Molecular Devices

SoftMax® Pro Microplate Data Acquisition and Analysis Software

Version 6

User Guide

Contents

Chapter 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 119.

The software provides 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 the 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 a PC-compatible computer using the Windows 7 or Windows XP operating system.

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 and Recommended Requirements

Software

- Windows XP, 32-bit (x86), with Service Pack 3

    Recommended: Windows 7, 32-bit or 64-bit (x86 or x64)

- .NET Framework 4.0[1]

- Internet connection or external USB drive

    Recommended: Internet connection

Hardware

- Computer that has a 2 GHz or faster processor

    Recommended: Quad core or faster CPU[2]

- RS-232 serial port or USB 2.0 port with a Keyspan USB-to-serial adapter[3]

    Recommended: RS-232 serial port

- 2 GB RAM

    Recommended: 4 GB RAM[2]

- 200 MB of available hard disk space

    Recommended: 500 MB of available hard disk space

- 1024 x 768 or higher-resolution display

    Recommended: 1280 x 1024 or higher-resolution display

- CD-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.

[3] Keyspan USB-to-serial adapters have been field tested and approved by Molecular Devices.

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 starts automatically.

2. If the installation program does not start, navigate to your CD-ROM drive and double-click **SoftMaxPro6.2.2Setup.exe**.

   To install the GxP version, double-click **SoftMaxPro6.2.2GxPSetup.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 6.2.2** and then click **Uninstall** or **Remove**.

   To uninstall the GxP version, click **SoftMax Pro 6.2.2 GxP**.

> **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.

Starting the Software

To start the software under normal conditions, wait for the connected instrument to complete its start-up sequence, and then double-click the **SoftMax Pro 6.2.2** 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 6.2.2 > SoftMax Pro 6.2.2**.

For the GxP version, double-click the **SoftMax Pro 6.2.2 GxP** icon or click **Start > All Programs > Molecular Devices > SoftMax Pro 6.2.2 GxP > SoftMax Pro 6.2.2 GxP**.

If you are using the SoftMax Pro GxP Software, you are asked to log on before you can continue. Type your administrator-assigned user ID and your password, and then click **Log On**. See Logging onto SoftMax Pro GxP Software on page 272.

> **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.

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 activate 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.

   On the Internet-enabled computer, go to: https://smplicensing.moleculardevices.com

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, see page 120

- SpectraMax Plus 384 Absorbance Microplate Reader, see page 120

- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 121

- SpectraMax M3 Multi-Mode Microplate Reader, see page 122

- SpectraMax M4 Multi-Mode Microplate Reader, see page 123

- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 124

- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 124

- SpectraMax 190 Absorbance Microplate Reader, see page 125

- Gemini XPS Fluorescence Microplate Reader, see page 125

- Gemini EM Fluorescence Microplate Reader, see page 126

- FilterMax F5 Multi-Mode Microplate Reader, see page 127

- FilterMax F3 Multi-Mode Microplate Reader, see page 128

- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 128

- Vmax Kinetic ELISA Microplate Reader, see page 129

- Emax Endpoint ELISA Microplate Reader, see page 129

- SpectraMax Paradigm Multi-Mode Detection Platform, see page 130

- StakMax Microplate Handling System, see page 141

When connected to a SpectraMax Paradigm instrument, see Selecting the SpectraMax Paradigm Detection Cartridge on page 161.

Setting Instrument Calibration Options

To calibrate the reader:

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

   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 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 the SoftMax Pro Software main window, and the correct instrument is shown as selected.

The instrument icon shows the connection status of the instrument.

Table 1-1: Instrument Connection Status

Icon	Status
	Instrument Connected
	Instrument Disconnected
	Instrument Simulated

If the instrument status shows as disconnected, check to make sure that the instrument is powered on and that the connections between the instrument and the computer are secure.

Unlocking the SpectraMax Paradigm Instrument

When a SpectraMax Paradigm Multi-Mode Detection Platform is first installed, the hardware transport locks need to be removed as described in the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*. As an additional safety precaution, internal locks controlled by the software prevent the drawers from opening until the instrument is detected and initialized.

> **Note:** Do not install detection cartridges until after you have completed the following instructions to unlock the drawers and initialize the instrument.

To unlock the drawers and initialize the instrument:

1. Remove the hardware transport locks as described in "Removing the Transport Locks" in the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*.

   > **CAUTION:** The instrument can be damaged if all the transport locks are not removed before unlocking the drawers and initializing the instrument.

2. Make sure that all drawers have been pushed back into the instrument, the front panels have been replaced, and the microplate door is closed.

3. Make sure that the instrument is connected to a power source as described in the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*.

4. Turn on the power switch on the back of the instrument.

5. If the **Standby** button on the front lower-right corner of the instrument is illuminated, press the button to take the instrument out of standby mode.

   The green and amber LEDs on the status panel flash and turn off, and then the red LED turns on indicating that the instrument drawers are locked.

6. Connect the instrument to the computer as described in the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*.

   > **Note:** If you are using a 9-pin serial-to-USB adapter cable, install the driver for the adapter on your computer before connecting the cable to the instrument. To make sure the adapter has been correctly installed, open the Windows Device Manager and check for the presence of a COM port, and that there are no conflicts with the COM port.

7. Start the SoftMax Pro Software. See Starting the Software on page 10.

   The red LED on the status panel turns off and the green LED turns on, indicating a successful connection between the instrument and the SoftMax Pro Software.

8. If the **Instrument Unlocking Procedure** wizard does not appear, then use the **Instrument Connection** dialog to select and connect to the instrument. See Selecting and Connecting to an Instrument on page 11.

   > **Note:** If the instrument does not appear in the **Available Instruments** list of the **Instrument Connection** dialog, then click **Refresh** above the list.

9. After successfully connecting to the instrument, the **Instrument Unlocking Procedure** wizard appears.

10. Follow the on-screen instructions to unlock the drawers and initialize the instrument.

After completing the **Instrument Unlocking Procedure** wizard, the instrument performs an initialization procedure that moves the optics and microplate drawers to home positions. The green LED on the status panel turns on, the amber LED flashes and turns off, and then the green LED remains on. You can install the desired detection cartridges in the instrument. See "Installing Detection Cartridges" in the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*.

To lock the drawers again for shipment, use the **Instrument Information** dialog. See Using the Instrument Information Dialog on page 260.

Getting Help

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 through 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.

  You can find topics through the table of contents and by using the search function. This document also features a linked index.

- Click **Release Notes** to view the *SoftMax Pro Software Release Notes* for this release of the software. This document is in PDF format and can be viewed using a PDF viewer like Adobe Reader.

- Click **Contact Us** to display the SoftMax Pro Software What's New page, view the Molecular Devices home page, access the knowledge base, or request technical support.

  The knowledge base contains documentation supporting Molecular Devices products. You can use the customer login tools and featured links to help you get the information you need.

- Click **Software License** and follow the instructions in the **Software License Activation** dialog to activate your SoftMax Pro Software license.

- Click **SoftMax Pro** in the **About** section to see release information about this release of the SoftMax Pro Software application.

Context-sensitive help is available for the dialogs that have a ![help icon] help button. Additional documentation in PDF format can be found in the Windows **Start** menu.

- To view the *SoftMax Pro User Guide*, the *Formula Reference Guide*, or the *Software Release Notes*, click **Start > All Programs > Molecular Devices > SoftMax Pro 6.2.2** and then click the document you want to view.

- To view user guides for the supported instruments, click **Start > All Programs > Molecular Devices > SoftMax Pro 6.2.2 > Hardware User Guides** and then click the document you want to view.

These documents are in PDF format and can be viewed using a PDF viewer like Adobe Reader.

Chapter 2: User Interface Overview

If you are familiar with earlier versions of SoftMax Pro Software, adapting to the new SoftMax Pro Software 6 user interface is quick and easy. The SoftMax Pro Software 6 user interface provides advanced features and state-of-the-art navigation, analysis, and data display tools, plus it also supports a version of the classic SoftMax Pro Software interface.

SoftMax Pro Software 6 improves the basic functionality of earlier versions of the software, and it adds new features that further contribute to the usability of SoftMax Pro Software's powerful computing engine.

Workflow Overview

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.

1. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

   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.

   After selecting a **Plate** section, you can use the **Plate Setup Helper** to help you set up a microplate experiment. See Using the Plate Setup Helper on page 19.

3. Create a protocol. Click the **Settings** button to set the instrument options. See Selecting Instrument Settings on page 160.

   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 162.

   Click **OK** to save the settings.

4. Click the **Read** ▶ button to run the protocol and have SoftMax Pro Software collect and store 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 Setting the Data Display Options on page 210, Performing Data Reduction on page 214, and Graphing Data on page 233.

   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 the file as 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 the Plate Setup Helper

When you open a new SoftMax Pro Software file, the **Plate Setup Helper** opens to help you set up a microplate experiment. You can also open the **Plate Setup Helper** by clicking **Plate Setup Helper** ![wand icon] in the toolbar at the top of the active **Plate** section.
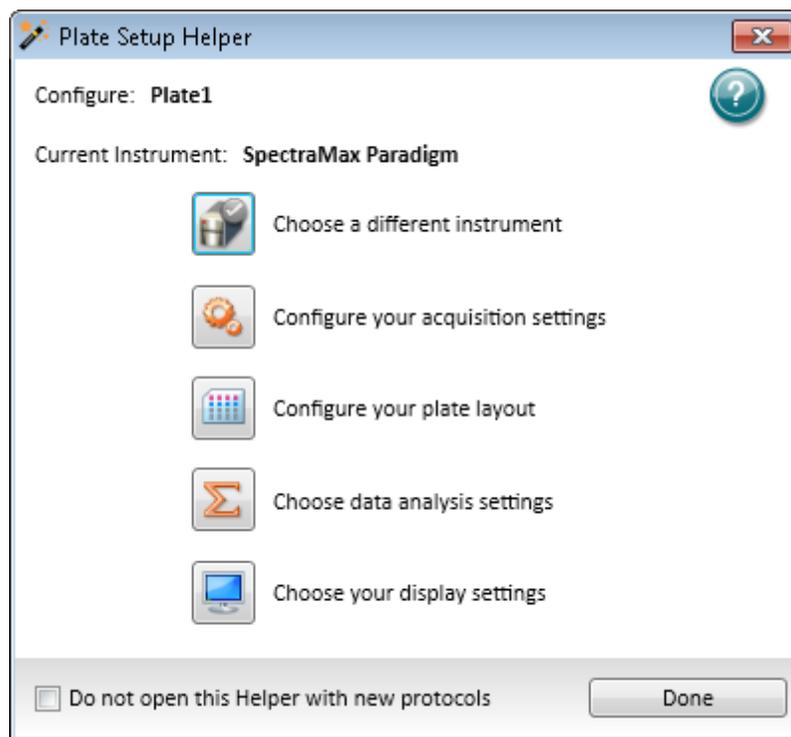
The wizard-like **Plate Setup Helper** visually guides you through the tasks available in the software to setup a microplate to acquire data. To complete a task, click its button. After you complete a task, the settings for the task are saved, and you return to the helper to select another task. The tasks do not need to be performed in the order they are presented in the helper. However, you must select the instrument before defining your acquisition settings, since the settings are specific to the instrument.

Configure tells you the name of the active **Plate** section. Acquisition settings are applied to the entire **Plate** section. If you have a plate clone selected, then the layout, analysis, and display settings are applied to the selected clone. For information about **Plate** sections, see Using a Plate Section on page 82.

Current Instrument tells you the name of the currently selected instrument. The image in the button below it shows you the instrument and its connection status. You must select the instrument before defining your acquisition settings, since the settings are specific to the instrument. To choose a different instrument or change its status, click the button. See Selecting an Instrument on page 26.

To complete a task, click its button.

- To configure your acquisition settings, click ⌖. See Instrument Settings on page 142.

- To configure your plate layout, click ⊞. See Configuring a Microplate Template on page 162.

- To choose data analysis settings, click Σ. See Performing Data Reduction on page 214.

- To choose your display settings, click ⬜. See Setting the Data Display Options on page 210.

To prevent the **Plate Setup Helper** from automatically starting each time you open a new protocol, select the **Do not open this Helper with new protocols** check box. You can enable this option again in the **SoftMax Pro Options** dialog. See Setting Application Options on page 69.

To close the **Plate Setup Helper**, click **Done**.

Main Window

The SoftMax Pro Software window has three main areas: the navigation tree on the left, the workspace area on the right, and the ribbon toolbar along the top. In addition, the application menu and quick-access toolbar in the upper-left corner give you easy access to file and application management features.
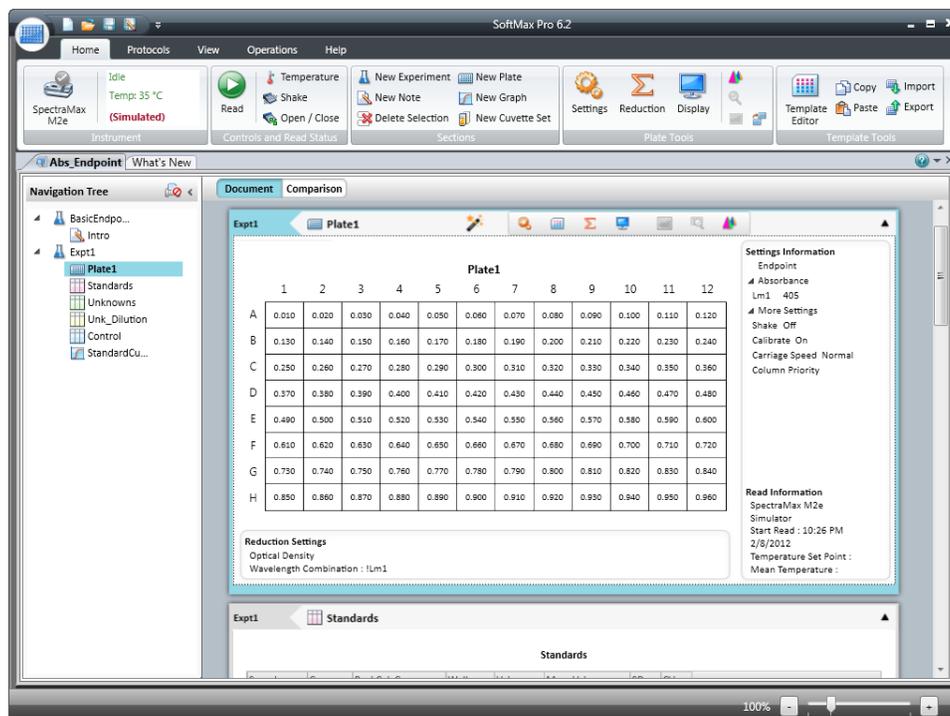
Navigation Tree

The **Navigation Tree** shows the sections in the data file that is currently open and that are available for display in your Workspace.
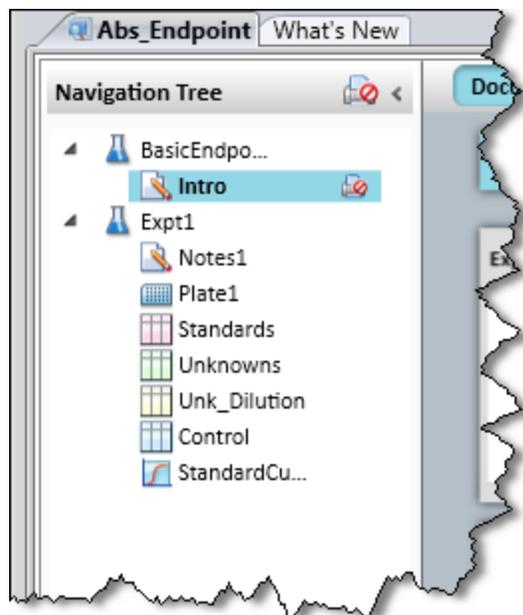
When you save a data file, the name that you give the file appears on the tab in the Navigation Tree. You can have more than one data file open at a time. Each data file can contain an unlimited number of experiments. Each experiment can contain an unlimited number sections. Section types include: Notes, Plate, Cuvette Set, Group, and Graph. See Workspace Sections on page 71.

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

Workspace Views

The SoftMax Pro Software workspace has two main views that change the way the sections appear in the workspace.

- The **Document** view provides you with a scrollable area for viewing the sections. See Document View on page 22.

- The **Comparison** view lets you move the sections around in the workspace to position them where they are most useful. See Comparison View on page 23.

For information about working with the sections in the workspace, see Workspace Sections on page 71.

Document View

Document view displays information in a manner that is similar to previous SoftMax Pro Software releases. The Document view provides you with a scrollable area for viewing the sections. To view a section either click on it in the navigation tree or use the scroll bar until the desired section comes into view.
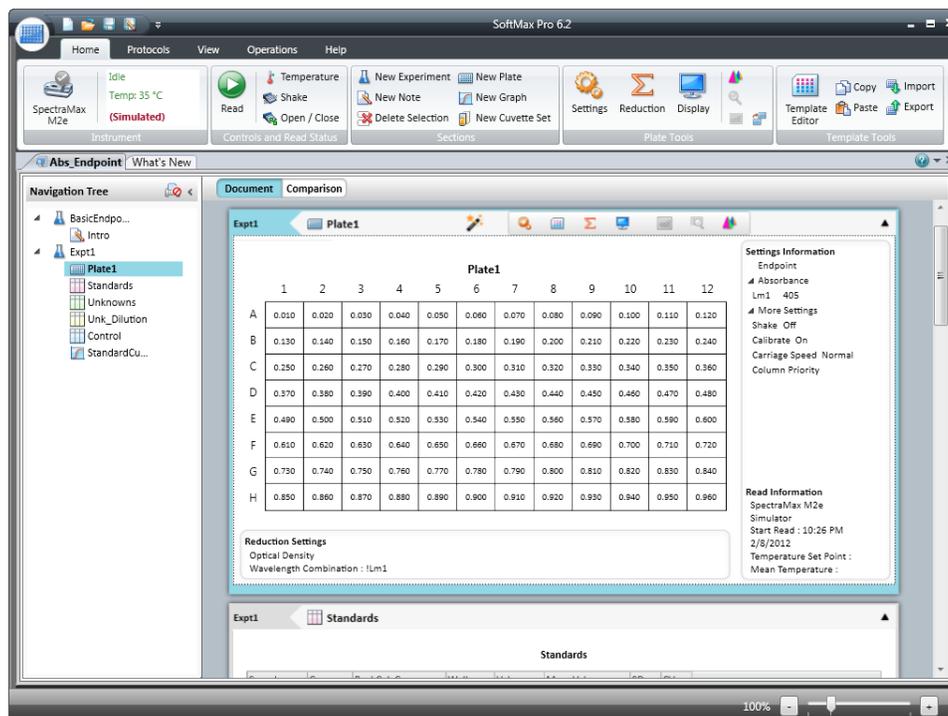
For information about working with the sections in the workspace, see Workspace Sections on page 71.

Comparison View

To change to the Comparison view, click the **Comparison** 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.
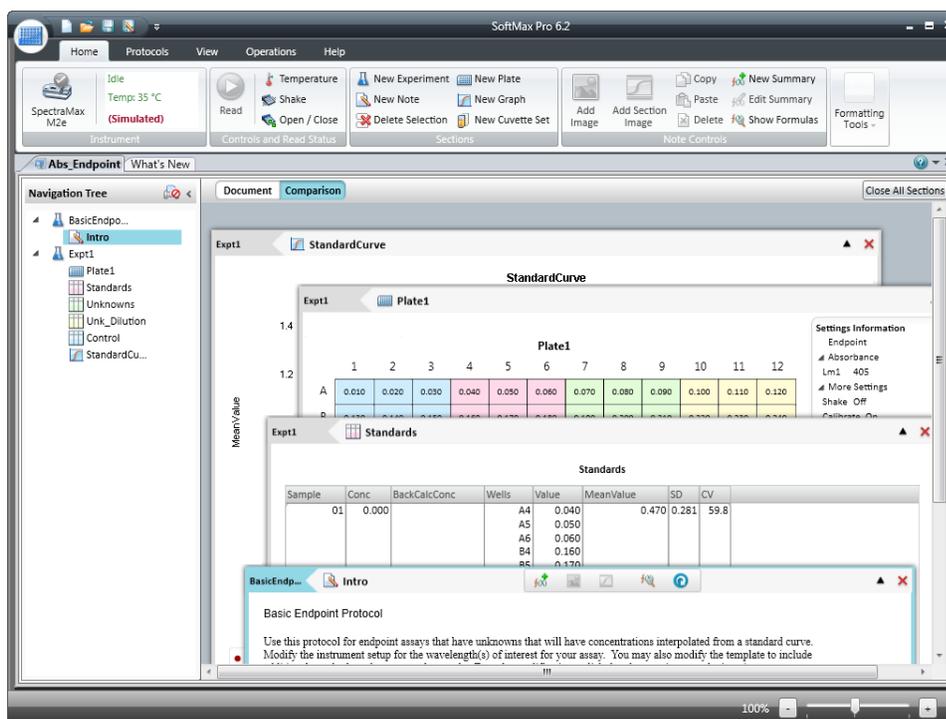
- You can drag sections from the navigation tree and position them in the workspace.

- Sections can overlap.

- Double clicking a section in the navigation tree adds that section to the bottom of the workspace.

For information about working with the sections in the workspace, see Workspace Sections on page 71.

Ribbon Toolbar

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

Home Tab

The Home tab always contains the following sections:

- Instrument

- Controls and Read Status

- Sections

See Using Instrument Controls in the Home Tab on page 26 and Using Section Controls in the Home Tab on page 29.

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

Notes Section Tools

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

- Notes Controls

- Formatting Tools

See Using a Notes Section on page 72.

Plate Section Tools

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

- Plate Tools

- Template Tools

See Using Plate Tools on page 83.

Cuvette Section Tools

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

- Cuvette Tools

- Template Tool

See Using Cuvette Tools on page 91.

Group Section Tools

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

- Column Tools

- Summary Tools

- Formatting Tools

See Using Column Tools on page 104.

Graph Section Tools

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

- Graph Tools

See .

Protocols Tab

The **Protocols** tab contains the following:

- Protocol Manager
- Community

See .

View Tab

The **View** tab contains the following:

- Show/Hide Panel
- Document View

See .

Operations Tab

The **Operations** tab contains the following:

- Instrument Tools
- Calculations
- Automation

See .

GxP Tab

The **GxP** tab is available only when using the SoftMax Pro GxP Software and contains the following:

- User Account
- Data

See .

Help Tab

The **Help** tab contains the following:

- Help
- About

See .

Using Instrument Controls in the Home Tab

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

Selecting 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, see page 120

- SpectraMax Plus 384 Absorbance Microplate Reader, see page 120

- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 121

- SpectraMax M3 Multi-Mode Microplate Reader, see page 122

- SpectraMax M4 Multi-Mode Microplate Reader, see page 123

- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 124

- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 124

- SpectraMax 190 Absorbance Microplate Reader, see page 125

- Gemini XPS Fluorescence Microplate Reader, see page 125

- Gemini EM Fluorescence Microplate Reader, see page 126

- FilterMax F5 Multi-Mode Microplate Reader, see page 127

- FilterMax F3 Multi-Mode Microplate Reader, see page 128

- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 128

- Vmax Kinetic ELISA Microplate Reader, see page 129

- Emax Endpoint ELISA Microplate Reader, see page 129

- SpectraMax Paradigm Multi-Mode Detection Platform, see page 130

- StakMax Microplate Handling System, see page 141

Reading Data

After you have your experiment defined and your prepared microplate or cuvette inserted in the reader, you can start a read.

To start a read, click the plate or cuvette section in the workspace, and then click the **Read** button in the **Home** tab of the ribbon.

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 in the ribbon.

 The **Temperature Control** dialog opens.
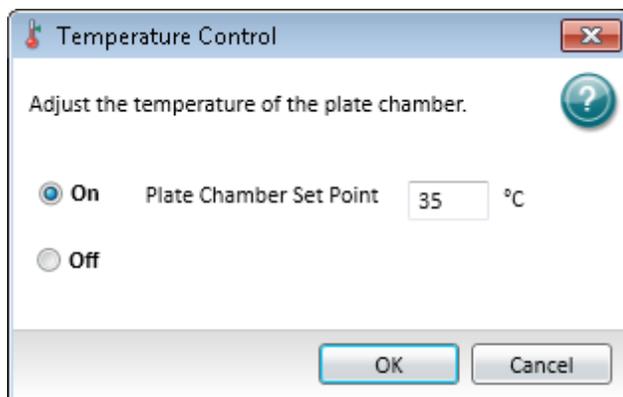
3. Click **On**.

4. Type the desired temperature in degrees Celsius.

5. Click **OK**.

With the SpectraMax M2, M2e, M5, M5e, and Plus 384, the **Instrument** 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 Microplate

If the selected instrument allows manual shaking, then the **Shake** button is available in the **Controls and Read Status** section on the **Home** tab in the ribbon.

To shake the plate:

1. Insert the prepared microplate into the connected instrument.

2. Click and hold **Shake** on the **Home** tab in the ribbon.

3. Release the mouse button to stop the shaking operation.

Opening and Closing the Microplate Drawer

To open and close the microplate drawer on the connected instrument:

1. Click **Open / Close** on the **Home** tab in the ribbon to open the microplate drawer.

2. Insert or remove the prepared microplate in the instrument.

3. With the microplate drawer open on the connected instrument, click **Open / Close** on the **Home** tab in the ribbon to close the microplate drawer.

Using Section Controls in the Home Tab

The Section controls appear on the Home tab in the ribbon. For information about the section controls, see the following topics:

- Creating an Experiment on page 29
- Creating a Notes Section on page 30
- Deleting a Section on page 30
- Creating a Plate Section on page 30
- Creating a New Graph Section on page 31
- Creating a Cuvette Set Section on page 31

Creating an Experiment

To create a new Experiment:

1. Open a data file in the workspace.

2. If you have more than one Experiment, select the Experiment that you want to duplicate to make the new Experiment.

3. Click **New Experiment** in **Sections** on the **Home** tab in the ribbon.

A duplicate of the selected Experiment appears below the selected Experiment in the **Navigation Tree**. The sections and settings are duplicated, but the data is not.

Creating a Notes Section

To create a **Notes** section:

1. Select the experiment where you want to add a **Notes** section in the **Navigation Tree**.

2. If you have more than one section in the Experiment, select the section above where you want the new **Notes** section to appear in the **Navigation Tree**.

3. Click **New Note** in **Sections** on the **Home** tab in the ribbon.

A new blank **Notes** section appears below the selected section in the **Navigation Tree**. See Using a Notes Section on page 72.

Deleting a Section

To delete a section:

1. Select the section or sections that you want to delete in either the **Navigation Tree** or a section in the Workspace. To delete and entire Experiment. select the Experiment in the **Navigation Tree**.

> **Note:** To select multiple sections, press and hold the **Ctrl** or **Shift** key and then click the sections you want to select in the **Navigation Tree**.

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

Deleting a section or experiment cannot be undone. When you delete a **Plate** section, the data from the **Plate** section is also deleted.

Creating a Plate Section

To create a new **Plate** section:

1. Select the Experiment where you want to add a **Plate** section in the **Navigation Tree**.

2. If you have more than one section in the Experiment, select the section above where you want the new **Plate** section to appear in the **Navigation Tree**.

3. Click **New Plate** in **Sections** on the **Home** tab in the ribbon.

A new blank **Plate** section appears below the selected section in the **Navigation Tree**. See Using a Plate Section on page 82.

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.

2. Click **New Graph** in **Sections** on the **Home** tab in the ribbon.

3. In the **New Graph** dialog, set the options for the graph. See Creating a New Graph on page 237.

4. When the graph options have been set, click **OK**.

A new **Graph** section appears below the selected section in the **Navigation Tree**. See Using a Graph Section on page 110.

Creating a Cuvette Set Section

To create a new **Cuvette Set** section:

1. Select the experiment you want to add the **Cuvette Set** section to in the **Navigation Tree**.

2. If you have more than one section in the Experiment, select the section above where you want the new **Cuvette Set** section to appear in the **Navigation Tree**.

3. Click **New Cuvette Set** in **Sections** on the **Home** tab in the ribbon.

A new blank **Cuvette Set** section appears below the selected section in the **Navigation Tree**. See Using a Cuvette Set Section on page 90.

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.

Protocol files are experiment template files that contain microplate well layout assignments and all other reader configuration information, but no data.

After the SoftMax Pro Software is installed, the Basic Endpoint protocol is set as the default protocol with the filename **default.spr**. Whenever you create a new document, the **default.spr** protocol file is opened as an untitled document that contains the settings from the default protocol file.

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 document, the settings in the **default.spr** file that you have saved are loaded into an untitled document.

A large number of predefined protocols are installed with SoftMax Pro Software and are placed 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 159. 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 or Removing a Folder in the Protocol Manager on page 50.

• Click **Save As Default** to overwrite the default.spr file with the protocol setting of the experiment that is active in your workspace. See Saving a Protocol as the Default Protocol on page 49.

> **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 49.

> **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.

Using the View Tab

The **View** tab in the ribbon contains two sections: **Show/Hide Panel** and **Document View**.

In the **Show/Hide Panel** section, select or clear the **Navigation Tree** check box to show or hide the **Navigation Tree** in the workspace.

In the **Document View** section, click an option to change the layout of the tabs in the workspace.

- Click **Single Tab Group** to view one tab group at a time.

- Click **New Horizontal Tab Group** to split the workspace horizontally. The selected tab moves below inactive tabs in the workspace. You can have as many horizontal areas as you have tabs in the workspace.

- Click **New Vertical Tab Group** to split the workspace vertically. The selected tab moves to the right of inactive tabs in the workspace. You can have as many vertical areas as you have tabs in the workspace.

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 259.

- **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 260.

- **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 260.

- **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.

For technical information about SoftMax Pro Software calculations, see Calculations and Numerical Precision on page 208.

Automation

Auto Read: You can automatically read plate sections as they appear within an experiment. See Enabling Auto Read on page 195.

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 141.

Automation Mode: You can place the software in automation mode, allowing the software to accept legacy commands from scripts written for the SoftMax Pro Software version 5.x automation interface. See Using Automation Mode on page 42.

Using the GxP Tab

The **GxP** tab is available only when using the SoftMax Pro GxP Software. See Using SoftMax Pro GxP Software Features on page 269.

User Account

The buttons in the **User Account** group provide access to information and management of the current user account.

- **Account Information**: The **User Account Information** dialog provides information about the current user. See Viewing the User Information on page 273.

- **User Log On**: Click the **Log On** button to log on after previously logging off. See Logging onto SoftMax Pro GxP Software on page 272.

- **User Log Off**: Click the **Log Off** button to log the current user off without closing the program.

- **Change Password**: The **Change Password** dialog allows the current user to change the password required for logging onto the software. See Changing the User Password on page 274.

- **Select Account Database**: The **User Accounts Connection** dialog allows the GxP Admin Software administrator to define the location of the user account file associated with this installation of the SoftMax Pro GxP Software. See Connecting a User Account File on page 270.

Data

The buttons in the **Data** group provide access to features that are unique to the SoftMax Pro GxP Software.

- **Statements**: The **Statements** dialog allows you to view, edit, and add statements. A user with the proper permission can add an electronic signature to statements. See Working with GxP Statements on page 275.

- **Audit Trail**: The **Audit Trail** dialog gives you read-only access to the audit trail for the open data file, and allows you to attach a note to the audit trail. See Viewing the GxP Audit Trail on page 281.

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 through 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.

  You can find topics through the table of contents and by using the search function. This document also features a linked index.

- Click **Release Notes** to view the *SoftMax Pro Software Release Notes* for this release of the software. This document is in PDF format and can be viewed using a PDF viewer like Adobe Reader.

- Click **Contact Us** to display the SoftMax Pro Software What's New page, view the Molecular Devices home page, access the knowledge base, or request technical support.

  The knowledge base contains documentation supporting Molecular Devices products. You can use the customer login tools and featured links to help you get the information you need.

- Click **Software License** and follow the instructions in the **Software License Activation** dialog to activate your SoftMax Pro Software license.

- Click **SoftMax Pro** in the **About** section to see release information about this release of the SoftMax Pro Software application.

Context-sensitive help is available for the dialogs that have a ⬤ help button. Additional documentation in PDF format can be found in the Windows **Start** menu.

- To view the *SoftMax Pro User Guide*, the *Formula Reference Guide*, or the *Software Release Notes*, click **Start > All Programs > Molecular Devices > SoftMax Pro 6.2.2** and then click the document you want to view.

- To view user guides for the supported instruments, click **Start > All Programs > Molecular Devices > SoftMax Pro 6.2.2 > Hardware User Guides** and then click the document you want to view.

These documents are in PDF format and can be viewed using a PDF viewer like Adobe Reader.

