fluorescence Polarization Mode protocol, follow these steps:

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.
3. With the plate or cuvette section active, click the **Settings** button on the Home tab in the ribbon.
4. When working with a SpectraMax Paradigm instrument, click the Fluorescence Polarization (FP) Detection Cartridge from the **Cartridges** list.
5. Click **FP** to select the Fluorescence Polarization read mode.
6. Click a read type.
7. Define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
8. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
9. Create a template, if applicable. See Configuring a Microplate Template on page 150.
10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
11. Set Display options, if applicable. See Viewing the Data Display on page 206.
12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

For more information about Fluorescence Polarization, see Fluorescence Polarization Mode Protocol Overview on page 183 and Optimizing Fluorescence Polarization Assays on page 184.

Fluorescence Polarization Mode Protocol Overview

By using a fluorescent dye to label a small molecule, its binding to a large molecule can be monitored through changes in its speed of rotation.

Fluorescence Polarization mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. These S and P values are used to calculate the Polarization (mP) and Anisotropy (r) values in SoftMax Pro Software. Although the Raw S&P values are the true actual values returned from the instrument, the calculated Polarization (mP) and Anisotropy (r) values are treated as the raw data; these become the basis for further reduction calculations. You can choose to display any of these data types in the Plate section.

When raw (S&P) is displayed, the mP value is by default used for all reduction calculations. When exporting, the displayed raw values are exported, whether mP, r, or Raw (S&P).

Polarization (mP) is calculated as follows:

$$mP = 1000 \times \frac{(parallel - (G \times perpendicular))}{(parallel + (G \times perpendicular))}$$

Anisotropy (r) is calculated as follows:

$$r = \frac{(parallel - (G \times perpendicular))}{(parallel + (2G \times perpendicular))}$$

Optimizing Fluorescence Polarization Assays

Fluorescence polarization for the SpectraMax M5 and M5e Multi-Mode Microplate Readers, the FilterMax F3 and F5 Multi-Mode Microplate Readers, and the SpectraMax Paradigm Multi-Mode Detection Platform can be read only from the top of a microplate. The plastic from which a microplate is formed affects the light polarization, precluding bottom reads and reading a covered plate.

Solid black plates are recommended for fluorescence polarization reads. If the assay components seem to bind to the microplate, as evidenced by poor mP dynamic range (small difference between bound and unbound tracer), we suggest using plates treated to minimize binding, or polypropylene plates and/or adding a very small amount of detergent, to the assay buffer.

Background wells, containing all assay components minus the fluorophore, should be tested. If the signal in the background wells is more than 1/10 the signal in the wells containing fluorophore, then background wells should be run on each assay plate. The average raw signal from the background's parallel and perpendicular readings should be subtracted from the raw parallel and perpendicular readings of each sample well before the mP calculation is performed.

For best precision in assays using a low amount of fluorophore (for example, <5 nm fluorescein), set the PMT sensitivity to High and the number of readings to 100. If faster read speed is required, be sure Settling Time is “Off” in the SoftMax Pro Software settings, and experiment with fewer flashes per well until acceptable speed and still enough precision are achieved.

Note: Fewer flashes result in a higher speed, but less precision is achieved.

Creating an AlphaScreen Mode Protocol

To create AlphaScreen Mode protocol, follow these steps:

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select a SpectraMax Paradigm instrument. See Selecting and Connecting to an Instrument on page 12.

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

3. With the plate or cuvette section active, click the **Settings** button on the Home tab in the ribbon.
4. Click one of the AlphaScreen Cartridges from the **Cartridges** list.
5. Click **Screen** to select the AlphaScreen read mode.
6. Click a read type and define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
7. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
8. Create a template, if applicable. See Configuring a Microplate Template on page 150.
9. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
10. Set Display options, if applicable. See Viewing the Data Display on page 206.
11. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

For more information see, AlphaScreen Mode Protocol Overview on page 186.

AlphaScreen Mode Protocol Overview

ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. AlphaScreen is a bead-based chemistry used to study molecular interactions between moieties A and B, for example. When a biological interaction between A and B brings beads (coated with A and B, respectively) together, a cascade of chemical reactions acts to produce a greatly amplified signal.

The cascade finally resulting in signal is triggered by laser excitation (680 nm), making a photosensitizer on the A-beads convert oxygen to an excited (singlet) state. That energized oxygen diffuses away from the A-bead. When reaching the B-bead in close proximity, it reacts with a thioxene derivative on the B-bead generating chemiluminescence at 370 nm. Energy transfer to a fluorescent dye on the same bead shifts the emission wavelength into the 520 nm to 620 nm range. The limited lifetime of singlet oxygen in solvent (~4 microseconds) allows diffusion reach only up to about 200 nm distance. Thus, only B-beads in the proximity of A-beads yield signal, which indicates binding between moieties A and B.

An AlphaScreen measurement includes a light pulse, by turning on the laser diode for a specified time, turning off the laser diode, followed by the measurement of the AlphaScreen signal, as specified in the measurement protocol timing parameters.

AlphaScreen beads are light sensitive. Beads are best handled under subdued (<100 lux) or green filtered (Roscolux filters #389 from Rosco, or equivalent) light conditions. Incubation steps should be performed in the dark.

Applications of AlphaScreen

AlphaScreen reagent and assays are used for drug discovery purposes. Examples of AlphaScreen assays include:

- G-protein coupled receptor (GPCR) assay kits, for cAMP quantification or IP3 quantification.
- Tyrosine Kinase assays.
- Cytokine detection kits, such as TNFa a detection (immunoassay).

Creating a FRET Mode Protocol

To create a Fluorescence Resonance Energy Transfer (FRET) protocol, follow these steps:

1. Start the SoftMax Pro Software. See Starting the Software on page 12.
2. Select an instrument. See Selecting and Connecting to an Instrument on page 12.

Note: It is not necessary to be physically connected to an instrument to create a protocol. If you are not connected to an instrument you can select the target instrument and work offline.

3. With the plate or cuvette section active, click the **Settings** button on the Home tab.
4. When working with a SpectraMax Paradigm instrument, click one of the Fluorescence Intensity cartridges from the **Cartridges** list.
5. Click **FRET** to select the FRET read mode.
6. Click a read type and define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.
7. After defining the setting for the protocol, click **OK** to close the **Settings** dialog.
8. Create a template, if applicable. See Configuring a Microplate Template on page 150.
9. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 208.
10. Set Display options, if applicable. See Viewing the Data Display on page 206.
11. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 188.

For more information about FRET, see the Fluorescence Resonance Energy Transfer Overview on page 188.

Fluorescence Resonance Energy Transfer Overview

Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule *without emission of a photon*.

FRET relies on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. Due to its sensitivity to distance, FRET has been used to investigate molecular interactions. FRET is the radiationless transmission of energy from a donor molecule to an acceptor molecule. The donor molecule is the dye or chromophore that initially absorbs the energy and the acceptor is the chromophore to which the energy is subsequently transferred. This resonance interaction occurs over greater than interatomic distances, without conversion to thermal energy, and without any molecular collision. The transfer of energy leads to a reduction in the donor's fluorescence intensity and excited state lifetime, and an increase in the acceptor's emission intensity. A pair of molecules that interact in such a manner that FRET occurs is often referred to as a donor/acceptor pair.

While there are many factors that influence FRET, the primary conditions that need to be met in order for FRET to occur are relatively few. The donor and acceptor molecules must be in close proximity to one another. The absorption or excitation spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor. The degree to which they overlap is referred to as the spectral overlap integral (J). The donor and acceptor transition dipole orientations must be approximately parallel.

Saving the Settings to a Protocol File

To save the settings including the template into a protocol file:

1. Click **File**.
2. Select **Save > Data file or Protocol**.

   The **Save as** dialog opens.
3. Navigate to the file system location where you want to save the protocol.
4. Type the name of the protocol in the **File name** field.
5. Select **Protocol files (*.spr)** in the **File Save Type** field.
6. Click **Save**.

Verifying Data Recovery

To verify data recovery:

1. Click the application  icon to open the **Application** menu.
2. Click the **Options** button to open the **Options** dialog.
3. Verify that **Save open files for data recovery every five minutes** is selected.
4. Click **Ok** to close the dialog.

When Data Recovery is enabled, open files are saved to a temporary file every five minutes.

Recovering Files

In the case of an application interruption, upon system restart SoftMax Pro Software presents the files available for recovery.

1. Select the file or files you want to recover.
2. Click **Close** to close the dialog.
3. The selected files open.

Files not selected from the list will be deleted when the dialog closes.

Saving a recovered file overwrites the original file. If an Untitled file is recovered and opened, SoftMax Pro Software prompts for a file name and location when the file is saved.

Setting Auto Save Options

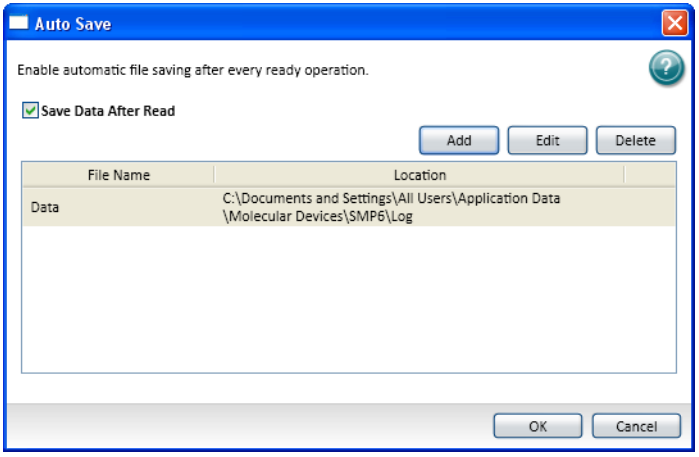
When this option is enabled, the collected data is saved automatically to a user-defined location immediately after each plate read is completed. Auto Save reduces the likelihood of lost data, particularly when Auto Save is set to save files to corporate network volumes that are backed up on a regular basis. This feature helps prevent data loss when used with automated reads, such as with the StakMax Microplate Handling System. See StakMax Microplate Handling System on page 132.

Note: Auto Save settings are saved with each document.

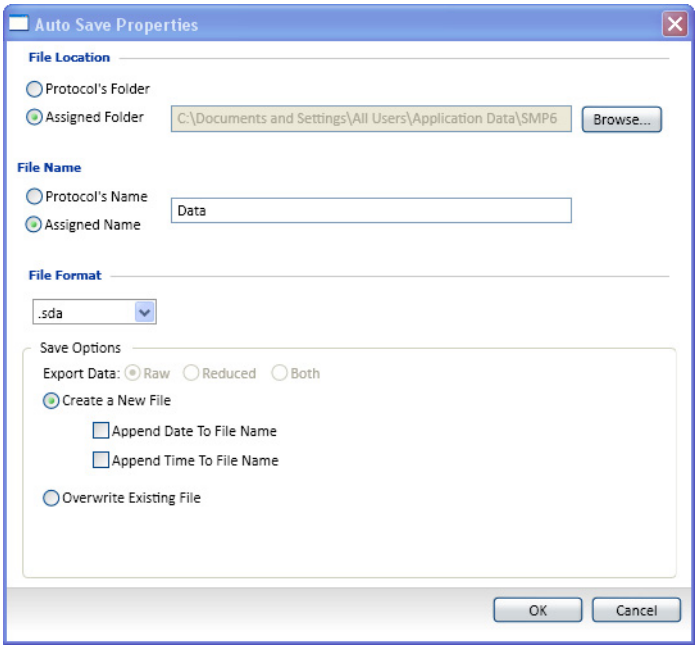
You can add as many Auto Save instances as desired, each with its own settings.

To set Auto Save options:

1. Click the application button in the ribbon to open the **Application** menu and click **Auto Save** to open the **Auto Save** dialog.



2. To enable Auto Save, select **Save Data After Read**.
3. To modify the auto save properties, click Add to add a new location or **Edit** to modify the existing location.



This dialog contains the following options:

File Location

You can choose to automatically save files to the current protocol's folder or to assign a different folder.

To assign a folder select **Assigned Folder** and use the **Browse** function to locate the target location.

File Name/Format

You can control the automatic naming of all automatically saved files by modifying the options listed in the File Name/Format section.

Name

To name the file with the current protocol's name select **Protocol's Name**.

To assign a name, select **Assigned Name**.

If selected, any text in this field is automatically inserted at the beginning of each automatically saved file name. Files are also numbered sequentially, for example “Data 1”, “Data 2”, etc.

Format

Select a data format from the menu. Options include:

- .sda
- .xml
- .txt (list)
- .txt (plate)
- .xls (list)
- .xls (plate)

Format options allow you to specify what type of file is automatically saved. Version 6 supports four file formats: SoftMax Pro, XML, and Tab-Delimited text (list and plate) and Excel (list and plate).

This applies to both SoftMax Pro and text file formats.

SoftMax Pro File Format

When selected, your data will be automatically saved as a proprietary SoftMax Pro Software file format: .sda (standard) format.

XML File

XML is supported for data export and Auto Save. XML is the optimal file format if you plan on importing all read data into other data collection and storage applications—specifically, LIMS (Laboratory Information Management System) or SDMS (Scientific Data Management System) packages.

Tab-Delimited File

When selected, your data will be automatically saved as a .txt file format, which can be opened by any word processor, spreadsheet, or database program. If you are saving data to a text file, use Append to File to save all data for all Plate sections to a single .txt file, or use Create New File to save the data from each plate to a new file.

For all data formats except .sda you can also choose the type of data to save. Options include:

- Export Raw Data
- Export Reduced Data
- Export Both

Excel

The Excel output has the same format as a tab-delimited file with the .xls file extension which allows the file to be opened easily by Excel.

Save Options

The save options include:

Create a new file

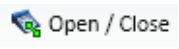
When you choose to create a new file you can also select to append the date and/or time to the file name.

- Append Date to File Name
  Checking Append Date adds the date to the Assigned Name or Protocol Name specified.
- Append Time to File Name
  If Append Time is not also checked, SoftMax Pro Software indexes the runs for that day starting with "1" and increments with each successive run. For example, the first file AutoSaved on November 18th, 2011 at 9:01am and 58 seconds would be named as "Data-11-18-05 1", while the second file AutoSaved would be named "Data-11-18-05 2", and so on.
- Overwrite the Existing file
  This option should be used carefully as overwriting a file can cause loss of data.
- Append to file
  If the .txt or .xls format is selected, you can choose to append the plate data to the current file. Enter the number of plates to append per file.

Collecting Data from a Microplate

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 instrument are raw absorbance, fluorescence, luminescence, or fluorescence polarization values and are not affected by settings in the Template Editor.

