SoftMax® Pro

Data Acquisition and Analysis Software

Version 7.0.2

User Guide

MOLECULAR DEVICES

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

Contents

Chapter 1: Installation and Setup

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SoftMax® Pro Data Acquisition and Analysis Software controls Molecular Devices® spectrophotometers and absorbance, luminescence, and fluorescence microplate readers and detection platforms. For a complete list of the instruments this release of the SoftMax Pro Software supports, see Supported Instruments on page 179.

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.

The software includes over 120 assay protocols to speed life science research and drug discovery assay development and screening. You 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 specialized data collection and analysis needs through custom assay development.

SoftMax Pro Software is widely integrated with industry-leading robotics systems.

The SoftMax Pro Software can collect data from one or more microplates and store it in a single data file, using the same or different instrument settings for different microplates. Assays that require a read in two or more read modes or read types can be combined in a single experiment and run with a single command in the software when you define separate microplate reads and enable Auto Read. See Enable Auto Read on page 301.

This guide describes features available in SoftMax Pro Software version 7.0.2 and later. SoftMax Pro Software 7.0.2 is available for a computer that uses the Windows 7, Windows 8, or Windows 10 operating system.

This section contains the following topics:

Computer System Requirements

You can install SoftMax Pro Software version 7.0.2 on a computer with the following system specifications.

Table 1-1: Minimum and Recommended Requirements for SoftMax Pro Software

Item	Minimum	Recommended
Processor	Single-core, 2 GHz or faster	Quad core or faster
Operating system	Windows 7 (SP1 or newer), Professional 32-bit or 64-bit (x86 or x64) and .NET Framework 4.6 (.NET Framework 4.6 is installed automatically by the SoftMax Pro Software installer if required.)	- Windows 7 (SP1 or newer) Professional or Enterprise 32-bit or 64-bit (x86 or x64) - Windows 8 Professional or Enterprise, 32-bit or 64-bit (x86 or x64) - Windows 10 Professional or Enterprise, 32-bit or 64-bit (x86 or x64) - The SoftMax Pro MiniMax Imaging Edition must be installed on a 64-bit operating system.
Data connection	RS-232 serial port or USB 2.0 port Network port (depending on the instrument)	USB 2.0 ports For instruments that require an RS-232 serial port, you can use a USB 2.0 port with a Keyspan USB-to-serial adapter. Keyspan USB-to-serial adapters have been field tested and are approved by Molecular Devices. Network port
Memory	2 GB RAM	4 GB RAM If run on a virtual machine, Molecular Devices recommends a minimum of 6 GB RAM. For automation, Molecular Devices recommends a minimum of 8 GB RAM.
Hard drive	500 MB of available space	1 GB of available space, or more
Graphics display	Graphics display adapter 1024 x 768 or higher-resolution display	32-bit graphics display with 256 MB video RAM 1280 x 1024 or higher-resolution display
Software installation and activation	CD/DVD drive Internet connection or external USB drive	CD/DVD drive Internet connection or external USB drive

> **Note:** Installation and usage of the SoftMax Pro Software on the Windows XP operating system is no longer supported. The software is neither tested nor validated on Windows XP.

To prevent data loss, turn off all sleep and hibernation settings for the hard disk, the CPU, and the USB ports. Disable automatic Windows Updates. Update Windows manually when the instrument is not used by the software. You can set these options in Windows Control Panel. See Required Computer Settings on page 11.

The SpectraMax MiniMax 300 Imaging Cytometer is shipped with a computer that meets greater minimum computer system specifications than those required for the standard installation of the SoftMax Pro Software. See Optimize Your Computer for Image Acquisition on page 298.

Install SoftMax Pro Software

> **Note:** Molecular Devices recommends that you disable your anti-virus program before you install the SoftMax Pro Software. Anti-virus programs can interfere with the installation process.

To install the SoftMax Pro Software:

1. Power off all instruments you connect to the computer.
2. Start the installation program.
   - If you download the software installation, double-click the **SoftMaxPro7.0.2Setup.exe** file to start the installation program.
   - If you install from a USB flash drive, insert the SoftMax Pro Software flash drive into a USB port. Navigate to the flash drive and double-click **SoftMaxPro7.0.2Setup.exe**.
   - If you install from a CD, insert the SoftMax Pro Software CD into the CD drive. If the installation program does not automatically start, navigate to the CD drive and double-click **SoftMaxPro7.0.2Setup.exe**.
3. Wait while the initialization process occurs until the Installation Wizard appears. On the Installation wizard Welcome page, click **Next** to display the License Agreement page.
4. Select the **I accept the terms of the license agreement** check box and click **Next**.

5. Select the SoftMax Pro Software edition to install.

> **Note:** You must have the correct license for the software edition you select to install.

- **SoftMax Pro Standard Edition** supports most Molecular Devices microplate readers. The Standard edition does not support the SpectraMax MiniMax 300 Imaging Cytometer. For a complete list of the instruments this release of the SoftMax Pro Software supports, see Supported Instruments on page 179.
- **SoftMax Pro MiniMax Imaging Edition** must be the edition you install to support the features of the SpectraMax MiniMax 300 Imaging Cytometer. The MiniMax Imaging edition requires a 64-bit operating system.
- **SoftMax Pro GxP Edition** supports the use of the software in a regulated environment. The GxP edition does not support the SpectraMax MiniMax 300 Imaging Cytometer. For information about how to use the features of this edition, see SoftMax Pro GxP Software on page 417.

6. Follow the prompts through the installation wizard to finish the software installation. Installation can take several minutes.

> **Note:** SoftMax Pro Software version 7.0.2 installation includes the QuickSync Tool for use with the SpectraMax® iD3 Multi-Mode Microplate Reader. The QuickSync Tool icon ⟳ appears along the bottom of your computer screen. See Use the QuickSync Tool on page 181.

7. Before you start the software, power on the instrument you connect to the computer.

> **Note:** You should add the SafeNet HASP License Manager Service (hasplms.exe) to the list of trusted applications for the anti-virus programs on the computer.

Install the Software Remotely or From a Command Line

You can install the SoftMax Pro Software silently from a command line. Make sure you install the correct edition of the software.

- To silently install the **SoftMax Pro Standard Edition**, type the command:
  `SoftMaxPro7.0.2Setup.exe /s`
- To silently install the **SoftMax Pro MiniMax Imaging Edition**, type the command:
  `SoftMaxPro7.0.2Setup.exe /s EDITIONIMAGING=TRUE`
- To silently install the **SoftMax Pro GxP Edition**, type the command:
  `SoftMaxPro7.0.2Setup.exe /s EDITIONGXP=TRUE`

To view and follow the installation wizard, omit `/s` in the command.

To uninstall all editions of the software, type the command:
`SoftMaxPro7.0.2Setup.exe UNINSTALL=YES`

> **Note:** Some IT environments, such as those that use remote installation with deployment tools like SCCM, require an installer in MSI format. To obtain a copy of the MSI installer, contact Molecular Devices technical support. See Obtain Support on page 23.

Required Computer Settings

If your computer hibernates or turns off during data acquisition, the transfer of data from the instrument to the software can be interrupted, which can result in data loss.

To prevent data loss, turn off all sleep and hibernation settings for the hard disk, the CPU, and the USB ports. Disable automatic Windows Updates. Update Windows manually when the instrument is not used by the software.

Set these options in Windows Control Panel.

1. Open **Control Panel**.
2. Click **Hardware and Sound**.
3. Under **Power Options**, click **Change when the computer sleeps**.
4. Click **Change advanced power settings**.
5. On the Power Options dialog, set **Hard disk > Turn off hard disk after** to **Never**.
6. Set **Sleep > Sleep after** to **Never**.
7. Set **Sleep > Hibernate after** to **Never**.
8. Set **USB settings > USB selective suspend setting** to **Disabled**.
9. Click **OK**.
10. Return to the Control Panel home page.
11. Click **System and Security**.
12. Under **Windows Update**, click **Turn automatic updating on or off**.
13. Under **Important Updates**, clear the **Install updates automatically** check box. Select one of the other options.
14. Click **OK**.
15. Close **Control Panel**.

Decimal Symbol Must be a Period

For the SoftMax Pro Software to properly parse or execute calculations, the regional options for the computer must use the period symbol ("."") for the decimal symbol. This can be an issue if the Region and Language setting for the computer is something other than English.

To set custom regional settings, go to **Control Panel > Regional and Language**.

Manually Install USB Drivers in Windows 7

For some Windows 7, 64-bit operating system installations, automatic installation of the USB instrument driver for some instruments does not occur due to elevated security settings.

If the SoftMax Pro Software cannot connect to your instrument after you install the software, shut down the SoftMax Pro Software and restart Windows.

If the Windows restart does not permit access to the instrument, then do the following to install the driver:

1. Open **Control Panel**.
2. Click **Hardware and Sound**.
3. Under **Devices and Printers**, click **Device Manager**.
4. On the Device Manager, double-click the unknown device with the yellow warning icon.
5. Select the **Driver** tab and click **Update Driver**.
6. Click **Browse my computer for driver software**.
7. Click **Browse** and select the SoftMax Pro Software installation folder.
   The default installation path is:
   C:\Program Files (x86)\Molecular Devices\SoftMax Pro 7.0.2.
8. On the Windows Security message, click **Install this driver software anyway**.

Grant TCP/IP Access to MDC File Server

When you use the SoftMax Pro GxP Software with MDC File Server on a Windows 7, 64-bit operating system, the Windows Firewall sometimes prevents user access to the MDC File Server through TCP/IP.

To grant user access through TCP/IP, create a new inbound rule and a new outbound rule in the Windows Firewall Advanced Settings.

Access Windows Firewall Advanced Settings

1. Open **Control Panel**.
2. Click **System and Security**.
3. Click **Windows Firewall**.
4. In the left pane, click **Advanced settings** to open the Windows Firewall with Advanced Security dialog.

Create an Inbound Rule

1. In left pane, on the Windows Firewall with Advanced Security dialog, click **Inbound Rules**.
2. In the right pane, click **New Rule**.
3. In the **New Inbound Rule Wizard**, click **Port**.
4. Click **Next**.
5. Click **TCP**.
6. Click **Specify local ports**.
7. Enter **9001** in the field.
8. Click **Next**.
9. Complete the rest of the wizard based on your network configuration and the access rules your system administrator defines.

Create an Outbound Rule

1. In left pane on the Windows Firewall with Advanced Security dialog, click **Outbound Rules**.
2. In the right pane, click **New Rule**.
3. In the **New Outbound Rule Wizard**, click **Port**.
4. Click **Next**.
5. Click **TCP**.
6. Click **Specify local ports**.
7. Enter **9001** in the field.
8. Click **Next**.
9. Complete the rest of the wizard based on your network configuration and access rules your system administrator defines.

Uninstall SoftMax Pro Software

Before you uninstall the program, back up your data and saved files to a folder outside of the SoftMax Pro Software folder.

1. Click **Start > Control Panel**.
2. Click **Programs and Features**.
3. In the program list, select **SoftMax Pro 7.0.2**.

   To uninstall the GxP edition, click **SoftMax Pro 7.0.2 GxP**.
4. Click **Uninstall** or **Remove**.
5. Follow the prompts to uninstall the program.

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

Start the Software

To start the software under normal conditions, wait for the connected instrument to complete its start-up sequence.

- **SoftMax® Pro Software**: Double-click the **SoftMax Pro 7.0.2** icon on the desktop or from the Windows Start menu, click **Start > All Programs > Molecular Devices > SoftMax Pro 7.0.2 > SoftMax Pro 7.0.2**.
- **SoftMax Pro GxP Software**: Double-click the **SoftMax Pro 7.0.2 GxP** icon on the desktop or from the Windows Start menu, click **Start > All Programs > Molecular Devices > SoftMax Pro 7.0.2 GxP > SoftMax Pro 7.0.2 GxP**.
  - Enter your user name and password, and then click **Log On**. See Log onto SoftMax Pro GxP Software on page 421.

> **Note:** When you connect to the SpectraMax i3x and i3 Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, or the FilterMax F3 and F5 Multi-Mode Microplate Readers for the first time, the latest firmware updates automatically install.

> **Note:** You can choose to run the SoftMax Pro Software with the computer connected to the instrument or with the computer not connected to the instrument. When you do not connect the computer to an instrument, you cannot acquire data. To do operations that require data you must open an existing data file.

Activate Software License

The software product key is included with the SoftMax Pro Software package. The instrument serial number is located on a label affixed to the rear of the instrument.

Activate Software License

If your license is inactive or is ready to expire, the Software License Activation dialog appears when you start the SoftMax Pro Software.

To update the license when the software is already running, select the **Help** tab in the ribbon and click **Software License** .

The Software License Activation dialog displays the current status for each available license. For information about the features of your license or to obtain a new license key, contact your Molecular Devices representative.

To activate the SoftMax Pro Software license, click **Activate** to open the Activate Online dialog.

- If you have access to the Internet, enter the **Product Key** in the field, click **Activate Online**, and then follow the prompts to activate the license.

- If you do not have access to the Internet, click **Activate Offline** and follow the prompts to activate the license.

  To activate offline, you need the product key, a computer that has access to the Internet, and a USB drive for the transfer of files between the computers.

  On the Internet-enabled computer, go to:

  https://smplicensing.moleculardevices.com

  Follow the prompts to activate the license.

Select an Instrument

The first thing you want to do is enable the communication between the computer that runs the SoftMax Pro Software and the instrument that is to read the microplates. See the instrument user guide for how to connect the cables between the computer and the instrument. You can run the SoftMax Pro Software offline or in simulation mode without physically connecting the computer to the instrument.

1. Select the **Home** tab in the ribbon and click **Instrument** to open the Instrument Connection dialog.

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

   If the instrument to which you want to connect does not appear in the list, click **Refresh**.

   > **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If you do not connect the computer to an instrument, select **Offline** and then select the instrument from the **Reader** list.

3. If you work offline, select **Offline** and then select the instrument from the **Reader** list.
4. Optionally, to work in simulation mode, select **Offline**, select the instrument from the **Reader** list, and then select the **Simulator On** check box.
5. Click **OK**. The Home tab displays an icon for the instrument you select.

   > **Note:** The ⓘ icon is for troubleshooting purposes only.

SpectraMax iD3 Multi-Mode Microplate Reader Considerations

When you use SoftMax Pro Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader, your company network configuration could prevent direct communication between the computer and the instrument.

You can use the Ethernet cable to connect the computer directly to the instrument or you can connect the SpectraMax iD3 to your company intranet. You may have to perform the following steps to select the instrument.

1. On the SpectraMax iD3 instrument touchscreen, navigate to the **Maintenance** page **Instrument** tab and note the instrument IP address.

2. In the SoftMax Pro Software, click to open the Application menu.

3. At the bottom of the Application menu, click the **Options** button to open the SoftMax Pro Options dialog.

4. In the list on the left, click **SpectraMax iD3 Control**.

5. Select the **Enables Control of SpectraMax iD3 Instruments** check box.

6. Enter the IP address of the instrument in one of the fields. Fields enable you to enter up to five IP addresses.

7. Click **OK**.

8. Select the **Home** tab in the ribbon and click **Instrument** to open the Instrument Connection dialog.

9. Click **Refresh** if needed and the SpectraMax iD3 Instrument should appear. If the instrument does not appear, you may have to work with your company network administrator to confirm that your company network configuration permits the communication between the computer and the instrument.

When you use a computer running the SoftMax Pro Software to operate the instrument, the instrument touchscreen is locked.

When you use SoftMax® Pro GxP Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader and you log on as a user with the Sign Statements permission, two additional icons appear on the GxP tab in the ribbon.

- Users with the Sign Statements permission click **GxP Mode On** to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and the instrument must be operated from a computer running SoftMax Pro GxP Software.

- Users with the Sign Statements permission click **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to perform reads from the instrument.

 Note: The instrument remains locked until the Sign Statements user clicks **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an SpectraMax iD3 instrument that is locked in GxP mode.

Supported Instruments

SoftMax Pro Software supports the following instruments:

- SpectraMax iD3 Multi-Mode Microplate Reader on page 180
- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184
- VersaMax Microplate Reader, see page 184
- SpectraMax Plus 384 Absorbance Microplate Reader, see page 185
- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M3 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 187
- SpectraMax 190 Absorbance Microplate Reader, see page 188
- Gemini XPS Microplate Reader, see page 188
- Gemini EM Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189
- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader, see page 190
- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 191
- Vmax Kinetic Microplate Reader, see page 191
- EMax Endpoint Microplate Reader, see page 191
- EMax Plus Microplate Reader, see page 192
- StakMax Microplate Handling System, see page 192

Some instruments use detection cartridges. Each detection cartridge contains its own independent light source, optics, and electrical components needed to do specific read modes for some applications.

- The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.
- The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

See Supported Detection Cartridges on page 194.

Troubleshoot Instrument Connections

After you connect the computer to the instrument and turn the instrument on, select the instrument in the in the Available Instruments list to display the icon for the instrument on the Home tab in the SoftMax Pro Software main window.

The instrument icon indicates the status of the connection between the computer and the instrument.

Table 1-2: Instrument Connection Status

Icon	Status
	Instrument Connected
	Instrument Disconnected
	Instrument Simulated

If the instrument to which you connect the computer does not appear on the Instrument Connection dialog, click **Refresh**.

If the status indicates the instrument is disconnected, make sure that the instrument is powered on and that the connections between the computer and the instrument are secure.

To troubleshoot Intranet connectivity for the SpectraMax iD3 Multi-Mode Microplate Reader instruments, see SpectraMax iD3 Multi-Mode Microplate Reader on page 180

Instrument Calibration

Some instruments do not require or do not support the air-calibration process. If the Calibration button does not appear or is not active, then either the computer is not connected to the instrument or the instrument does not support the air-calibration process.

> **Note:** Air-calibration applies to absorbance reads. You do not need a microplate or cuvette for instrument calibration. If you do a cuvette calibration, the cuvette chamber must be empty.

During calibration, the instrument steps through all available wavelengths one-by-one to measure the signal that reaches the detector when there is no sample in the optical path. The software calculates air-calibration values and stores these values in the firmware. The instrument uses the stored air-calibration values when you enable Speed Read and when the Calibrate icon is disabled on the Settings dialog. See Speed Read on page 231 and Calibrate on page 249.

To calibrate the microplate reader:

1. Select the **Operations** tab in the ribbon and click **Calibration** to display the Calibration dialog.

2. Select **Plate** to calibrate for microplates and/or select **Cuvette** to calibrate for cuvettes.

3. Click **Calibrate Now**.

4. When the calibration indicator shows that the calibration is complete, click **Close**.

Barcode Reader Usage

A barcode reader is an input device similar a keyboard. When you place the cursor in a text field and then use the barcode reader to read a barcode, the barcode reader passes the text embedded in the barcode to the text field.

Before you use a barcode reader with the SoftMax Pro Software, make sure that the correct driver is installed and that the barcode reader functions correctly. To test a barcode reader, open a text editor, such as Notepad, and then use the barcode reader to read a barcode. If the reader is functioning correctly, the text embedded in the barcode appears in the text editor.

Barcodes can have several different formats, such as MSI or Pharmacode. Make sure that the barcode reader you use supports the type of barcode that you need to read. See the documentation that comes with your barcode reader.

Use of a barcode reader to input text can be useful to name a Plate section to match the barcode on a microplate. See Rename Experiments or Sections on page 31.

Unlock SpectraMax Paradigm Instruments

When you first install a SpectraMax Paradigm Instrument, you need to remove hardware transport locks as described in the SpectraMax Paradigm Instrument *User Guide or Unpacking Guide*. As a safety precaution, the SoftMax Pro Software controls internal locks to prevent the drawers from opening until the software detects and initializes the instrument.

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

To unlock the drawers and initialize the instrument:

1. Remove the physical instrument hardware transport locks. *See the* SpectraMax Paradigm Instrument *User Guide or Unpacking Guide.*

> **CAUTION!** The instrument can be damaged if you do not remove all physical transport locks before you use the software to unlock the drawers and initialize the instrument.

2. Push all drawers back into the instrument, replace the front panels, and close the microplate door.

3. Connect the computer to the instrument and to a power source. Install the SoftMax Pro Software on the computer. See the SpectraMax Paradigm Instrument *Unpacking Guide or User Guide.*

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

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

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

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

5. Start the SoftMax Pro Software. See Start the Software on page 14.

   The amber LED on the status panel turns off and the green LED turns on to indicate a successful connection between the instrument and the computer.

6. After the computer and the instrument successfully connect, the SoftMax Pro Software displays the Instrument Unlocking Procedure wizard.

7. If the Instrument Unlocking Procedure wizard does not appear, use the Instrument Connection dialog to select and connect to the instrument. See Select an Instrument on page 16.

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

8. Follow the prompts in the Instrument Unlocking Procedure wizard to unlock the drawers and initialize the instrument.

After you complete the Instrument Unlocking Procedure wizard, the instrument does 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 now install detection cartridges in the instrument. See "Installing Detection Cartridges" in the *SpectraMax Paradigm Instrument User Guide*.

To lock the drawers for shipment, use the Instrument Information dialog. See Instrument Information on page 409.

Get Help

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

The Help tab in the ribbon provides access to the SoftMax Pro Software comprehensive help library.

- Click **SoftMax Pro Help** to view the *SoftMax Pro Software 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 through the search function. This document also features a linked index and glossary.

- Click **Formula Reference** to view the *SoftMax Pro Software Formula Reference Guide*. This document has four main sections:
    - **Introduction** provides a general introduction to SoftMax Pro Software formulas.
    - **Operators** describes the types of operators you can use to assemble formulas.
    - **Functions** describes the types of functions you can use to assemble formulas.
    - **Accessors** describes special functions that provide access to other information.

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

- Click **Release Notes** to view the *SoftMax Pro Software Release Notes* for this software release.

- Click **Contact Us** to display a menu with the following options:
    - Select **What's New** to view information about what is new in the SoftMax Pro Software.
    - Select **Home Page** to view the Molecular Devices home page.
    - Select **Knowledge Base** to view the Molecular Devices on line knowledge base.
    - Select **Request Support** to access the Molecular Devices support request web page.

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

- In the About section, click **SoftMax Pro** to view the SoftMax Pro Software version number and release information.

You can find more documentation from the Windows Start menu.

- To view the *SoftMax Pro Software User Guide*, the *Formula Reference Guide*, or the *Software Release Notes*, click **Start > All Programs > Molecular Devices > SoftMax Pro 7.0.2** and click the document to view.

- To view user guides for the supported instruments, click **Start > All Programs > Molecular Devices > SoftMax Pro 7.0.2 > Hardware User Guides** and click the document to view.

Obtain Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support, has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you seek, follow the links to the Technical Support Service Request Form to send an email to our technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (North America only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

To find regional support contact information, visit www.moleculardevices.com/contact.

Please have your instrument serial number or Work Order number and your software version number available when you call.
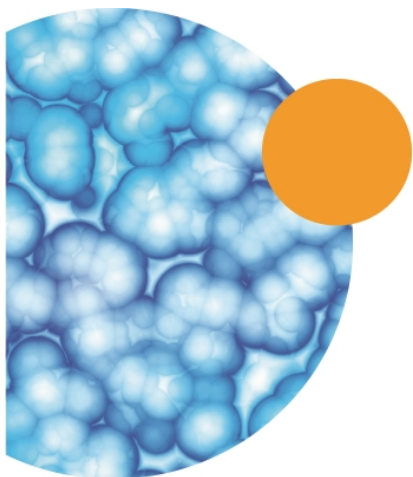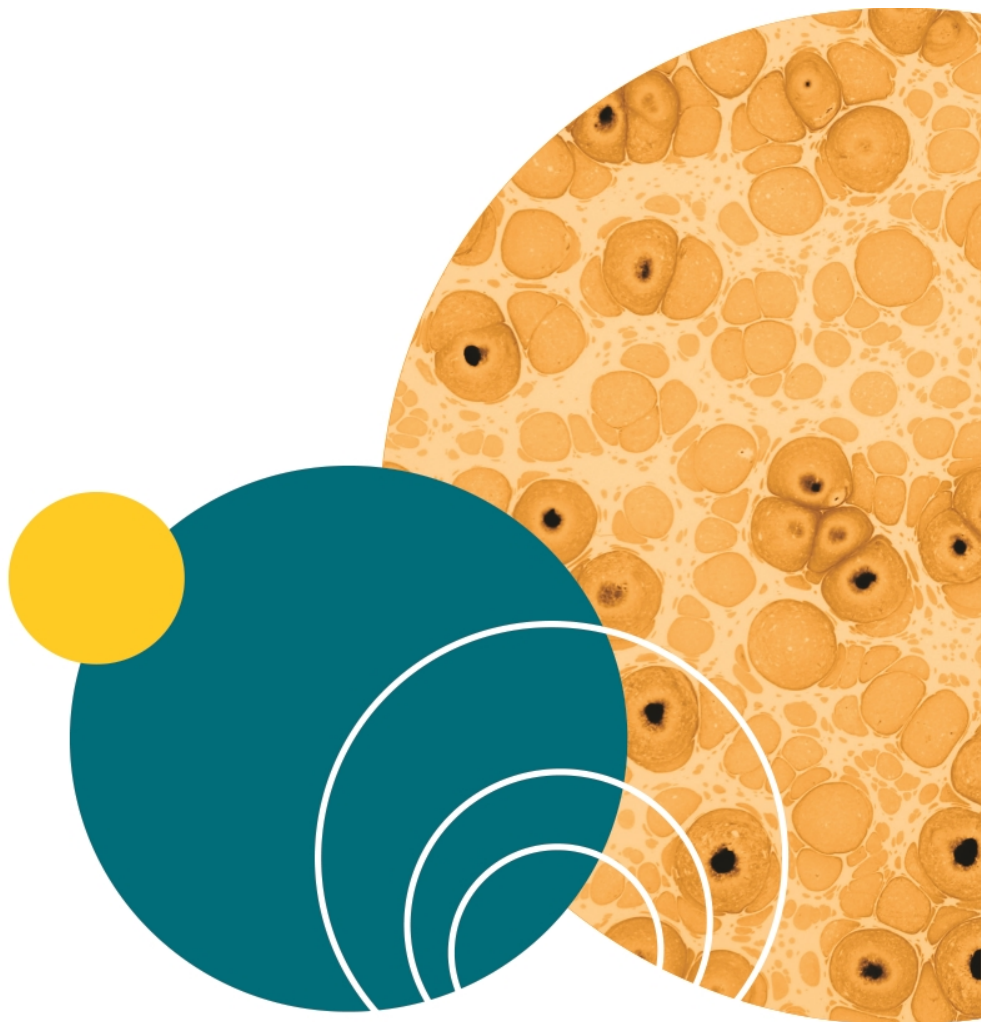
Chapter 2: User Interface Overview

2

The SoftMax Pro Software user interface enables you to perform state-of-the-art analysis from an informative data display. The first time you login to the software the following appears.
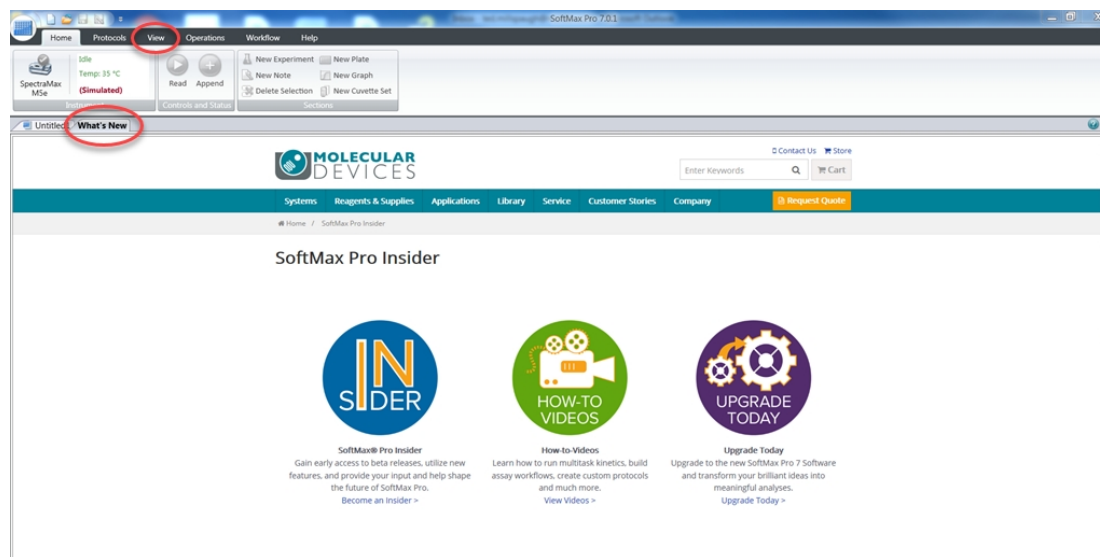
Each of the following items is detailed in the documentation.

- The **What's New** section is actually an integrated web browser. The Help tab in the ribbon toolbar enables you change what appears in the web browser. See What's New - Web Browser on page 25.
- The **View** tab in the ribbon toolbar enables you to rearrange the location of the sections. See View Tab on page 43.

Your protocols appear as tabs to the left of the What's New tab above the workspace. When you login for the first time a tab displays **Untitled1**.

What's New - Web Browser

The Web Browser tab in the document section appears each time you start the SoftMax Pro Software. What's New information appears by default to keep you informed of new or updated products. The content of the Web Browser tab changes as you select the different options from the Contact Us button on the Help tab in the ribbon and can display the Molecular Devices Home page, Knowledge Base, or Support Request page.

The icon on the very top left (left of the Home tab) enables you to change options including the ability to hide the Web Browser tab. See Application Settings on page 92.

Main Window

The SoftMax Pro Software window has three main areas: the ribbon toolbar along the top, the navigation tree on the left, and the workspace area on the right. The application menu and quick-access toolbar on the top left provide access to file and application management features.
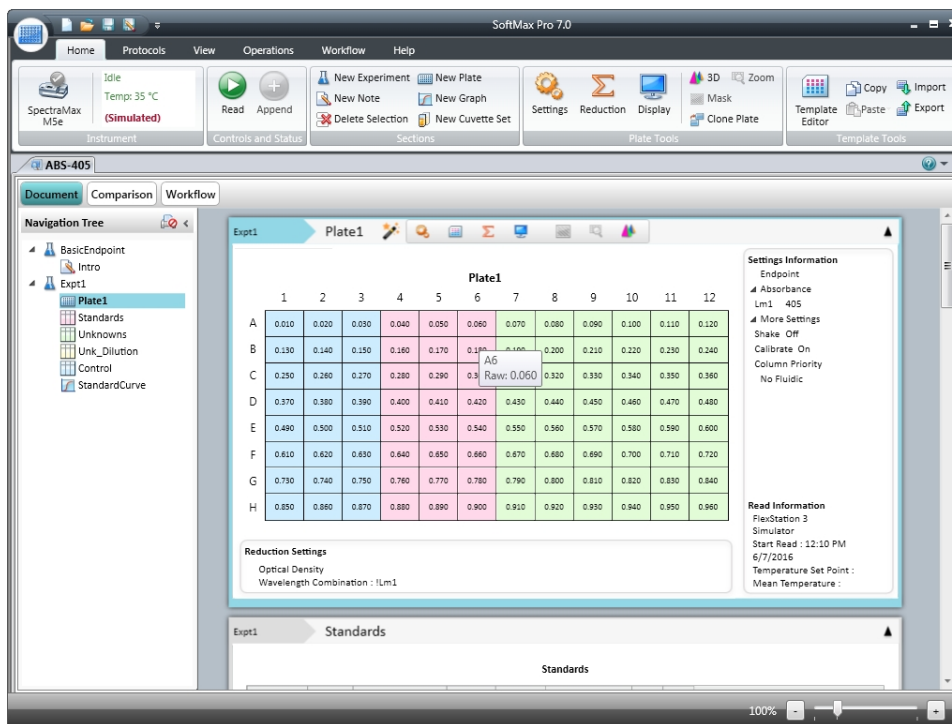
General Workflow Overview

SoftMax Pro Software enables you to set up and run complete protocols for the supported instruments. You can save instrument settings as protocol (template) files to repeatedly read different microplates or cuvettes. You can control most standalone instrument functions by the SoftMax Pro Software.
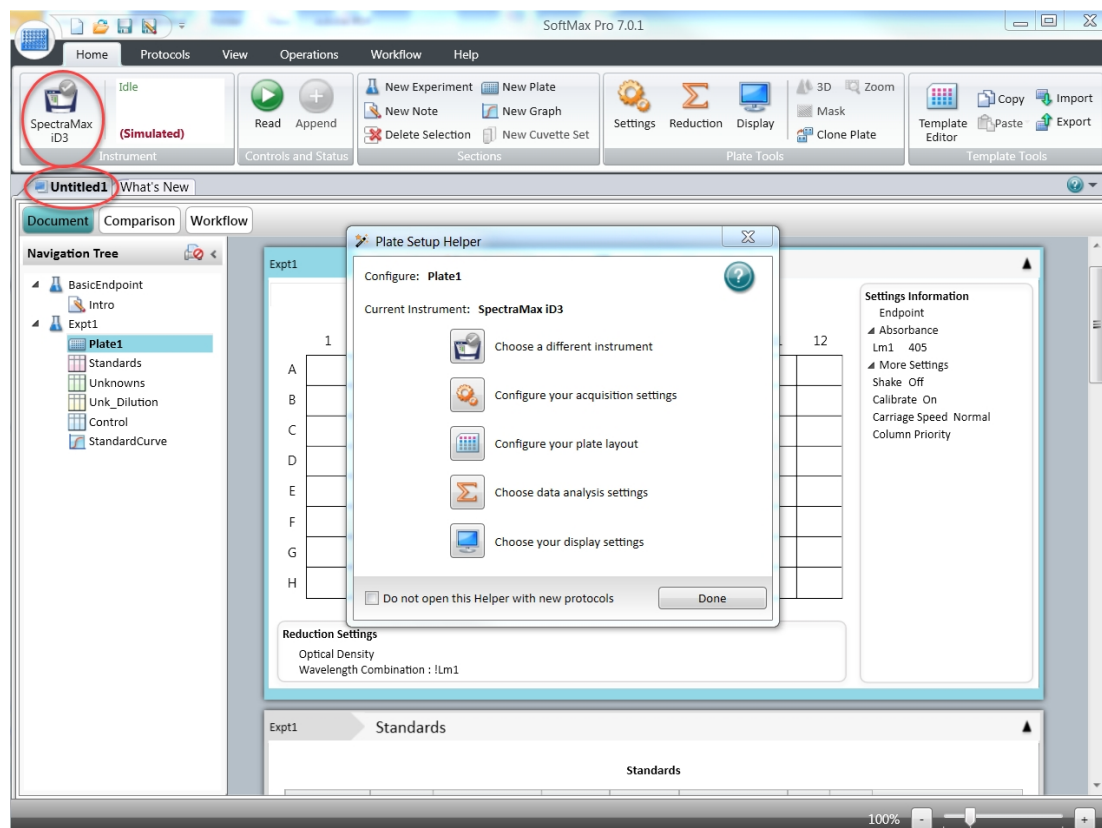
The following is a high level overview of the initial workflow to acquire and analyze microplate data. Your workflow will vary as it applies to your specific application and the standard operating procedures of your laboratory. Use the links in this document for details of each step.

1. On the Home tab in the ribbon, select an instrument. See Select an Instrument on page 16.

2. Select the **Untitled1** tab above the Workspace. The Workspace changes to enable you to define a protocol and the Plate Setup Helper dialog displays. See Plate Setup Helper on page 29.

3. Add Note, Plate or Cuvette Set, Group, and Graph sections to the protocol in the Navigation Tree. See Workspace Sections on page 95

4. The Ribbon toolbar changes depending on the instrument you select and the section you select in the Navigation Tree. Define the settings for the instrument to perform including the read mode, read type, etc. on the Home tab, **Settings** button. See Select Instrument Settings on page 206.

5. As you create the protocol, on the Home tab, you can click the **Template Editor** button to add a microplate template that includes the definition of groups, blank cells, and a sample series for your application. See Configure Microplate Templates on page 264.

6. Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

7. On the Home tab, click **Read** to run the protocol. SoftMax Pro Software collects and stores all raw data received from the instrument. See Collect Data from Microplates on page 300, Collect Data from Cuvettes on page 310, or Collect Data from Membrane Holders on page 312.

   As information is read, the SoftMax Pro Software displays updates that show the new data. Data displays in a grid format that corresponds to the wells in a microplate or individual cuvettes.

   During the read, the Read button changes to . Click to stop the read.

   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 that contain different samples can be read using the same or different modes, all within the same experiment.

8. View and analyze the data. See Data Display Settings on page 318, Data Reduction on page 325, Graph Data on page 343, and View Western Blot Membrane Data on page 395.

   You can manipulate or reduce the raw data using built-in formulas or you can define your own analysis structure to summarize the raw data. More than one reduction can display and you can compare results from different microplates and cuvettes within the same experiment.

 Note: After you define instrument settings and customize the 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 repeatable data collection and analysis.

Plate Setup Helper

The Plate Setup Helper dialog enables you to setup a microplate to acquire data.

When you open a new protocol file, the Plate Setup Helper dialog displays to help you set up a microplate experiment. Click 🪄 in the Workspace toolbar above an active Plate section to display the Plate Setup Helper dialog.
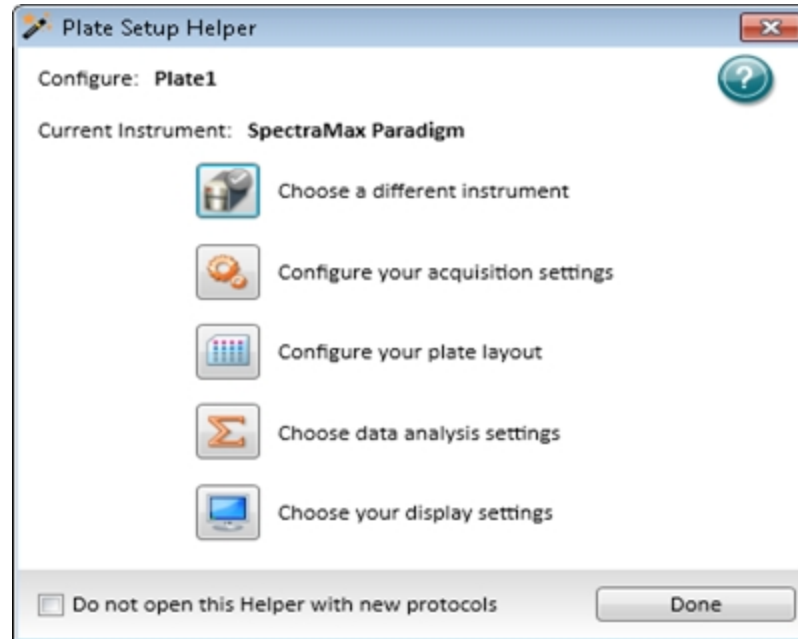
The **Configure** caption displays the name of the plate. The **Current Instrument** caption displays the instrument to perform the read. Acquisition settings are applied to the entire Plate section. If you select a plate clone, the layout, analysis, and display settings apply to the clone you select. See Plate Sections on page 105.

- ▪ 🔧 - Click to select a different instrument to do the read. The icon changes to reflect the instrument you select and the connection status between the computer and the instrument. After you select the instrument, do the following steps in any sequence. The following steps are instrument specific and are reset if you change the instrument. See Select an Instrument on page 37.

- ▪ 🔍 - Click to configure the acquisition settings. See Instrument Settings on page 216.

- ▪ 🔲 - Click to configure the plate layout. See Configure Microplate Templates on page 264.

- ▪ Σ - Click to choose data analysis settings. See Data Reduction on page 325.

- ▪ 🖥 - Click to choose display settings. See Data Display Settings on page 318.

To prevent the Plate Setup Helper dialog from displaying each time you open a new protocol, select the **Do not open this Helper with new protocols** check box. You can enable this option on the SoftMax Pro Options dialog. See Application Settings on page 92.

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

Navigation Tree

The Navigation Tree displays the sections in the data file for the tab you select above the Navigation Tree. Click a section in the Navigation Tree to display the section settings in the Workspace on the right.
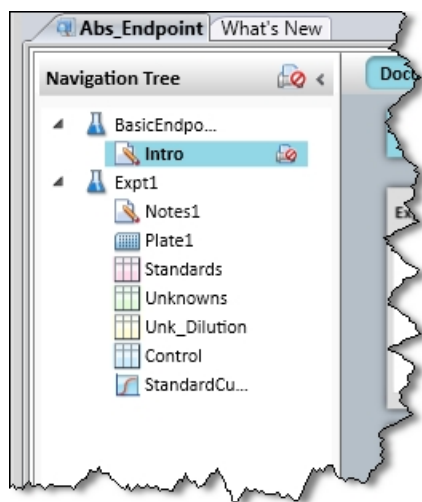
When you save a data file, the data file name displays on the tab above the Navigation Tree. You can open more than one data file at a time. Each data file can contain an unlimited number of experiments. Each experiment can contain an unlimited number sections. Section types include: Note, Plate, Cuvette Set, Group, and Graph. See Workspace Sections on page 95.

To change the sequence of the sections in an experiment, drag the section name to the desired location. You can move the sections within an experiment and cannot move sections between experiments.

Right-click an experiment or section in the Navigation Tree to rename, duplicate, or delete the section or experiment.

When you duplicate an experiment, all sections in the experiment are duplicated in a new experiment without data. When you duplicate a Note or Graph section, a duplicate is made of the section. When you duplicate a Group section, a new group is created with the same properties as the original section. When you duplicate a Plate section, the new section contains all the settings from the original section without data.

To rename an experiment or a section, see Rename Experiments or Sections on page 31.

Rename Experiments or Sections

To rename an experiment or section, right-click the experiment or section in the Navigation Tree and select **Rename**.
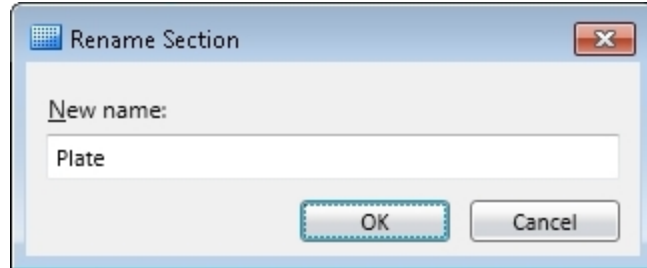
Edit the text in the **New Name** field and click **OK**.