Viewing the On-Screen Help

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

Application Menu

The **Application** menu in the upper-left corner of the window contains options for working with files, printing, and managing the application.
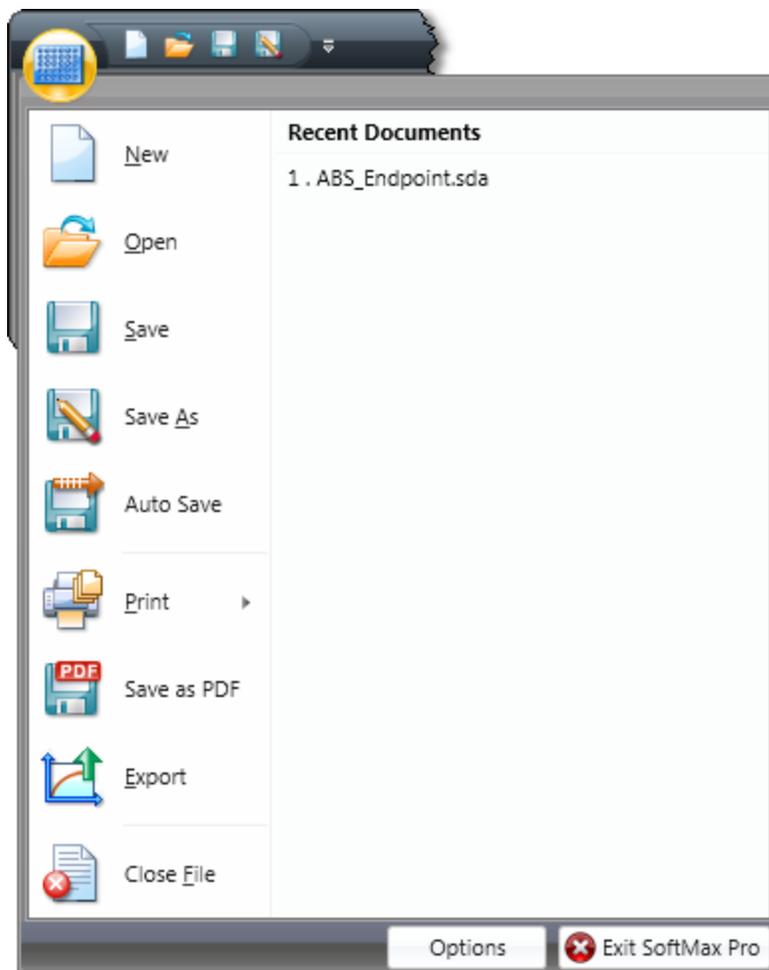
Click the **Application** [icon] button to open the **Application** menu and then click a menu option.

- To create a new blank data file or protocol file, click **New**. See Creating a New Data File on page 45 or Creating a New Protocol File on page 53.

- To open an existing data file or protocol file, click **Open** or select the file from the list of **Recent Documents** on the right. See Opening a Data File on page 46 or Opening a Protocol File on page 54.

- To save an open data file or protocol file, click **Save** or **Save As**. See Saving Data Files on page 47 or Saving Protocol Files on page 55.

- To set up automatic saving options, click **Auto Save**. See Setting Auto Save Options on page 56.

- To print the sections of an experiment, preview a print job, or set printing options, click **Print** and then click the option you want on the right. See Printing on page 60.

- To save the defined printed output of a file in portable document format (PDF), click **Save As PDF**. See Saving Data in a PDF File on page 65.

- To export the data in a data file, click **Export**. See Exporting Plate Data on page 68.

- To close the currently open document in the workspace, click **Close File**.

- To set application options, click **Options** at the bottom of the menu. See Setting Application Options on page 69.

- To exit the program, click **Exit SoftMax Pro** at the bottom of the menu.

Quick-Access Toolbar

The Quick-Access toolbar is available for your customization. The Quick-Access toolbar initially opens directly to the right of the Application button in the upper-left corner of the SoftMax Pro Software application window.
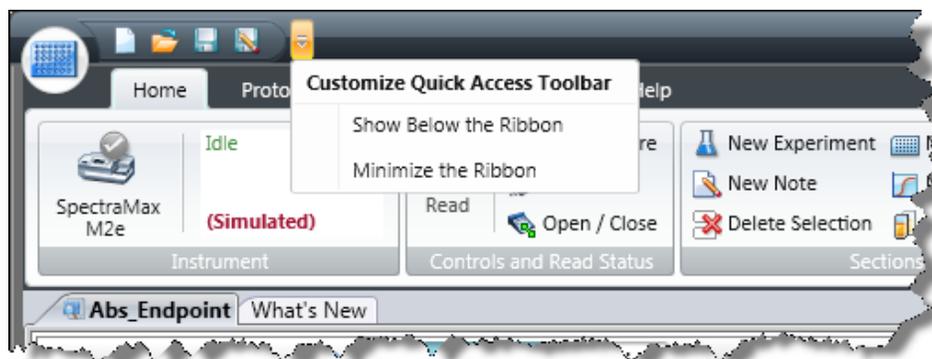
You can move the location to above or below the ribbon and add any function buttons that you want to have quick-access to.

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

- Create a new data file.

- Open a data file.

- Save a data file.

- Save (Save as) a data file or protocol file with a new name.

- The actions button.

Repositioning the Quick-Access Toolbar

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

To reposition the Quick-Access toolbar:

1. Click the actions button to the right of the Quick-Access toolbar.

2. From the menu that appears, click either **Show Below the Ribbon** or **Show Above the Ribbon**.

Adding a Button

To add a function button to the Quick-Access toolbar:

1. Right-click the button you want to add in the ribbon.

2. From the menu that appears, click **Add to Quick-Access Toolbar**.

Removing a Button

To remove a function button from the Quick-Access toolbar:

1. Right-click the button you want to remove in the Quick-Access toolbar.

2. From the menu that appears, click **Remove from Quick-Access Toolbar**.

Minimizing the Ribbon

To minimize the ribbon display:

1. Click the actions button to the right of the Quick-Access toolbar.

2. From the menu that appears, click **Minimize the Ribbon**.

To return the minimized ribbon to the display:

1. Click the actions button to the right of the Quick-Access toolbar.

2. From the menu that appears, click **Minimize the Ribbon** to remove the check mark next to the option.

Integrated Web Browser

The **What's New** tab in the document area contains a built-in web browser to keep you informed of new or updated products and to give you access to the technical support knowledge base.

This tab automatically starts each time you start the SoftMax Pro Software. To hide this tab on start up, select the **Hide web browser tab when application starts** check box in the **SoftMax Pro Options** dialog. See Setting Application Options on page 69.

Using Automation Mode

The SoftMax Pro Software automation mode prevents user interaction when the software is being controlled by the automation interface through third-party automation software or the StakMax Software. To write scripts to use with the SoftMax Pro Software version 6 automation interface, Molecular Devices recommends that you use the most recent version of the automation commands as described in the *SoftMax Pro Software Automation API Reference Guide*.

To show that the SoftMax Pro Software is in automation mode, a dialog appears in front of the main window.

Application is in Automation Mode

User input has been disabled

Automation Messages:

Client connection:
IP Address: ::1 Port: 61518

Terminate   Will forcefully close client connection.
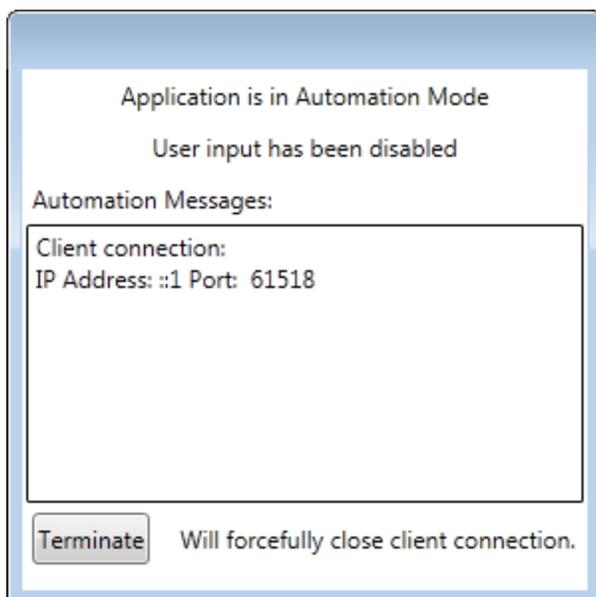
When the automation software acquires data, the related **Plate** section appears in the SoftMax Pro Software main window behind the automation mode dialog and displays the acquired data.

If the automation software encounters an error, an error message appears in the **Automation Messages** area of the automation mode dialog.

If you need to stop the automation process and regain control of the SoftMax Pro Software, click **Terminate**.

To allow the software to accept legacy commands from scripts written for the SoftMax Pro Software version 5.x automation interface, go to the **Operations** tab in the ribbon, and then in the **Automation** section click **Automation Mode** . The legacy script must use the Windows message WM_COPYDATA command. The WM_SETTEXT command is not supported. Legacy scripts might not work correctly with the new automation interface. If your legacy script does not work properly, Molecular Devices recommends that you rewrite the script using the most recent version of the automation commands as described in the *SoftMax Pro Software Automation API Reference Guide*.

For more information on automation, see StakMax Microplate Handling System on page 141 or the *SoftMax Pro Software Automation API Reference Guide*.

Chapter 3: File and Application Management

The **Application** menu in the upper-left corner of the SoftMax Pro Software window contains options for working with files, printing, and managing the application.

Click the **Application** button to open the **Application** menu and then click a menu option. See Application Menu on page 39.

File Types

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

Data files

Data files contain the experiment settings, the raw data collected by your reader, and 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.

You can open data files that have the following file extensions:

- .sda (SoftMax Pro Software 6 data files)
- .sdax (SoftMax Pro GxP Software 6 data files)
- .pda (SoftMax Pro Software 4 and 5 data files)
- .eda (SoftMax Pro GxP Software 4 and 5 data files)

See Opening a Data File on page 46.

All SoftMax Pro Software 6 data files are saved with the .sda or .sdax extension. See Saving Data Files on page 47.

The Auto Save feature 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 56.

Data Export Formats

You can export the data from a SoftMax Pro Software data file using the following file extensions:

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

See Exporting Plate Data on page 68.

Protocol Files

Protocol files are experiment template files that contain microplate well layout assignments and all other reader configuration information, but no data.

You can open protocol files that have the following file extensions:

- .spr (SoftMax Pro Software 6 protocol files)

- .sprx (SoftMax Pro GxP Software 6 protocol files)

- .ppr (SoftMax Pro Software 4 and 5 protocol files)

- .epr (SoftMax Pro GxP Software 4 and 5 protocol files)

See Opening a Protocol File on page 54.

All SoftMax Pro Software 6 protocol files are saved with the .spr or .sprx extension. Saving a data file as a protocol file removes any data in the file, leaving only the configuration information. See Saving Protocol Files on page 55.

Predefined Protocols

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. Whenever you open a new file, the new file contains the settings from the default protocol file. To change the default protocol file, see Saving a Protocol as the Default Protocol on page 49.

You can use the features available in the **Protocols** tab in the application ribbon to manage and share protocol files. See Using the Protocols Tab to Manage Protocols on page 32.

Creating a New Data File

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

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

To create a new data file:

1. Click the **Application** ▦ button to open the **Application** menu.

2. Click **New**. A new blank file opens and a new tab appears above the navigation tree.

    > 📋 **Note:** Molecular Devices recommends that you name the file before you configure settings and collect data.

3. Click the **Application** button to open the **Application** menu.

4. Click **Save as** to open the **Save As** dialog.

5. Navigate to where you want to save the file.

6. Type the file name in the **File name** field.

7. In the **Save as type** list, select **Data Files (*.sda)**.

    If you are working with the SoftMax Pro GxP Software, select **Data Files (*.sdax)**.

8. Click **Save**.

    The name of the data file appears on the tab above 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.

You can open data files that have the following file extensions:

- .sda (SoftMax Pro Software 6 data files)

- .sdax (SoftMax Pro GxP Software 6 data files)

- .pda (SoftMax Pro Software 4 and 5 data files)

- .eda (SoftMax Pro GxP Software 4 and 5 data files)

> **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 to open the **Application** menu.

2. Select **Open**. The **Data Files** file type is selected by default.

   SoftMax Pro Software 6 can open files created by version 4.0 or later.

   When you open a file from a previous version, 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 above the navigation tree, and the file opens in the workspace.

Saving Data Files

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

If you want to save data in PDF format, see Saving Data in a PDF File on page 65.

To save a data file:

1. Click the **Application** ▦ button to open the **Application** menu.

2. Click **Save** (for first time file saving) or **Save As**.

3. In the **Save As** dialog, navigate to where you want the file to be saved.

4. In the **File name** field, type a name for the file.

5. From the **Save as type** field, select **Data Files (*.sda)**.

   If you are working with the SoftMax Pro GxP Software, select **Data Files (*.sdax)**.

6. Click **Save**.

Molecular Devices recommends that you save your data file frequently. When you want to save the data file, click the **Application** ▦ button to open the **Application** menu and then click **Save** to save the file without opening the **Save As** dialog.

Copying and Pasting Data

You can copy plate data from one **Plate** section to another within the same data file or between different data files. The format of the copied plate data must match the format of the target **Plate** section. If you want to copy plate data between **Plate** sections in the same file, it might be easier to use the **Clone Plate** feature. See Cloning a Plate Section on page 89.

In some cases, you can paste endpoint plate data copied from an external program, such as a spreadsheet or text editor. When pasting data from an external program, the format of the copied endpoint data must exactly match the format of the target **Plate** section. Only endpoint data can be pasted from an external program.

For more information about **Plate** sections, see Using a Plate Section on page 82.

To copy data from a **Plate** section:

1. View the **Plate** section that has the data you want to copy.

2. Right-click the plate data area and click **Copy Plate Data** to copy all of the data in the entire plate.

To paste data in a **Plate** section:

1. View the **Plate** section where you want to paste the data.

2. Right-click the plate data area and click **Paste Plate Data**.

   If you are pasting data from an external program, click **Paste Data**.

3. When pasting over existing data, click **Yes** in the message box that appears.

   If the format of the data does not match the format of the target plate, a message appears informing you of this. Click **OK** to close the message without pasting the data.

After the data is pasted into the **Plate** section, the **Read Information** on the right indicates that the data was pasted and shows the time and date of when it was pasted.

Managing Protocol Files

Protocol files are experiment template files that contain microplate well layout assignments and all other reader configuration information and reduction parameters, but no data. Protocol files can be useful if you repeat a particular type of experiment frequently.

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. See Using the Protocols Tab to Manage Protocols on page 32.

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 the SoftMax Pro Software can further enhance any specialized data collection and analysis needs through custom assay development.

The predefined protocols installed with SoftMax Pro Software are placed in the SoftMax Pro Software Protocols folder. You can use the Protocol Manager to quickly find and open a protocol. See Using the Protocol Manager to Open a File on page 49.

During software installation, the Basic Endpoint protocol is set as the default protocol. Whenever you open a new file, the new file contains the settings from the default protocol file. You replace the default protocol with the settings from an open file so that future new files will use those settings. See Saving a Protocol as the Default Protocol on page 49.

Saving a data file as a protocol file removes any data in the file, leaving only the configuration information. You can add or remove folders in the Protocol Manager to help you find the protocols you use most often. See Adding or Removing a Folder in the Protocol Manager on page 50.

Using the Protocol Manager to Open a File

The predefined protocols installed with SoftMax Pro Software are placed in the SoftMax Pro Software Protocols folder. You can use one of the predefined protocols, or you can create your own protocol. For more information on protocols, see Creating a Protocol on page 159.

You can use the Protocol Manager to quickly find and open a protocol.

To open a protocol file in the Protocol Manager:

1. Click **Protocol Manager** on the **Protocols** tab to open the Protocol Manager where you can select a protocol to be assigned to a new file.

2. Select **Protocol Library >** *foldername* to view the available protocols in that folder.

   To open the default protocol, click **Default** at the bottom of the Protocol Library.

3. Click 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 configure thee instrument settings using the **Settings** dialog. See Instrument Settings on page 142.

Saving a Protocol as the Default Protocol

After the SoftMax Pro Software is installed, the Basic Endpoint protocol is set as the default protocol with the filename **default.spr**. Whenever you create a new document, the **default.spr** protocol file is opened as an untitled document that contains the settings from the default protocol file.

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 document, the settings in the **default.spr** file that you have saved are loaded into an untitled document.

Saving a Default Protocol

1. Click **Save as Default** ![icon] 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 47.

Adding or Removing a Folder in the Protocol Manager

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**. See Using the Protocol Manager to Open a File on page 49.

Adding a Protocol Folder Location

To add a protocol folder location:

1. Click **Folder Locations** ![icon] to open the **Folder Locations** dialog.

2. Click **Add** to browse the file system to the folder to be added.
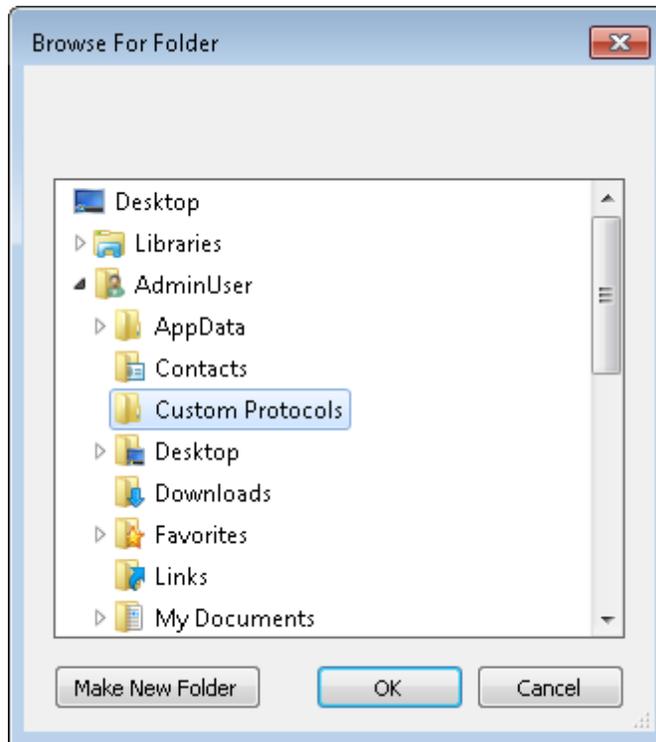
3. Select the folder where you want to store protocols.

4. Optionally, click **Make New Folder** to add a New Folder inside the selected folder and then type a name for the 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 the folder location you want to remove.
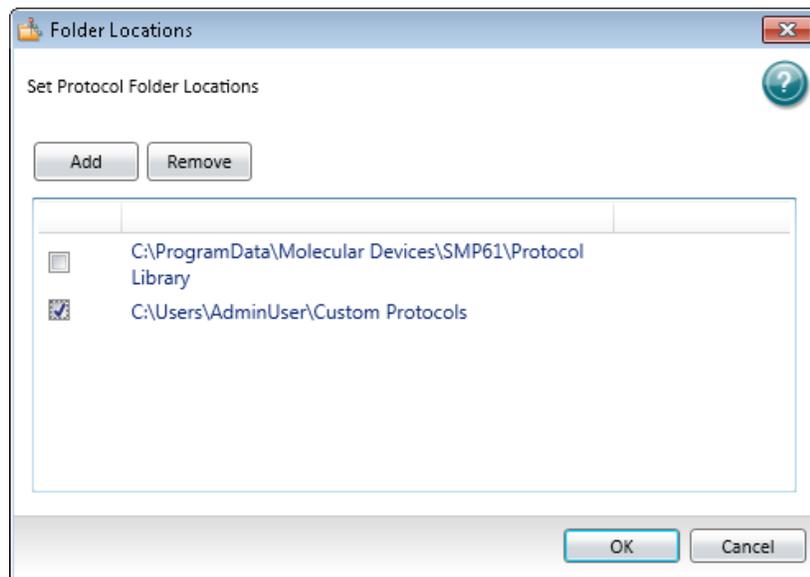
3. Click **Remove**. The selected folder is removed from the folder list in the **Folder Locations** dialog.

4. Click **OK** to close the **Folder Locations** dialog.

Creating a New Protocol File

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

To create a new protocol file from a predefined protocol in the Protocol Manager, see Using the Protocol Manager to Open a File on page 49.

To create a new protocol file:

1. Click the **Application** button to open the **Application** menu.

2. Click **New**. A new blank file opens and a new tab appears above the navigation tree.

3. Configure the protocol settings, see Creating a Protocol on page 159.

   > **Note:** Molecular Devices recommends that you name the protocol file before you collect data with the settings.

4. Click the **Application** button to open the **Application** menu.

5. Click **Save as** to open the **Save As** dialog.

6. Navigate to where you want to save the protocol file.

7. Type the file name in the **File name** field.

8. In the **Save as type** list, select **Protocol Files (*.spr)**.

   If you are working with the SoftMax Pro GxP Software, select **Protocol Files (*.sprx)**.

9. Click **Save**.

Before using the protocol to collect data, save it as a data file. See Saving Data Files on page 47.

Opening a Protocol File

To open a supported protocol 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.

You can open protocol files that have the following file extensions:

- .spr (SoftMax Pro Software 6 protocol files)

- .sprx (SoftMax Pro GxP Software 6 protocol files)

- .ppr (SoftMax Pro Software 4 and 5 protocol files)

- .epr (SoftMax Pro GxP Software 4 and 5 protocol files)

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 protocol 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 predefined protocol in the Protocol Manager, see Using the Protocol Manager to Open a File on page 49.

To open a protocol file:

1. Click the **Application** button to open the **Application** menu.

2. Select **Open**. The **Data Files** file type is selected by default.

3. Select a protocol file type from the list.

   SoftMax Pro Software 6 can open files created by version 4.0 or later.

   When you open a file from a previous version, it is converted to SoftMax Pro Software version 6 format.

4. Navigate to the protocol file you want to open.

5. Select the file.

6. Click **Open**.

   An untitled file opens in the workspace with the settings of the selected protocol applied to it. Before using the protocol to collect data, save it as a data file. See Saving Data Files on page 47.

Saving Protocol Files

Use the **Application** menu **Save As** command to save a file as a protocol file.

1. Click the **Application** button to open the **Application** menu.

2. Click **Save As**.

3. In the **Save As** dialog, navigate to where you want the protocol file to be saved.

4. In the **File name** field, type a name for the file.

5. From the **Save as type** field, select **Protocol Files (*.spr)**.

   If you are working with the SoftMax Pro GxP Software, select **Protocol Files (*.sprx)**.

6. Click **Save**.

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

Before using the protocol to collect data, save it as a data file. See Saving Data Files on page 47.

Setting Auto Save Options

When Auto Save 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.

You can add as many Auto Save instances as desired, each with its own settings.

Note: Auto Save settings are saved with each document.

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 141.

To set Auto Save options:

1. Click the **Application** button in the ribbon to open the **Application** menu and then click **Auto Save** to open the **Auto Save** dialog.
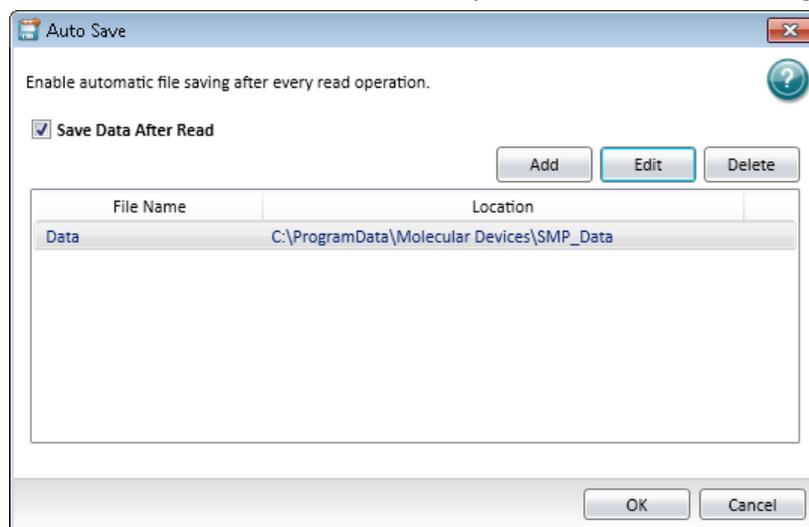
2. To enable Auto Save, select **Save Data After Read**.

3. To create a new Auto Save location, click **Add**.

4. To modify an existing Auto Save location, select the location in the list and then click **Edit**.

5. When you are finished setting Auto Save options, click **OK**.

Defining Auto Save Properties
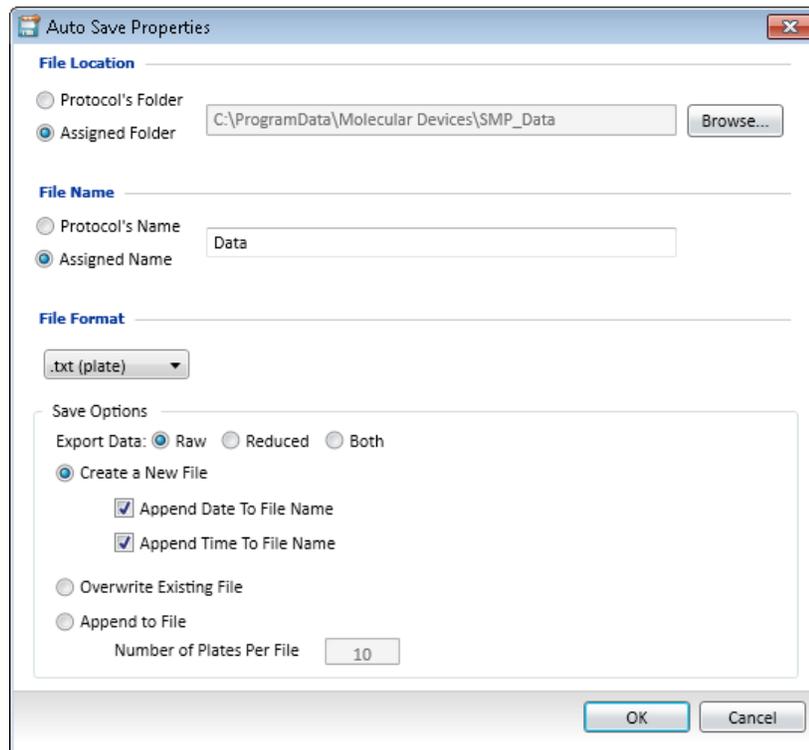
The **Auto Save Properties** dialog contains the following options:

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 click **Assigned Folder** and then click the **Browse** button to locate the folder where you want to save the data.

File Name

To name the automatically saved file with the current protocol's name, select **Protocol's Name**.

To assign a name to the automatically saved file, select **Assigned Name** and then type a name for the file. The text in this field is automatically inserted at the beginning of each automatically saved file name.

File Format

Select a data format from the menu. Options include:

- .sda or .sdax

- .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 the following file formats: SoftMax Pro (.sda), XML (.xml), tab-delimited text (.txt) in list or plate layouts, and Excel (.xls) in list or plate layout.

SoftMax Pro File Format

When **.sda** or **.sdax** is selected, your data is automatically saved as a proprietary SoftMax Pro Software file format.

XML File

When **.xml** is selected, you data is automatically saved in XML format. XML is supported for data export and Auto Save. XML is the best file format if you plan to import 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 either **.txt (list)** or **.txt (plate)** is selected, your data is automatically saved as a tab-delimited text file, which can be opened by most word processor, spreadsheet, or database programs.

Excel

When either **.xls (list)** or **.xls (plate)** is selected, your data is automatically saved in a format that can be opened by Microsoft 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 available save options depend on the selected file format for the automatically saved file.

Export Data

For all data formats except .sda, you can you can specify the type of data to save.

- Select **Raw** to export only the raw data.

- Select **Reduced** to export only the reduced data.

- Select **Both** to export both the raw and the reduced data in the same file.

Create a New File

When you choose to create a new file after each read, you can also choose to append the date and time to the file name.

- Select **Append Date to File Name** to add the date after the specified file name.

- Select **Append Time to File Name** to add the time after the specified file name.

If you select to append both the date and the time to the file name, the time follows the date.

If you do not select to append the date or the time to the file name, files with the same name are numbered sequentially, for example **Data 1**, **Data 2**, and so on.

If you select to append only the date to the file name, SoftMax Pro Software indexes the runs for that day starting with **1** and increments with each successive run. For example, the first file named **Data** that is automatically saved on November 18th, 2012 would be named as **Data-11-18-12 1**, while the second automatically saved file would be named **Data-11-18-12 2**, and so on.

Overwrite Existing File

This option should be used carefully as overwriting a file can cause loss of data. To help ensure against data loss, a file can overwrite only itself. If another file with the same name exists in the defined autosave directory, the SoftMax Pro Software appends a number to the end of the file name.

Append to File

If either a .txt or .xls format is selected, you can choose to append the plate data to the current file. Enter the maximum number of plates to append per file.

If you are reading 10 microplates using the StakMax Microplate Handling System, you can use **Append to File** to save the data from all 10 microplates in a single data file.

Printing

You can print the contents of the currently selected file. You can print all the sections, or select the sections that you want to print. You can preview the print job before you print it. You can define the header and footer of the pages and the print quality in the **Printing Options** dialog.

To print the file or set the printing options, Click the **Application** button to open the **Application** menu, and then click **Print** . The following options appear on the right:

- Click **Print All** to print all the included sections in the file. See Printing All Included Sections on page 61.

- Click **Print Preview** to preview the print job before printing it. See Previewing a Print Job on page 61.

- Click **Print Selected** to select the sections that you want to print for the print job. See Printing Selected Sections on page 62.

- Click **Printing Options** to define the header and footer of the pages and the print quality for your print jobs. See Setting Printing Options on page 63.

In addition, you can predefine the sections that you want to exclude from your future print jobs by disabling printing for the sections in the **Navigation Tree**. See Disabling Sections from Printing on page 64.

To print to a PDF file, select the **Molecular Devices(PDF Writer)** in the **Print** dialog or open the **Application** menu and click **Save As PDF**. See Defining PDF File Settings on page 65.

Printing All Included Sections

When you select to print all the sections in the file, all the included sections print in the order they are listed in the **Navigation Tree**. If you have excluded a section from printing by disabling printing for the section in the **Navigation Tree**, the section does not print. See Disabling Sections from Printing on page 64.

To print all the included sections in the file:

1. Click the **Application** button to open the **Application** menu, and then click **Print** .

2. From the list on the right, click **Print All** .

3. In the **Print** dialog, select the printer you want to use and set any print settings available for that printer, including the number of copies that you want to print.

   To print to a PDF file, select the **Molecular Devices (PDF Writer)**. See Defining PDF File Settings on page 65.

4. Click **Print**.

Previewing a Print Job

To preview the print job before printing it:

1. Click the **Application** button to open the **Application** menu, and then click **Print** .

2. From the list on the right, click **Print Preview** .

3. In the **Print Preview** dialog, you can scroll through the pages and zoom in and out.

4. Click the **Print** button in the toolbar at the top of the dialog.

5. In the **Print** dialog, select the printer you want to use and set any print settings available for that printer, including the number of copies that you want to print.

   To print to a PDF file, select the **Molecular Devices (PDF Writer)**. See Defining PDF File Settings on page 65.

6. Click **Print**.

Printing Selected Sections

To select the sections that you want to print:

1. Click the **Application** ▦ button to open the **Application** menu, and then click **Print** 🖨 .

2. Select **Print Selected** 🖨 .
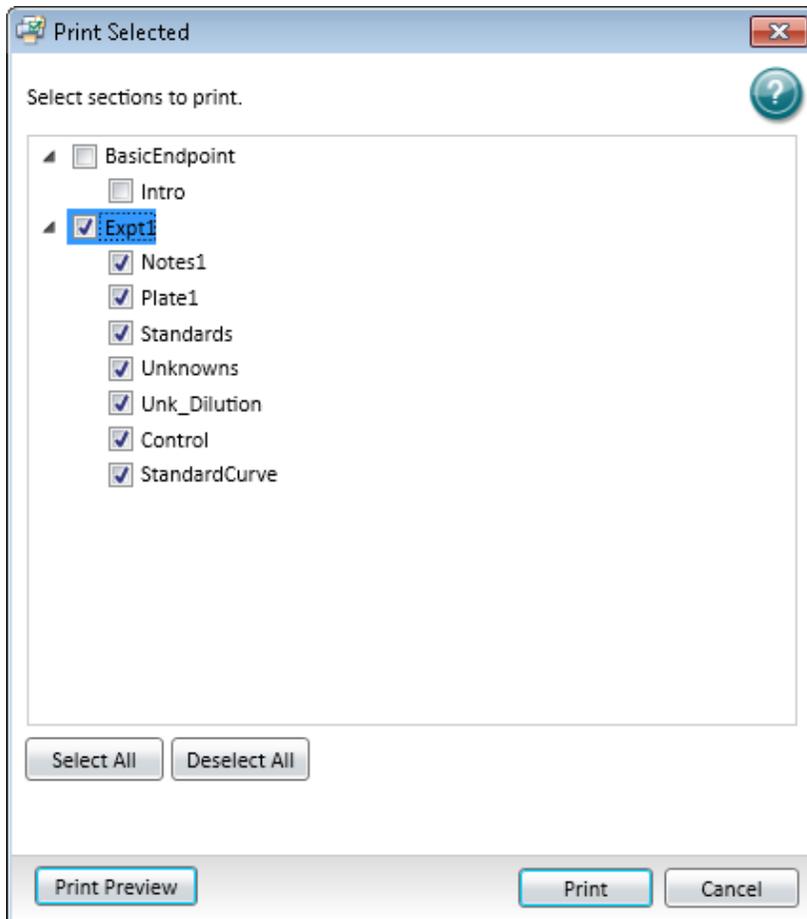
3. In the **Print Selected** dialog, select the check boxes next to the sections you want to print and clear the check boxes next to the sections that you do not want to print.