To read a microplate:

1. Open the drawer of the instrument by clicking the **Open/Close** 

button in the Home tab.
2. Insert the prepared microplate matching well A1 with position A1 in the drawer. Make sure the microplate is flat against the drawer bottom or against the adapter.
3. Click the **Open/Close** button to close the drawer.
4. Open a SoftMax Pro Software data file or protocol file that contains the appropriate experiment settings for the plate read. Alternatively, create new settings by selecting the Plate section in the SoftMax Pro Software program and configuring the instrument settings using the **Settings** dialog. For More information, see Selecting Instrument Settings on page 148.
5. Click the **Read** button in the **Home** tab.
6. The active Plate section is read.
   - If you are using a SpectraMax Paradigm Multi-Mode Detection Platform and selected to perform pre-read optimizations, the Pre-Read Optimization Options dialog appears. See Performing Pre-Read Optimization on page 195.
   - If you are using a SpectraMax Paradigm Tunable Wavelength (TUNE) Detection Cartridge and selected to run spectral optimization, the Spectral Optimization Wizard runs before the microplate is read. See Spectral Optimization on page 201.
7. When a reading begins, the **Read** button changes to **Stop**, allowing you to terminate a reading if desired.
8. When reading is complete, the drawer of the instrument opens, allowing you to remove the microplate. If the incubator is on, the drawer closes again after approximately 10 seconds.

Note: If you return to the instrument and find the drawer closed after a reading has finished, press the **Drawer** key. After the drawer opens, you can remove the microplate.

Next, go to Analyzing Data on page 205.

Performing Pre-Read Optimization

When using a SpectraMax Paradigm Multi-Mode Detection Platform, you can use pre-read optimization options. Using this function allows you to perform microplate optimization and read height adjustment.

The **Pre-Read Optimization Options** dialog appears when the **Show Pre-Read Optimization Options** check box is selected on the **More Settings** tab of the Settings dialog for a SpectraMax Paradigm detection cartridge. The available optimization options are dependant on the read mode.

Note: If you are using a SpectraMax Paradigm Tunable Wavelength (TUNE) Detection Cartridge and selected to run spectral optimization, the Spectral Optimization Wizard runs before the Pre-Read Optimization Options dialog appears. See Spectral Optimization on page 201.

To select pre-read optimization options:

1. Select the **Run Microplate Optimization** check box to run the Microplate Optimization Wizard before reading the microplate. See Microplate Optimization on page 196.
2. Select the **Run Read Height Adjustment** check box to run the Read Height Optimization Wizard before reading the plate. See Read Height Optimization on page 199.
3. If the microplate has a lid on it, select the **Plate is Lidded** check box.
4. Optionally, you can select the orientation for the microplate:
   - Landscape
   - Portrait
   - Opposite Landscape
   - Opposite Portrait
5. Click **Run Optimization** to run the selected optimization options.
6. Click **Read Plate** to read the microplate without running the selected optimization options.

Microplate Optimization

Microplate dimensions can vary slightly between production lots, which potentially affects measurement accuracy. SoftMax Pro Software allows microplate dimensions to be optimized by determining the centers of the four corner wells on the plate. Each time a microplate is optimized, a new microplate definition is created with dimensions specific to that lot.

Note: If a microplate type is to be used in different plate orientations for measurements, microplate optimization must be done for each plate orientation separately.

The microplate is optimized using the Microplate Optimization Wizard.

You can select to run the Microplate Optimization Wizard in the Pre-Read Optimization Options dialog that appears if the **Show Pre-Read Optimization Options** check box is selected on the **More Settings** tab of the **Settings** dialog for a SpectraMax Paradigm detection cartridge. See Selecting Instrument Settings on page 148.

When selected, the **Pre-Read Optimization Options** dialog runs after the **Read** button is clicked.

To perform microplate optimization:

1. In the **Pre-Read Optimization Options** dialog, select the **Run Microplate Optimization before reading the plate** check box.
2. Click **Run Optimization**.

The Microplate Optimization Wizard takes you through the optimization process:

- Insert the Microplate on page 197
- Running Optimization on page 197
- Selecting the Centers of the Four Corner Wells on page 198
- Verifying Microplate Dimensions on page 198

Insert the Microplate

Microplate dimensions are optimized by reading the four corner wells of the plate. The **Insert the Microplate** step provides controls to load and eject the microplate from the instrument and to select the orientation of the plate in the microplate drawer.

To insert the prepared microplate for optimization:

1. Click **Open the Microplate Drawer** to move the microplate drawer carrier outside the instrument.
2. Fill the corner wells of the plate with identical samples. To ensure accuracy, samples must be appropriate for the selected read mode detection method. Sample concentration and volume must be identical in each well.
3. Place the prepared microplate on the microplate drawer.
4. Click **Close the Microplate Drawer** to load the microplate into the instrument.
5. In **Select the Microplate Orientation**, select the orientation of the plate on the microplate drawer. The selected orientation is displayed graphically on the right, with well A1 marked in red. Make sure this orientation is the same as the orientation of the microplate in the drawer selected in the **Pre-Read Optimization Options** dialog.
6. Click **Next** to start the optimization. See Running Optimization on page 197. The optimization read begins automatically.

Running Optimization

Optimization in Progress displays the status of the optimization read and provides the ability to cancel the optimization in progress. The optimization read requires several minutes to complete.

To cancel the optimization process and close the **Microplate Optimization** wizard without saving the optimization data, click the **Cancel** button.

When the optimization read is complete, you can select the centers of each of the four corner wells. See Selecting the Centers of the Four Corner Wells on page 198.

Selecting the Centers of the Four Corner Wells

Use the **Select the Center** steps to precisely define the centers of the corner wells read in the optimization. Each **Select the Center** step displays an image of the well generated by the optimization read. Define the well centers by dragging the cross hairs to the position visually identified as the center. Perform a **Select the Center** step for each corner well individually.

To define the centers of the wells:

1. Place the pointer in the well image.
2. Click-and-drag the cross hairs to the desired center of the well.

Note: The cross hairs appear after the cursor is placed in the well image.

3. Click **Next** to define the centers of the remaining well reads.

After all four well centers are defined, the **Verify Microplate Dimensions** step appears. See Verifying Microplate Dimensions on page 198.

Verifying Microplate Dimensions

Use the **Verify Microplate Dimensions** step to verify that the x and y offsets and distances between rows and columns are correct. The offsets, distances, and lot name can be edited in the **Verify Microplate Dimensions** step.

1. If necessary, click the + next to **Microplate Dimensions** to display the fields in the category.
2. Verify that the x and y offset and distances between rows and columns are correct. If necessary, you can edit the dimension. All offsets and well-spacing dimensions are defined in millimeters.
3. If necessary, click the + next to **Microplate Name** to display the default name assigned to the new microplate definition.
4. Type a new Microplate Name, if desired.
5. Click **Save** to save the optimization data and create the new Microplate definition.

This new microplate definition can be selected from the plate types listed in the Plate Type Library. For additional information, see Plate Type Library on page 135.

If you have selected the **Run Read Height Adjustment** check box in the **Pre-Read Optimization Options** dialog, the **Read Height Optimization Wizard** runs before reading the plate. See Read Height Optimization on page 199.

Return to Collecting Data from a Microplate on page 194.

Read Height Optimization

The SpectraMax Paradigm Multi-Mode Microplate reader features an objective lens that can be moved up and down to optimize the read height used in luminescence, fluorescence intensity top, fluorescence polarization, and time-resolved fluorescence protocols. Read height is the distance between the top (using top reading) or bottom (using bottom reading) surface of the microplate being read and the surface of the objective lens. Optimizing read height matches the focus of the optics with the sample volume. This maximizes the raw signal, which yields the highest precision and maximum sensitivity.

A single sample with a known maximum signal and volume is placed on the same type of microplate used in the protocol. The sample is measured using the same, or very similar, read mode used in the protocol.

The optimized read height is saved in the protocol and is used for all subsequent runs of the protocol until reset by performing a new optimization or manually selecting a read height option.

You can select to run the **Read Height Optimization Wizard** in the **Pre-Read Optimization** Options dialog that appears if the **Show Pre-Read Optimization Options** check box is selected on the **More Settings** tab of the **Settings** dialog for a SpectraMax Paradigm detection cartridge. See Selecting Instrument Settings on page 148.

When selected, the **Pre-Read Optimization Options** dialog runs after the **Read** button is clicked.

To perform Read Height optimization:

1. Select the **Run Read Height Adjustments before reading the plate** check box.
2. Select **Microplate options**.
3. Click **Run Optimization**.

Running Read Height Optimization

Select the well to be measured to perform the optimization.

Use a standard or sample position with a good signal or pipette liquid with a known maximum signal to a well on the microplate used in the optimization. The concentration of the optimization sample should be at least ten times greater than the detection limit. The sample volume should be the same as that of samples measured in the protocol. If using a layout with standards, the standard well closest to the center of the plate is pre-selected. If not using a layout with standards, the first sample is pre-selected.

Note: When optimizing reading height for a fluorescence protocol, make sure the optimization sample is the same fluorescent substance that the detection method is configured to detect.

1. Load the plate into the instrument.
2. In the **Select Well** step, select the well containing the optimization sample.
3. Click **Next** to start the optimization.

   The **Optimization in Progress** step appears. The optimization can take from several seconds to several minutes depending on the detection methods used.
4. When the read is finished, the **Optimization Complete** step appears, displaying the Optimized Read Height. To specify a different read height, type a value in the **Custom Read Height** field. The read height is measured in millimeters.
5. Click **Save** to save the specified read height in the protocol. The specified read height is used for all subsequent runs of the protocol until reset by performing a new optimization or manually selecting a read height option.

When **Read Height Optimization** is complete and you return to the **Pre-Read Optimization** dialog, you can choose to change the microplate options and rerun Microplate Optimization or Read Height Optimization. When all you have completed all the desired optimization, click **Read Plate** to start reading the plate.

Spectral Optimization

Spectral optimization in spectral assays improves accuracy while reducing data acquisition and computational burden. Spectral optimization can help to get the maximum signal-to-background ratio for virtually any fluorophore or luminescence label compatible with the wavelength ranges of the cartridge.

Spectral Optimization is available for the SpectraMax Paradigm Multi-Mode Detection Platform with the following detection cartridge:

- Tunable Wavelength (TUNE) Detection Cartridge

To run the **Spectral Optimization Wizard**, select **Unknown** on the **Wavelengths** tab in the **Settings** dialog.

When **Unknown** is selected, the **Spectral Optimization Wizard** dialog opens after the **Read** button is clicked.

Running Spectral Optimization

1. In the **Read Settings** step, specify the wavelength ranges and other parameters for the optimization.

   The **Start Emission Wavelength** value must be at least 20 nm greater than the **Start Excitation Wavelength** value. Molecular Devices recommends using an emission value that is at least 40 nm greater than the excitation value.

   To perform Spectral Optimization, there must be at least 10 data points in the range for both the excitation and emission wavelengths. Make sure that the Wavelength Increment allows a minimum of 10 data points in each range.

   For a luminescence read, only emission values can be specified.
2. To specify the **Reading Height** or **Integration Time**, click **Advanced parameters** and then type the desired values in the fields.

   For Time-Resolved Fluorescence (TRF) reads, you can also specify **Pulse Length**, **Number of Pulses**, and **Measurement Delay**.
3. Click **Next** to continue.
4. In the **Sample Well** step, select the well in the plate layout that corresponds with the well in the microplate that contains your sample.
5. Click **Next** to continue.
6. Wait while the indicator in the **Optimization in Progress** step displays the operation's progress.

7. In the **Optimization Complete** step, a 3-dimensional heat map image appears. The heat map is generated using the formula (S – B) / B, where S = signal and B = background.

Note: For a luminescence read, the Spectral Optimization Wizard generates a 2-dimensional graph.

The cross hair in the image indicates the optimized peak wavelengths. To change the wavelengths for the read, drag the cross hair to a new location or type values in the fields.
The Emission value must be at least 20 nm greater than the Excitation value. Molecular Devices recommends using an emission value that is at least 40 nm greater than the excitation value.
For a luminescence read, only emission values can be specified.
To change the way the a 3-dimensional heat map image appears, select or clear the **Use LogScale** check box.

- If you have edited the Wavelength (Custom) values and want to reset the values to the wizard-defined optimized peak, click **Reset to Optimized Peak**.
- If you want to redefine the settings and run the wizard again, click **Restart Wizard**.
- If you want to save the wavelength values without immediately reading the microplate, click **Cancel**, and then click **Save** in the message that appears.

Right-click on the image to display the pop-up menu. You can select from the following options:

- **Copy Image** copies the image into a bitmap file that can be pasted into a graphic application.
- **Save Image to Disk** saves the image into a .bmp file.

8. Click **Read** to save the protocol with the specified excitation and emission wavelengths and use these values to read the microplate.

If you selected to perform pre-read optimizations, the Pre-Read Optimization Options dialog appears before the plate is read. See Performing Pre-Read Optimization on page 195.

Collecting Data From a Cuvette

Two types of data collection are possible using a cuvette: a reference and a sample reading.

You can start a reading at any time after defining instrument settings. It is not necessary to define groups and assign cuvettes within the Template Editor first, but you can use the Template Editor to create the appropriate number of cuvettes in the section. Alternatively, you can create a small number of cuvettes one at a time with the New Cuvette Set button on the Home tab.

Instrument settings must be the same for all cuvettes in a Cuvette Set section.

Sample Reading

You can select any cuvette in the Cuvette Set section and choose to read it by clicking the **Read**  button on the ribbon.

The Read button changes to **Stop**  , allowing you to terminate a reading if desired.

If you select more than one cuvette, the software starts with the left-most selected cuvette that does not contain sample read data.

Cuvette Set sections have a limit of 96 cuvettes. If this limit is reached, create a new Cuvette Set section.

Reference Reading

As in a spectrophotometer, taking a reference reading can be done either on air or using a cuvette containing the buffer of your sample.

The reference can be read before or after reading samples.

If no reference has been taken in a Cuvette Set section before samples were read and you then read a reference, the following occurs:

- The reference reading is applied to all cuvettes in the Cuvette Set section if it is the first reference reading.
- In subsequent reference readings if you select one or more cuvettes it will selectively replace the reference reading for those selected cuvettes.
- The time and date of the reading are displayed under each cuvette in the section.
- The reference is applied to any new cuvettes subsequently created in that section.

You can have more than one reference per Cuvette Set section. If you have cuvettes to which you want to apply a different reference:

1. Highlight the cuvettes in the Cuvette Set section.
2. Place the appropriate reference in the cuvette port.
3. Click the Ref button.

The time and date stamp of the reference for the selected cuvettes changes.

Any new cuvettes created in the Cuvette Set section after reading a second reference use the new reference reading.

The reference reading is applied to all cuvettes in the Cuvette Set section, unless you have highlighted specific cuvettes. The reference value is not displayed in SoftMax Pro Software. If you want to see the reference value, you can use one of the following procedures.

Procedure 1:

1. Create a cuvette in the CuvetteSet section and Ref on air (empty cuvette port).
2. Read a cuvette containing the blanking solution.

   If you want to have both reference and read values available, do the following:
3. Create a Cuvette Set with up to the maximum of 96 cuvettes.
4. Define a template blank in the Cuvette Set section.
5. Place buffer in the cuvette and select Read.
6. Click the Read button to read the remaining samples.

The optical density of the cuvette designated as the template blank is subtracted from the optical density of all the other cuvettes in the Cuvette Set section.

Procedure 2:

1. Create a Cuvette Set with up to the maximum of 96 cuvettes.
2. Reference and read the cuvette.
3. Create a template with the appropriate groups.
4. Create a column in each Group table with the formula !wellprereadLm1.

If you have a multiple-wavelength reading and want to see the reference at each wavelength, create additional columns with the formula !wellprereadLmx where “x” is 2, 3, or 4, corresponding to the wavelength for which you want to see a reference.