The new name displays in the Navigation Tree and at the top of the section in the Workspace.

To rename an experiment or section using the automation interface, see the description of the setTitles() command in the *SoftMax Pro Software Automation API Reference Guide*.

> ✳ **Tip:** You can use a barcode reader to input the text embedded in a barcode. See Barcode Reader Usage on page 20.

Workspace Views

The Workspace has three views that change the way the sections display. The View tab in the ribbon enables you to split the Workspace to view multiple data files at the same time. See View Tab on page 43.

- The **Document** view enables you to scroll through the sections in the protocol. See Document View on page 32.
- The **Comparison** view enables you to move the sections around in the Workspace to where they are most useful. See Comparison View on page 33.
- The **Workflow** view enables you to drag-and-drop protocol steps to define how to run a multi-step protocol workflow. Workflow View on page 34.

For information about how to work with the sections in the Workspace, see Workspace Sections on page 95.

Document View

The Document view provides a scrollable area that enables you to view the sections.

The Document view displays by default. Click the **Document** button above the Navigation Tree, if needed.
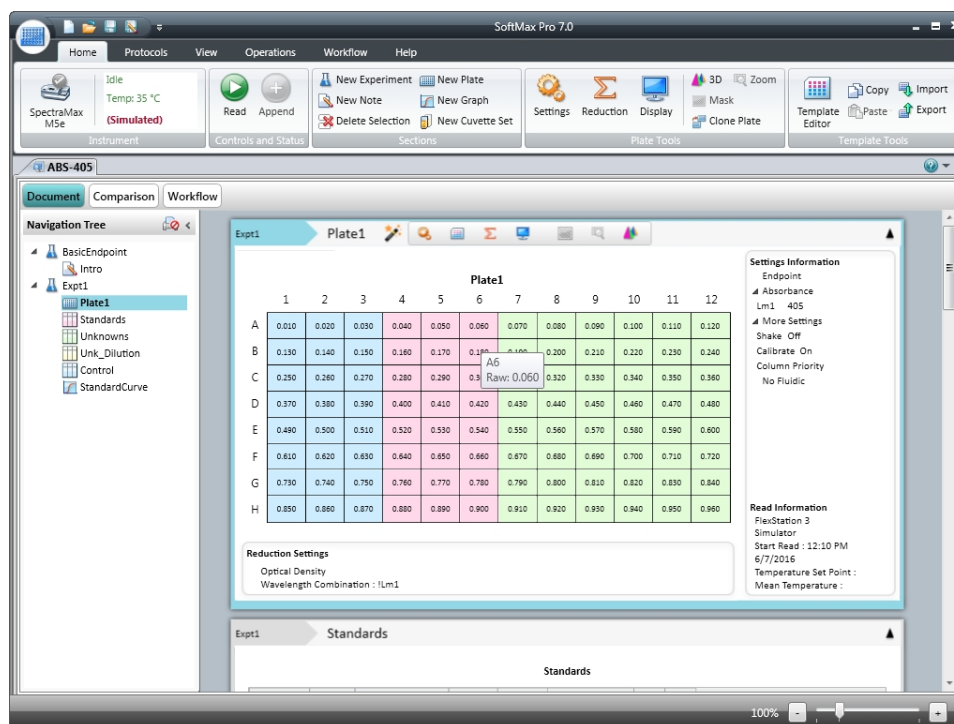
To view a section either click the section in the Navigation Tree or use the scroll bar until the section comes into view.

For information about how to work with the sections in the Workspace, see Workspace Sections on page 95.

Comparison View

The Comparison view provides flexible display options that enable you to move the sections around in the Workspace to position them where they are most useful.

To use the Comparison view, click the **Comparison** button above the Navigation Tree.
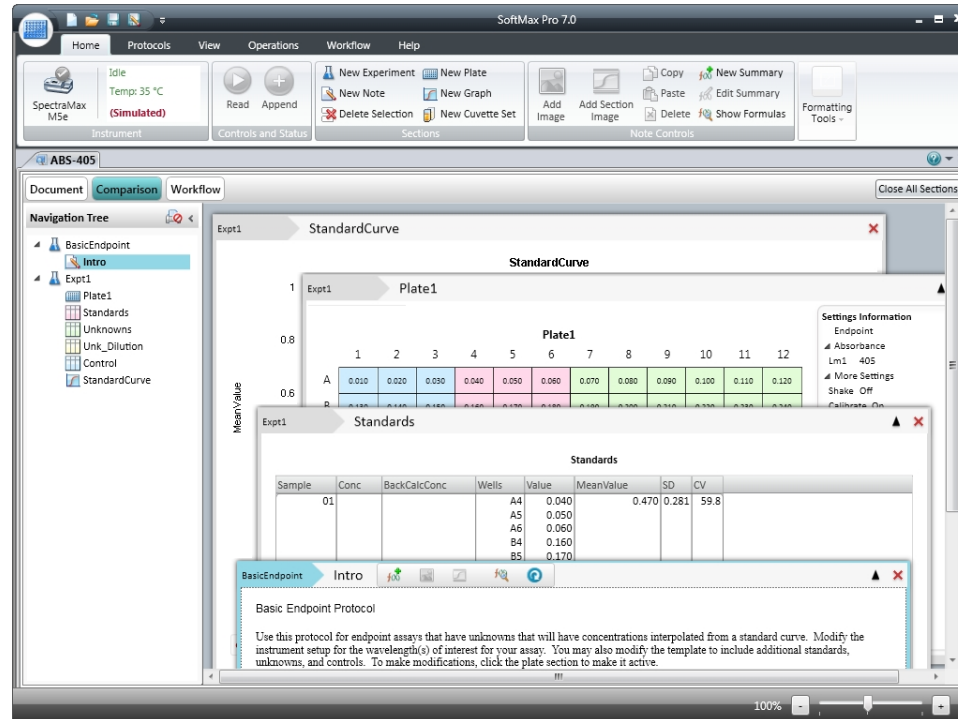
- Drag sections from the Navigation Tree and position them in the Workspace.
- Sections can overlap.
- Double-click a section in the Navigation Tree to add that section to the bottom of the Workspace.

For information about how to work with the sections in the Workspace, see Workspace Sections on page 95.

Workflow View

The Workflow view provides a drag-and-drop workflow editor and tools to run and monitor a workflow. In Workflow view you can define a workflow that uses the defined Plate sections and instrument settings to run multitask kinetic reads that last hours or days.

To use the Workflow view, click the **Workflow** button above the Navigation Tree.
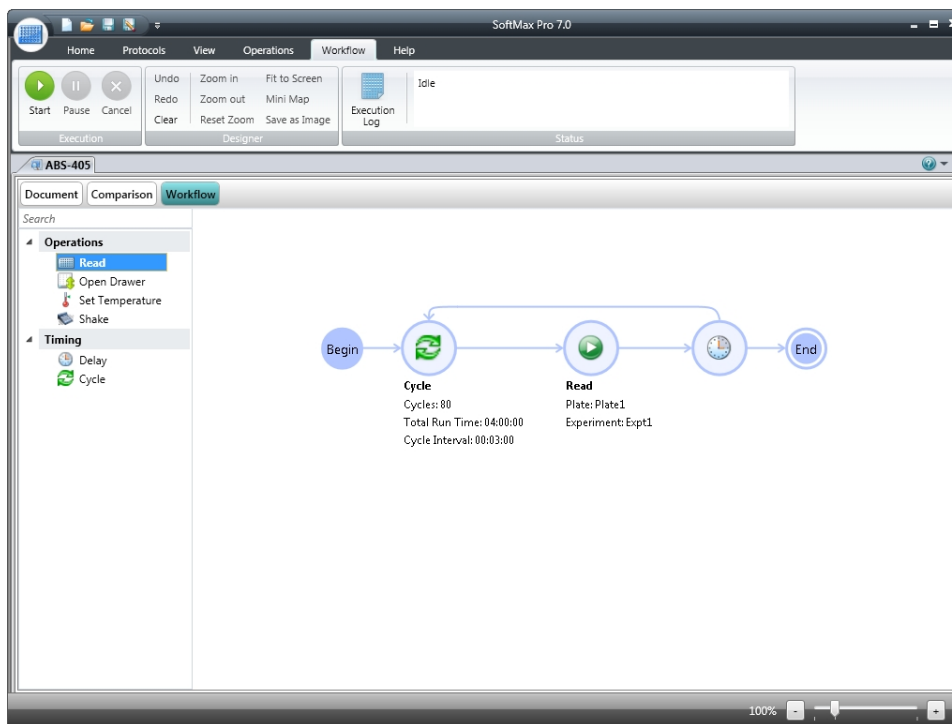
- Drag tasks from the menu on the left to the timeline on the right.
- To define repeated tasks, place the tasks in a Cycle task.
- Click the workflow options on the Workflow tab in the ribbon to start, pause, resume, and cancel the workflow. See Workflow Tab on page 49.

For more information, see Define and Run Workflows on page 260.

Ribbon Toolbar

The Ribbon toolbar contains tabs with the tools you need to work with SoftMax Pro Software. The tool vary depending on the instrument to which you connect the computer and your context withing the SoftMax Pro Software.

Home Tab

The Home tab contains the following sections:

- Instrument
- Controls and Read Status
- Sections

See Home Tab on page 37 and Home Tab Section Controls on page 40.

The contents of the Home tab change depending on the section type you select in the Workspace.

Notes Section Tools

When you select a Note section, the Home tab contains:

- Notes Controls
- Formatting Tools

See Note Sections on page 96.

Plate Section Tools

When you select a Plate section, the Home tab contains:

- Plate Tools
- Template Tools

See Plate Sections on page 105.

Cuvette Section Tools

When you select a Cuvette Set section, the Home tab contains:

- Cuvette Tools
- Template Tool

See Cuvette Set Sections on page 115.

Group Section Tools

When you select a Group section, the Home tab contains:

- Column Tools
- Summary Tools
- Formatting Tools

See Group Sections on page 123.

Graph Section Tools

When you select a Graph section, the Home tab contains:

- Graph Type
- Graph Tools

See Graph Sections on page 132.

Protocols Tab

The Protocols tab contains the following:

- Protocol Manager
- Community

See Protocols Tab on page 42.

View Tab

The View tab contains the following:

- Show/Hide Panel
- Window
- Expand/Collapse Sections

See View Tab on page 43.

Operations Tab

The Operations tab contains the following:

- Instrument Commands
- Instrument Tools
- Calculations
- Automation

See Operations Tab on page 43.

Workflow Tab

The Workflow tab contains the following:

- Execution
- Designer
- Status

See Workflow Tab on page 49.

GxP Tab

The GxP tab is available when you use the SoftMax Pro GxP Software and contains the following:

- User Account
- Data

See GxP Tab on page 48.

Help Tab

The Help tab contains the following:

- Help
- About

See .

Home Tab

The **Instrument** and **Controls and Status** options on the **Home** tab contain the following controls:

-
-

Select an Instrument

The first thing you want to do is enable the communication between the computer that runs the SoftMax Pro Software and the instrument that is to read the microplates. See the instrument user guide for how to connect the cables between the computer and the instrument. You can run the SoftMax Pro Software offline or in simulation mode without physically connecting the computer to the instrument.

1. Select the **Home** tab in the ribbon and click **Instrument** to open the Instrument Connection dialog.

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

   If the instrument to which you want to connect does not appear in the list, click **Refresh**.

   Note: It is not necessary to physically connect the computer to an instrument to create a protocol. If you do not connect the computer to an instrument, select **Offline** and then select the instrument from the **Reader** list.

3. If you work offline, select **Offline** and then select the instrument from the **Reader** list.

4. Optionally, to work in simulation mode, select **Offline**, select the instrument from the **Reader** list, and then select the **Simulator On** check box.

5. Click **OK**. The Home tab displays an icon for the instrument you select.

> **Note:** The ⓘ icon is for troubleshooting purposes only.

SoftMax Pro Software supports the following instruments:

- SpectraMax iD3 Multi-Mode Microplate Reader on page 180
- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184
- VersaMax Microplate Reader, see page 184
- SpectraMax Plus 384 Absorbance Microplate Reader, see page 185
- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M3 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 187
- SpectraMax 190 Absorbance Microplate Reader, see page 188
- Gemini XPS Microplate Reader, see page 188
- Gemini EM Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189
- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader, see page 190
- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 191
- Vmax Kinetic Microplate Reader, see page 191
- EMax Endpoint Microplate Reader, see page 191
- EMax Plus Microplate Reader, see page 192
- StakMax Microplate Handling System, see page 192

Read Data

After you define your experiment and you insert the prepared microplate or cuvette in the microplate reader, you can start a read.

> **Note:** Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

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

As information is read, the SoftMax Pro Software displays updates that show the new data. The data display in a grid format that corresponds to the wells in a microplate or individual cuvettes.

During the read, the read button changes to . You can click this button to stop the read operation. The read stops immediately.

To stop and restart a Kinetic read while the **Plate** section is being read, click **Interrupt**

to pause the read, and then click **Append** to continue the Kinetic read from where it left off. When you interrupt a read, the instrument finishes reading all the selected wells in the microplate and then waits for user input before continuing. This type of discontinuous kinetic read can help with acquisition development.

After a Kinetic read ends successfully, you can read the same Plate section again and append

the data to the data that already exists in the Plate section by clicking **Append** .

> **Note:** If you stop a Kinetic read before it completes, you cannot restart the read or append data to the read. To use these features, you must either let the read finish on its own or click **Interrupt** to pause the read.

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

Home Tab Section Controls

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

- New Experiment on page 40
- New Note on page 40
- Delete Section on page 40
- New Plate on page 41
- New Graph on page 41
- New Cuvette Set on page 41

New 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 Experiment you select displays below the selected Experiment in the Navigation Tree. The sections and settings are duplicated, but the data is not.

To change the name of the experiment, right-click the experiment in the Navigation Tree and select **Rename**. See Rename Experiments or Sections on page 31.

New Note

To create a Note section:

1. Select the experiment where you want to add a Note section in the Navigation Tree.
2. If there is more than one section in the Experiment, select the section above where you want the new Note section to display in the Navigation Tree.
3. Click **New Note** in **Sections** on the **Home** tab in the ribbon.

A new blank Note section displays below the section you selected in the Navigation Tree. See Note Sections on page 96.

To change the name of the Note section, right-click the Note section in the Navigation Tree and select **Rename**. See Rename Experiments or Sections on page 31.

Delete 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 an 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.

You cannot undo the deletion of a section or experiment. When you delete a Plate section, the data from the Plate section is also deleted.

New Plate

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 be displayed in the Navigation Tree.
3. Click **New Plate** ▦ in **Sections** on the **Home** tab in the ribbon.

A blank Plate section displays in the Workspace. You can add as many Plate sections as you need to your data file. See Plate Sections on page 105.

To change the name of the Plate section, right-click the Plate section in the Navigation Tree and select **Rename**. See Rename Experiments or Sections on page 31.

New Graph

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 Create Graphs on page 345.
4. When the graph options have been set, click **OK**.

A new Graph section displays below the selected section in the Navigation Tree. See Graph Sections on page 132.

To change the name of the Graph section, right-click the Graph section in the Navigation Tree and select **Rename**. See Rename Experiments or Sections on page 31.

New Cuvette Set

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 display in the Navigation Tree.
3. Click **New Cuvette Set** ▯ in **Sections** on the **Home** tab in the ribbon.

A new blank Cuvette Set section displays below the selected section in the Navigation Tree. See Cuvette Set Sections on page 115.

To change the name of the Cuvette Set section, right-click the Cuvette Set section in the Navigation Tree and select **Rename**. See Rename Experiments or Sections on page 31.

Protocols Tab

The **Protocols** tab in the ribbon provides access to protocol files that are stored in the file system. It also gives you access to the SoftMax Pro Software user 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.

Several 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 Create Protocols on page 205. When you create protocols, save them into folders on your file system. Name the folders in such a way that you can find the protocols later.

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

- Click **Folder Locations** to add the folders that contain the protocols you want to use to the short cut menu you can access by clicking **Protocol Manager**. See Add or Remove Folders in the Protocol Manager on page 65.

- Click **Save As Default** to overwrite the default.spr file with the protocol setting of the experiment that is active in your workspace. See Save a Protocol as the Default Protocol on page 64.

  > **Note:** When you save the file as the default protocol, the data you have collected in the current experiment remains unchanged. 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 Protocol Manager - Open Files on page 64.

  > **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.

The following 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 **Note** section has a clear description of the protocol, since the first **Note** 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 SoftMax Pro Software community web site.

View Tab

The **View** tab in the ribbon contains three sections: **Show/Hide Panel**, **Window**, and **Expand/Collapse Sections**.

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.

In the **Expand/Collapse Sections** section, click an option to **Collapse All Sections** or to **Expand All Sections** in the workspace.

Operations Tab

Instrument Commands

The buttons that display in the **Instrument Commands** group depend on the currently selected instrument.

- **Temperature**: When connected to an instrument with temperature control, you can set the temperature in the microplate or cuvette chamber in degrees Celsius. See Temperature Settings on page 45.
- **Shake**: If the selected instrument permits manual shaking of the microplate, then the **Shake** button is available. See Shake the Microplate on page 46.
- **Open / Close**: For most instruments, you can open and close the microplate drawer using the software. For some instrument, other drawers can be opened and closed using the software. See Open and Close a Drawer on page 47.

Instrument Tools

The buttons that display 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 Calibrate Microplate Readers on page 407.

- **Filters**: The positions of the filters in the EMax and Vmax Microplate Readers can be defined using the **Filter Configuration** dialog. For instructions, see Configure Filters for EMax and Vmax Microplate Readers on page 408.EMax Plus Microplate Reader

- **Configure**: In the **Configuration** dialog, you can adjust the intensity of the lamp for each of the installed filters in the EMax Plus Microplate Reader. You can view the positions and wavelengths of the installed filters. This dialog also lists other instrument information like the serial number and firmware version. See Adjust the Lamp for EMax Plus Microplate Reader on page 408.

- **Initialize**: To return EMax Plus Microplate Reader microplate carrier to its home position, click the **Initialize** button.

- **Info**: In the **Instrument Information** dialog, you can view a list of the installed detection cartridges in a SpectraMax i3x or i3 Multi-Mode Detection Platform or in a SpectraMax Paradigm Multi-Mode Microplate Reader, or view 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 Instrument Information on page 409.

- **Refresh**: To update the software with the currently installed detection cartridges in a SpectraMax i3x or i3Multi-Mode Detection Platform or in a SpectraMax Paradigm Multi-Mode Microplate Reader, or with the currently installed filter slides in FilterMax F3 and F5 Multi-Mode Microplate Readers, click the **Refresh** button.

Calculations

By default, SoftMax Pro Software does continuous recalculation of the data when you read plates, create or change formulas, or change settings that have an effect on data. At certain times, it is useful to disable recalculations so that you can edit the Experiment without waiting for recalculations to complete.

- **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 displays in the workspace footer.

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

For technical information about SoftMax Pro Software calculations, see Calculations and Numerical Precision on page 316.

Automation

The following automation commands are available.

- **Auto Read**: You can automatically read plate sections as they are displayed within an experiment. See Enable Auto Read on page 301.
- **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 192.
- **Automation Mode**: You can place the software in automation mode to let the software accept legacy commands from scripts written for the SoftMax Pro Software version 5.x automation interface. See Automation Mode on page 54.

Temperature Settings

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 **Operations** tab in the ribbon.
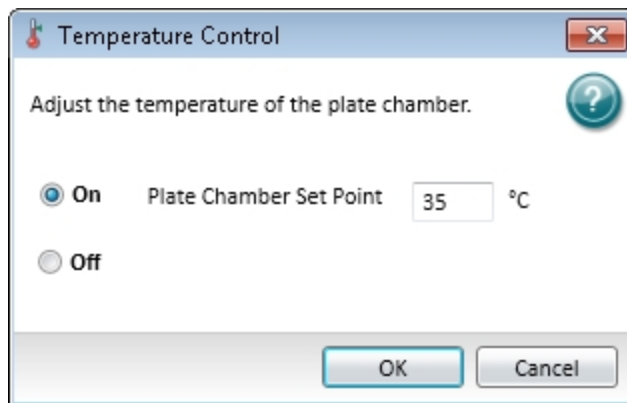
3. In the **Temperature Control** dialog, click **On**.
4. Enter the temperature in degrees Celsius.
5. Click **OK**.

The instrument monitors the temperature of the air inside the microplate chamber, and the software displays the air temperature of the chamber on the **Instrument** section on the **Home** tab. The temperature display is continuously updated with the current air temperature.

You can set the temperature 2°C or more above the current air temperature in the chamber. After you turn on the temperature control, the chamber can take from 10 to 30 minutes to reach the set temperature, depending on the difference between the start temperature and the set temperature.

For the SpectraMax M2, M2e, M3, M4, M5, M5e, and Plus 384 instruments, the **Instrument** section on the **Home** tab displays the temperature within the microplate chamber. This can be different from the front panel on the instrument that displays the temperature within the cuvette chamber. The readings should be similar to each other after both chambers reach equilibrium. During warm-up, you might notice a discrepancy in temperatures, which is normal.

> **Note:** The temperature sensors detect the temperature of the air inside the chamber, not the temperature of the samples in the microplate. If you use the instrument to warm the samples, Molecular Devices recommends that you use a seal or lid on the microplate to prevent evaporation of the sample. Using a seal or lid also helps to maintain uniform temperature. Letting the prepared sample equilibrate inside the microplate chamber can take an hour or more. You can speed up equilibration by pre-warming the sample and the assay reagents to the desired temperature before placing the microplate in the chamber. Validate the data quality to determine whether the seal or lid can stay on the microplate for the read.

> **CAUTION!** The SpectraMax Paradigm, SpectraMax i3x, SpectraMax iD3 , and FilterMax F5 instruments have an automatic read-height (Z-height) adjustment for fluorescence and luminescence reads. For top reads, the lens or the microplate can be damaged if the microplate height is not set properly. If the microplate has a lid on top, you must indicate that the microplate is lidded and verify that the microplate height with a lid is set accurately on the Plate Editor dialog before you start a read.

After you turn off the temperature control, open the microplate drawer to help the microplate chamber cool to the ambient temperature.

Shake the Microplate

If the selected instrument permits manual shaking of the microplate, then the **Shake** button is available in the **Instrument Commands** section on the **Operations** tab in the ribbon.

To shake the plate:

1. Insert the prepared microplate into the connected instrument.
2. Click and hold **Shake** on the **Operations** tab in the ribbon.
3. Release the mouse button to stop the shake operation.

> **Tip:** You can also have the instrument shake the microplate by holding down the **F8** key.

Open and Close a Drawer

For most instruments, you can open and close the microplate drawer using the software.

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

1. Click **Open / Close** on the **Operations** 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 **Operations** tab in the ribbon to close the microplate drawer.

> **Note:** The microplate carrier on the EMax Plus Microplate Reader moves in and out of the read chamber during the read, and then returns to the home position after the read ends. If the microplate carrier does not return to the home position, you can initialize the carrier to return it to its home position. Click **Initialize** on the **Operations** tab in the ribbon. See Operations Tab on page 43.

For some instruments, the software can open and close other drawers. For example, the three drawers on the FlexStation 3 reader can be opened using the software. Go to the **Operations** tab in the ribbon and then click **Tips** for the tip rack drawer, **Reagent** for the compound plate drawer, or **Open / Close** for the read chamber drawer.

Use the **Instrument Information** dialog to open and close the cartridge drawers on the SpectraMax i3x Instrument or SpectraMax Paradigm Instrument or to open and close the filter drawers on the FilterMax F3 and F5 Instruments. See Instrument Information on page 409.

GxP Tab

The **GxP** tab is available when you use the SoftMax Pro GxP Software. See SoftMax Pro GxP Software on page 417.

User Account

The buttons in the **User Account** group enable you to manage the current user account.

- **Account Information**: The **User Account Information** dialog provides information about the current user. See View User Information on page 422.
- **User Log On**: Click the **Log On** button to log on after you log off. See Log onto SoftMax Pro GxP Software on page 421.
- **User Log Off**: Click the **Log Off** button to log the current user off without closing the program.
- **Change Password**: The **Change Password** dialog lets the current user change the password required to log onto the software. See Change User Password on page 424.
- **Select Account Database**: The **User Accounts Connection** dialog lets the GxP Admin Software administrator define the location of the User Accounts file related to this installation of the SoftMax Pro GxP Software. See Connect a User Accounts File on page 419.

Data

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

- **Statements**: The **Statements** dialog lets you view, edit, and add statements. A user with the proper permission can add an electronic signature to statements. See Work with GxP Statements on page 425.
- **Audit Trail**: The **Audit Trail** dialog gives you read-only access to the audit trail for the open data file, and lets you attach a note to the audit trail. See View GxP Audit Trail on page 432.

Instrument

When you use SoftMax® Pro GxP Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader and you log on as a user with the Sign Statements permission, two additional icons appear on the GxP tab in the ribbon.

- Users with the Sign Statements permission click **GxP Mode On** to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and the instrument must be operated from a computer running SoftMax Pro GxP Software.

- Users with the Sign Statements permission click **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to perform reads from the instrument.

> **Note:** The instrument remains locked until the Sign Statements user clicks **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an SpectraMax iD3 instrument that is locked in GxP mode.

Workflow Tab

The Workflow tab in the ribbon contains three sections: Execution, Designer, and Status. Use the Workflow tab in conjunction with the Workflow view of the Workspace. See Define and Run Workflows on page 260.

- The **Execution** group enables you to start, pause, resume, and cancel the workflow.
- The **Designer** group enables you to undo or redo actions, clear all tasks from the editor, and zoom in and out of the editor. Click **Mini Map** to open the Overview dialog that helps you navigate a long timeline. To export an image of the timeline in JPG, PNG, GIF or XPS format, click **Save as Image**.
- The **Status** group displays the current status of the workflow. Click **Execution Log** to view the status of the tasks to run a workflow in real time.

Help Tab

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

The Help tab in the ribbon provides access to the SoftMax Pro Software comprehensive help library.

- Click **SoftMax Pro Help** to view the *SoftMax Pro Software 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 through the search function. This document also features a linked index and glossary.

- Click **Formula Reference** to view the *SoftMax Pro Software Formula Reference Guide*. This document has four main sections:
    - **Introduction** provides a general introduction to SoftMax Pro Software formulas.
    - **Operators** describes the types of operators you can use to assemble formulas.
    - **Functions** describes the types of functions you can use to assemble formulas.
    - **Accessors** describes special functions that provide access to other information.

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

- Click **Release Notes** to view the *SoftMax Pro Software Release Notes* for this software release.

- Click **Contact Us** to display a menu with the following options:
  - Select **What's New** to view information about what is new in the SoftMax Pro Software.
  - Select **Home Page** to view the Molecular Devices home page.
  - Select **Knowledge Base** to view the Molecular Devices on line knowledge base.
  - Select **Request Support** to access the Molecular Devices support request web page.
- Click **Software License** and follow the instructions on the Software License Activation dialog to activate your SoftMax Pro Software license.
- In the About section, click **SoftMax Pro** to view the SoftMax Pro Software version number and release information.

You can find more documentation from the Windows Start menu.

- To view the *SoftMax Pro Software User Guide*, the *Formula Reference Guide*, or the *Software Release Notes*, click **Start > All Programs > Molecular Devices > SoftMax Pro 7.0.2** and click the document to view.
- To view user guides for the supported instruments, click **Start > All Programs > Molecular Devices > SoftMax Pro 7.0.2 > Hardware User Guides** and click the document to view.

View Help

On-screen help displays 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 to work with files, print, and manage the application.
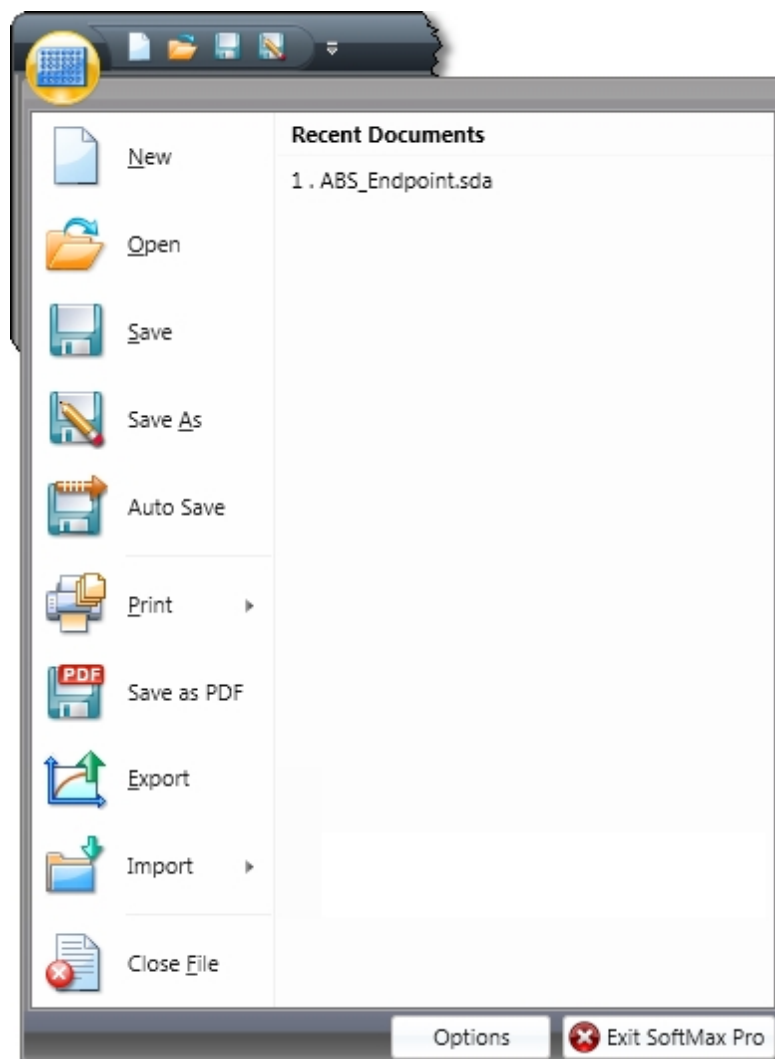
Click the **Application** [icon] button to open the **Application** menu and then click a menu option.

- To create a new blank data file or protocol file, click **New**. See Create Data Files on page 60 or Create Protocol Files on page 67.
- 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 Open Data Files on page 61 or Open Protocol Files on page 67.
- To save an open data file or protocol file, click **Save** or **Save As**. See Save Data Files on page 62 or Save Protocol Files on page 68.
- To set up automatic saving options, click **Auto Save**. See Auto Save Settings on page 69.

- To print the sections of an experiment, preview a print job, or set print options, click **Print** and then click the option you want on the right. See Printing on page 72.
- To save the defined printed output of a file in portable document format (PDF), click **Save As PDF**. See Save Data as a PDF on page 401.
- To export the data in a data file, click **Export**. See Export Plate Data on page 76.
- To import data to a data file, click **Import**. See Import Microplate Data on page 88.

  The data import feature requires a separate license. To obtain a license for this feature, contact your Molecular Devices representative.

- 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 Application Settings on page 92.
- 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 displays directly to the right of the Application button in the upper-left corner of the SoftMax Pro Software application window.
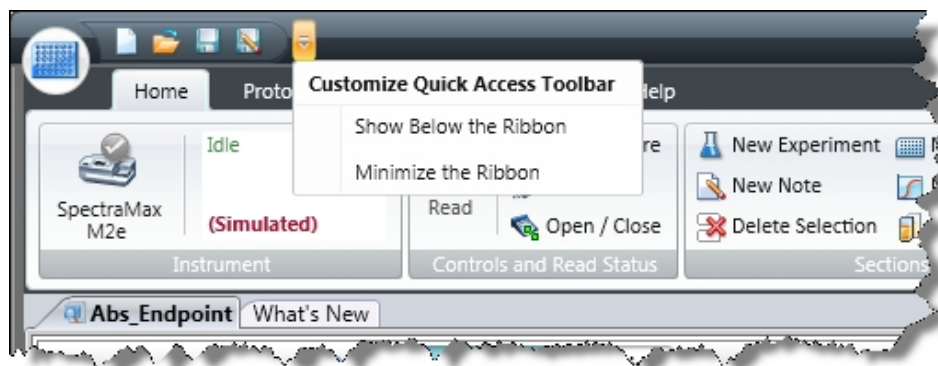
You can move the location to above or below the ribbon and add function buttons that you want to have quick-access to.

The Quick Access Toolbar initially has buttons to do the following tasks:

- 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

Reposition the Quick-Access Toolbar

The Quick-Access toolbar can display 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 displays, click either **Show Below the Ribbon** or **Show Above the Ribbon**.

Add Buttons

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 displays, click **Add to Quick-Access Toolbar**.

Remove Buttons

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 is displayed, click **Remove from Quick-Access Toolbar**.

Minimize 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 displays, 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 displays, click **Minimize the Ribbon** to remove the check mark next to the option.

Automation Mode

The SoftMax Pro Software automation mode prevents user interaction when the software is controlled by the automation interface through third-party automation software or the StakMax Software. To write scripts to use with the SoftMax Pro Software 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.
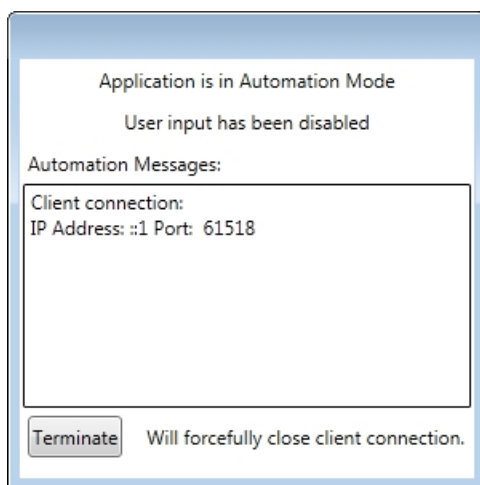
When the automation software acquires data, the related **Plate** section displays in the SoftMax Pro Software main window behind the automation mode dialog and displays the acquired data.

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 let the software 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 Write and Run Automation Scripts on page 55, StakMax Microplate Handling System on page 192, or the *SoftMax Pro Software Automation API Reference Guide*.

Write and Run Automation Scripts

The automation commands in the SoftMax Pro Software can be scripted and run through the automation API. If you are an experienced automation programmer, consult the *SoftMax Pro Software Automation API Reference Guide* for information about the available commands and their parameters. If you use the StakMax Microplate Handling System, the StakMax Software has a built in scripting tool. See StakMax Microplate Handling System on page 192. The SoftMax Pro Software also supports a Microsoft Excel Add-In to write and run Excel Workflows.

SoftMax Pro Excel Workflows

The SoftMax Pro Excel workflows are designed to expand the industry-leading handling of Plate Format Data by the SoftMax Pro Software with Excel-based handling of List Format Data. You can use SoftMax Pro Excel workflows to run discontinuous kinetic reads, multiplexed reads, kinetic well scan reads, and temperature-triggered reads.

The SoftMax Pro Automation SDK is the underlying mechanism used by SoftMax Pro Excel workflows to access SoftMax Pro Software functionality. If you want to write your own workflows, you need to be familiar with the available Automation Commands. See the *SoftMax Pro Software Automation API Reference Guide*.

Before you can use Excel to create and run automated workflows, you must install the custom Add-In in Excel. With the Add-In installed you can run or edit the provided workflows or create your own.

The provided Excel workflows are installed in the same folder as the Excel Add-In files.

- For a 32-bit installation, the workflows are in the following folder:
  C:\Program Files\Molecular Devices\SoftMax Pro 7.0.2 Automation SDK\ExcelAddIn
- For a 64-bit installation, the workflows are in the following folder:
  C:\Program Files (x86)\Molecular Devices\SoftMax Pro 7.0.2 Automation SDK\ExcelAddIn

The Excel workflow files have an extension of **.xlsm**.

Before you run or edit a provided Excel workflow, save a copy of the workflow on your computer.

For complete information about how to setup and use SoftMax Pro Excel Workflows, see the *SoftMax Pro Software Automation API Reference Guide*.
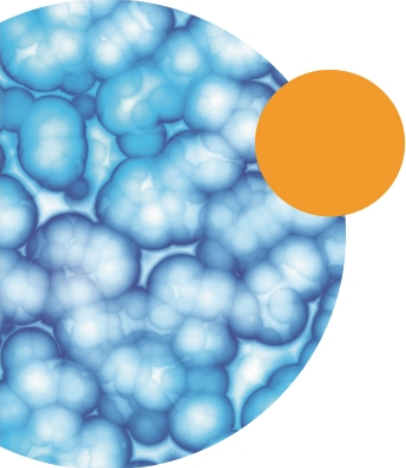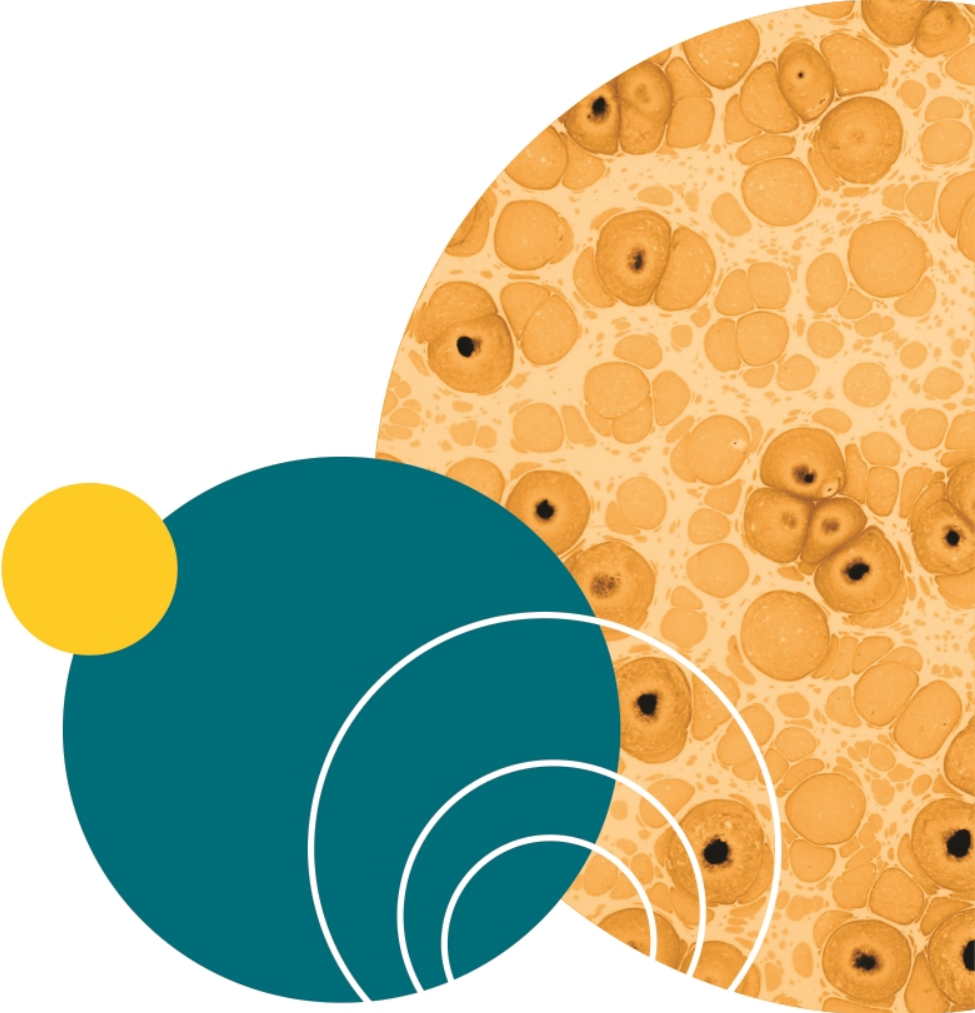
Chapter 3: File and Application Management

The **Application** menu in the upper-left corner of the SoftMax Pro Software window contains options to work with files, print, and manage the application.

Click the **Application** [icon] button to open the **Application** menu and then click a menu option. See Application Menu on page 51.

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 the data analysis you might have completed.

SoftMax Pro Software version 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.

> **Note:** Files with data acquired from a FlexStation® 3 Multi-Mode Microplate Reader can be opened for data analysis only.

You can open data files that have the following file extensions:
- .sda (SoftMax Pro Software 6 and 7 data files)
- .sdax (SoftMax Pro GxP Software 6 and 7 data files)
- .pda (SoftMax Pro Software 4 and 5 data files)
- .eda (SoftMax Pro GxP Software 4 and 5 data files)

See Open Data Files on page 61.

All SoftMax Pro Software version 6 data files are saved with the .sda or .sdax extension. See Save Data Files on page 62.

When you create a data file for an imaging experiment or a Western Blot experiment, the SoftMax Pro Software creates a folder with the same name as the data file. See Image Data Files and Folders on page 59.

You can use the Auto Save feature to prevent data loss. For more information and a description of the data file types, see Auto Save Settings on page 69.

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 Export Plate Data on page 76.

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 and 7 protocol files)
- .sprx (SoftMax Pro GxP Software 6 and 7 protocol files)
- .ppr (SoftMax Pro Software 4 and 5 protocol files)
- .epr (SoftMax Pro GxP Software 4 and 5 protocol files)

See Open Protocol Files on page 67.

All SoftMax Pro Software version 6 protocol files are saved with the .spr or .sprx extension. Saving a data file as a protocol file removes the data in the file and leaves only the configuration information. See Save Protocol Files on page 68.

Predefined Protocols

Several 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 Save a Protocol as the Default Protocol on page 64.

You can use the features available on the **Protocols** tab in the application ribbon to manage and share protocol files. See Protocols Tab on page 42.

Image Data Files and Folders

Before you start an image acquisition for Imaging mode or a Western Blot scan, you must save the data file in a location with enough capacity for the image files. You create and save data files for image-based experiments the same as you do for other experiments. See Create Data Files on page 60.

For best results, save your imaging data file on a secondary internal hard drive. You can use an external hard drive, but this can slow the data acquisition and is not recommended.

Note: To prevent data loss, do not save your imaging data file on a USB flash drive or to a network location. These methods are not supported by Molecular Devices.