   • To select all the check boxes, click **Select All**.

   • To clear all the check boxes, click **Deselect All**.

   • To preview the print job before printing, click **Print Preview**. See Previewing a Print Job on page 61.

4. Click **Print** to print the selected sections.

5. In the **Print** dialog, select the printer you want to use and set any print settings available for that printer, including the number of copies that you want to print.

   To print to a PDF file, select the **Molecular Devices (PDF Writer)**. See Defining PDF File Settings on page 65.

6. Click **Print**.

Setting Printing Options

To define the contents of the header and footer and the quality of the printed output:

1. Click the **Application** button to open the **Application** menu, and then click **Print** .

2. Click **Printing Options** .

3. In the **Printing Options** dialog, define the header and footer of the pages.

   • To include the predefined text, select the check box next to the text.

   • To include custom text in the header, type the text in the fields.

   • To add a JPEG image, such as a logo, to the right side of the header, select the **Add Image** check box and then click the **Browse** button to locate and select the image file. When printing, the selected image is sized to fit in the image area in the upper-right area of the page.

4. Adjust the print quality by dragging the **Print Quality** slider to the desired quality level.

5. Click **OK** to save the printing options and close the dialog.

Disabling Sections from Printing

To predefine the sections that you want to exclude from your future print jobs:

1. In the **Navigation Tree**, click the section you want to exclude from printing.

2. Click the **Disable printing for the selection** icon in the upper-right corner of the **Navigation Tree**.

   The icon appears to the right of the section in the **Navigation Tree**.
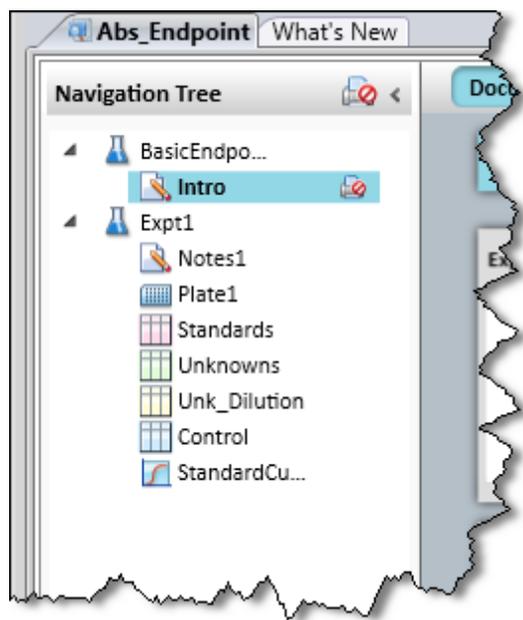
To enable printing the selected section, click the **Disable printing for the selection** icon again.

To disable or enable all the sections in an Experiment for printing, click the Experiment to select it and then click the **Disable printing for the selection** icon in the upper-right corner of the **Navigation Tree**.

Saving Data in a PDF File

You can save the contents of the currently selected file in PDF format. Before saving the PDF file, you can define the header and footer of the pages and the print quality in the **Printing Options** dialog and predefine the sections that you want to exclude from the PDF file by disabling printing for the sections in the **Navigation Tree**. See and .

To save the data in a PDF file:

1. Click the **Application** 🔳 button to open the **Application** menu.

2. Click **Save As PDF**.

   You can also select the **Molecular Devices (PDF Writer)** in the **Print** dialog.

3. In the **Molecular Devices (PDF Writer) - Create File** dialog, name the file and define the PDF options. See .

4. Click **Save** to save the PDF file.

Defining PDF File Settings

Table 3-1: PDF File Settings

Tab	Option	Description
General	Option Set	Select a pre-defined set of PDF settings from this list. To define a new set, select **<Edit>**.
	Format	To create a PDF file, select PDF from this list. You can also select from this list to create output in image file formats.
	File Name	Type the file name in the path or click [...] to browse for a folder and name the file.
	Append if output exists	Select this check box to append this file to an existing file instead of overwriting an existing file.
	Open destination folder after creation	Select this check box to open the folder where the file is saved after saving the file.
	Open the document after creation	Select this check box to open the file after saving the file.
Document	Author	Type the name of the author in this field for displaying in the document properties.
	Title	Type the title of the document in this field for displaying in the document properties.
	Subject	Type the subject of the document in this

Table 3-1: PDF File Settings (continued)

Tab	Option	Description
		field for displaying in the document properties.
	Keywords	Type keywords for the document in this field for displaying in the document properties.
	Quality	Select a quality option from this list to define the size of the PDF file.
	Initial zoom level	Select a zoom level from this list to define how the document appears each time it is opened.
	Compatibility Level	Select a format from this list to make the PDF file compatible with older versions of PDF readers. Some options might not be supported in older version.
	PDF/A-1b	Select this check box to use the PDF/A-1b archiving standard.
Image	DPI	When creating an image file, select the resolution for the image in dots per inch.
	Text Alpha Bits	When creating an image file, select the number of bits to use for the alpha channel for text.
Watermark	Text	Type the text that you want to appear in the watermark.
	Font	Select the typeface for the watermark.
	Color	Click ... to select the color for the text.
	Size	Type the size of the text in points.
	Outline width	Type the width of the text outline in pixels to have the text appear in outlines. To have the text appear as a solid color, leave this field blank.
	Layer	To have the watermark text appear on top of the document text, select **Stamp**. To have the watermark text appear behind the document text, select **Watermark**.
	Rotation	Select to display the text upward from the lower left-corner or downward from the upper-left corner.
	Vertical position	Select to display the text on the top, center,

Table 3-1: PDF File Settings (continued)

Tab	Option	Description
		or bottom of the document.
	Vertical adjustment	Type a percentage offset for the vertical watermark position.
	Horizontal position	Select to display the text on the left, center, or right of the document.
	Horizontal adjustment	Type a percentage offset for the horizontal watermark position.
Merge	Append PDF	Type the name of the file to which you want to append this file. To browse for the file, click [...].
	Position	To append the new file to the end of the old file, select **Bottom**. To append the new file to the beginning of the old file, select **Top**.
	Background PDF	If you want to print the data on an existing file, type the file name in this field. This can be used to print on company letterhead. To browse for the file, click [...].
	Layer	To have the new file appear on top of the old file, select **Stamp**. To have the new file appear behind the old file, select **Watermark**.
Security	Owner password	Type the password that gives owner permissions for the file.
	User password	Type the password that gives user permissions for the file.
	Key Length	Select the length of the key to be used for document encryption.
	Set Permissions	Select this check box to limit access to the document for the user level.
	Print	Select whether or not to allow printing and what print quality is allowed.
	Copy to clipboard	Select this check box to allow users to copy text from the file to the clipboard.

Exporting Plate Data

The **Export** feature creates a data file from the selected sections in a SoftMax Pro Software file.

The precision of the data displayed in a **Plate** section varies based on the amount of space available to view the characters.

For example, for a plate with 96 wells or fewer, each well is limited to 5 digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to 4 characters, including the decimal point.

When SoftMax Pro Software exports plate data, the values are not limited by space and can have more digits for each well.

The values displayed in a **Plate** section are only a representation of the data. In both the **Plate** section and in the exported data, the last digit displayed or exported is rounded based on the actual data value.

If you want to export data in PDF format, see Saving Data in a PDF File on page 65.

To export the data from a file:

1. Click the **Application** button to open the **Application** menu.

2. Click **Export** .

3. In the **Export** dialog, select the sections to export:

    - To include a section in the export, select the check box next to the section.

    - To exclude a section from the export, clear the check box next to the section.

    - To export all plate data, select the **All Plates** check box.

    - To export all group data, select the **All Groups** check box.

    - To export all notes data, select the **All Notes** check box.

4. Click one of the following the **Plate Data Options**:

    - **Raw**

    - **Reduced**

    - **Both**

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

5. Click the **Output Format** you want for the export file:

- **Columns** exports data in single column of text for each well.

- **Plate** exports data in a text matrix corresponding to a microplate grid.

- **XML** exports data in an XML file format. For a copy of the SoftMax Pro XML schema, contact Molecular Devices Technical Support.

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

6. Click **OK** to export the data.

7. In the **Save As** dialog, navigate to the folder where you want to save the exported data.

8. In the **File name** field, type a name for the export file.

9. From the **Save as type** list, select the file type for the export file. For XML exports, only **.xml** is available in this list.

10. Click **Save** to save the file with the exported data.

Setting Application Options

Click the **Application** button to open the **Application** menu, and then click the **Options** button at the bottom of the menu.

The **SoftMax Pro Options** dialog contains settings for the following options:

- Data Recovery

  The Data Recovery option contains an informational setting to inform you that opened files will automatically be saved to a temporary file every five minutes to assist with data recovery. This option cannot be turned off. For more information, see Enabling Data Recovery on page 70.

- Web Browser

  The Web Browser option contains a setting to allow you to hide the integrated web browser tab when the SoftMax Pro Software starts. The web browser automatically starts with the program when this check box is cleared. For more information, see Integrated Web Browser on page 41.

- Plate Setup Helper

  The Plate Setup Helper option contains a setting to prevent the Plate Setup Helper from automatically starting each time you open a new protocol. The Plate Setup Helper automatically opens each time you open a new SoftMax Pro Software file when this check box is cleared. For more information, see Using the Plate Setup Helper on page 19.

To close the **SoftMax ProOptions** dialog and save your settings, click **OK**.

Enabling Data Recovery

To verify data recovery:

1. Click the **Application** button to open the **Application** menu.

2. Click the **Options** button at the bottom of the menu.

3. In the **SoftMax Pro Options** dialog, 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, opened files are automatically saved to a temporary file every five minutes to assist with data recovery.

Recovering Files

If the SoftMax Pro Software experiences an unexpected application interruption, then when the SoftMax Pro Software restarts, it lists the files that are 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 in the SoftMax Pro Software.

The files that are not selected from the list are deleted after 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.

Chapter 4: 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. Notes sections can contain text and graphics, and can contain images of the other sections in the file. You can have as many not sections as you want in the file. For more information, see Using a Notes Section on page 72.

- **Plate** sections are used to collect data from the instrument, and to define data display and data reduction. You can have as many **Plate** sections as you want, and each **Plate** section can have different instrument settings from the other **Plate** sections in the file. For more information, see Using a Plate Section on page 82.

- **Cuvette Set** sections are used to collect data from a cuvette port. You can have as many **Cuvette Set** sections as you want, and each **Cuvette Set** section can have different instrument settings from the other **Cuvette Set** sections in the file. For more information see Using a Cuvette Set Section on page 90.

- 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 a Group Section on page 100.

- **Graph** sections are used to plot information from groups as scatter plots or bar graphs. You can have as many **Graph** section as you want in a file. For more information, see Using a Graph Section on page 110.

The sections are contained in an **Experiment**. Experiments can be used as an organizational tool within the file to maintain separate data sets. You can have as many Experiments as you want in a file.

When you use the **AutoRead** feature, only the **Plate** sections within the selected **Experiment** are run. See Enabling Auto Read on page 195.

In the SoftMax Pro GxP Software, you can lock the individual sections in an experiment to prevent changes to them. See Locking and Unlocking Sections on page 283.

Using a 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 create a **Notes** section:

1. Select the experiment where you want to add a **Notes** section in the **Navigation Tree**.

2. If you have more than one section in the Experiment, select the section above where you want the new **Notes** section to appear in the **Navigation Tree**.

3. Click **New Note** in **Sections** on the **Home** tab in the ribbon.

A new blank **Notes** section appears below the selected section in the **Navigation Tree**.

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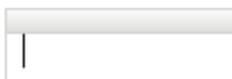
    Note: Each image must be placed in its own frame, and the frame cannot contain any text.

2. Click **Add Image** in **Note Controls** on the **Home** tab in the ribbon or in the toolbar at the top of the **Notes** section.

3. In the **Open** dialog, 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.
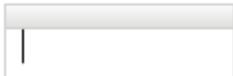
   > 📝 **Note:** Each image must be placed in its own frame, and the frame cannot contain any text.

2. Click **Add Section Image** in **Note Controls** on the **Home** tab in the ribbon 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. 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 the text.
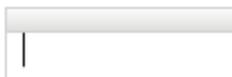
2. Type the text you want to appear.

3. 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, click to select the line of text to be aligned, or click and drag to select multiple lines of text.

The following alignment options are in **Formatting Tools** on the **Home** tab in the ribbon:

- To align the line to the left edge, click **Align Left** ≣.

- To center the line, click **Align Center** ≣.

- To align the line to the right edge, click **Align Right** ≣.

- To left and right justify the line, click **Justify** ≣.

- To outdent an indented line, click **Decrease Indent** ≢.

- To indent the selected line, click **Increase Indent** ≢.

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 select the text to be edited.

2. Type the revised text.

   The selected text is replaced with the text you 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 select the text to be copied.

2. Right-click and then click **Copy**, or press **Ctrl+C**.

   The selected 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 and then click **Paste**, or press **Ctrl+V**.

   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 select the text to be cut.

2. Right-click and then click **Cut**, or press **Ctrl+X**.

   The selected text is cut from the text frame and placed in the clipboard.

Deleting Text

To delete text from a text frame:

1. Click on the word or click and drag on a group of words to select the text to be deleted.

2. Press **Delete**.

   The selected text is removed 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 **Note Controls** on the **Home** tab in the ribbon.

Click and drag or double-click text in a text frame to select the text and then click the formatting tools to perform the following functions:

- Select a font face Calibri ▼

- Select a font size 14 ▼

- Create **Subscript** x_2 or **Superscript** x^2 characters

- Format **Bold** B, **Italic** *I*, or **Underline** U characters

- Add **Bullets**

    To add a blank line between lines of bulleted text, click at the end of a line and then press **Shift+Enter** to add a soft line break.

- Add **Numbering**

    To add a blank line between lines of numbered text, click at the end of a line and then press **Shift+Enter** to add a soft line break.

- Change the **Font Color**

You can also click **Undo Previous Action** or **Redo Previous Action** for quick formatting changes.

Positioning a Text Frame in a Notes Section

You can manually position a text frame 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.

To automatically position text frames within a **Notes** section:

1. Click on a text frame border to select the text frame.

   You can also select multiple text frames, summaries, and images by pressing **Ctrl** and then clicking on the frame borders.
2. Click an alignment option in **Note Controls** on the **Home** tab in the ribbon.

   - To align a single text frame to the left edge of the **Notes** section or align the left edges of multiple frames to the left-most text frame, click **Align Lefts** .

   - To align a single text frame to the right edge of the **Notes** section or align the right edges of multiple frames to the right-most text frame, click **Align Rights** .

   - To align a single text frame to the top edge of the **Notes** section or align the top edges of multiple frames to the top-most text frame, click **Align Tops** .

   - To align a single text frame to the bottom edge of the **Notes** section or align the bottom edges of multiple frames to the bottom-most text frame, click **Align Bottoms** .

Resizing a Text Frame

To resize a text frame:

1. Click the text frame to select it.
2. Click and hold on the right or left side border of the text frame.

   A double-ended arrow appears.
3. Drag the box to the desired size and release the mouse button.

Deleting a Text Frame

To delete a text frame:

1. Click on the border of the text frame to select the text frame.
2. Right-click and then click **Delete**, or press **Delete** on your keyboard.

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

Adding a New Summary Formula

To add a new Summary Formula to a **Notes** section:

1. Click the **Notes** section to make it active in the Workspace.

2. Click **New Summary** 🎯 in **Note Controls** on the **Home** tab in the ribbon.

3. In the **Formula Editor** dialog, type a short descriptive name for the summary formula in the **Name** field.

> 📓 **Note:** If you plan to reference this formula in another formula, for example referencing the formula in a column of a group table, make sure that you do not use reserved characters or names of operators, functions, or accessors in this name. For information about reserved characters and words, see "Rules to Follow When Writing Formulas" in the *SoftMax Pro Software Formula Reference Guide*.

4. Optionally, click **Hide Name** to hide the name of the Summary in the **Notes** section or **Group** section.

5. Optionally, type a brief text **Description** of the formula.

6. Optionally, click **Syntax Helper** to turn on the formula completion tool. See Enabling Syntax Helper on page 80.

7. Type a **Formula** to be used for the Summary or column.

8. Optionally, click **Check Syntax** check the validity of the formula. A message appears to the right of the button to help you troubleshoot the syntax of the formula.

9. Specify the **Data Display** characteristics:

   • For **Precision**, click **Decimal Places** and type the number of places to display or click **Significant Figures** and type the number of figures to display.

   • For **Format**, click either **Numeric Notation** or **Scientific Notation**.

   A preview of the **Data Display** appears in the **Sample** on the right.

10. Click **OK**.

For a complete guide to the formulas used in the SoftMax Pro Software, click

Formula Reference on the **Help** tab in the ribbon.

Enabling Syntax Helper

For assistance creating formulas in the **Formula Editor** dialog, click **Syntax Helper**. To disable the Syntax Helper, click **Syntax Helper** to clear the check box.

When you first start typing a formula, a pop-up 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.

Editing a Summary Formula

To edit an existing summary formula:

1. Click the summary formula text frame in the **Notes** section to select the text frame.

2. Click **Edit Summary** in **Note Controls** on the **Home** tab in the ribbon.

   You can also double-click the formula to open the **Formula Editor** dialog.

3. In the **Formula Editor** dialog, make the required edits. See Adding a New Summary Formula on page 79.

4. Click **OK**.

Showing or Hiding the Formula in a Summary Formula

Generally, the summary formula in the **Notes** section displays the result of the formula. You can show or hide the formula in addition to the result.

To show the formula in the summary formula:

1. Click the summary formula text frame.

2. Click **Show Formulas** in the **Note Controls** section of the **Home** tab in the ribbon.

To hide the formula in the summary formula:

1. Click the summary formula text frame.

2. Click **Hide Formulas** in the **Note Controls** section of the **Home** tab in the ribbon.

Deleting a Summary Formula

To delete an existing summary formula:

1. Click the summary formula text frame.

2. Click **Delete** in the **Note Controls** section of the **Home** tab in the ribbon.

Using a Plate Section

A **Plate** section is 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 want to have two **Plate** sections. You can create as many **Plate** sections as you need.

The precision of the data displayed in a **Plate** section varies based on the amount of space available to view the characters.

For example, for a plate with 96 wells or fewer, each well is limited to 5 digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to 4 characters, including the decimal point.

When SoftMax Pro Software exports plate data, the values are not limited by space and can have more digits for each well.

The values displayed in a **Plate** section are only a representation of the data. In both the **Plate** section and in the exported data, the last digit displayed or exported is rounded based on the actual data value.

To change the properties of the plate, such as the number of wells, click **Settings** on the **Home** tab in the ribbon to open the **Settings** dialog, and then click the **Plate Type** category to view a list of the available plate definitions. See Plate Type Settings on page 144.

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

Plate sections have the following areas:

- Tool bar (above the data display)

- Data display (in a microplate grid format)

- Instrument Settings Information and Read Information (to the right of the data display)

- Reduction settings (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.

For information about using a **Plate** section, see the following topics:

- Adding a Plate Section on page 83

- Using Plate Tools on page 83

- Using Template Tools on page 89

Adding a Plate Section

To add a **Plate** section:

1. Select the experiment where you want to add a **Plate** section in the **Navigation Tree**.

2. If you have more than one section in the Experiment, select the section above where you want the new **Plates** section to appear in the **Navigation Tree**.

3. Click **New Plate** in **Sections** on the **Home** tab in the ribbon.

A blank **Plate** section opens in the Workspace. You can add as many **Plate** sections as you need to your data file.

Using Plate Tools

When a **Plate** section is active in the Workspace, **Plate Tools** are available on the **Home** tab in the ribbon.

Many of the **Plate Tools** available in **Home** tab are also available in the toolbar at the top of the **Plate** section.

For information about using the **Plate Tools**, see the following topics:

- Modifying Instrument Settings on page 84
- Modifying Data Reduction Settings on page 84
- Modifying the Data Display on page 85
- Showing Reduced Data in a Three-Dimensional Graph on page 86
- Zooming the Well Display on page 87
- Masking Wells on page 88
- Cloning a Plate Section on page 89

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 in the ribbon.

The **Settings** dialog appears.
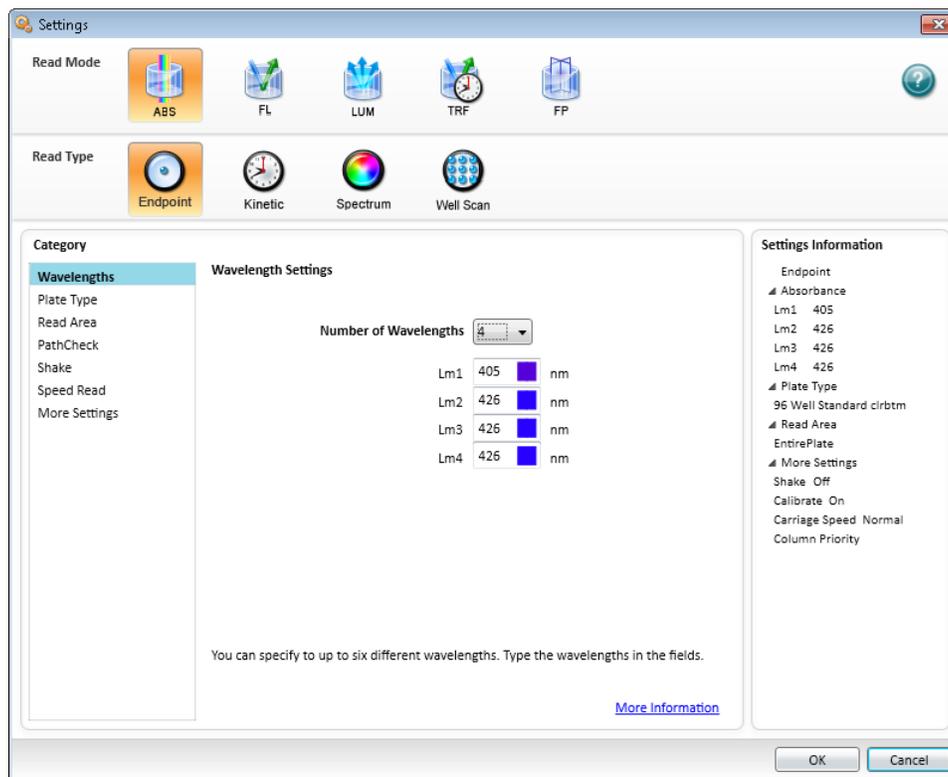
Use the options in the **Settings** dialog to define the parameters for acquiring data from a plate or cuvette. See Selecting Instrument Settings on page 160.

Modifying Data Reduction Settings

To modify the data reduction settings for a plate or cuvette, click **Reduction** on the **Home** tab in the ribbon to view the **Data Reduction** dialog.

The **Data Reduction** dialog displays only the reduction options that are available for the instrument you are using, the options that have been selected in the **Settings** dialog, and the options defined in the template.

For more information, see Performing Data Reduction on page 214.

Modifying the Data Display

At any time in an active **Plate** section or **Cuvette Set** section you can change how

the data is presented by clicking **Display** on the **Home** tab in the ribbon or in
the toolbar at the top of the section.

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

Raw Data

Selecting **Raw Data** 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 display is based on the selections made in the **Data Reduction** dialog. See Performing Data Reduction on page 214.

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

- Select **Number** to view only the reduced number.

- Select **Plot** for Kinetic reads to display a plot of the raw and reduced data. To also view the reduced number, select the **Reduced Number** check box.

- Select **Grayscale** to display 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. To define the **High Limit** and the **Low Limit** for the grayscale map, type values in the fields. To also view the reduced number, select the **Reduced Number** check box.

- Select **Color Map** to display 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. To define the **High Limit** and the **Low Limit** for the color map, type values in the fields. To also view the reduced number, select the **Reduced Number** check box.

You can view both raw and reduced data or multiple reductions of the same data by cloning a plate. See Cloning a Plate Section on page 89.

Showing Reduced 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. See Viewing Data in a Three-Dimensional Graph on page 226.
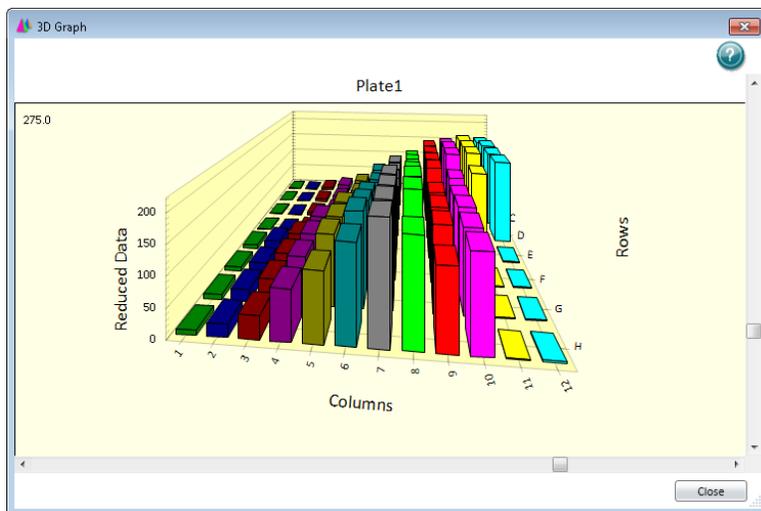
To close the **3D Graph** dialog, click **Close**.

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 Well** dialog:

1. Click the well or cuvette that you want to zoom.

2. Click **Zoom** 🔍 on the **Home** tab in the ribbon or **Zoom** 🔍 in the toolbar at the top of the section.

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

3. To print the graph window, click **Print**.

4. To scale the display 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 Performing Data Reduction on page 214.

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 and choose the minimum and maximum values for the selected axis. You can also add or remove grid lines from the display.

To save the settings and return to the **Zoom Well** dialog, click **OK**. Only the well graph being viewed is affected by these changes.

8. To close the **Zoom Well** dialog, click **Close**.

Masking Wells

The Mask feature hides selected data so that they are not used for calculations and are not reported. Masking is commonly used to suppress outliers from data reduction calculations.

Masking can be used as a "what if?" tool. For example, if you have included a group blank in a template and want to see the data both with and without the blank, masking the group blanks suppresses the blanking function, while unmasking them enables it again.

To mask wells:

1. In an active **Plate** section, click to select a single well or click and drag to select multiple wells.

2. Click **Mask** ▦ in **Plate Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the **Plate** section.

   You can also right-click a well and select **Mask** from the shortcut menu.

To unmask masked wells, select the wells and then click **Mask**.

Cloning a Plate Section

Cloning a plate causes a duplicate copy of the active plate to be added to the plate section. This can allow you to apply multiple templates and reductions to the same set of raw data.

To clone a plate:

1. With the **Plate** section you want to clone active in the workspace, click **Clone Plate** on the **Home** tab in the ribbon.

2. The cloned plate is added to the active plate section.
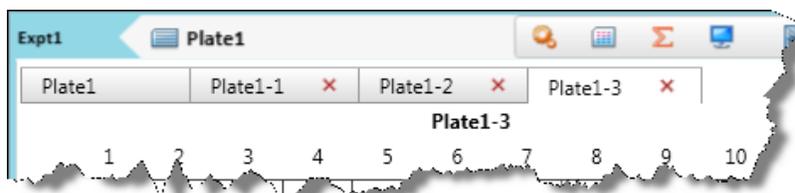
You can add as many plate clones as needed for your experiment. The cloned plates are shown in tabs at the top of the **Plate** section. The first tab on the left is the original plate. Clones are listed with the original plate name and a number corresponding to the number of the clone.

   • To make a cloned plate active in the section, click the tab for the cloned plate.

   • To delete a cloned plate, click the **X** in the tab for the cloned plate.

Using Template Tools

When a **Plate** section is active in the Workspace, **Template Tools** are available on the **Home** tab in the ribbon.

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

Each template contains 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 contains seven samples named STD01, STD02, …, STD07, and a group named "Unknowns" that contains five samples named 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 and **Cuvette Set** sections as well as within a single **Plate** section or **Cuvette Set** 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 data file.

For information about using the **Template Tools**, see Configuring a Microplate Template on page 162.

Using a Cuvette Set Section

Cuvette Set sections are used to collect data from the cuvette port of the SpectraMax M2 and M2e Multi-Mode Microplate Readers, SpectraMax M5 and M5e Multi-Mode Microplate Readers, and the SpectraMax Plus 384 Absorbance Microplate Reader. You can use a **Cuvette Set** section 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.

You can add up to 96 cuvettes in a single **Cuvette Set** section or create as many **Cuvette Set** sections as you need. Cuvettes are read one at a time.

Cuvette Set sections have the following areas:

- Tool bar (above the data display)

- Cuvette data display

- Instrument Settings Information and Read Information (to the right of the data display)

- Reduction settings (below 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.

For information about using a **Cuvette Set** section, see the following topics:

- Adding a Cuvette Set Section on page 91

- Using Cuvette Tools on page 91

- Using the Template Tool with a Cuvette Set on page 99

Adding a Cuvette Set Section

To add a new **Cuvette Set** section:

1. Select the experiment where you want to add a **Cuvette Set** section in the **Navigation Tree**.

2. If you have more than one section in the Experiment, select the section above where you want the new **Cuvette Set** section to appear in the **Navigation Tree**.

3. Click **New Cuvette Set** in **Sections** on the **Home** tab in the ribbon.

A new **Cuvette Set** section opens in the Workspace. You can add as many **Cuvette Set** sections as you need to your data file. Each **Cuvette Set** section can have up to 96 cuvettes defined in the section.

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

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:

- Modifying Instrument Settings for Cuvettes on page 92
- Modifying Data Reduction Settings for Cuvettes on page 92
- Modifying the Cuvette Data Display on page 93
- Zooming the Cuvette Display on page 95
- Masking Cuvettes on page 96
- Adding a Cuvette to a Cuvette Set on page 97
- Deleting a Cuvette from a Cuvette Set on page 97
- Copying a Cuvette Set Section on page 97
- Copying and Pasting Cuvette Data on page 98

Modifying Instrument Settings for Cuvettes

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

To change the instrument settings:

1. Click the **Cuvette Set** section to make it active in the workspace.

2. Click **Settings** in the **Cuvette Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the **Cuvette Set** section. The **Settings** dialog opens.

3. Set the instrument settings options as needed. See Selecting Instrument Settings on page 160.

4. Click **OK**.

Modifying Data Reduction Settings for Cuvettes

To change the data reduction settings:

1. Click **Reduction** in the **Cuvette Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the **Cuvette Set** section. The **Data Reduction** dialog opens.

   The **Data Reduction** dialog displays only the reduction options that are available for the instrument you are using, the options that have been selected in the **Settings** dialog, and the options defined in the template.

2. Change the reduction settings as needed. See Performing Data Reduction on page 214.

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 Cuvette Data Display

At any time in an active **Plate** section or **Cuvette Set** section you can change how

the data is presented by clicking **Display** on the **Home** tab in the ribbon or in the toolbar at the top of the section.

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

Raw Data

Selecting **Raw Data** 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 display is based on the selections made in the **Data Reduction** dialog. See Performing Data Reduction on page 214.

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

- Select **Number** to view only the reduced number.

- Select **Plot** for Kinetic reads to display a plot of the raw and reduced data. To also view the reduced number, select the **Reduced Number** check box.

- Select **Grayscale** to display 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. To define the **High Limit** and the **Low Limit** for the grayscale map, type values in the fields. To also view the reduced number, select the **Reduced Number** check box.

- Select **Color Map** to display 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. To define the **High Limit** and the **Low Limit** for the color map, type values in the fields. To also view the reduced number, select the **Reduced Number** check box.

You can view both raw and reduced data or multiple reductions of the same data by cloning a plate. See Cloning a Plate Section on page 89.