The OD/RFU/RLU with the reference subtracted is displayed in the Values column. The reference value for each sample is displayed in the custom column you have created.

Analyzing Data

5

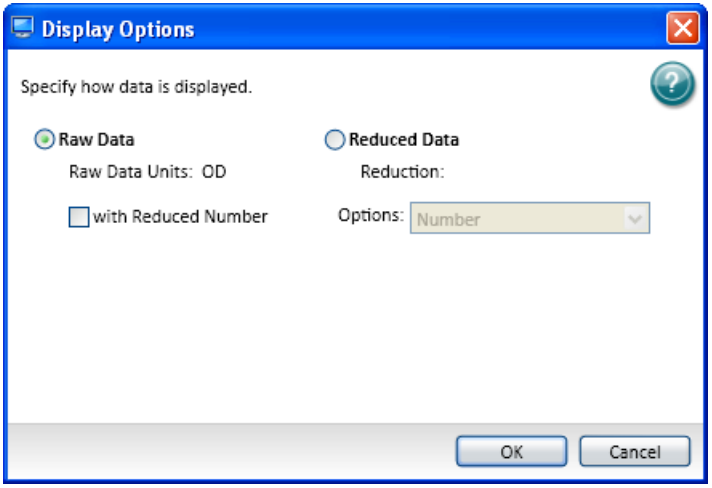
Any SoftMax Pro Software data file has a minimum of one Plate section or one Cuvette Set section. Most files also have an assigned plate template and associated Group, Graph, and Notes sections. However, depending upon the protocol and how you want to report the data, only some of these sections might be present in every file. Some simple protocols, such as reading the absorbance of proteins at 280 nm in a cuvette (or in a microplate with PathCheck Pathlength Measurement Technology), allow you to calculate concentration in the Plate or Cuvette Set sections. In such an example, Group and Graph sections are necessary only if you want to display the data in tables or graphically. Many common protocols consist of data acquired in a Plate section or Cuvette Set section. Standards and unknowns are defined in a template. Unknowns are then interpolated from a standard curve.

For more information see:

- Viewing the Data Display on page 206
- Performing Data Reduction on page 208
- Viewing Data in a Three-Dimensional Graph on page 223
- Graphing Data on page 230

Viewing the Data Display

At any time in an active **Plate** or **Cuvette** section you can click the **Display** button on the Home tab to change how the data is presented.



Choices available in the **Display Options** dialog include the selection between **Raw** and **Reduced Data**.

Raw Data

Selecting Raw displays the default data type for the selected read type:

- **Endpoint**: Raw absorbance, fluorescence, or luminescence values.
- **Kinetic**: The change in raw OD/RFU/RLU values over time, displayed as a plot.
- **Spectrum**: Raw OD/RFU/RLU values for the range of wavelengths, displayed as a plot.
- **Well Scan**: Raw OD/RFU/RLU values as shades of blue to red.

To see a reduced number, click **with Reduced Number**.

Reduced Data

The reduced data number is a combination of plate blank subtraction in the Plate section, wavelength reduction, and if applicable, a Spectrum, Well Scan, or Kinetic reduction.

The reduced number is reported in the Group section when a template has been defined.

- **Number**
  To view by reduced number alone, select **Number**.
- **Plot**
  For Kinetic reads, the reduced number and a plot of the data can be selected for display.
- **Grayscale**
  Grayscale 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).
- **Color Map**
  Color Map presents the raw data in eight colors, changing from blue (for values less than or equal to the low limit) to red (for values greater than or equal to the high limit).

Viewing Reduced Data

To display reduced data:

1. Click **Display** on the Home tab to open the **Display Options** dialog.
2. Click **Reduced Data**.
3. Select an option from the **Options** list.
4. Click **OK**.

Viewing Raw Data

To display raw data:

1. Click **Display** on the Home tab to open the Display Options dialog.
2. Click **Raw Data**.
3. Optionally, click **with Reduced Number** to include the reduced data number on the display.
4. Click **OK**.

Zooming the Display

To zoom the display to enlarge a well:

1. Select the well or cuvette to zoom.

Note: This function works with Well Scan, Spectrum, and Kinetic reads only, and is not allowed for Endpoint reads.

2. Click **Zoom** on the **Home** tab.
3. Click **Close** to close the zoomed well.

Zoom Window Actions

- Click **Print** to print the zoomed window.
- Click the **Show Reduced/Show Raw** button to toggle between reduced data and raw data.
- Click **Reduction** to open the **Data Reduction** dialog where you can change the characteristics of the reduced data display.
- Click **Options** to open **Zoom Well Options** where you can change the graph type settings and the characteristics of the zoomed display.

Masking Wells or Cuvettes

The **Mask** button allows you to remove selected wells from the data analysis.

Mask is a toggle. Selecting masked wells and clicking the **Mask** button unmasks them.

To mask a well:

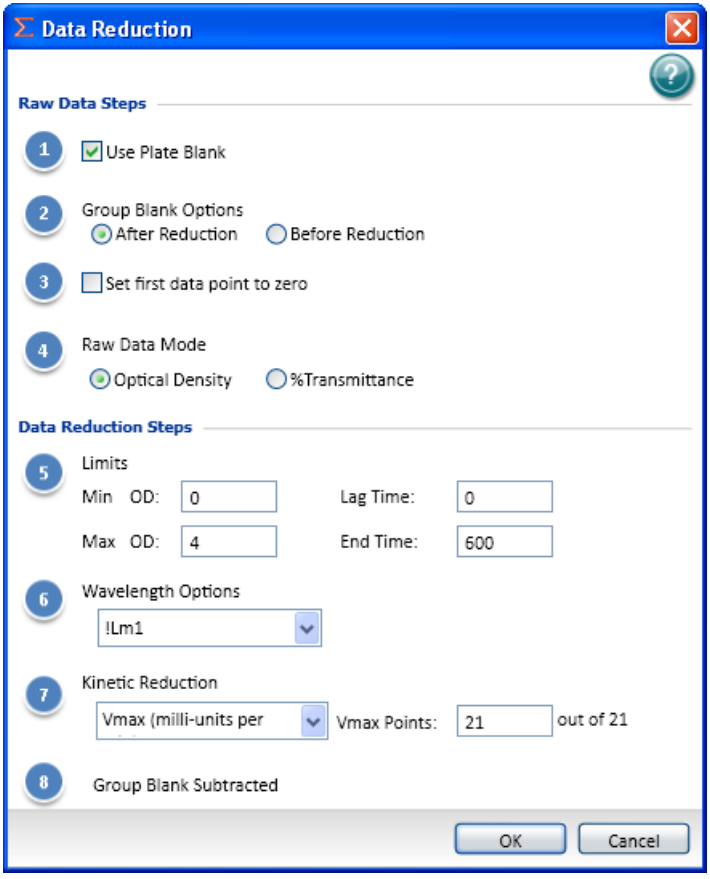
1. Select the well to be masked.
2. Click **Mask**.

Performing Data Reduction

The reduction process in SoftMax Pro Software is based on formulas that reduce the raw data to show a single number for each well or cuvette. Further analysis of this reduced number is done in **Group** and **Graph** sections.

When reducing raw information collected from the instrument, SoftMax Pro Software performs calculations hierarchically. Only the data reduction calculations that apply to the data that is read according to the instruments mode, type, and settings are performed.

Click the **Reduction** button on the **Home** tab or the **Reduction** shortcut button on the window header bar to open the **Data Reduction** dialog.

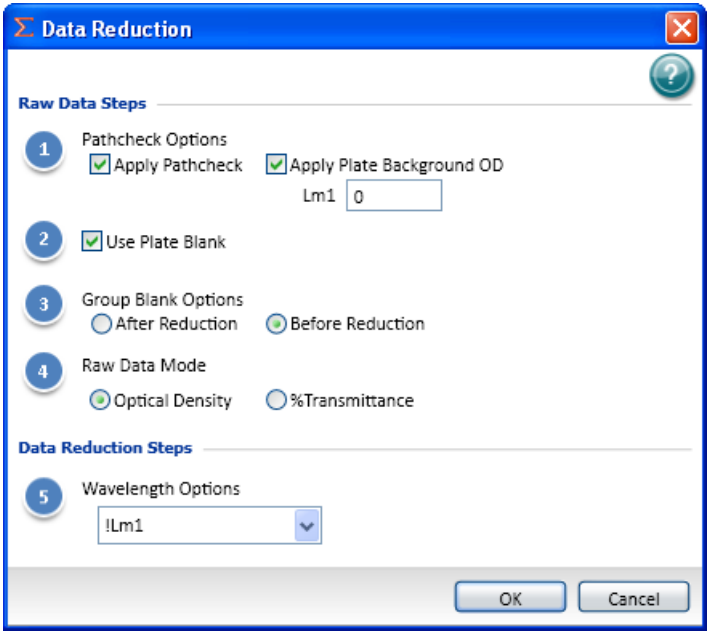


SoftMax Pro software performs the applicable calculations in the **Plate** section or **Cuvette Set** section in the order shown below. If an option has either not been selected in the **Instrument Settings** dialog, is not available for the instrument you are using, or has not been defined in the template, SoftMax Pro software continues with the next applicable calculation.

Raw Data Steps

Raw data displays are calculated using the following steps that apply to the selected mode, type and settings:

- **Apply PathCheck normalization** (Absorbance Endpoint only)
  PathCheck Pathlength Measurement Technology normalization calculations apply to the data only when PathCheck has been enabled in the Absorbance mode/Endpoint type PathCheck settings. When PathCheck is enabled, this calculation can be disabled in the Data Reduction dialog. For more information, see PathCheck Pathlength Measurement Technology on page 138.



- **Subtract Plate Background constant** (Absorbance Endpoint only)
  Subtraction of the Plate Background constant applies to the data only when PathCheck has been enabled in the Absorbance mode/Endpoint type PathCheck settings. When PathCheck is enabled, this calculation can be disabled in the Data Reduction dialog.

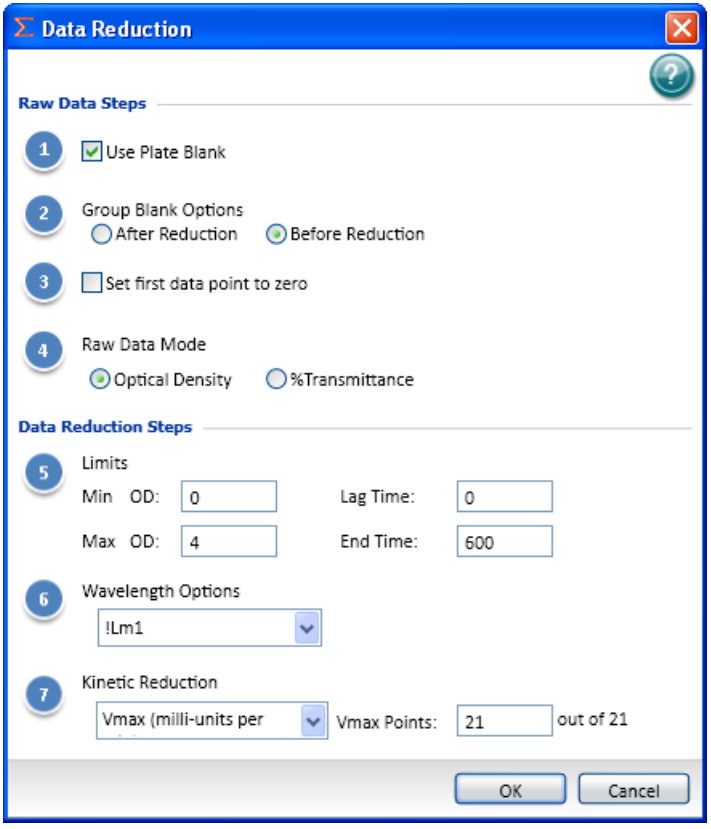
- **Use Plate Blank**
  The **Use Plate Blank** calculation is available when a plate blank is specified in the template settings for the plate. To enable subtraction of the plate blank value, check **Use Plate Blank**. This calculation can be disabled in the Data Reduction dialog. For more information about plate blanks, see Blanking on page 157.
- **Group Blank Option**
  The **Group Blank Option** calculation is available when a group blank is specified in the template settings for the plate. The subtraction of the group blank value can be selected as a pre-reduction action or a post-reduction action.
  To enable the group blank value subtraction calculation as a pre-reduction action, select **Before Reduction**.
  To enable the group blank value subtraction calculation as a post-reduction action, select **After Reduction**. When After Reduction is selected, the last step in the Data Reduction dialog shows **Group Blank Subtracted**.
  For more information about group blanks, see Blanking on page 157.
- **Polarization** or **Anisotropy** (Fluorescence Polarization only)
  Fluorescence Polarization mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. These S and P values are used to calculate the **Polarization** (mP) and **Anisotropy** (r) values in SoftMax Pro Software.
  G-factor (Grating factor) is used in fluorescence polarization to correct polarization data for optical artifacts, converting relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency.
  For more information, see Fluorescence Polarization (RFUs) on page 222.
- **Set first data point to zero** (Kinetic only)

- **Raw Data Display Mode**
  Select either Optical Density or % Transmittance. For more information see Data Mode (%Transmittance/Absorbance) on page 222.
  - **Optical Density**: The amount of light passing through a sample to a detector relative to the total amount of light available. Optical Density includes absorbance of the sample plus light scatter from turbidity.
  - **% Transmittance** is the ratio of transmitted light to the incident light (for absorbance readings).

Data Reduction Steps

Data Reduction display includes one or more of the following calculations:

- **Limits**
  Apply Reduction Limits (!Lm1 & !Well Lm1 after this step)



- **Wavelength Options**
  Apply Wavelength Combination (!CombinedPlot & !WellcombinedPlot after this step)
  Choose !Lm1 or Custom. For information about custom reduction formulas, see Data Reduction Formulas on page 213.
- **Kinetic** or **Spectrum Reduction**
  Select a Kinetic reduction mode from the list. For more information, see Kinetic Reads (Data Reduction) on page 217.
  Select a Spectrum reduction mode from the list. For more information see, Spectrum Reads (Data Reduction) on page 220.
  The number of Vmax Points is determined by the Timing settings.
- **Group Blank Subtracted**
  This step is displayed when the Group Blank Option is set to After Reduction.

For additional information about data reduction, see the following topics:

- Endpoint Reads (Data Reduction) on page 217
- Well Scan Reads (Data Reduction) on page 221

When all applicable data reduction options have been set, click **OK**.

Data Reduction Formulas

The reduction process in SoftMax Pro Software is based on formulas that reduce the raw data to show a single number for each well or cuvette. Further analysis of this reduced number is done in Group and Graph sections.

Custom Reduction Formulas

If the predefined reduction formulas do not meet your needs, you can create one or more different reduction formulas for any read type (Endpoint, Kinetic, Spectrum, or Well Scan). Choosing Custom from any of the menus or dialogs displays a Formula button with which you can open a Formula dialog.

Examples of some formulas that can be used when combining multiple wavelengths in a **Formula** dialog are given in the following table.