When you create a data file for an imaging experiment or a Western Blot experiment, the SoftMax Pro Software creates a folder with the same name as the data file. For example, if you save the data file with the name **Imaging_Data**, then a new folder named **Imaging_Data** is also created in the same folder where the data file is saved. This folder stores the acquired images and analysis results.

```
Imaging_Experiments
    Imaging_Data.sda
    Imaging_Data
        Expt1
            Plate1
                IMAGES
                RESULTS
```
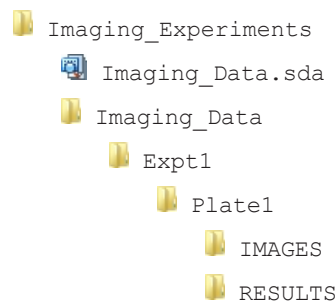
The acquired images are stored separately for each microplate in the **IMAGES** folder in TIFF format. The image analysis data are stored in the **RESULTS** folder. For a Western Blot scan, the **RESULTS** folder is not created or used.

CAUTION! Do not manually rename the data file. Do not manually rename, move, or delete the related folders or files. If you make changes to the structure of these files and folders, the SoftMax Pro Software may not be able open the data file or can display inaccurate results.

To make a copy of the data file, image files, and analysis data files, click the **Application** button in the ribbon to open the **Application** menu, and then click **Save As**. See Save Data Files on page 62.

To delete the acquired images or the analysis data for an Imaging mode read of a microplate, in the **Navigation Tree** right-click the **Plate** section and then click **Delete Images** or **Delete Cell Data**. See Navigation Tree on page 30.

If you delete the TIFF file for a Western Blot scan, the SoftMax Pro Software recreates the TIFF image from the intensity data stored in the data file.

Molecular Devices recommends that you always use the file-management tools in the SoftMax Pro Software to copy or delete your imaging files.

> **Note:** When you save an imaging experiment as a protocol, only the protocol file is saved. No other folders or files are created. See Create Protocol Files on page 67.

Acquiring images in Imaging read mode requires a large portion of computer memory and resources.

Each acquired image file for an Imaging mode read can be larger than 2 megabytes. Acquiring the image of a single site in each well of a 96-well microplate can generate 300 megabytes of image data. A 384-well microplate can generate 1 gigabyte of image data. Acquiring images of multiple sites increases the data-storage requirement. Molecular Devices recommends that you use a data management system.

Before you start an image acquisition, minimize the demands on computer memory and resources by turning off all other programs. When the SoftMax Pro Software has limited access to computer memory and resources, image acquisition can take a long time. In some cases, images of some of the wells can be lost.

To prevent data loss, turn off all sleep and hibernation settings for the hard disk, the CPU, and the USB ports. Disable automatic Windows Updates. Update Windows manually when the instrument is not used by the software. You can set these options in Windows Control Panel. See Optimize Your Computer for Image Acquisition on page 298.

Molecular Devices recommends that you use Auto Save with imaging data for saving to text files only. If you use Auto Save to save multiple imaging data files, each new data file is saved without the related folders required for saving images and analysis results. See Auto Save Settings on page 69.

Create Data Files

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 displays above the navigation tree.

   > **Note:** Molecular Devices recommends that you name and save 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. Enter the file name in the **File name** field.

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

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

8. Click **Save**.

   The name of the data file displays on the tab above the navigation tree.

When you create a data file for an imaging experiment or a Western Blot experiment, the SoftMax Pro Software creates a folder with the same name as the data file. See Image Data Files and Folders on page 59.

Open Data Files

To open a supported protocol or data file, use the **Application** menu. SoftMax Pro Software version 7 can open files created by version 4.0 or newer. 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 and 7 data files)
- .sdax (SoftMax Pro GxP Software 6 and 7 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.

Files with data acquired from a FlexStation® 3 Multi-Mode Microplate Reader can be opened for data analysis only.

SoftMax Pro Software version 7 can open files created by version 4.0 or newer. 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 newer, and then save it as a version 4.0 or newer file. Instrument compatibility requirements also must be met.

This version of the SoftMax Pro Software saves the new file with a different file extension from the extension used in previous versions. The file created with the older version can continue to be used with the older version without affecting the contents of the new file.

After you open a file from a previous version, it is good practice to review the file to make sure that the conversion went well. In some cases, the file conversion might change instrument settings, curve fits, or other items that have been updated for version 7.

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 version 7 can open files created by version 4.0 or newer.

   When you open a file from a previous version, it is converted to SoftMax Pro Software version 7 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.

Save 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 Save Data as a PDF on page 401.

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, enter a name for the file.
5. From the **Save as type** field, select **Data Files (*.sda)**.

   If you use 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.

When you create a data file for an imaging experiment or a Western Blot experiment, the SoftMax Pro Software creates a folder with the same name as the data file. See Image Data Files and Folders on page 59.

Copy and Paste Data

You can copy plate data from one **Plate** section to a different **Plate** section 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 Clone Plate Sections on page 112.

In some cases, you can paste endpoint plate data copied from an external program, such as a spreadsheet or text editor. When you paste 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 Plate Sections on page 105.

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 paste data from an external program, click **Paste Data**.
3. When you paste over existing data, click **Yes** in the message box that displays.

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

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

Manage 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 community for protocol sharing, exchanging tips and tricks, and networking with other experienced users. See Protocols Tab on page 42.

The software includes over 120 assay protocols to speed life science research and drug discovery assay development and screening. You 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 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 find and open a protocol. See Protocol Manager - Open Files on page 64.

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 Save a Protocol as the Default Protocol on page 64.

Saving a data file as a protocol file removes the 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 Add or Remove Folders in the Protocol Manager on page 65.

Protocol Manager - Open Files

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 Create Protocols on page 205.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined 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 the protocol to assign to a new file.

2. Select **Protocol Library >** *foldername* to view the 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 section or Cuvette Set section to an experiment, you can configure the instrument settings using the **Settings** dialog. See Instrument Settings on page 216.

Save 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.

Save a Default Protocol

1. Click **Save as Default** to overwrite the default.spr file with the protocol setting of the experiment that is active in your workspace.

2. Click **OK** to overwrite the default protocol file with this file. With the exception of acquired data, all settings and sections (with their contents) are saved as part of the new default protocol. When you create a new file, it will initially be identical to this current data file (minus existing data).

> **Note:** When you save the file as the default protocol, the data you have collected in the current experiment remains unchanged. If you want to keep the data, you need to save the data into a data file. See Save Data Files on page 62.

Add or Remove Folders in the Protocol Manager

You can add folders in your file system to those folders where protocols can be stored. See Add Protocol Folder Locations on page 65 and Delete Protocol Folder Locations on page 66.

Whenever you store protocol files in the added folder, those protocols are available in the **Protocol Manager**. See Protocol Manager - Open Files on page 64.

Add Protocol Folder Locations

To add a protocol folder location to the Protocol Manager:

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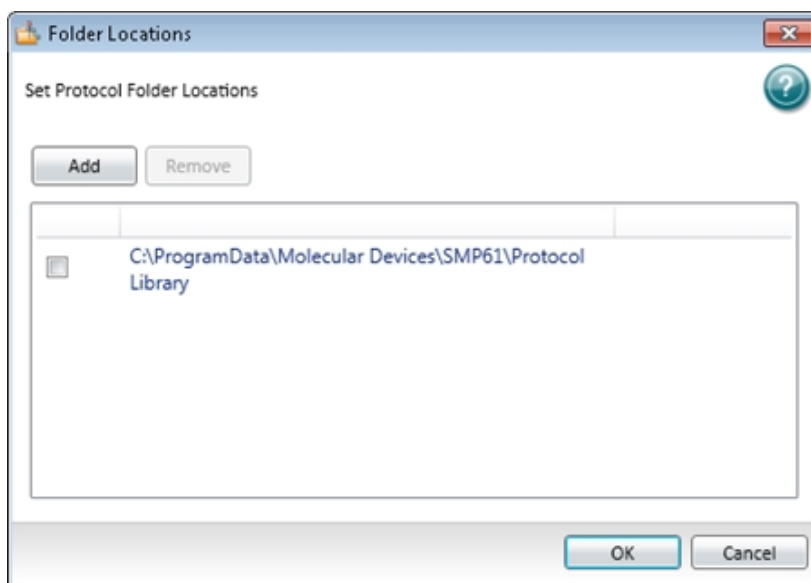
2. Click **Add** to browse the file system to the folder to be added.
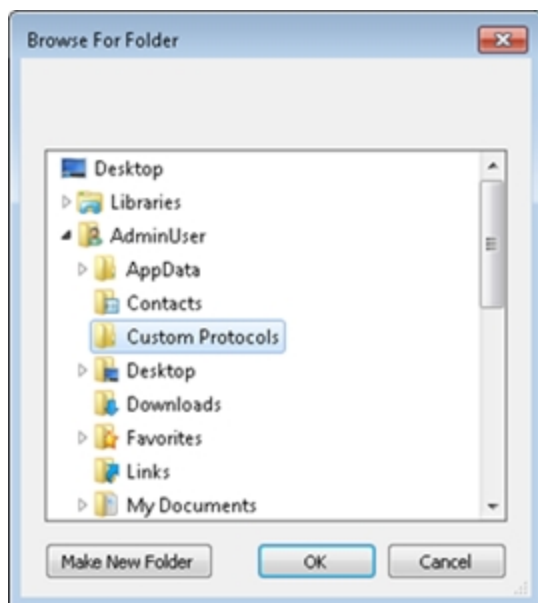
3. Select the folder where you want to store protocols.

4. Optionally, click **Make New Folder** to add a New Folder inside the selected folder and then enter the name for the folder.

5. Click **OK** to add the selected folder to the folder list in the **Folder Locations** dialog.

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

Delete Protocol Folder Locations

To remove a protocol folder location from the Protocol Manager:

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

2. Select the check box for the folder location to remove.
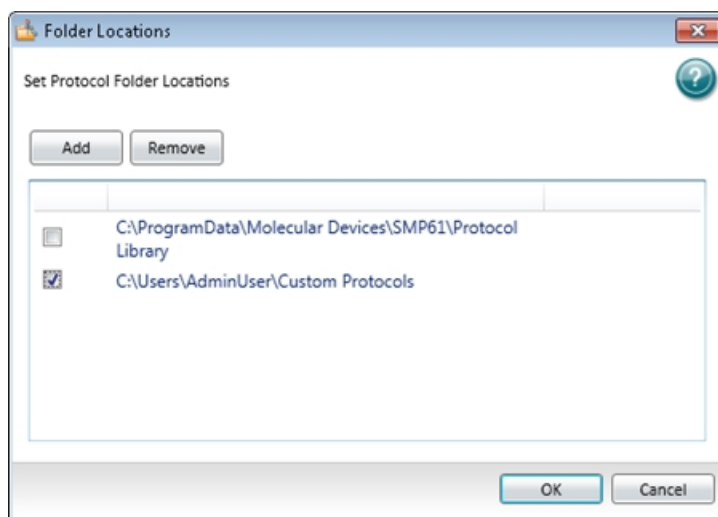
3. Click **Remove** to remove the selected folder from the folder list in the **Folder Locations** dialog.

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

> **Note:** Removing a protocol folder location from the Protocol Manager does not remove the folder or the files from your computer.

Create Protocol Files

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 Protocol Manager - Open Files on page 64.

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 displays above the navigation tree.

3. Configure the protocol settings, see Create Protocols on page 205.

> **Note:** Molecular Devices recommends that you save and 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. Enter the file name in the **File name** field.

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

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

9. Click **Save**.

Before you use the protocol to collect data, save it as a data file. See Save Data Files on page 62.

Open Protocol Files

To open a supported protocol file, use the **Application** menu. SoftMax Pro Software version 7 can open files created by version 4.0 or newer. However, after you save a file, the new file is readable only by the current version.

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

- .spr (SoftMax Pro Software 6 and 7 protocol files)
- .sprx (SoftMax Pro GxP Software 6 and 7 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 version 7 can open files created by version 4.0 or newer. 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 newer 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.

After you open a file from a previous version, it is good practice to review the file to make sure that the conversion went well. In some cases, the file conversion might change instrument settings, curve fits, or other items that have been updated for version 7.

To open a predefined protocol in the Protocol Manager, see Protocol Manager - Open Files on page 64.

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 version 7 can open files created by version 4.0 or newer.

   When you open a file from a previous version, it is converted to SoftMax Pro Software version 7 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 you use the protocol to collect data, save it as a data file. See Save Data Files on page 62.

Save 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 Applications menu.
2. Click **Save As**.
3. In the **Save As** dialog, navigate to where you want to save the protocol file.
4. In the **File name** field, enter 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 the data in the file, leaving only the configuration information.

Before you use the protocol to collect data, save it as a data file. See Save Data Files on page 62.

Auto Save Settings

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 192.

Molecular Devices recommends that you use Auto Save with imaging data for saving to text files only. If you use Auto Save to save multiple imaging data files, each new data file is saved without the related folders required for saving images and analysis results. See Image Data Files and Folders on page 59.

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.
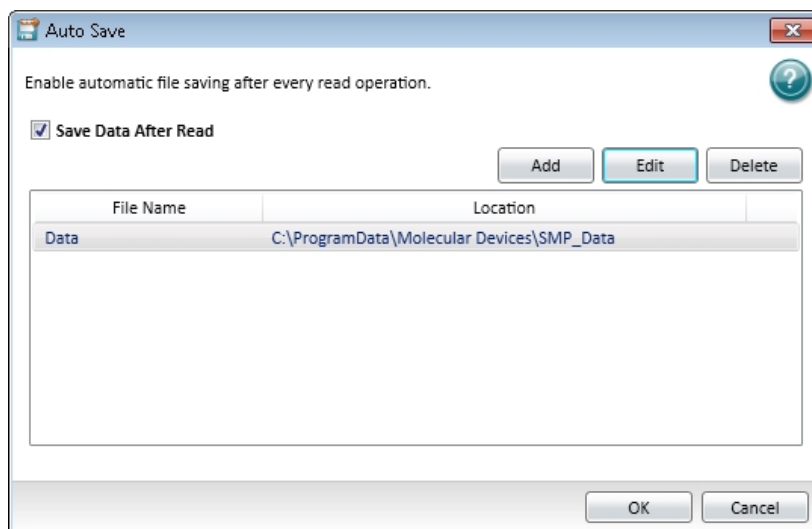
2. To enable Auto Save, select **Save Data After Read**.
3. To create a new Auto Save location, click **Add**.

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

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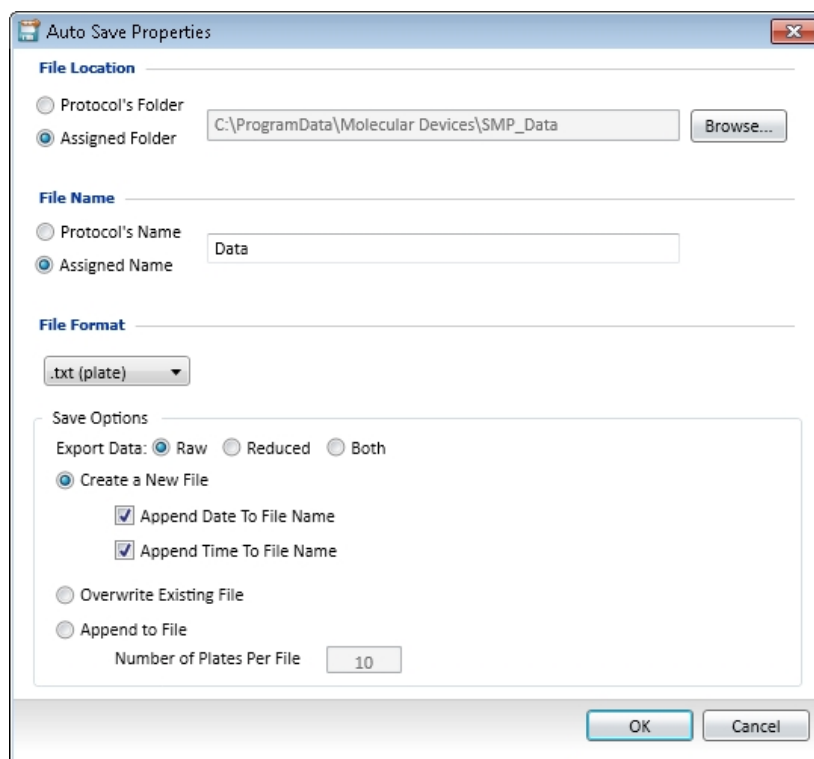
The **Auto Save Properties** dialog contains the following options:

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 enter a name for the file. The text in this field is automatically inserted at the start 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 let you specify what type of file is saved. Version 7 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 you select **.sda** or **.sdax**, data is saved in a proprietary SoftMax Pro Software file format.

XML File

When you select **.xml**, data is 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 you select either **.txt (list)** or **.txt (plate)**, data is saved as a tab-delimited text file, which can be opened by most word processor, spreadsheet, or database programs.

Excel

When you select either **.xls (list)** or **.xls (plate)**, data is 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 lets the file be opened easily by Excel.

Save Options

The available save options depend on the selected file format for the 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 February 12th, 2017 would be named as **Data-02-12-17 1**, while the second saved file would be named **Data-02-12-17 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 a different 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 you select either a .txt or .xls format, you can choose to append the plate data to the current file. Enter the maximum number of plates to append per file.

If you read 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 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 display on the right:

- Click **Print All** to print all the included sections in the file. See Print All Included Sections on page 73.

- Click **Print Preview** to preview the print job before you print. See Preview Print Jobs on page 73.

- Click **Print Selected** to select the sections to print. See Print Selected Sections on page 73.

- Click **Printing Options** to define the header and footer of the pages and the print quality for the print job. See Print Settings on page 75.

You can predefine the sections to exclude from your future print jobs by disabling printing for the sections in the **Navigation Tree**. See Disable Sections from Print Jobs on page 76.

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 PDF File Settings on page 403.

Print 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 exclude a section from printing by disabling printing for the section in the **Navigation Tree**, the section does not print. See Disable Sections from Print Jobs on page 76.

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 to use and set the 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 PDF File Settings on page 403.

4. Click **Print**.

Preview Print Jobs

To preview the print job before you print:

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, 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 to use and set the 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 PDF File Settings on page 403.

6. Click **Print**.

Print Selected Sections

To select the sections to print:

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

2. Click **Print Selected** ![icon].
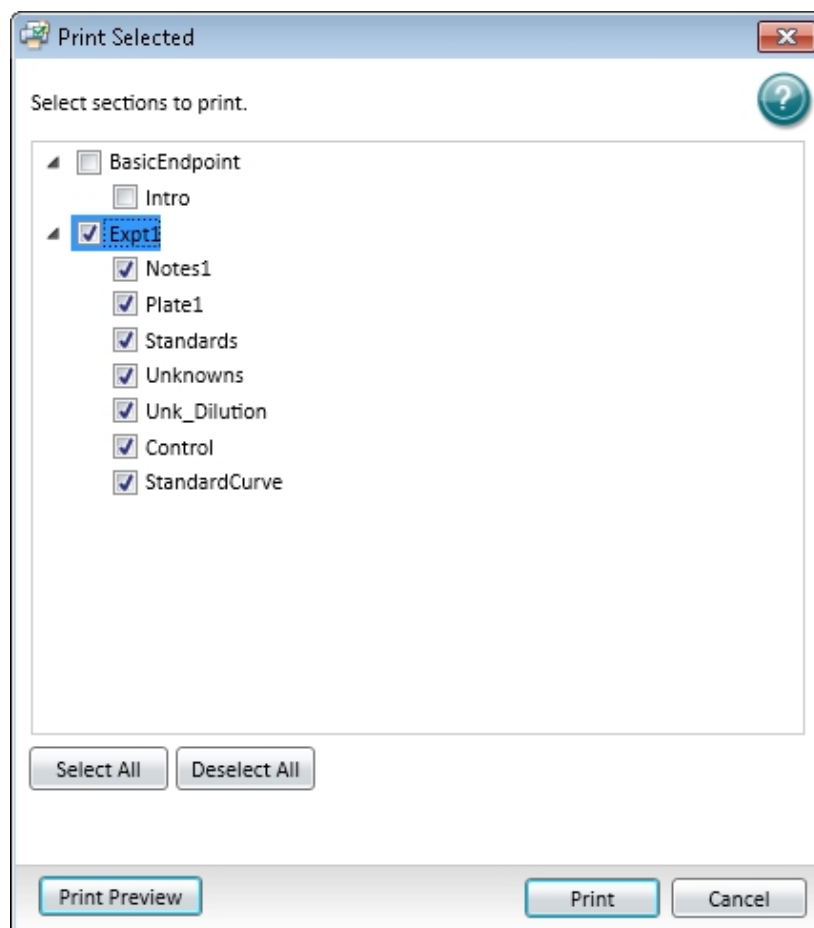
3. In the **Print Selected** dialog, select the check box for each section to print and clear the check box for each section to not print.

   - To select all the check boxes, click **Select All**.
   - To clear all the check boxes, click **Deselect All**.
   - To preview the print job before you print, click **Print Preview**. See Preview Print Jobs on page 73.

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

5. In the **Print** dialog, select the printer to use and set the 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 PDF File Settings on page 403.

6. Click **Print**.

Print Settings

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** .
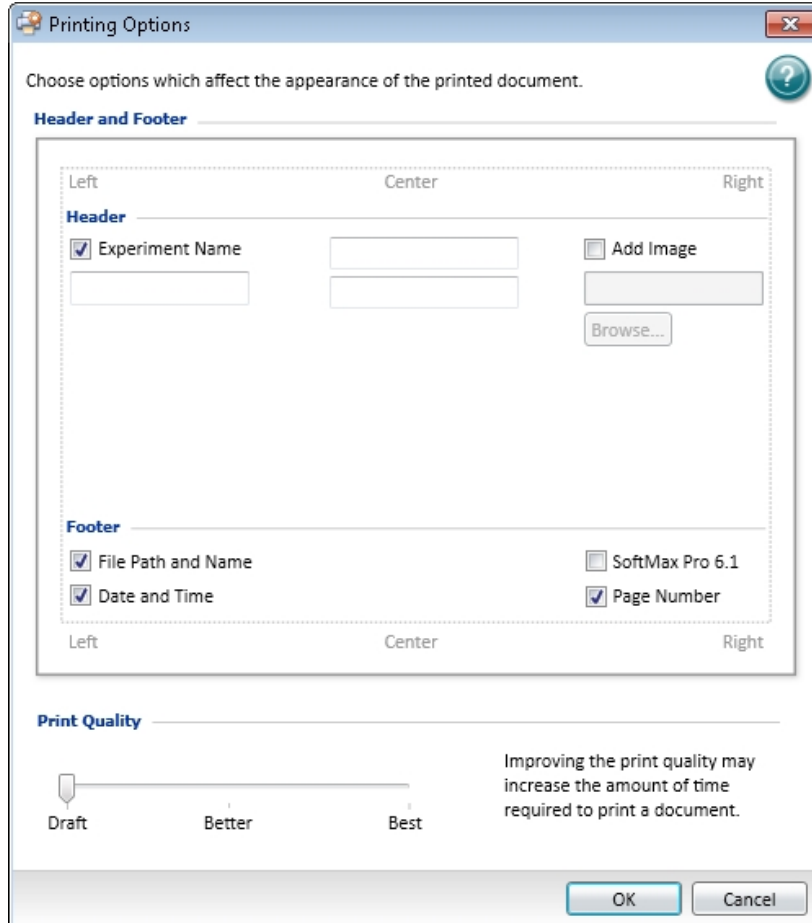
3. In the **Printing Options** dialog, define the header and footer of the pages.
   - To include the predefined text, select the check box next to the text.
   - To include custom text in the header, enter the text in the fields.
   - To add a JPEG image, such as a logo, to the right side of the header, select the **Add Image** check box and then click the **Browse** button to locate and select the image file. When printing, the 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**.

Disable Sections from Print Jobs

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

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

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

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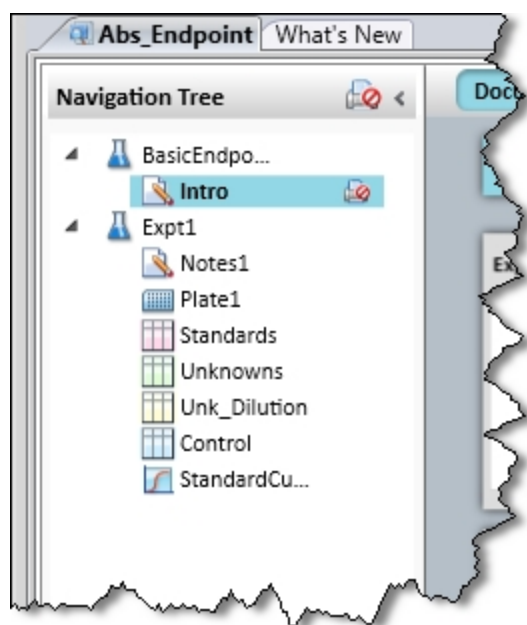
To enable printing the selected section, click the **Disable printing for the selection** icon again.

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**.

Export 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 space available to view the characters.

You can customize the data display to **Numeric notation** or **Scientific notation** format, and specify the number of **Significant Figures** or **Decimal Places**. These options can be set separately for raw data and reduced data.

When the number of digits is increased, the font size of the values in the Plate section is decreased. If the value becomes too long, then the numbers are cut off at the edge of the wells.

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.

For information on how to export membrane data, see View Western Blot Membrane Data on page 395.

For information on how to export Imaging data to Microsoft Excel or PowerPoint, see View Imaging Data in Wells on page 322.

If you want to export data in PDF format, see Save Data as a PDF on page 401.

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 for each section to export.
   - To exclude a section from the export, clear the check box for each section to not export.
   - 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 **Plate Data Options**:
   - **Raw**
   - **Reduced**
   - **Both**

   > **Note:** These options apply only if you select one or more **Plate** sections.

5. Click the **Output Format** you want for the export file:
   - **Columns** exports data in a single column of text for each well.
   - **Plate** exports data in a text matrix that corresponds 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 you select one or more plate sections.

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, enter 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**.

Export File Formats

Whether you save the export as a .txt or .xls, the information is presented as tab-separated values. This topic describes the basic information that is included in these two export formats.

For a copy of the SoftMax Pro XML schema, contact Molecular Devices Technical Support.

The first line of an export file defines the number of sections (or "Blocks") that are included in the export using the following format:

- ##BLOCKS= *n*

    where *n* is the number of sections that are included in the export file.

The selected sections are exported in the order that they display in the SoftMax Pro Software data file.

Sections are presented in the following format:

- Header line

    The header line contains the type of section, such as **Note**, **Plate**, or **Group**, and the title of the section as defined in the SoftMax Pro Software data file. **Plate** sections contain more information in the header line.

- Data lines

    The data lines immediately follow the header line and contain the text or data from the exported section. The layout and format of the data lines are dependent on many factors, such as the section type, export type, read mode, and read type.

- End line

    The end line of each section is defined by the text: **~End**

The final line of an export file contains a single field with the original name given to the export file, and the date and time that the file was last saved.

For more detailed information on export formats, see the following topics:

-
-
-

Plate Export Formats

This topic describes the format of **Plate** sections that are exported as a .txt or .xls file. For a copy of the SoftMax Pro XML schema, contact Molecular Devices Technical Support.

- The header line contains the type of section, such as **Plate**, and the title of the section as defined in the SoftMax Pro Software data file. **Plate** sections contain more information in the header line. See .

- The data lines follow the header line and contain the data from the exported section. The layout and format of the data lines are dependent on many factors, such as the section type, export type, read mode, and read type. See Plate Data Lines on page 86.

Plate Header Fields

The fields in the header line for a **Plate** section help define the data that is presented in the section. The order and content of the fields are different depending on the read mode you use to collect the data.

The tables that follow define the fields in the header line for the different read modes:

Table 3-1: Header Fields for Absorbance (ABS) Read Mode

Position	Name	Description
1.	Section type	Contains the type of section such as **Plate**
2.	Section name	Contains the title of the section as defined in the SoftMax Pro Software data file
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains **TimeFormat** for Columns output format or **PlateFormat** for Plate output format as defined in the **Export** dialog
5.	Read type	Contains the read type used for the acquisition, such as **Endpoint**, **Kinetic**, **Spectrum**, or **Well Scan**
6.	Read mode	Contains the read mode used for the acquisition, such as **Absorbance**
7.	Data type	Contains **Raw** or **Reduced** to define the type of data in the export file
8.	Pre-read	Always **FALSE** Pre-read is not a function of SoftMax Pro Software version 6 or newer
9.	Kinetic points	Contains the number of reads done during the acquisition For Endpoint reads the number is **1**
10.	Read time Read pattern	Contains the read time in seconds for a Kinetic read Contains the scan pattern for a Well Scan read Contains no value (blank) for other read types

Table 3-1: Header Fields for Absorbance (ABS) Read Mode (continued)

Position	Name	Description
11.	Kinetic interval Well Scan density	Contains the interval between reads in seconds for a Kinetic read Contains the scan density for a Well Scan read Contains no value (blank) for other read types
12.	Start wavelength	Contains the starting wavelength for a Spectrum read Contains no value (blank) for other read types
13.	End wavelength	Contains the ending wavelength for a Spectrum read Contains no value (blank) for other read types
14.	Wavelength step	Contains the wavelength increment or step for a Spectrum read Contains no value (blank) for other read types
15.	Number of wavelength	Contains the number of wavelengths read for the acquisition Contains no value (blank) for Spectrum reads
16.	Wavelengths	Contains the wavelengths used for the acquisition For multiple-wavelength reads, the values are separated by a space Contains no value (blank) for Spectrum reads
17.	First column	Contains the number of the first column read during the acquisition
18.	Number of columns	Contains the number of columns read during the acquisition
19.	Number of wells	Contains the total number of wells in the microplate, such as **96** or **384**
20.	First row	Contains a number corresponding with the first row read during the acquisition
21.	Number of rows	Contains the number of rows read during the acquisition
22.	Time tags	Contains time-tag information if the exported data has read-time tags

Table 3-2: Header Fields for Fluorescence Intensity (FL) and AlphaScreen (ALPHA) Read Modes

Position	Name	Description
1.	Section type	Contains the type of section such as **Plate**
2.	Section name	Contains the title of the section as defined in the SoftMax Pro Software data file
3.	Export version	Contains the version of the export application used for this export

Table 3-2: Header Fields for Fluorescence Intensity (FL) and AlphaScreen (ALPHA) Read Modes (continued)

Position	Name	Description
4.	Export format	Contains **TimeFormat** for Columns output format or **PlateFormat** for Plate output format as defined in the **Export** dialog
5.	Read type	Contains the read type used for the acquisition, such as **Endpoint**, **Kinetic**, **Spectrum**, or **Well Scan**
6.	Read mode	Contains the read mode used for the acquisition, such as **Fluorescence** or **Luminescence**
7.	Bottom read	Contains **TRUE** if reading from the bottom of the microplate and **FALSE** if reading from the top
8.	Data type	Contains **Raw** or **Reduced** to define the type of data in the export file
9.	Pre-read	Always **FALSE** Pre-read is not a function of SoftMax Pro Software version 6 or newer
10.	Kinetic points	Contains the number of reads done during the acquisition For Endpoint reads the number is **1**
11.	Read time Read pattern	Contains the read time in seconds for a Kinetic read Contains the scan pattern for a Well Scan read Contains no value (blank) for other read types
12.	Kinetic interval Well Scan density	Contains the interval between reads in seconds for a Kinetic read Contains the scan density for a Well Scan read Contains no value (blank) for other read types
13.	Start wavelength	Contains the starting wavelength for a Spectrum read Contains no value (blank) for other read types
14.	End wavelength	Contains the ending wavelength for a Spectrum read Contains no value (blank) for other read types
15.	Wavelength step	Contains the wavelength increment or step for a Spectrum read Contains no value (blank) for other read types
16.	Number of wavelengths	Contains the number of wavelengths read for the acquisition Contains no value (blank) for Spectrum reads

Table 3-2: Header Fields for Fluorescence Intensity (FL) and AlphaScreen (ALPHA) Read Modes (continued)

Position	Name	Description
17.	Wavelengths	Contains the wavelengths used for the acquisition For multiple-wavelength reads, the values are separated by a space For Fluorescence reads, contains the emission wavelengths Contains no value (blank) for Spectrum reads or Ex/Em sweeps
18.	First column	Contains the number of the first column read during the acquisition
19.	Number of columns	Contains the number of columns read during the acquisition
20.	Number of wells	Contains the total number of wells in the microplate, such as **96** or **384**
21.	Excitation wavelengths	Contains the excitation wavelengths used for a Fluorescence read For multiple-wavelength reads, the values are separated by a space Contains no value (blank) for Spectrum reads or Ex/Em sweeps
22.	Cutoff	Contains **Manual** or **Automatic** as defined in the **Settings** dialog
23.	Cutoff filters	Contains the wavelengths of the cutoff filters with the values separated by a space
24.	Sweep wave	Contains **Excitation Sweep** or **Emission Sweep** as defined in the **Settings** dialog
25.	Sweep fixed wavelength	Contains the fixed wavelength of an Ex/Em sweep
26.	Reads per well	Contains the number of times a well is read during a single reading
27.	PMT gain	Contains **Automatic**, **High**, **Medium**, or **Low** as defined in the **Settings** dialog
28.	Start integration time	Contains the time to start integration for a TRF read. Contains no value (blank) for other read modes
29.	End integration time	Contains the time to end integration for a TRF read. Contains no value (blank) for other read modes
30.	First row	Contains a number corresponding with the first row read during the acquisition
31.	Number of rows	Contains the number of rows read during the acquisition
32.	Time tags	Contains time-tag information if the exported data has read-time tags

Table 3-3: Header Fields for Luminescence (LUM), Time-Resolved Fluorescence (TRF), and Fluorescence Polarization (FP) Read Modes

Position	Name	Description
1.	Section type	Contains the type of section such as **Plate**
2.	Section name	Contains the title of the section as defined in the SoftMax Pro Software data file
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains **TimeFormat** for Columns output format or **PlateFormat** for Plate output format as defined in the **Export** dialog
5.	Read type	Contains the read type used for the acquisition, such as **Endpoint**, **Kinetic**, **Spectrum**, or **Well Scan**
6.	Read mode	Contains the read mode used for the acquisition, such as **Luminescence** or **Time Resolved**
7.	Data type	Contains **Raw** or **Reduced** to define the type of data in the export file
8.	Pre-read	Always **FALSE** Pre-read is not a function of SoftMax Pro Software version 6 or newer
9.	Kinetic points	Contains the number of reads done during the acquisition For Endpoint reads the number is **1**
10.	Read time Read pattern	Contains the read time in seconds for a Kinetic read Contains the scan pattern for a Well Scan read Contains no value (blank) for other read types
11.	Kinetic interval Well Scan density	Contains the interval between reads in seconds for a Kinetic read Contains the scan density for a Well Scan read Contains no value (blank) for other read types
12.	Start wavelength	Contains the starting wavelength for a Spectrum read Contains no value (blank) for other read types
13.	End wavelength	Contains the ending wavelength for a Spectrum read Contains no value (blank) for other read types
14.	Wavelength step	Contains the wavelength increment or step for a Spectrum read Contains no value (blank) for other read types
15.	Number of wavelengths	Contains the number of wavelengths read for the acquisition Contains no value (blank) for Spectrum reads

Table 3-3: Header Fields for Luminescence (LUM), Time-Resolved Fluorescence (TRF), and Fluorescence Polarization (FP) Read Modes (continued)

Position	Name	Description
16.	Wavelengths	Contains the wavelengths used for the acquisition For multiple-wavelength reads, the values are separated by a space For Time Resolved reads, contains the emission wavelengths Contains no value (blank) for Spectrum reads or Ex/Em sweeps
17.	First column	Contains the number of the first column read during the acquisition
18.	Number of columns	Contains the number of columns read during the acquisition
19.	Number of wells	Contains the total number of wells in the microplate, such as **96** or **384**
20.	Excitation wavelengths	Contains the excitation wavelengths used for a Time Resolved read For multiple-wavelength reads, the values are separated by a space Contains no value (blank) for Spectrum reads or Ex/Em sweeps
21.	Cutoff	Contains **Manual** or **Automatic** as defined in the **Settings** dialog
22.	Cutoff filters	Contains the wavelengths of the cutoff filters with the values separated by a space Contains no value (blank) if no cutoff filter is used
23.	Sweep wave	Contains **Excitation Sweep** or **Emission Sweep** as defined in the **Settings** dialog
24.	Sweep fixed wavelength	Contains the fixed wavelength of an Ex/Em sweep
25.	Reads per well	Contains the number of times a well is read during a single reading
26.	PMT gain	Contains **Automatic**, **High**, **Medium**, or **Low** as defined in the **Settings** dialog
27.	Start integration time	Contains the time to start integration for a Time Resolved read. Contains no value (blank) for other read modes
28.	End integration time	Contains the time to end integration for a Time Resolved read. Contains no value (blank) for other read modes
29.	First row	Contains a number corresponding with the first row read during the acquisition

Table 3-3: Header Fields for Luminescence (LUM), Time-Resolved Fluorescence (TRF), and Fluorescence Polarization (FP) Read Modes (continued)

Position	Name	Description
30.	Number of rows	Contains the number of rows read during the acquisition
31.	Time tags	Contains time-tag information if the exported data has read-time tags
Position	**Name**	**Description**
1.	Section type	Contains the type of section, such as **Plate**
2.	Section name	Contains the title of the section as defined in the SoftMax Pro Software data file
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains **TimeFormat** for Columns output format or **PlateFormat** for Plate output format as defined in the **Export** dialog
5.	Read type	Contains the read type used for the acquisition, such as **Endpoint**
6.	Read mode	Contains the read mode used for the acquisition, such as **Imaging**
7.	Data type	Contains **Raw** or **Reduced** to define the type of data in the export file
8.	Pre-read	Always **FALSE** Pre-read is not a function of SoftMax Pro Software version 6 or newer
9.	Kinetic points	Contains the number of reads done during the acquisition For Endpoint reads the number is **1**
10.	Read time	Contains no value (blank) for Imaging reads
11.	Kinetic interval	Contains no value (blank) for Imaging reads
12.	Start wavelength	Contains no value (blank) for Imaging reads
13.	End wavelength	Contains no value (blank) for Imaging reads
14.	Wavelength step	Contains no value (blank) for Imaging reads
15.	Number of wavelengths	Contains the number of wavelengths, or channels, read for the acquisition
16.	Emission wavelengths	Contains the emission wavelengths used for the acquisition For multiple-wavelength reads, the values are separated by a space

Table 3-3: Header Fields for Luminescence (LUM), Time-Resolved Fluorescence (TRF), and Fluorescence Polarization (FP) Read Modes (continued)

Position	Name	Description
17.	First column	Contains the number of the first column read during the acquisition
18.	Number of columns	Contains the number of columns read during the acquisition
19.	Number of wells	Contains the total number of wells in the microplate, such as **96** or **384**
20.	First row	Contains a number corresponding with the first row read during the acquisition
21.	Number of rows	Contains the number of rows read during the acquisition
22.	Excitation wavelength	Contains the excitation wavelengths used for the acquisition For multiple-wavelength reads, the values are separated by a space
23.	Analysis type	Contains the analysis type used, such as **Discrete Object Analysis**
24.	Classification	Contains **On** if classification is used and **Off** if it is not used
25.	Find on	Contains the emission wavelength used for finding objects or fields
26.	Measurement	Contains the type of measurement selected in the **Display Options** dialog, such as **Cell Count**

Plate Data Lines

The data lines immediately follow the header line.

- The first column shows the time points for a kinetic read, the data points in a well scan read, or the wavelengths in a spectrum read. For endpoint reads, this column is blank.
- The second column shows the temperature for the read, if you use the incubator for the read. If the incubator is off, the value is zero.

The read data starts in the third column. When both raw and reduced data are exported, the reduced data displays below the raw data.

The format of the data depends on whether the data was exported in column format or plate format.

For column formats, the data displays in a single row. The data for multiple time points for kinetic reads, multiple data points for well scan reads, or multiple wavelengths for spectrum reads is listed in separate rows. For endpoint, kinetic, or well scan reads with multiple wavelengths, the data for each wavelength displays in subsequent rows below the previous wavelength. For imaging reads, the data for each measurement is in a separate row.

Time	Temperature	A1	A2	A3	...	B1	B2	B3	...	
0:00:00	##	1.596	2.479	2.95	...	3.002	3.103	1.39	...	wavelength 1
0:01:58	##	1.564	3.344	2.378	...	2.744	0.113	3.872	...	
...	##	...	...	...		...	...	...	...	
0:00:00	##	3.928	3.645	1.956	...	3.622	2.195	0.519	...	wavelength 2
0:01:58	##	2.891	3.824	2.782	...	3.203	1.105	0.281	...	
...	##	...	...	...		...	...	...	...	

Raw Data

A1	A2	A3	...	B1	B2	B3	...
203.45455	-18.91525	-28.13251			106.53005	82.585516	...

Reduced Data

Figure 3-1: Kinetic data exported in column format

For plate formats, the data displays in a table to indicate the columns and rows of the microplate. The data for multiple time points for kinetic reads, multiple data points for well scan reads, or multiple wavelengths for spectrum reads is listed in separate tables below the previous table. For endpoint, kinetic, or well scan reads with multiple wavelengths, the data for each wavelength displays in separate tables to the right of the previous table. For imaging reads, the data for each measurement is in a separate table to the right of the previous table.

wavelength 1

Temperature	1	2	3	...
0:00:00 ##	1.596	2.479	2.95	...
	1.024	0.308	3.925	...
	0.195	0.287	2.638	...
	...	...	...	...

0:01:58 ##	1.564	3.344	2.378	...
	0.82	1.797	1.064	
	0.421	3.066	3.906	
	...	...	...	...

... ##	1.169	1.092	3.969	...
	1.105	3.022	2.845	...
	3.602	2.033	3.737	
	...	...	...	...

wavelength 2

1	2	3	...
3.103	1.39	1.97	...
2.381	1.957	3.722	...
0.967	1.049	1.422	...
...	...	...	...

0.113	3.872	0.637	...
3.58	2.603	0.996	
0.915	0.57	2.251	...
...	...	...	...

1.126	0.004	1.934	...
1.933	2.834	0.657	...
0.437	3.242	1.337	...
...	...	...	...

Raw Data

1	2	3	...
203.45455	-18.91525	-28.13251	
74.597843	107.62096	-88.65948	
-134.6718	68.810478	-52.5208	...
...	...	...	...