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

Zooming the Cuvette Display

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

To display the **Zoom Well** dialog:

1. Click the well or cuvette that you want to zoom.

2. Click **Zoom** 🔍 on the **Home** tab in the ribbon or **Zoom** 🔍 in the toolbar at the top of the section.

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

3. To print the graph window, click **Print**.

4. To scale the display 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 Performing Data Reduction on page 214.

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 and choose the minimum and maximum values for the selected axis. You can also add or remove grid lines from the display.

To save the settings and return to the **Zoom Well** dialog, click **OK**. Only the well graph being viewed is affected by these changes.

8. To close the **Zoom Well** dialog, click **Close**.

Masking Cuvettes

The Mask feature hides selected data so that they are not used for calculations and are not reported. Masking is commonly used to suppress outliers from data reduction calculations.

Masking can be used as a "what if?" tool. For example, if you have included a group blank in a template and want to see the data both with and without the blank, masking the group blanks suppresses the blanking function, while unmasking them enables it again.

To mask cuvettes:

1. In an active **Cuvette Set** section, click to select a single cuvette or hold down the **Ctrl** key and click to select multiple cuvettes.

2. Click **Mask** ▦ in **Cuvette Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the **Cuvette Set** section.

   You can also right-click a cuvette and select **Mask** from the shortcut menu.

To unmask masked cuvettes, select the cuvettes and then click **Mask**.

Adding a Cuvette to a Cuvette Set

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

1. Click on a **Cuvette Set** section to make it active in the workspace.

2. Click **New** in **Cuvette Tools** on the **Home** tab in the ribbon.

Up to 96 cuvettes can be added to one **Cuvette Set** section.

The cuvettes in a **Cuvette Set** are numbered as if they were wells in a microplate. The first 12 cuvettes are numbered A1 through A12, and then the next new cuvette is numbered B1. This representation provides easy access to the data analysis capabilities of the SoftMax Pro Software.

You can also add cuvettes with the Template Editor. See Using the Template Tool with a Cuvette Set on page 99.

Deleting a Cuvette from a Cuvette Set

To delete a cuvette from a **Cuvette Set** section:

1. In an active **Cuvette Set** section, click to select a single cuvette or hold down the **Ctrl** key and click to select multiple cuvettes.

2. Click **Delete** in **Cuvette Tools** on the **Home** tab in the ribbon.

Copying a Cuvette Set Section

To make a duplicate copy of a **Cuvette Set** section:

1. Click the **Cuvette Set** section you want to duplicate to make it active in the workspace.

2. Click **New Cuvette Set** in **Sections** on the **Home** tab in the ribbon.

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 Navigation Tree.

A new **Cuvette Set** section opens in the Workspace that is a duplicate copy of the active **Cuvette Set** section.

You can add as many **Cuvette Set** sections as you need to your data file. Each **Cuvette Set** section can have up to 96 cuvettes defined in the section.

Copying and Pasting Cuvette Data

You can copy cuvette data from one or more cuvettes in a **Cuvette Set** section to a cuvette or cuvettes in the same or another **Cuvette Set** section. Data can be copied and pasted within the same data file or between different data files.

If you want to copy all the cuvette data from one **Cuvette Set** section to all the cuvettes in another **Cuvette Set** section in the same file, it might be easier to make a duplicate copy of the **Cuvette Set** section. See Copying a Cuvette Set Section on page 97.

To copy cuvette data:

1. In an active **Cuvette Set** section, click to select a single cuvette or hold down the **Ctrl** key and click to select multiple cuvettes.

2. Right-click and select **Copy Cuvette Data** from the shortcut menu.

3. The data is copied into the clipboard.

To paste cuvette data that is stored in the clipboard:

1. Click the cuvette that the data should be pasted into.

2. If you copied data from multiple cuvettes, click the first cuvette where you want to paste the data. SoftMax Pro Software pastes cuvette data into consecutive cuvettes. For example, if you copied data from three cuvettes and then click cuvette A6 to paste the data, the data will be pasted into cuvettes A6, A7, and A8.

3. Right-click and select **Paste Cuvette Data** from the shortcut menu.

After data is pasted into a cuvette, the **Read Information** on the right indicates that the data was pasted and shows the time and date of when it was pasted.

Using the Template Tool with a Cuvette Set

When a **Cuvette Set** section is active in the Workspace, the **Template Tool** is available on the **Home** tab in the ribbon.

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

Each template contains 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 contains seven samples named STD01, STD02, …, STD07, and a group named "Unknowns" that contains five samples named 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 and **Cuvette Set** sections as well as within a single **Plate** section or **Cuvette Set** 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 data file.

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

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 microplate. The cuvettes 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 SoftMax Pro Software.

For information about using the **Template Tools** with a **Cuvette Set** section, see .

Using a Group Section

Unlike other section types, **Group** sections are created automatically when you create and assign a **Group** to a **Plate** section or **Cuvette Set** section from within the **Template Editor** dialog. See Configuring a Microplate Template on page 162.

Reduced numbers from the **Plate** section or **Cuvette Set** section appear 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*.

Reusing Formulas

Formulas are used in **Group** columns and custom reductions. Sometimes it is useful to copy an existing formula or 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.

Formulas in contiguous column formulas can be copied and then pasted as a unit.

When a **Group** section is active in the workspace **Column Tools**, **Summary Tools**, and **Formatting Tools** appear on the **Home** tab in the ribbon.

For information about using a **Group** section, see the following topics:

- Using the Group Settings Dialog on page 100
- Using Column Tools on page 104
- Using Summary Tools on page 108
- Using Formatting Tools in a Group Section on page 110

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.

For information about the column formats, see Group Table Column Format on page 103.

Defining a Group

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Template Editor** ⊞ in **Template Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the active section. This opens the **Template Editor**.

   The **Template Editor** contains a representation of the rows and columns of the microplate in the active **Plate** 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 microplate. The cuvettes 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 SoftMax Pro Software.

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

4. In the **Groups** area, select an existing group and click **Edit** or click **Add** to open the **Group Settings** dialog.

5. Type the name to assign to the group in the **Group Name** field.

6. If you want to set a color for the group, click **Set Color** and select the color to be displayed for the group.

7. In the **Group Table Layout** area, click **Standards**, **Unknowns**, or **Custom** to specify the column format.

   For information about the column formats, see Group Table Column Format on page 103.

   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.

8. In the **Add Columns** area, if it is not already selected, click **Sample Descriptor1** to apply a descriptor and its units to the group.

9. Type a short descriptive name for the sample descriptor in the **Column Name** field.

   > **Note:** If you plan to reference the name of this column in a formula, make sure that you do not use reserved characters or names of operators, functions, or accessors in this name. For information about reserved characters and words, see "Rules to Follow When Writing Formulas" in the *SoftMax Pro Software Formula Reference Guide*.

10. Select a set of units from the list or type them directly in the **Units** field.

11. Optionally, click **Sample Descriptor2** to define a second sample descriptor.

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

12. Click **OK** to close the **Group Settings** dialog.

    The selected wells are placed in the newly defined group.

13. Click **OK** to close the **Template Editor** and apply the template.

    > **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 on page 104.

Group Table Column Format

The type of Group Table Layout 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, Unknown, and Custom), creates a different set of columns in a new or existing group. The following table shows the default columns created with each selection type.

Table 4-1: Group Type Column Format

Name	Formula	Standard	Unknown	Custom
Sample	!SampleNames	X	X	X
Wells	!WellIDs	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

Using Column Tools

When a column or data is selected in the group table, 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 shortcut menu.

For information about using the **Column Tools** on the **Home** tab, see the following topics:

- Adding a New Column on page 104
- Modifying a Column on page 105
- Copying Column Contents on page 106
- Pasting Column Contents on page 106
- Cutting Column Content on page 106
- Deleting a Column on page 107
- Autosizing a Column on page 107
- Hiding Columns on page 107
- Showing Hidden Columns on page 108
- Hiding Replicates on page 108

Adding a New Column

To add a new column to a group:

1. Click a **Group** section to make it active in the workspace.

2. Click **New** in **Column Tools** on the **Home** tab in the ribbon.

3. In the **Formula Editor** dialog, type a short descriptive name for the summary formula in the **Name** field.

    > **Note:** If you plan to reference this formula in another formula, for example referencing the formula in a column of a group table, make sure that you do not use reserved characters or names of operators, functions, or accessors in this name. For information about reserved characters and words, see "Rules to Follow When Writing Formulas" in the *SoftMax Pro Software Formula Reference Guide*.

4. Optionally, click **Hide Name** to hide the name of the Summary in the **Notes** section or **Group** section.

5. Optionally, type a brief text **Description** of the formula.

6. Optionally, click **Syntax Helper** to turn on the formula completion tool. See Enabling Syntax Helper on page 80.

7. Type a **Formula** to be used for the Summary or column.

8. Optionally, click **Check Syntax** check the validity of the formula. A message appears to the right of the button to help you troubleshoot the syntax of the formula.

9. Specify the **Data Display** characteristics:

   • For **Precision**, click **Decimal Places** and type the number of places to display or click **Significant Figures** and type the number of figures to display.

   • For **Format**, click either **Numeric Notation** or **Scientific Notation**.

   A preview of the **Data Display** appears in the **Sample** on the right.

10. Click **OK**.

    • If no columns are selected, the column is added at the end of the group on the right.

    • If you select a column first, the new column is added to the right of the selected 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. For a complete discussion, see the **SoftMax Pro Formula Reference Guide** help.

Modifying a Column

To modify a column formula:

1. Click a **Group** section to make it active in the workspace.

2. To modify a column formula, double click the column heading to open the **Formula Editor** dialog. See Adding a New Summary Formula on page 79.

3. To resize the column width, click and hold the column border and then drag it to the desired column width.

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 **Column Tools**.

To copy content to the clipboard:

1. Click a **Group** section to make it active in the workspace.

2. Select the content to be copied.

3. Click **Copy** in **Column Tools** on the **Home** tab in the ribbon.

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 a **Group** section to make it active in the workspace.

2. Click to select the target column location.

3. Click **Paste** in **Column Tools** on the **Home** tab in the ribbon.

> **Note:** If the Paste button is disabled when you attempt this operation, there is no appropriate content present in the clipboard. You must first copy content to the clipboard.

Cutting Column Content

To cut content out of a column:

1. Click a **Group** section to make it active in the workspace.

2. Select the content to be removed.

3. Click **Cut** in **Column Tools** on the **Home** tab in the ribbon.

> **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.

Deleting a Column

To delete a column:

1. Click a **Group** section to make it active in the workspace.

2. Click a column title to select the column.

   To select multiple columns, hold down the **Shift** key and click to select a range of columns or hold down the **Ctrl** key and click to select non-contiguous columns.

3. Click **Delete** in **Column Tools** on the **Home** tab in the ribbon, or right-click and select **Delete Column** from the shortcut menu.

Autosizing a Column

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

1. Click a **Group** section to make it active in the workspace.

2. Click a column title to select the column.

   To select multiple columns, hold down the **Shift** key and click to select a range of columns or hold down the **Ctrl** key and click to select non-contiguous columns.

3. Click **Auto Size** in **Column Tools** on the **Home** tab in the ribbon, or right-click and select **Auto Size** from the shortcut menu.

Hiding Columns

To hide one or more columns:

1. Click a **Group** section to make it active in the workspace.

2. Click **Show/Hide** in **Column Tools** on the **Home** tab in the ribbon or above the right side of the group table.

3. In the **Show Hide group table columns** dialog, clear the check boxes next to the columns that you want to hide.

4. Click **OK**.

Showing Hidden Columns

To show hidden columns:

1. Click a **Group** section to make it active in the workspace.

2. Click **Show/Hide** in **Column Tools** on the **Home** tab in the ribbon or above the right side of the group table.

3. In the **Show Hide group table columns** dialog, select the check boxes next to the columns that you want to show.

4. Click **OK**.

Hiding Replicates

To hide data for all replicates:

1. Click a **Group** section to make it active in the workspace.

2. Click **Hide Replicates** in the **Column Tools** on the **Home** tab in the ribbon.

Using Summary Tools

When a **Group** section is active in the workspace, the **Home** tab in the ribbon displays the **Summary Tools**.

For information about using the **Summary Tools**, see the following topics:

Copying and Pasting a Formula

To copy a formula and paste it into a new summary:

1. Click a **Group** section to make it active in the workspace.

2. Select the Summary formula to be copied.

3. Click **Copy** in **Summary Tools** on the **Home** tab in the ribbon.

4. Click the location where you want to paste the copy of the Summary formula.

5. Click **Paste** in **Summary Tools** on the **Home** tab in the ribbon.

Deleting a Formula

To delete a formula:

1. Click 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** on the **Home** tab in the ribbon.

Adding a New Formula

To add a new formula to a Group section:

1. Click on a **Group** section to make it active in the workspace.

2. Click **New Summary** 🗗 in **Summary Tools** on the **Home** tab in the ribbon to open the **Formula Editor** dialog. For more information, see Adding a New Summary Formula on page 79.

3. Type the formula to be added and click **OK**.

Editing a Formula

To edit a formula in a Group section:

1. Click a **Group** section to make it active in the workspace.

2. Click the formula to be changed to select it.

3. Click **Edit Summary** 🗗 in **Summary Tools** on the **Home** tab in the ribbon to open the **Formula Editor** dialog. For more information, see Adding a New Summary Formula on page 79.

4. Type the changes to formula and click **OK**.

Showing and Hiding the Formulas in a Group Section

Generally, the summary formulas in the **Group** section display the results of the formulas. You can show or hide the formulas in addition to the results.

To show the formulas in the Group section:

1. Click a **Group** section to make it active in the workspace.

2. Click **Show Formulas** 🗗 in **Summary Controls** on the **Home** tab in the ribbon.

To hide the formula in the Group section:

1. Click a **Group** section to make it active in the workspace.

2. Click **Hide Formulas** 🗗 in **Summary Controls** on the **Home** tab in the ribbon.

Using Formatting Tools in a Group Section

When a **Group** section is active in the workspace, the **Home** tab in the ribbon displays the Formatting Tools.

You can format the text in the summaries and text frames in the **Group** section using the available **Formatting Tools**. Formatting text in a **Group** section is similar to formatting text in a **Notes** section. See the following topics:

- Adding Text to a Notes Section on page 75

- Aligning Text on page 75

- Editing Text in a Text Frame on page 76

- Formatting Text in a Text Frame on page 77

- Positioning a Text Frame in a Notes Section on page 78

- Resizing a Text Frame on page 78

- Deleting a Text Frame on page 78

Using a Graph Section

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

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

After 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. The grid lines for the graph can be enabled or disabled. The default setting has grid lines enabled.

Graph sections are divided into the following areas:

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

- The Legend.

For more information see Working with Graphs on page 234.

Chapter 5: Instrument and Detection Settings

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 the SoftMax Pro Software can further enhance any specialized data collection and analysis needs through custom assay development.

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 instrument collects data over time with multiple readings taken at regular intervals. To achieve the shortest possible interval for Kinetic readings, choose wavelengths in ascending order.

The values calculated based on raw kinetic data are VMax, VMax per Sec, Time to VMax, and Onset Time. Kinetic readings can be single-wavelength or multiple-wavelength readings.

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

A Well Scan read can take readings 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.

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.

The options available for Endpoint reads are available for Well Scan reads.

Supported Read Modes

SoftMax Pro Software supports the following Read Modes:

- ABS Read Mode on page 113
- FL Read Mode on page 114
- LUM Read Mode on page 114
- TRF Read Mode on page 115
- FP Read Mode on page 116
- AlphaScreen Read Mode on page 117
- FRET Read Mode on page 118

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. To measure absorbance accurately, it is necessary to eliminate light scatter. In the absence of turbidity, absorbance = optical density.

$$A = \log_{10}(I_0 / I) = -\log_{10}(I/I_0)$$

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.

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.

For Absorbance reads, you can choose whether to display absorbance data as Optical Density or %Transmittance in the Reduction dialog.

%Transmittance is the ratio of transmitted light to the incident light for absorbance reads.

$$T = I/I_0$$

$$\%T = 100T$$

where I is the intensity of light after it passes through the sample and I_0 is incident light before it enters the sample.

Optical Density and %Transmittance are related by the following formulas:

$$\%T = 10^{2-OD}$$

$$OD = 2 - \log_{10}(\%T)$$

The factor of two comes from the fact that %T is expressed as a percent of the transmitted light and $\log_{10}(100) = 2$.

When in %Transmittance analysis mode, the SoftMax Pro Software converts the raw OD values reported by the instrument to %Transmittance using the above formula. All subsequent calculations are performed on the converted numbers.

For information about setting up an Absorbance mode protocol, see Creating an Absorbance Mode Protocol on page 174.

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 measures 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 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.

For information about setting up a Fluorescence Intensity mode protocol, see Creating a Fluorescence Intensity Mode Protocol on page 176.

LUM Read Mode

In the Luminescence (LUM) read mode provides measurements in Relative Luminescence Units (RLUs).

Luminescence 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.

For information about setting up a Luminescence mode protocol, see Creating a Luminescence Mode Protocol on page 184.

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.

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 FL detection. 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.

The use of long-lived fluorophores combined with time-resolved detection (a delay between excitation and emission detection) minimizes interference from fluorescence excitation light. 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 Multi-Mode Microplate Reader and the SpectraMax Paradigm Multi-Mode Detection Platform with the Cisbio HTRF Detection Cartridge.

For information about setting up a TRF mode protocol, see Creating a Time-Resolved Fluorescence Mode Protocol on page 186.

FP Read Mode

Fluorescence Polarization (FP) mode measures the relative change of polarization of emitted fluorescent compared to excitation light.

Fluorescence polarization detection is similar to fluorescence intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Plate readers measure FP of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation.

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.

When molecules are excited with polarized light, the polarization of the emitted light depends on the size of the molecule to which the fluorophore is bound. Larger molecules emit a higher percentage of polarized light, while smaller molecules emit a lower percentage of polarized light because of their rapid molecular movement. For this reason FP is typically used for molecular binding assays in HTS.

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 either of these data types on the **Plate** section by selecting **Reduced Data** in the **Display** dialog and then selecting the data type in the **Data Reduction** dialog. When Raw (S&P) is displayed, the mP value is by default used for all reduction calculations. See Setting the Data Display Options on page 210 and Performing Data Reduction on page 214.

When exporting, you can export either mP or r as reduced data.

Polarization (mP) is calculated as follows:

$$mP = 1000 * \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (G * \text{perpendicular}))}$$

Anisotropy (r) is calculated as follows:

$$r = \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (2G * \text{perpendicular}))}$$

G factor, or 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. See Performing Data Reduction on page 214.

For information about setting up a Fluorescence Polarization mode protocol, see Creating a Fluorescence Polarization Mode Protocol on page 188.

AlphaScreen Read 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 Multi-Mode Detection Platform with an AlphaScreen Detection Cartridge.

For information about setting up an AlphaScreen mode protocol, see Creating an AlphaScreen Mode Protocol on page 190.

FRET Read 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 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).

For information about setting up a FRET mode protocol, see Creating a FRET Mode Protocol on page 192.

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 6.2.2 > Hardware User Guides**.

SoftMax Pro Software supports the following instruments:

- VersaMax ELISA Microplate Reader, see page 120
- SpectraMax Plus 384 Absorbance Microplate Reader, see page 120
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 121
- SpectraMax M3 Multi-Mode Microplate Reader, see page 122
- SpectraMax M4 Multi-Mode Microplate Reader, see page 123
- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 124
- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 124
- SpectraMax 190 Absorbance Microplate Reader, see page 125
- Gemini XPS Fluorescence Microplate Reader, see page 125
- Gemini EM Fluorescence Microplate Reader, see page 126
- FilterMax F5 Multi-Mode Microplate Reader, see page 127
- FilterMax F3 Multi-Mode Microplate Reader, see page 128
- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 128
- Vmax Kinetic ELISA Microplate Reader, see page 129
- Emax Endpoint ELISA Microplate Reader, see page 129
- SpectraMax Paradigm Multi-Mode Detection Platform, see page 130
- StakMax Microplate Handling System, see page 141

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 113.

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 113.

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 113.

- Fluorescence Intensity (FL), see FL Read Mode on page 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

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 113.

- Fluorescence Intensity (FL), see FL Read Mode on page 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

Features of the SpectraMax M3 Multi-Mode Microplate Reader include:

- Three-mode cuvette port for assay development

- Dual monochromator tunability

- Automated Absorbance Pathlength Correction with PathCheck Pathlength Measurement Technology

- 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 113.

- Fluorescence Intensity (FL), see FL Read Mode on page 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

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, spectrum, and well scan 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 113.

- Fluorescence Intensity (FL), see FL Read Mode on page 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

- Fluorescence Polarization (FP), see FP Read Mode on page 116.

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 113.

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 113.

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 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

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 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

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 113.

- Fluorescence Intensity (FL), see FL Read Mode on page 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

- Fluorescence Polarization (FP), see FP Read Mode on page 116.

- Fluorescence Resonance Energy Transfer (FRET), see FRET Read Mode on page 118.

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 read height (Z-height) optimization for 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 262.

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 113.

- Fluorescence Intensity (FL), see FL Read Mode on page 114.

- Luminescence (LUM), see LUM Read Mode on page 114.

- Fluorescence Resonance Energy Transfer (FRET), see FRET Read Mode on page 118.

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 262.

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 128.

- For information about the DTX 880 Multi-Mode Microplate Reader, see FilterMax F5 Multi-Mode Microplate Reader on page 127.

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 113.

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 113.

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 131.

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

- Array of detection cartridge capabilities
- Automatic read height (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, see page 132
- Tunable Wavelength (TUNE) Detection Cartridge, see page 132
- Multi-Mode (MULTI) Detection Cartridge, see page 133
- AlphaScreen Detection Cartridges , see page 134
- Cisbio HTRF Detection Cartridge on page 135
- Time Resolved Fluorescence (TRF) Detection Cartridge, see page 136
- Fluorescence Intensity (FI) Detection Cartridges, see page 137
- Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge, see page 138
- Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge, see page 138
- Fluorescence Polarization (FP) Detection Cartridge, see page 139
- Glow Luminescence (LUM) Detection Cartridges, see page 140
- Dual Color Luminescence (LUM) (BRET2) Detection Cartridge, see page 140
- Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge, see page 140

For complete descriptions and specifications for each of the supported detection cartridges, see the *SpectraMax Paradigm Multi-Mode Detection Platform User Guide*.

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 (ABS-DET) which measures the absorbance, and an excitation component (ABS-MONO) 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), see FL Read Mode on page 114.

- Glow Luminescence (LUM), see LUM Read Mode on page 114.

- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

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. See Spectral Optimization on page 202.

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), see FL Read Mode on page 114.
- Glow Luminescence (LUM), see LUM Read Mode on page 114.
- Time-Resolved Fluorescence (TRF), see TRF Read Mode on page 115.

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

The SpectraMax® Paradigm® AlphaScreen® Detection Cartridges use 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.

The following AlphaScreen Detection Cartridges are available:

- AlphaScreen 384 Std Detection Cartridge

- AlphaScreen 384 HTS Detection Cartridge

- AlphaScreen 1536 HTS Detection Cartridge

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 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.

The following Fluorescence Intensity (FI) Detection Cartridges are available:

- Fluorescence Intensity (FI) (coum-fluor) Detection Cartridge
- Fluorescence Intensity (FI) (fluor-rhod) Detection Cartridge
- Fluorescence Intensity (FI) (Cy3-Cy5) Detection Cartridge
- Fluorescence Intensity (FI) (CFP-YFP) Detection Cartridge

Other Fluorescence Intensity (FI) Detection Cartridges include the Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge on page 138 and the Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge on page 138.

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 GeneBLAzer assays from Invitrogen. 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 MultiTox-Fluor assays from Promega. 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 Multi-Mode Detection Platform 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: 96-well, 384-well, or 1536-well.

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 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.

The 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.

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 **Plate Stacker**, the SoftMax Pro Software is place in automation mode, and then 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 56.

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.

For information about the SoftMax Pro Software automation mode, see Using Automation Mode on page 42.

Instrument Settings

Each **Plate** section or **Cuvette Set** section in a file or experiment must use the same instrument, but can have different parameters for the instrument settings.

Use the **Settings** dialog to configure all parameters for a microplate or cuvette read, such as the read type (Endpoint, Kinetic, Spectrum, Well Scan), the read mode (Absorbance, Fluorescence, Luminescence, Time Resolved Fluorescence, Fluorescence Polarization, ALPHAScreen, FRET), wavelength settings, microplate type, wells to read, PathCheck technology, optics settings, shake parameters, timing for Kinetic runs, speed read, and more.

To open the **Settings** dialog:

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Settings** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

The available options in the **Settings** dialog depend on the instrument that is selected. For information about the available settings, see the following topics:

- Wavelength Settings on page 143
- Plate Type Settings on page 144
- Read Area Settings on page 147
- PathCheck Pathlength Measurement Technology on page 148
- TRF Settings on page 152
- PMT and Optics Settings on page 152
- Timing Settings on page 153
- Well Scan Settings on page 154
- Shake Settings on page 155
- Speed Read on page 155
- More Settings on page 156

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, Kinetic, or Well Scan Reads

Specify the number of wavelengths to read, and then type a wavelength value within the available range for the instrument in the field for each wavelength.

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

For Spectrum Reads

Choose a **Start** and a **Stop** wavelength value within the available range for the instrument, and choose a wavelength **Step** for each increment between reads.

The minimum selectable **Step** increment is 1 nm.

Cutoffs

The choices in this portion of the Wavelengths setting depend on 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 a Luminescence read mode is selected. With other read modes, 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 on the wavelengths chosen for reading. When a Spectrum read type is selected, 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 on the known value of the Stokes shift, which 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 about cutoff filter settings.

Plate Type Settings

The Plate Type settings determines the display of the wells in the **Plate** section. Set it to match the microplate type, the number of wells, the clarity or opaqueness of the wells, and the round or square shape of the wells in the microplate to be read.

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 145.

When using a SpectraMax Paradigm Multi-Mode Detection Platform, you can define the plate orientation.

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.

Changing the Plate Type within a **Plate** section causes the 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.

Using the Plate Editor

Use the Plate Editor to modify the definition of an existing microplate, or to add a new microplate definition to the Plate Library.

To view the Plate Editor:

1. Click a **Plate** section to make it active in the workspace.

2. Click **Settings** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. In the **Settings** dialog, click the **Plate Type** category.

4. In the **Plate Type Settings**, click **Edit Plate**.

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

> **Note:** SoftMax Pro Software comes with plate definitions for the µMax 24-well low-volume microplates and µMax 64-well low-volume microplates.

Modifying the Dimension of an Existing Plate

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

1. In the **Plate Type Settings**, click the plate in the list and then click **Edit Plate**.

2. Type 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. In the **Plate Type Settings**, click any plate in the plate list then and click **Edit Plate**.

   Defining a new plate can be easier if you select a similar plate from the list before opening the Plate editor.

2. In the **Plate Editor**, in the **Plate Name** field, type a name for the new plate definition.

3. Type the dimensions of the new plate as needed.

4. Click **Save**.

The new plate name appears in the plate list.

Table 5-1: Plate Editor Tools

Field Name	Image	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 a round well or the width of a square well.
Well Shape Square	Check box	The wells have a square shape, rather than round. This setting is available only with the SpectraMax Paradigm Multi-Mode Detection Platform and the FilterMax F3 and F5 Multi-Mode Microplate Readers.
Well Bottom Type Clear well bottom	Check box	The wells have a clear bottom, rather than opaque.
Plate Height		The height of the microplate used to determine the read height for top reads. This setting is available only with the SpectraMax Paradigm Multi-Mode Detection Platform and the FilterMax F3 and F5 Multi-Mode Microplate Readers.

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.

Microplate definitions are saved in the **CustomPlates.plt** file in the **Molecular Devices** folder. A custom microplate definition is specific to the computer on which it is saved, when a protocol that has a custom microplate selected is opened on another computer, the SoftMax Pro Software gives an option to save the custom microplate definition on the new computer

Importing a Plate Definition

For the SpectraMax Paradigm Multi-Mode Detection Platform, you can import a previously saved microplate definition.

1. In the **Plate Type Settings**, 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)** file type.

4. Select the plate definition file.

5. Click **Open** to add the plate definition to the list.

Read Area Settings

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.

You can choose to read an entire plate or a subset of wells. Drag the cursor to select the wells to be read.
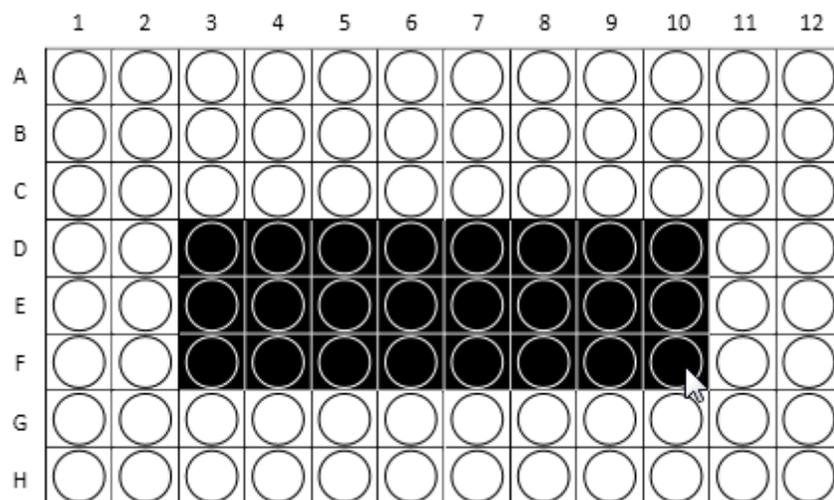
You can select a contiguous, rectangular region on the plate. Columns do not need to start with "1" but must be contiguous.

To read all the wells in the plate, select the **Select All** check box.

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, however, are present in the Template Editor. See Configuring a Microplate Template on page 162.

PathCheck Pathlength Measurement Technology

You can enable PathCheck technology in the **Settings** dialog by selecting the **PathCheck** check box in the **PathCheck Settings** for instruments that support this technology.

The PathCheck® Pathlength Measurement Technology 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 absorptivity 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.
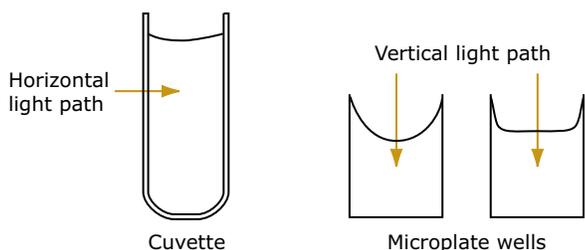
Figure 5-1: Cuvette and Microplate Well Light Paths

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.

Water Constant

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.

Cuvette Reference

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 151. 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*.

> **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.

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 technology calculation to get valid results that have been normalized by the PathCheck technology. There are two ways to accomplish this, described below, beginning with the easiest.

Use a Plate Blank

This method can be used only if all samples in the microplate are the same volume and you are not depending on the PathCheck technology 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 169).

2. Pipette buffer (for example, your sample matrix) into those wells and read along with your samples. SoftMax Pro 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. See Performing Data Reduction on page 214.

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 that 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 technology normalized values that are lower than 1 cm cuvette values. Omitting the Background Constant results in values that have been normalized by the PathCheck technology and are higher than 1 cm cuvette values.

Interfering Substances

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

Turbidity is the most common interference. If you can detect any turbidity in your sample, you should not use the PathCheck 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) spectrum and can interfere with the PathCheck 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 technology.

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 technology. Use of Cuvette Reference on supported 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 technology 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 nm and 1000 nm, the interference would be similar to the interference of highly colored samples as previously described. 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 technology.

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. See TRF Read Mode on page 115.

PMT and Optics Settings

The sensitivity settings can be set in **PMT and Optics Settings** in the **Settings** dialog.

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, the **Automatic** setting allows 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 Multi-Mode Microplate Readers, the PMT Sensitivity is always set to **Automatic** for Luminescence (LUM) and Time Resolved Fluorescence (TRF) read modes.

For the SpectraMax Paradigm Multi-Mode Detection Platform, you can select **On the Fly Detection** options.

On the Fly Detection, when enabled, yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to when it is disabled (**Off-stop and go**) and the plate stops moving for each read.

To use On the Fly Detection, select an option to specify whether the detection method should be optimized for **Performance** or **Speed**.

- 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.

For the SpectraMax Paradigm and FilterMax instruments, you can set the **Integration Time**. Type a value in the **Integration Time** field in milliseconds, to specify the measurement time per well to be validated.

Select the **Read From Bottom** check box to have an instrument with bottom-read capability read up through the bottom of the microplate rather than down from the top.

For the SpectraMax Paradigm Multi-Mode Detection Platform and the FilterMax F5 Multi-Mode Microplate Reader, you can specify the **Read Height**.