Table 5-1 Wavelength Reduction Formula Examples

2 Wavelengths	3 Wavelengths	4 to 6 Wavelengths
!Lm1 + !Lm2	Lm1 + !Lm2 + !Lm3	!Lm1 + !Lm2 + ... + !Lmn
!Lm1 - !Lm2	(!Lm1 – !Lm3) / (!Lm2 – !Lm3)	(!Lm1 – !Lm6) /(!Lm2 – !Lm5) / (!Lm3 – !Lm4)
!Lm1 / !Lm2	!Lm1 / !Lm3	!Lm1 / !Lmn
!Lm1 * !Lm2	!Lm1 * !Lm3	!Lm1 * !Lmn
Log10(!Lm1/!Lm2)	Log10(!Lm1/!Lm3)	Log10(!Lm1/!Lmn)
!Pathlength	!Pathlength	!Pathlength

Table 5-1 Wavelength Reduction Formula Examples (cont'd)

2 Wavelengths	3 Wavelengths	4 to 6 Wavelengths
The following Wavelength Reduction Formulas apply to all wavelength combinations.		
!Lmx/constant	For example, Lm1/1.44 for quantitating a polyclonal antibody by measuring the absorbance at A280 with PathCheck on.	
Average(!Lm1&!Lm2&!Lm3)	Averages together the optical densities for multiple readings at the same wavelength (for example, if you read the well six times at 280 nm).	
Min(!Lm1&!Lm2&!Lm3)	Reports the minimum OD/RFU/RLU recorded for multiple wavelength readings in each well.	
Max(!Lm1&!Lm2&!Lm3)	Reports the maximum OD/RFU/RLU recorded for multiple wavelength readings in each well.	
If(!Lmz<A,makeerr(118), (if(!Lmx>B, makeerr(117), !Lm1)))	Reports "low" for any well with an OD/RFU/RLU less than A, "high" for any OD/ RFU/RLU greater than B, and the OD/ RFU/RLU of any well that lies between A and B.	

Custom reduction formulas using mathematical operators or terms can be used to obtain specific types of data. The following table provides some examples of such formulas for Kinetic and Spectrum readings.

Table 5-2 Kinetic and Spectrum Reduction Formula Examples

Kinetic	
Vmaxcorr(!combinedplot, !Vmaxpoints,!readinterval)	Reports Vmax correlation coefficient for plots in all 96 wells.
Vmax(Delta(!combinedplot) , !Vmaxpoints,!readinterval)	Reports the Vmax Rate of the delta between each time point.
Spectrum or Kinetic	
Nthitem(!Lm1,X)	Reports the optical density at item X in the list of readings. For example, if you have a Kinetic run with 20 time points and X is 10, it reports the OD/RFU/RLU of the 10th time point. Similarly, if you have a Spectrum scan with 20 measurements and X is 10, it reports the OD/RFU/RLU of the 10th wavelength measured.

For a full discussion of custom formulas, see the *SoftMax Pro Software Formula Reference Guide* help.

When you finish your entry in the Calculation dialog and click OK, the formula is displayed and becomes the default selection for the Custom option.

Endpoint Reads (Data Reduction)

The default reductions for Endpoint readings are !Lm1, !Pathlength (only when PathCheck is enabled), and Custom for all instruments when you read at a single wavelength or if you choose 1-Ref. The !Pathlength reduction reports the pathlength in each well. Additional wavelength combinations become available depending on the number of wavelengths chosen.

You can choose Custom to create different reduction formulas. See the *SoftMax Pro Software Formula Reference Guide* help for more on custom formulas.

Note: The wavelength reduction display is not instrument-dependent in SoftMax Pro Software, so all selections are always shown, regardless of the instrument.

Kinetic Reads (Data Reduction)

Kinetic read data reduction options include the following:

Wavelength Combination

The wavelength combination choices depend on the number of wavelengths chosen in the protocol. For example, if you read at two wavelengths, choices default to !Lm1 and !Lm2, along with Custom.

Kinetic Reduction

Kinetic reductions are applied to the value at each time point after the wavelength combination formula is applied.

Vmax per min and Vmax per sec reductions are available for all instruments that are capable of Kinetics; the default Kinetic reduction is Vmax.

Vmax

Vmax is the maximum slope of the Kinetic display of mOD/min or RFU/RLU per second. Vmax is calculated by measuring the slopes of a number of straight lines, where Vmax Points determines the number of contiguous points over which each straight line is defined.

This is an alternative method for analyzing non-linear Kinetic reactions that 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.

Time to 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 changes 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 reach a specified OD or RFU/RLU (onset OD/RFU/RLU).

This elapsed time data is useful for cascade reactions including clot formation (endotoxin testing, for example) and clot lysis applications where the change in reagent concentration does not affect the maximum optical density change but changes the time required for the reaction to reach completion.

Time at Minimum

This setting reports the time at the minimum OD, RFU/RLU, or %T that falls within the reduction limits.

Time at Maximum

This setting reports the time at the maximum OD, RFU/RLU, or %T that falls within the reduction limits.

Time at 1/2 Maximum

This setting reports the time at the half of the maximum OD, RFU/RLU, or %T that falls within the reduction limits.

To calculate this reduction, SoftMax Pro Software determines the Kinetic point (within the reduction limits) that has the maximum signal level (OD or %T) and divides it by 2 to get the 1/2 Maximum value. Then it finds the time value at the 1/2 Maximum.

Area Under Curve

This reduction estimates the area under the curve as defined by the data plots 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.

Slope

This setting determines the slope of the combined plot (for example, the slope of the line using linear regression after the wavelength combination reduction). It is calculated using all visible time points in the reduction window.

Slope is the same as Vmax Rate when Vmax Rate is set to the same number of points as the run, but is different if you have modified Vmax Points.

Vmax Points

This setting defines the maximum size of the line segment used to determine the slope of the line used in calculating the rate of the reaction. The default is the total number of points taken in the reading.

The first slope is calculated for a line drawn beginning at the first reading as defined by Lag Time (described below) and ending at a total number of readings equal to the Vmax Points setting. The second and any subsequent slopes are calculated beginning at the second time point and ending at a total number of readings equal to Vmax Points. The steepest positive or negative slope is reported as Vmax.

If the data plot displays fewer time points (data points) than Vmax Points, all of the time points are used to determine the slope of the data.

Kinetic Limits

Limits define the data that are viewed and included in data reduction. If you alter a limit to show less data, you can always display the excluded data again by changing the limit.

The display of OD/RFU/RLU values is 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.

MaxOD/RFU/RLU

The limit for the maximum value to report. Any values from the reading that are above this limit are excluded from data reduction. The default for MaxOD is 1.0, while it is 200,000 for RFU/RLU.

The MaxOD/RFU/RLU 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 MaxOD/RFU/RLU 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.

MinOD/RFU/RLU

The limit for the minimum value to report. Any values from the reading that are under this limit are not shown and are excluded from data reduction. The default is 0 OD or RFU/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, but does not prevent data from being collected. Kinetic plots do not display the data collected prior to the Lag Time. The default is 0.

End Time

Specifies the time at which to stop showing data in the display and exclude from data reduction. Any values occurring after this limit are not reported in the display and are excluded from data reduction. The default setting is the total assay time.

Set First Data Point to Zero

When selected, this setting offsets the first point to (0,) and shifts all other data accordingly. Deactivating this setting can cause some data points that do not fall within the default limits to disappear.

To see more data, yet still display absolute values, increase the limits for MinOD/RLU/RFU and MaxOD/RLU/RFU.

Spectrum Reads (Data Reduction)

The Spectrum reduction formula is applied to the list of numbers in each well (values at each wavelength) after the wavelength combination formula is applied. The default reduction for Spectrum readings is Lambda at Maximum.

Maximum

Reports the maximum absorbance (optical density) or percent transmittance (%T), RFU or RLU within the reduction limits.

Minimum

Reports the minimum absorbance (optical density) or percent transmittance (%T), RFU or RLU within the reduction limits.

Lambda at Maximum

Reports the wavelength at which maximum absorbance (optical density) or percent transmittance (%T), RFU or RLU within the reduction limits.

Lambda at Minimum

Reports the wavelength of minimum absorbance (optical density) or percent transmittance (%T), RFU or RLU within the reduction limits.

Area Under Curve

Estimates the area under the curve as defined by the data plots (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.

Spectrum Limits

Limits define the data that are viewed and included in data reduction. If you alter a limit to show less data, you can always display the excluded data again by changing the limit.

Start (Wavelength) nm

Specifies the limit for the minimum wavelength setting to report. Any values from the reading that are under this limit are not shown and are excluded from data reduction.

End (Wavelength) nm

Specifies the limit for the maximum wavelength setting to report. Any values from the reading that are above this limit are not shown and are excluded from data reduction.

Well Scan Reads (Data Reduction)

Well Scan read data reduction options include the following:

Wavelength Combination

When one wavelength is chosen, !Lm1 is the only choice besides Custom. When more than one wavelength is specified additional choices are offered.

Well Scan Reduction

Choices are Maximum, Minimum, Average (default), and Custom. Average provides the average value for all points in the Well Scan.

Data Mode (%Transmittance/Absorbance)

For Absorbance reads only, you can choose whether to display absorbance data as %Transmittance or Absorbance (OD).

Separate mathematical calculations are used for handling OD and %T calculations for Pre-read plate blanking, PathCheck, and Reference, because OD calculations are performed on a linear scale, whereas %T calculations are performed on a logarithmic scale. However, SoftMax Pro Software does not perform other calculations differently for absorbance and %T modes. Because of this, Molecular Devices recommends that %T be used only for raw OD, raw OD with Pre-read plate blank subtraction, or raw OD readings which are normalized by the PathCheck program. Use caution when using %T on reduced numbers or any readings that apply other calculations since the data might not be calculated correctly.

Fluorescence Polarization (RFUs)

By using a fluorescent dye to label a small molecule, its binding to a large molecule can be monitored through its speed of rotation.

Fluorescence Polarization mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. These S and P values are used to calculate the Polarization (mP) and Anisotropy (r) values in SoftMax Pro Software. You can choose to display any of these data types on the Plate section by selecting Reduced Data in the **Display** dialog. When Raw (S&P) is displayed, the mP value is by default used for all reduction calculations.

When exporting, you can export either mP, or r as reduced data.

Polarization (mP) is calculated as follows:

$$mP = 1000 \times \frac{(parallel - (G \times perpendicular))}{(parallel + (G \times perpendicular))}$$

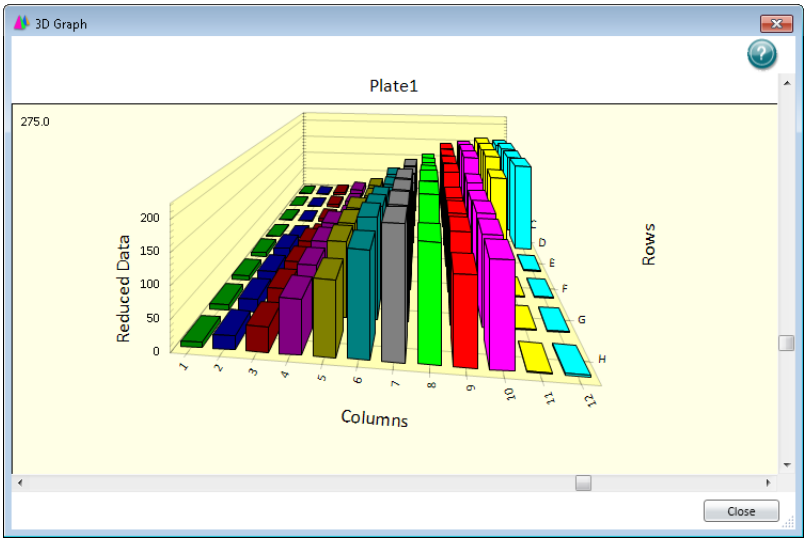
Anisotropy (r) is calculated as follows:

$$r = \frac{(parallel - (G \times perpendicular))}{(parallel + (2G \times perpendicular))}$$

Viewing Data in a Three-Dimensional Graph

To view a three-dimensional graphical representation of reduced plate data, view the **Plate** section and then click the **3D Graph** button in the **Plate Tools** area of the **Home** tab in the ribbon, or in the toolbar at the top of the **Plate** section.

You can rotate a 3D graph vertically and horizontally.



To rotate the 3D graph:

- To rotate the 3D graph vertically, drag the vertical scroll bar located to the right of the 3D graph.
- To rotate the 3D graph horizontally, drag the horizontal scroll bar located below the 3D graph.
- To start or stop a continuous animated horizontal rotation of the 3D graph, double-click the 3D graph.

You can also resize the **3D Graph** dialog by dragging a side or a corner of the dialog.

For customization and export options, right-click anywhere on the 3D graph.

You can quickly choose a customization option from the menu, or click **Customization Dialog** to change several options at the same time. See Customizing a Three-Dimensional Graph on page 224.

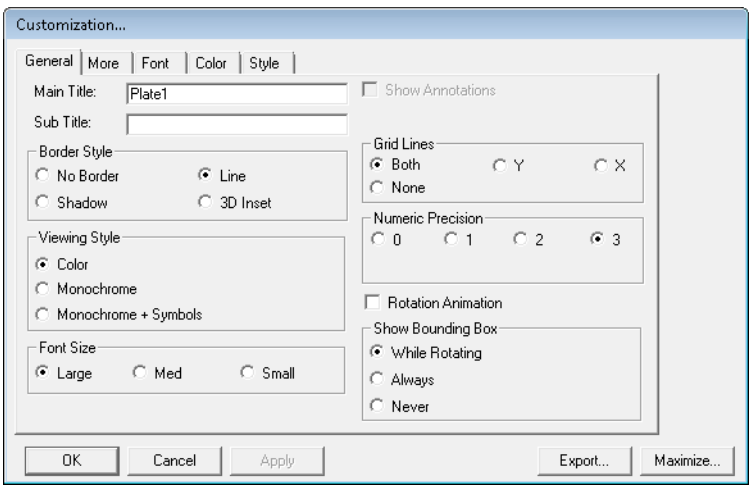
Other options include:

- **Numeric Precision** allows you to show no decimals or up to 3 decimal places in the data.
- **Plotting Method** allows you to view the graphed data in **Wire Frame**, **Surface**, **Surface with Shading** views.
- **Maximize** expands the 3D graph to a full screen view. To return to the normal view, click the title bar or press **Esc**.
- **Export Dialog** opens the **Export Control** dialog allowing you to export the graphical image to your choice of formats, or export the data to a text format. See Exporting a Three-Dimensional Graph on page 228.

To close the **3D Graph** dialog, click **Close**.

Customizing a Three-Dimensional Graph

The customization dialog has five tabs that contain customization options for the current 3D graph.



Click a tab to work with the options under that tab.

- For the **General** tab, see General Customization Options for a 3D graph on page 225.
- For the **More** tab, see More Customization Options for a 3D graph on page 226.
- For the **Font** tab, see Font Customization Options for a 3D graph on page 226.
- For the **Color** tab, see Color Customization Options for a 3D graph on page 227.

- For the **Style** tab, see Style Customization Options for a 3D graph on page 227.

After making changes to the options in this dialog, you can click **Apply** to view how the changes affect the 3D graph.

To close the **Customization** dialog and save any unapplied changes, click **OK**.

Click **Export** to close the **Customization** dialog and open the **Export Control** dialog allowing you to export the graphical image to your choice of formats, or to export the data to a text format. See Exporting a Three-Dimensional Graph on page 228.