1	2	3	...
106.53005	82.585516	121.96918	...
...376	-77.66718	-83.94453	...
246.51772	60.674884	48.51772	...
...	...	...	...

Reduced Data

Figure 3-2: Kinetic data exported in plate format

Group Section Export Formats

This topic describes the format of **Group** sections that are exported as a .txt or .xls file. For a copy of the SoftMax Pro XML schema, contact Molecular Devices Technical Support.

The header line contains the type of section, such as **Group**, and the title of the section as defined in the SoftMax Pro Software data file.

The data lines immediately follow the header line and contain the text and data from the exported section.

- The data from the group table displays in a table that contains the column headings in the first row followed by the data from the table.
- Group summaries, if present, display in the rows at the end of the section.

Note Section Export Formats

This topic describes the format of **Note** sections that are exported as a .txt or .xls file. For a copy of the SoftMax Pro XML schema, contact Molecular Devices Technical Support.

The header line contains the type of section, such as **Note**.

The data lines follow the header line and contain text and summary formulas from the exported section.

Images and section images from the Note section are not included in the export file.

Import Microplate Data

Note: The data import feature requires a separate license. To obtain a license for this feature, contact your Molecular Devices representative.

Templates to import data in the Plate format and a schema for the XML format are installed with the software.

- To import data from another program in Plate format, the tab-delimited text file must match the format of an import template. See Use Plate Format Templates to Import Data on page 89.
- To import data from another program in XML format, the XML file must be formatted using the SoftMax Pro Software XML schema. See Use the XML Schema to Import Data on page 91.

When you import microplate data, the SoftMax Pro Software creates a new **Plate** section using the section name from the import file. If the named **Plate** section already exists, then the imported data overwrites the existing data in the section.

When you import microplate data into an existing **Plate** section, the SoftMax Pro Software microplate template and reduction settings in the existing **Plate** section are preserved if the imported data is compatible with the settings in the existing section.

If you want to preserve the template and reduction settings, make sure that the name of the **Plate** section in the import file exactly matches the name of the **Plate** section in the SoftMax Pro Software protocol into which you plan to import the data. If the name of the **Plate** section in the text file does not match the name of a **Plate** section in the protocol, then the import process creates a new **Plate** section in the protocol.

Another way to preserve template and reduction settings is to copy the imported data from the new **Plate** section and then paste the data into an existing **Plate** section to use the template and reduction settings in the section where you pasted the data. See Copy and Paste Data on page 63.

Note: To maximize the number of data points used for reduction, the end time reduction parameter is changed to match the read time from the import file.

To import the microplate data:

1. Open or create a new protocol file.

2. If required, define a **Plate** section for importing the microplate data with the same name as the **Plate** section in the import file.

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

4. Click **Import** and then click the data format of the import file.

   - To import tab-delimited text data, click **Plate Format**.
   - To import XML data, click **XML Format**.

5. In the **Open** dialog, locate and select the file to import.

6. Click **Open**.

   The **Plate** section displays the imported data.

7. Save the file as a data file. See Save Data Files on page 62.

After the data imports into the **Plate** section, the **Read Information** on the right indicates that the data was imported and shows the time and date of the import. For the GxP edition, the **Read Information** includes the name of the user who imported the data.

Use Plate Format Templates to Import Data

Before you can import microplate data that was exported in a Plate format from version 5.x and 6.x of the SoftMax Pro Software or acquired from a different software program, you must first use that software program to export the data into a compatible format. Templates to import data in the Plate format and a schema for the XML format are installed with the software. To import data from another program in XML format, the XML file must be formatted using the SoftMax Pro Software XML schema. See Use the XML Schema to Import Data on page 91.

The data import templates are installed with the SoftMax Pro Software, and can be found in a sub-folder of the Program Files folder where the SoftMax Pro Software program is installed. The default installation path is as follows:

C:\Program Files (x86)\Molecular Devices\SoftMax Pro 7.0.2\Plugins\Import-PlateFormat\Templates

The templates are supplied in .xlsx format, but must be saved in .txt format with tab-separated values before you import the data.

The cells in the template are color-coded. Do not alter the data in cells that have a white background. You can edit cells with colored backgrounds. A green background color indicates cells that require information, and a light yellow background color indicates cells that optionally can contain information.

You can paste your data directly into the template, or use the template as a model for a script that converts your data to the acceptable format for import.

Plate Data Layout

The first line of an import template defines the number of Plate sections (or "Blocks") that are included in the data file.

- ##BLOCKS= 1

Each Plate section is presented in the following format:

- Header lines

The first header line contains descriptive titles for the data in the second header line. The second header line contains the type of section, Plate, and the title to be given to the Plate section in the SoftMax Pro Software data file.

The fields in the header lines define the type of read, the number of wells in the microplate, and the wavelengths used for the read, among other relevant information. The layout and format of the header lines are dependent on the read type. For more information, see the template for the read type of your data.

- Data lines

The data lines follow the header lines with a blank row between the header lines and data lines. The layout and format of the data lines are dependent on the read mode and read type.

  - The first column shows the time points for a kinetic read, the data points in a well scan read, or the wavelengths in a spectrum read. For endpoint reads, this column is blank.

  - The second column shows the temperature for the read, if an incubator is used for the read. If the incubator is off, the value is zero.

  - The third column is where the read data starts.

The data displays in a table to indicate the columns and rows of the microplate used for the read. The data for multiple time points for kinetic reads, multiple data points for well scan reads, or multiple wavelengths for spectrum reads is listed in separate tables below the previous table. For endpoint, kinetic, or well scan reads with multiple wavelengths, the data for each wavelength displays in separate tables to the right of the previous table.

			wavelength 1					wavelength 2		
Temperature		1	2	3	…		1	2	3	…
0:00:00	##	1.596	2.479	2.95	…		3.103	1.39	1.97	…
		1.024	0.308	3.925	…		2.381	1.957	3.722	…
		0.195	0.287	2.638	…		0.967	1.049	1.422	…
		…	…	…	…		…	…	…	…
0:01:58	##	1.564	3.344	2.378	…		0.113	3.872	0.637	…
		0.82	1.797	1.064	…		3.58	2.603	0.996	…
		0.421	3.066	3.906	…		0.915	0.57	2.251	…
		…	…	…	…		…	…	…	…
…	##	1.169	1.092	3.969	…		1.126	0.004	1.934	…
		1.105	3.022	2.845	…		1.933	2.834	0.657	…
		3.602	2.033	3.737	…		0.437	3.242	1.337	…
		…	…	…	…		…	…	…	…

Figure 3-3: Kinetic data in Plate format to import

- End line

The end line follows the data lines with a blank row between the data lines and end line. The end line of each Plate section is defined by the text: **~End**

To import data from more than one Plate section in a single text file, include the header lines, the data lines, and the end line for each Plate section after the end line of the previous Plate section.

The final line of an import file contains a single field with the original name given to the import file, and the date and time that the file was saved. This information follows the end line for the last Plate section in the file.

Use the XML Schema to Import Data

To import data in XML format, the XML file must be formatted using the SoftMax Pro Software XML schema from software version 6.4.1 or newer.

Before you can import microplate data that was acquired from a different software program, you must first use that software program to export the data into a compatible format. Templates to import data in the Plate format and a schema for the XML format are installed with the software. To import data from another program in Plate format, the tab-delimited text file must match the format of an import template. See Use Plate Format Templates to Import Data on page 89.

The XML schema file for data import (XMLImportSchema.xsd) is installed with the SoftMax Pro Software, and can be found in a sub-folder of the Program Files folder where the SoftMax Pro Software program is installed. The default installation path is as follows:

C:\Program Files (x86)\Molecular Devices\SoftMax Pro 7.0.2\Plugins\Import-XML\Schema

For information about the XML elements and their usages, see the comments in the schema file.

To import data from more than one **Plate** section in a single XML file, make sure that all the **Plate** sections are between the `<PlateSections>` tags and that each **Plate** section is defined between a set of `<PlateSection>` tags.

```
<PlateSections>
    <PlateSection>
        [Data from the first Plate section]
    </PlateSection>
    <PlateSection>
        [Data from the second Plate section]
    </PlateSection>
</PlateSections>
```

Figure 3-4: Import data from more than one Plate section in XML format

Application Settings

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 Enable Data Recovery on page 93.

- **Web Browser**

  The **Web Browser** option contains a setting to let you 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 What's New - Web Browser on page 25.

- **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 appears each time you open a new SoftMax Pro Software file when you clear this check box. For more information, see Plate Setup Helper on page 29.

- **Plate Height Warning**

  The **Plate Height Warning** option contains a setting to prevent the plate height warning from automatically being displayed when you start a read. To prevent damage to the SpectraMax i3 Multi-Mode Detection Platform, the plate height warning displays as a reminder to make sure that the **Plate Height** for the microplate is defined correctly. The plate height warning automatically displays when you start a read if you clear this check box. For more information, see Plate Editor on page 221.

- **Plate Eject After Read**

  The **Plate Eject After Read** option contains a setting to prevent the microplate drawer from automatically opening after you finish a read. When you clear this check box, the microplate drawer opens after a read completes. When you select this check box, the microplate drawer remains closed after a read completes.

- **Remote Control**

  The **Remote Control** option contains settings for connecting an Apple mobile device to control the running software and the connected microplate reader. To allow control by the mobile app, select the **Enable Remote Control** check box, make sure that both the app and the software use the same **Port**, and enter the **Host** name or IP address of the computer into the settings in the app. You can download the **SoftMax Pro Remote** app from the Apple App Store.

- **Calculation**

  The **Calculation** option enables the software to do data calculations of the kinetic data while a kinetic acquisition is in progress. To enable kinetic data calculations during a read, select the **Calculate during acquisition** check box and then enter the number of kinetic reads between successive calculations in the **Calculate every ___ cycle(s)** field.

- **SpectraMax iD3 Control**

  The **SpectraMax iD3 Control** option enables the software to connect to the SpectraMax iD3 Multi-Mode Microplate Reader using a specific IP address. If the Instrument Connection dialog does not detect the instrument automatically, enter the IP address of the instrument. You can enter up to five different IP addresses so you can connect the computer to multiple instruments on your network. You can find the IP address of the instrument on the touchscreen in Maintenance > Instrument.

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

Enable Data Recovery

To verify that data recovery is enabled:

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.

Recover Files

If the SoftMax Pro Software experiences an unexpected application interruption, when the SoftMax Pro Software restarts, it lists the files that are available for recovery.

1. Select the file or files to recover.
2. Click **Close** to close the dialog.
3. The selected files open in the SoftMax Pro Software.

The files that you do not select are deleted when the dialog closes.

Saving a recovered file overwrites the original file. If an "untitled" file is recovered and opened, the SoftMax Pro Software prompts for a file name and location when the file is saved.
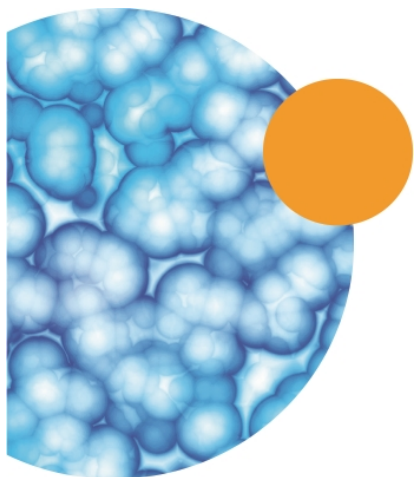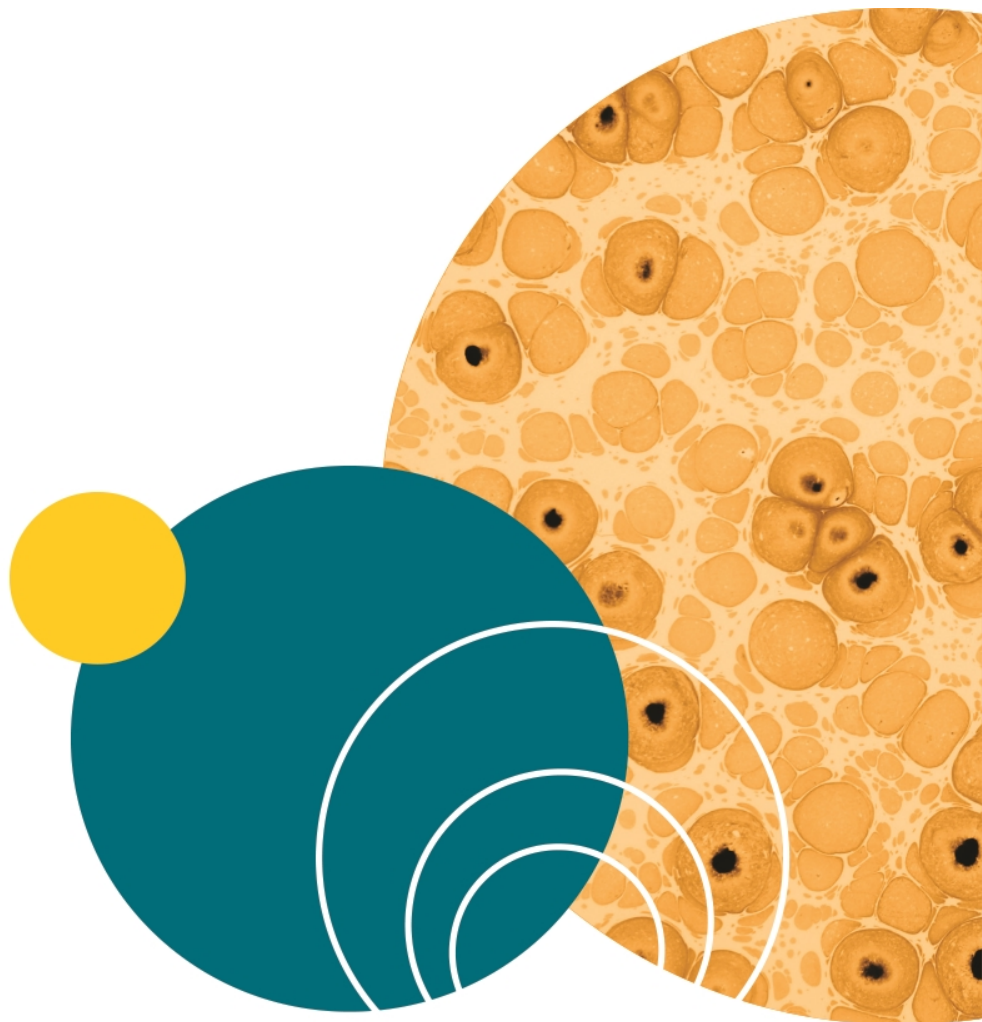
Chapter 4: Workspace Sections

The workspace can contain five different types of sections.

- **Note** sections are used to record text or to report summary data that pertains to the experiment. **Note** sections can contain text and graphics, and can contain images of the other sections in the file. You can have as many **Note** sections as you want in the file. For more information, see Note Sections on page 96.

- **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 Plate Sections on page 105.

- **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 Cuvette Set Sections on page 115.

- 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 you click **Template** in a **Plate** section). For more information, see Group Sections on page 123.

- **Graph** sections are used to plot information from groups as scatter plots or bar graphs. You can have as many **Graph** sections as you want in a file. For more information, see Graph Sections on page 132.

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.

Experiments and sections are listed in the **Navigation Tree**. See Navigation Tree on page 30.

To rename an experiment or a section, see Rename Experiments or Sections on page 31.

When you use the **AutoRead** feature, only the **Plate** sections within the selected **Experiment** are run. See Enable Auto Read on page 301.

In the SoftMax Pro GxP Software, you can lock the individual sections in an experiment to prevent changes to them. See Lock and Unlock Sections on page 434.

Note Sections

Note sections are used to record text or to report summary data that pertains to the experiment.

You can enter text in a Note section, add images, and create summaries that contain formulas to display reduced data.

- A Summary is part of a Note 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 Note 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 Note 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 text.

You can create multiple Note sections within the same experiment.

To work in a Note section, see the following topics:

Add Note Sections

To create a Note section:

1. Select the experiment where you want to add a Note section in the Navigation Tree.
2. If there is more than one section in the Experiment, select the section above where you want the new Note section to display in the Navigation Tree.
3. Click **New Note** in **Sections** on the **Home** tab in the ribbon.

A new blank **Note** section displays below the section you select in the **Navigation Tree**.

To change the name of the **Note** section, right-click the **Note** section in the **Navigation Tree** and then click **Rename**. See Rename Experiments or Sections on page 31.

Insert Images

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

1. With a **Note** section active in the workspace, click in a blank area of the **Note** section to place the cursor in a new frame where you want to insert the image.
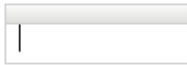
> **Note:** Each image must be placed in its own frame, and the frame cannot contain text.

2. Click **Add Image** in **Note Controls** on the **Home** tab in the ribbon or in the toolbar at the top of the **Note** section.
3. In the **Open** dialog, browse to locate the image to insert.
4. Select the image and click **Open**.

   The image displays in the **Note** section.

Delete Images

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

1. Click the image to delete.
2. Right-click the image and then click **Delete**.

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

Insert Images of Sections

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

1. With a **Note** section active in the workspace, click in a blank area of the **Note** section to place the cursor in a new frame where you want to insert the section image.
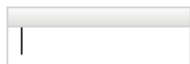
> **Note:** Each image must be placed in its own frame, and the frame cannot contain text.

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

   The section image displays in the **Note** 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.

Refresh Section Images

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

All the section images in the **Note** section update to their current state. For example, if you insert an image of a **Graph** section and then make changes to the graph section, you can refresh the **Graph** section image in the **Note** section.

> **Note:** The section images automatically refresh 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.

Delete Section Images

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

1. Click the section image to delete.
2. Right-click the section image and click **Delete**.

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

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

Add Text to Note Sections

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

1. With a **Note** section active in the workspace, click in a blank area of the **Note** section to place the cursor in a new frame where you want the text.
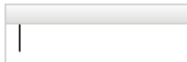
2. Enter the text you want to display.
3. To exit from the text frame, click somewhere else in the **Note** section.

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

Align 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 align, 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** 􀅵.

Edit Text in Text Frames

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 edit.
2. Enter the revised text.

The selected text is replaced with the text you enter.

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

The selected text is placed in the clipboard.

Paste Text

To paste text from the clipboard into a text frame:

1. Copy text onto 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 pastes into the text frame.

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

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

Delete 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 delete.
2. Press **Delete**.

The selected text is removed from the text frame and not placed on the clipboard.

Format Text in Text Frames

You can format text in a text frame using the formatting tools found 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 do 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** ., or **Underline** . 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**

Click **Undo Previous Action** or **Redo Previous Action** for quick formatting changes.

Position Text Frames in Note Sections

You can position a text frame within the **Note** section:

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

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

To position text frames within a **Note** 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 **Note** 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 **Note** 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 **Note** 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 **Note** section or align the bottom edges of multiple frames to the bottom-most text frame, click **Align Bottoms** .

Resize Text Frames

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 displays.
3. Drag the box to the desired size and release the mouse button.

Delete Text Frames

To delete a text frame:

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

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

Add Summary Formulas

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

1. Click the **Note** 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, in the **Name** field, enter a short descriptive name for the formula.

> **Note:** If you plan to reference this formula in a different 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 **Note** section or **Group** section.

5. Optionally, enter a brief text **Description** of the formula to be displayed in the section.

6. Optionally, enter text **Comments** to annotate the formula.

7. Optionally, click **Syntax Helper** to turn on the formula completion tool. See Enable Syntax Helper on page 103.

8. Enter a **Formula** to be used for the Summary or column.

9. Optionally, click **Check Syntax** check the validity of the formula. A message displays to the left of the button to help you troubleshoot the syntax of the formula.

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

    - From the drop-down list select either **Numeric notation** or **Scientific notation**.
    - Click **Decimal Places** and enter the number of places to display or click **Significant Figures** and type the number of figures to display.

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

11. 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.

Enable 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 start to enter a formula, a list displays below the 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 enables you to see what formulas are available and what parameters these formulas expect to receive.

By default, Syntax Helper is enabled when you start the SoftMax Pro Software. You can enable and disable this feature whenever you are on the Formula Editor dialog.

Edit Summary Formulas

To edit a summary formula:

1. Click the summary formula text frame in the **Note** 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 edits. See Add Summary Formulas on page 102.
4. Click **OK**.

Show or Hide the Formula in Summary Formulas

Generally, the summary formula in the **Note** section displays the result of the formula. You can show or hide the formula with 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 on 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 on the **Home** tab in the ribbon.

Delete Summary Formulas

To delete a summary formula:

1. Click the summary formula text frame.
2. Click **Delete** ⊠ in the **Note Controls** section on the **Home** tab in the ribbon.

Plate Sections

A Plate section is used to collect data from the instrument, and to define data display and data reduction. If you need to read the same physical plate twice with different instrument settings, then use 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 space available to view the characters.

You can customize the data display to **Numeric notation** or **Scientific notation** format, and specify the number of **Significant Figures** or **Decimal Places**. These options can be set separately for raw data and reduced data.

When the number of digits is increased, the font size of the values in the Plate section is decreased. If the value becomes too long, then the numbers are cut off at the edge of the wells.

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 220.

The **Template Editor** in the Plate section enables you 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:

- Add Plate Sections on page 106
- Plate Tools on page 106
- Template Tools on page 114

Add Plate Sections

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 Plate section to be displayed in the Navigation Tree.

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

A blank Plate section displays in the Workspace. You can add as many Plate sections as you need to your data file. See Plate Sections on page 105.

To change the name of the Plate section, right-click the Plate section in the Navigation Tree and select **Rename**. See Rename Experiments or Sections on page 31.

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** on **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:

- Modify Instrument Settings on page 107
- Modify Data Reduction Settings on page 107
- Modify Data Display on page 108
- Show Reduced Data in Three-Dimensional Graphs on page 110
- Zoom Well Display on page 111
- Mask Wells on page 112
- Clone Plate Sections on page 112
- View Membrane Images on page 113

Modify Instrument Settings

Instrument settings are part of the protocol used to collect data. To modify settings for the selected instrument, select a **Plate** section or **Cuvette Set** section in the Workspace and then

click **Settings** on the **Home** tab in the ribbon.

The **Settings** dialog appears.
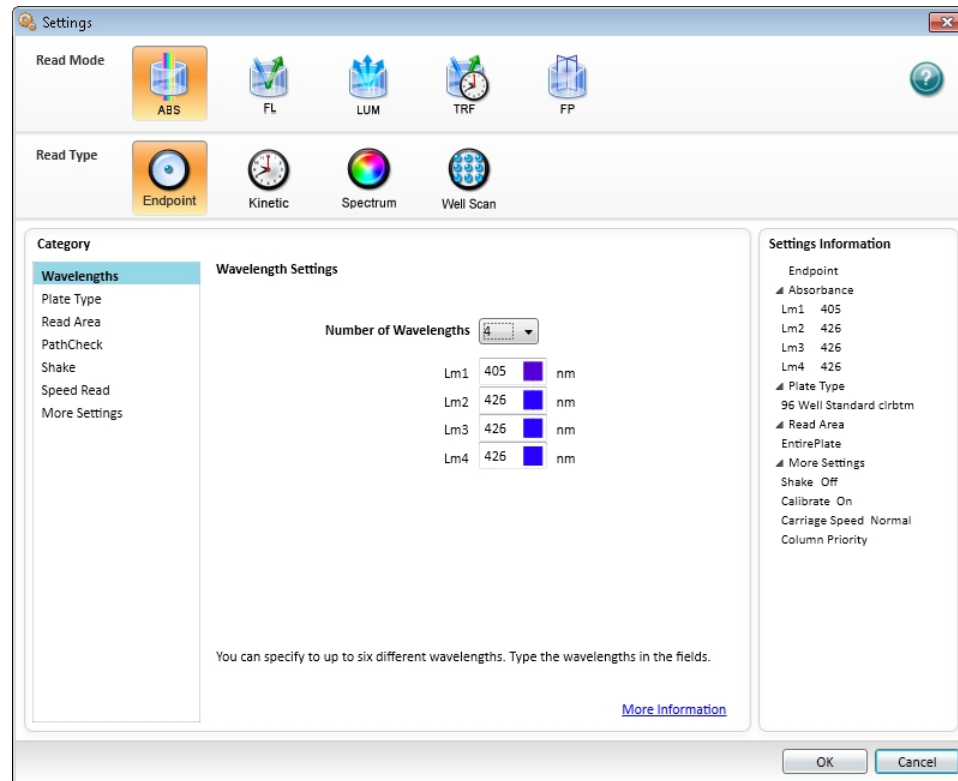
Use the options in the **Settings** dialog to define the parameters to acquire data from a microplate or cuvette. See Select Instrument Settings on page 206.

Modify 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 Data Reduction on page 325.

Modify Data Display

In an active Plate section or Cuvette Set section, you can change how the data is presented.

Click **Display** ![monitor icon] on the **Home** tab in the ribbon or in the toolbar at the top of the section.

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

For Imaging read mode **Imaging Data** replaces the **Raw Data** option.

You can customize the data display to **Numeric notation** or **Scientific notation** format, and specify the number of **Significant Figures** or **Decimal Places**. These options can be set separately for raw data and reduced data.

The **Default** format determines the number of decimal places to display based on the space available. For example, for a plate with 96 wells or fewer, each well is limited to five digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to four characters, including the decimal point. If the value requires greater precision than can fit in the available space, the format changes to scientific notation.

Raw Data

Select **Raw Data** to display 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, select **with Reduced Number**.

Reduced Data

The reduced data display is based on the selections made in the Data Reduction dialog. See Data Reduction on page 325.

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, enter 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, enter 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 Clone Plate Sections on page 112.

Imaging Data

The **Imaging Data** option is available for Imaging read mode only, and replaces the **Raw Data** option.

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

The **Data Type** list contains the measurement selections from the Image Analysis Settings on the Settings dialog. See View and Select Measurement Statistics on page 239.

The available **Data Types** for imaging data are as follows:

- **Object Count** gives the total number of objects detected in the image. This measurement is not used for field analysis of confluent areas.
- **Field Count** gives the total number of confluent areas detected in the image. This measurement is not used for discrete object analysis.
- **Object Percentage** gives the percentage the objects detected in the image by classification. This measurement is not used for field analysis of confluent areas.
- **Covered Area** gives the combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area** gives the average area of the objects detected in the image expressed in μm^2. This measurement is not used for field analysis of confluent areas.
- **Field Area** gives the average area of the confluent areas detected in the image expressed in μm^2. This measurement is not used for discrete object analysis.
- **Object Roundness** gives the average roundness of each object detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for field analysis of confluent areas.

- **Field Roundness** gives the average roundness of each confluent area detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for discrete object analysis.

- **Object Average Intensity** gives the average fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).

- **Field Average Intensity** gives the average fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).

- **Object Intensity** gives the average total fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).

- **Field Intensity** gives the average total fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).

- **Total Intensity** gives the combined total fluorescent signal intensity of the objects or confluent areas detected in the image expressed in million intensity counts. This measurement is not used for transmitted light (TL).

Show Reduced Data in Three-Dimensional Graphs

To view a three-dimensional graphical representation of reduced plate data, view the **Plate** section and click the 🔺 **3D Graph** button in the **Plate Tools** area on the **Home** tab in the ribbon or in the toolbar at the top of the **Plate** section. See View Data in Three-Dimensional Graphs on page 337.
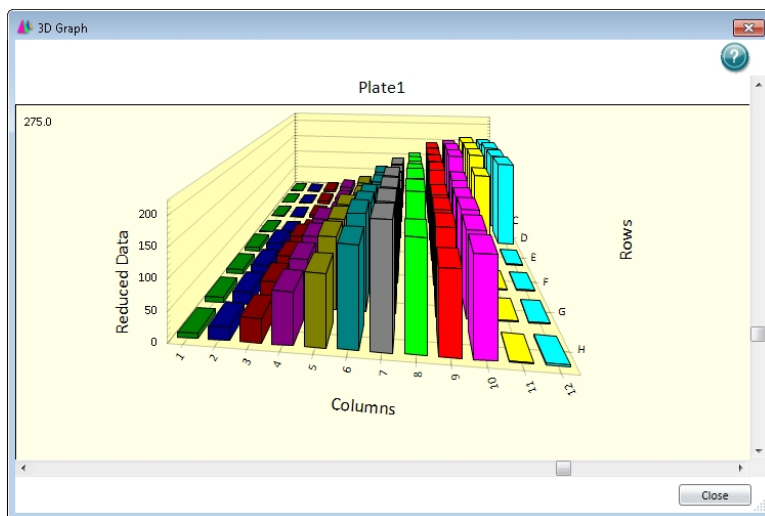
To close the **3D Graph** dialog, click **Close**.

Zoom Well Display

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

To magnify the display of imaging data in a well, see View Imaging Data in Wells on page 322.

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. You can also double-click the well to open the Zoom Well dialog.

   The data from the selected well or cuvette is displayed 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 displays. See Data Reduction on page 325.

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

- **Graph Type Settings** lets you enable or disable connected points or plotted symbols on the graph.
- **Characteristics** lets you 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**. These changes have an effect on only the well graph being viewed.

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

Mask 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**.

Clone Plate Sections

Cloning a plate adds a duplicate copy of the active plate to the **Plate** section. This enables 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 display 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 followed by 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.

View Membrane Images

After you scan a membrane for Western Blot data, the data displays in the SoftMax Pro Software as an image. Use the image tools in the **Plate** section to zoom, crop, colorize, and adjust the intensity of the image. You can select a region of interest (ROI) and rescan the membrane at a higher resolution. See View Western Blot Membrane Data on page 395.

Western Blot membrane data are saved as a TIFF image that can be analyzed by the image-analysis tool of your choice. The SoftMax Pro Software comes with an installed version of the ImageJ software from U.S. National Institute of Health (NIH). See Analyze Western Blot Images on page 400.

To view the membrane image in the ImageJ software, click in the toolbar at the top of the section and then click **ImageJ Editor**.

To view the membrane image in your selected image-analysis tool, click in the toolbar at the top of the section and then click **External Image Editor**. This option is available only if you have .tif files associated with a program other than ImageJ software. The icon used for this tool changes depending on the selected image-analysis tool. See Define the Membrane Image-Analysis Tool on page 401.

> **Note:** The image tools are available only when viewing a **Plate** section that contains membrane data.

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 related to 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 related **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 Configure Microplate Templates on page 264.

Cuvette Set Sections

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 are displayed 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:

- Add Cuvette Set Sections on page 115
- Cuvette Set Tools on page 116
- Use the Template Tool with Cuvette Sets on page 122

Add Cuvette Set Sections

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

A new **Cuvette Set** section displays 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.

To change the name of the **Cuvette Set** section, right-click the **Cuvette Set** section in the **Navigation Tree** and then click **Rename**. See Rename Experiments or Sections on page 31.

Note: When you create 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.

Cuvette Set Tools

Cuvette Tools display on the **Home** tab when a **Cuvette Set** section is active in the workspace. **Cuvette Tools** include the following:

- Modify Instrument Settings for Cuvettes on page 116
- Modify Cuvette Data Reduction Settings on page 116
- Modify Cuvette Data Display on page 117
- Zoom Cuvette Display on page 118
- Mask Cuvettes on page 120
- Add Cuvettes to Cuvette Sets on page 120
- Delete Cuvettes from Cuvette Sets on page 121
- Copy Cuvette Set Sections on page 121
- Copy and Paste Cuvette Data on page 121

Modify 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 appears.

3. Set the instrument settings options as needed. See Select Instrument Settings on page 206.

4. Click **OK**.

Modify Cuvette Data Reduction Settings

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 is displayed.

   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 Data Reduction on page 325.

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.

Modify Cuvette Data Display

In an active Plate section or Cuvette Set section, you can change how the data is presented.

Click **Display** on the **Home** tab in the ribbon or in the toolbar at the top of the section.
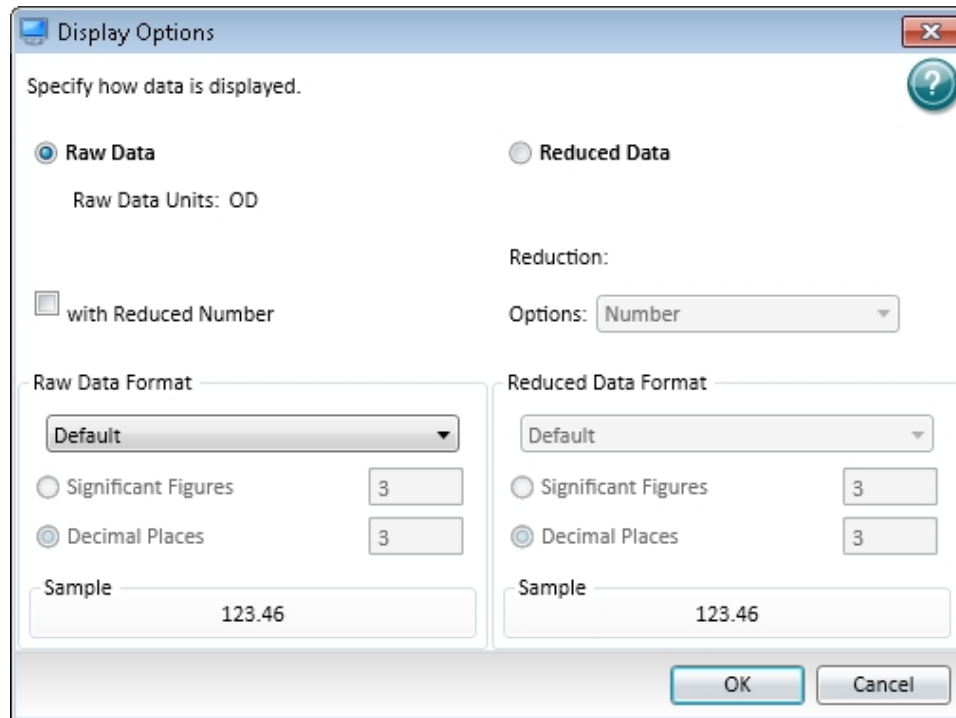
The choices available in the Display Options dialog include selecting between **Raw Data** and **Reduced Data**.

You can customize the data display to **Numeric notation** or **Scientific notation** format, and specify the number of **Significant Figures** or **Decimal Places**. These options can be set separately for raw data and reduced data.

The **Default** format determines the number of decimal places to display based on the space available. For example, for a plate with 96 wells or fewer, each well is limited to five digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to four characters, including the decimal point. If the value requires greater precision than can fit in the available space, the format changes to scientific notation.

Raw Data

Select **Raw Data** to display 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, select **with Reduced Number**.

Reduced Data

The reduced data display is based on the selections made in the Data Reduction dialog. See Data Reduction on page 325.

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, enter 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, enter 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 Clone Plate Sections on page 112.

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

Zoom 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. You can also double-click the well to open the Zoom Well dialog.

   The data from the selected well or cuvette is displayed 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 displays. See Data Reduction on page 325.

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

- **Graph Type Settings** lets you enable or disable connected points or plotted symbols on the graph.
- **Characteristics** lets you 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**. These changes have an effect on only the well graph being viewed.

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

Mask 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**.

Add Cuvettes to Cuvette Sets

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.

You can add up to 96 cuvettes 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 Use the Template Tool with Cuvette Sets on page 122.

Delete Cuvettes from Cuvette Sets

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.

Copy Cuvette Set Sections

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

1. Click the **Cuvette Set** section 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 displays in the Navigation Tree.

A new **Cuvette Set** section displays 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.

Copy and Paste 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 a different **Cuvette Set** section. You can copy and paste data 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 a different **Cuvette Set** section in the same file, it might be easier to make a duplicate copy of the **Cuvette Set** section. See Copy Cuvette Set Sections on page 121.

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 onto the clipboard.

To paste cuvette data that is stored on the clipboard:

1. Click the cuvette into which to paste the data.

2. If you copy 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 copy data from three cuvettes and then click cuvette A6 to paste the data, the data is pasted into cuvettes A6, A7, and A8.

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

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

Use the Template Tool with Cuvette Sets

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 related to 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 related **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 adds cuvettes to the active **Cuvette Set** section.

You can add up to 96 cuvettes to one **Cuvette Set** section.

When you open the Template Editor with a Cuvette Set section active in the workspace, the Template Editor 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 Configure Microplate Templates on page 264.

Group Sections

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 Configure Microplate Templates on page 264.

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

Reuse Formulas

Formulas are used in Group columns and custom reductions. Sometimes it is useful to copy a formula or column from one area of the program to a different area, or from one Experiment to a different Experiment, 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** display on the **Home** tab in the ribbon.

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

- Group Settings on page 123
- Column Tools on page 126
- Summary Tools on page 130
- Format Tools in Group Sections on page 131

Group Settings

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

Whenever you create a group (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.

To define a new group, see Create Groups on page 124.

For information about the column formats, see Group Table Column Format on page 126.

Create Groups

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 to open the Template Editor dialog.

    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 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. Use your mouse to drag the pointer to select one or more wells to be defined as a group.

4. In the **Groups** area, click **Add**.

    To modify an existing group, select the group and click **Edit**.

5. In the **Group Settings** dialog, in the **Group Name** field, enter the name to assign to the group.

6. If you want to set a color for the group, click **Set Color** and select the color to display 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 126.

   When the **Standards** group type is selected, **Sample Descriptor1** is activated and the assumption is that it will be used for concentration information. However, it could be used for all 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 selected, click **Sample Descriptor1** to apply a descriptor and its units to the group.

9. Enter 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 enter them in the **Units** field.

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

    The **Group Table Column Preview** displays 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.

    To define a series for the selected wells, click **Series**. See Create a Series on page 267.

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, but the information does not display automatically in the **Group** section. To see it, create a new column that contains the formula **!Sampledescriptor**, **!factor**, or **!concentration**. For more information about how to create columns, see Add Columns on page 127.

Group Table Column Format

The type of **Group Table Layout** defines the default columns for the data calculated and reported in the related **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	✓	✓	✓
Wells	!WelllDs	✓	✓	✓
Sample#	Index			
Concentration	!Concentration	✓		
Mean Value	Average(Values)	✓		
Values	!WellValues	✓		
R (Outside Standard Range)	If(Values)>='MinStd @Standards' and Values<='MaxStd@ Standards',"","R"		✓	✓
Result	InterpX(STD@ StandardCurve, Values)		✓	✓
MeanResult	Average(Result)		✓	✓
Std.Dev.	Stdev(Result)	✓	✓	✓
CV%	Cv(Result)	✓	✓	✓
Dilution	!Factor			✓
Adj.Result	MeanResult*!Factor			✓

Column Tools

When a column (or the data in a column) is selected in the group table, the **Column Tools** that can be used on the selected items are active. Actions not applicable for a selected item are disabled.

After selecting a column or data in a group table, functions applicable 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:

- Add Columns on page 127
- Modify Columns on page 128
- Copy Column Content on page 128
- Paste Column Content on page 128
- Cut Column Content on page 128

Add Columns

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, in the **Name** field, enter a short descriptive name for the formula.

> **Note:** If you plan to reference this formula in a different 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, enter text **Comments** to annotate the formula.
5. Optionally, click **Syntax Helper** to turn on the formula completion tool. See Enable Syntax Helper on page 103.
6. Enter a **Formula** to be used for the Summary or column.
7. Optionally, click **Check Syntax** check the validity of the formula. A message displays to the left of the button to help you troubleshoot the syntax of the formula.
8. Specify the **Data Display** characteristics:
   - From the drop-down list select either **Numeric notation** or **Scientific notation**.
   - Click **Decimal Places** and enter the number of places to display or click **Significant Figures** and type the number of figures to display.

   A preview of the **Data Display** displays in the **Sample** on the right.
9. 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 a different group, 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 to create and edit column formulas. For a complete discussion, see the *SoftMax Pro Formula Reference Guide*.

Modify Columns

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 Add Summary Formulas on page 102.
3. To resize the column width, click and hold the column border and then drag it to the desired column width.

Copy Column Content

To paste content into a column you must first copy applicable content onto 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 copy.
3. Click **Copy** in **Column Tools** on the **Home** tab in the ribbon.

Paste Column Content

The content to paste into a column must be copied from a different column location and be present on 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 try this operation, there is no applicable content present on the clipboard. You must first copy content to the clipboard.

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

> **Note:** Content that is cut from a column is not placed on the clipboard. To place content on the clipboard, you must copy the content.

Delete Columns

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.

Autosize Columns

You can autosize a column to set the width of the selected column to hold 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.

Hide 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 box for each column to hide.

    - To clear all the check boxes, click **Hide All**.
    - To select all the check boxes, click **Show All**.
4. Click **OK**.

Show 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 box for each columns to show.

    - To select all the check boxes, click **Show All**.
    - To clear all the check boxes, click **Hide All**.

4. Click **OK**.

Hide 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.

Summary Tools

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

For information about how to use the **Summary Tools**, see the following topics:

- Copy and Paste Formulas on page 130
- Delete Formulas on page 130
- Add Formulas on page 130
- Edit Formulas on page 131
- Show or Hide the Formulas in Group Sections on page 131

Copy and Paste Formulas

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 copy.
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.

Delete Formulas

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 delete.
3. Click **Delete** ❌ in **Summary Tools** on the **Home** tab in the ribbon.

Add Formulas

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 Add Summary Formulas on page 102.
3. Type the formula to add and click **OK**.

Edit Formulas

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 change 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 Add Summary Formulas on page 102.
4. Type the changes to formula and click **OK**.

Show or Hide the Formulas in Group Sections

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

Format Tools in Group Sections

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 **Note** section. See the following topics:

- Add Text to Note Sections on page 99
- Align Text on page 99
- Edit Text in Text Frames on page 100
- Format Text in Text Frames on page 100
- Position Text Frames in Note Sections on page 101
- Resize Text Frames on page 101
- Delete Text Frames on page 102

Graph Sections

Graph sections enable you to plot information from groups as scatter plots or bar graphs.

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

After you create a graph, you can add and delete plots, customize the axes, and change the size and type of graph. You can enable and disable the grid lines for the graph. Grid lines are enabled by default.

Graph sections are divided into the following areas:

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

For more information see Graph Tools on page 343.

Chapter 5: Read Modes and Read Types

5

Use the SoftMax Pro Software to define the parameters for the read mode and read type of your assay.