Read Height specifies the distance in millimeters between the objective lens and the microplate. You can type a value for the read height in this field.

To have SoftMax Pro Software find the optimized read height before the read, use the **Read Height Optimization** feature in the **Pre-Read Optimization** dialog by selecting the **Show Pre-Read Optimization Options** check box in the **More Settings** tab.

Read Height is available for the FilterMax F5 Multi-Mode Microplate Reader for top-read only.

Timing Settings

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

Type a **Total Run Time** and the **Interval** between readings in **hh:mm:ss** format.

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

The minimum allowable interval values are shown below the **Interval** field.

The **Number of Reads** is calculated based on the values in the **Total Run Time** and **Interval** fields.

The minimum allowable 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. Maximum read intervals also vary by instrument, for example the SpectraMax M2 Multi-Mode Microplate Reader has a maximum read interval of 2 hours and 45 minutes, and the SpectraMax Paradigm Multi-Mode Detection Platform has a maximum read interval of 18 hours and 12 minutes. If you type a non-allowed read interval, a message appears defining the minimum or maximum allowable value for the read interval.

Well Scan Settings

A Well Scan read can take readings 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.

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.

The options available for Endpoint reads are also available for Well Scan reads.

Well Scan reads allow readings to be taken at more than one location within a well. Well Scan settings allow you to specify how a Well Scan is performed.

You can specify the scanning pattern and the scanning density.

For some instruments, only odd numbers of points are included in density settings.

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

Up to four scanning patterns are available, depending on the selected instrument:

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

The fill pattern can be either round or square to match the shape of the well. The image in the Well Scan settings shows the shape of the well as defined for the selected microplate. See Plate Type Settings on page 144.

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.

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 and the instrument.

Before First Read

This setting can be used with any read type. Whether you read at a single wavelength or at multiple wavelengths, selecting the 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 a Kinetic read type 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 Multi-Mode Detection Platform 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. The 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.

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.

More Settings

The **More Settings** tab in the **Settings** dialog provides additional settings options. The options in this tab vary depending on the selected instrument, read mode, and read type.

Calibrate

For Absorbance reads, the Calibrate setting tells the instrument to make an air calibration measurement at each wavelength before reading the microplate, as determined by the type of instrument and the read settings. For kinetic readings this measurement is sometimes taken between readings during a run.

For Fluorescence instruments, the measurement occurs before reads, and sometimes between kinetic reads if the interval is long enough.

If you turn calibration off, SoftMax Pro Software 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.

These new calibrations overwrite the calibration setting stored in firmware for the current data acquisition read only. When the instrument is powered off and on, the calibrations revert to those stored in firmware. The calibration values stored in the firmware can be updated using the **Calibration** button on the **Operations** tab in the application ribbon.

Carriage Speed

Carriage Speed can be slowed down to accommodate samples (such as, fragile clots) that could be disrupted by rapid carriage movement. Select a **Carriage Speed** of Normal or Slow.

Read Order Column/Wavelength

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.

Settling Time

To specify a settling time, select the **Settling Time** check box, and then type a time between 100 and 1000 milliseconds. The specified delay is added between the reading of each column. There is no delay between each well in a column.

More Settings for the SpectraMax Paradigm and FilterMax Instruments

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.

Read Height Optimization is available for the FilterMax F5 Multi-Mode Microplate Reader for top-read only.

Read Height Optimization is not available for the FilterMax F3 Multi-Mode Microplate Reader.

Attenuation

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

Normalization of AlphaScreen Data

The conversion rate of photons to counts is individual for each detector. Therefore, raw data from the same plate can appear to be different from one instrument to the next. In addition, the data format used by instrument manufacturers can be counts normalized-per-second or not-normalized counts, therefore raw data can be different by several orders of magnitude. It is important to know that the number of counts and the size of figures is in no way an indication of sensitivity.

The raw data can be normalized to counts per second by selecting the **Normalization** option.

Background Correction with Interlaced Readings

Although background is significantly lower with AlphaScreen than with fluorescence intensity measurements, Molecular Devices recommends that you use blank or assay controls for background correction. The background can be effectively measured using blank replicates. When reading a sample with small signal, an interference can occur from the afterglow of a very strong emitting adjacent sample that was measured just before. Such cross talk can occur through the wall of a white 384-well microplate. To prevent such interference, you can select the **Interlaced Reading** option, which reads only every other well in a checkerboard pattern, and adds another plate run to read the previously omitted wells.

For improved results, Molecular Devices recommends that you use replicates for all blanks, controls, and samples.

Chapter 6: Collecting Data

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

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

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 54.

    • To set up a new protocol, see Creating a Protocol on page 159.

4. Prepare the microplate or the cuvette 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 207.

7. Save the acquired data, see Saving Data Files on page 47.

Creating a Protocol

Protocols contain instrument settings, template maps, Data Reduction, and Display options. Templates are maps to the contents of a microplates or the cuvettes in a cuvette set, and how the data will be collected and displayed.

You can manage protocols using the **Protocol Manager** on the **Protocols** tab in the ribbon. See Using the Protocols Tab to Manage Protocols on page 32.

You can create as many different protocols as you need. You can create a new protocol from a blank file or by modifying an existing protocol 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 10.

2. Select the instrument to be used with the protocol. See Selecting and Connecting to an Instrument on page 11.

3. Define the instrument settings to use with the protocol. See Selecting Instrument Settings on page 160.

4. Create a template, if applicable. See Configuring a Microplate Template on page 162.

5. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

6. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

7. Set Display options, if applicable. See Setting the Data Display Options on page 210.

Selecting Instrument Settings

Use the options in the **Settings** dialog to define the parameters for acquiring data from a plate or cuvette using the selected instrument.

To select and connect to an instrument see, Selecting and Connecting to an Instrument on page 11.

To select instrument settings:

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Settings** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, select a detection cartridge. See Selecting the SpectraMax Paradigm Detection Cartridge on page 161.

4. Select a **Read Mode**. See Selecting the Read Mode on page 161.

5. Select a **Read Type**. See Selecting the Read Type on page 162.

6. After defining the settings 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 162.

• Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

• Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

Selecting the SpectraMax Paradigm Detection Cartridge

1. Select the SpectraMax Paradigm Multi-Mode Detection Platform using the **Instrument Connection** dialog. See Selecting and Connecting to an Instrument on page 11.

2. Click a **Plate** section to make it active in the workspace.

3. Click **Settings** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

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 Multi-Mode Detection Platform, you see only the cartridges that are installed in the instrument.

For information on the available detection cartridges, see SpectraMax Paradigm Detection Cartridges on page 131.

Selecting the Read Mode

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Settings** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, select a detection cartridge. See Selecting the SpectraMax Paradigm Detection Cartridge on page 161.

4. Click a **Read Mode** button.

For information on the available read modes, see Supported Read Modes on page 112.

Selecting the Read Type

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Settings** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, select a detection cartridge. See Selecting the SpectraMax Paradigm Detection Cartridge on page 161.

4. Select a **Read Mode**. See Selecting the Read Mode on page 161.

5. Click a **Read Type** button.

For information on the available read types, see Supported Read Types on page 111.

Configuring a Microplate Template

The **Template Editor** is a representation of the microplate or 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.

When you select multiple wells in the **Template Editor**, the selection must be a rectangular group of contiguous wells.
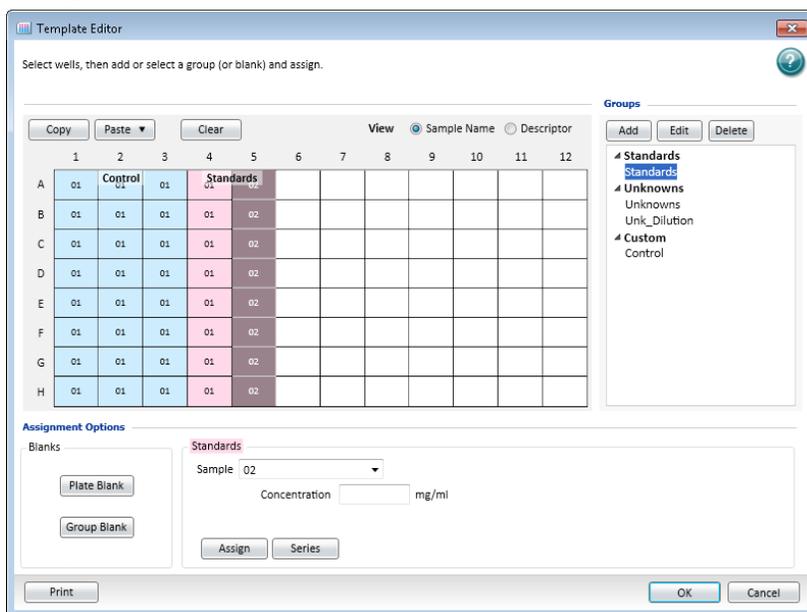
1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Template** in the **Template Tools** section of the **Home** tab in the ribbon or in the toolbar at the top of the section. This opens the **Template Editor**.
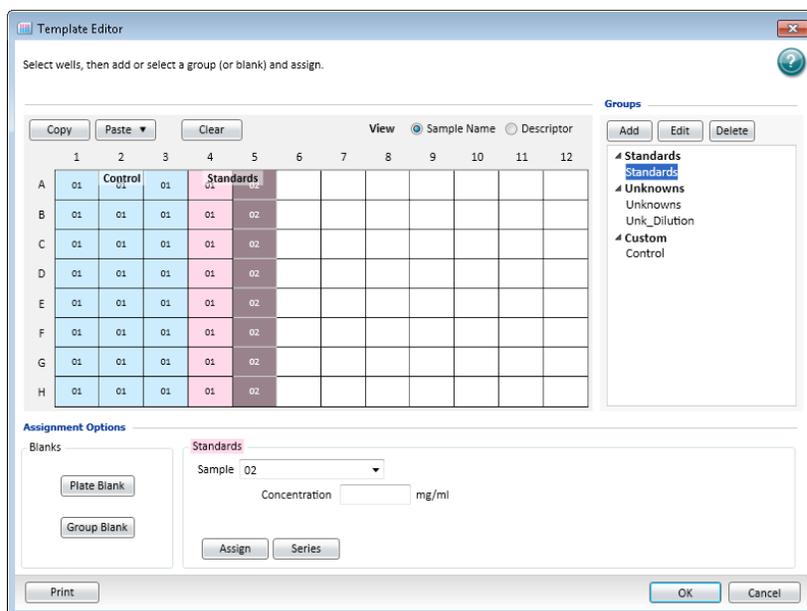
The **Template Editor** contains a representation of the rows and columns of the microplate in the active **Plate** section.

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 microplate. The cuvettes 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 SoftMax Pro Software.

3. Define a group of wells, see Defining a Group on page 164.

   • To assign wells to an existing group, select the wells to add to the group, click the group name, and then click **Assign**.

   • To specify wells as Plate Blank, select the wells and then click **Plate Blank**.

   • To specify wells as Group Blank, select the wells, click **Group Blank**, and then click **Assign**.

   > 📝 **Note:** For more information about using blanks, see Blanking on page 169.

   • To remove wells from a group, select the wells to be removed and then click **Clear**.

   • To print the **Template Editor** dialog, click the **Print** button.

4. To assign wells to a series, select the wells and then click the **Series** button. See Creating a Series on page 165.

5. Click **OK** to close the **Template Editor** and apply the template.

To save the template in a protocol file for use on future experiments, see Saving Protocol Files on page 55.

Modifying a Template

To modify a template:

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Template Editor** ⊞ in **Template Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. In the **Template Editor**, modify the template as needed. See Configuring a Microplate Template on page 162.

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

Defining a Group

Wells can be assigned to group types using the Template Editor. Depending on the protocol, certain group types, such as Standard or Unknown, can be created automatically. You can create other groups as required.

To define the group for selected wells:

1. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Template Editor** ⊞ in **Template Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the active section. This opens the **Template Editor**.

The **Template Editor** contains a representation of the rows and columns of the microplate in the active **Plate** 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 microplate. The cuvettes 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 SoftMax Pro Software.

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

4. In the **Groups** area, select an existing group name.

   To create a new group, click **Add** to open the **Group Settings** dialog. See Using the Group Settings Dialog on page 100.

5. If a sample descriptor has been defined for the selected group, type an appropriate value in the field for the descriptor.

6. Click **Assign** to assign the group to the selected wells.

   To define a series for the selected wells, click **Series**. See Creating a Series on page 165.

7. Click **OK** to close the **Template Editor** and apply the template.

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. This 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 series name can be incremented as well.

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. To create replicates, click the Assign button or use 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. Click a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Template Editor** in **Template Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. In the **Template Editor** dialog, click and drag the mouse to select one or more wells to be part of the series.

4. In the **Groups** area, select an existing group name.

> **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. To create a new group, click **Add** to open the **Group Settings** dialog. See Using the Group Settings Dialog on page 100.

5. Click the **Series** button.

6. 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. For example, if you are filling down from the top 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 that includes all the wells in the microplate, select all the wells, select the fill direction, and then set the replicates to 1.

   • Optionally, click **Reverse Series Order** to reverse the order of the series.

7. 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.

8. If a sample descriptor has been defined for the selected group, type an aopriate value in the **Starting Value** field for the descriptor.

9. From the **Step by** list, select the operator for the series (+, -, *, or /) and then 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, 0.125, and so on.

10. 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. If a sample descriptor has been defined for the selected group, clear the check box next to the descriptor.

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. In the **Starting Value** field for the sample descriptor, type a value for the concentration or dilution.

3. From the **Step by** list, select the multiplication operator (*) and then enter a value of 1 for the increment.

   If sample names are set to increment automatically using the Series function, be aware that SoftMax Pro Software automatically truncates 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 need to set up your replicates manually.

Copying and Pasting Template Contents

The content of a template can be copied and pasted to another section within the same experiment or between different experiments. Only contiguous subsets can be copied in the template.

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, with the 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 that the group names and sample names of the pasted template are identical to those from the source template. Since they are in different Experiments, however, 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

Only contiguous subsets can be copied and pasted in a template.

To copy and paste a region of a template:

1. Click the source **Plate** section to make it active in the workspace.

2. Click **Template Editor** in **Template Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the active section.

3. Click and drag to select the wells to copy.

4. Click the **Copy** button on the left above the template.
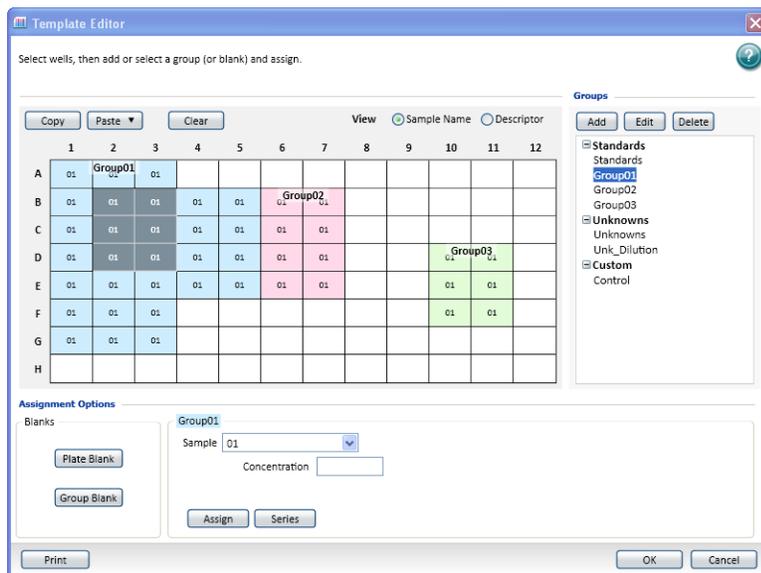
5. Click another location in the same template and then click **Paste**.

   Note: You can select the same footprint, or click 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 the following 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 a **Plate** section to make it active in the workspace.

2. Click **Template Editor** on the **Home** tab in the ribbon or in the toolbar above the active section.

3. In the **Template Editor**, select the wells to be used for the **Plate Blank**.

4. 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 **Data Reduction** dialog so that the data can be reviewed with or without **Plate Blank** subtraction. See Performing Data Reduction on page 214.

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 a **Cuvette Set** section to make it active in the workspace.

2. Click **Template Editor** on the **Home** tab in the ribbon or in the toolbar above the active section.

3. In the **Template Editor**, select the cuvettes to be used for the Template Blank.

4. Click **Blank**.

The average value of all the cuvettes in a Template Blank group is subtracted from individual cuvette readings in the same Cuvette Set 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 a **Plate** section or **Cuvette Set** section to make it active in the workspace.

2. Click **Template Editor** on the **Home** tab in the ribbon or in the toolbar above the active section.

3. In the **Template Editor**, select the wells or cuvettes to be used for the group blank. These should be wells or cuvettes that are already assigned to a Group, but not the "Blank" group.

4. Select a group from the **Group Types** list.

5. Click **Group Blank** 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 Pathlength Measurement Technology, 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 214.

Group blanks apply to all wells or cuvettes in a group and can be subtracted from wells or cuvettes in more than one **Plate** section or **Cuvette Set** section.

Exporting a Template

To export template information from a **Plate** section:

1. Click the **Plate** section with the template to be exported to make it active in the workspace.

2. Click **Export** in **Template Tools** on the **Home** tab in the ribbon.

3. In the **Save As** dialog, navigate to the location in the file system where you want to save the template file.

4. Type a name for the file in the **File name** field

5. From the **Save as type** list, select **Text files (*.txt)** or **Excel files (*xls)**.

   Text files are save in tab-delimited format.

6. Click **Save**.

For information about the format of an imported or exported template file, see Template File Formats on page 172.

Importing a Template

Template information can be imported into a **Plate** section from a tab-delimited text file. Template files can be prepared outside SoftMax Pro Software and then imported as needed when ready to run samples.

For information about the format of an imported or exported template file, see Template File Formats on page 172.

Note: A good 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.

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1. Click the **Plate** section where you want to import template information to make it active in the workspace.

2. Click **Import** in **Template Tools** on the **Home** tab in the ribbon.

3. In the **Open** dialog, navigate to and select the file to be imported.

4. Click **Open**.

Template File Formats

Template information saved as a tab-delimited ASCII text file can be imported to or exported from Plate sections. A template file consists ten columns of data separated by tabs. Each line of the file provides information for a single well in the microplate.

When template information is exported, the first line of data contains template information for well A1, the second line of data contains template information for well A2, and so on, until a line has been created in the export file for each well in the microplate. If no template information exists for a well in a plate, 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, providing a well with no template setting.

Table 6-1: 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.
G	Descriptor1 Units	Assigned in the Group Settings dialog.

Table 6-1: ASCII Template File Columns and Descriptions (continued)

Column	Column Entry	Description
		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.

A tab-delimited ASCII file can be opened with a spreadsheet program, such as Microsoft Excel. If the file is edited in Microsoft Excel, it cannot be imported back into SoftMax Pro Software.

Creating an Absorbance Mode Protocol

To create an Absorbance Mode protocol:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

    > **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 **Settings** on the **Home** tab in the ribbon.

4. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, 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 settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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.

$$A = \log_{10}(I_0 /I) = -\log_{10}(I/I_0)$$

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 Scan** reads.

It is always good scientific practice to designate wells in the microplate to serve as plate blanks or group blanks. This can be done in the **Template Editor** dialog of the **Plate** section in the SoftMax Pro Software. See Configuring a Microplate Template on page 162.

If desired, the PathCheck Pathlength Measurement Technology feature can be used to normalize the data to a 1 cm pathlength. See PathCheck Pathlength Measurement Technology on page 148.

> **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:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

> 📋 **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 **Settings** on the **Home** tab in the ribbon.

4. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, 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 settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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 difference between the wavelengths of the excitation and emission maxima (the Stokes shift) is generally fairly small, about 30 nm. There is considerable overlap between the excitation and emission spectra (gray area) when a small Stokes shift is present.
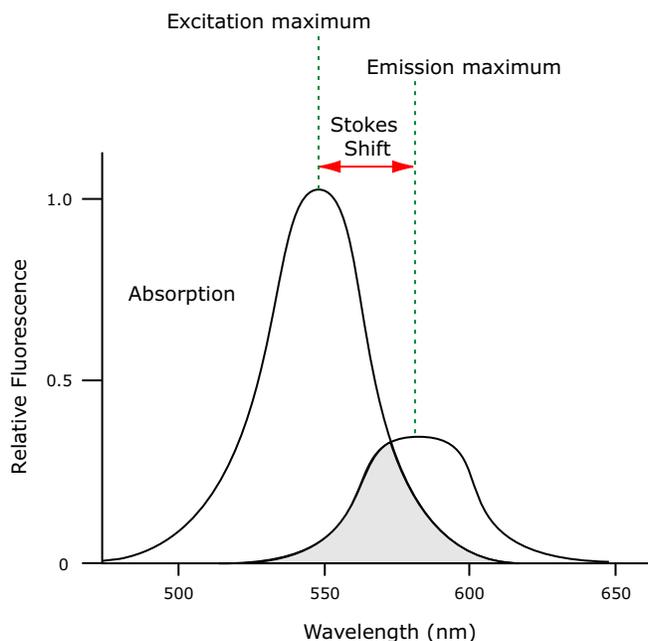
Figure 6-1: Excitation and Emission Spectra

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.
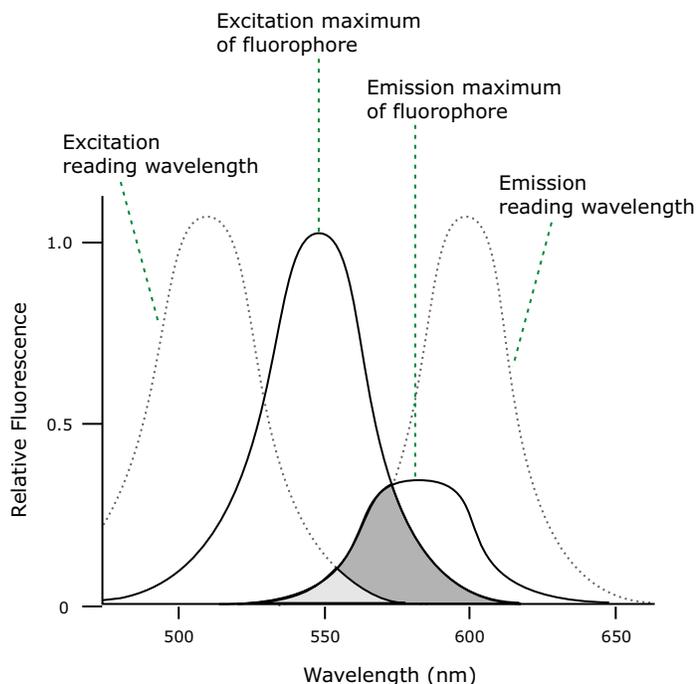
Figure 6-2: Optimized Excitation and Emission Reading Wavelengths

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, which is the difference between the wavelengths of the excitation and emission maxima. 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 180.

- 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 180.

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 202.

Pipette 200 µL of sample that includes the fluorophore and 200 µL of a buffer control into separate wells of a microplate.

Perform the Excitation Scan

1. 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.

2. 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.

3. 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).

4. 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.

5. 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.

6. 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.
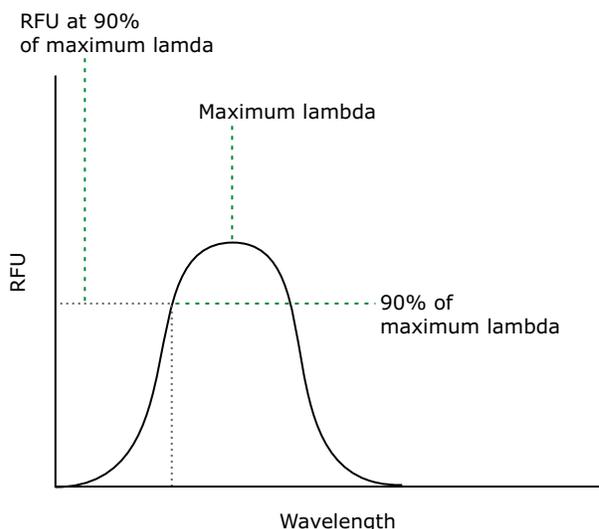
Figure 6-3: Plot of RFU and Wavelength

Perform Emission Scan #1

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.

2. Set the excitation wavelength to the value determined in Perform the Excitation Scan on page 180.

3. 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.

4. Set the step increment to 2 nm 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 nm to 3 nm increment.)

5. Perform the scan and view the results as a plot of fluorescence vs. emission wavelength.

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.

Perform Emission Scan #2

1. In the SoftMax Pro Software, set up a third plate section for an emission scan as specified in Perform Emission Scan #1 on page 181, except selecting Manual Cutoff Filter and setting the wavelength to that determined in Choose the Emission Filter on page 182.

2. 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).

3. 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 Choose the Emission Filter on page 182 and Perform Emission Scan #2 on page 182 with another choice of cutoff filter.

Results

The optimal excitation and emission wavelengths are those determine in Perform the Excitation Scan on page 180 and Perform Emission Scan #2 on page 182.

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 the previously described optimization procedure. 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 6-2: Emission cutoff filter default settings for SpectraMax M2, M2e, M3, M4, M5, and M5e readers

#	Automatic Cutoff Selection Wavelength (nm)	Endpoint and Kinetic Modes 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:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

> 📋 **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 **Settings** on the **Home** tab in the ribbon.

4. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, click a Luminescence detection cartridge from the **Cartridges** list.

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 settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

> **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 **Settings** on the **Home** tab in the ribbon.

4. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, click a TRF-capable 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 settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

   > 📋 **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 **Settings** on the **Home** tab in the ribbon.

4. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, click a 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 settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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:

$$mP = 1000 * \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (G * \text{perpendicular}))}$$

Anisotropy (r) is calculated as follows:

$$r = \frac{(\text{parallel} - (G * \text{perpendicular}))}{(\text{parallel} + (2G * \text{perpendicular}))}$$

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 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 an AlphaScreen Mode protocol:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select the SpectraMax Paradigm Multi-Mode Detection Platform. See Selecting and Connecting to an Instrument on page 11.

> **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 section active, click **Settings** on the **Home** tab in the ribbon.

4. Click one of the AlphaScreen Detection Cartridges from the **Cartridges** list.

5. Click **Screen** to select the AlphaScreen 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 settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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 TNF-alpha detection (immunoassay).

Creating a FRET Mode Protocol

To create a Fluorescence Resonance Energy Transfer (FRET) protocol:

1. Start the SoftMax Pro Software. See Starting the Software on page 10.

2. Select an instrument. See Selecting and Connecting to an Instrument on page 11.

📝 **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.

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3. With the plate or cuvette section active, click **Settings** ⚙ on the **Home** tab in the ribbon.

4. When working with a SpectraMax Paradigm Multi-Mode Detection Platform, 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.

7. Define the settings.

   For more information about the settings, see the **SoftMax Pro Application Help**.

8. After defining the settings for the protocol, click **OK** to close the **Settings** dialog.

9. Create a template, if applicable. See Configuring a Microplate Template on page 162.

10. Set Data Reduction parameters, if applicable. See Performing Data Reduction on page 214.

11. Set Display options, if applicable. See Setting the Data Display Options on page 210.

12. Save the settings to a protocol file. See Saving the Settings to a Protocol File on page 193.

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 for the experiment into a protocol file:

1. Click the **Application** button to open the **Application** menu.

2. Click **Save As**.

3. In the **Save As** dialog, navigate to where you want the protocol file to be saved.

4. In the **File name** field, type a name for the file.

5. From the **Save as type** field, select **Protocol Files (*.spr)**.

   If you are working with the SoftMax Pro GxP Software, select **Protocol Files (*.sprx)**.

6. Click **Save**.

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

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 in the Template Editor first. The values received from the instrument are raw values and are not affected by the settings in the Template Editor.

To automatically read multiple **Plate** sections in the same experiment, see Enabling Auto Read on page 195.

To read a microplate:

1. Open the drawer of the instrument by clicking **Open/Close** on the **Home** tab in the ribbon.

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 **Open/Close** 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 160.

5. Click **Read** on the **Home** tab in the ribbon.

   The Read button changes to **Stop** , allowing you to terminate a reading if desired.

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 196.

   • 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 202.

7. When reading is complete, the drawer of the instrument opens, allowing you to remove the microplate. If temperature control 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** button on the instrument. After the drawer opens, you can remove the microplate.

After the reading is complete, save the data. See Saving Data Files on page 47.

For information on the data-analysis tools in the SoftMax Pro Software, see Analyzing Data on page 207.

Enabling Auto Read

AutoRead enables automatic reading of **Plate** sections in the order they appear within a single Experiment. When you start a read for any **Plate** section in the Experiment that is enabled for automatic reading, all enabled **Plate** sections following the selected **Plate** section are read. Enabled **Plate** sections above the selected **Plate** section are not read. You can set intervals (delay times) between the plate readings, if desired.

1. In the **Navigation Tree** or the workspace, select the Experiment that has the **Plate** sections that you want to automatically read.

2. Click **Auto Read** 🔲 in the **Operations** tab of the ribbon.
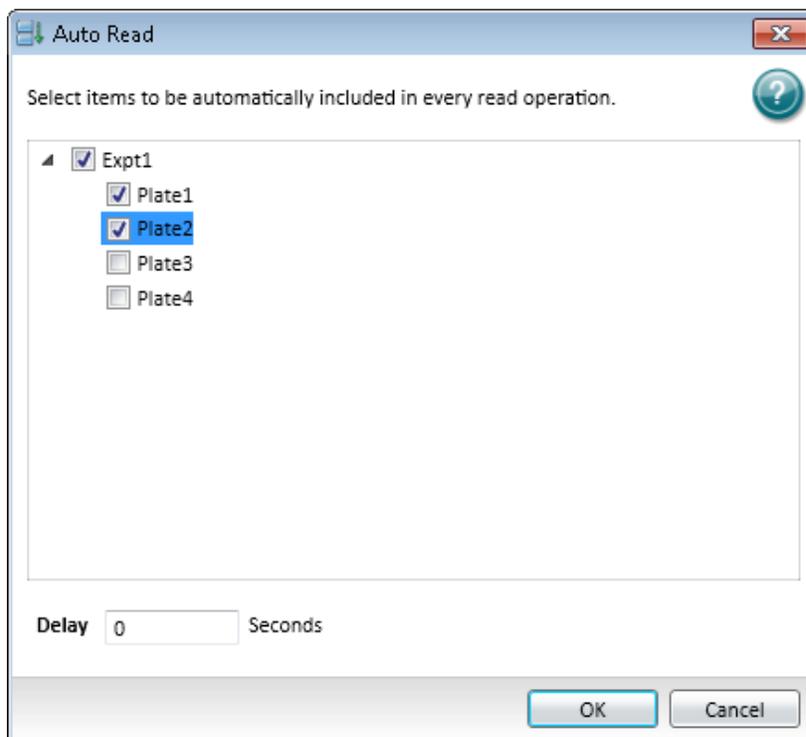
2. In the **AutoRead** dialog, select the **Plate** sections that you want to automatically read.

3. Optionally, type the number of seconds to delay (the interval) between reading each **Plate** section.

4. Click **OK** to close the dialog.

> 📋 **Note:** If you have non-enabled **Plate** sections following the last enabled **Plate** section, the first non-enabled **Plate** section following the last enable **Plate** section is also read. For example, if you have four **Plate** sections (Plate 1, Plate 2, Plate 3, and Plate 4) with Plate 1 and Plate 2 enabled for AutoRead and then start a Read operation for Plate 1, then Plate 1, Plate 2, and Plate 3 are read in sequence.

Performing Pre-Read Optimization

When using the SpectraMax Paradigm Multi-Mode Detection Platform or the FilterMax F3 and F5 Multi-Mode Microplate Readers, 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 the SpectraMax Paradigm detection cartridges or the FilterMax F3 and F5 Multi-Mode Microplate Readers. 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 202.

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 197.

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 200.

3. Click **Run Optimization** to run the selected optimization options.

4. If the microplate has a lid on it, select the **Plate is Lidded** check box.

5. Optionally, you can select the orientation for the microplate:

- Landscape

- Portrait

- Opposite Landscape

- Opposite Portrait

6. Click **Read Plate** to start the read.

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 160.

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 198

- Running Optimization on page 198

- Selecting the Centers of the Four Corner Wells on page 199

- Verifying Microplate Dimensions on page 199

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 198. 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 199.

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 199.

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 Settings on page 144.

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 200.

Read Height Optimization

The SpectraMax Paradigm Multi-Mode Detection Platform 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 160.

When selected, the **Pre-Read Optimization Options** dialog opens 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 read 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.

After the wizard closes, the **Pre-Read Optimization Options** dialog appears.

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 **Read 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 shortcut 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 196.

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 in 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 **New Cuvette Set** on the **Home** tab in the ribbon.