Click **Maximize** to close the **Customization** dialog and expand the 3D graph to a full screen view. To return to the normal view, click the title bar or press **Esc**.

General Customization Options for a 3D graph

Click the **General** tab to customize the 3D graph with the following options:

- **Main Title**: Type the text you want for the title of the 3D graph.
- **Sub Title**: Type the text you want below the title of the 3D graph.
- **Show Annotations**: To show annotations, select this check box. This option is not available if there are no annotations available.
- **Border Style**: To change the border around the outside of the graphical image, click **No Border**, **Line**, **Shadow**, or **3D Inset**.
- **Viewing Style**: To change the display of the image background, click **Color**, **Monochrome**, or **Monochrome + Symbols**.
- **Font Size**: To change the font size for all the text, click **Large**, **Med**, or **Small**.
- **Grid Lines**: To view both the Y and X grid lines, click **Both**. To view only the grid lines for the Y-axis, click **Y**. To view only the grid lines for the X-axis, click **X**. To remove the grid lines, click **None**.
- **Numeric Precision**: Selections in this area have no effect on the 3D graph display.
- **Rotation Animation**: To start a continuous animated horizontal rotation of the 3D graph, select this check box.
- **Show Bounding Box**: To display a wire-frame box around the 3D graph under specific conditions, click **While Rotating**, **Always**, or **Never**.

More Customization Options for a 3D graph

Click the **More** tab to customize the 3D graph with the following options:

- **Rotation Detail**: To change the display style when the 3D graph rotates, click **Wireframe** to display only the outlines of the 3D graph, **Plotting Method** to display the data plot in full 3D while displaying only outlines for the grid, or **Full** to display the entire 3D graph in full 3D during rotation.
- **Rotation Increment**: To control the speed and direction of the animated rotation, select an increment from the list. Values closer to zero rotate slower, while values farther from zero rotate faster. To rotate counter-clockwise, select a negative value.
- **Shading Style**: To change the display of the shading, click **White** or **Color**. This option has no effect on the display if there is no shading in the 3D graph.
- **Plotting Method**: To change the display of the data plot, click **Wire Frame**, **Surface**, or **Surface with Shading**.

Font Customization Options for a 3D graph

Click the **Font** tab to customize the text in the 3D graph:

- **Main Title** includes only the title of the 3D graph.
- **Sub-Title** includes only the text directly below the title of the 3D graph, if applicable.
- **Subset/Point/Axis Labels** includes all of the other text in the 3D graph.

For each text area, you can select the font and select a check box to add **bold**, **italic**, and **underline** styles to the text.

A **sample** appears at the bottom to help you with your selections.

Color Customization Options for a 3D graph

Click the **Color** tab to customize the colors used in the 3D graph.

Changes in the **Color** tab are automatically applied to the 3D graph as they are selected.

You can click an option from the **Graph Attributes** group and then click a color to apply to the selected attribute:

- **Desk Foreground** includes the text of the title and subtitle of the 3D graph.
- **Desk Background** includes the area behind the title and subtitle of the 3D graph.
- **Shadow Color** includes the border shadowing when Shadow is select for the Border Style in the General tab.
- **Graph Foreground** includes all the text other than the title and subtitle, and the border around the 3D graph.
- **Graph Background** includes the area within the border and behind the 3D graph.
- **X and Z Axis Plane** includes the floor of the 3D graph.
- **Y Axis Plane** includes the walls of the 3D graph.

Quick Styles group allows you to select from pre-defined styles for the 3D graph:

- Select the **Bitmap/Gradient Styles** check box to add texture to the 3D graph.
- Click the **Inset**, **Shadow**, **Line**, and **No Border** options in the **Light**, **Medium**, and **Dark** columns to view how the selected styles affect the 3D graph.

Style Customization Options for a 3D graph

Click the **Style** tab to customize colors used in the 3D graph.

Changes in the **Style** tab are automatically applied to the 3D graph as they are selected.

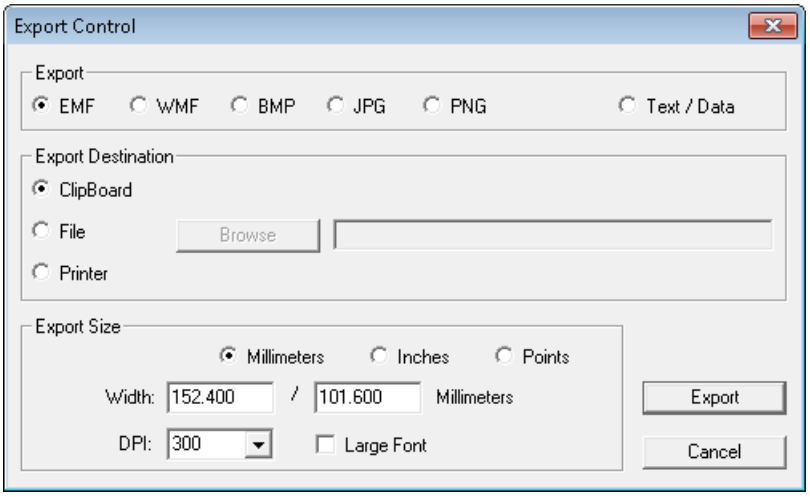
The customizable items in the list on the left are dependent on the some of the selections in the other tabs, and on the type of data.

Click an item in the list, and then click a color to apply to it.

Exporting a Three-Dimensional Graph

Before exporting an image of a 3D graph, make sure that you have rotated the 3D graph to the view that you want and that you have set your customization options. See Customizing a Three-Dimensional Graph on page 224.

For export options, right-click anywhere on the 3D graph and then click **Export Dialog** to open the **Export Control** dialog.



Click the graphic format from the **Export** area. If you select **Text/Data**, only the data is exported. For more information about exporting the data, see Exporting Data from a Three-Dimensional Graph on page 229.

In the **Export Destination** area, select how you want to export the image or data:

- Click **ClipBoard** to export the image or data to the Windows clipboard so that you can paste it into another program like a word processor or a spreadsheet.
- Click **File** to save the image or data in a file on your computer or network. Click **Browse** to choose the destination and give the file a name.
- Click **Printer** to export the image or data directly to a printer. After you click **Print**, you can select the printer and printer options.

The options in the **Export Size** area are dependent on the selected **Export** format and the **Export Destination**:

- **Full Page** is available for exporting to a Printer.
- **No Specific Size** is available for the WMF format exported to the clipboard or a file. Click this option to let the export operation select the appropriate size for the image.
- **Pixels** is available for BMP, JPG, and PNG formats exported to the clipboard or a file.
- **Millimeters**, **Inches**, and **Points** are available for EMF and WMF formats exported to the clipboard or a file, and for all graphic formats exported to a printer.
- If you have selected measurement units, type the width and height for the exported image.
- For all graphic formats exported to the clipboard or a file, select the **DPI** (dots per inch) for the exported image.
- For all graphic formats exported to the clipboard or a file, You can select the **Large Font** check box to increase the size of the text in the exported image.

When you have finished selecting your export options, click **Export** or **Print**.

If you are exporting the data as text, then the Export dialog opens with additional options for formatting the exported data. See Exporting Data from a Three-Dimensional Graph on page 229.

Exporting Data from a Three-Dimensional Graph

To export just the data from a 3D graph:

1. Right-click anywhere on the 3D graph and then click **Export Dialog**.
2. In the **Export Control** dialog, click **Text/Data**.
3. In the **Export Destination** area, select how you want to export the data:

- Click **ClipBoard** to export the data to the Windows clipboard so that you can paste it into another program like a word processor or a spreadsheet.
- Click **File** to save the data in a file on your computer or network. Click **Browse** to choose the destination and give the file a name.

4. Click **Export**.
5. In the **Export** dialog, in the **Select Subsets and Points** area, choose to export **All Data** or **Selected Data**.

If you choose **Selected Data**, you can export columns of data by clicking the numbers that correspond to the column numbers on the microplate, or you can export rows of data by clicking the letters that correspond to the row letters on the microplate. If you want to export a single well or a group of wells, click the column numbers and row letters that correspond to the well or group of wells on the microplate. To select more than one column or row in each list, hold down the **Shift** or **Ctrl** key as you click.

1. In the **Export What** area choose to export just the **Data** or the **Data and Labels**.
2. If you have multiple values available in your data, choose an option from the **Data to Export** area.
3. In the **Export Style** area, click **List** or **Table**.

- When you choose to export a **List**, define the delimiter for the data in each line by clicking **Tab** or **Comma**. The type of delimiter you choose must match the requirements of the program where you want to use the exported data.
- When you choose to export a **Table**, you can click **Subsets/Points** to place the data from the microplate column numbers into the rows of the table and the data from the microplate row letter into the columns of the table, or click **Point/Subsets** to place the data from the microplate row letters into the rows of the table and the data from the microplate column numbers into the columns of the table.

4. In the **Numeric Precision** area, click **Current Precision** to export the data using the rounding defined in the 3D graph, or click **Maximum Precision** to export the rounding available in the data file.
5. Click **Export** to export the data to the format defined in Step 3.

Note: If you exported the data to the Windows clipboard, you need to immediately open the program where you want to use the data and paste the data into that program.

Graphing Data

The **New Graph** button opens the Plot Editor dialog which enables you to create a graph from the collected data.

Double-clicking a well creates a zoomed well plot of the data in that well.

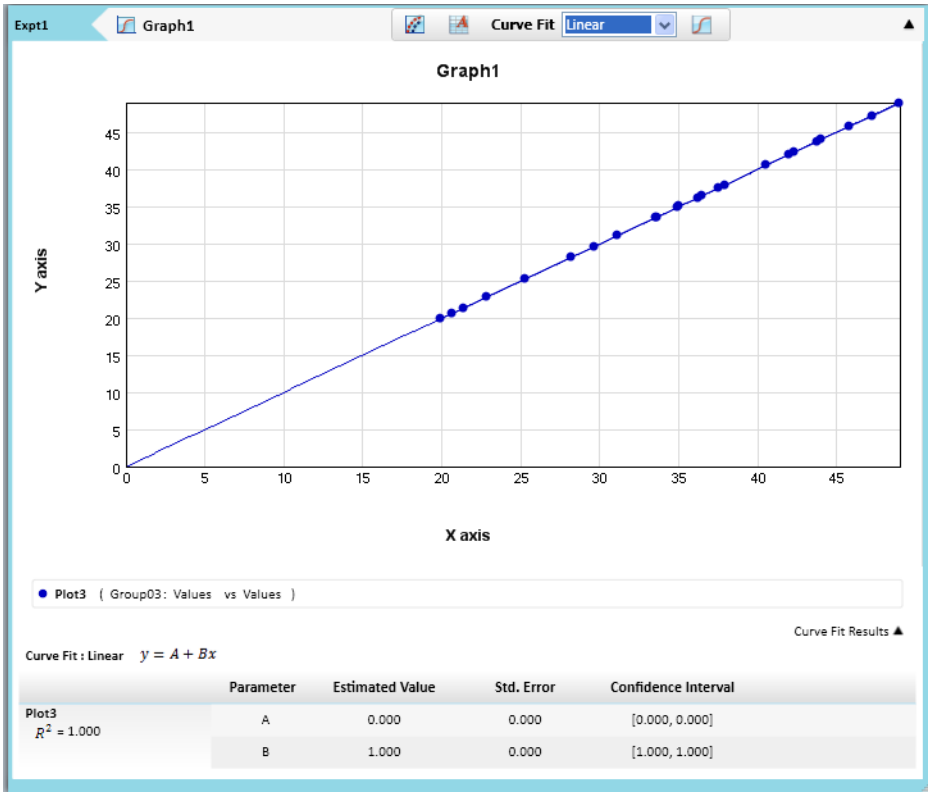
For more information about graphing data see Working with Graphs on page 231.

To view a three-dimensional graphical display of the data, see Viewing Data in a Three-Dimensional Graph on page 223.

Working with Graphs

Graph sections are used to plot information from groups as scatter plots.

Once a graph has been created, new plots can be added and deleted, the axes can be customized, and the size of the graph can be changed. Grid lines can be enabled or disabled (default is enabled).



Graph sections are divided into:

- The body of the **Graph** section.
- The Legend, see Graph Legend on page 232.

You can create more than one **Graph** section within an Experiment data file, and plots in the **Graph** section can be created from any Experiment in the file.

See the following sections for information about:

- Creating a new graph, see Creating a New Graph on page 235.
- Changing the name of the graph, see Changing the Graph Name on page 234.
- Changing the display, see Zooming the Graph Display on page 237.
- Changing the graph appearance, see Changing the Graph's Appearance on page 237.
- Editing an existing graph, see Editing an Existing Graph on page 240.
- Toggling the Log/Linear Functions, see Toggling the Log/Linear Functions on page 239.
- Exporting the graph to a .png file, see Exporting a Graph on page 234

Graph Legend

The graph legend contains information about the contents of the graph.

Independent Fits

- The formula(s) for the selected curve fit(s)
- For each plot:
- R^2 value
- EC50 value (4P and 5P only)
- For each parameter:
- Estimated Value
- Std. Error of the estimate
- The confidence interval for a given confidence level (chosen in the Statistics tab of the curve settings dialog) is a range such that the true value lies within the range with the desired probability.

- Independence: Parameter *Independence* is one way to examine the suitability of a given curve fit for the data set. It is a measure of the extent to which the best value of one parameter depends on the best values of the other parameters, and is a number between 0 and 1, with 1 being the ideal. In the graph fit legend, parameter independence has been translated into bars, where 10 bars indicate a high degree of independence; because only very small values indicate a problem, a nonlinear transformation is used for this translation. If one or more parameters have few bars or no bars, the curve fit might not be appropriate for the data set.

  For example, if the data set is sigmoidal with clear lower and upper asymptotes, 4-parameter fits should be appropriate with many bars for all parameters. However, if one or both of the asymptotes is missing, the A or the D parameter will have few bars, indicating that reliable values cannot be deduced from the data set.
- The confidence interval for a given confidence level (chosen in the Statistics tab of the curve settings dialog) is a range such that the true value lies within the range with the desired probability.

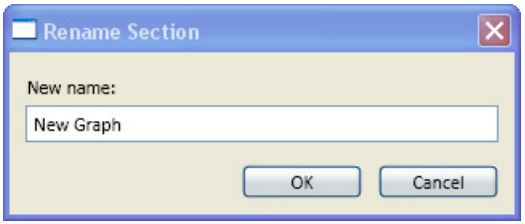
Global Fits

- The formula for the selected curve fit.
- For each plot:
- Estimated Relative Potency (Estimated Rel. Pot.) –
- Standard Error (Std. Error) of the estimate
- Confidence Interval of the estimate (see explanation above)
- Independence (see explanation above)
- EC50 (4P or 5P only)
- R^2 value
- For each parameter:
- Estimated Value
- Std. Error of the estimate
- Confidence Interval of the estimate
- Independence

Changing the Graph Name

To change the name of a graph.

1. Make the **Graph** section active in the workspace.
2. Double click on the graph name in the header bar to open the **Rename Section** dialog.