For more information on read modes, see the following topics:

- Absorbance Read Mode on page 137
- Fluorescence Intensity Read Mode on page 143
- Luminescence Read Mode on page 149
- Time-Resolved Fluorescence Read Mode on page 154
- HTRF Read Mode on page 160
- FRET Read Mode on page 159
- Fluorescence Polarization Read Mode on page 164
- AlphaScreen Read Mode on page 168
- Western Blot TRF Read Mode on page 171
- Imaging Read Mode on page 174

Application notes with specific application protocol suggestions can be found in the Information Center and the Knowledge Base on the Molecular Devices web site at www.moleculardevices.com.

Supported Read Types

For most read modes, endpoint, kinetic, multi-point well-scan, and spectrum microplate applications can be set up and run with the touchscreen or optionally with the SoftMax Pro Software. In addition, the FlexStation 3 reader support the flex read type that can integrate fluid transfer with a kinetic read.

For more information on read types, see the following topics:

- Endpoint Read Type on page 134
- Kinetic Read Type on page 134
- Well Scan Read Type on page 134
- Spectrum Read Type on page 134
- Flex Read Type on page 135
- Membrane Read Type on page 135

Endpoint Read Type

In an endpoint read, a reading of each microplate well is taken 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 (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

Kinetic Read Type

In a kinetic read, the instrument collects data over time with multiple readings taken in the center of each well at regular intervals. To get the shortest possible interval for multiple-wavelength Kinetic readings, choose wavelengths in ascending order.

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

Kinetic analysis can collect data points in time intervals of seconds, minutes, hours, or days.

Kinetic analysis has many advantages when determining the relative activity of an enzyme in different types of microplate assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis is capable of providing improved dynamic range, precision, and sensitivity relative to endpoint analysis.

Peak Pro™ Analysis functions provide advanced peak detection and characterization for applicable kinetic reads. See the *SoftMax Pro Software Formula Reference Guide*.

Spectrum Read Type

Depending on the read mode selected, a spectrum read measures optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU) across a spectrum of wavelengths.

Spectrum readings are made using the scanning monochromators of the instrument and can measure across the spectrum of absorbance wavelengths 230 nm to 1000 nm. Fluorescent intensity reads scan excitation wavelengths between 250 nm to 830 nm and emission wavelengths between 270 nm to 850 nm, where the emission wavelength must be a minimum of 20 nm greater than the excitation wavelength. Luminescence reads scan emission wavelengths between 300 nm to 850 nm.

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 permit 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 following scanning patterns are available:

- A horizontal line
- A vertical line
- A cross pattern
- 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.

You can set the density of the well scan to determine the number of points to read in a line pattern or the maximum number of horizontal and vertical points included in a cross or fill pattern.

Depending on the read mode selected, the values are reported as optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

Flex Read Type

The fluidics module in the FlexStation 3 reader is designed to aspirate fluids from a compound source plate and dispense them into an assay plate. Fluid transfer is made possible with an 8-channel or 16-channel pipettor that is fully automated, including changing the tips from a tip rack.

In the Flex read type, one to eight or one to sixteen wells in one column of the assay plate are read repeatedly for a selected total experimental time. At a preselected point or points during that time sequence, the pipettor can transfer up to three reagents from the compound plate to the assay plate. The instrument continues to read at the preselected time intervals before and after each fluid transfer. After completion of reading the column (or partial column) for a preselected time, the instrument can repeat this cycle with other columns. All the data are collected in one data file represented as a 96-well or 384-well microplate.

For example, an experiment with a two-minute run time accommodates a 96-well microplate in about 24 minutes.

Run time × Number of columns = Plate time

2 minutes × 12 columns = 24 minutes

Membrane Read Type

The Membrane read type is used for a time-resolved fluorescence reading of a Western Blot membrane. The selected area is read, and a TIFF image is generated with the results of the read.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that is incorporated into a SpectraMax® Paradigm® or SpectraMax® i3x Multi-Mode Detection Platform. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. For more information, contact your Molecular Devices representative or search the knowledge base for **ScanLater** or **Western Blot** at www.moleculardevices.com/support.

If you use the SoftMax Pro GxP Software, you must enable the following permissions to complete a Western Blot membrane read:

- **Read empty plates/cuvettes** to start a read.
- **Save data files** to save the data file before the read.
- **Overwrite plate/cuvette data** to do a high-resolution ROI read.

See SoftMax Pro GxP Software on page 417.

Absorbance Read Mode

In the Absorbance (ABS) read mode, the instrument measures the Optical Density (OD) of the sample solutions.

Absorbance is the quantity of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. If there is no turbidity, then 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.

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

Optical Density

Optical density (OD) is the quantity of light passing through a sample to a detector relative to the total quantity of light available. Optical Density includes absorbance of the sample plus light scatter from turbidity and background. You can compensate for background using blanks.

A blank well contains everything used with the sample wells except the chromophore and sample-specific compounds. Do not use an empty well for a blank.

Some applications are designed for turbid samples, such as algae or other micro-organisms in suspension. The reported OD values for turbid samples are likely to be different when read by different instruments.

For optimal results, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can be subtracted is the average value of all blanks.

% 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.

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 instrument converts the raw OD values reported by the instrument to %Transmittance using the above formula. All subsequent calculations are done on the converted numbers.

Applications of Absorbance

Absorbance-based detection has been commonly used to evaluate changes in color or turbidity, permitting 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 micro-volume measurements, you can use SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates.

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

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org).

For information about setting up an Absorbance mode protocol, see Create Absorbance Mode Protocols on page 274.

Absorbance Instruments

The following instruments have absorbance read mode capability:

- SpectraMax iD3 Multi-Mode Microplate Reader, see page 180
- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  To do absorbance reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires the Absorbance Detection Cartridge, see page 195.

- VersaMax Microplate Reader, see page 184
- SpectraMax Plus 384 Absorbance Microplate Reader, see page 185
- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M3 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 187
- SpectraMax 190 Absorbance Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189
- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader, see page 190
- Vmax Kinetic Microplate Reader, see page 191
- EMax Endpoint Microplate Reader, see page 191
- EMax Plus Microplate Reader, see page 192

PathCheck Pathlength Measurement Technology

You can enable PathCheck technology in the SoftMax Pro Software **Settings** dialog by selecting the **PathCheck** check box in the **PathCheck Settings** for absorbance endpoint reads.

The temperature-independent PathCheck® Pathlength Measurement Technology normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

The Beer–Lambert law states that absorbance is proportional to the distance that light travels through the sample:

$$a - b = c\,d\,e + f$$

where a is absorbance, b is blank, c is concentration, d is the depth of sample layer, e is extinction (coefficient of…), and f is further terms, e.g., non-linearity caused from turbidity.

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 do extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis for quantifying the unique absorptivity properties of compounds in solution. Quantitative analysis can be done 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 directly proportional to concentration when there is no background interference.

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 occur 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 way of correcting the microwell absorbance values is accurate to within ±4% of the values obtained directly in a 1 cm cuvette.
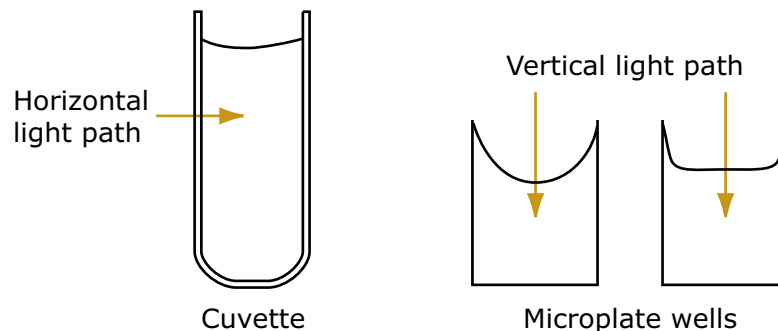
Figure 5-1: Cuvette and Microplate Well Light Paths

The 1 cm values can be obtained by using the factory installed *Water Constant*. 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 for absorbance endpoint reads.

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 sufficient. The Water Constant is determined for each instrument 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. 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 Cuvette Set 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 Cuvette Set 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 *SoftMax Pro Software Formula Reference Guide*.

Note: After you read a plate with PathCheck technology turned on, PathCheck information is stored permanently in the data file. You have the option to apply, or not apply, 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.

Eliminate the Pathlength-Independent Component

Raw OD measurements of microplate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of microplate material). The pathlength-independent component must be eliminated from the calculation to get valid results that have been normalized by the PathCheck technology. You can do this using a plate blank or using a plate background constant.

Using a Plate Blank

This method can be used 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 a minimum of one well (preferably several) as Plate Blank.
2. Pipette buffer (for example, your sample matrix) into those wells and read along with your samples. Do not use an empty well for a blank.

   The instrument 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.

Using a Plate Background Constant

If your sample volumes are not identical or if you choose not to use a Plate Blank, then you must use a Plate Background Constant. Omitting a Plate Background Constant results in artificially high values after being normalized by the PathCheck technology.

To determine the Plate Background Constant:

1. Fill a clean microplate with water.
2. Read at the wavelengths that you will be reading your samples.

The average OD value is the Plate Background Constant. If you intend to read your samples at more than one wavelength, there should be a corresponding number of Plate Background Constant values for each wavelength.

Note: It is important that you put water in the wells and not read a dry microplate for the Plate Background Constant. A dry microplate has a slightly higher OD value than a water-filled microplate because of differences in refractive indices. Using a dry microplate results in PathCheck technology normalized values that are lower than 1 cm cuvette values.

Interfering Substances

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 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 recommended to not use the PathCheck technology.

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. 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 should run a Spectrum scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck technology.

Fluorescence Intensity Read Mode

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

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 5-2: 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, you must have sufficient spectral separation to reduce the interference of the excitation light with detection of the emitted light.

✳ **Tip:** If the Stokes shift is small, you should 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, permitting better selection and quantitation of the emitted light.

The **Spectral Optimization Wizard** provides the best settings for maximizing the signal to background window, (S-B)/B, while minimizing the optimization time. You can use this wizard with the SpectraMax i3x Multi-Mode Detection Platform or with a Tunable Wavelength (TUNE) Detection Cartridge installed in the SpectraMax Paradigm Multi-Mode Microplate Reader. See Spectral Optimization on page 307.
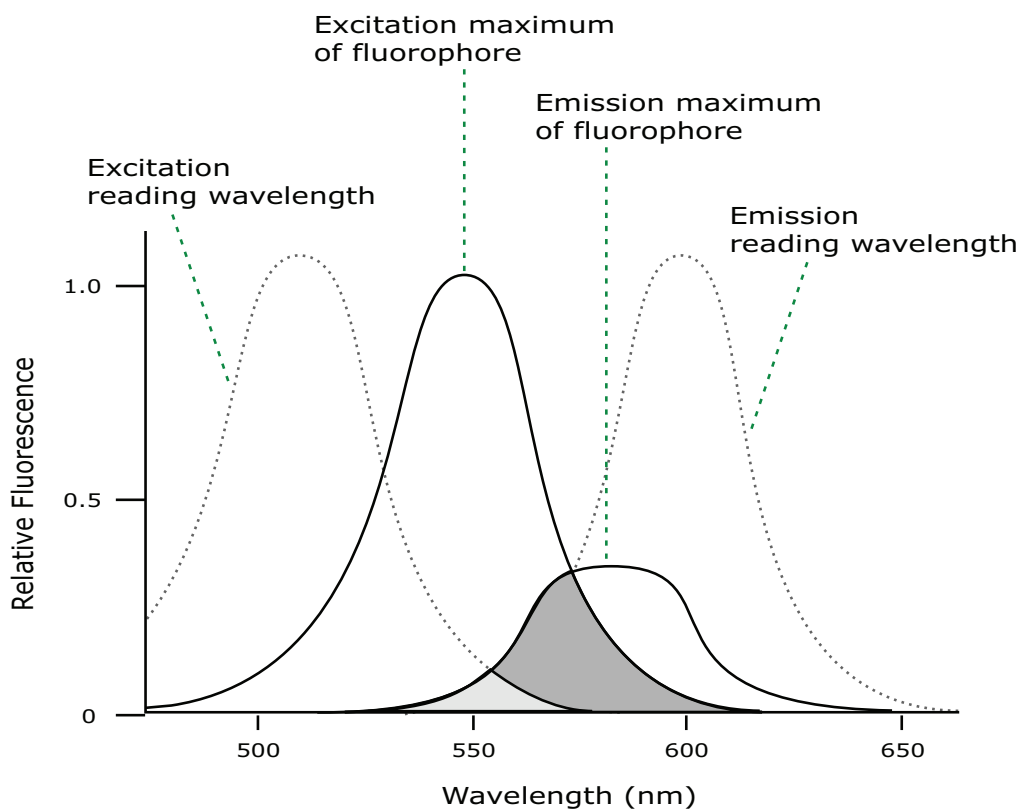
Figure 5-3: Optimized Excitation and Emission Reading Wavelengths

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 of the excitation and emission spectra of the fluorophore. When the reading wavelengths for excitation and emission are separated, a smaller quantity 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 instrument permits scanning of both excitation and emission wavelengths, using separate tunable dual monochromators. One benefit of scanning emission spectra is that you can determine 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. One more benefit is that you can find excitation and emission wavelengths that prevent 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 optimal setting is where the ratio of the sample emission to background emission is at the maximum.

Fluorescence intensity data is dependent on a number of variables. See Analyze Fluorescence Intensity Data on page 146.

Applications of Fluorescence Intensity

Fluorescence intensity is used widely in applications such as fluorescent ELISAs, protein assays, nucleic acid quantitation, reporter gene assays, cell viability, cell proliferation, and cytotoxicity. One more major application of this mode is to study the kinetics of ion release.

Some assays use a fluorescent label to selectively attach to certain compounds. The quantity or concentration of the compound can then be quantified by measuring the fluorescence intensity of the label, which is attached to the compound. Such methods are often used to quantify low concentrations of DNA or RNA, for example.

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

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org).

For information about setting up a fluorescence intensity mode protocol, see Create Fluorescence Intensity Mode Protocols on page 276.

Fluorescence Intensity Instruments and Detection Cartridges

The following instruments have fluorescence intensity read mode capability:

- SpectraMax iD3 Multi-Mode Microplate Reader, see page 180
- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

   The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

   The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M3 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- Gemini XPS Microplate Reader, see page 188
- Gemini EM Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189

- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader, see page 190

The following detection cartridges have fluorescence intensity read mode capability:

- Tunable Wavelength (TUNE) Detection Cartridge, see page 196
- Multi-Mode (MULTI) Detection Cartridge, see page 197
- Fluorescence Intensity (FI) Detection Cartridges, see page 199
- Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge, see page 199
- Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge, see page 200

Note: For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

Fluorescence Intensity Reads with Injectors

Injectors in a microplate reader are used to automatically deliver a specified volume of a reagent to the wells of a microplate. They are generally used when delivery of the reagent initiates a reaction that occurs rapidly and results in a luminescent or fluorescent signal that must be detected quickly.

The SpectraMax i3x Instrument with the Injector Cartridge installed can be set up to inject and read well by well to reduce signal loss.

Common inject-and-read assays include calcium flux assays.

Analyze Fluorescence Intensity Data

Fluorescence intensity data is dependent on a number of variables. Raw data is compared to a standard curve with known concentrations of a reference label.

A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of a reference label.

Analyzing and validating fluorescence intensity data generally consists of the following:

- Background Correction and Quantification on page 146
- Detection Limit on page 147
- Linearity and the Linear Dynamic Range on page 147

Background Correction and Quantification

A blank well contains everything used with the sample wells except the label and sample-specific compounds. Do not use an empty well for a blank.

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label, and is generally subtracted before data reduction is done.

Within the linear detection range, the blank-subtracted raw data are proportional to the quantity of label in a sample such that the label concentration is quantified by the following equation.

$$\text{conc}_{label} = \frac{(\text{sample} - \text{blank})}{\dfrac{\text{std} - \text{blank}}{\text{conc}_{std}}}$$

where $conc_{std}$ is the concentration of the *standard*, and *sample*, *blank*, and *standard* are average values of replicates for the sample, blank, and standard wells. In the general case where the standard curve covers a concentration range of more than a few linear logs, *(standard – blank) / conc_{std}* is equivalent to the slope of the standard curve, and so the concentration of the label is determined by *(sample – blank) / (slope of standard curve)*.

For optimal results, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can be subtracted is the average value of all blanks.

Detection Limit

The detection limit is the smallest sample concentration that can be measured reliably above the blank. Determining the detection limit requires taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection threshold is defined as the average blank plus three standard deviations. If the average sample value measures above the threshold, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{Det Limit} = \frac{3\,\text{Stdev}_{blank}}{\dfrac{\text{std} - \text{blank}}{\text{conc}_{std}}}$$

where $conc_{std}$ is the concentration of the standard, $StDev_{Blank}$ is the standard deviation of the blank replicates, and *blank* and *Std* are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks to calculate their standard deviation.

Linearity and the Linear Dynamic Range

Within a wide range at moderately high concentrations, blanked raw data is proportional to the quantity of label in a sample.

The linear dynamic range (LDR) is defined by:

$$\text{LDR} = \log_{10}\left(\frac{\text{max conc lin}}{\text{detection limit}}\right)$$

where *LDR* is expressed as a log, and *max conc lin* is the highest concentration in the linear range that can be quantified.

When the standard curve after blank reduction is not linear in concentration at the lower end, there might be an incorrect or contaminated blank.

When the standard curve levels are off at the highest concentrations, this can be addressed to the inner filter effect: excitation does not reach as deep into the sample for lower concentrations, without being more significantly attenuated (absorbance) layer by layer.

Luminescence Read Mode

In luminescence read mode, no excitation is necessary as the species being measured emit light naturally. For this reason, the lamp does not flash, so no background excitation interference occurs.

In the Luminescence (LUM) read mode, the instrument 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.

To help eliminate background luminescence from a microplate that has been exposed to light, Molecular Devices recommends dark adaptation of the microplate by placing the sample-loaded microplate in the instrument for several minutes before starting the read.

For some monochromator-based instruments, the default setting for luminescence is the "zero order" position where the grating monochromator acts as a mirror that reflects all light to the PMT detector.

The instrument bypasses the emission monochromator for luminescence reads that detect all wavelengths.

If wavelength selection is desired, you can choose the wavelength where peak emission is expected to occur. Also, multiple wavelength choices let species with multiple components be differentiated and measured easily.

Luminescence can be read from the top or the bottom of a microplate. Solid white microplates or white microplates with clear bottoms are recommended for luminescence reads.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls. See Analyze Luminescence Data on page 151.

Applications of Luminescence

Chemiluminescent or bioluminescent reactions can be induced to measure the quantity of a particular compound in a sample. Examples of luminescent assays include the following:

- Reporter gene assays (the measurement of luciferase gene expression)
- Quantitation of adenosine triphosphate (ATP) as an indication of cell counts with cell-proliferation, cytotoxicity, and biomass assays
- Enzyme measurements with luminescent substrates, such as immunoassays

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

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org).

For information about setting up a luminescence mode protocol, see Create Luminescence Mode Protocols on page 284.

Luminescence Instruments and Detection Cartridges

The following instruments have luminescence read mode capability:

- SpectraMax iD3 Multi-Mode Microplate Reader, see page 180
- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M3 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- Gemini XPS Microplate Reader, see page 188
- Gemini EM Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189
- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader, see page 190

The following detection cartridges have luminescence read mode capability:

- Tunable Wavelength (TUNE) Detection Cartridge, see page 196
- Multi-Mode (MULTI) Detection Cartridge, see page 197
- Glow Luminescence (LUM) Detection Cartridges, see page 200
- Dual Color Luminescence (LUM) (BRET2) Detection Cartridge, see page 201
- Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge, see page 202

Note: For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

Luminescence Reads with Injectors

Injectors in a microplate reader are used to automatically deliver a specified volume of a reagent to the wells of a microplate. They are generally used when delivery of the reagent initiates a reaction that occurs rapidly and results in a luminescent or fluorescent signal that must be detected quickly.

The SpectraMax i3x Instrument with the Injector Cartridge installed can be set up to inject and read well by well to reduce signal loss.

Common inject-and-read assays include luciferase reporter assays.

The Injector Cartridge is DLReady™ certified by Promega for the Dual-Luciferase Reporter (DLR™) assay system.

DLReady, DLR, and the DLReady logo are trademarks of Promega Corporation.

Analyze Luminescence Data

The conversion rate of photons to counts is individual for each reader. Therefore, raw data from the same plate can seem significantly different from one instrument to the next. Also, the data format used by other manufacturers might not be counts per second and 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. See Detection Limit on page 152.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls. A standard curve consists of, at a minimum, a blank sample and a reference standard sample of known concentration. The raw data can then be expressed in equivalent concentration of a reference label. The raw data is normalized to counts per second by dividing the number of counts by the read time per well.

Analyzing and validating luminescence data generally consists of the following:

- Background Correction on page 151
- Detection Limit on page 152
- Sample Volumes and Concentration of Reactants on page 152
- Data Optimization on page 152

Background Correction

The light detected in a luminescent measurement generally has two components: specific light from the luminescent reaction and an approximately constant level of background light caused by various factors, including the plate material and impurities in the reagents. The background can be effectively measured using blank replicates. Blanks should include the luminescent substrate (chemical energy source) but not the luminescence agent (generally an enzymatic group which makes the substrate glow).

A blank well contains everything used with the sample wells except the label and sample-specific compounds. Do not use an empty well for a blank.

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label, and is generally subtracted before data reduction is done.

For optimal results, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can be subtracted is the average value of all blanks.

To help eliminate background luminescence from a microplate that has been exposed to light, Molecular Devices recommends dark adaptation of the microplate by placing the sample-loaded microplate in the instrument for several minutes before starting the read.

Detection Limit

The detection limit is the smallest sample concentration that can be measured reliably above the blank. Determining the detection limit requires taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection threshold is defined as the average blank plus three standard deviations. If the average sample value measures above the threshold, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{Det Limit} = \frac{3\ \text{Stdev}_{blank}}{\dfrac{\text{std} - \text{blank}}{\text{conc}_{std}}}$$

where $conc_{std}$ is the concentration of the standard, $StDev_{Blank}$ is the standard deviation of the blank replicates, and *blank* and *Std* are average values of the replicates for the blank and standard wells.

Determining the detection limit for an assay requires multiple blanks to calculate their standard deviation.

Sample Volumes and Concentration of Reactants

The concentration of the luminescent agent impacts the quantity of light output in a luminescent reaction. Light is emitted as a result of a reaction between two or more compounds. Therefore, the quantity of light output is proportional to the quantity of the limiting reagent in the sample.

For example, in an ATP/luciferin-luciferase system, when total volume is held constant and ATP is the limiting reagent, the blanked light output is proportional to the concentration of ATP in the sample, at very high concentrations of ATP. Substrate can be used up and become rate-limiting, providing it is the rate-limiting component. In this case, the non-linearity is an effect of the assay and not caused by the microplate reader.

> **Note:** Very bright samples can exceed the linear dynamic range of the instrument. If so, reading can be done using an attenuation filter.

Data Optimization

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. Therefore, it is important to specify the read time when comparing measurements.

All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

Z′ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

The value of Z′ can be determined using the following formula:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|\, Mean_{c+} - Mean_{c-} \,|}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c–** is the negative control.

A Z′ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. Higher values might be desired when results are more critical.

Z′ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z′ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

Time-Resolved Fluorescence Read Mode

Time-resolved fluorescence (TRF) is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cut off of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the microplate) vanishes while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period with the added benefit of reduced background.

In Time-Resolved Fluorescence read mode, the instrument detects the extremely long emission half-lives of rare earth elements called lanthanides such as europium (lifetime of about 700 µs), samarium (lifetime of about 70 µs), or terbium (lifetime of about 1000 µs).

Applications of Time-Resolved Fluorescence

Time-resolved fluorescence (TRF) is widely used in high throughput screening applications such as kinase assays, and is useful in some fluorescence immunoassays, such as DELFIA (dissociation-enhanced enzyme linked fluorescence immunoassay). TRF is also useful in some assay variants of TR-FRET (time-resolved fluorescence resonance energy transfer) in which the FRET acceptor label acts as a quencher only and does not emit fluorescence. The proximity between donor label and acceptor (quencher) is then quantified by the intensity decrease of the donor label.

DELFIA requires washing steps as in an ELISA, but the TR-FRET assay involving quenching is a homogeneous microplate assay technique and requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

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. See HTRF Read Mode on page 160.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

For information about setting up a time-resolved fluorescence mode protocol, see Create Time-Resolved Fluorescence Mode Protocols on page 286.

Time-Resolved Fluorescence Instruments and Detection Cartridges

The following instruments have time-resolved fluorescence read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  The SpectraMax i3x Instrument requires detection cartridges to do time-resolved fluorescence reads.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- Gemini XPS Microplate Reader, see page 188
- Gemini EM Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189
- FilterMax F5 Multi-Mode Microplate Reader, see page 189

The following detection cartridges have time-resolved fluorescence read mode capability:

- Tunable Wavelength (TUNE) Detection Cartridge, see page 196
- Multi-Mode (MULTI) Detection Cartridge, see page 197
- Cisbio HTRF Detection Cartridge on page 198
- Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge, see page 198

> **Note:** For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

Analyze Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of pulses. Each pulse consists of turning the light source on and off (Excitation Time), pausing for a specified length of time (Measurement Delay), and measuring the fluorescence intensity of the sample for a specified length of time (Integration Time). These pulses are repeated several times, as specified in the protocol parameters.

Analyzing and interpreting TRF data generally consists of the following:

- Blank Correction on page 155
- Data Normalization on page 156
- Data Optimization on page 156

Blank Correction

Although background is significantly lower than with fluorescence intensity measurements, Molecular Devices recommends that you use blanks or assay controls.

A blank well contains everything used with the sample wells except the label and sample-specific compounds. Do not use an empty well for a blank.

The blank sample reveals the offset underlying each data sample. This offset does not carry information on the label, and is generally subtracted before data reduction is done.

For optimal results, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can be subtracted is the average value of all blanks.

Data Normalization

TRF raw data changes in magnitude when the timing parameters are changed. However, TRF data are normalized for a number of 1000 pulses. This means that the sample raw data does not change when only the number of pulses is changed.

When selecting a fast read mode, the raw data becomes slightly lower because during the continuous plate movement, some signal is collected under non-optimal focusing conditions.

Data Optimization

There are two timing parameters which can be optimized to adjust the performance of the measurement as desired: time per well and integration time per cycle.

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. Therefore, it is important to specify the read time when comparing measurements. For TRF, the read time per well increases with the selected number of pulses. The time between pulses and the intensity of each pulse, however, can be different on various systems.

All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

To further optimize measurement results, optimize the timing parameters. The following table and figure can be used as guidelines for the selection of timing parameters.

Table 5-1: Time-resolved fluorescence timing parameters example

Parameter	Value	Comment
Pulse length	0.100 ms	The period for excitation of the sample, shown as t_1 in the following figure. This is the suggested value for the TUNE, MULTI, and TRF detection cartridges.
Measurement delay	0.010 ms	The delay to ensure the excitation pulse is no longer detectable, shown as t_2 in the following figure. This is the suggested value for the TUNE, MULTI and TRF detection cartridges.

Table 5-1: Time-resolved fluorescence timing parameters example (continued)

Parameter	Value	Comment
Integration time per cycle (pulse)	0.890 ms	The period for accumulating the signal, shown as t_3 in the following figure. This is the suggested value for the TUNE and MULTI detection cartridges.
Integration time per cycle (pulse)	1.890 ms	The period for accumulating the signal, shown as t_3 in the following figure. This is the suggested value for the TRF detection cartridge.
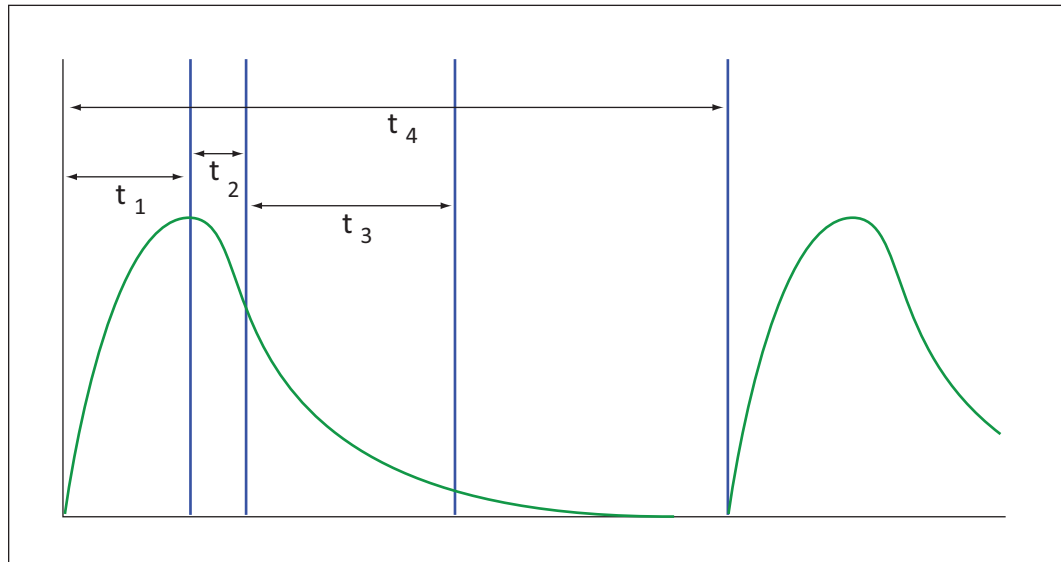
Figure 5-4: Timing parameters for time-resolved fluorescence

When neglecting the time delay t_2 compared to the integration time window t_3, the accumulated signal A can be approximated with the following equation:

$$A / A_{max} = (1 - \exp(-M)) \times 100\%$$

In the previous equation, M is the size of the time window (or integration time) divided by the exponential decay time constant (or the fluorescence lifetime of the label).

$$M = (\text{integration time}) / (\text{fluorescence lifetime})$$

For example, using Europium, which has a fluorescence lifetime of 700 µs, and the suggested integration time per cycle of 1.890 ms (or 1890 µs), $M = 1890 / 700 = 2.7$. Inserting this value of M into the first equation yields $A / A_{max} = 93\%$.

To optimize the integration time per cycle (pulse), the integration time should be set such that the value of M produces the desired signal. For example, to get more than 86% signal, select an integration time such that M is greater than 2.0. Using the previous Europium example and solving for the integration time, the integration time can be set to M (2.0) times the fluorescence lifetime (700 µs), or 1400 µs (1.4 ms).

Table 5-2: Achievable accumulated signal percentage compared to M

M	0.25	0.50	0.75	1.00	1.25	1.50	2.00	3.00
A / A$_{max}$[%]	22	39	53	63	71	78	86	95

M can be technically limited by the time between pulses. Further gain in signal above some value of M can be negligible to improve results.

When performing a dual-label Europium-Samarium measurement, there are more timing parameters. There is some residual cross-talk of the Samarium signal captured in the Europium emission channel. Samarium has a much shorter fluorescence lifetime, so to reduce the cross-talk of Samarium in the Europium channel, Europium is measured in a time window shifted away from the time window for Samarium. This lets the Europium be quantified without cross contamination from the Samarium. The known Europium concentration can then be used to remove the Europium cross-contamination in the Samarium channel.

Suggested timing parameters for a dual-label Europium-Samarium measurement are listed in the following table.

Table 5-3: Time-resolved fluorescence timing parameters for dual-label Europium-Samarium

Parameter	Value	Comment
Pulse length	0.100 ms	The time interval for flash monitoring This is the suggested value for the TRF detection cartridge.
Measurement delay (first window)	0.010 ms	The delay to ensure the excitation pulse is no longer detectable This is the suggested value for the TRF detection cartridge.
Integration time (first window)	0.100 ms	The period for accumulating the Samarium signal This is the suggested value for the TRF detection cartridge.
Measurement delay (second window)	0.140 ms	The read out of the Samarium signal This is the suggested value for the TRF detection cartridge.
Integration time (second window)	0.750 ms	The period for accumulating the Europium signal This is the suggested value for the TRF detection cartridge.

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 a molecular collision. The transfer of energy leads to a reduction in the fluorescence intensity and excited state lifetime of the donor, and an increase in the emission intensity of the acceptor. 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 each other.
- 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 must be approximately parallel.

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

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org).

For information about setting up a FRET mode protocol, see Create FRET Mode Protocols on page 289.

FRET Instruments and Detection Cartridges

The following instruments have FRET read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  To do HTRF reads, the SpectraMax i3x Instrument requires the Cisbio HTRF Detection Cartridge, see page 198.
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  To do HTRF reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires the Cisbio HTRF Detection Cartridge, see page 198.
- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader on page 190

Note: For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

HTRF Read Mode

Homogeneous time-resolved fluorescence (HTRF) is a measurement technique based on fluorescence resonance energy transfer (FRET) using the advantages of time-resolved fluorescence (TRF) reading.

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 a molecular collision. The transfer of energy leads to a reduction in the fluorescence intensity and excited state lifetime of the donor, and an increase in the emission intensity of the acceptor. 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 each other.
- 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 must be approximately parallel.

HTRF uses a donor fluorophore with a long fluorescence lifetime, such as Europium. The acceptor fluorophore acts as if it also has a long fluorescence lifetime. This lets the time-gating principle of time-resolved fluorescence be applied to the acceptor emission to separate specific signal from background and signal caused by compound interference.

Time-gating electronics introduce a delay between the flashes and the start of signal collection. During the delay, the unspecific fluorescence caused by test compounds, assay reagents, and the microplate vanishes while only a small portion of the specific fluorescence from the acceptor fluorophore is sacrificed. Enough of the specific signal remains, with the benefit of reduced background.

Applications of Homogeneous Time-Resolved Fluorescence

Homogeneous time-resolved fluorescence (HTRF) is used in competitive assays to quantify the binding between two labeled molecules, or the disintegration of a bound complex. Binding partners can have similar molecular weights as opposed to fluorescence polarization read modes. HTRF is a homogeneous assay that requires only mixing and measuring—no wash steps are required. It can also be miniaturized, which makes it useful for high-throughput screening applications.

The fluorescence ratio related to the HTRF readout is a correction method developed by Cisbio, for which Cisbio has granted a license to Molecular Devices. Its application is strictly limited to the use of HTRF reagents and technology, excluding other TR-FRET technologies such as IMAP TR-FRET calculations of acceptor to donor ratios.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

HTRF Instruments and Detection Cartridges

The following instruments have HTRF read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  To do HTRF reads, the SpectraMax i3x Instrument requires the Cisbio HTRF Detection Cartridge, see page 198.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  To do HTRF reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires the Cisbio HTRF Detection Cartridge, see page 198.

Note: For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

HTRF is a registered trademark of Cisbio Bioassays.

Analyze HTRF Data

A Homogeneous Time-Resolved Fluorescence (HTRF) measurement includes a number of flash intervals. Each flash interval consists of flashing the lamp, pausing for a specified length of time, and measuring the fluorescence intensity of the sample. These flash intervals are repeated several times, as specified in the protocol parameters. See Data Optimization on page 162.

Analyzing and interpreting HTRF data generally consists of the following:

- Data Reduction on page 161
- Data Optimization on page 162

Data Reduction

Data reduction for HTRF reads consists of two steps.

First, a ratio of the signal measured by the emission from the acceptor label at 665 nm to the signal measured by the emission of the donor label at 616 nm is calculated and multiplied by a factor of 10,000. This generates what is called the HTRF ratio.

In the second step, ratios are calculated that represent the relative change in the HTRF signal compared to that of the assay background, represented by assay controls potentially named negative or Standard 0. This relative response ratio is called the Delta F and is formatted as a percentage, although values greater than 100 can be generated.

Data Optimization

The measurement noise is dependent on the read time per sample (time per plate or time per well). In particular, the detection limit improves when the read time is increased. Therefore, it is important to specify the read time when comparing measurements. For TRF, the read time per well increases with the selected number of pulses. The time between pulses, however, can be different on various systems.

Table 5-4: HTRF timing parameters example

Parameter	Value	Comment
Number of pulses	30	The number of flashes per read.
Measurement delay	30 μs	The delay to ensure the excitation pulse is no longer detectable.
Integration time per cycle (pulse)	400 μs	The period for accumulating the signal.

Defining the number of flashes (pulses) cannot be used for comparative purposes because the flash and intensity rate varies from system to system.

There are two timing parameters which can be optimized to adjust the performance of the measurement as desired: time per plate or time per well, and integration time per cycle.

All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as far as is acceptable from throughput and sample stability considerations.

As the number of flashes (read time per well) is increased, several aspects of the data improve:

- Delta F values show less variability (better CVs).
- Small Delta F values are better distinguished from noise.
- Noise of background is reduced.

The second timing parameter which can be optimized is the Integration time per cycle. Care must be taken in optimizing the integration time to consider noise. Delta F is higher at low integration times, but noise is also high at low integration times. The optimal integration time is where noise is minimized while maximizing Delta F.

In the following example, the optimal integration time (read time per cycle) is displayed to be in the 500 μs to 1000 μs range, as noise is minimized and Delta F is still relatively high. Going greater than 1000 μs shows a sharp decline in Delta F without apparent improvement in noise.
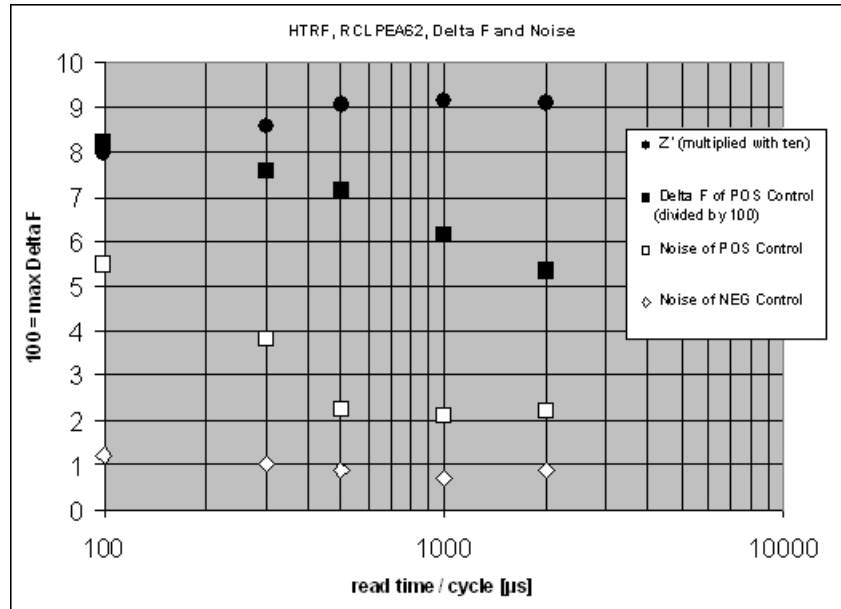
Figure 5-5: Relationship Between Integration Time, Noise, and Delta F

Z´ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

The value of Z´ can be determined using the following formula:

$$Z´ = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|\,Mean_{c+} - Mean_{c-}\,|}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c−** is the negative control.

A Z´ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. Higher values might be desired when results are more critical.

Z´ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z´ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

Fluorescence Polarization Instruments and Detection Cartridges

The following instruments have fluorescence polarization read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  To do fluorescence polarization reads, the SpectraMax i3x Instrument requires a Fluorescence Polarization (FP) Detection Cartridges, see page 201.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  To do fluorescence polarization reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires a Fluorescence Polarization (FP) Detection Cartridges, see page 201.

- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185

- FlexStation 3 Multi-Mode Microplate Reader, see page 189

- FilterMax F5 Multi-Mode Microplate Reader, see page 189

Note: For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

Analyze Fluorescence Polarization Data

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 the SoftMax Pro Software.

FP assays in microplates are generally designed with two control samples:

- LOW control sample: minimal polarization value resulting from unbound labeled ligand only

- HIGH control sample: maximum polarization value resulting from bound labeled ligand only

The FP data for a sample is evaluated based on its relative position between the low and high control values. Total intensity can also be determined from the raw data and is proportional to the quantity of label in a sample.

Analyzing and interpreting fluorescence polarization data generally consists of the following:

- Blank Correction on page 166
- Data Reduction on page 166
- Data Qualification and Validation on page 167

Blank Correction

Many fluorescence polarization assays use small fluorescent label concentrations in the lower nm range. In this range, blank controls become significant when compared to samples.

A blank well contains everything used with the sample wells except the label and sample-specific compounds. Do not use an empty well for a blank.

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 must be subtracted from the raw parallel and perpendicular readings of each sample well before the mP calculation is done.

For optimal results, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can be subtracted is the average value of all blanks.

Data Reduction

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 the SoftMax Pro Software.

Although the raw S and 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 and become the basis for further reduction calculations.

Polarization (mP) is calculated as follows:

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

Anisotropy (r) is calculated as follows:

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

The 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.

Data Qualification and Validation

When validating the data of a fluorescence polarization measurement and the assay, the two factors to look at are the precision value and the Z′ parameter.

The FP precision value is a measure of replicate uniformity determined by the standard deviation of replicates at a label concentration of 1 nM. Since the precision of a measured signal also depends on the read time, the read time must also be specified. A longer read time leads to a lower (better) precision value.

Z′ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

The value of Z′ can be determined using the following formula:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|\,Mean_{c+} - Mean_{c-}\,|}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c–** is the negative control.

A Z′ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. Higher values might be desired when results are more critical.

Z′ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z′ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

The assay window is dependent on the fluorophore lifetime and relative size of the receptor to the ligand. Precision values are better (lower) at higher signals, which normally come from higher label concentrations.

For a given assay window, Z′ is a downward sloping linear function. That is, as precision values get higher (worse), the Z′ value gets lower (worse).

Precision is dependent upon assay characteristics (sample volume, label concentration) and read time. In many assays, the characteristics are defined and cannot be changed. In this case, the only way to improve precision is to increase the read time per well.

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 moves beads (coated with A and B, respectively) together, a cascade of chemical reactions 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) lets diffusion reach up to only around 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 beads are light sensitive. Beads are best handled under subdued (<100 lux) or green filtered (Roscolux filters #389 from Rosco, or equivalent) light conditions. Do incubation steps in the dark.

The raw data can be normalized to counts per second. See Analyze AlphaScreen Data on page 169.

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).

AlphaScreen read mode can also capture the Europium emission line of AlphaLISA®.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

For information about setting up a fluorescence polarization mode protocol, see Create Fluorescence Polarization Mode Protocols on page 291.