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 **Read** on the **Home** tab in 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

A reference reading can be taken 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.

To apply a different reference to cuvettes:

1. Select 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 selected 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 **Read** 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.
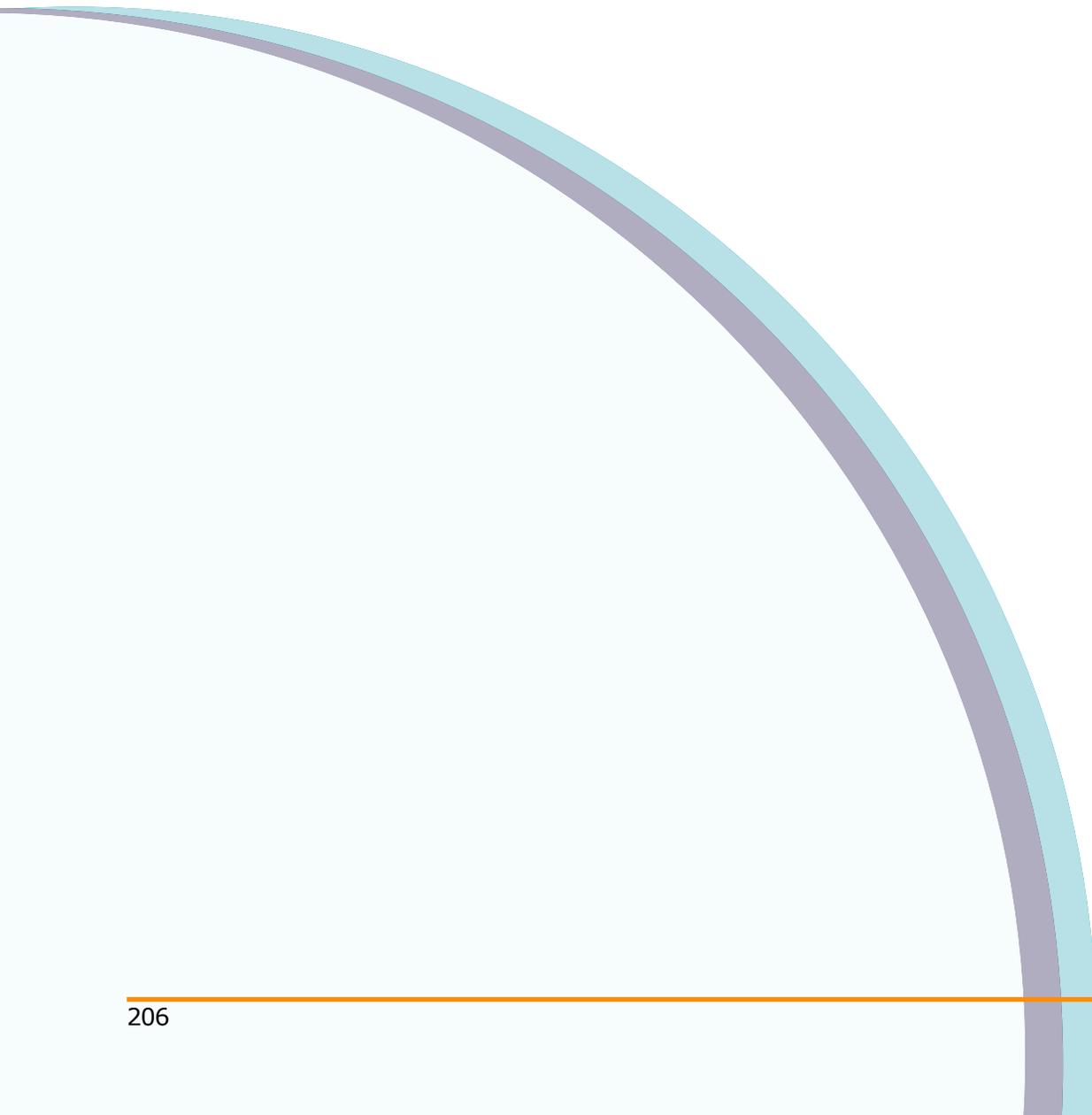
Chapter 7: Analyzing Data

Each 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** sections, **Graph** sections, 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** section or **Cuvette Set** section. In such an example, **Group** sections 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:

- Calculations and Numerical Precision on page 208
- Setting the Data Display Options on page 210
- Performing Data Reduction on page 214
- Viewing Data in a Three-Dimensional Graph on page 226
- Graphing Data on page 233

Calculations and Numerical Precision

The calculations performed by the SoftMax Pro Software use 64-bit double-precision real numbers (15+ digits of precision) as defined by IEEE standard 754 for binary floating-point arithmetic.

Numbers from **Plate** sections or **Cuvette Set** sections are presented, printed, and exported with three to four digits after the decimal point depending on the read mode and the instrument in use. Numbers from Group sections and Summaries are presented, printed, and exported with as many as nine digits after the decimal point, as specified by the user. All numbers saved in the SoftMax Pro Software data files are stored with full 64-bit precision.

The precision of the data displayed in a **Plate** section varies based on the amount of space available to view the characters.

For example, for a plate with 96 wells or fewer, each well is limited to 5 digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to 4 characters, including the decimal point.

When SoftMax Pro Software exports plate data, the values are not limited by space and can have more digits for each well.

The values displayed in a **Plate** section are only a representation of the data. In both the **Plate** section and in the exported data, the last digit displayed or exported is rounded based on the actual data value.

Although the SoftMax Pro Software can display readings and numbers derived from those readings with more than four decimal places, the resolution of the reader ultimately determines the precision of the calculations. For more information about the numerical resolution of a reader, see the documentation for that reader.

For information on suspending and resuming calculations, see Using the Operations Tab on page 34.

Calculation Hierarchy

SoftMax Pro Software performs calculations hierarchically when reducing the data collected from the instrument. If an option either has not been selected in the **Settings** dialog, is not available for the instrument being used, is not applicable to the type of read being performed, or has not been defined in the **Template** dialog or the **Reduction** dialog, SoftMax Pro Software continues with the next listed calculation.

The following is the general hierarchy of calculations:

1. Apply Well Masking

2. Subtract Plate Background constant

3. Apply PathCheck Technology normalization

4. Subtract Plate Blank

5. Subtract Group Blank (pre-reduction option)

6. Calculate mP or Anisotropy

7. Set first data point to zero

8. Convert OD to %T

9. Apply Reduction Limits

10. Apply Wavelength Combination

11. Apply Kinetic or Spectrum Reduction

12. Subtract Group Blank (post-reduction option)

More information on SoftMax Pro Software calculations can be found in the following topics or references:

- For information on Optical Density and %Transmittance calculations, see ABS Read Mode on page 113.

- For information on Fluorescence Polarization calculations, see FP Read Mode on page 116.

- For information on pathlength calculations, see PathCheck Pathlength Measurement Technology on page 148.

- For information on kinetic and spectrum reductions, see Kinetic Data Reduction Options on page 223 and Spectrum Data Reduction Options on page 225.

- For information on statistical functions, see the *SoftMax Pro Software Formula Reference Guide*.

- For information on curve fit calculations, see Curve Fit Functions on page 245.

Setting the Data Display Options

At any time in an active **Plate** section or **Cuvette Set** section you can change how the data is presented by clicking **Display** on the **Home** tab in the ribbon or in the toolbar at the top of the section.
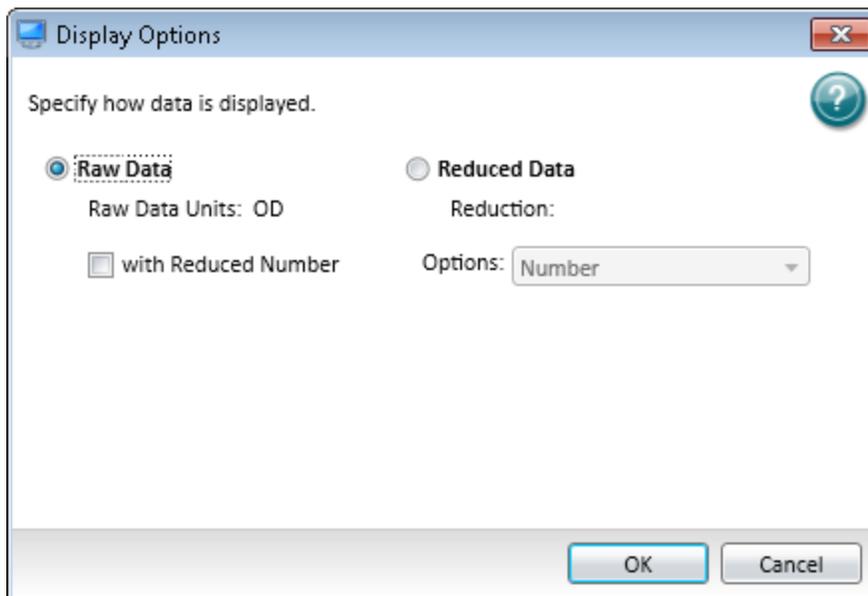
Choices available in the **Display Options** dialog include selecting between **Raw Data** and **Reduced Data**.

You can view both raw and reduced data or multiple reductions of the same data by cloning a plate. See Cloning a Plate Section on page 89.

The precision of the data displayed in a **Plate** section varies based on the amount of space available to view the characters.

For example, for a plate with 96 wells or fewer, each well is limited to 5 digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to 4 characters, including the decimal point.

When SoftMax Pro Software exports plate data, the values are not limited by space and can have more digits for each well.

The values displayed in a **Plate** section are only a representation of the data. In both the **Plate** section and in the exported data, the last digit displayed or exported is rounded based on the actual data value.

Viewing Raw Data

1. Click **Display** on the **Home** tab in the ribbon or in the toolbar at the top of the section.

2. In the **Display Options** dialog, click **Raw Data**.

3. Optionally, click **with Reduced Number** to include the reduced data number on the display.

4. Click **OK**.

Raw Data Options

Selecting **Raw Data** 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**.

Viewing Reduced Data

1. Click **Display** on the **Home** tab in the ribbon or in the toolbar at the top of the section.

2. In the **Display Options** dialog, click **Reduced Data**.

3. Select an option from the **Options** list.

4. Click **OK**.

Reduced Data Options

The reduced data display is based on the selections made in the **Data Reduction** dialog. See Performing Data Reduction on page 214.

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

- Select **Number** to view only the reduced number.

- Select **Plot** for Kinetic reads to display a plot of the raw and reduced data. To also view the reduced number, select the **Reduced Number** check box.

- Select **Grayscale** to display 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. To define the **High Limit** and the **Low Limit** for the grayscale map, type values in the fields. To also view the reduced number, select the **Reduced Number** check box.

- Select **Color Map** to display 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. To define the **High Limit** and the **Low Limit** for the color map, type values in the fields. To also view the reduced number, select the **Reduced Number** check box.

Zooming the Display of a Well or Cuvette

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

To display the **Zoom Well** dialog:

1. Click the well or cuvette that you want to zoom.

2. Click **Zoom** 🔍 on the **Home** tab in the ribbon or **Zoom** 🔍 in the toolbar at the top of the section.

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

3. To print the graph window, click **Print**.

4. To scale the display 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 Performing Data Reduction on page 214.

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 and choose the minimum and maximum values for the selected axis. You can also add or remove grid lines from the display.

To save the settings and return to the **Zoom Well** dialog, click **OK**. Only the well graph being viewed is affected by these changes.

8. To close the **Zoom Well** dialog, click **Close**.

Masking Wells or Cuvettes

The Mask feature hides selected data so that they are not used for calculations and are not reported. Masking is commonly used to suppress outliers from data reduction calculations.

Masking can be used as a "what if?" tool. For example, if you have included a group blank in a template and want to see the data both with and without the blank, masking the group blanks suppresses the blanking function, while unmasking them enables it again.

To mask wells or cuvettes:

1. In an active **Plate** section or **Cuvette Set** section, select a well or cuvette or select multiple wells or cuvettes.

   • To select multiple wells in a **Plate** section, click and drag across the desired wells.

   • To select multiple cuvettes in a **Cuvette Set** section, hold down the **Ctrl** key and click the desired wells.

2. Click **Mask** in **Plate Tools** or **Cuvette Tools** on the **Home** tab in the ribbon or in the toolbar at the top of the section.

   You can also right-click a well or cuvette and select **Mask** from the shortcut menu.

To unmask masked wells or cuvettes select them and then 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.

The **Data Reduction** dialog displays only the reduction options that are available for the instrument you are using, the options that have been selected in the **Settings** dialog, and the options defined in the template.

SoftMax Pro Software performs the applicable calculations in the **Plate** section or **Cuvette Set** section in the order displayed in the **Data Reduction** dialog. 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 (Absorbance Endpoint only)

PathCheck Pathlength Measurement Technology normalization calculations apply to the data only when PathCheck has been enabled in the PathCheck settings for an Absorbance Endpoint read. When PathCheck is enabled, this calculation can be disabled in the **Data Reduction** dialog by clearing the **Apply Pathcheck** check box. For more information, see PathCheck Pathlength Measurement Technology on page 148.
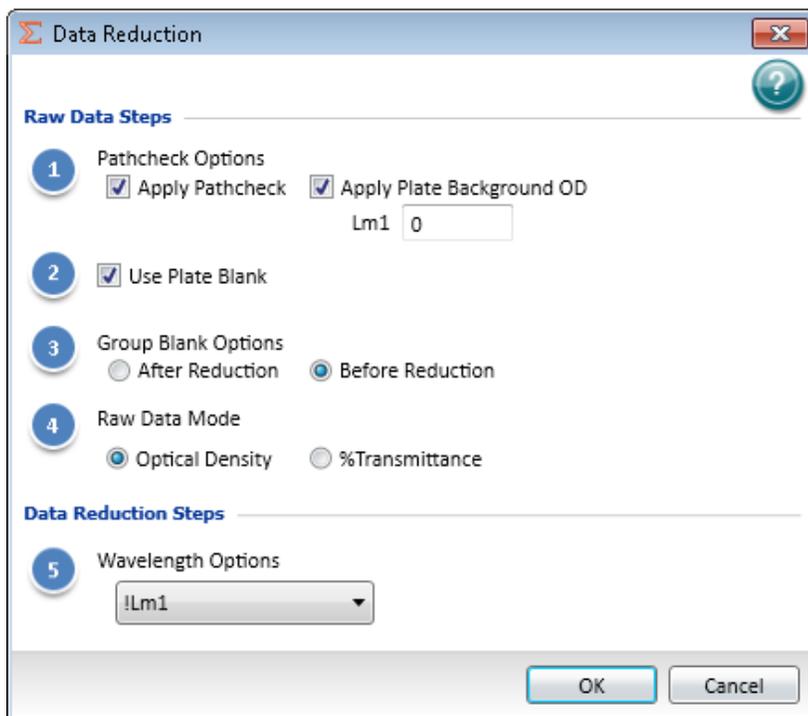
Apply Plate Background OD (Absorbance Endpoint only)

Subtraction of the Plate Background constant applies to the data only when PathCheck has been enabled in the PathCheck settings for an Absorbance Endpoint read. To define the constant, type a value in the field. When PathCheck is enabled, this calculation can be disabled in the **Data Reduction** dialog by clearing the **Apply Plate Background OD** check box.

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, select the **Use Plate Blank** check box. For more information about plate blanks, see Blanking on page 169.

Group Blank Options

The **Group Blank Options** 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, click **Before Reduction**.

- To enable the group blank value subtraction calculation as a post-reduction action, click **After Reduction**. When **After Reduction** is selected under **Group Blank Options**, the last step in the **Data Reduction** dialog shows **Group Blank Subtracted**.

For more information about group blanks, see Blanking on page 169.

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. Click the data type to use as the raw data for the reduction.

G Factor

G factor, or 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. To define the **G Factor**, type a value in the field.

Set first data point to zero (Kinetic only)

Select this check box to offset the first data point to (0,) and shift all other data accordingly. Clearing this check box can cause some data points that do not fall within the reduction limits to disappear.

To see more data and still display absolute values, increase the reduction limits for **Min OD**, **Min RLU**, or **Min RFU** and the **Max OD**, **Max RLU**, or **Max RFU**.

Raw Data Display Mode (Absorbance only)

For Absorbance reads, you can choose whether to display absorbance data as Optical Density or %Transmittance.

- **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).

Click the data type to use as the raw data for the reduction.

> **Note:** Separate mathematical calculations are used for handling Optical Density (OD)and %Transmittance (%T) calculations for Pre-read plate blanking, PathCheck Pathlength Measurement Technology, and Reference, because OD calculations are performed on a linear scale, while %T calculations are performed on a logarithmic scale. However, SoftMax Pro Software does not perform other calculations differently for OD 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 Pathlength Measurement Technology. Use caution when using %T on reduced numbers or any readings that apply other calculations since the data might not be calculated correctly.

Data Reduction Steps

Data Reduction display includes one or more of the following calculations:

Limits

Apply the specified reduction limits to the reduced data after this step. To specify the limits, type values in the fields.

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, or 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.

- **Min OD**, **Min RFU**, or **Min RLU** defines 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).

- **Max OD**, **Max RFU**, or **Max RLU** defines 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** in a kinetic read defines the period of very slow growth of microorganisms or the rate of reaction that can precede the rapid or linear phase of reaction.The 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.

- **End Time** in a kinetic read specifies the time at which to stop showing data in the display and exclude it 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 for the kinetic read.

- **Start** in a spectrum read specifies the limit in nm 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** in a spectrum read 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.

Wavelength Options

Select an available wavelength formula from the list. To use a custom reduction, select **Custom** from the list and then, click [icon] to open the **Formula Editor**. See Data Reduction Formulas on page 221.

Kinetic Reduction

Select a Kinetic reduction mode from the list. To use a custom reduction, select **Custom** from the list and then, click [icon] to open the **Formula Editor**. See Data Reduction Formulas on page 221.

For more information about kinetic reductions, see Kinetic Data Reduction Options on page 223.

The number of Vmax Points is determined by the Timing settings.

Spectrum Reduction

Select a Spectrum reduction mode from the list. To use a custom reduction, select **Custom** from the list and then, click [icon] to open the **Formula Editor**. See Data Reduction Formulas on page 221.

For more information about spectrum reductions, see Spectrum Data Reduction Options on page 225.

Well Scan Reduction

Select **Maximum**, **Minimum**, **Average**, or **Custom** from the list. **Average** provides the average value for all points in the Well Scan. To use a custom reduction, select **Custom** from the list and then, click [icon] to open the **Formula Editor**. See Data Reduction Formulas on page 221.

Group Blank Subtracted

When **After Reduction** is selected under **Group Blank Options**, the last step in the **Data Reduction** dialog shows **Group Blank Subtracted**.

After 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** sections and **Graph** sections. See Using a Group Section on page 100 and Graphing Data on page 233.

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 tables.

Table 7-1: Wavelength Reduction Formula Examples for Specific Wavelength Combinations

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 7-2: Wavelength Reduction Formula Examples for all Wavelength Combinations

Formula	Description
!Lmx / constant	For example, Lm1 / 1.44 for quantitating a polyclonal antibody by measuring the absorbance at A280 with PathCheck Technology 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 7-3: Kinetic Reduction Formula Examples

Formula	Description
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.

Table 7-4: Spectrum or Kinetic Reduction Formula Examples

Formula	Description
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*.

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.

Kinetic Data Reduction Options

Kinetic reductions are applied to the value at each time point after the wavelength combination formula is applied.

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.

Vmax Rate is reported as signal/min (milli-OD, RFU, or RLU units per minute) for a Kinetic read. 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 can also be reported as units per second (the default for Fluorescence and Luminescence modes).

Vmax (milli-units per min) and **Vmax (units per sec)** reductions are available for all instruments that are capable of Kinetics. The default Kinetic reduction is **Vmax (milli-units per min)**.

Time to Vmax 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.

The number of available Vmax Points is determined by the Timing settings. Typing a value in the **Vmax Points** field 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** 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 . 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.

Onset Time

Onset time is a 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 (such as, endotoxin testing) 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

The slope reduction option 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 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.

To use a custom reduction, select **Custom** from the list and then, click to open the **Formula Editor**. See Data Reduction Formulas on page 221.

Spectrum Data Reduction Options

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 a Spectrum read is **Lambda at Maximum**.

Maximum

This reduction reports the maximum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.

Minimum

This reduction reports the minimum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.

Lambda at Maximum

This reduction reports the wavelength at which maximum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.

Lambda at Minimum

This reduction reports the wavelength of minimum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.

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.

To use a custom reduction, select **Custom** from the list and then, click to open the **Formula Editor**. See Data Reduction Formulas on page 221.

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.
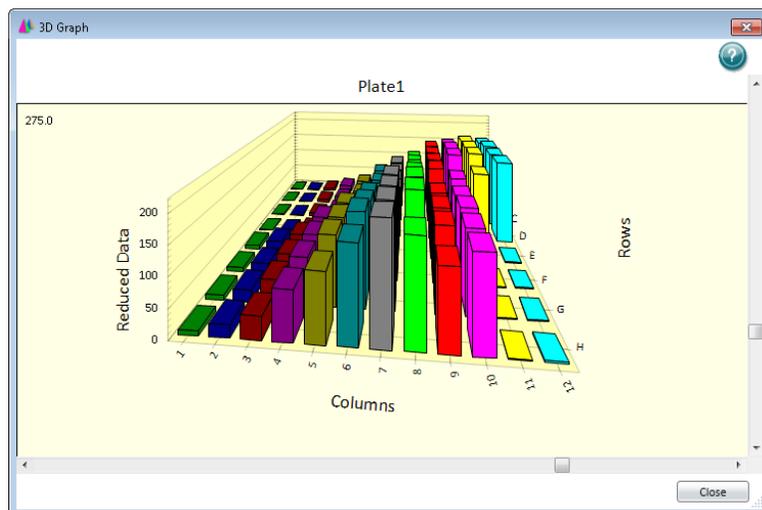
To rotate the 3D graph:

- 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 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 227.

Other options in the 3D graph dialog include:

- **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 230.

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.
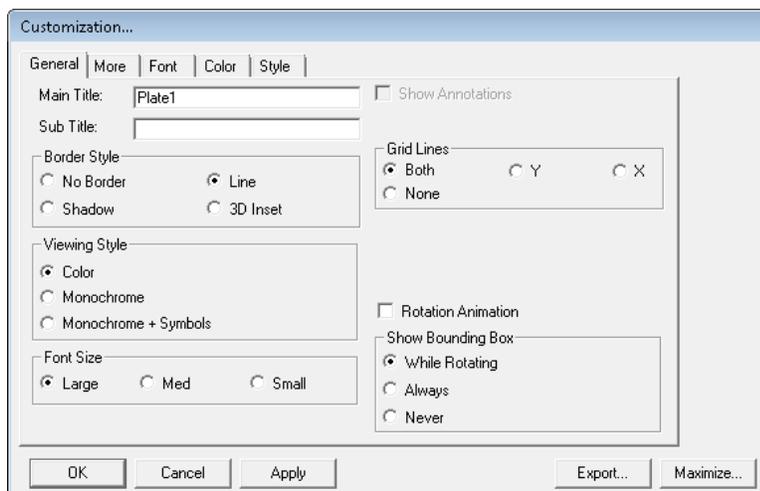
Click a tab to work with the options under that tab.

- For the **General** tab, see General Customization Options for a 3D graph on page 228.

- For the **More** tab, see More Customization Options for a 3D graph on page 228.

- For the **Font** tab, see Font Customization Options for a 3D graph on page 229.

- For the **Color** tab, see Color Customization Options for a 3D graph on page 229.

- For the **Style** tab, see Style Customization Options for a 3D graph on page 230.

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 230.

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**.

- **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.

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 227.

For export options, right-click anywhere on the 3D graph and then click **Export Dialog** to open the **Export Control** dialog.
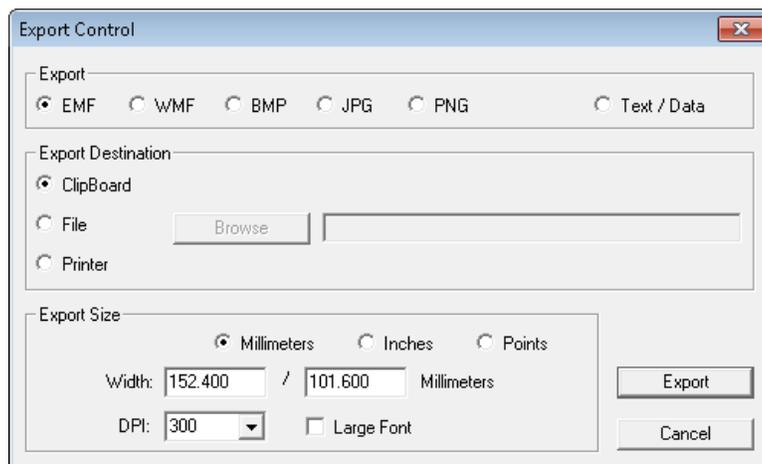
Click the graphic format from the **Export** area. If you select **Text/Data**, only the data is exported. For more information about exporting the data, see Exporting Data from a Three-Dimensional Graph on page 232.

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 232.

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.

6. In the **Export What** area choose to export just the **Data** or the **Data and Labels**.

7. If you have multiple values available in your data, choose an option from the **Data to Export** area.

8. 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.

10. 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.

11. 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

Click **New Graph** in **Sections** on the **Home** tab in the ribbon to open the **Plot Editor** dialog where you can create a graph from the collected data.

After 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. The grid lines for the graph can be enabled or disabled. The default setting has grid lines enabled.

To view a zoomed well plot of the data in a well, double-click the well. See Zooming the Display of a Well or Cuvette on page 212.

To view a three-dimensional graphical display of the data, see Viewing Data in a Three-Dimensional Graph on page 226.

Working with Graphs

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

You can create more than one **Graph** section within a data file, and plots in the **Graph** section can be created from any Experiment in the file.
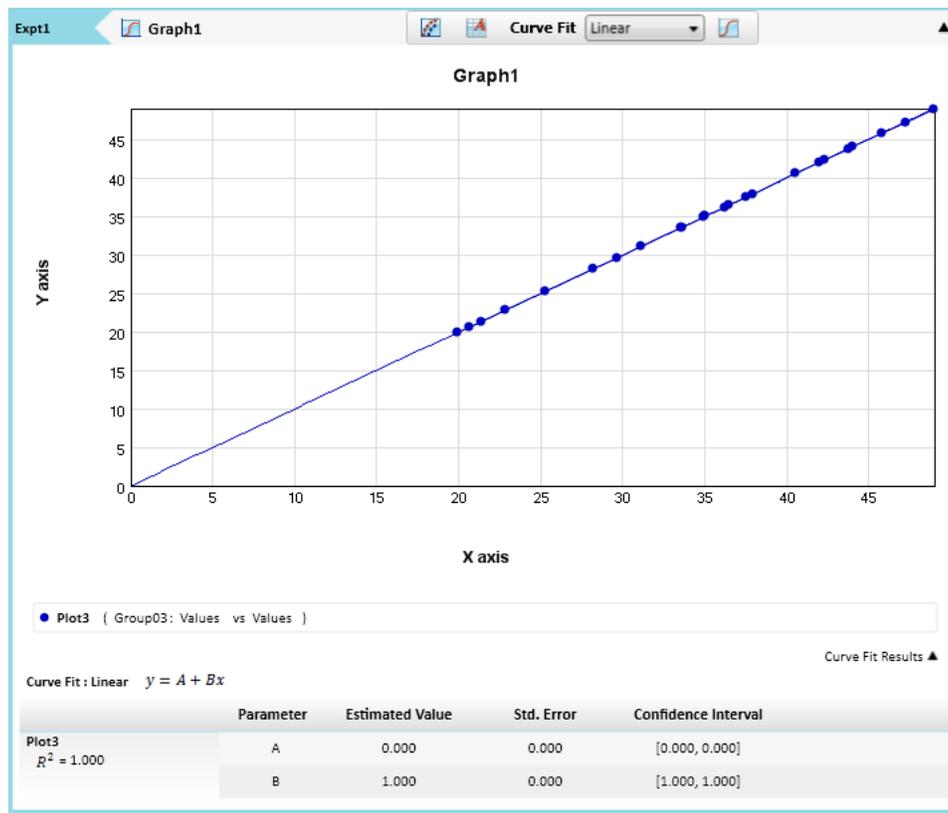
Graph sections are divided into the following areas:

- The body of the **Graph** section.
- The Legend. See Graph Legend on page 235.

For more information about graphs, see the following topics:

- Creating a New Graph on page 237
- Editing an Existing Graph on page 239
- Selecting Curve Fit Settings on page 239
- Changing the Name of the Graph Section on page 255
- Changing the Appearance of a Graph on page 256
- Zooming the Graph Display on page 258
- Exporting a Graph on page 258

Graph Legend

The graph legend contains information about the contents of the graph. To view or hide the graph legend, click **Curve Fit Results** on the right below the graph.

Independent Fits

- The formulas for the selected curve fits

- For each plot:

  - R^2 value

  - EC50 value (4-Parameter and 5-Parameter only)

- For each parameter:

  - Estimated Value

  - Standard Error (Std. Error) of the estimate

  - The confidence interval for a given confidence level is a range such that the true value lies within the range with the desired probability. The confidence level is usually specified as a percentage. The greater the confidence level, the wider the resulting confidence interval. Calculation of the confidence interval is based on sampling statistics. 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. Select the Confidence Interval Method in the Statistics tab of the Curve Fit Settings dialog. See Curve Fit Statistics Tab on page 252.

  - 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.

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 under Independent Fits on page 235)

  - Independence (see explanation under Independent Fits on page 235)

  - EC50 value (4-Parameter and 5-Parameter only)

  - R^2 value

- For each parameter:

  - Estimated Value

  - Standard Error (Std. Error) of the estimate

  - Confidence Interval of the estimate

  - Independence

Creating a New Graph

Click **New Graph** in **Sections** on the **Home** tab in the ribbon to open the **Plot Editor** dialog where you can 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 read.

In the **Plot Editor** dialog, you can name the graph, assign specific information to be plotted on the X and Y axes, and choose which icon and color to use.

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.
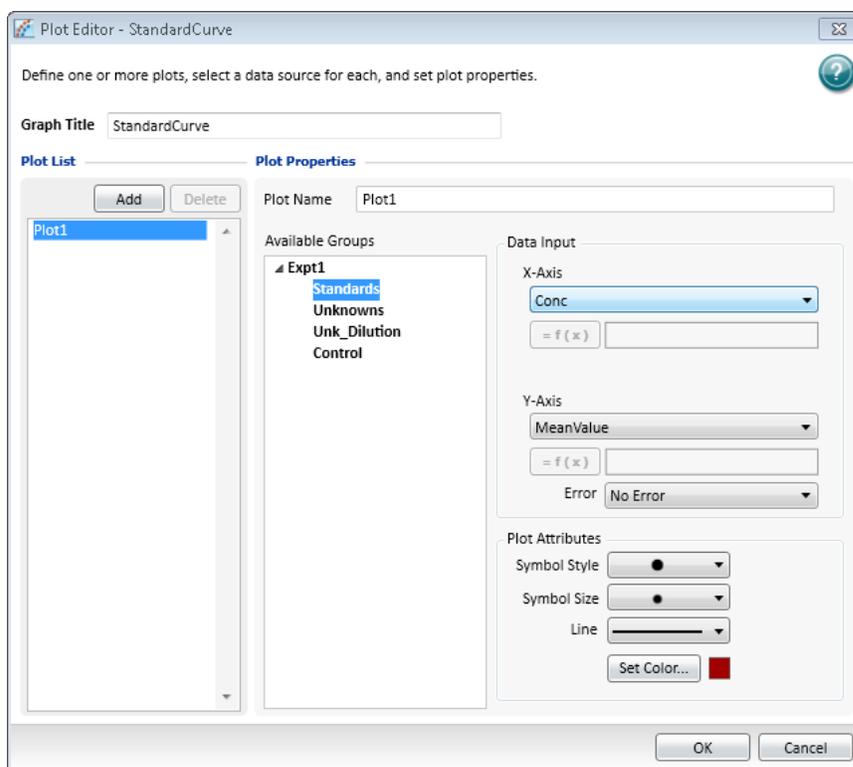
2. Type the name of the graph in the **Graph Title** field.

3. Select a plot.

   The **Plot List** shows the existing plots. One plot is shown by default.

   • To add a new plot to the graph, click **Add**.

   • To remove a plot from the list, select a plot and then click **Delete**.

   When you select a plot from the list, the parameters for the selected plot appear in the **Plot Properties** area of the dialog.

4. Optionally, type the name of the plot in the **Plot Name** field. 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 164.

6. In the **Data Input** area, select column types from those listed to assign to the plot in the X-Axis and Y-Axis. The list contains only the group column names available for the group that is selected in Available Groups. When you select the **Custom** type, the Formula Editor opens for you to define the custom formula. See Using the Formula Editor.