3. Type the name in the **New Name** field and click **OK**.

Exporting a Graph

To export a graph to a .png file:

1. Click on the graph section to want to export to make it active in the workspace.
2. Click **Export Graph**  in the **Graph Tools** section of the **Home** tab.
3. The **Save As** dialog opens.
4. Browse to the file location where you want to save the graph file and type the name of the graph in the **Filename** field.
5. Click **OK**.
6. The active graph section is exported and saved to specified filename.

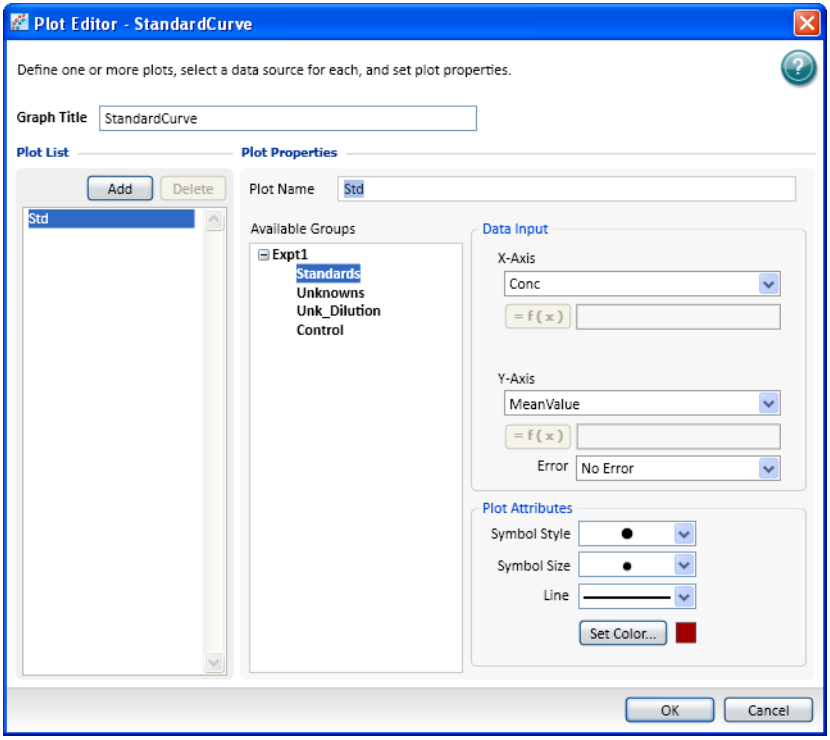
Creating a New Graph

The **New Graph** button opens the **Plot Editor** dialog which enables you to create a graph from the collected data. Graphing is enabled both during data collection and after data has been acquired for a Kinetic, Spectrum, and Well Scan reading.

The **Plot Editor** dialog lets you name the graph, assign specific information to be plotted on the X- and Y- axes, and choose which icon and color will be used.

All groups that have been created or assigned within the file are listed on the side in the Source field. To create a new graph:

1. Click **New Graph** on the **Plate** section **Home** tab to open the Plot Editor dialog.



2. Type the name of the graph in the **Graph Title** field.

3. Select a plot.

   The **Plot List** shows the existing plots. One plot is shown by default.

   - Click **Add** to add a new plot to the graph.
   - Select a plot and click **Delete** to remove a plot from the list.

   When you select a plot from the list, the parameters for the selected plot appear on the **Plot Properties** section of the dialog.

4. Optionally, type the name of the plot in the **Plot Name** field and the plot name changes in the plots list.
5. Select a group from the **Available Groups** list. Data from the selected group will be plotted in the graph.

Note: Groups are defined using the template editor. If no groups appear in the list you must go to the Template editor dialog and define one or more groups. See Defining a Group on page 153 for more information

6. In the **Data Input** area, select columns types from those listed to assign to the plot in the X-Axis and Y-Axis. When you select the **Custom** type you can enter a custom formula. Data Input displays group column names from the group that is selected in the Available Groups. Then you can select a column to use as the source for either the X-axis or Y-axis
   - Click the **New Formula** button for the X-axis and/or Y-axis to open a **Formula Editor** dialog.
   - Enter a formula into the **Formula Editor** dialog.
   - Click **OK** to display the selected formula in the **Formula** field.
7. Select a column or **No Error** in the **Error** field to toggle the display of error bars.

Note: When data is displayed as a scatter graph, you can choose to display error bars for the plot of the data for the X- and 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.

8. You can use the **Plot Attributes** area to change the graph's appearance:
   - Select the **Symbol Style** for the icon to be used in the plot.
   - Select the **Symbol Size** for the icon to be used in the plot.
   - Select the **Line Style** for the line to be used in the plot.
   - Click **Set Color** and select the color from the color picker for the icon to be used in the plot.

   The Selected Icon graphic changes to show the style, size, and color of selected for the plot icon.
9. Click **OK** to apply the settings and close the **New Graph** dialog.

A new graph section is added to the experiment with the resulting data plots displayed as specified.

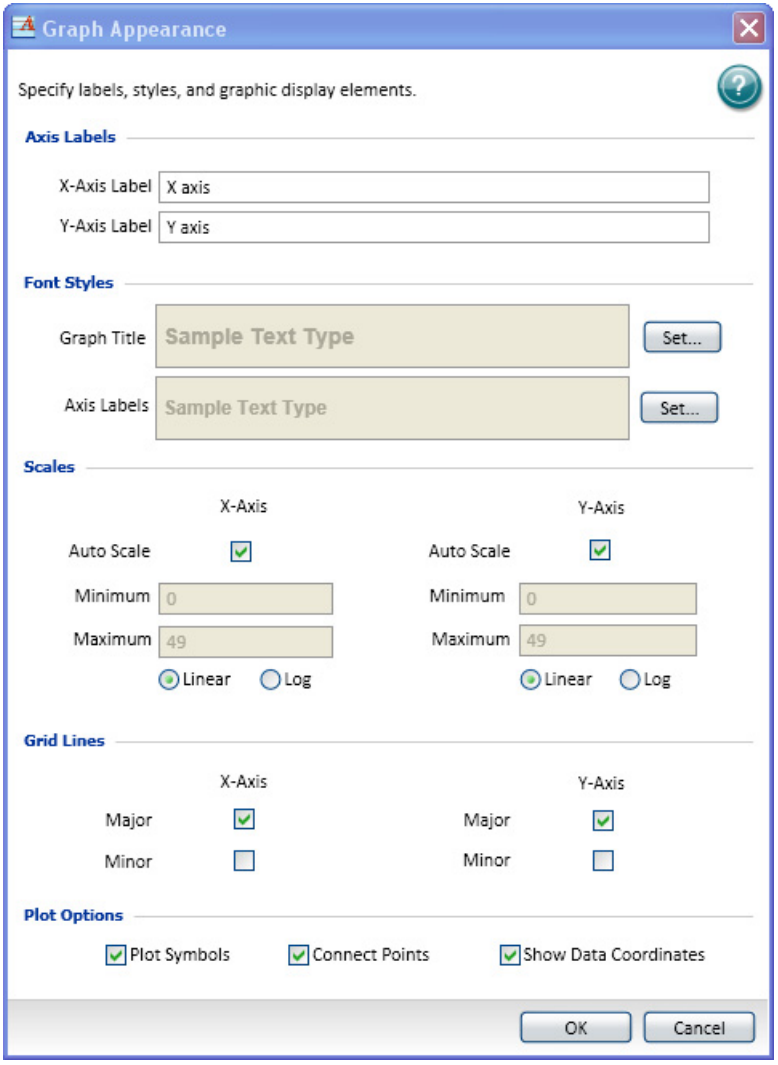
Zooming the Graph Display

Use the mouse to select a portion of a graph and the display zooms into the selected region.

Once you zoom in you can double click the graph body to go back to the default original scale.

Changing the Graph's Appearance

With a **Graph** section active in the workspace, click **Graph Appearance** in the **Graph Tools** section of the **Home** tab or click the **Appearance**  shortcut button on the graph header bar. The **Graph Appearance** dialog appears.



In the Graph Appearance dialog you can specify labels, styles, and graphic display options.

Axis Labels

You can apply labels to the X-Axis and Y-Axis by typing the text of the label into the entry fields. These labels will be displayed in the font specified the Font Styles.

Font Styles

Font Styles provides control over the font that is used for the Graph Title and Axes Labels.

Scales

You can choose the Auto Scale function for the X-Axis and Y-Axis individually or enter Minimum and Maximum scaling values. You can also select either a log or a linear display of each axis.

Grid Lines

You can choose to display the major and minor grid lines for the X-Axis and the Y-Axis individually.

Plot Options

In the Plot Options area you can select the following for display on a plot:

- Plot Symbols
- Connect Points
- Show Data Coordinates

Toggling the Log/Linear Functions

The log/linear display can be toggled in the **Appearance** dialog.

1. With a **Graph** section active in the workplace, click **Graph Appearance**  .
2. In the **Scales** section of the **Graph Appearance** dialog, select either the **Log** or **Linear** button. The log function is enabled by default.
3. You can change the representation individually for the X-Axis and the Y-Axis of the graph.

Editing an Existing Graph

You can change a graph by editing its appearance or by using the **Plot Editor** dialog to change the plots and the data or plot attributes.

To change the graphs appearance, see Changing the Graph's Appearance on page 237.

To edit the content of the graph

1. With a **Graph** section active in the workspace, click **Plot Editor**  on the **Home** tab or click the **Plot Editor** shortcut on the **Graph** section header bar.
2. To change the name of the graph, select the text in the **Title** field and type a new name.
3. Select a plot.

   The Plots list shows the existing plots. One plot is shown by default.
   - Click **Add** to add a new plot to the graph.
   - Click on a plot and click **Delete** to remove a plot from the list.

   When you select a plot from the list, the parameters for the selected plot appear on the Source section of the dialog.
4. Optionally, type the name of the plot in the **Name** field and the plot name changes in the plots list.
5. Select a group from the **Source** list. Data from the selected group will be plotted in the graph.
6. In the **Data Input** area, select columns types from those listed to assign to the plot in the X-Axis and Y-Axis. When you want to add a custom formula, select **Custom**.
   - Click the **New Formula** button for the X-axis or Y-axis to open a **Formula** dialog.
   - Enter a formula into the New Formula dialog.
   - To plot a Summary, enter the **Summary** name in the **Formula** dialog.
   - Click **OK** to display the selected formula in the **Formula** field.
7. Select a column or **No Error** in the **Error** field to toggle the display of error bars.

8. To change the graph's appearance, use the **Plot Attributes** area:
   - Select the **Symbol Style** for the icon to be used in the plot.
   - Select the **Symbol Size** for the icon to be used in the plot.
   - Select the **Line Style** for the line to be used in the plot.
   - Click **Set Color** and select the color from the color picker fir the icon to be used in the plot.

   The Selected Icon graphic changes to show the style, size, and color of selected for the plot icon.
9. Click **OK** to apply the settings and close the **Plot Editor** dialog.

Note: You can add as many plots as you need during one editing session. To add additional plots click **New**. To delete a plot from the graph select the plot and click **Delete**.

Selecting Curve Fit Settings

When you first create a graph of the data, it does not have a fit associated with it. You can fit any plot to one curve fit selection. These selections are shown in the Curve Fit list in the Graph section tool bar. All plots on a graph must have the same type of fit.

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

After you have selected a fit type, SoftMax Pro Software determines the parameter values that best fit the data. The function with these parameters is drawn on the graph.

Note: Ideally, the type of fit used should be determined by the underlying chemistry of the assay and should be set before data is read.

When a fit is performed, the parameter values are tabulated in the legend at the bottom of the graph. The parameter table also contains information regarding the uncertainty of the parameters, specifically standard errors and (optionally) confidence intervals.

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).

Judging a Good Curve Fit

Visual inspection is always useful for assessing a curve fit. The legend at the bottom of the graph contains information summarizing the numerical output of the fit. This includes the coefficient of determination R^2, a traditional measure of goodness of fit, which is the fraction of the variance of the y-values explained by the fit, and will be 1 for a perfect fit. However this should not be used to assess the statistical significance of the fit, or to compare the suitability of different curve fit functions. For such purposes statistics such as chi-squared or Fisher-F can be used, and these can be obtained from the formula system.

Even for a good fit (as judged by eye or R^2) some parameters might have high uncertainty.

The **Curve Fit Settings** dialog has three tabs available for defining the curve fit:

- **Curve Fit**, see Curve Fit Tab on page 242.
- **Weighting**, see Curve Fit Weighting Tab on page 251.
- **Statistics**, see Curve Fit Statistics Tab on page 253.

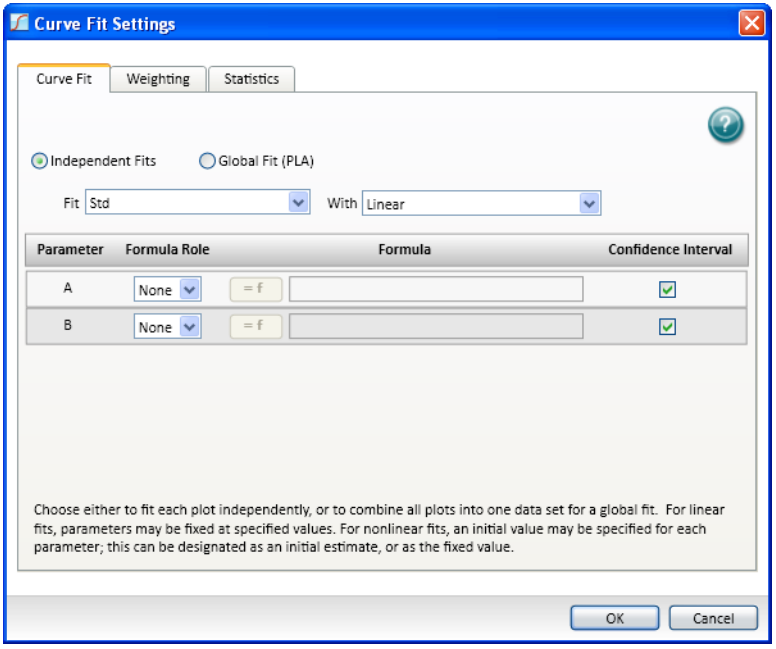
After you finish defining the curve fit, click **OK**.

Curve Fit Tab

When you first create a graph of the data, it does not have a fit associated with it. You can fit any plot to one of the pre-defined curve-fitting functions.

These selections are shown in the **Curve Fit** list in the **Graph** section header bar.

You can also select a plot from the graph and a curve fit to apply to that plot from the list on the **Curve Fit** tab in the **Curve Fit Settings** dialog.



All plots on a graph can have the same type of fit or each plot can have a different fit applied individually. The **Curve Fit** tab of the **Curve Fit Setting** dialog provides the ability to set a different fit to individual plots.

To assign plot fits, you use the **Curve Fit** button on the **Graph** section and choose to fit each plot independently, or to combine all plots into one data set for a global fit.

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

For information about the curve fit functions see Curve Fitting Functions on page 247.

Once you have selected a fit type, SoftMax Pro Software determines the parameter values that best fit the data. The function with these parameters is drawn on the graph.

Note: 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).

- For linear fits, parameters can be fixed at specified values.
- For nonlinear fits, an initial value can be specified for each parameter; this can be designated as an initial estimate (the seed value)`, or as the fixed value.
- Also, specify whether a confidence interval should be calculated for each parameter.