AlphaScreen Instruments and Detection Cartridges

The following instruments have AlphaScreen read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  To do AlphaScreen reads, the SpectraMax i3x Instrument requires AlphaScreen Detection Cartridges , see page 197.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  To do AlphaScreen reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires AlphaScreen Detection Cartridges , see page 197.

> **Note:** For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

For more information, go to www.perkinelmer.com.

ALPHASCREEN and ALPHALISA are registered trademarks of PerkinElmer, Inc.

Analyze AlphaScreen Data

The conversion rate of photons to counts and relative fluorescence units (RFU) is individual for each reader. Therefore, raw data from the same microplate can seem to be different from one instrument to the next. Also, the data format used by instrument manufacturers might be counts normalized per second or not normalized counts, and therefore the 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. See Detection Limit on page 170.

The raw data can be normalized to counts per second by selecting the **Normalization** option in the **Settings** dialog. See Create Protocols on page 205.

Analyzing and validating AlphaScreen data can consist of the following:

- Background Correction on page 169
- Detection Limit on page 170
- Data Qualification and Validation on page 170

Background Correction

Although background is significantly lower than with fluorescence intensity measurements, Molecular Devices recommends that you use blanks 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 plate. To prevent such interference, you can select the **Interlaced Reading** option in the SoftMax Pro Software Settings dialog. This option reads only every other well in a checkerboard pattern, and then reads the microplate again to read the previously omitted wells.

A blank well contains everything used with the sample wells except the label and sample-specific compounds. Do not use an empty well for a blank.

For optimal results, Molecular Devices recommends that you run replicates for all blanks, controls, and samples. In this case, the blank value that can be subtracted is the average value of all blanks.

Detection Limit

The detection limit is the smallest sample concentration that can be measured reliably above the blank. Determining the detection limit requires taking a number of blank measurements and calculating an average value and standard deviation for the blanks. The detection threshold is defined as the average blank plus three standard deviations. If the average sample value measures above the threshold, the sample can be detected at a statistically significant level.

The detection limit can be described by the following equation:

$$\text{Det Limit} = \frac{3\,\text{Stdev}_{blank}}{\dfrac{std - blank}{conc_{std}}}$$

where $conc_{std}$ is the concentration of the standard, $StDev_{Blank}$ is the standard deviation of the blank replicates, and $blank$ and Std are average values of the replicates for the blank and standard wells.

Data Qualification and Validation

Z´ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

The value of Z´ can be determined using the following formula:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|\,\text{Mean}_{c+} - \text{Mean}_{c-}\,|}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c–** is the negative control.

A Z´ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. Higher values might be desired when results are more critical.

Z´ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z´ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

> ⚠ **CAUTION!** The assay plate and the instrument should be kept at room temperature, since temperature variations cause fluctuations in signal.

Western Blot TRF Read Mode

Protein detection is an important task for pharmaceutical and clinical research, and Western Blots (WB), or protein immunoblots, are one of the most common methods employed for this purpose. Various techniques are used to detect proteins on Western Blot membranes including fluorescence, silver staining, and chemiluminescence. However, each technique has its limitations, and there is a continuous need to improve quantitation, accuracy, and dynamic range of Western Blots.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that is incorporated into a SpectraMax® Paradigm® or SpectraMax® i3x Multi-Mode Detection Platform. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. Europium has a long fluorescence lifetime, on the order of 1 msec, and detection is done in Time Resolved Fluorescence (TRF) mode which significantly reduces background from auto-fluorescence or other sources of short lifetime emissions. The membranes are placed into a microplate reader where they are scanned with the ScanLater Western Blot (WB) Detection Cartridge.

The method does not involve enzyme detection, and the Eu-chelates are resistant to photo-bleaching, so the signal remains stable for long periods of time (weeks to months). This permits repeat reading of membranes and the potential for comparison of band intensities to known standards for more accurate quantitation.

The TRF detection employs photon counting, so the theoretical dynamic range is $>10^{5}$. In practice, however, dynamic range is limited by saturation of binding sites on high-abundant bands and non-specific binding to background membrane. There is also no camera blooming, as can occur with chemiluminescence or fluorescence detection, so the system gives sharp bands and excellent image quality.

The SpectraMax Western Blot system is a simple, sensitive, and stable platform that provides excellent Western Blot capability in a SpectraMax Paradigm or SpectraMax i3x Multi-Mode Detection Platform extending the uses of these essential laboratory instruments.

Applications

- Identify the nature of the protein or epitope effectively. Also, it can be used as a tool for quantitative analysis of protein.
- Use for chromatography components analysis, sucrose gradient analysis.
- Test the endogenous or exogenous expression of phosphoprotein so as to detect the phosphorylation signal.
- Protein resilience in the function experiment.
- Structure domain analysis.
- Analysis of the protein expression level.
- Analysis of protein content in the serum.
- Analysis of regulatory proteins expressed in the cell cycle.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

For information about setting up a Western Blot TRF mode protocol, see Create Western Blot TRF Mode Protocols on page 295.

If you use the SoftMax Pro GxP Software, you must enable the following permissions to complete a Western Blot membrane read:

- **Read empty plates/cuvettes** to start a read.
- **Save data files** to save the data file before the read.
- **Overwrite plate/cuvette data** to do a high-resolution ROI read.

See SoftMax Pro GxP Software on page 417.

Western Blot TRF Instruments and Detection Cartridges

The following instruments have Western Blot TRF read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  To do Western Blot TRF reads, the SpectraMax i3x Instrument requires the ScanLater Western Blot (WB) Detection Cartridge.

- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184

  To do Western Blot TRF reads, the SpectraMax Paradigm Multi-Mode Microplate Reader requires the ScanLater Western Blot (WB) Detection Cartridge.

The ScanLater Western Blot (WB) Detection Cartridge can be used for top reads only.

Analyze Western Blot TRF Data

After you scan a membrane for Western Blot data, the data displays in the SoftMax Pro Software as an image. Use the image tools in the **Plate** section to zoom, crop, colorize, and adjust the intensity of the image. You can select a region of interest (ROI) and rescan the membrane at a higher resolution. See View Western Blot Membrane Data on page 395.

Western Blot membrane data are saved as a TIFF image that can be analyzed by the image-analysis tool of your choice. The SoftMax Pro Software comes with an installed version of the ImageJ software from U.S. National Institute of Health (NIH). See Analyze Western Blot Images on page 400.

For best results, use the Molecular Devices ScanLater™ Western Blot Assay Kit that matches your application.

To ensure successful acquisition of Western Blot data, see the following topics:

- Block Nonspecific Binding on page 172
- Handle Membranes on page 173

Block Nonspecific Binding

To reduce noise, use blocking buffer to reduce non-specific protein from binding with the membrane.

No single-blocking reagent is optimal for every antigen-antibody pair. Some primary antibodies can exhibit greatly reduced signal or different nonspecific binding in different blocking solutions. If you have difficulty detecting your target protein, changing the blocking solution can dramatically improve the performance. If the primary antibody has worked well in the past using chemiluminescent detection, try that same blocking solution for detection. Other commonly used blocking buffers other than BSA are 3% casein and 5% non-fat milk.

Milk-based blockers can contain IgG that can crossreact with anti-goat antibodies. This can significantly increase background and reduce antibody titer. Milk-based blockers can also contain endogenous biotin or phosphoepitopes that can cause higher background.

To prevent background speckles on blots, use high-quality, ultra-pure water for buffers.

Do not over-block. Extended blocking times can cause loss of target protein from the membrane.

Handle Membranes

To scan the membrane in a SpectraMax Paradigm or SpectraMax i3x Instrument, you must place the membrane in a Molecular Devices ScanLater™ membrane holder. See Load the Membrane into the Membrane Holder on page 296.

> **Note:** Handle membranes by their edges only, using clean forceps. Do not touch the membrane with gloved or bare hands.

The maximum size for a membrane is 109 mm x 77 mm to let it fit in the membrane holder.

The Western Blot should be prepared using standard blotting procedures for the membrane being used. For optimal results, use Millipore Immobilon FL (IPFL00010). If using PVDF, pre-wet the membrane in 100% methanol.

Use enough antibody volume so that the entire membrane surface is sufficiently covered with liquid at all times. Use heat-seal bags if the volume is limiting. Do not let an area of the membrane dry out. Use agitation for all antibody incubations.

Small proteins can pass through the membrane during transfer ("blow-through"). To prevent this, use a membrane with a smaller pore size or reduce the transfer time.

Allow the blot to dry for a minimum of 1 hour before detection.

Do not wrap the membrane in plastic when scanning.

Imaging Read Mode

To use this read mode, you must have the SpectraMax MiniMax 300 Imaging Cytometer installed with the SpectraMax i3x Instrument.

Imaging read mode does whole-cell imaging assays.

Whole-cell imaging assays are cell-based, or object-based, rather than the single-point measurements found in other types of microplate reads. These types of assays can yield more biologically meaningful results that can discriminate the fluorescence related to objects, such as cells or beads, from the bulk solution within a microplate well.

The measurement is primarily fluorescent with quantification of cell size, shape, area, and intensity. Label-free quantification is also supported through brightfield, transmitted light imaging. The camera resolution in the SpectraMax MiniMax 300 Imaging Cytometer is sufficient to determine the approximate shape of small 8 micron objects, such as blood cells.

Other advantages of these assays include the direct interrogation of individual cells instead of whole well or cell lysates that permit controlling for cell numbers and heterogeneity in cell-based assays. Also, increased sensitivity by both detecting a few fluorescent cells per well, as well as detecting cells that are, on average, less fluorescent.

The SpectraMax® MiniMax™ 300 Imaging Cytometer 300 is capable of acquiring image data using transmitted light and two fluorescent channels during the same microplate read. In the SoftMax Pro Software **Settings** dialog, you can select the channels that you want to use for the acquisition. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

You can obtain imaging data through visual inspection of the image or by using the analysis tools available in the SoftMax Pro Software. See Analyze Imaging Data on page 175.

Applications of Whole-Cell Imaging

Whole-cell imaging assays measure a diverse set of cellular responses, such as fluorescent protein expression (phosphorylated and total), cell viability (cell toxicity), cell apoptosis, and cell cycle analysis.

Supported dyes include the following:

- EarlyTox™ Cell Integrity Kit
- Fluorescein isothiocyanate (FITC)
- Calcein AM
- Alexa Fluor 488 or Alexa Fluor 647
- Cy5
- DRAQ5

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

For information about creating Imaging read mode protocols, see Create Imaging Mode Protocols on page 297.

Imaging Instruments and Detection Cartridges

The following instrument has Imaging read mode capability:

- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182

  The SpectraMax® MiniMax™ 300 Imaging Cytometer adds imaging capability to the SpectraMax i3x Instrument to visually inspect your sample and to run cell-based assays at cellular or whole-cell resolution.

  To do brightfield, transmitted-light imaging, you must install the SpectraMax Transmitted Light (TL) Detection Cartridge in the detection cartridge drawer. See Transmitted Light (TL) Detection Cartridge on page 204.

Analyze Imaging Data

You can obtain imaging data through visual inspection of the image or by using the analysis tools available in the SoftMax Pro Software.

In fluorescent imaging, the fluorescence in the sample is excited with light of a specified wavelength. The fluorophore emits at a longer wavelength that is captured in the image.

The software measures the intensity of the emitted fluorescence and estimates the intensity of the background in the image.

You can select one of the following image analysis types.

Discrete Object Analysis

Discrete Object Analysis uses proprietary algorithms to analyze separate objects, or cells, based on multiple parameters including the signal intensity over the background and the size of the objects.

When you click **Discrete Object Analysis**, the **Wavelength for Finding Objects** list appears below the list of analysis types. Select the wavelength from this list to use to identify the objects in the well images. You can use transmitted light or a fluorescent wavelength to find nuclei or easily separable objects in the image. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

Click the **Image Analysis Settings** category on the left to display the following subcategories:

- Click **Find Objects** to enter size and intensity values or to use proprietary algorithms with the drawing tools to graphically define the objects and the background areas for the analysis. See Find Discrete Objects or Confluent Areas on page 234.
- Click **Classify Objects** to define separate measurements for different types of found objects, or cells. See Classify Discrete Objects on page 237.
- Click **Select Measurements** to view the results of the analysis for the wells you select. See View and Select Measurement Statistics on page 239.
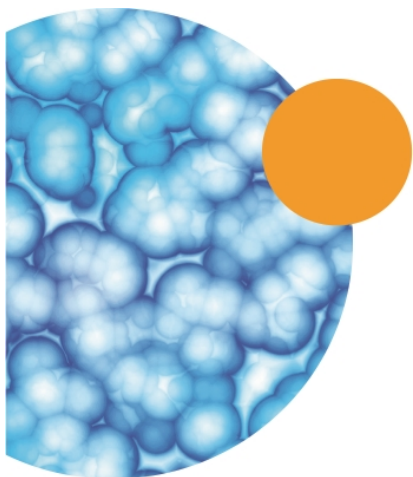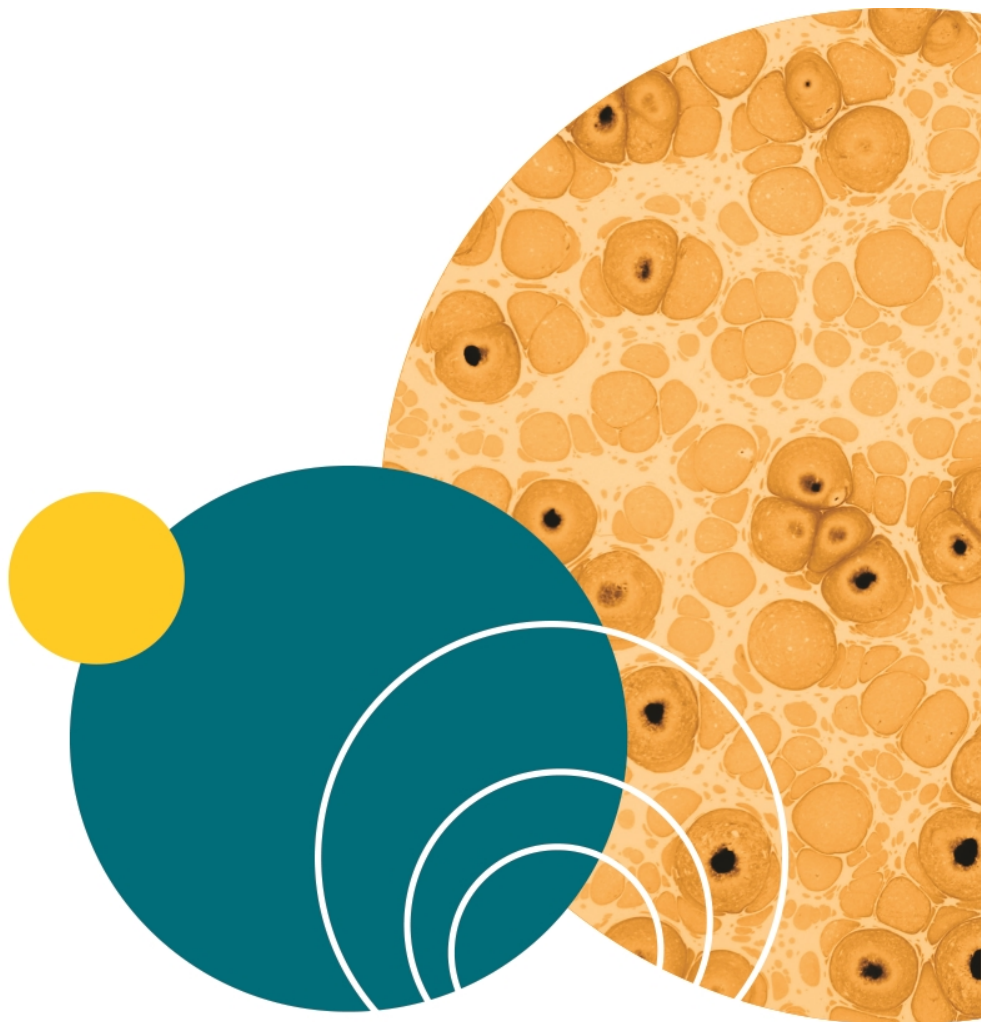
Field Analysis

Field Analysis uses proprietary algorithms to analyze confluent areas based on multiple parameters including the signal intensity over the background and the size of the areas.

When you click **Field Analysis**, The **Wavelength for Finding Confluent Areas** list appears below the list of analysis types. Select the wavelength from this list to use to identify the confluent areas in the well images. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

The **Image Analysis Settings** category on the left expands to show the subcategories that follow:

- Click **Find Confluent Areas** to use proprietary algorithms with the provided drawing tools to graphically define the confluent areas and the background areas for the analysis. See Find Discrete Objects or Confluent Areas on page 234.
- Click **Select Measurements** to view the results of the analysis for the selected wells. See View and Select Measurement Statistics on page 239.

The SoftMax Pro Software proprietary algorithms with the provided drawing tools help the software learn the patterns of the objects that you want to find. Depending on the complexity of the object patterns, this teaching process can take several iterations before you get your desired results. See Resolve Issues with Object Drawing and Finding on page 241.

Analysis Measurement Parameters

Depending on the needs of your application, you can use one or more of the following analysis measurement parameters for the region of interest (ROI) in your experiment:

- **Object Count** gives the total number of objects detected in the image. This measurement is not used for field analysis of confluent areas.
- **Field Count** gives the total number of confluent areas detected in the image. This measurement is not used for discrete object analysis.
- **Object Percentage** gives the percentage the objects detected in the image by classification. This measurement is not used for field analysis of confluent areas.
- **Covered Area** gives the combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area** gives the average area of the objects detected in the image expressed in μm^2. This measurement is not used for field analysis of confluent areas.
- **Field Area** gives the average area of the confluent areas detected in the image expressed in μm^2. This measurement is not used for discrete object analysis.
- **Object Roundness** gives the average roundness of each object detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for field analysis of confluent areas.

- **Field Roundness** gives the average roundness of each confluent area detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for discrete object analysis.
- **Object Average Intensity** gives the average fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).
- **Field Average Intensity** gives the average fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).
- **Object Intensity** gives the average total fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).
- **Field Intensity** gives the average total fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).
- **Total Intensity** gives the combined total fluorescent signal intensity of the objects or confluent areas detected in the image expressed in million intensity counts. This measurement is not used for transmitted light (TL).

Chapter 6: Supported Instruments and Detection Cartridges

6

SoftMax® Pro Data Acquisition and Analysis Software controls Molecular Devices® spectrophotometers and absorbance, luminescence, and fluorescence microplate readers and detection platforms.

Some instruments use detection cartridges. Each detection cartridge contains its own independent light source, optics, and electrical components needed to do specific read modes for some applications.

- The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.
- The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

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 7.0.2 > Hardware User Guides**.

SoftMax Pro Software supports the following instruments:

- SpectraMax iD3 Multi-Mode Microplate Reader on page 180
- SpectraMax i3x and i3 Multi-Mode Detection Platforms, see page 182
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 184
- VersaMax Microplate Reader, see page 184
- SpectraMax Plus 384 Absorbance Microplate Reader, see page 185
- SpectraMax M5 and M5e Multi-Mode Microplate Readers, see page 185
- SpectraMax M4 Multi-Mode Microplate Reader, see page 186
- SpectraMax M3 Multi-Mode Microplate Reader, see page 186
- SpectraMax M2 and M2e Multi-Mode Microplate Readers, see page 187
- SpectraMax 340PC 384 Absorbance Microplate Reader, see page 187
- SpectraMax 190 Absorbance Microplate Reader, see page 188
- Gemini XPS Microplate Reader, see page 188
- Gemini EM Microplate Reader, see page 188
- FlexStation 3 Multi-Mode Microplate Reader, see page 189
- FilterMax F5 Multi-Mode Microplate Reader, see page 189
- FilterMax F3 Multi-Mode Microplate Reader, see page 190
- DTX 800 and DTX 880 Multi-Mode Microplate Readers, see page 191
- Vmax Kinetic Microplate Reader, see page 191
- EMax Endpoint Microplate Reader, see page 191

- EMax Plus Microplate Reader, see page 192
- StakMax Microplate Handling System, see page 192

Some instruments use detection cartridges. Each detection cartridge contains its own independent light source, optics, and electrical components needed to do specific read modes for some applications.

- The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.
- The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

See Supported Detection Cartridges on page 194.

SpectraMax iD3 Multi-Mode Microplate Reader

The SpectraMax® iD3 Multi-Mode Microplate Reader is a monochromator-based detection platform. The instrument has a touchscreen interface that enables you to run protocols without a connected computer. The SpectraMax iD3 Multi-Mode Microplate Reader can export results over your company intranet or to a computer that is directly connected to the instrument for statistical data analysis. You must use the SoftMax Pro Software to operate the instrument for protocols that use the injectors.

The instrument supports the following read modes:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.

For more information on supported read types:

- Endpoint Read Type on page 134
- Kinetic Read Type on page 134
- Well Scan Read Type on page 134
- Spectrum Read Type on page 134

You can operate the instrument via the SoftMax Pro Software. The SoftMax Pro Software version 7.0.2 installation includes the QuickSync Tool for use with the SpectraMax® iD3 Multi-Mode Microplate Reader. The QuickSync Tool icon ⟳ appears along the bottom of your computer screen. See Use the QuickSync Tool on page 181.

> **Note:** When you operate the instrument via a computer running the SoftMax Pro Software, the instrument touchscreen is disabled.

SpectraMax iD3 Multi-Mode Microplate Reader Considerations

When you use SoftMax Pro Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader, your company network configuration could prevent direct communication between the computer and the instrument.

You can use the Ethernet cable to connect the computer directly to the instrument or you can connect the SpectraMax iD3 to your company intranet. You may have to perform the following steps to select the instrument.

1. On the SpectraMax iD3 instrument touchscreen, navigate to the **Maintenance** page **Instrument** tab and note the instrument IP address.

2. In the SoftMax Pro Software, click to open the Application menu.

3. At the bottom of the Application menu, click the **Options** button to open the SoftMax Pro Options dialog.

4. In the list on the left, click **SpectraMax iD3 Control**.

5. Select the **Enables Control of SpectraMax iD3 Instruments** check box.

6. Enter the IP address of the instrument in one of the fields. Fields enable you to enter up to five IP addresses.

7. Click **OK**.

8. Select the **Home** tab in the ribbon and click **Instrument** to open the Instrument Connection dialog.

9. Click **Refresh** if needed and the SpectraMax iD3 Instrument should appear. If the instrument does not appear, you may have to work with your company network administrator to confirm that your company network configuration permits the communication between the computer and the instrument.

When you use SoftMax® Pro GxP Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader and you log on as a user with the Sign Statements permission, two additional icons appear on the GxP tab in the ribbon.

- Users with the Sign Statements permission click **GxP Mode On** to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and the instrument must be operated from a computer running SoftMax Pro GxP Software.

- Users with the Sign Statements permission click **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to perform reads from the instrument.

 Note: The instrument remains locked until the Sign Statements user clicks **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an SpectraMax iD3 instrument that is locked in GxP mode.

Use the QuickSync Tool

The QuickSync tool enables you to use the raw data the SpectraMax iD3 Multi-Mode Microplate Reader exports for further analysis.

The instrument acquires an IP address when you connect the instrument to your intranet. After you synchronize the computer with the instrument, the instrument exports result data to the computer for further analysis.

The QuickSync icon appears in your computer taskbar area, with the other programs running on your computer, at the bottom of the screen. You might have to look in the Show Hidden Icons area.

1. Click the big in the taskbar to display a smaller version of the in the computer tray near the clock at the bottom of the computer screen.

2. Right-click in the tray to display a menu and select **Available Services** to display the list of SpectraMax iD3 Multi-Mode Microplate Reader instruments on your intranet and/or the instrument to which you connect the computer through an Ethernet cable.
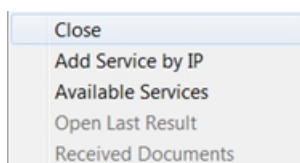
3. Click the name of instruments to which to synchronize the computer. A check mark appears next to each instrument name to which the computer is synchronized.

   If the name of the instrument does not appear in the list of available services, right-click and select **Add Service by IP** and enter the IP address of the instrument to which to connect.

4. When the instrument is set to export read data, after the instrument performs a read, right-click and select **Open Last Result**. From the instrument touchscreen, you can touch **Export** to send result data to the QuickSync tool.

SpectraMax i3x and i3 Multi-Mode Detection Platforms

The SpectraMax i3x Multi-Mode Detection Platform is a monochromator-based, multi-mode detection platform. An external computer running the SoftMax Pro Software provides integrated instrument control, data display, and statistical data analysis.

The built-in read modes include:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.

The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities. Some instruments use detection cartridges. Each detection cartridge contains its own independent light source, optics, and electrical components needed to do specific read modes for some applications. Up to four detection cartridges can be installed in the detection cartridge drawer. For information about the different detection cartridges, see Supported Detection Cartridges on page 194.

For most read modes, endpoint, kinetic, multi-point well-scan, and spectrum microplate applications can be set up and run with the SoftMax Pro Software.

For more information on read types, see the following topics:

- Endpoint Read Type on page 134
- Kinetic Read Type on page 134
- Well Scan Read Type on page 134
- Spectrum Read Type on page 134
- Flex Read Type on page 135
- Membrane Read Type on page 135

The SpectraMax® MiniMax™ 300 Imaging Cytometer adds imaging capability to the SpectraMax i3x Instrument to visually inspect your sample and to run cell-based assays at cellular or whole-cell resolution. See SpectraMax MiniMax 300 Imaging Cytometer on page 183.

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well microplates. For micro-volume measurements, the instrument supports the SpectraDrop 24-well Micro-Volume Microplates and the SpectraDrop 64-well Micro-Volume Microplates. With some detection cartridges, the instrument is capable of reading 1536-well microplates.

Features of the SpectraMax i3x Multi-Mode Detection Platform include:

- Built-in, 2x2 monochromators for absorbance, fluorescence, and luminescence reads
- Top and bottom read capability
- More capabilities with detection cartridges, including AlphaScreen
- Adjustable read height (Z-height) for top reads
- Up to 1536-well format with some detection cartridges
- Temperature control
- PathCheck Pathlength Measurement Technology

SpectraMax MiniMax 300 Imaging Cytometer

The SpectraMax® MiniMax™ 300 Imaging Cytometer adds imaging capability to the SpectraMax i3x Instrument to visually inspect your sample and to run cell-based assays at cellular or whole-cell resolution. The cytometer uses solid-state illumination, a digital camera, a 4x objective lens, laser auto-focus, and auto-exposure to capture fluorescent or label-free images of a sample at the bottom of the microplate wells. The cytometer supports 96-well and 384-well, flat-bottom, clear-bottom microplates.

The measurement is primarily fluorescent with quantification of cell size, shape, area, and intensity. Label-free quantification is also supported through brightfield, transmitted light imaging. The camera resolution in the SpectraMax MiniMax 300 Imaging Cytometer is sufficient to determine the approximate shape of small 8 micron objects, such as blood cells.

The SpectraMax® MiniMax™ 300 Imaging Cytometer 300 is capable of acquiring image data using transmitted light and two fluorescent channels during the same microplate read. In the SoftMax Pro Software **Settings** dialog, you can select the channels that you want to use for the acquisition. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

To do brightfield, transmitted-light imaging, you must install the SpectraMax Transmitted Light (TL) Detection Cartridge in the detection cartridge drawer. See Transmitted Light (TL) Detection Cartridge on page 204.

SpectraMax Paradigm Multi-Mode Microplate Reader

The SpectraMax Paradigm Multi-Mode Microplate Reader is a modular multi-mode microplate reader. User-installable detection cartridges let the microplate reader be configured for specific applications, and they expand the capabilities of the microplate reader. The measurement capabilities of the microplate 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 Microplate Reader.

Most detection cartridges start 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 Supported Detection Cartridges on page 194.

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

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

VersaMax Microplate Reader

The VersaMax™ 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 standard 96-well microplates.

Detection mode includes:

- Visible Absorbance (ABS), see Absorbance Read Mode on page 137.

VersaMax 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.
- 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 a 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 Absorbance Read Mode on page 137.

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 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 Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.
- Fluorescence Polarization (FP), see Fluorescence Polarization Read Mode on page 164.

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 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 Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.

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 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 offers a triple-mode cuvette port, accurate temperature control, and microplate shaking capability.

Detection modes include:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.

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 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 PathCheck® Pathlength Measurement Technology let homogeneous and heterogeneous microplate assays be done in one flexible system.

Detection modes include:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.

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

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

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 standard 96-well or 384-well microplates.

Detection mode includes:

- Visible Absorbance (ABS), see Absorbance Read Mode on page 137.

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 standard 96-well microplates.

Detection mode includes:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 137.

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 Microplate Reader

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

Detection modes include:

- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.

Gemini XPS Microplate Reader features include:

- Dual monochromators let you select wavelengths 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 Microplate Reader

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

Detection modes include:

- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.

Gemini EM Microplate Reader features include:

- Dual monochromators let you select wavelengths in 1 nm increments.
- Cutoff filters reduce stray light and minimize background interference.
- Wavelength scanning ensures that the most sensitive assay conditions are used.

FlexStation 3 Multi-Mode Microplate Reader

The FlexStation® 3 Multi-Mode Microplate Reader combines the performance of a dual-monochromator, multi-mode microplate reader and an integrated 8-channel or 16-channel pipettor into one compact bench-top reader. This integrated system provides a multi-detection platform capable of increasing liquid-handling throughput and flexibility for biochemical-based and cell-based assays. The system is equally amenable to agonist and antagonist assay formats. Combining fluid transfer with multi-detection optics provides a microplate reader capable of doing a broad span of applications for the drug discovery and research environments.

Detection modes include:

- UV and Visible Absorbance (ABS), see Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Luminescence (LUM), see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.
- Fluorescence Polarization (FP), see Fluorescence Polarization Read Mode on page 164.

The dual monochromators in the FlexStation 3 reader let you target the optimal assay excitation and emission wavelengths, while eliminating the need to change expensive band pass filters between experiments.

For most read modes, endpoint, kinetic, multi-point well-scan, and spectrum microplate applications plus the dedicated flex read type can be set up and run with the SoftMax Pro Software.

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) at wavelengths between 230 nm and 650 nm, see Absorbance Read Mode on page 137.

- Fluorescence Intensity (FL) at wavelengths between 230 nm and 700 nm for top reading, or between 300 nm and 650 nm for bottom reading, see Fluorescence Intensity Read Mode on page 143.
- Glow-type Luminescence (LUM) at wavelengths between 400 nm and 700 nm, see Luminescence Read Mode on page 149.
- Time-Resolved Fluorescence (TRF) at wavelengths between 340 nm and 700 nm, see Time-Resolved Fluorescence Read Mode on page 154.
- Fluorescence Polarization (FP) at wavelengths between 300 nm and 700 nm, see Fluorescence Polarization Read Mode on page 164.
- Fluorescence Resonance Energy Transfer (FRET), see FRET Read Mode on page 159.

Other features of the FilterMax F5 Multi-Mode Microplate Reader include:

- A deuterium light source that extends sensitivity into the deep UV band.
- Endpoint, kinetic, multiple wavelength, linear scan, and area scan read types
- Measurements on samples in 6, 12, 24, 48, 96, 384, 1536-well microplates.
- PathCheck Pathlength Measurement Technology
- Automatic read height (Z-height) optimization for top read
- Temperature control in the microplate chamber during measurements.
- Comprehensive Data Analysis with SoftMax Pro Software

The filters used to do 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 Configure Filter Slides on page 410.

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) at wavelengths between 340 nm and 650 nm, see Absorbance Read Mode on page 137.
- Fluorescence Intensity (FL) for top reading at wavelengths between 340 nm and 650 nm, see Fluorescence Intensity Read Mode on page 143.
- Glow-type Luminescence (LUM) at wavelengths between 400 nm and 650 nm, see Luminescence Read Mode on page 149.
- Fluorescence Resonance Energy Transfer (FRET), see FRET Read Mode on page 159.

Other features of the FilterMax F3 Multi-Mode Microplate Reader include:

- Endpoint, kinetic, multiple wavelength, linear scan, and area scan read types.
- Measurements on samples in 96-well and 384-well microplates.

- Linear and orbital microplate shaking.
- Comprehensive Data Analysis with the SoftMax Pro Software.

The filters used to do 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 Configure Filter Slides on page 410.

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 190.
- For information about the DTX 880 Multi-Mode Microplate Reader, see FilterMax F5 Multi-Mode Microplate Reader on page 189.

Vmax Kinetic Microplate Reader

The Vmax Kinetic Microplate Reader measures optical density (OD) and can do 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 standard 96-well microplates.

Detection mode includes:

- Visible Absorbance (ABS), see Absorbance Read Mode on page 137.

Vmax Kinetic Microplate Reader features include:

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

EMax Endpoint Microplate Reader

The EMax Endpoint 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 standard 96-well microplates.

Detection mode includes:

- Visible Absorbance (ABS), see Absorbance Read Mode on page 137.

EMax Endpoint Microplate Reader features include:

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

EMax Plus Microplate Reader

The EMax® Plus Microplate Reader is a filter-based absorbance microplate reader, with eight standard filters for a wide range of applications. The instrument can read standard 96-well microplates.

The instrument has a built-in read mode for visible absorbance (ABS) measurements, see Absorbance Read Mode on page 137.

Endpoint and kinetic read types are supported. See Endpoint Read Type on page 134 and Kinetic Read Type on page 134.

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 placed in automation mode, and then the StakMax Software starts and automatically connects to the StakMax Microplate Handling System. The current status of the instrument is displayed in the status line of the StakMax Software window.
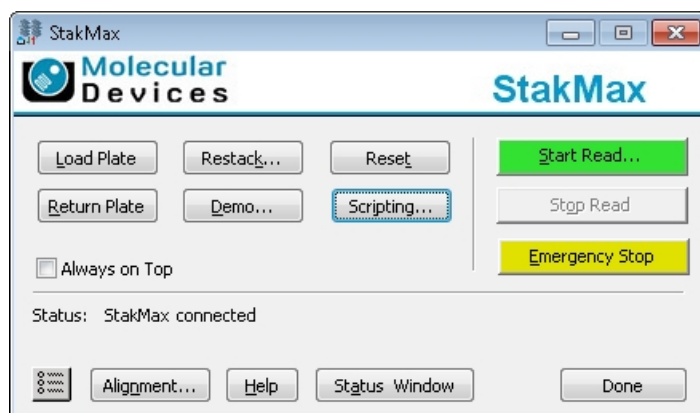
CAUTION! To prevent data loss, make sure that **Auto Save** is enabled for each protocol you want to run before you start the StakMax Software to run automated reads. See Auto Save Settings on page 69.

To exit the StakMax Software, click **Done** and then click **Terminate** in the SoftMax Pro Software Automation dialog.

Compatible Instruments

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

- SpectraMax® iD3 Multi-Mode Microplate Reader
- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® Paradigm® Multi-Mode Microplate Reader
- 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
- SpectraMax® L Microplate Reader
- Gemini™ EM and XPS Microplate Readers

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™ 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 Automation Mode on page 54.

Supported Detection Cartridges

Some instruments use detection cartridges. Each detection cartridge contains its own independent light source, optics, and electrical components needed to do specific read modes for some applications.

- The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.
- The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

Note: For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

The SoftMax Pro Software supports the following detection cartridges:

- Absorbance Detection Cartridge on page 195
- Tunable Wavelength (TUNE) Detection Cartridge, see page 196
- Multi-Mode (MULTI) Detection Cartridge, see page 197
- AlphaScreen Detection Cartridges , see page 197
- Cisbio HTRF Detection Cartridge on page 198
- Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge, see page 198
- Fluorescence Intensity (FI) Detection Cartridges, see page 199
- Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge, see page 199
- Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge, see page 200
- Fluorescence Polarization (FP) Detection Cartridges, see page 201
- Glow Luminescence (LUM) Detection Cartridges, see page 200
- Dual Color Luminescence (LUM) (BRET2) Detection Cartridge, see page 201
- Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge, see page 202
- ScanLater Western Blot (WB) Detection Cartridge on page 202
- Injector Cartridge on page 203
- Transmitted Light (TL) Detection Cartridge on page 204

For complete descriptions and specifications for each of the supported detection cartridges, see the *SpectraMax i3x Multi-Mode Detection Platform User Guide* or the *SpectraMax Paradigm Multi-Mode Microplate Reader User Guide*.

Custom detection cartridges can be designed to meet the specific needs of your application. For more information, see your Molecular Devices representative.

Absorbance Detection Cartridge

The 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 do 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. The two components must be installed to do absorbance measurements with the SpectraMax Paradigm Multi-Mode Microplate Reader.

The SpectraMax i3x Multi-Mode Detection Platform does not support the Absorbance Detection Cartridge.

Typical Applications

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

You can create protocols that use the Absorbance Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Tunable Wavelength (TUNE) Detection Cartridge

The Tunable Wavelength (TUNE) Detection Cartridge enables several detection modes, including:

- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.
- Luminescence (LUM), see Luminescence Read Mode on page 149.

The spectral optimization feature of the Tunable Wavelength (TUNE) Detection Cartridge can help to get the maximum signal-to-background ratio for most fluorophores or luminescence labels that are compatible with the wavelength ranges.

Using a Spectrum read, you can define a fixed excitation wavelength and scan the emission wavelengths, or define a fixed emission wavelength and scan the excitation wavelengths. The spectral optimization helps to get the maximum signal-to-background ratio from a fluorescent or fluorescently labeled analyte. For more information on using the spectral optimization feature, see the SoftMax Pro Software application help or user guide.

The Tunable Wavelength (TUNE) Detection Cartridge occupies three (3) slots in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.
- Spectrum, see Spectrum Read Type on page 134.

You can create protocols that use the Tunable Wavelength (TUNE) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Multi-Mode (MULTI) Detection Cartridge

Optimized for 96-well and 384-well plates, the Multi-Mode (MULTI) Detection Cartridge enables several detection modes, including:

- Fluorescence Intensity (FL), see Fluorescence Intensity Read Mode on page 143.
- Time-Resolved Fluorescence (TRF), see Time-Resolved Fluorescence Read Mode on page 154.
- Luminescence (LUM), see Luminescence Read Mode on page 149.

The Multi-Mode (MULTI) Detection Cartridge occupies three (3) slots in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Multi-Mode (MULTI) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

AlphaScreen Detection Cartridges

The AlphaScreen® Detection Cartridges use a 680 nm laser diode to provide a sensitive reading system for AlphaScreen assays. The design isolates each well and enables the optimal performance for AlphaScreen assays. The detection cartridges also capture the Europium emission line of AlphaLISA®.

The following AlphaScreen Detection Cartridges are available:

- AlphaScreen 384 Std Detection Cartridge
- AlphaScreen 384 HTS Detection Cartridge
- AlphaScreen 1536 HTS Detection Cartridge

Each AlphaScreen Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The AlphaScreen Detection Cartridges can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the AlphaScreen Detection Cartridges in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

ALPHASCREEN and ALPHALISA are registered trademarks of PerkinElmer, Inc.

Cisbio HTRF Detection Cartridge

The Cisbio HTRF Detection Cartridge uses a high-energy Xenon flash lamp for sensitive reading of HTRF reagents. Also, the standard dual-emission design gives the most accurate results in short overall read time. This detection cartridge enables time-resolved fluorescence read modes for europium cryptate and europium acceptors.

The fluorescence ratio related to the HTRF readout is a correction method developed by Cisbio, for which Cisbio has granted a license to Molecular Devices. Its application is strictly limited to the use of HTRF reagents and technology, excluding other TR-FRET technologies such as IMAP TR-FRET calculations of acceptor to donor ratios.

The Cisbio HTRF Detection Cartridge is a dual-emission detection cartridge and occupies two (2) slots in the detection cartridge drawer.

The Cisbio HTRF Detection Cartridges can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Cisbio HTRF Detection Cartridges in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

HTRF is a registered trademark of Cisbio Bioassays.

Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge

The Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge enables time-resolved fluorescence read modes for Europium and Samarium. It is ideal for protein interaction, GPCR, and enzyme activity applications.

The Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

Well scan is not available for dual-label assays.

You can create protocols that use the Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Fluorescence Intensity (FI) Detection Cartridges

The Fluorescence Intensity (FI) Detection Cartridges use an ultra high power LED for the excitation of various fluorescent labels, enabling fluorescence intensity read modes. Also, the standard dual-emission design enables simple, straightforward fluorescence resonance energy transfer (FRET) 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 199 and the Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge on page 200.

Each Fluorescence Intensity (FI) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The Fluorescence Intensity (FI) Detection Cartridges can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Fluorescence Intensity (FI) Detection Cartridges in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge

The Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge is designed for use with GeneBLAzer assays from Invitrogen. Also, the standard dual-emission design enables fluorescence resonance energy transfer (FRET) measurement.

The Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.

- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Glow Luminescence (LUM) Detection Cartridges

The Glow Luminescence (LUM) Detection Cartridges enable luminescence read modes.

The three Glow Luminescence (LUM) Detection Cartridges are individually optimized for 1536-well, 384-well, or 96-well microplate formats:

- Glow Luminescence (LUM) Detection Cartridge
- Glow Luminescence (LUM) Detection Cartridge (384)
- Glow Luminescence (LUM) Detection Cartridge (96)

For dual-color luminescence, see the Dual Color Luminescence (LUM) (BRET2) Detection Cartridge on page 201 and the Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge on page 202.

Each Glow Luminescence (LUM) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The Glow Luminescence (LUM) Detection Cartridges can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Glow Luminescence (LUM) Detection Cartridges in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

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

The Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge uses ultra high-powered LEDs for the excitation of a dual set of fluorescent labels. This detection cartridge has been optimized for detection of the label set used in the MultiTox-Fluor Multiplex Cytotoxicity Assay from Promega:

- AFC (amino-fluorocoumarin)
- R110 (rhodamine 110)

The Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Fluorescence Polarization (FP) Detection Cartridges

The Fluorescence Polarization (FP) Detection Cartridges enable fluorescence polarization read modes for specific labels, depending on the cartridge.

Fluorescence Polarization (FP) Detection Cartridges are available for the following labels:

- Fluorescence Polarization (FP) (Fluorescein) Detection Cartridge
- Fluorescence Polarization (FP) (Rhodamine) Detection Cartridge

Each Fluorescence Polarization (FP) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.

You can create protocols that use the Fluorescence Polarization (FP) Detection Cartridges in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Dual Color Luminescence (LUM) (BRET2) Detection Cartridge

The Dual Color Luminescence (LUM) (BRET2) Detection Cartridge enables read modes for luminescence and is designed to provide the optimal performance when used with Perkin Elmer BRET^2 reagents.