7. Select a column or **No Error** in the **Error** field to define 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-axis 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. Use the **Plot Attributes** area to change the graph's appearance:

   • Select the **Symbol Style** to define the symbol to be used to mark each data point.

   • Select the **Symbol Size** to define the size of the selected symbol.

   • Select the **Line Style** to define the line to be used between data points.

   • Click **Set Color** and select the color from the color picker to define the color of the symbol and line in the plot.

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.

Editing an Existing Graph

You can change a graph by editing the appearance of the graph or by using the **Plot Editor** dialog to change the plots and the data or plot attributes.

To edit the appearance of the graph, see Changing the Appearance of a Graph on page 256.

To use the **Plot Editor** dialog to change the plots and the data or plot attributes:

1. With a **Graph** section active in the workspace, click **Plot Editor** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section.

2. Make the required changes to the graph definitions. See Creating a New Graph on page 237.

3. 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 **Add**. 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 toolbar at the top of the **Graph** section. 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 the 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 is determined by the underlying chemistry of the assay and can 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. R^2 is the fraction of the variance of the y-values explained by the fit, and is 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. These statistics 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.

To define a curve fit for the plots in a graph, click **Curve Fit** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section.

The **Curve Fit Settings** dialog has three tabs available for defining the curve fit:

- **Curve Fit**, see Curve Fit Tab on page 241.

- **Weighting**, see Curve Fit Weighting Tab on page 251.

- **Statistics**, see Curve Fit Statistics Tab on page 252.

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-fit 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.
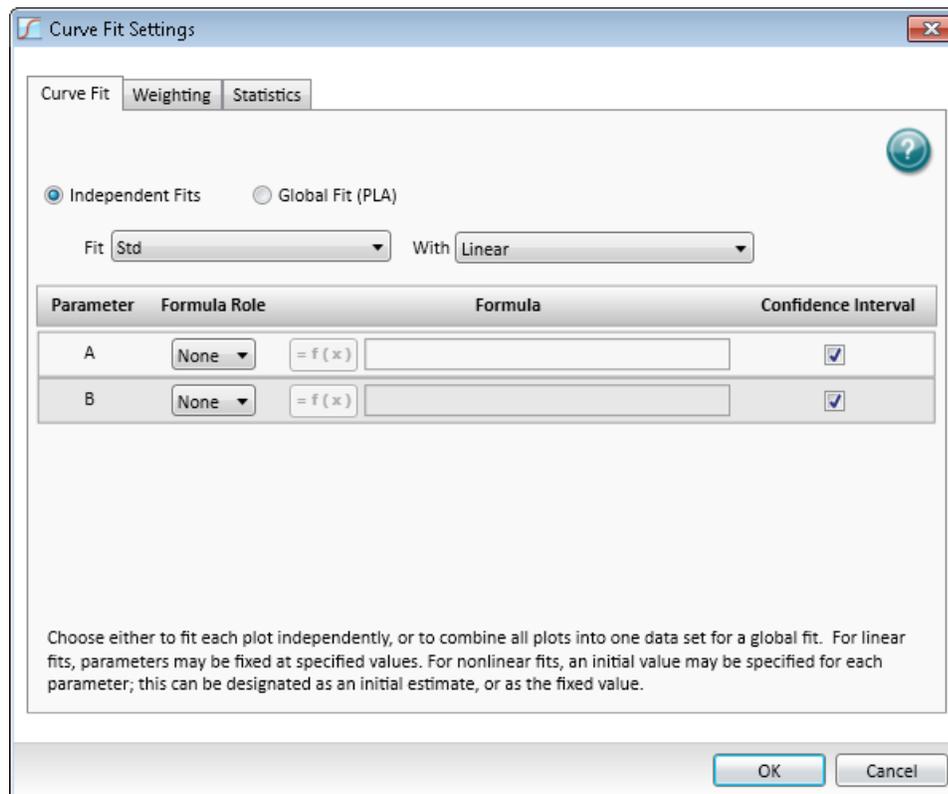
All plots on a graph can have the same type of fit or each plot can have a different fit applied individually. The **Curve Fit** tab of the **Curve Fit Settings** dialog provides the ability to set a different fit to individual plots.

To define a curve fit for the plots in a graph, click **Curve Fit** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section. You can choose to fit each plot independently or to combine all plots into one data set for a global fit.

- To fit each plot independently, see .

- To combine all plots into one data set, see .

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

For information about the available curve fit functions, see .

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 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.

You can specify whether a Confidence Interval should be calculated for each parameter.

The confidence interval for a given confidence level is a range such that the true value lies within the range with the desired probability. The confidence level is usually specified as a percentage. The greater the confidence level, the wider the resulting confidence interval. Calculation of the confidence interval is based on sampling statistics. 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.

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 **Curve Fit** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section.

3. In the **Curve Fit Settings** dialog in the **Curve Fit** tab, 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. See Curve Fit Weighting Tab on page 251.

8. If applicable, click the **Statistics** tab. See Curve Fit Statistics Tab on page 252.

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** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section.

3. In the **Curve Fit Settings** dialog, select **Global Fit (PLA)**.
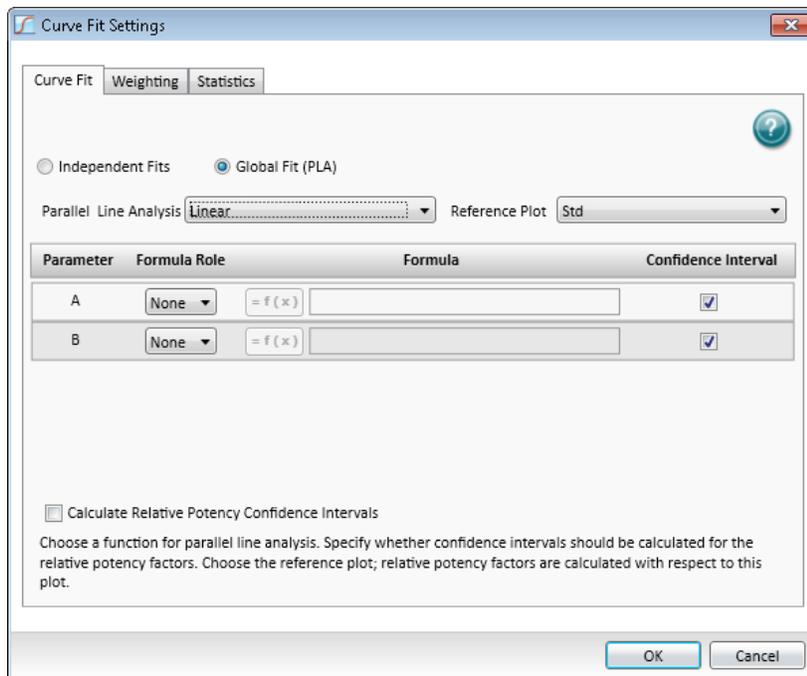
The available global fit is parallel line analysis. See Parallel Line Analysis on page 245.

6. Select the plot from the **Fit** list.

7. Select any option except point-to-point or cubic spline from the **With** list.

8. If applicable, select **Relative Potency Confidence Interval**.

9. Select a plot for the **Reference Plot** list.

10. If applicable, click the **Weighting** tab. See Curve Fit Weighting Tab on page 251.

11. If applicable, click the **Statistics** tab. See Curve Fit Statistics Tab on page 252.

12. When all curve fit options have been set, click **OK**.

Parallel Line Analysis

Parallel line analysis (PLA) is available for all Global fits except point-to-point and cubic spline fits.

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-parameter 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.

Function operators are included with SoftMax Pro Software that can be used to summarize this information in a Notes section. For additional details, see the *SoftMax Pro Formula Reference Guide*.

Curve Fit 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 the following curve-fit functions.

These selections are shown in the **Curve Fit** list in the toolbar at the top of the **Graph** section.

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 is determined by the underlying chemistry of the assay and can 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

Select **No Fit** to apply no curve fit, and to plot the scatter of the data points.

Linear

Select a **Linear** curve fit to fit 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.

Use a linear fit when the values appear to lie on or are scattered around a straight line.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a linear curve fit algorithm is 2.

Semi-Log

Select a **Semi-Log** curve fit to fit 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.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a semi-log curve fit algorithm is 2.

Log-Log

Select a **Log-Log** curve fit to fit the best straight line to the set of data that 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.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a log-log curve fit algorithm is 2.

Quadratic

Select a Quadratic curve fit to fit 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.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a quadratic curve fit algorithm is 3.

Cubic

Select a **Cubic** curve fit to fit the best third-order polynomial to the data, based on the equation:

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

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a cubic curve fit algorithm is 4.

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 a **4-Parameter**, **5-Parameter**, or **Log-Logit** curve fit.

Both the 4-parameter 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. Generally, B has a magnitude of about 1 for inhibitory experiments and -1 for excitatory experiments.

The difference between the 4-parameter fit and the log-logit 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 the *SoftMax Pro Software 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.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a 4-parameter curve fit algorithm or a log-logit curve fit algorithm is 4. For a 5-parameter curve fit algorithm minimum is 5.

The fits for these logistic curves are based on the assumption that the curve has a high and a low asymptote, and a certain steepness for the linear portion around the inflection point between the asymptotes. An insufficient number of points defining the inflection point and the asymptotes results in an inappropriate fit. For best results, make sure that the logistic fit is appropriate for your assay and that the entire range of the assay is represented in your standards.

If your standards do not clearly define both a high and low asymptote, the cubic spline curve fit might be more appropriate for your assay. See Cubic Spline on page 249.

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

Select a **Cubic Spline** curve fit to generate 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.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a cubic spline curve fit algorithm is 4.

Exponential

The exponential function used to generate the **Exponential** 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.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for an exponential curve fit algorithm is 3.

Point-to-Point

A **Point-to-Point** curve fit uses a linear equation to fit each pair of data points. This 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.

As with a **Cubic Spline** curve fit, this is not a least-squares fit, so no parameter is shown and statistics are not available.

For the best results, you should always exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a point-to-point curve fit algorithm is 2.

Rectangular Hyperbola

A **Rectangular Hyperbola** curve fit generates a hyperbola for which the asymptotes are perpendicular, also called an equilateral hyperbola or right hyperbola. This occurs when the semi-major and semi-minor axes are equal.

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 generally 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 generally 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.

Gaussian

The function used to generate the **Gaussian** curve fit is:

$$y = A * e^{-\left(\frac{x - B}{C}\right)^2} + D$$

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. In the **Curve Fit Settings** dialog, click the **Weighting** tab.
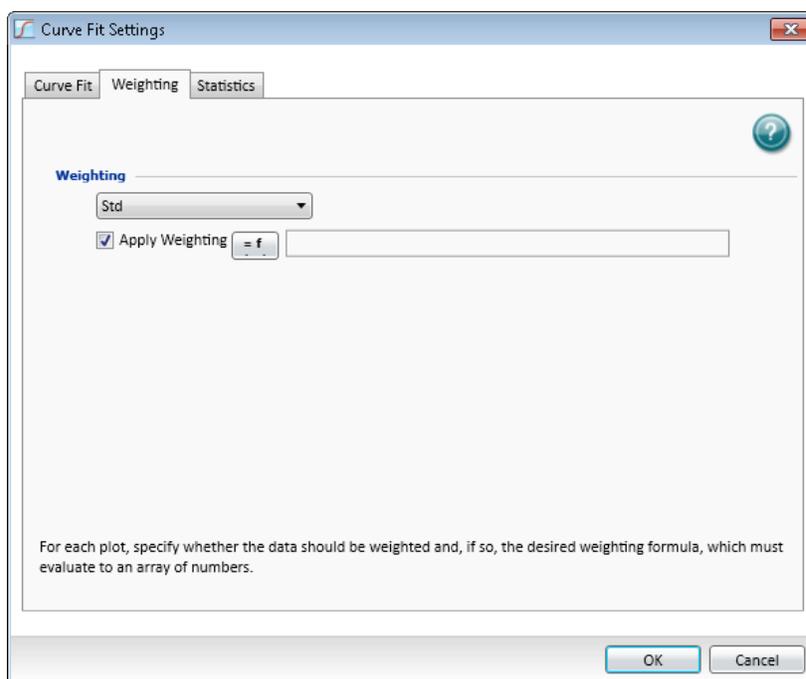
3. Select a plot from the **Weighting** list.

4. Select **Apply Weighting**.

5. Click the **Formula** button.

6. In the **Formula** field, type the formula to be applied as the weighting.

7. Click **OK**.

Curve Fit Statistics Tab

In the **Statistics** tab, you can specify the statistics display.
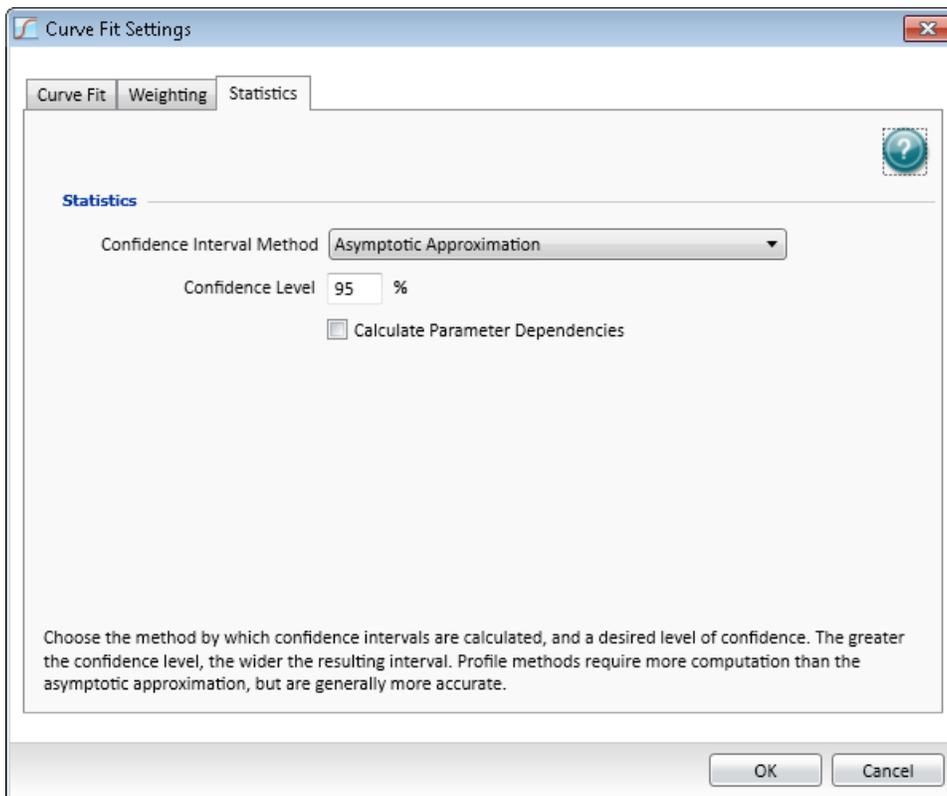
Choose the method by which confidence intervals are calculated, and a desired level of confidence.

Confidence Interval Method

The confidence interval for a given confidence level is a range such that the true value lies within the range with the desired probability. The confidence level is usually specified as a percentage. The greater the confidence level, the wider the resulting confidence interval. Calculation of the confidence interval is based on sampling statistics. 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 7-5: 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 distribution)	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 usually widens 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 .

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.

Changing the Name of the Graph Section

To change the name of a graph.

1. Click the **Graph** section to make it active in the workspace.

2. Double click the name of Graph section in the header bar to open the **Rename Section** dialog.

   You can also right-click the graph in the **Navigation Tree** and then click **Rename**.
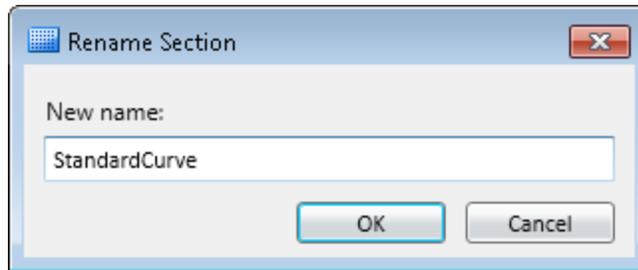
3. Type the name in the **New name** field.

4. Click **OK**.

This changes the name of the **Graph** section, but does not change the title of the graph. To change the title of the graph, see Editing an Existing Graph on page 239.

Changing the Appearance of a Graph

With a **Graph** section active in the workspace, click **Graph Appearance** [icon] in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the section.

In the **Graph Appearance** dialog, you can specify labels, styles, and graphic-display options.
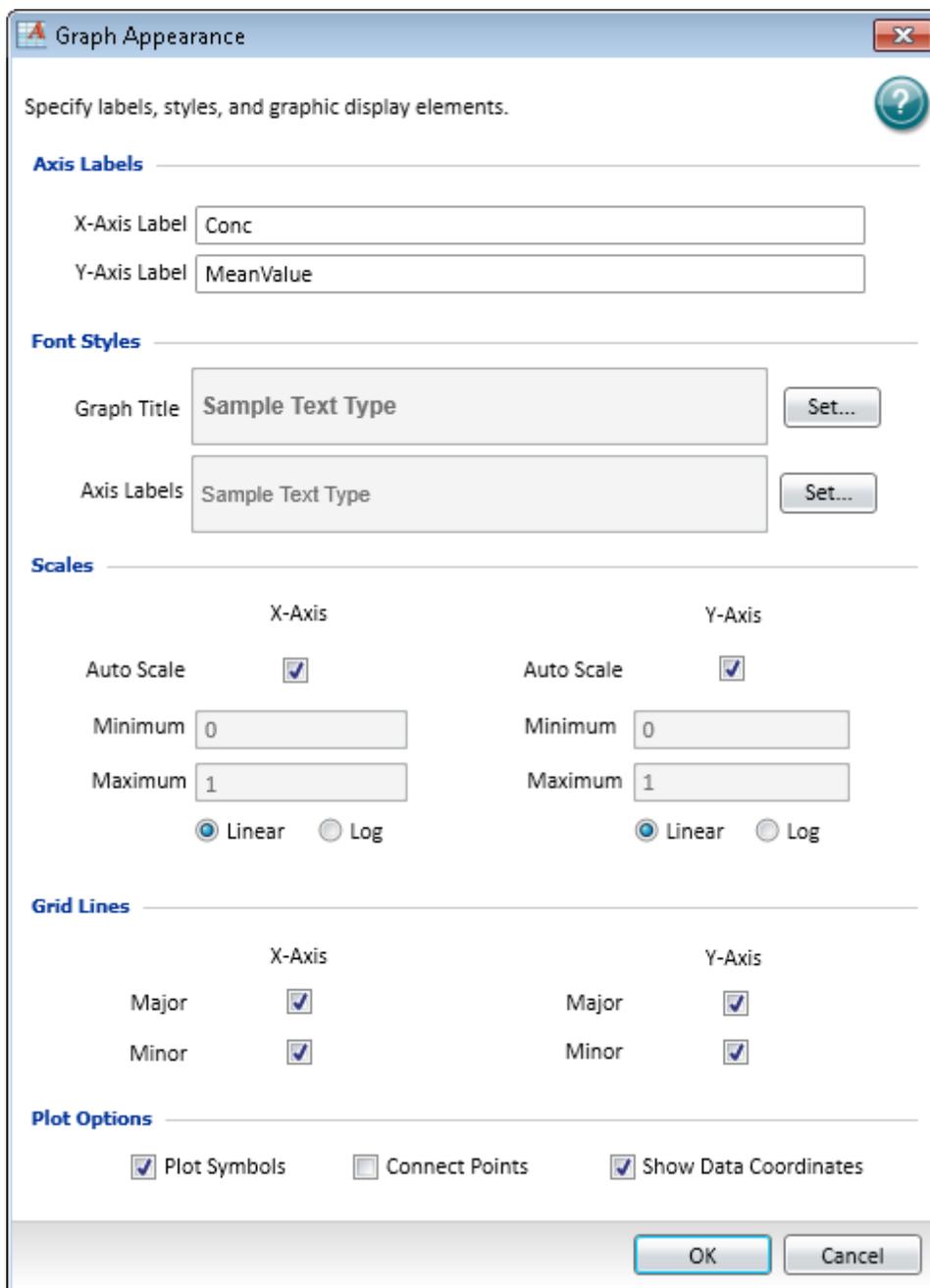
Axis Labels

Define labels for the X-Axis and Y-Axis by typing the text of the labels in the fields. These labels are displayed in the font specified in the Font Styles area.

Font Styles

Font Styles provides separate control of the fonts used for the Graph Title and Axis Labels. Click **Set** to open the **Font Style** dialog.
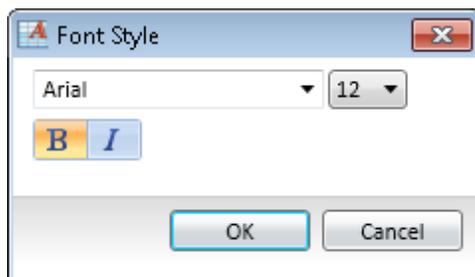
After you have finished defining the font style, click **OK**. The defined font style appears in the **Sample Text Type** example text.

Scales

To automatically scale one or both axes, select the **Auto Scale** check box for the axis.

Alternatively, you can define **Minimum** and **Maximum** scaling values.

You can also select either a **Linear** or a **Log** display of each axis. Log is the default selection.

Grid Lines

You can choose to display the major and minor grid lines for the X-Axis and the Y-Axis individually. To display a grid line, select the related check box.

Plot Options

In the Plot Options area you can select the following for display on a plot:

- Select **Plot Symbols** to display the defined symbol for each data point.

- Select **Connect Points** to display a line connecting each of the data points.

- Show **Data Coordinates** to display the coordinates for each data point when you use your mouse to hover the pointer over the data point.

To define the size and style of the symbol and the color of the symbol and the line in the **Plot Editor** dialog. See .

Zooming the Graph Display

To zoom in on a section of a graph, use your mouse to draw a rectangle over the section. After you release the mouse button, the display zooms into the selected region.

To return to the default view, double-click the graph body.

Exporting a Graph

To export an image of a graph to a .png file:

1. Click the **Graph** section to make it active in the workspace.

2. Make sure that the graph appears the way you want, including whether or not the graph legend is displayed. See Graph Legend on page 235.

3. Click **Export Graph** in the **Graph Tools** section on the **Home** tab in the ribbon.

4. In the **Save As** dialog, browse to the file location where you want to save the .png file.

5. In the **File name** field, type a name for the file.

6. Click **Save**.

An image of the active **Graph** section is saved as a .png file.

Chapter 8: Maintaining and Calibrating an Instrument

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

Information on configuration, maintenance, and calibration tasks that can be performed using the SoftMax Pro Software can be found in the following topics:

- Calibrating the Reader on page 259
- Configuring the Filters for Emax and Vmax ELISA Microplate Readers on page 260
- Using the Instrument Information Dialog on page 260
- Viewing Instrument Software Support on page 268

Calibrating the Reader

To calibrate the reader:

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

   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 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.

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 174.

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 [icon] 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** and follow the on-screen instructions. For information about preparing an instrument for shipment, contact Molecular Devices support.

For information about unlocking the drawers, see Unlocking the SpectraMax Paradigm Instrument on page 13.

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 [icon] 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 262.

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 263.

- Click **Remove Slide** to delete the selected filter slide. See Removing a Filter Slide on page 264.

- Click **Export selected Slide** to export the filter definitions for the selected filter slide. See Exporting a Filter Slide on page 264.

- Click **Import Slides** to import a filter slide definition file that defines one or more filter slides. See Importing Filter Slides on page 265.

- Click **Load Slides** to replace all the current filter slide definitions with the imported definitions. See Replacing the Filter Slide Definitions on page 266.

- Click **Export Slides** to export all the defined filter slides. See Exporting the Filter Slide Definitions on page 267.

📝 **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 264.

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 267.

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\SMP621\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\SMP621\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 264 or Exporting the Filter Slide Definitions on page 267.

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 the XML file is saved and select the file.

   The default location for filter slide definitions is:
   C:\ProgramData\Molecular Devices\SMP62\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 .

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:\Program Data\Molecular Devices\SMP621\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.

Viewing Instrument Software Support

Each of the supported instruments has related "plug-in" support that defines the interaction between the instrument and the SoftMax Pro Software interface. You can view a list of the of these plug-ins for troubleshooting purposes. Generally, you would view this list after contacting Molecular Devices technical support.
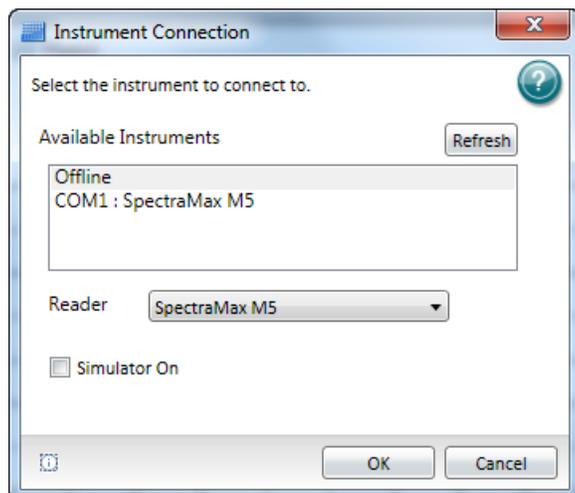
Click the **Information** button on the lower-left of the **Instrument Connection** dialog to open a list of the plug-ins currently installed with the SoftMax Pro Software.

For a list of the instruments that are supported by this version of the SoftMax Pro Software, see Supported Instruments on page 119.

Chapter 9: Using SoftMax Pro GxP Software Features

Molecular Devices provides a fully integrated hardware and software solution for adherence to Good Manufacturing Practices (GMP), Good Laboratory Practices (GLP), and many other regulatory compliance requirements including 21 CFR Part 11. SoftMax® Pro GxP Microplate Data Compliance Software extends SoftMax Pro Software into regulated laboratories working under GMP, GLP, and 21 CFR Part 11 guidelines for secure electronic records. SoftMax Pro GxP Software provides a method for electronically signing data files, and also automatically maintains a document-based audit trail for electronic records. This audit trail preserves user actions and modifications for review even after microplate data is collected and analyzed.

This integrated solution consists of the following components:

- A Molecular Devices microplate reader.

- SoftMax Pro GxP Software for data collection and analysis.

- GxP Admin Software for user management in SoftMax Pro GxP Software.

- MDC File Server (optional) for TCP/IP-based file serving of the User Account files.

The SoftMax Pro GxP Software connects to and controls the Molecular Devices microplate reader, and analyzes all collected data.

Use the GxP Admin Software to set up User Accounts that can be linked to the SoftMax Pro GxP Software through a Network File Server or the optional MDC File Server. See Setting Up User Accounts for SoftMax Pro GxP Software on page 270.

The SoftMax Pro GxP Software is installed on individual computers that are used for data collection or data analysis. Each user account must be linked to a User Accounts file before users can log onto the SoftMax Pro GxP Software and use the software to collect and analyze microplate data.

Setting Up User Accounts for SoftMax Pro GxP Software

Use GxP Admin Software to create and maintain User Account (.edb) files. SoftMax Pro GxP Software uses these User Account files to regulate user access to the features of SoftMax Pro GxP Software and to provide traceability and accountability in electronic records. The User Account file records all authorized users and their associated information, including the following:

- User names, user logon IDs, and passwords.

- Assigned user permissions to SoftMax Pro GxP Software features.

- Global settings pertaining to all listed users, such as password aging, non-network use, guest access, and so on.

- Audit histories of all administrator actions GxP Admin, such as user creation, modification, and so on.

If set by the administrator, SoftMax Pro GxP Software users do not require continual access to a User Accounts (.edb) file in order to use the SoftMax Pro GxP Software for data collection and analysis when no network connection is available, such as during a corporate network failure or using SoftMax Pro GxP Software while traveling.

SoftMax Pro GxP Software users can be connected to a User Account file in one of two ways:

- Network File Server (File Path connection) where the User Account file is placed on a secure, shared network file server, and the system browser is used to link each SoftMax Pro GxP Software installation directly to the defined User Account file.

- MDC File Server (TCP/IP Server connection) where the User Account file is placed on a secure network location and each SoftMax Pro GxP Software installation is linked indirectly to the defined User Account file using a TCP/IP address provided by the MDC File Server.

For information about setting up User Account files, see the *GxP Admin Software User Guide*.

Connecting a User Account File

Before users can log onto the SoftMax Pro GxP Software, the installed program needs to be connected to a User Account file. You can also change the associated User Account file.

To define the User Account file connection:

1. Start the SoftMax Pro GxP Software.

2. In the **Log On** dialog, click the **Change** button.

   If the software is already running with a user logged on, then go to the **GxP** tab in the ribbon. In the **User Account** area, click **User Log Off** and then click **Select Account Database** .

3. In the **User Accounts Connection** dialog, click the **Connection Type** for either a **File Path** or **TCP/IP Server** connection.

   • If you are setting up a **File Path** connection, then click the **Browse** button and use the **Open** dialog to locate the User Account (.edb) file on your network. Click **Open** to return to the **User Accounts Connection** dialog where the selected file path is shown.

   • If you are setting up a **TCP/IP Server** connection, then type the server computer **Name or IP Address** and the **Port Number** in the fields.

   For information about setting up User Account files, see the *GxP Admin Software User Guide*.

4. Click **Connect**.

5. In the **Log On** dialog, type your user name and password.

6. Click **Log On**.

After establishing the User Account file connection, the defined users can log on and begin acquiring and analyzing data with the SoftMax Pro GxP Software.

Logging onto SoftMax Pro GxP Software

Each time you start the SoftMax Pro GxP Software, you are asked to log on before you can continue. If the software is running and the user has logged off, go to the **GxP** tab in the ribbon, and then in the **User Accounts** section, click **User Log On** .

In the **Log On** dialog, type your user ID and your password and then click **Log On**.

> **Note:** Although the user ID is not case sensitive, the password is.

If you want to associate the installed program with a User Account file, or change the associated User Account file, click the **Change** button. See Connecting a User Account File on page 270.

If the GxP Admin Software administrator has allowed it, those without an account can log on as a guest. Guests can open, view, and print any existing data files or protocols. They are not able to modify or save changes to any existing data file or protocol. If guest log on is allowed, the **Log On** dialog explicitly states this, and a **Guest** button is available for logging on as a guest. For more information, see the *GxP Admin Software User Guide*.

Viewing the User Information

To view information about the current user, go to the **GxP** tab in the ribbon, and

then in the **User Accounts** section, click **Account Information** .

The **User Account Information** dialog provides information about the current user.
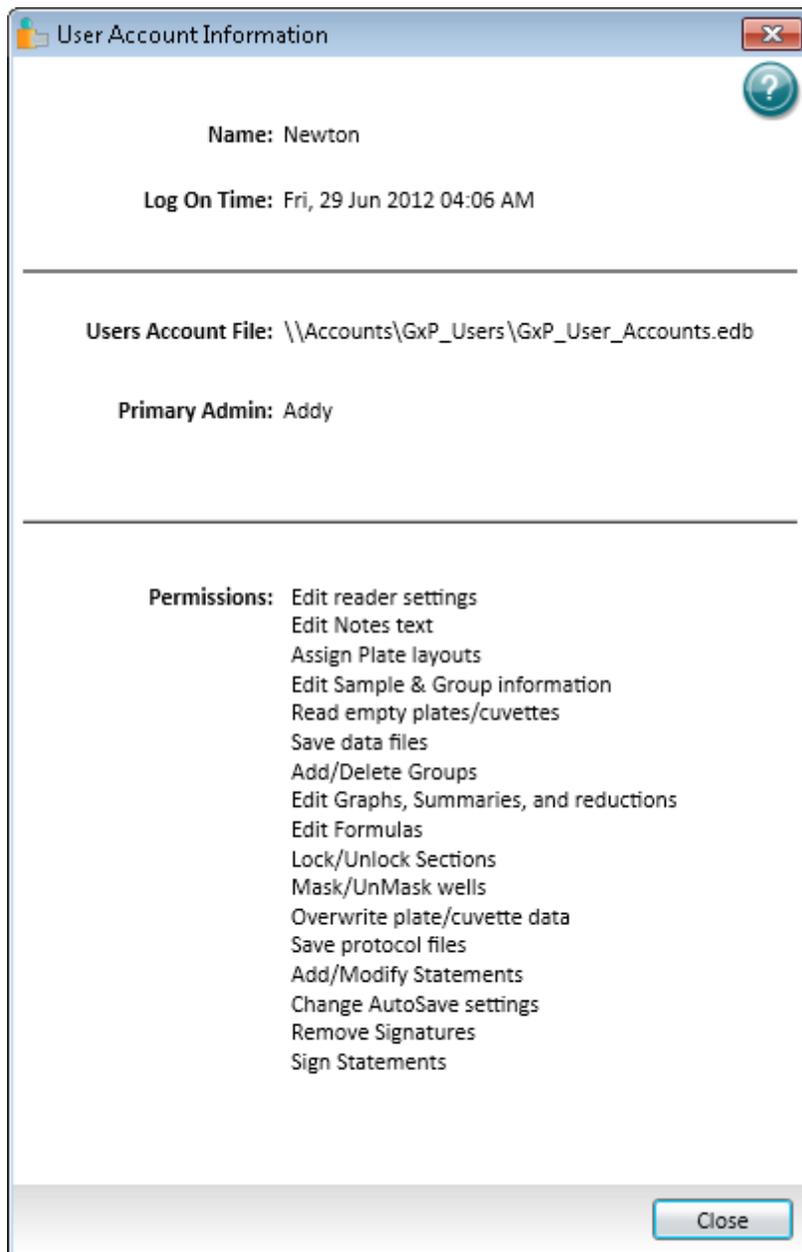
- The top section shows the user name and the time that the user logged on.