Independent Fits

When plots in a graph are to be fit with curve fit functions independently:

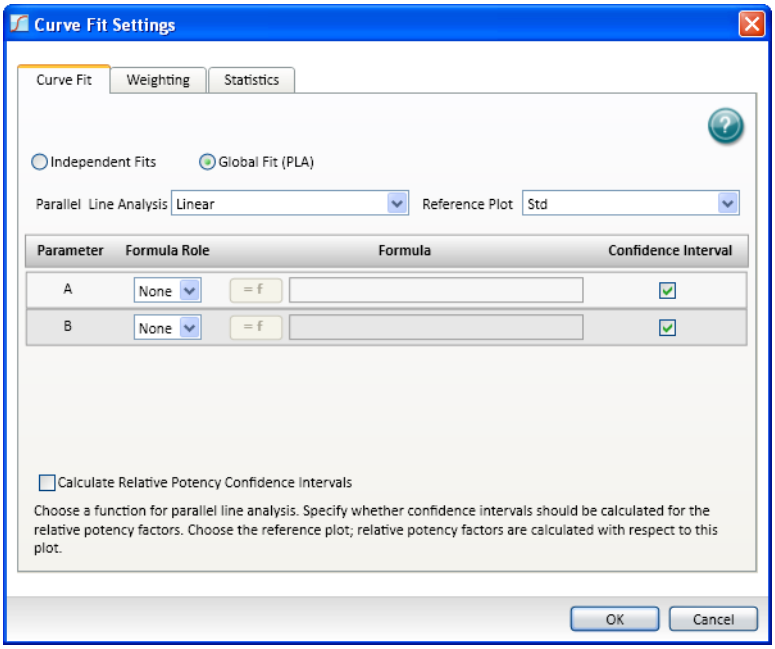
1. Open a **Graph** section with multiple plots in the workspace.
2. Click **Settings** on the window header bar.
3. Select **Independent Fits**.
4. Select a plot from the **Fit** list.
5. Select which curve fit function to apply to the plot from the **With** list.
6. Continue selecting plots and apply curve fit functions as needed.
7. If applicable, click the **Weighting** tab.
8. If applicable, click the **Statistics** tab.
9. When all curve fit options have been set, click **OK**.

Note: For independent fits (and global to a limited extent), you have the ability to fix values. Curve fit parameter values can either be fixed, or "manually seeded" to start the curve fit algorithm search.

Global Fit (PLA)

When all the plots in a graph are to have the same curve fit functions applied:

1. Open a **Graph** section with multiple plots in the workspace.
2. Click **Curve Fit Settings** on the window header bar.
3. Select **Global Fit (PLA)**.



Currently the only global fit available is parallel line analysis, described in the following section.

Parallel Line Analysis

This feature, accessible through the Curve Fit Settings dialog, is available for all except point-to-point and cubic spline fits. This is set using the Settings button in the Graph section. The fit type should be Global.

Determining parallelism between a standard and test compound, and subsequent estimation of relative potency, are fundamentals of bioassay use in biopharmaceutical development and testing. For linear data, parallelism can be evaluated by examining the similarity between the slopes of straight lines.

Bioassay data and immunoassays in particular, have been demonstrated to fit well with a sigmoidal shape defined by a 4- or 5-parameter curve fit. The basic question of whether a curve with a specified shape can fit both standard and test data sets when shifted along the X-axis can be addressed following a method detailed by Gottschalk and Dunn (Journal of Biopharmaceutical Statistics, 15:437-463, 2005).

The parameters are those for the fit function, plus a relative potency factor for each data set relative to one designated as a reference. Beneath the graph are displayed their values, standard errors, and (optionally) confidence intervals.

New function operators have been added to SoftMax Pro Software which can be used to summarize this information in a Notes section. Please see the *SoftMax Pro Formula Reference Guide* help for additional details.

Using a Standard Curve

To use parallel line analysis with a standard curve:

1. You need to have a Graph section containing at least two plots, one a Standard and one a Test sample.
2. Click **Settings**.
3. On the **Curve Fit** tab, click **Global**.
4. Select the plot from the **Fit** list.
5. Select any option except point-to-point or cubic spline from the **With** list.
6. If applicable, select **Relative Potency Confidence Interval**.
7. Select a plot for the **Reference Plot** list.
8. If applicable click the **Weighting** tab.
9. If applicable click the **Statistics** tab.
10. When all curve fit settings options have been set, click **OK**.

Curve Fitting Functions

When you first create a graph of the data, it does not have a fit associated with it. You can fit any plot to one of ten curve-fitting functions:

- No Fit on page 247
- Linear on page 248
- Semi-Log on page 248
- Log-Log on page 248
- Quadratic on page 248
- Cubic on page 248
- 4-Parameter, 5-Parameter, and Log-Logit on page 249
- Point-to-Point on page 250
- Cubic Spline on page 250
- Exponential on page 250
- Rectangular Hyperbola on page 250
- 2-Parameter Exponential on page 251
- Bi-Exponential on page 251
- Bi-Rectangular Hyperbola on page 251
- Two-Site Competition on page 251

These selections are shown in the Fit list in the Graph section tool bar.

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

After you have selected a fit type, SoftMax Pro Software determines the parameter values that best fit the data. The function with these parameters is 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.

When a fit is performed, the parameter values are displayed in the legend at the bottom of the graph. The correlation coefficient describes how well a change in x values correlates with a change in the y values. The R^2 value should be used only for linear curve fits.

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).

No Fit

SoftMax Pro Software allows for no curve fit to be applied, and the scatter of the data points plotted.

Linear

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.

Cubic

The Cubic function fits the best 3rd-order polynomial to the data, based on the equation:

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

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:

$$y = A + B * Log(x)$$

where A is the y-intercept of the line at x=1 and B is the slope (log in this equation is the common or base-10 logarithm).

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 is a straight line with both axes drawn in logarithmic scale. The equation for the log-log fit is:

$$Log(y) = A + B * Log(x)$$

where A is the log(y)-intercept of the line for log(x) = 0, and B is the slope (log in this equation is the common or base-10 logarithm).

Quadratic

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

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

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.

4-Parameter, 5-Parameter, and Log-Logit

If the standard curve has a sigmoidal shape when plotted on the semi-log axes, it can be appropriate to use either the log-logit, 4-parameter, or 5-parameter fit. Both logistic and log-logit fits are based on the equation:

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

where D is the Y-value corresponding to the asymptote 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, commonly called the IC50 or EC50. The coefficient B describes how rapidly the curve makes its transition from the asymptotes in the center of the curve, and is commonly known as the slope factor. A large value of B describes a sharper transition. Typically, B has a magnitude of about 1 for inhibitory experiments and –1 for excitatory experiments.

Both the log-logit and 4-parameter equations are displayed as:

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

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.

The 5-parameter logistic equation has an extra exponential term G that is sometimes described as an asymmetry factor:

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

As with the 4-parameter logistic equation, D is the high asymptote, A is the low asymptote and B is the slope factor. Unlike the 4-parameter logistic equation, however, C is not the IC50 value. The IC50 value needs to be calculated separately. This is described in Chapter 3 of the Formula Reference Guide.

Use the 5-parameter logistic equation instead of the 4-parameter logistic equation when you need to fit to a non-symmetrical sigmoidal shape. The non-symmetry is typically seen in the rate of change of the slope of the curve as it approaches the asymptote. With 4-parameter fits, the rate of change of the slope is the same at the lower asymptote as at the upper asymptote. With 5-parameter fits, the upper and lower portions of the curve can have very different shapes.

The minimization method for the 4-parameter and 5-parameter equations is based on the Levenberg-Marquardt Method.

Discussion of these methods can be found in *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.

Cubic Spline

This curve fit generates a piece-wise fit to a cubic equation between each pair of adjacent data points. The equations are computed with the requirement that the first and second derivatives of the curve are continuous at the data points, with the additional constraints that the second derivatives are zero at the Endpoints. As this is not a least-squares fit, parameters are not shown with the graph and there are no available statistics.

Exponential

The exponential function used to generate this curve fit is:

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

In previous versions of SoftMax Pro Software it was possible to select a linearized version of this fit; this option is not available in SoftMax Pro Software 6.

Point-to-Point

Point-to-point uses a linear equation to fit each pair of data points.As with cubic spline, this is not a least-squares fit, so no parameter is shown and statistics are not available. The point-to-point curve fit is composed of multiple linear pieces joining the points. The line segment defined by each pair is used to interpolate data between those points.

Rectangular Hyperbola

The function used to generate the Rectangular Hyperbola curve fit is:

$$y = \frac{Ax}{x + B}$$

This fit is applicable to both the Michaelis-Menten model for enzyme kinetics and the one-site binding model.

2-Parameter Exponential

The function used to generate the 2-Parameter Exponential curve fit is:

$$y = A * e^{Bx}$$

This fit would typically be used for a simple exponential decay.

Bi-Exponential

The function used to generate the Bi-Exponential curve fit is:

$$y = Ae^{Bx} + Ce^{Dx} + G$$

This fit would typically be used for a rate process with two exponential phases.

Bi-Rectangular Hyperbola

The function used to generate the Bi-rectangular Hyperbola curve fit is:

$$y = \frac{Ax}{x+B} + \frac{Cx}{x+D}$$

This fit is applicable to equilibrium binding of a ligand to two binding sites with different affinities, and to two enzymes catalyzing a chemical reaction with different Michaelis constants.

Two-Site Competition

The function used to generate the Two-site Competition curve fit is:

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

This fit is applicable to competition studies involving two receptor binding sites.

Curve Fit Weighting Tab

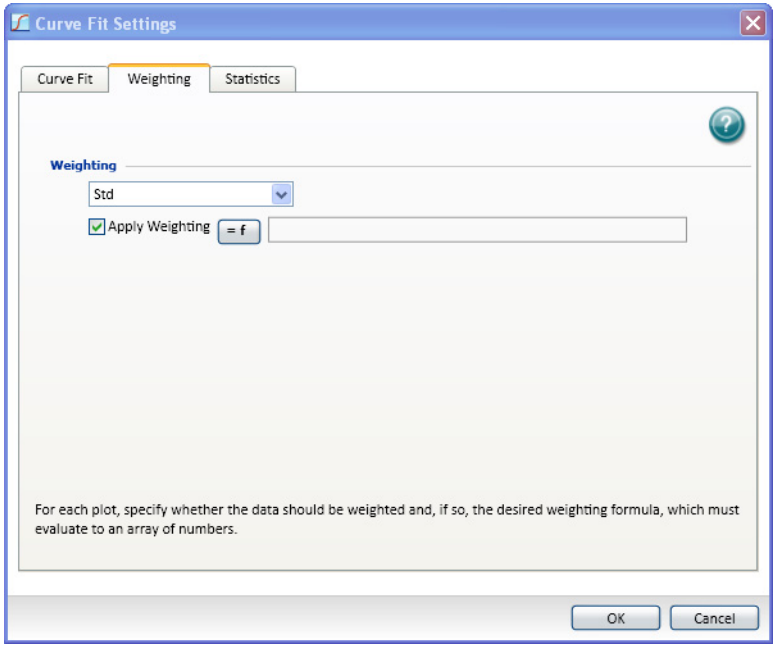
For each plot, specify whether the data should be weighted and, if so, the desired weighting formula, which must evaluate to an array of numbers.

Least-squares fitting is based on the assumption that errors are normally distributed; if the variance of each point in the fit is known, or can be estimated by some means, its inverse should be specified as the weight for the point. If the variances are not known, but are believed to be non-uniform across the data, that is, a known function of y, then a weighting function should be used to reflect this information. If a formula for the weights is not provided, then all points have equal weight.

The weighting feature allows independent weighting of individual plots within a **Graph** section.

To apply weighting to a plot:

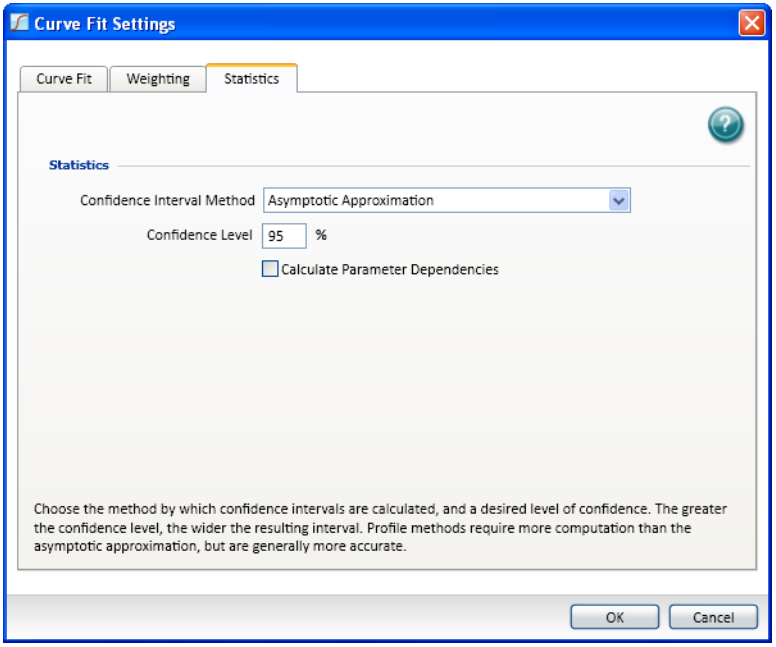
1. On the graph, select **Settings**.
2. Click the **Weighting** tab.



3. Select a plot from the **Weighting for** list.
4. Click **Apply Weighting**.
5. Click the **New Formula** button.
6. Type the formula to be applied as the weighting in the input field.
7. Click **OK**.

Curve Fit Statistics Tab

The **Statistics** tab lets you to specify the statistics display.



Choose the method by which confidence intervals are calculated, and a desired level of confidence.

Confidence Interval Method

A confidence interval is a range with a specified confidence level, usually specified as a percentage. For example, suppose that many data sets are obtained, and the confidence interval is calculated for each data set according to a chosen statistic. The percentage of those confidence intervals containing the true value is the specified confidence level.

To specify the method used to establish the confidence interval, select a confidence interval method from the list.

Table 5-3 Confidence Interval Method

Confidence Interval Method	Description
Asymptotic Approximation	Calculates Wald confidence interval, which is simply related to the standard error.
Profile Method (F-Distribution)	Based on model comparison with F-statistic.
Profile Method (Chi-Squared)	Based on model comparison with chi-squared-statistic.
Profile Method (T-Distribution)	Based on T-profile function.

Note: Profile methods require more computation than the asymptotic approximation, but are generally more accurate.

Confidence Level

How likely the interval is to contain the parameter is determined by the **Confidence Level**. Increasing the desired confidence level will widen the confidence interval.