For luminescence measurements that do not require emission filters, see Glow Luminescence (LUM) Detection Cartridges on page 200.

The Dual Color Luminescence (LUM) (BRET2) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Dual Color Luminescence (LUM) (BRET2) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

BRET^2 is a trademark of PerkinElmer, Inc.

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

The Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge enables read modes for luminescence and is designed to provide the optimal performance when used with Promega Chroma-Glo™ reagents.

For luminescence measurements that do not require emission filters, see Glow Luminescence (LUM) Detection Cartridges on page 200.

The Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

The can do the following read types:

- End Point, see Endpoint Read Type on page 134.
- Kinetic, see Kinetic Read Type on page 134.
- Well Scan, see Well Scan Read Type on page 134.

You can create protocols that use the Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Chroma-Glo is a trademark of Promega Corporation.

ScanLater Western Blot (WB) Detection Cartridge

The ScanLater™ Western Blot (WB) Detection Cartridge enables time-resolved fluorescence read mode for Western Blot membranes.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that is incorporated into a SpectraMax® Paradigm® or SpectraMax® i3x Multi-Mode Detection Platform. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. For more information, contact your representative or search the knowledge base for **ScanLater** or **Western Blot** at www.moleculardevices.com/support.

The ScanLater Western Blot (WB) Detection Cartridge occupies two (2) slots in the detection cartridge drawer.

Western Blot reads use time-resolved fluorescence read mode with a membrane read type. See Membrane Read Type on page 135.

You can create protocols that use the ScanLater Western Blot (WB) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

If you use the SoftMax Pro GxP Software, you must enable the following permissions to complete a Western Blot membrane read:

- **Read empty plates/cuvettes** to start a read.
- **Save data files** to save the data file before the read.
- **Overwrite plate/cuvette data** to do a high-resolution ROI read.

See SoftMax Pro GxP Software on page 417.

Injector Cartridge

The SpectraMax® Injector Cartridge adds injector capability to the SpectraMax i3x Instrument. You can use the injectors and optics in the Injector Cartridge for luminescence reads from the top of the sample, or use the injectors in the cartridge along with the built-in Monochromator in the SpectraMax i3x Instrument for fluorescence intensity reads from the bottom of the sample. To do a read with injection, you must first install the Injector Cartridge in the detection cartridge drawer.

The Injector Cartridge is installed in the two rear-most slots (3 and 4) in the detection cartridge drawer.

You can create protocols that use the Injector Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

To define the settings for a read with injection, you must use the **Acquisition View** of the **Settings** dialog. See Acquisition View on page 210.

Transmitted Light (TL) Detection Cartridge

To do brightfield, transmitted-light imaging, you must install the SpectraMax Transmitted Light (TL) Detection Cartridge in the detection cartridge drawer. The Transmitted Light (TL) Detection Cartridge provides white LED illumination for transmitted-light imaging when used with the SpectraMax MiniMax 300 Imaging Cytometer. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

The Transmitted Light (TL) Detection Cartridge occupies one (1) slot in the detection cartridge drawer.

You can create protocols that use the Transmitted Light (TL) Detection Cartridge in the SoftMax Pro Software. See Create Protocols on page 205.

You can use the Protocol Manager in the SoftMax Pro Software to quickly find and open a predefined protocol. See Protocol Manager - Open Files on page 64.

More protocols and updated protocols can be downloaded from the protocol sharing web site (www.softmaxpro.org). See Protocols Tab on page 42.

Chapter 7: Collect Data

A general process to prepare, collect, and analyze data is as follows:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select an instrument. See Select an Instrument on page 16.

3. Choose a data acquisition protocol. You can use an existing protocol or create a new one.
   - To use an existing protocol, see Open Protocol Files on page 67.
   - To set up a new protocol, see Create Protocols on page 205.

4. Prepare the microplate or the cuvette and insert it into the instrument.

5. Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

6. Click **Read** ▶ to read the microplate or the cuvette. See Collect Data from Microplates on page 300, Collect Data from Cuvettes on page 310, or Collect Data from Membrane Holders on page 312.

7. Analyze the data. See Analyze Data on page 315.

8. Save the acquired data, see Save Data Files on page 62.

Create Protocols

Protocols contain instrument settings, template maps, data reduction, and display options. Templates are maps to the contents of a microplate or the cuvettes in a cuvette set, and how to collect and display data.

Application notes with specific application protocol suggestions can be found in the Information Center and the Knowledge Base on the Molecular Devices web site at www.moleculardevices.com.

You can manage protocols using the **Protocol Manager** on the **Protocols** tab in the ribbon. See Protocols Tab on page 42.

You can create as many different protocols as you need. You can create a new protocol from a blank file or you can modify an existing protocol and save it with a new name.

To create a protocol, follow these steps:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select the instrument to use with the protocol. See Select an Instrument on page 16.

3. Define the instrument settings to use with the protocol. See Select Instrument Settings on page 206.

4. Create a template, if applicable. See Configure Microplate Templates on page 264.

5. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.

6. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

7. Set Display options, if applicable. See Data Display Settings on page 318.

Select Instrument Settings

The **Settings** dialog enables you to configure all parameters for a microplate or cuvette read, such as the read type (Endpoint, Kinetic, Spectrum, Well Scan, Membrane), the read mode (Absorbance, Fluorescence, Luminescence, Time Resolved Fluorescence, Fluorescence Polarization, ALPHAScreen, FRET, Imaging), wavelength settings, microplate type, wells to read, PathCheck technology, optics settings, shake parameters, timing for Kinetic runs, speed read, and more.

The **Plate** sections or **Cuvette Set** sections in a file or experiment generally use the same instrument, but they can have different parameters for the instrument settings.

1. In the navigation tree, click a **Plate** section or **Cuvette Set** section to make it active in the workspace.
2. On the Home tab in the ribbon or in the toolbar above the active section, click **Settings** to display the Settings dialog.

Settings and available options are dependent on the instrument you select. See Select an Instrument on page 16.

The available options in the **Settings** dialog depend on the instrument, read mode, and read type you select.

For the SpectraMax i3, i3x, and iD3 instruments, the **Settings** dialog provides two methods to configure a microplate read.

- The **Standard View** uses a table design, with selectable categories to configure settings. See Standard View on page 207.
- The **Acquisition View** uses a workflow design, with drag-and-drop tools to configure settings. See Acquisition View on page 210.

To switch between the views, click **Standard View** or **Acquisition View** in the lower-left corner of the **Settings** dialog.
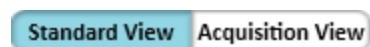
After you define the protocol settings, click **OK** to close the Settings dialog.

After you define the settings you can do the following:

- Create a template, if applicable. See Configure Microplate Templates on page 264.
- Set Data Reduction parameters, if applicable. See Data Reduction on page 325.
- Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

Standard View

The Standard View uses a table design where you select a category on the left to configure applicable settings. The Standard View is view is available for all instruments that SoftMax Pro Software supports. However, you must use the Acquisition view to define the settings for reads with injection. See Acquisition View on page 210.

Note: If you define an Imaging read or a ScanLater Western Blot read in the Standard View and then switch to the Acquisition View, your settings are lost.

For the SpectraMax Paradigm, SpectraMax i3, and i3x Instruments, select the optical configuration or detection cartridge to use. See Select Optical Configuration or Detection Cartridge on page 208.

For all instruments, select a read mode and a read type. See Select a Read Mode on page 209 and Select a Read Type on page 209.

From the **Category** list on the left, select a category to display the corresponding settings in the center section. The **Settings Information** section displays the setting you define.

The options that display in the center section depend on the instrument, read mode, and read type you select. See the following topics:

- Wavelength Settings on page 218
- Plate Type Settings on page 220
- Read Area Settings on page 225

After you define the protocol settings, click **OK** to close the Settings dialog.

Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

Select Optical Configuration or Detection Cartridge

The Settings dialog enables you to select an **Optical Configuration** or a **Cartridge**.

Some instruments use detection cartridges. Each detection cartridge contains its own independent light source, optics, and electrical components needed to do specific read modes for some applications.

- The SpectraMax i3x Instrument supports user-installable detection cartridges to expand its read capabilities.
- The SpectraMax Paradigm Multi-Mode Microplate Reader requires detection cartridges to do reads.

For the SpectraMax i3x Instrument, you can select **Monochromator** to use the built-in read modes. To use an installed SpectraMax MiniMax 300 Imaging Cytometer for imaging reads, select **MiniMax**. For transmitted light imaging, you need to install the Transmitted Light (TL) Detection Cartridge, but do not need to select it in the **Settings** dialog.

> 📝 **Note:** When you select to work offline on the Instrument Connection dialog, all detection cartridges that the SpectraMax i3x Multi-Mode Detection Platform or SpectraMax Paradigm Multi-Mode Microplate Reader supports appear for selection.
>
> When you connect the computer to an instrument, the detection cartridges that are installed in the instrument appear for selection.

For the SpectraMax i3x Multi-Mode Detection Platform, you can use the detection cartridges for top reads only.

For detection cartridge information, see Supported Detection Cartridges on page 194.

After you select the optical configuration or detection cartridge, select the read mode and read type for the protocol. See Select a Read Mode on page 209 and Select a Read Type on page 209.

Select a Read Mode

The Settings dialog enables you to select the Read Mode for the protocol. Your instrument selection determines which Read Modes are available.

For more information on read modes, see the following topics:

- Absorbance Read Mode on page 137
- Fluorescence Intensity Read Mode on page 143
- Luminescence Read Mode on page 149
- Time-Resolved Fluorescence Read Mode on page 154
- HTRF Read Mode on page 160
- FRET Read Mode on page 159
- Fluorescence Polarization Read Mode on page 164
- AlphaScreen Read Mode on page 168
- Western Blot TRF Read Mode on page 171
- Imaging Read Mode on page 174

After you select the Read Mode, select a Read Type. See Select a Read Type on page 209.

Select a Read Type

After you select a Read Mode on the Settings dialog, select a Read Type. Your instrument selection determines which Read Types are available.

For more information on read types, see the following topics:

- Endpoint Read Type on page 134
- Kinetic Read Type on page 134
- Well Scan Read Type on page 134
- Spectrum Read Type on page 134
- Flex Read Type on page 135
- Membrane Read Type on page 135

After you select the Read Mode and Read Type, select the **Category** from the list to define its settings.

After you define the protocol settings, click **OK** to close the Settings dialog.

Acquisition View

The Acquisition View is available for the SpectraMax i3, i3x, and iD3 Instruments, however, you must use the Standard View to define the settings for Imaging reads and ScanLater Western Blot reads. See Standard View on page 207.

The Acquisition View uses a workflow design that enables you to create a time line for multi-step protocol reads.

Note: If you define a read with injection and then switch to the Standard View, your settings are lost.

In the top section, select the **Plate Size**, **Plate Name**, **Read Area**, and **Read Order**. See the following topics:

- Plate Type Settings on page 220
- Read Area Settings on page 225
- Read Order on page 249

The Acquisition Plan section displays the time line of steps for the protocol. The steps occur in sequence from left to right between the **Begin** node and the **End** node. When a step has multiple settings, these setting display below the step in the time line.

1. To start from scratch, click **Clear All**. For the SpectraMax i3, i3x and iD3 instruments, you can click the **Load Predefined Acquisition Template** drop-down and select a predefined acquisition plan.

2. In the Toolbox section on the left, either double-click or drag **Acquisitions**, **Actions**, and **Loops** to the time line. The software lets you drop nodes only in an appropriate place for the step. For example, you can place the Optimization node only at the beginning of the time line.

3. When you add an Acquisition node, a prompt appears:

   - Some Acquisition nodes require you to select hardware for the optical configuration. This can be the Monochromator or a detection cartridge for an i3x instrument. See Supported Detection Cartridges on page 194.

   - All Acquisition nodes require you to select a Read Type. See Endpoint Read Type on page 134, Kinetic Read Type on page 134, Spectrum Read Type on page 134, and Well Scan Read Type on page 134.

4. For details about Actions and Loops, see Add Actions and Loops to the Time Line on page 212.

5. For details about injector steps, see Define Reads with Injection on page 213.

6. Click an editable node to display .

   - Click the pencil to open a settings dialog related to the node.
   - Click the red X to delete the node.

7. For information about the settings, see the following topics:

   - Wavelength Settings on page 218
   - Timing Settings on page 228
   - Shake Settings on page 230
   - Speed Read on page 231
   - PathCheck Technology Settings on page 226
   - Well Scan Settings on page 229
   - PMT and Optics Settings on page 227
   - Normalization of AlphaScreen Data on page 252 (for i3x)
   - Background Correction with Interlaced Readings on page 252

8. After you define the settings for a node, click the workspace outside of the settings

dialog to close the dialog.

9. After you define the protocol settings, click **OK** to close the Settings dialog.

Before you start a read, it is a good practice to save your data file. See Save Data Files on page 62.

Add Actions and Loops to the Time Line

The Acquisition View Settings dialog enables you to place Actions and Loops into a protocol read time line. The software lets you drop nodes only in appropriate places for the specific action or loop. For example, you can place the Plate Optimizer node only at the beginning of the time line.

When you double-click an Action or a Loop in the Toolbox, the node appears in the first appropriate position in the time line.

The instrument you select determines which of the following Actions and Loops are available in the Toolbox.

Table 7-1: Available Actions and Loops

Node	Description
	Shake enables you define options to shake the microplate in the chamber. See Shake Settings on page 230. To shake the microplate at the same time as an injection step, use the **SmartInject** Action.
	Plate Optimizer causes the **Pre-Read Optimization Options** dialog to open before the microplate is read. You can place this step only at the beginning of the time line. See Show Pre-Read Optimization Options on page 250.
	Delay enables you to define the number of seconds to wait between steps in the time line. You can set the delay from 0.001 seconds to 100 seconds. See Define Reads with Injection on page 213.
	Inject adds a step for injection. See Define Reads with Injection on page 213.
	SmartInject™ technology enables you to add a step for injection while the microplate is shaking. The microplate continues to shake through the following delay step, if included, and stops shaking before the well is read. See Define Reads with Injection on page 213.
	Baseline takes the number of baseline reads you define before the **Inject** or **SmartInject** step of a kinetic read with injection. You can then subtract the baseline data from the reads that follow the injection. See Define Reads with Injection on page 213.
	For Each Well runs the steps in the time line for each well individually with all the injections you define, delays, and then reads before the steps are run again for the next well. The software adds the **Next Well** node to the end of the time line.

Define Reads with Injection

The Acquisition View on the Settings dialog enables you to define the settings for a read with injection.

iD3

For a SpectraMax iD3 instrument equipped with the SpectraMax Injector System, the injectors in the instrument enable you to deliver a reagent to the wells of a microplate. You use the injectors to deliver a specific volume of reagent to initiate a reaction that occurs rapidly and results in a luminescent or fluorescent signal that must be detected quickly. The injector system is method independent which means you can run injector protocols for all read modes: Absorbance, Luminescence (all wavelength), Luminescence Monochromator, Fluorescence Intensity top, and Fluorescence Intensity bottom.

i3x

For a SpectraMax i3x Instrument, you must first install the Injector Cartridge into the cartridge drawer. You can use the Injector Cartridge for luminescence reads, or use the built-in Monochromator in the SpectraMax i3x Instrument for fluorescence intensity reads with injection.

- When you add a luminescence (LUM) step to the time line, select **INJECTOR** from the **Select Hardware** list to use the Injector Cartridge for the read. A luminescence read with injection is taken from the top of the microplate.
- When you add a fluorescence intensity (FL) step to the time line select **MONO** from the **Select Hardware** list to use the built-in Monochromator for the read. A fluorescence intensity read with injection is taken from the bottom of the microplate.

Acquisition View Settings

Reads with injection are generally done well-by-well. Double-click **For Each Well** in the Toolbox to run all the steps in the time line for each well individually. All the injections, delays, and reads you define are done on the first well before the steps are run again for the next well. The software adds the **Next Well** node to the end of the time line. Add the steps for your assay between these nodes.

To add a simple injection step, drop the **Inject** node in the time line before the **LUM Read** node or the **FL Read** node.

- To shake the microplate after the injection, add a **Shake** step between the injection step and the read step.
- To shake the microplate during the injection, use the **SmartInject** node instead of the **Inject** node, and do not add a **Shake** step. The SmartInject™ technology shakes the microplate during the injection and continues to shake through the following delay step, if a **Delay** node follows the **SmartInject** node. The microplate stops shaking before the well is read.

To define the settings for the injection step, click the **Inject** node or the **SmartInject** node and then click the pencil icon to open a settings window. Select the injector to use and enter the number of microliters (µL) to dispense. You can dispense from 1 µL to the maximum allowable volume of the well, based on the microplate type you select. Define the dispense volume based on the requirements of your assay and the net volume available in the well.

You can add a **Delay** step after the injection ends and before the read begins. You can set the delay from 0.001 seconds to 100 seconds.

iD3 Delay

- For absorbance reads there is an 800 msec delay after injection for both injectors.
- For luminescence top reads there is a 500 msec delay after injection for both injectors.
- For fluorescence top and bottom reads there is a 500 msec delay when the injector 1 injection starts and a 500 msec delay after the injector 2 ends.

i3x Delay

- For luminescence reads, there is a delay of 0.3 seconds after the injection ends and before the read begins to allow time for the detector to move into position for the read.
- For fluorescence intensity reads, there is no delay for injector number 1. The read starts at the same time as the injection unless you add a Delay step. For injector number 2, there is a delay of 0.3 seconds after the injection ends and before the read begins.

The Delay step compensates for the hardware delay. If the delay you define is longer than the hardware delay, then the actual delay time is the delay time you define. However, if the delay you define is shorter than the hardware delay, then the actual delay time is the hardware delay time. For example, using an i3x instrument, if you define a luminescence read delay of 1 second, then the actual delay time between the end of the injection and the beginning of the read is 1 second. However, if you define a luminescence read delay of 0.05 seconds, then the actual delay time between the end of the injection and the beginning of the read is 0.3 seconds due to the hardware delay.

To take baseline reads before the injection step of a kinetic read, double-click **Baseline** in the Toolbox. The **Baseline** step appears in the time line before the injection step. Click the pencil icon and enter the number of baseline reads. You can define from 1 to 999 baseline reads. Baseline reads use the settings you define in the ABS Read (iD3 only), LUM Read, or FL Read step. You can subtract the baseline data from the reads that follow the injection. You can use the Formula Editor to automate baseline subtraction. See Add Summary Formulas on page 102. You can find more information about baseline subtraction in the *Formula Reference Guide*.

The following illustration shows a time line for a luminescence kinetic read with SmartInject technology. It starts with microplate optimization and does 10 baseline reads before the injection step. It then delays 1 second after the injection ends before starting the read.
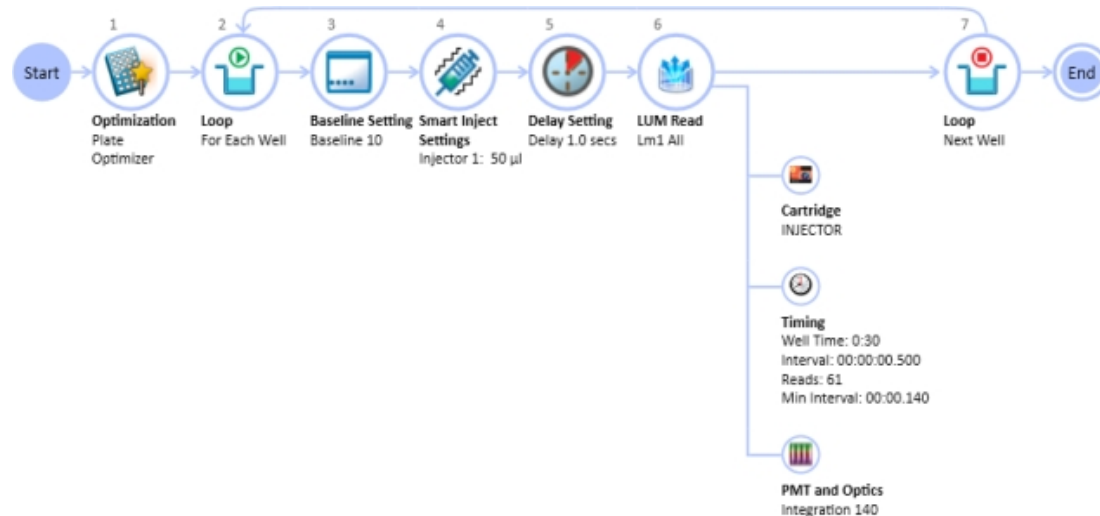
Note: With SmartInject™ technology, the microplate shakes during the injection and continues to shake through the following delay step. The microplate stops shaking before the well is read.

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.

The Settings dialog enables you to configure the parameters for a microplate or cuvette read, such as the read type (Endpoint, Kinetic, Spectrum, Well Scan, Membrane), the read mode (Absorbance, Fluorescence, Luminescence, Time Resolved Fluorescence, Fluorescence Polarization, ALPHAScreen, FRET, Imaging), wavelengths, microplate type, wells to read, PathCheck technology, optics, shake, timing for Kinetic runs, speed read, and more.

To open the Settings dialog:

1. In the navigation tree, 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 above the active section.

The options in the Settings dialog depend on the instrument, read mode, and read type you select.

For the SpectraMax i3, i3x, and iD3 Instruments, the Settings dialog provides two methods to configure a microplate read.

- The **Standard View** uses a table-like design, with selectable categories where you can configure your settings. See Standard View on page 207.
- The **Acquisition View** uses a workflow design, with drag-and-drop tools to configure your settings. See Acquisition View on page 210.

To switch between the views, click **Standard View** or **Acquisition View** in the lower-left corner of the Settings dialog.

For information about the available settings, see the following topics:

- Wavelength Settings on page 218
- Plate Type Settings on page 220
- Read Area Settings on page 225
- PathCheck Technology Settings on page 226
- TRF Settings on page 226
- PMT and Optics Settings on page 227
- Timing Settings on page 228
- Well Scan Settings on page 229
- Shake Settings on page 230
- Speed Read on page 231
- Well Area Settings on page 231
- Image Acquisition Settings on page 231
- Image Analysis Settings on page 233

Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

Wavelength Settings

Wavelength settings are dependent on the instrument, read mode, and read type you select.

The user guide for each instrument that supports this function and describes the procedures to optimize excitation and emission wavelengths for a protocol. For more information, see the instrument user guide.

Endpoint, Kinetic, Well Scan, or Flex Reads

Specify the number of wavelengths to read and enter or select a wavelength value within the available range for the instrument for each wavelength.

For filter-based instruments, select the appropriate filters.

Bandwidth

Bandwidth selection is available for fluorescence intensity (FL) and luminescence (LUM) reads when the SpectraMax i3 or SpectraMax i3x Multi-Mode Detection Platforms use the built-in Monochromator.

Select the spectral bandwidth of the wavelength for the read. The instrument uses the bandwidth you select for all the wavelengths in a multiple wavelength read.

For fluorescence intensity reads, select separate bandwidths for the excitation and emission wavelengths.

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 you select the Luminescence read mode. 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 you enable Auto Cutoff, the instrument sets the cutoffs based on the wavelengths you choose to read. When you select the Spectrum read type, 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 is the difference between the wavelengths of the excitation and emission maxima. If the Stokes shift is small, 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, permitting better selection and quantitation of the emitted light. See the instrument user guide for more information about cutoff filter settings.

Imaging Reads

Select one or more wavelengths, or channels, to use for the acquisition. Images are acquired separately for each wavelength or channel, and can be analyzed separately.

The SpectraMax MiniMax 300 Imaging Cytometer captures images from the bottom of each microplate well. You can illuminate the sample with white transmitted light from the top of the microplate when you use the Transmitted Light (TL) Detection Cartridge, or you can use fluorescent excitation from the bottom of the microplate.
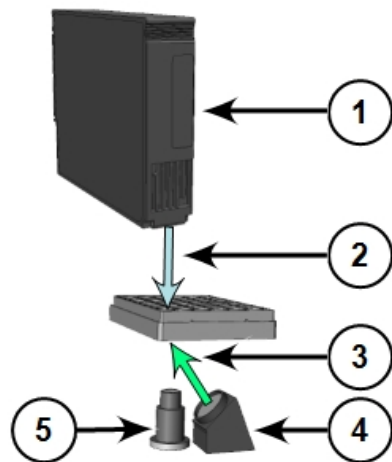
Figure 7-1: Path of Selected Light Sources

Item	Description
1	Transmitted Light (TL) Detection Cartridge
2	Path of white light from the Transmitted Light (TL) Detection Cartridge
3	Path of fluorescent excitation
4	Light source for fluorescent excitation
5	Camera lens

To illuminate the sample from the top, you need to install the Transmitted Light (TL) Detection Cartridge in the detection cartridge drawer. See Transmitted Light (TL) Detection Cartridge on page 204.

For best results with transmitted-light reads, use a microplate with no cover. You can use a clear cover, if required. For fluorescent reads, you can use a solid cover on the microplate.

Plate Type Settings

The Plate Type settings determine the display of the wells in the Plate section. Set the plate type 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 read.

If you change the Plate Type for a Plate section, the well assignments stored in the previous template is discarded. The groups created previously remain, however, so you can select new wells and apply existing groups to these wells.

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

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

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

- To edit the dimensions of a microplate, select a microplate from the list and click **Edit Plate** to open the Plate Editor dialog.

  In the Acquisition View, click ![icon] to open the Plate Editor dialog.

- To add a new microplate to the library, select a microplate from the list and click **Edit Plate** or click ![icon]. Enter a name for the microplate in the **Plate Name** field on the Plate Editor dialog.

- To include a microplate from a predefined microplate description, click **Import Plate** and use the file system browser to locate the microplate description file.

  > **Note:** The Import Plate option is available only in the Standard view for the SpectraMax Paradigm Multi-Mode Microplate Reader, the SpectraMax i3x Multi-Mode Detection Platform, and the FilterMax F3 and F5 Multi-Mode Microplate Readers.

- To delete a microplate from the microplate library, select the microplate to remove and click **Remove**.

  In the Acquisition View, click ![icon]. On the Plate Editor dialog, make sure the name of the microplate to remove is in the Plate Name field and click **Delete**.

For information about how to define custom microplate dimensions, see Plate Editor on page 221.

> **Note:** Only the custom microplate definitions created or edited in Imaging mode can be used for Imaging reads. Microplate definitions created, edited, or optimized for other read modes are not included in the list of available microplates for Imaging reads.

For a SpectraMax Paradigm Multi-Mode Microplate Reader you can define the microplate orientation.

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

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

If the microplate has a lid, select **Is Lidded** in the Standard view, or **Plate has a lid on top** in the Acquisition view.

> ⚠️ **CAUTION!** If the microplate has a lid on top, you must indicate that the microplate is lidded and verify that the microplate height with a lid is set accurately on the Plate Editor dialog before you start a read.

Plate Editor

The Plate Editor dialog enables you to modify the definition of a microplate or to add a microplate definition to the Plate Library.

If you open the Plate Editor from the Acquisition view, you can remove the custom microplate that is named in the **Plate Name** field by clicking **Delete**. The **Delete** button is available only for saved custom microplate definitions.

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 to open the Settings dialog.
3. Select the **Plate Type** category.
4. In the Plate Type Settings, click **Edit Plate**.

   In the Acquisition view, click 🖼️ to open the Plate Editor dialog.

The Plate Editor has fields and check boxes to define the microplate dimensions, in millimeters.

> 📓 **Note:** SoftMax Pro Software comes with plate definitions for the SpectraDrop 24-well Micro-Volume Microplates and SpectraDrop 64-well Micro-Volume Microplates.

Modify the Dimension of a Plate

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

1. In the Plate Type Settings, select a plate in the **Select Specific** list and click **Edit Plate**.

   In the Acquisition view, click to open the Plate Editor dialog.

2. Enter the custom dimensions in the available fields, as needed.

3. Click **Save**.

The plate you change displays an asterisk at the end of the plate name.

Import a Plate Definition

The ability to import a previously saved microplate definition is available only for the SpectraMax i3 and i3x Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers. For the SpectraMax i3x Multi-Mode Detection Platform, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers.

1. In the Plate Type Settings section, click **Import Plate**.

2. On the **Open** dialog, navigate to the microplate definition file (Microplate File (*.plt) or Multimode File (*.xml) file type) and select the plate definition file.

3. Click **Open** to add the plate definition to the list.

Create a New Plate

Defining a new plate can be easier if you select a similar plate from the list before you open the Plate editor.

1. In the Plate Type Settings section, select a plate in the **Select Specific** list and click **Edit Plate** to open the Plate Editor dialog.

   In the Acquisition view, click to open the Plate Editor dialog.

2. In the **Plate Name** field, enter the new plate name.

3. Enter the dimensions of the plate in the available fields. See Below.

4. Click **Save**.

The plate name displays in the plate list.

Table 7-2: Plate Editor Tools

Field Name	Image	Description
Left Edge to Left Well Center		Enter the distance from the left edge of the plate to the center of well A1 in millimeters.
Horizontal Center to Center		Enter the horizontal distance between well centers in millimeters.
Top Edge to Top Left Well Center		Enter the distance from the top edge of the plate to the center of well A1 in millimeters.
Vertical Center to Center		Enter the vertical distance between well centers in millimeters.
Well Diameter and Well Shape		Enter the diameter of a round well or the width of a square well in millimeters. Select the **Well Shape Square** check box if the wells have a square shape, rather than round. The setting is available only for the SpectraMax i3 and i3x Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers.
Well Bottom Type / Thickness		Select the **Clear well bottom** check box if the wells have a clear bottom, rather than opaque. For the SpectraMax i3x Multi-Mode Detection Platform used with a SpectraMax MiniMax 300 Imaging Cytometer, enter the thickness of the bottom of the well of the microplate. This setting is used to determine the initial focus distance for Imaging reads.
Well Volume / Depth		Enter the depth of the well in millimeters. This setting is available only for the SpectraMax i3 and i3x Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers. For the SpectraMax i3x Multi-Mode Detection Platform used with a SpectraMax Injector Cartridge, enter the working volume of the well for the microplate in microliters. This setting is used to determine the maximum allowed dispense volume for reads with injection.

Table 7-2: Plate Editor Tools (continued)

Field Name	Image	Description
Plate Height		Enter the height of the microplate in millimeters and also the height of the microplate with a lid in millimeters. This setting is used to determine the read height for top reads, and is available only for the SpectraMax i3 and i3x Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers.
Plate Length		Enter the length of the microplate in millimeters. This setting is available only for the SpectraMax i3 and i3x Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers.
Plate Width		Enter the width of the microplate in millimeters. This setting is available only for the SpectraMax i3 and i3x Multi-Mode Detection Platforms, the SpectraMax Paradigm Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers.

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

To determine the correct settings for these fields, measure the applicable areas of the microplate in the lab.

CAUTION! To prevent damage to the instrument, the microplate height and read height must be set accurately before you start a read for a SpectraMax i3 Multi-Mode Detection Platform.

Microplate definitions are saved in a *.plt file in a sub-folder of the ProgramData folder. A custom microplate definition is specific to the computer on which it is saved. When you open a protocol that has a custom microplate selected on a different computer, the SoftMax Pro Software enables you to save the custom microplate definition on the new computer.

Read Area Settings

Partial-microplate reading is available for all read types, although it is most useful for fast kinetic analysis or image acquisition. The time required for kinetic readings or acquiring images can be significantly reduced when you use this setting since the instrument does not have to read the entire microplate.

You can read an entire microplate or a subset of wells.

To select a contiguous, rectangular region on the microplate, use your mouse to drag the pointer and select the wells to read.
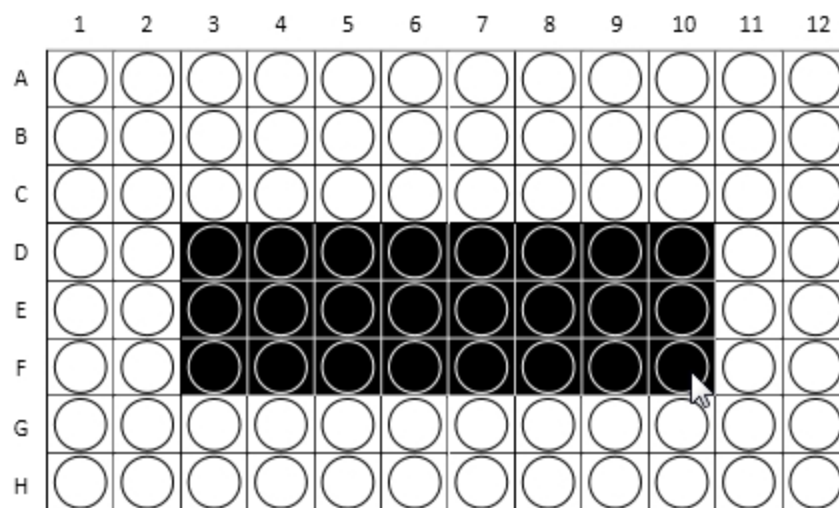
Columns do not need to start with "1" but must be contiguous.

To read all the wells in the microplate, select the **Select All** check box.

For the SpectraMax i3, iD3, and SpectraMax i3x Instruments, you can select non-contiguous wells if you use a Well read order in the Standard View, or you add a For Each Well loop in the Acquisition View. To select non-contiguous wells, press the **CTRL** key as you click each well.

If you select a partial microplate 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 display in the Template Editor. See Configure Microplate Templates on page 264.

Western Blot Membrane Read Area Settings

To scan the membrane in a SpectraMax Paradigm or SpectraMax i3x Instrument, you must place the membrane in a Molecular Devices ScanLater™ membrane holder. See Load the Membrane into the Membrane Holder on page 296.

A high-resolution scan of the entire surface can take approximately 15 minutes. To reduce the scanning time, you can define a smaller area for the scan.

Select the area to scan from the **Target Read Area** list.

- Select **Mini Membrane** to scan an area 85 mm x 65 mm.
- Select **Whole Membrane Holder** to scan an area 109 mm x 77 mm.

- Select **ROI Selection** to scan an area based on the region of interest (ROI) you define and acquire in the Plate section.

  If you do not define and acquire a region of interest in the Plate section, the ROI Selection option is not available.

The example in the Read Area Settings displays the approximate area to scan.

PathCheck Technology Settings

The Settings dialog enables you to use PathCheck Technology for absorbance endpoint reads.

Select the **PathCheck** category on the left and then select the **PathCheck** check box in the PathCheck Settings.

The temperature-independent PathCheck® Pathlength Measurement Technology normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

The Beer–Lambert law states that absorbance is proportional to the distance that light travels through the sample:

a - b = c d e + f

where *a* is absorbance, *b* is blank, *c* is concentration, *d* is the depth of sample layer, *e* is extinction (coefficient of...), and *f* is further terms, e.g., non-linearity caused from turbidity.

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 do extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis for quantifying the unique absorptivity properties of compounds in solution. Quantitative analysis can be done 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 directly proportional to concentration when there is no background interference.

See PathCheck Pathlength Measurement Technology on page 139.

TRF Settings

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

- The integration delay is the amount of time that elapses between the flash of the lamp (excitation) and the beginning of data acquisition from the well (emission).
- The integration time is the amount of time that the well is read.

See Time-Resolved Fluorescence Read Mode on page 154.

PMT and Optics Settings

The Settings dialog enables you to define sensitivity settings in the PMT and Optics category.

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

To define the exact voltage to use for the PMT Gain, select **Manual** and enter a value in the field.

The **Automatic** setting, when available, enables the instrument to adjust the PMT voltage automatically for varying concentrations of sample in the microplate.

The SpectraMax i3x Instrument uses two light sources. The Spectral Fusion™ Illumination uses the spectral range of a high power Xenon flash lamp intensified by LEDs in the visible range. Automatic LED power adjustment is used with high PMT Gain when the excitation wavelength is between 430 nm and 680 nm for high sensitivity across multiple fluorophores. In multiple-wavelength reads, the PMT Gain is set to High and cannot be changed.

For some instruments, the PMT sensitivity is always set to Automatic for Luminescence (LUM) and Time Resolved Fluorescence (TRF) read modes. For these instruments, the PMT Gain category is not available for these read modes.

For Western Blot membrane scans, you can choose the resolution for the full scan and for a secondary scan of a region of interest.

- The **Default Scan Resolution** applies to the entire read area of the membrane you define in the Read Area category settings.
- The **ROI Scan Resolution** applies to the region of interest (ROI) you define in the Plate section.

A High resolution scan provides the best results, while a Low resolution scan provides the fastest read.

The general workflow to acquire Western Blot data is to scan the defined read area of the membrane with a low resolution and then define a region of interest (ROI) to scan at a high resolution.

For some instruments in certain read modes, you can enter the desired **Integration Time** or number of **Flashes per read**.

For the SpectraMax Paradigm Multi-Mode Microplate Reader, and for some detection cartridges with the SpectraMax i3x Multi-Mode Detection Platform, you can select **On the Fly Detection** options.

On-the-Fly Detection yields faster read times while the microplate moves continuously as each well is measured. When you disable On-the-Fly Detection (**Off-stop and go**), the microplate 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.

- Select **Performance** for 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.
- Select **Speed** for the fastest possible read time per microplate. 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 Multi-Mode Microplate Reader and the FilterMax F5 Multi-Mode Microplate Reader, and some detection cartridges with the SpectraMax i3x Instrument, you can set the Integration Time. Enter a value in the **Integration Time** field in milliseconds, to specify the measurement time per well to validate.

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 i3x Instrument, the SpectraMax Paradigm Multi-Mode Microplate Reader, 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. Enter a value for the read height in the **Read Height** field.

The Read Height Optimization feature enables the software to find the optimized read height before the read. On the More Settings tab, on the Pre-Read Optimization dialog, select the **Show Pre-Read Optimization Options** check box.

Read Height is available for the SpectraMax i3x Multi-Mode Detection Platform and the FilterMax F5 Multi-Mode Microplate Reader for top-read only.

> ⚠️ **CAUTION!** To prevent damage to the instrument, the microplate height and read height must be set accurately before you start a read for a SpectraMax i3 Multi-Mode Detection Platform.

Timing Settings

For Kinetic and Flex reads you can define the total run time and the time interval between reads.

Enter values for the **Total Run Time** and the **Interval** between readings in hh:mm:ss format.

For the SpectraMax i3x, SpectraMax iD3 Multi-Mode Microplate Reader, or i3 Multi-Mode Detection Platform and the SpectraMax Paradigm Multi-Mode Microplate Reader, select a Well read order in the Standard View or add a For Each Well loop in the Acquisition View, for a kinetic read to define timing parameters in milliseconds.

The minimum permitted interval values display below the Interval field.

The software calculates the **Number of Reads** based on the values you enter in the **Total Run Time** field and the **Interval** field.

To read only one time point, click **Single Point Read** to set the Total Run Time and the Interval to the same value. This is useful when you run a multitask kinetic read with alternating read modes within a cycle. See .

The minimum permitted read interval is determined by the instrument and depends on many factors including the number of wavelengths, the number of wells to read, and the distance the instrument's filter wheel must move. Maximum read intervals 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 Microplate Reader has a maximum read interval of 18 hours and 12 minutes. If you enter an invalid read interval, a message appears to indicate the minimum or maximum allowable value for the read interval.

✳ **Tip:** In some cases, choosing wavelengths in ascending order can get the shortest possible 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 permit 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.

Well Scan reads let readings be taken at more than one location within a well. Well Scan settings let you specify how a Well Scan is done.

You can specify the scanning pattern and the scanning density.

The number of points that are available depend on the well size of the microplate you select.

Up to four scanning patterns are available, depending on the instrument you select:

- 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 220.

The following are determined by density:

- The number of points that are read in the line patterns.
- The maximum number of horizontal and vertical points that are included in the cross or fill pattern.

Shake Settings

Shake lets you set options to shake the microplate in the chamber. Shake options are dependent on the instrument and the read type you select.

Before First Read

The Before First Read setting is available for all read types. Whether you read at a single wavelength or at multiple wavelengths, when you select **Before First Read** the instrument shakes the plate for the set quantity of time before the first wavelength reading only.

Between Reads

The Between Reads option is available when you select the Kinetic read type.

For readings at a single wavelength, when you enable **Between Reads** the instrument shakes the plate for the set quantity of time before each reading at that wavelength.

For readings at multiple wavelengths, when you enable **Between Reads** the instrument shakes the plate for the set quantity of time before each reading at the first wavelength only.

> **Note:** The Before First Read and Between Reads options are available in the Standard View. To enable shaking in the Acquisition View, place the Shake node in the time line where you want the shake to occur.

For the SpectraMax i3x Multi-Mode Detection Platform and the SpectraMax Paradigm Multi-Mode Microplate Reader, you can specify the method for the microplate shake operation. The microplate shake options include the following:

- **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.

For the SpectraMax iD3 Multi-Mode Microplate Reader, you can specify the method for the microplate shaking operation. The microplate shaking options include the following:

- **Intensity:** Low, Medium, or High. The actual shake speed is based on the microplate format.
- **Direction:** Orbital or Double Orbital pattern.
- **Duration:** Length of time in seconds.

Speed Read

Enable Speed Read for an Absorbance read to read the plate faster by decreasing the number of lamp flashes.

The Speed Read option is useful in Spectrum scans and can greatly reduce the time needed to run the protocol.

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

Well Area Settings

The Well Area Settings for Imaging reads let images be acquired at more than one location, or site, within a well to form a single seamless tiled image.

Select the number of sites that you want to acquire.

Well Area Settings
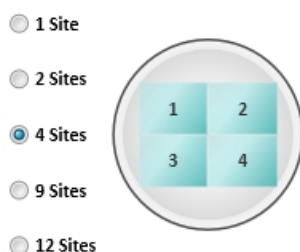
If you select to acquire images of more than one site in the well, the images are "tiled" together into a single image for analysis.

Image acquisition time increases with each site you select. For faster image acquisition, select fewer sites.

Image Acquisition Settings

In the Image Acquisition Settings, you can define the exposure time and focus adjustment for two sample wells. The instrument uses these settings for every well in the entire microplate during acquisition. The exposure and focus adjustment can be set separately for each wavelength or channel.

Acquire Images for Maximum Intensity and Minimum Intensity

Only one site is acquired in each well. If you select to acquire more than one site, the first site is acquired.