- The middle section shows the path to the associated User Account file and the name of the GxP Admin Software administrator.

- The lower section shows the permissions assigned to the user by the GxP Admin Software administrator.

For more information about user accounts, see the *GxP Admin Software User Guide*.

To close the **User Account Information** dialog, click **Close**.

Changing the User Password

Users cannot change their user names, but they can change their passwords. The GxP Admin Software administrator can also define rules for requiring password changes.

To change your password:

1. Go to the **GxP** tab in the ribbon, and then in the **User Accounts** section, click

   Change Password .

2. In the **Change Password** dialog, type your old password in the first field.
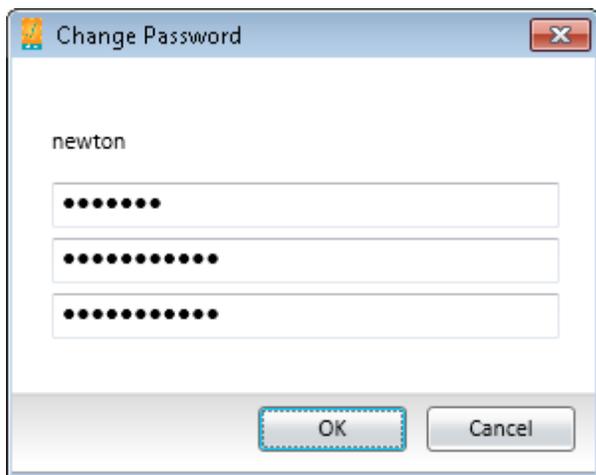
3. Type your new password in the second field, and then type it again in the third field to confirm it.

   The software compares the second and third field to make sure that they match exactly before the new password is saved.

   > **Note:** The password must be between 6 and 32 characters with at least one alpha character and at least one numeric character. The password is case sensitive.

4. Click **OK**.

The next time you log onto the software, you will need to use the new password.

Working with GxP Statements

A user with the correct permission can view, add, and edit GxP statements to a SoftMax Pro GxP Software data file. You can use GxP statements to designate that an Experiment has been approved, reviewed, or verified.

A user with the correct permission can apply an electronic signature to a statement. See Signing a GxP Statement on page 278. After a GxP statement is signed, the SoftMax Pro GxP Software locks the data file to prevent any further changes to the file. To unlock the file, remove all the statement signatures. See Removing Signatures from All GxP Statements on page 279.

To work with GxP statements, go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** .
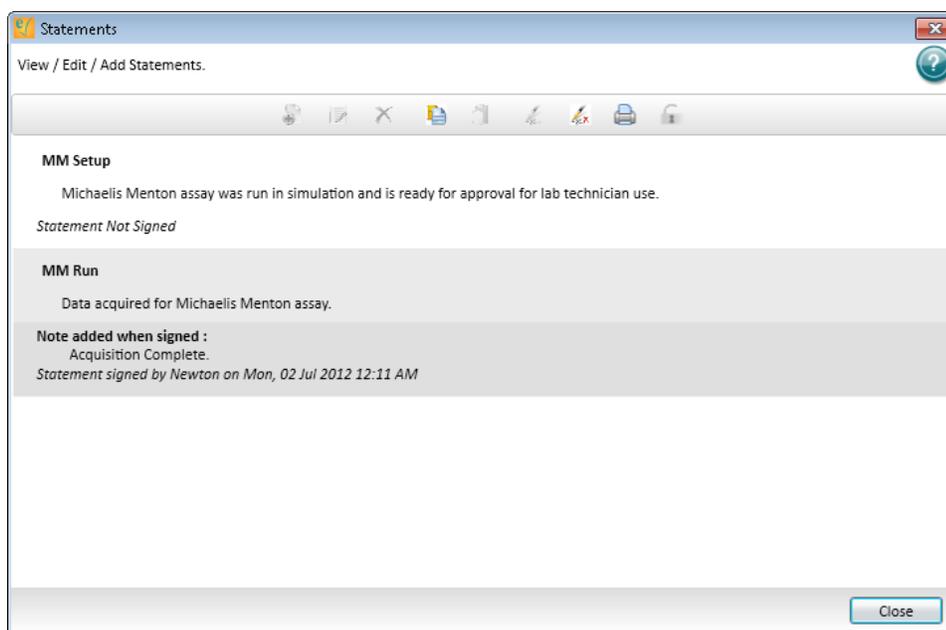
In the **Statements** dialog, you can work with GxP statements by clicking the tools in the toolbar at the top of the dialog.

To close the **Statements** dialog, click **Close**.

The statements status button in the upper-right corner of the work area displays the number of statements in the file, and how many statements are signed.

To quickly open the **Statements** dialog, click the statements status button.

Adding a GxP Statement

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** .

2. If there is more than one statement displayed in the **Statement** dialog, click the statement directly above where you want to add the new statement.

3. Click **Add Statement** in the toolbar at the top of the dialog.
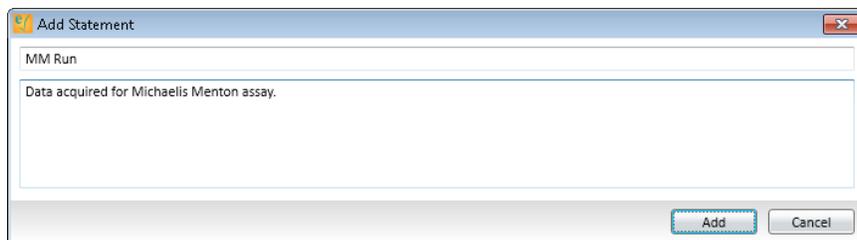
4. In the **Add Statement** dialog, click in the upper field and type a title for the statement.

    A title is required for each statement.

5. Click in the lower field and type the content of the statement.

6. Click **Add**.

Editing a GxP Statement

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** .

2. In the **Statement** dialog, click the statement that you want to edit.

3. Click **Edit Statement** in the toolbar at the top of the dialog.

4. In the **Edit Statement** dialog, click in the upper field and edit the title for the statement.

5. Click in the lower field and edit the content of the statement.

6. Click **Save**.

Deleting All GxP Statements

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click

   Statements .

2. Click **Delete Statements** ✗ in the toolbar at the top of the dialog.

   > ⚠ **CAUTION:** Deleting statements deletes all the GxP statements in the
   > SoftMax Pro GxP Software data file. There is no undo for this. After
   > deleting the statements, they cannot be retrieved.
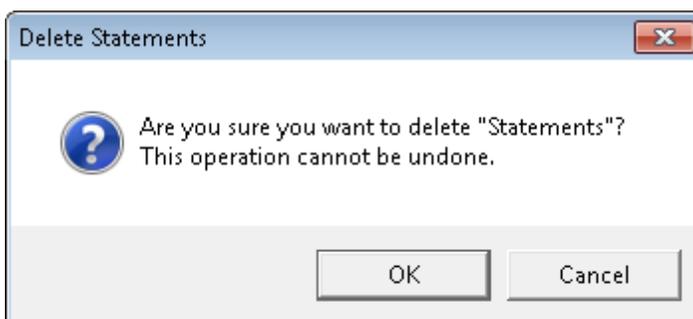
3. In the **Delete Statements** message, click **OK** to delete all the GxP statements
   in the SoftMax Pro GxP Software data file.

Duplicating a GxP Statement

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click

   Statements .

2. In the **Statement** dialog, click the statement that you want to duplicate.

3. Click **Copy Statement** 📄 in the toolbar at the top of the dialog.

4. Click the statement directly above where you want to paste the copied
   statement.

5. Click **Paste Statement** 📋 in the toolbar at the top of the dialog.

To edit the pasted statement, click the statement to select it and then click **Edit
Statement** 📝 in the toolbar at the top of the dialog. See
.

Signing a GxP Statement

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** .

2. In the **Statement** dialog, click the statement that you want to sign.

> 📓 **Note:** If the statements are locked, they cannot be signed. To unlock the statements, click **Unlock Statements** 🔒 in the toolbar at the top of the **Statements** dialog.

3. Click **Sign Statement** ✍ in the toolbar at the top of the dialog.
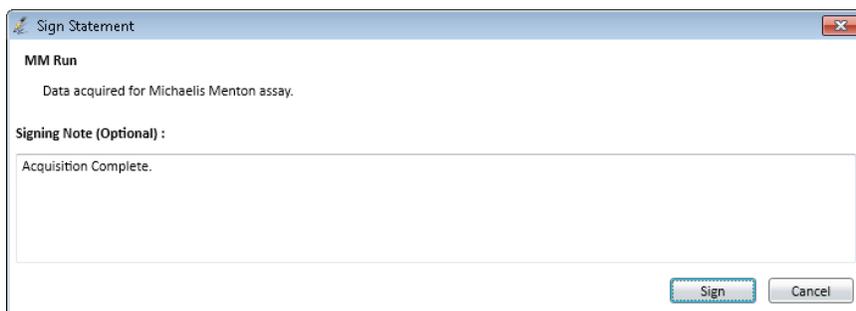
4. In the **Sign Statement** dialog, verify that the text at the top is the statement that you want to sign.

5. Click in the lower field and type a signing statement, if applicable.

6. Click **Sign**.
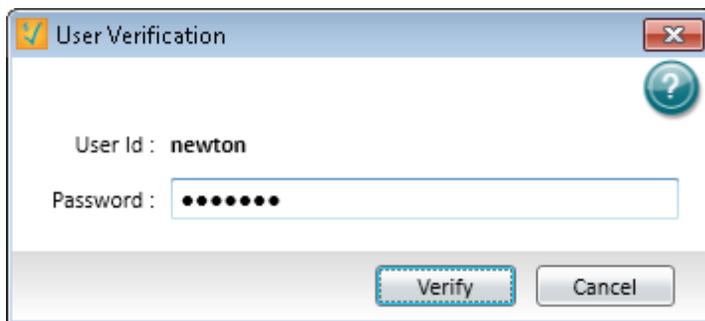
7. In the **User Verification** dialog, type your password.

8. Click **Verify**.

> 📓 **Note:** After a GxP statement is signed, the SoftMax Pro GxP Software locks the data file to prevent any further changes to the file. To unlock the file, remove all the statement signatures. See Removing Signatures from All GxP Statements on page 279.

Removing Signatures from All GxP Statements

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** .

2. Click **Remove all signatures** in the toolbar at the top of the dialog.

> ⚠️ **CAUTION:** Removing signatures removes the signatures from all the GxP statements in the SoftMax Pro GxP Software data file. There is no undo for this. After the signatures are removed, the data file is unlocked and changes can be made to the file.
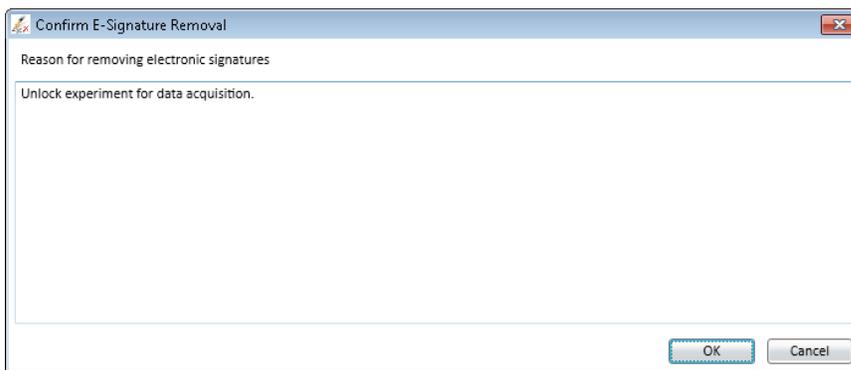
3. In the **Confirm E-Signature Removal** dialog, type a reason for removing the signatures.

4. Click **OK**.
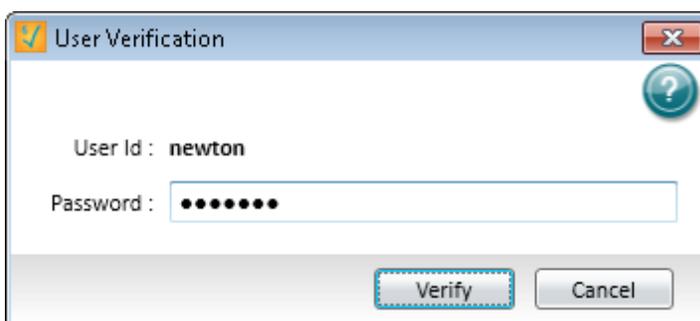
5. In the **User Verification** dialog, type your password.

6. Click **Verify**.

Printing GxP Statements

By default, GxP statements do not print when users print the contents of the data file. You can allow the statements to print or block the statements from printing in the **Statements** dialog .

To change the GxP printing options, go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** to open the **Statements** dialog.

- To allow the statements to be printed with the data file, click **Enable printing of Statements** in the toolbar at the top of the dialog.

- To prevent the statements from printing with the data file, click **Disable printing of Statements** in the toolbar at the top of the dialog.

The button switches between and to indicate whether or not statements will be printed.

For information about printing the contents of a data file, see Printing on page 60.

Locking and Unlocking GxP Statements

You can lock the statements to prevent changes to the **Statements** dialog.

You cannot lock signed statements. After a GxP statement is signed, the SoftMax Pro GxP Software locks the data file to prevent any further changes to the file. To unlock the file, remove all the statement signatures. See Removing Signatures from All GxP Statements on page 279.

To change the GxP statements lock options, go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Statements** to open the **Statements** dialog.

- To prevent changes to the statements, click **Lock Statements** in the toolbar at the top of the dialog.

- To allow changes to the statements, click **Unlock Statements** in the toolbar at the top of the dialog.

The button switches between and to indicate whether or not statements are locked.

All the statements are locked or unlocked at the same time.

With the statements locked, you can still change the printing options. See Printing GxP Statements on page 280.

Viewing the GxP Audit Trail

The **Audit Trail** dialog gives you read-only access to the audit trail for the open data file, and allows you to attach a note to the audit trail.

To view the audit trail, go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Audit Trail** .
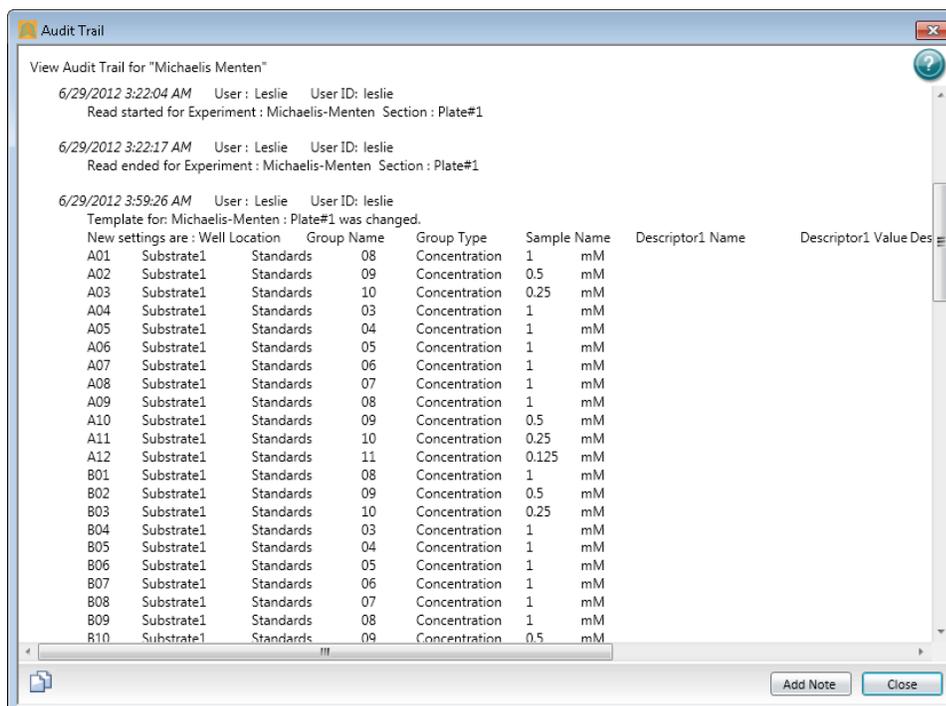
Each entry in the audit trail has a time-and-date stamp, and identifies the user performing the task.

To add a note to the audit trail, see Adding a Note to the GxP Audit Trail on page 282.

To copy the entire audit trail to the Windows clipboard, click **Copy to clipboard** in the lower-left corner of the dialog. You can then paste the audit trial into a text editor or word processor for saving or printing.

To close the **Audit Trail** dialog, click **Close**.

Adding a Note to the GxP Audit Trail

1. Go to the **GxP** tab in the ribbon, and then in the **Data** section, click **Audit Trail** .

2. In the **Audit Trail** dialog, click **Add Note**.
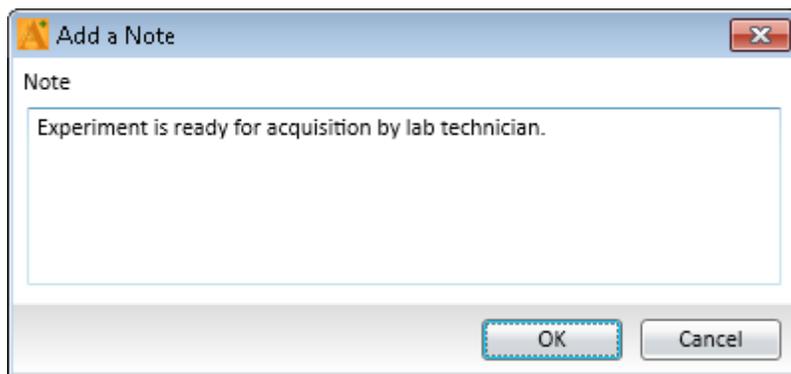
3. In the **Add a Note** dialog, type the content of the note.

4. Click **OK**.

The note is added to the end of the audit trail and becomes part of the permanent record for the open data file.

Locking and Unlocking Sections

In the SoftMax Pro GxP Software, you can lock the individual sections in an experiment to prevent changes to them. A locked section cannot be deleted nor have its data altered. When a locked section is duplicated, the duplicate section is unlocked by default. Locked sections can be printed with the data file.

To lock or unlock a section, click the section to select it and then click **Lock Selection** 🔒 at the top of the Navigation Tree.
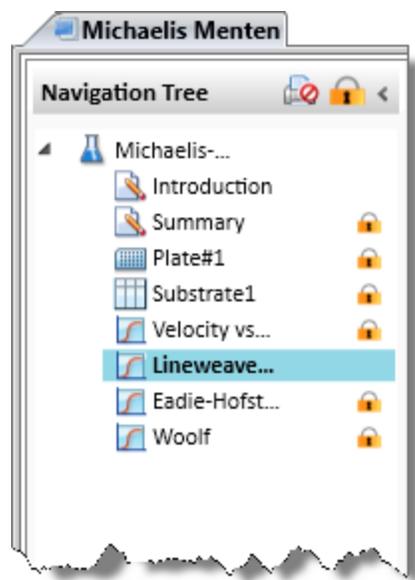
To lock or unlock all the sections in an Experiment, click the Experiment to select it and then click **Lock Selection** 🔒 at the top of the Navigation Tree.

When a section is locked, no direct changes can be made to it, with the following exceptions:

- In a **Plate** section, the data cannot be overwritten. However, a **Plate** section that has no data can have data acquired and added to the section.

- The name of a **Group** section can be changed indirectly in the **Group Settings** dialog.
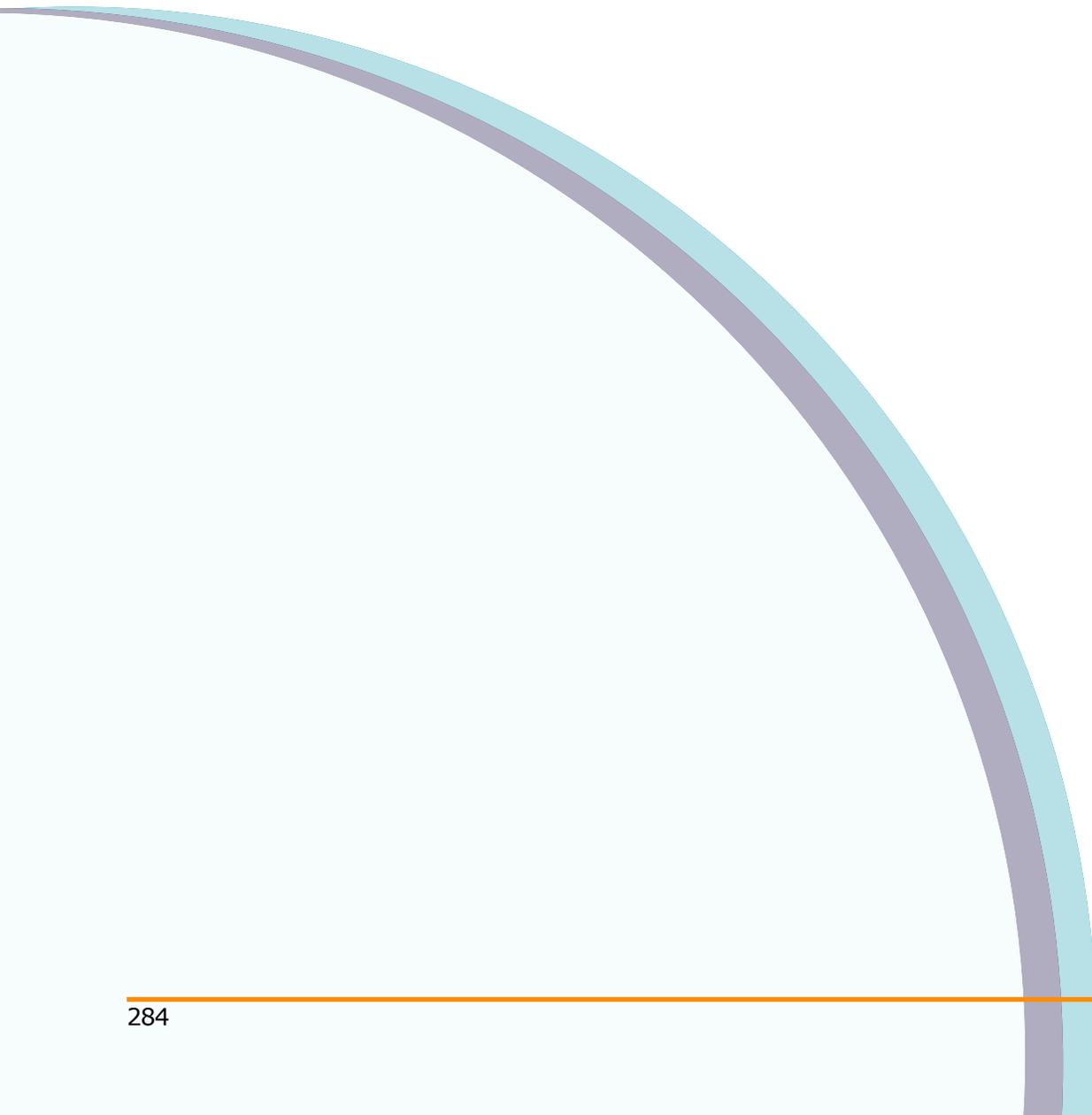
Glossary

Numerics

21 CFR Part 11

The FDA guidelines for trustworthy electronic records, known as 21 CFR Part 11, requires companies to employ procedures and controls designed to ensure the authenticity, integrity, and when appropriate the confidentiality of electronic records, and to ensure that the signer cannot readily repudiate the signed record as not genuine. To satisfy this requirement persons must, among other things, employ procedures and controls that include the use of computer-generated time stamps.

> **Note:** No software by itself can be 21 CFR Part 11 compliant. The SoftMax Pro GxP Software provides features that allow users to demonstrate compliance with these regulations.

See Using SoftMax Pro GxP Software Features on page 269.

A

Absorbance

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.

$$A = \log_{10}(I_0 /I) = -\log_{10}(I/I_0)$$

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.

See ABS Read Mode on page 113.

AutoRead

AutoRead enables automatic reading of **Plate** sections in the order they appear within a single Experiment. When you start a read for any **Plate** section in the Experiment that is enabled for automatic reading, all enabled **Plate** sections following the selected **Plate** section are read. Enabled **Plate** sections above the selected **Plate** section are not read. You can set intervals (delay times) between the plate readings, if desired.

See Enabling Auto Read on page 195.

Auto Save

When Auto Save 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.

You can add as many Auto Save instances as desired, each with its own settings.

> **Note:** Auto Save settings are saved with each document.

See Setting Auto Save Options on page 56.

B

Bottom Read

You can set up a bottom read in the **PMT and Optics** category of the **Settings** dialog. Select the **Read From Bottom** check box to have an instrument with bottom-read capability read up through the bottom of the microplate rather than down from the top.

See PMT and Optics Settings on page 152.

C

Calibrate

For Absorbance reads, the Calibrate setting tells the instrument to make an air calibration measurement at each wavelength before reading the microplate, as determined by the type of instrument and the read settings. For kinetic readings this measurement is sometimes taken between readings during a run.

For Fluorescence instruments, the measurement occurs before reads, and sometimes between kinetic reads if the interval is long enough.

If you turn calibration off, SoftMax Pro Software uses the calibration values stored in the firmware of the instrument.

See More Settings on page 156.

Cutoff

The term cutoff refers to filters used to block unwanted residual excitation light and minimize background interference.

No emission cutoff filter is used when a Luminescence read mode is selected. With other read modes, 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 on the wavelengths chosen for reading. When a Spectrum read type is selected, a manual setting for the emission monochromator is required, with the default being no cutoff filter.

See Wavelength Settings on page 143.

Cuvette Set Section

Cuvette Set sections are used to collect data from the cuvette port of the SpectraMax M2 and M2e Multi-Mode Microplate Readers, SpectraMax M5 and M5e Multi-Mode Microplate Readers, and the SpectraMax Plus 384 Absorbance Microplate Reader. You can use a **Cuvette Set** section 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.

See Using a Cuvette Set Section on page 90.

D

Default Protocol

After the SoftMax Pro Software is installed, the Basic Endpoint protocol is set as the default protocol with the filename **default.spr**. Whenever you create a new document, the **default.spr** protocol file is opened as an untitled document that contains the settings from the default protocol file.

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 document, the settings in the **default.spr** file that you have saved are loaded into an untitled document.

See Saving a Protocol as the Default Protocol on page 49.

E

Emission Spectrum Scan

An emission spectrum scan measures fluorescence or luminescence across a spectrum of wavelengths for the emitted light from a fixed excitation wavelength. The default value reported for each well is the wavelength of maximum emission in either RFU for fluorescence mode or RLU for luminescence mode.

See Excitation Spectrum Scan on page 288.

End Time

End Time in a kinetic read specifies the time at which to stop showing data in the display and exclude it 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 for the kinetic read.

See Performing Data Reduction on page 214.

Endpoint

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.

See Endpoint Read Type on page 111.

Excitation Spectrum Scan

An emission spectrum scan measures fluorescence at a single emission wavelength for the emitted light across a spectrum of excitation wavelengths. The default value reported for each well is the wavelength of maximum fluorescence excitation in RFU.

See Emission Spectrum Scan on page 288.

F

Fluorescence

Fluorescence occurs when absorbed light is re-radiated at a longer wavelength. Fluorescence intensity measures 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

See FL Read Mode on page 114.

Fluorescence Polarization

Fluorescence polarization detection is similar to fluorescence intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Plate readers measure FP of the sample by detecting light emitted both parallel and perpendicular to the plane of excitation.

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.

See FP Read Mode on page 116.

Fluorophore

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

See FL Read Mode on page 114, TRF Read Mode on page 115, and FP Read Mode on page 116.

G

G Factor

G factor, or 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.

See Performing Data Reduction on page 214.

Gain

Gain is the amount of increase in signal power expressed as the ratio of output to input for a photomultiplier tube (PMT).

See PMT and Optics Settings on page 152.

Group

Wells can be assigned to group types using the Template Editor. Depending on the protocol, certain group types, such as Standard or Unknown, can be created automatically. You can create other groups as required.

See Defining a Group on page 164.

K

Kinetic

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

The values calculated based on raw kinetic data are VMax, VMax per Sec, Time to VMax, and Onset Time. Kinetic readings can be single-wavelength or multiple-wavelength readings.

See Kinetic Read Type on page 111.

L

Lag Time

Lag Time in a kinetic read defines the period of very slow growth of microorganisms or the rate of reaction that can precede the rapid or linear phase of reaction.The 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.

See Performing Data Reduction on page 214.

Luminescence

Luminescence 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.

See LUM Read Mode on page 114.

M

Mask

The Mask feature hides selected data so that they are not used for calculations and are not reported. Masking is commonly used to suppress outliers from data reduction calculations.

Masking can be used as a "what if?" tool. For example, if you have included a group blank in a template and want to see the data both with and without the blank, masking the group blanks suppresses the blanking function, while unmasking them enables it again.

See Masking Wells or Cuvettes on page 214.

O

Onset Time

Onset time is a 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).

See Kinetic Data Reduction Options on page 223.

Optical Density (OD)

Optical density (OD) is 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.

P

PathCheck Technology

The PathCheck® Pathlength Measurement Technology 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 absorptivity 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.

See PathCheck Pathlength Measurement Technology on page 148.

Photomultiplier Tube (PMT)

A Photomultiplier Tube (PMT) is a vacuum tube that can detect light 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

A **Plate** section is 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 want to have two **Plate** sections. You can create as many **Plate** sections as you need.

See Using a Plate Section on page 82.

Protocol File

Protocol files are experiment template files that contain microplate well layout assignments and all other reader configuration information and reduction parameters, but no data. Protocol files can be useful if you repeat a particular type of experiment frequently.

See Managing Protocol Files on page 48.

R

Raw Data

Raw data is the signal reported from the instrument with no alteration. This is reported as OD, RFU, or RLU, depending on the read mode.

Raw Data Mode

In Absorbance (ABS) read mode or Fluorescence Polarization (FP) read mode, you can select the type of raw data to display and use for reduction calculations.

For Absorbance reads, you can choose whether to display absorbance data as Optical Density or %Transmittance.

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.

See .

Read Mode

SoftMax Pro Software supports the following Read Modes:

-
-
-
-
-
-
-

Read Type

SoftMax Pro Software supports the following Read Types:

-
-
-
-

Reduced Data

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

See Performing Data Reduction on page 214.

Reference

A reference reading can be taken either on air or using a cuvette containing the buffer of your sample.

See Collecting Data From a Cuvette on page 203.

S

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. This 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 series name can be incremented as well.

See Creating a Series on page 165.

Slope

The slope reduction option 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 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.

See Kinetic Data Reduction Options on page 223.

Spectrum

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.

See Spectrum Read Type on page 111, Emission Spectrum Scan on page 288, and Excitation Spectrum Scan on page 288.

Stokes Shift

The Stokes shift is the difference between the wavelengths of the excitation and emission maxima, or peaks.

See Emission Spectrum Scan on page 288 and Excitation Spectrum Scan on page 288.

T

Template Editor

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

The **Template Editor** is a representation of the microplate or 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.

See Configuring a Microplate Template on page 162.

Time-Resolved Fluorescence (TRF)

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 FL detection. 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.

See TRF Read Mode on page 115.

%Transmittance

%Transmittance is the ratio of transmitted light to the incident light for absorbance reads.

$$T = I/I_0$$

$$\%T = 100T$$

where I is the intensity of light after it passes through the sample and I_0 is incident light before it enters the sample.

V

Vmax Points

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.

See Kinetic Data Reduction Options on page 223.

Vmax Rate

Vmax Rate is reported as signal/min (milli-OD, RFU, or RLU units per minute) for a Kinetic read. 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 can also be reported as units per second (the default for Fluorescence and Luminescence modes).

See Kinetic Data Reduction Options on page 223.

W

Well Scan

A Well Scan read can take readings 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.

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.

See Well Scan Read Type on page 112.

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