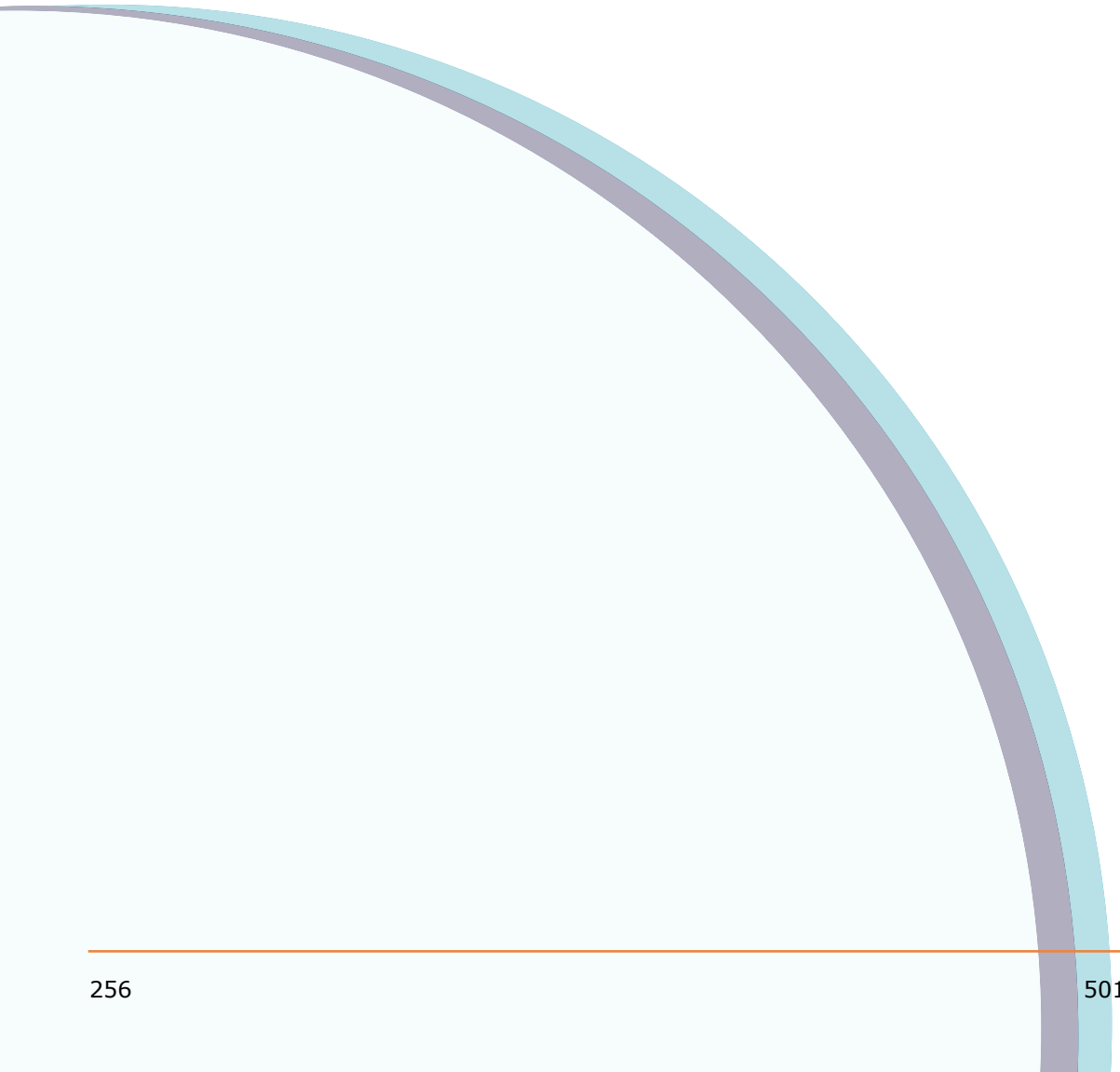
Calculate Parameter Dependencies

To calculate dependencies between parameters, select **Calculate Parameter Dependencies**.

With this option selected, the graphical representation in the graph legend represents the parameter independence of the curve fit. See Graph Legend on page 232.

Parameter *Independence* is one way to examine the suitability of a given curve fit for the data set. It is a measure of the extent to which the best value of one parameter depends on the best values of the other parameters, and is a number between 0 and 1, with 1 being the ideal. In the graph fit legend, parameter independence has been translated into bars, where 10 bars indicate a high degree of independence; because only very small values indicate a problem, a nonlinear transformation is used for this translation. If one or more parameters have few bars or no bars, the curve fit might not be appropriate for the data set.

For example, if the data set is sigmoidal with clear lower and upper asymptotes, 4-parameter fits should be appropriate with many bars for all parameters. However, if one or both of the asymptotes is missing, the A or the D parameter will have few bars, indicating that reliable values cannot be deduced from the data set.



Glossary

6

%

%Transmittance
Transmittance is the ratio of transmitted light to the incident light (for absorbance readings).

A

Absorbance
Absorbance is the amount of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter.

Area Under Curve
Reduction formula for a Kinetic or Spectrum scan that determines the area under the Kinetic or Spectrum plot, or under a plot in a Graph section.

Auto Save
Files can be set up to be saved at a predefined interval into a predefined location. This operation occurs automatically without user interaction.

AutoCalibrate
For Absorbance instruments, an air reference reading is taken before runs (and sometimes between Kinetic readings during a run) as determined by the type of instrument and read settings. For Fluorescence instruments, the measurement occurs before reads (and sometimes between Kinetic reads if the interval is long enough). Calibration data is stored in memory and is used by SoftMax® Pro software until the instrument is powered down or the wavelength is changed. AutoCalibration can be turned off.

Automix
The Automix function determines how often, if at all, automated shaking of the microplate is performed during a reading.

AutoRead
This feature enables automatic reading of subsequent Plate sections in the order in which they appear within an Experiment. You can set intervals (delay times) between the plate readings, if desired.

B

Baud
A data transmission rate (measured in bits/second) for communication devices.

Bottom Read
Available in the Instrument Settings, this selection causes instruments with bottom read capability to read up through the bottom of the microplate rather than down from the top.

C

Compound Source
Compounds are transferred from the compound source plate to the assay plate.

Concatenation
An operation in which a number of conceptually related components are linked together to form a larger, organizationally similar entity.

Cubic Spline
This curve fitting method 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.

Cutoff
A filter used to condition the light entering or exiting the monochromators. In automatic mode, the instrument sets the cutoffs automatically based upon the wavelengths chosen for reading. With some read modes and types, you can choose a different filter wavelength (manual setting) for the emission monochromator.

CuvetteSet Section

CuvetteSet sections determine how the cuvettes will be read, display data as it is acquired from the instrument, and define how the data will be displayed and used in reduction. CuvetteSet sections can display samples in three different ways: three samples per row, one sample per row, and in a grid of cells corresponding to a microplate format.

D

Data Mode

Reduction option to view absorbance data as OD (absorbance) or %Transmittance, fluorescence data as RFU (relative fluorescence units), or luminescence data as RLU (relative luminescence units).

Default Protocol

A protocol file, included with the SoftMax Pro program or created by a user, that resides in the default folder. The Default Protocol file defines the initial information that appears when you open SoftMax Pro software or when you create a new file from within the program. This file is called "Default Protocol" and has the 3-character ".spr" extension.

E

Emission Spectrum Scan

Measures fluorescence or luminescence across a spectrum of wavelengths for emitted light for a fixed excitation wavelength. The default value reported for each well is the wave-length of maximum emission (either fluorescence or luminescence).

End Point

A single reading made at one or more wavelengths.

End Time

Reduction parameter used to omit the end of a Kinetic, Spectrum, or Flex run from data reduction.

Excitation Spectrum Scan

Measures fluorescence at a single emission wavelength across a spectrum of excitation wavelengths. The default value reported for each well is the excitation wavelength of maximum fluorescence.

Exponential (Curve Fit)

The exponential function used to generate this curve fit is: y = A + B * (1 – exp^(–x / C))

F

Fluorescence

The light emitted by certain substances resulting from the absorption of incident radiation. To measure fluorescence accurately, it is necessary to reduce light scatter. The governing equation for fluorescence is: Fluorescence = extinction coefficient * concentration * quantum yield * excitation intensity * pathlength * emission collection efficiency

Fluorescence Polarization

By using a fluorescent dye to label a small molecule, its binding to another molecule of equal or greater size can be monitored through its speed of rotation. SpectraMax® M5 and M5e Fluorescence Polarization read mode has two raw data modes you can apply in the reduction settings: Polarization (mP) and Anisotropy (r). These values can be viewed as reduced data.

Fluorophore

A material that 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

G

Gain

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

G-Factor

G-factor (Grating factor) is used in fluorescence polarization to correct polarization data for optical artifacts, converting relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. G-factor corrects this instrumental bias.

Grayscale 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 may create others as required.

I

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.

Instrument Icon

Located in the Home Tab, the instrument icon shows the status of the connection between the computer and the instrument. If the icon has a circle with a diagonal line through it, the connection is not functioning or no instrument is connected; if the instrument icon appears without a check mark the connection is working properly.

Interleaved Display

A display choice in which 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. This display is most useful when the 384-well plate comprises four daughter plates of 96 wells each.

K

Kinetic

During Kinetic readings, data is collected over time, with multiple readings made at regular intervals (measured in seconds). The values calculated based on raw Kinetic data are VMax, VMax per Sec, Time to VMax, and Onset Time. Kinetic readings can be single- or multiple-wavelength readings (up to six, if using a SpectraMax® instrument).

L

Lag Time

Lag time in a Kinetic protocol is the period of very slow growth (microorganisms) or the rate of reaction that can precede the rapid/linear phase of reaction. When a lag time is specified in SoftMax Pro software, data collected prior to the lag time is not included in data reduction.

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.

Log-Logit (Curve Fit)

The Log-Logit is also called a two-parameter curve fit. The equation used to generate this curve fit is: $y = ((A - D) / (1 + (X / C)B)) + D$

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.

M

Mask
Lets you hide selected data so that they are not used for calculations and are not reported. The Mask function is commonly used to suppress outliers.

Maximum
Reduction for a Spectrum scan that reports the maximum signal within the reduction parameters.

MaxOD
The limit for the maximum value you want to use for displaying and analyzing Kinetic or Spectrum data. Any values above these limits are not shown and are excluded from data reduction.

MaxRFU
The limit for the maximum value you want to use for displaying and analyzing Kinetic or Spectrum data. Any values above these limits are not shown and are excluded from data reduction.

MaxRLU
The limit for the maximum value you want to use for displaying and analyzing Kinetic or Spectrum data. Any values above these limits are not shown and are excluded from data reduction.

Minimum
Reduction for a Spectrum scan that reports the minimum signal within the reduction parameters.

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

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

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

N

Normal Display
The default display for 384-well plates in the Plate section.

O

OD
The amount of light passing through a sample to a detector relative to the total amount of light available. Optical Density plus reflection loss at the air to sample, sample to plate, and plate bottom to air interfaces is subtracted from the ODs absorbance of the sample plus light scatter from turbidity.

Onset OD
The change in signal required to compute the onset time (for Kinetic readings).

Onset RFU
The change in signal required to compute the onset time (for Kinetic readings).

Onset RLU
The time it takes for a given increase in signal, called the onset OD (or RFU or RLU) to occur.

Onset Time
The time it takes for a given increase in signal, called the onset OD (or RFU or RLU) to occur.

Optical Density
The amount of light passing through a sample to a detector relative to the total amount of light available. Optical Density plus reflection loss at the air to sample, sample to plate, and plate bottom to air interfaces is subtracted from the ODs absorbance of the sample plus light scatter from turbidity.

P

PathCheck Pathlength Measurement Technology
Instrument Settings option within SoftMax Pro Software for selected readers that allows data collected in a Plate section to be normalized to a 1-cm pathlength.

Photomultiplier Tube (PMT)
A vacuum tube that detects light especially from dim sources through the use of photo emission 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 are 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 that determines the type of microplate being read. The specification of the plate type depends on the well format and manufacturer of the microplate.

Point Count Number of Reads per Well
The number of integrations performed on a single well in a Fast Kinetic read.

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

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

Q

Quadratic (Curve Fit)
A mathematical description of a parabola: y = A + Bx + Cx2

R

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 from the instrument with no alteration. This is reported as OD, RFU, or RLU, depending on the instrument type.

Read Interval
The interval of time between the start of one reading and the start of the next reading during a Kinetic run.

Read Mode
The type of reading performed in the instrument: Fluorescence, Time Resolved Fluorescence, Luminescence, Absorbance or Fluorescence Polarization.

Read Type
The method used to read the microplate or cuvette: Endpoint, Kinetic, Spectrum, Well Scan, or Flex. Not all types are available with all instruments.

Readings per Well
The number of times (programmable by the user) that readings are taken on a well in Fluorescence and Time Resolved Fluorescence modes. This setting also determines the amount of time that data is collected in Luminescence mode.

Rectangular Hyperbola
A hyperbola for which the asymptotes are perpendicular, also called an equilateral hyperbola or right hyperbola. This occurs when the semimajor and semiminor axes are equal.

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 reduction for Kinetic, Spectrum, Well Scan, or Flex readings.

Reference
A reading of buffer or water taken in the cuvette that is used as I0 to calculate Absorbance or %Transmittance of sample read in the cuvette port.

S

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.

Series
The Series function allows you to work with groups of wells in the Template so that the standard value or dilution factor increases or decreases in specified steps. The name can be incremented as well.

Slope
A reduction option that determines the slope of the combined plot (for example, the slope of the line using linear regression after the wavelength combination reduction). This reduction uses all time points visible in the reduction window. Slope is the same as VMax Rate when VMax Rate is set to the same number of points as the run (for example, the default). Slope is not the same as VMax Rate if you have modified the number of VMax Points.

Spectrum
A read type that allows you collect absorbance or fluorescence data across a spectrum of wavelengths. This data is displayed as OD, RFU, or RLU versus wavelength. The default reduction displays the wavelength that corresponds to the maximum signal.

Standard Curve
A mathematically idealized representation of the relationship between the concentration of a standard calibrator (X-value) and the OD/RFU/RLU (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.

Stokes Shift
The difference between the wavelengths of the excitation and emission peaks.

T

Template Editor
A representation of the microplate or a set of cuvettes 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 various wells of the microplate or cuvette.

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 Maximum
Reduction for a Kinetic or Flex run that reports the time at the maximum signal within the reduction limits.

Time at Minimum
Reduction for a Kinetic or Flex run that reports the time at the minimum signal within the reduction limits.

Time at one half Maximum

Reduction for a Kinetic or Flex run that reports the time at half the maximum signal within the reduction limits as follows: SoftMax Pro software first determines the point (within the reduction limits) that has the maximum signal level. It then scans the 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 Resolved Fluorescence

Most fluorescent substances are not suitable for this type of reading. However, the fluorescence emitted by lanthanide dyes is delayed long enough to measure fluorescence after the lamp is turned off. Time resolved fluorescence is used to reduce the amount of background noise interfering with fluorescence. The excitation lamp flashes and, after it is off, the delayed emission is collected before the lamp flashes again.

Time to VMax

The elapsed time until the maximum reaction rate of a Kinetic or Flex reading is reached, reported in seconds

V

VMax Points

The number of points in the Kinetic or Flex 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 Rate

The rate reported as signal/min (milli-OD, RFU, or RLU units per minute) for a Kinetic or Flex reading. 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. It may also be reported as units per second (the default for Fluorescence and Luminescence modes).

W

Well Scan

Some applications involve the detection of whole cells in large-area tissue culture plates. Well Scan reads can be used with such microplates to allow maximum surface area detection in whole-cell protocols. Since many cell lines tend to grow as clumps or in the corners of microplate wells, you can choose from several patterns and define the number of points to be scanned to work best with your particular application.

Index

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

W

X

Z