To acquire the image for maximum intensity:

1. Click ⋯ to the right of the **Well** field above the maximum intensity image area.

2. Click the microplate well that you expect to have the brightest image.

   The well filled with red is used to determine maximum intensity, and the well filled with blue is used to determine minimum intensity.

3. Click **Acquire Max Image** and wait for the image to be acquired.

To acquire the image for minimum intensity:

1. Click ⋯ to the right of the **Well** field above the minimum intensity image area.

2. Click the microplate well that you expect to have the darkest image.

   The well filled with red is used to determine maximum intensity, and the well filled with blue is used to determine minimum intensity.

3. Click **Acquire Min Image** and wait for the image to be acquired.

For some multi-channel experiments, the brightest and darkest images for one channel can be different than the brightest and darkest images for another channel. In this case, try to locate the wells that give you the best results between the different channels.

To view the image for a specific wavelength, click the tab above the image for that wavelength. To view an image with all the wavelengths, click **Composite**.

To zoom the acquired image in and out, right-click the image and select **Zoom In** or **Zoom Out**. You can also hold the **CTRL** key down and press **+** or **-**, or use the mouse wheel to zoom in or out. Drag the scroll bars to move the zoomed image in the image area, or click the image and drag it into position. To view a larger version of the image area, double-click the image to open the Zoom Well dialog. See .

Set Exposure Time and Focus Adjustment

The exposure and focus adjustment can be set separately for each wavelength, or channel. To view the image for a specific wavelength, click the tab above the image for that wavelength. To view an image with all the wavelengths, click **Composite**. Set the exposure and focus adjustment in the row designated for the specific channel.

To have the SoftMax Pro Software determine the best exposure, click **Auto Expose**. The new value in the **Exposure** field is based on the perceived best intensity for the Max Intensity or Min Intensity image, whichever is brightest. To view what effect the new exposure value has on the images, acquire the maximum intensity and minimum intensity images again.

To continue to adjust the intensity of the images, enter a value in the **Exposure** field for the number of milliseconds to hold the camera shutter open. Increase the exposure time to increase the intensity of the image or decrease the exposure time to decrease the intensity of the image. After each adjustment, acquire the images again to view what effect the change has on their intensity.

The system optics use a laser automatic focus system and software algorithms to find the initial focus distance for the cells in the microplate wells. To adjust the focus, enter a value in the **Focus Adjustment** field for the number of microns to move the objective lens closer to or farther away from the sample.

- Increase the **Focus Adjustment** value to move the objective lens closer to the sample.
- Decrease the **Focus Adjustment** value to move the objective lens farther away from the sample.

After each adjustment, acquire the images again to view what effect the change has on their focus.

For transmitted light images, make sure that enough contrast exists between the objects and the background. The sharper the focus is, the less contrast exists. For best results when you use a preset drawing analysis, make sure that the objects are brighter than the background.

The instrument uses these settings for every well in the entire microplate during acquisition.

Image Analysis Settings

In the **Image Analysis Settings** category, select an analysis type to use.

To acquire images without analysis, click **No Analysis**.

Discrete Object Analysis

Discrete Object Analysis uses proprietary algorithms to analyze separate objects, or cells, based on multiple parameters including the signal intensity over the background and the size of the objects.

When you click **Discrete Object Analysis**, the **Wavelength for Finding Objects** list appears below the list of analysis types. Select the wavelength from this list to use to identify the objects in the well images. You can use transmitted light or a fluorescent wavelength to find nuclei or easily separable objects in the image. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

Click the **Image Analysis Settings** category on the left to display the following subcategories:

- Click **Find Objects** to enter size and intensity values or to use proprietary algorithms with the drawing tools to graphically define the objects and the background areas for the analysis. See Find Discrete Objects or Confluent Areas on page 234.
- Click **Classify Objects** to define separate measurements for different types of found objects, or cells. See Classify Discrete Objects on page 237.
- Click **Select Measurements** to view the results of the analysis for the wells you select. See View and Select Measurement Statistics on page 239.

Field Analysis

Field Analysis uses proprietary algorithms to analyze confluent areas based on multiple parameters including the signal intensity over the background and the size of the areas.

When you click **Field Analysis**, The **Wavelength for Finding Confluent Areas** list appears below the list of analysis types. Select the wavelength from this list to use to identify the confluent areas in the well images. The StainFree™ Cell Detection Algorithm eliminates cell staining for cell counting and confluency measurements using proprietary transmitted light analysis technology.

The **Image Analysis Settings** category on the left expands to show the subcategories that follow:

- Click **Find Confluent Areas** to use proprietary algorithms with the provided drawing tools to graphically define the confluent areas and the background areas for the analysis. See Find Discrete Objects or Confluent Areas on page 234.
- Click **Select Measurements** to view the results of the analysis for the selected wells. See View and Select Measurement Statistics on page 239.

The SoftMax Pro Software proprietary algorithms with the provided drawing tools in the **Find Objects** or **Find Confluent Areas** settings help the software learn the patterns of the objects that you want to find. Depending on the complexity of the object patterns, this teaching process can take several iterations before you get the desired results. See Resolve Issues with Object Drawing and Finding on page 241.

Find Discrete Objects or Confluent Areas

The Find Objects or Find Confluent Areas settings display the minimum and maximum images from the Image Acquisition Settings.

To make it easier to select the objects or areas in the images, view this page in full screen.

- Click ⬚ to view the page in full screen.
- Click ⬚ to restore the page to its original size.

In full-screen view, click the tabs at the top of the page to switch between the different pages of the analysis settings.

To zoom the acquired image in and out, right-click the image and select **Zoom In** or **Zoom Out**. You can also hold the **CTRL** key down and press **+** or **-**, or use the mouse wheel to zoom in or out. Drag the scroll bars to move the zoomed image in the image area, or click the image and drag it into position.

Click the buttons above the image to switch the view of the image between wavelengths, and to show or hide found objects (or confluent areas) and drawings.

- When a buttons is filled in, the related wavelength, objects (or confluent areas), and drawings display in the image.
- When a button is outlined, the related wavelength, objects (or confluent areas), and drawings do not display in the image.

When you click a button above one of the images, it affects the display of both images.

Use the controls below the image to separately adjust the intensity of each wavelength.

From the list on the left, select the wavelength that you want to adjust.

Drag the sliders to adjust the intensity range of the image.

- The bar on the left controls the low level of intensity.
- The bar on the right controls the high level of intensity.

When you drag a bar the intensity level displays above the bar.

To move the sliders to the outside edges, click the double-arrow button on the left.

From the list on the right, select the color for the view.

For fluorescent acquisition and discrete object analysis, select a **Finding Method**:

- Select **Set Size and Intensity** to define the intensity threshold above the background intensity to be applied separately to each object in the image that meets the defined size range. This type of analysis helps to detect objects in areas of the image where the intensity is uneven.
- Select **Draw on Images** to use the drawing tools to help the software learn the patterns of the objects that you want to find.

The **Set Size and Intensity** settings are available for fluorescent acquisition and discrete object analysis. Click and then click four or more representative objects on the images that you want to detect as cells. In the **Min Width** and **Max Width** fields, enter values to define the size range in microns of the objects to be found. In the **Intensity Above Background** field, enter a value to indicate the intensity threshold above the background intensity at which to detect the dimmest object. For example, if the background intensity is 1000 and the intensity of the dimmest object is 1200, enter 200. After you enter new settings, click **Apply**.

The **Grow** setting enables you to define the number of pixels to increase the diameter of the found objects in one wavelength, so that they can be measured at the new size in another wavelength of the acquisition. For example, if you use a stain for nuclei, you can find the object in the first wavelength and then increase the diameter to find the protein of interest in another fluorescence wavelength. The innovative "grow without touching" algorithm increases the size of the measured areas without letting the areas touch or overlap their edges.

Draw on Images is available for every type of image acquisition and analysis. Use the drawing tools to help the software learn the patterns of the objects or confluent areas you want to find. Depending on the complexity of the object or area patterns, this teaching process can take some time before you get the desired results.

To use a preset drawing analysis, select the preset from the **Settings** list. Each preset drawing analysis is designed to work with specific cell types. Select the preset analysis that works best with your images. Click **Apply** to find objects or confluent areas using the preset.

After you acquire data, select **Stored Setting** from the **Settings** list to use the previously defined analysis settings.

To start from scratch, select **Create New Setting** from the **Settings** list.

To select the objects for fluorescent acquisition and discrete object analysis, click and then click a line width. Click four or more representative objects on the images. This method is not available for transmitted light acquisition or for confluent area analysis.

To define the objects or confluent areas, click the object or area pen and then click a line width.

- For discrete object analysis, draw inside the object up to the edge, but do not include the edge. When the object is filled in with yellow, the drawing is outlined in green. For best results, fill in four or more representative objects.
- For field analysis, draw across confluent areas, avoiding background. For best results, draw across four or more representative areas.
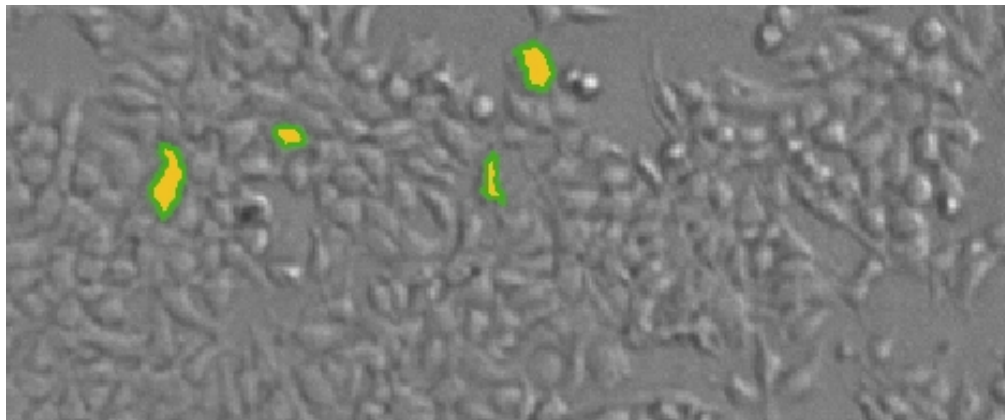
To define the background, click the background pen and then click a line width. Draw on the image to define artifacts and background areas in blue. For best results, define four or more representative background areas.
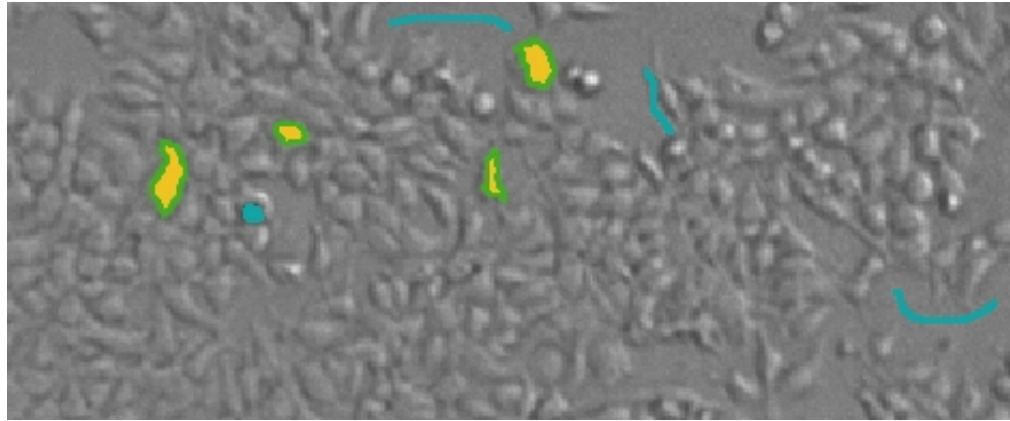
If you draw an object or area incorrectly, click **Undo** to remove it. If you undo something that you want to keep, click **Redo** to restore it. To clear the drawings from the image, click **Start Over**.

To erase parts of drawings, click the erase and then click a line width. Drag the eraser across drawings to remove them.

After you define the objects and areas, click **Apply** to find the objects or confluent areas in the image. Continue to draw and to apply until you get satisfactory results. See Resolve Issues with Object Drawing and Finding on page 241.
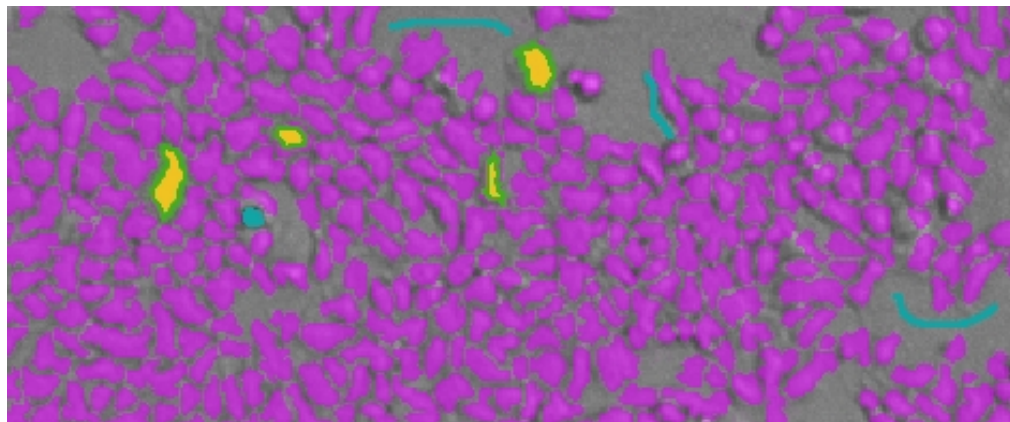
After you are satisfied with the found objects or confluent areas, you can classify objects or view the measurement statistics. See Classify Discrete Objects on page 237 or View and Select Measurement Statistics on page 239.

Classify Discrete Objects

The Classify Objects settings display the minimum and maximum images from the Image Acquisition settings display along with the found objects from the Find Objects settings. The Classify Objects settings are not available for field analysis of confluent areas.

Note: Before you can classify objects, you must first find objects in the Find Objects settings. See Find Discrete Objects or Confluent Areas on page 234.

To make it easier to select the objects in the images, view this page in full screen.

- Click ⬚ to view the page in full screen.
- Click ⬚ to restore the page to its original size.

In full-screen view, click the tabs at the top of the page to switch between the different pages of the analysis settings.

To zoom the acquired image in and out, right-click the image and select **Zoom In** or **Zoom Out**. You can also hold the **CTRL** key down and press **+** or **-**, or use the mouse wheel to zoom in or out. Drag the scroll bars to move the zoomed image in the image area, or click the image and drag it into position.

Click the buttons above the image to switch the view of the image between wavelengths, and to show or hide classified objects or selections.
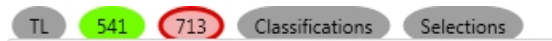
- When buttons are filled in, the related wavelengths, classifications, or selections are displayed in the image.
- When a button is outlined, the related wavelengths, classifications, or selections are not displayed in the image.

When you click a button above one of the images, it affects the display of both images.

Use the controls below the image to separately adjust the intensity of each wavelength.

From the list on the left, select the wavelength that you want to adjust.

Drag the sliders to adjust the intensity range of the image.

- The bar on the left controls the low level of intensity.
- The bar on the right controls the high level of intensity.

When you drag a bar the intensity level displays above the bar.

To move the sliders to the outside edges, click the double-arrow button on the left.

From the list on the right, select the color for the view.

Use the selection tools at the bottom of the page to classify the found objects in the images into two separate classifications.
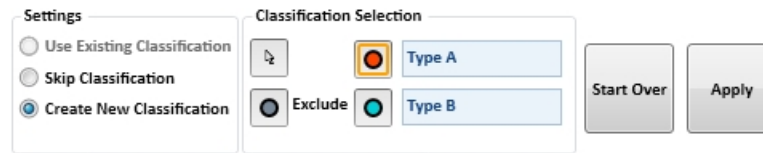
Re-Analysis mode enables you to select and apply **Use Existing Classification** to use the previously defined classification settings.

If you do not want to use classification in your analysis, click **Skip Classification**.

You can replace the Type A and Type B titles with your own titles for the classifications. Titles must use only letters, numbers, and spaces, and can be up to 16 characters long, including spaces. They must start and end with a letter. The titles cannot be the same as each other. You cannot use "Exclude" as a title, since this is a reserved word.

To classify objects from scratch, click **Create New Classification**.

- To select objects for the first classification, click the red button and then click the objects in the images.
- To select objects for the second classification, click the blue button and then click the objects in the images.
- To exclude objects from being classified, click the gray button and then click the objects in the images. If you exclude objects, you must also use at least one of the classifications types to include objects.

To deselect a selected object click it again. To remove all the selections from the images, click **Start Over**.

After you select several classified objects, click **Apply** to find and classify the objects in the image. Continue to select and to apply until you get satisfactory results.

After you are satisfied with the classified objects, you can view the measurement statistics. View and Select Measurement Statistics on page 239.

View and Select Measurement Statistics

The Measurement Selections settings table displays the analysis results for the minimum intensity and maximum intensity images.

To make it easier to view the measurements table, view this page in full screen.

- Click ⊠ to view the page in full screen.
- Click ⊠ to restore the page to its original size.

In full-screen view, click the tabs at the top of the page to switch between the different pages of the analysis settings.

The results display separately for each measured wavelength and classification, if applicable. Each measurement row displays two bars and numerical data that represents the **Max Intensity** or **Min Intensity** images.

The possible included measurements are as follows:

- **Object Count** gives the total number of objects detected in the image. This measurement is not used for field analysis of confluent areas.
- **Field Count** gives the total number of confluent areas detected in the image. This measurement is not used for discrete object analysis.
- **Object Percentage** gives the percentage the objects detected in the image by classification. This measurement is not used for field analysis of confluent areas.
- **Covered Area** gives the combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area** gives the average area of the objects detected in the image expressed in μm^2. This measurement is not used for field analysis of confluent areas.
- **Field Area** gives the average area of the confluent areas detected in the image expressed in μm^2. This measurement is not used for discrete object analysis.
- **Object Roundness** gives the average roundness of each object detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for field analysis of confluent areas.
- **Field Roundness** gives the average roundness of each confluent area detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for discrete object analysis.
- **Object Average Intensity** gives the average fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).
- **Field Average Intensity** gives the average fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).
- **Object Intensity** gives the average total fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).
- **Field Intensity** gives the average total fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).
- **Total Intensity** gives the combined total fluorescent signal intensity of the objects or confluent areas detected in the image expressed in million intensity counts. This measurement is not used for transmitted light (TL).

To select the measurements that you want to include in the analysis, click **Select Measurements** in the lower-right area of the page. This places a **Remove** button next to each measurement in the table.

- To exclude a measurement, click **Remove**.
- To include a measurement, click **Add**.
- To exclude all measurements, click **Remove All**.
- To include all measurements, click **Add All**.

After you select the measurements to include and exclude, click **Done**.

The selected measurements are added to the data reduction list that you use to view data in the **Plate** section or to add to a **Group** section for further analysis. See Plate Sections on page 105 or Group Sections on page 123.

To view the measurements in an interactive image, data table, and chart, click **Show Details**. See View Imaging Data in Wells on page 322.

If no measurements display and you have not made changes to the analysis settings since the last measurement, click **Measure** to analyze the selected wells. This is useful if you change acquisition wells and want to analyze the new wells.

Resolve Issues with Object Drawing and Finding

The SoftMax Pro Software proprietary algorithms provide drawing tools in the Find Objects or Find Confluent Areas settings to help the software learn the patterns of the objects that you want to find. Depending on the complexity of the object patterns, this teaching process can take several iterations before you get the desired results.

The SoftMax Pro Software uses a complex algorithm to learn the properties of the cells or confluent areas in the image. The algorithm examines each pixel and how it relates to its surrounding area. Cells change shape and size under different conditions such as confluency. Artifacts can look like cells or confuse the algorithm. Cells that are touching are more difficult to find than sparse cells in an image. Complex images can require more drawing examples to create a good training set.

Some common issues with finding cells and confluent areas are as follows:

- Under splitting finds multiple cells or confluent areas that can also include background. See Resolve Under-Splitting Issues on page 242.
- Over splitting finds portions of cells or confluent areas and can include several found objects within a single cell or confluent area. See Resolve Over-Splitting Issues on page 243.
- Artifacts can be found and counted as cells or confluent areas. See Resolve Artifact Issues on page 243.
- Ghosting finds background areas next to cells or confluent areas . See Resolve Ghosting Issues on page 244.

Molecular Devices recommends that you start with a preset drawing analysis. Select the preset from the **Settings** list and then click **Apply** to find objects or confluent areas using the preset. Each preset drawing analysis is designed to work with specific cell types. Select the preset analysis that works best with your images.

For transmitted light images, make sure that enough contrast exists between the objects and the background. The sharper the focus, the less contrast exists. To increase the contrast between the objects and the background, move the lens closer to or farther away from the microplate.

Adjust the focus in the **Image Acquisition Settings**. Increase the **Focus Adjustment** value to move the objective lens closer to the sample. Decrease the **Focus Adjustment** value to move the objective lens farther away from the sample. In general, moving the lens closer makes the objects darker than the background, while moving the lens farther away makes the objects brighter than the background.

> **Note:** For best results when you use a preset drawing analysis, make sure that the objects are brighter than the background.

To create an iterative training set for complex images can require the following steps:

1. Carefully draw four or more cells or confluent areas that best represent your target population. Draw inside the cell or confluent area up to the edge, but do not include the edge.
2. Define the artifacts and background areas in the image.
3. Apply the drawing and then try to fix the problems.
4. Continue to adjust the drawings. After you fix some problems, the results can actually look worse as the algorithm emphasizes the fixes. Keep making adjustments to create a complete training set.
5. After the result looks reasonable, scroll and look around the image for other issues.
6. Stop when you are satisfied or you get diminishing returns. As the training data set grows, more drawings will have less impact. At some point changes will be of little benefit.

If, at the end of the process, it is still not right, start again and repeat all the steps. Sometimes there is something that had an unexpected influence on the algorithm, and starting over can produce a better result.

If, after you try multiple iterations, ask someone else with experience to try. This person can possibly notice specific issues, or will draw larger or smaller.

Resolve Under-Splitting Issues

Under splitting finds multiple cells or confluent areas that can also include background.
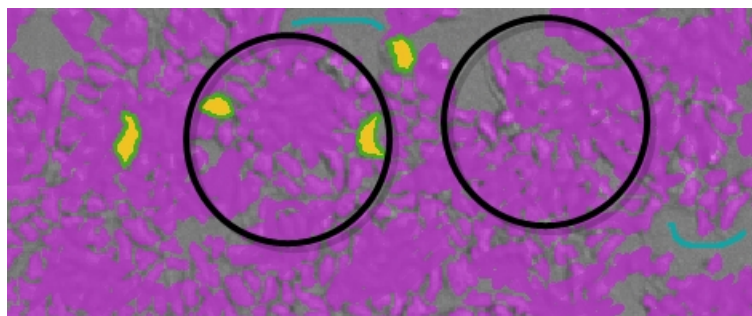
Under splitting can be caused by drawing outside of the edge of the cells or confluent areas. The lines between the cells or confluent areas are interpreted as being part of the cell or confluent-area structure.

To resolve under-splitting issues, draw inside the cell or confluent area up to the edge, but do not include the edge. You can also use the background pen to draw on the lines between the cells or confluent areas.
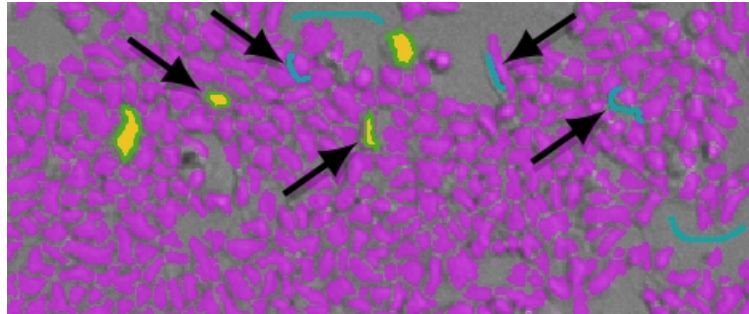
Resolve Over-Splitting Issues

Over splitting finds portions of cells or confluent areas and can include several found objects within a single cell or confluent area.
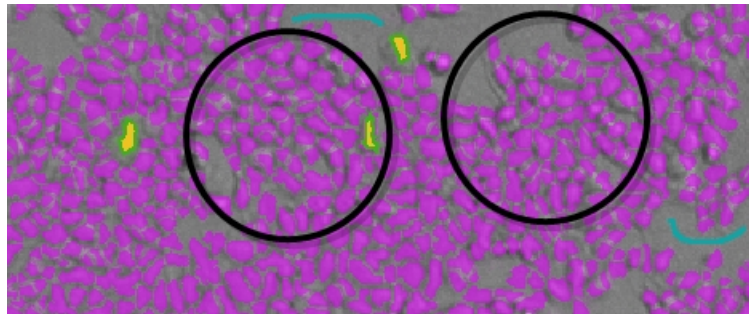
Over splitting can be caused by drawing too small within the cells or confluent areas. Sometimes over splitting can happen after using the background pen to remove an artifact.

To resolve over-splitting issues, draw inside the cell or confluent area up to the edge, but do not include the edge.
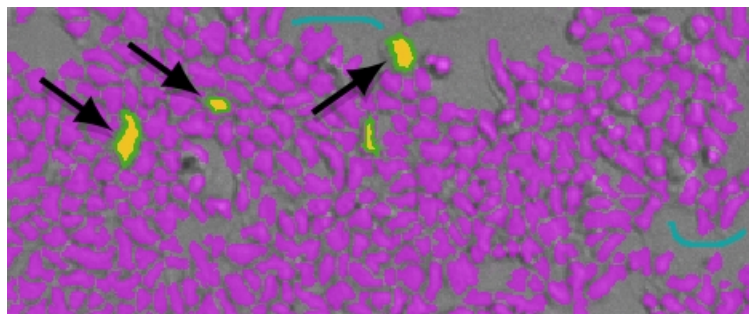
If over splitting is the result of artifact removal, try erasing the blue from the artifact defined by the background pen.

Resolve Artifact Issues

Artifacts can be found and counted as cells or confluent areas.
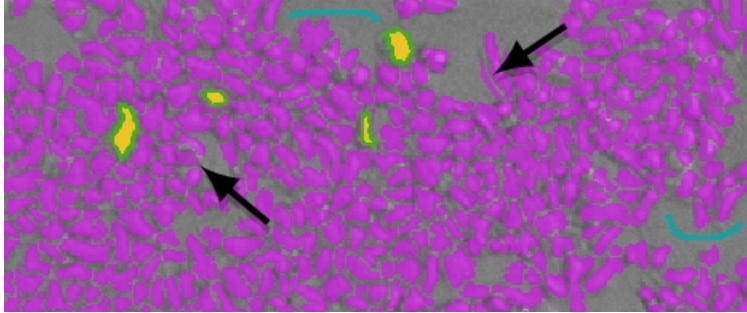
Artifact issues can be caused by debris or other artifacts in the image where the edges and surrounding pixels confuse the software.

To resolve artifact issues, use the background pen to define artifacts as background and not cells or confluent areas.
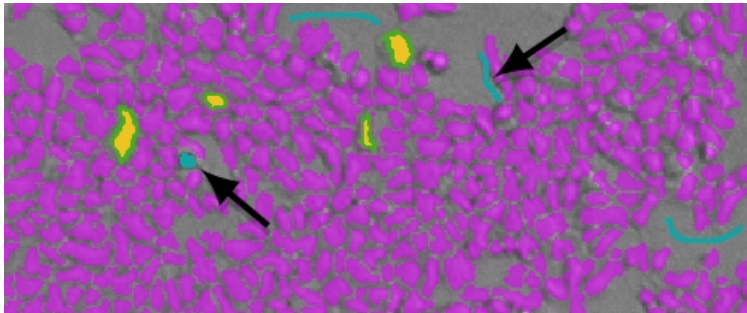
Resolve Ghosting Issues

Ghosting finds background areas next to cells or confluent areas .
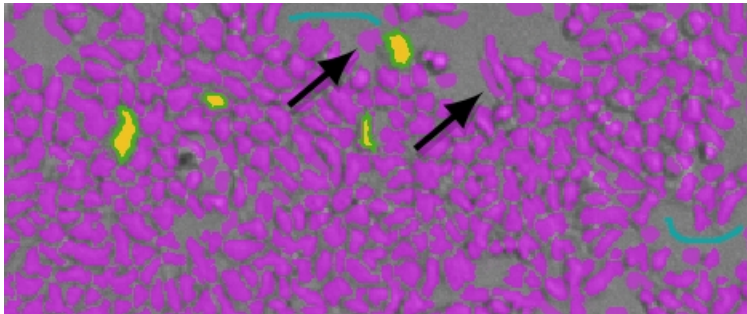
Ghosting can be caused by the edges of cells or confluent areas and the surrounding pixels that confuse the software.

To resolve ghosting issues, use the background pen to draw over the area of the ghost. Draw this area right to the edge of the cell or confluent area. If necessary, use the object pen to draw on the cell or confluent area so that the drawing goes out to the edge of the cell or confluent area.
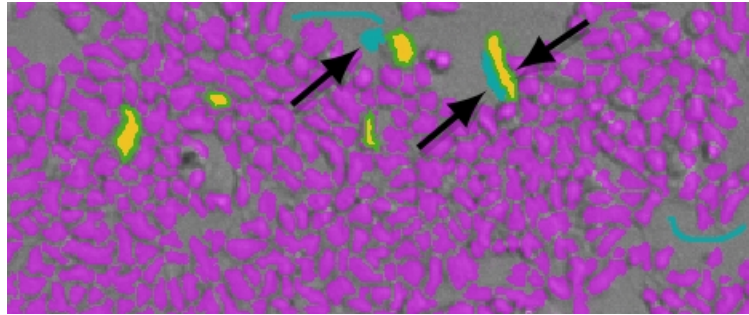
Region of Interest Settings

You can define a region of the images in all the wells to analyze, if needed for your application. Only the selected region of interest (ROI) is used for the analysis. This is useful if cells tend to congregate in the same region of the wells.

To select the well to use to define the region of interest, click [...] to the right of the **Well** field above the image area. The well filled with red is used to determine the region of interest. Select the well to use and click **Acquire All Sites**.

To view the image for a specific wavelength, click the tab above the image for that wavelength. To view an image with all the wavelengths, click **Composite**.

To zoom the acquired image in and out, right-click the image and select **Zoom In** or **Zoom Out**. You can also hold the **CTRL** key down and press **+** or **-**, or use the mouse wheel to zoom in or out. Drag the scroll bars to move the zoomed image in the image area, or click the image and drag it into position.

To define a region of interest:

1. Select the **Enable Region Selection** check box.

   A rectangle displays on the image.

2. Click **Resize** and then drag the corners of the rectangle redefine the size of the region.

3. To start over, click **Draw** and then drag a new rectangle on the image to define the region.

To use the entire image for the analysis, clear the **Enable Region Selection** check box.

Settings for Reads with Injection

To define the settings for a read with injection, you must use the Acquisition View on the Settings dialog.

iD3

For a SpectraMax iD3 instrument equipped with the SpectraMax Injector System, the injectors in the instrument enable you to deliver a reagent to the wells of a microplate. You use the injectors to deliver a specific volume of reagent to initiate a reaction that occurs rapidly and results in a luminescent or fluorescent signal that must be detected quickly. The injector system is method independent which means you can run injector protocols for all read modes: Absorbance, Luminescence (all wavelength), Luminescence Monochromator, Fluorescence Intensity top, and Fluorescence Intensity bottom.

i3x

For a SpectraMax i3x Instrument, you must first install the Injector Cartridge into the cartridge drawer. You can use the Injector Cartridge for luminescence reads, or use the built-in Monochromator in the SpectraMax i3x Instrument for fluorescence intensity reads with injection.

* When you add a luminescence (LUM) step to the time line, select **INJECTOR** from the Select Hardware list to use the Injector Cartridge for the read. A luminescence read with injection is taken from the top of the microplate.
* When you add a fluorescence intensity (FL) step to the time line select **MONO** from the Select Hardware list to use the built-in Monochromator for the read. A fluorescence intensity read with injection is taken from the bottom of the microplate.

Acquisition View Settings

The following options are specific to reads with injections.

For Each Well and Next Well

Reads with injection are generally done well-by-well. Double-click **For Each Well** in the Toolbox to run all the steps in the time line for each well individually. All the injections, delays, and reads you define are done on the first well before the steps are run again for the next well. The software adds the **Next Well** node to the end of the time line. Add the steps for your assay between these nodes.

The following illustration shows a time line for a luminescence kinetic read with SmartInject technology. It starts with microplate optimization. All the acquisition steps are between the **For Each Well** and **Next Well** nodes.
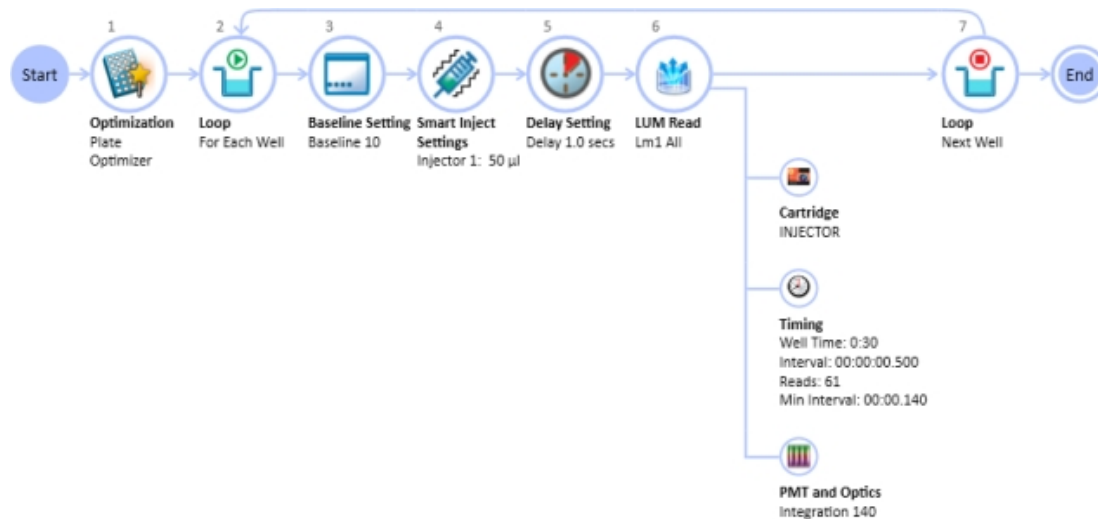
Inject

To add a simple injection step, drag the **Inject** node to the time line and drop the node before the ABS Read (iD3 only), LUM Read, or FL Read step.

- To shake the microplate after the injection, add a **Shake** step between the injection step and the read step. See Shake Settings on page 230.
- To shake the microplate during the injection, use the **SmartInject**™ node instead of the Inject node, and do not add a Shake step. The microplate shakes during the injection, continues to shake through the following delay step, if a **Delay** node follows the **SmartInject** node, and then stops shaking before the well is read. See SmartInject Technology on page 247.

To define the settings for the injection step, click the **Inject** node and then click the pencil icon to open a settings dialog. Select the injector to use and enter the number of microliters (µL) to dispense. You can dispense from 1 µL to the maximum allowable volume of the well, based on the microplate type you select. Define the dispense volume based on the requirements of your assay and the net volume available in the well.

You can add a **Delay** step after the injection ends and before the read begins. You can set the delay from 0.001 seconds to 100 seconds. See Delay on page 248.

SmartInject Technology

To shake the microplate during the injection, drag the **SmartInject** node to the time line and drop the node before the ABS Read (iD3 only), LUM Read, or FL Read step.

The SmartInject™ technology shakes the microplate during the injection and continues to shake through the following delay step, if a **Delay** node follows the **SmartInject** node. The microplate stops shaking before the well is read.

To define the settings for the injection step, click the **SmartInject** node and then click the pencil icon to open a settings dialog. Select the injector to use and enter the number of microliters (µL) to dispense. You can dispense from 1 µL to the maximum allowable volume of the well, based on the microplate type you select. Define the dispense volume based on the requirements of your assay and the net volume available in the well.

You can add a **Delay** step after the injection ends and before the read begins. You can set the delay from 0.001 seconds to 100 seconds. See .

Delay

You can add a **Delay** step after the injection ends and before the read begins. You can set the delay from 0.001 seconds to 100 seconds.

iD3 Delay

- For absorbance reads there is an 800 msec delay after injection for both injectors.
- For luminescence top reads there is a 500 msec delay after injection for both injectors.
- For fluorescence top and bottom reads there is a 500 msec delay when the injector 1 injection starts and a 500 msec delay after the injector 2 ends.

i3x Delay

- For luminescence reads, there is a delay of 0.3 seconds after the injection ends and before the read begins to allow time for the detector to move into position for the read.
- For fluorescence intensity reads, there is no delay for injector number 1. The read starts at the same time as the injection unless you add a Delay step. For injector number 2, there is a delay of 0.3 seconds after the injection ends and before the read begins.

The Delay step compensates for the hardware delay. If the delay you define is longer than the hardware delay, then the actual delay time is the delay time you define. However, if the delay you define is shorter than the hardware delay, then the actual delay time is the hardware delay time. For example, using an i3x instrument, if you define a luminescence read delay of 1 second, then the actual delay time between the end of the injection and the beginning of the read is 1 second. However, if you define a luminescence read delay of 0.05 seconds, then the actual delay time between the end of the injection and the beginning of the read is 0.3 seconds due to the hardware delay.

Baseline

To take baseline reads before the injection step of a kinetic read, double-click **Baseline** in the Toolbox. The **Baseline** step appears in the time line before the injection step. Click the pencil icon and enter the number of baseline reads. You can define from 1 to 999 baseline reads. Baseline reads use the settings you define in the ABS Read (iD3 only), LUM Read, or FL Read step. You can subtract the baseline data from the reads that follow the injection. You can use the Formula Editor to automate baseline subtraction. See . You can find more information about baseline subtraction in the *Formula Reference Guide*.

More Settings

The **More Settings** category on the Settings dialog provides more settings options. The options vary depending on the instrument you select, 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. If you turn calibration off, SoftMax Pro Software uses the air-calibration values stored in the instrument firmware or the values from the previous calibration.

For Fluorescence and Luminescence reads, the instrument makes a reference read on the internal reference chip at each wavelength before reading the microplate. If you turn calibration off, SoftMax Pro Software uses the default values stored in the instrument firmware or the values from the previous calibration.

For kinetic readings the calibration measurement is made between readings during a run, unless the read interval is too short to allow calibration.

If **Calibrate** is set to **On** on the More Settings tab, the instrument runs calibration for the wavelengths you specify in the protocol before each read.

The new calibrations overwrite the calibration setting stored in firmware for the current data acquisition read, and for any subsequent read with calibration turned off. When the instrument is powered off and on, the calibrations reset to those stored in firmware.

The air-calibration values stored in the firmware for Absorbance reads can be updated using the **Calibration** button on the Operations tab in the ribbon. See Calibrate Microplate Readers on page 407.

Turning off calibration allows the instrument to begin or complete readings more quickly. Molecular Devices recommends that you calibrate at your assay wavelengths for at least one microplate before you turn off calibration.

Carriage Speed

Carriage Speed can be slowed down for samples (such as, fragile clots) that can be disrupted by rapid carriage movement. Select a **Carriage Speed** of Normal or Slow.

Read Order

The Read Order options in the More Settings category on the Settings dialog vary depending on the instrument you select, read mode, and read type.

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 the entire microplate (or chosen number of strips) at the first wavelength, and then goes back and reads the microplate at the second and other selected wavelengths.
- In wavelength 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, and so on.

Microplate Read Order

- Select **Row** to read the entire microplate row-by-row.
- Select **Column** to read the entire microplate column-by-column.
- Select **Well** to read each well individually with all wavelengths and intervals you define for the read before reading the next well. This is useful for spectral scans, area scans, and specialized, fast-kinetic applications.

  For the SpectraMax i3x, SpectraMax iD3 Multi-Mode Microplate Reader, or i3 Multi-Mode Detection Platform and the SpectraMax Paradigm Multi-Mode Microplate Reader, select a Well read order in the Standard View or add a For Each Well loop in the Acquisition View, for a kinetic read to define timing parameters in milliseconds.

Settling Time

For some instruments, the **Settling Time** option is available in the More Settings category on the Settings dialog.

Settling Time is the delay between the motion of the reader and the start of the read. This delay allows time for the meniscus motion to cease, potentially improving precision, especially in the low-density, high-volume microplates with fewer than 96 wells.

To specify a settling time, select the **Settling Time** check box and enter 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.

Show Pre-Read Optimization Options

For some instruments, you can select to **Show Pre-Read Optimization Options** in the More Settings category on the Settings dialog.

In the Acquisition View, you can add Plate Optimization to the beginning of the time line.

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

Microplate Optimization

Microplate dimensions can vary slightly between production lots, which can have a potential effect on measurement accuracy. SoftMax Pro Software enables you to optimize labware dimensions by determining the centers of the four corner wells on the plate. Each time you optimize a labware type 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 iD3 Multi-Mode Microplate Reader, SpectraMax i3x Multi-Mode Detection Platform, and the SpectraMax Paradigm Multi-Mode Microplate Reader feature an objective lens that can be moved up and down to optimize the read height used in luminescence, fluorescence intensity, 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 SpectraMax i3x Multi-Mode Detection Platform for top-read only.

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. To reduce the intensity, use an attenuation filter for the read.

Normalization of AlphaScreen Data

For AlphaScreen reads, the **Normalization** option is available. In the Standard View, this option is available in the More Settings category on the Settings dialog.

The conversion rate of photons to counts is individual for each detector. Therefore, raw data from the same plate can seem to be different from one instrument to the next. 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.

To normalize raw data to counts per second, select the **Normalization** option.

Background Correction with Interlaced Readings

For AlphaScreen Endpoint reads, the **Interlaced Reading** option is available. In the Standard View, this option is available in the More Settings category on the Settings dialog.

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 a different plate run to read the previously omitted wells.

For improved results, Molecular Devices recommends that you use replicates for all blanks, controls, and samples.

This setting is available for the SpectraMax i3x Multi-Mode Detection Platform and the SpectraMax Paradigm Multi-Mode Microplate Reader with an AlphaScreen Detection Cartridge installed.

Compound Transfer Settings

Compound transfer settings are supported by the FlexStation 3 Multi-Mode Microplate Reader.

The **Compound Transfer** settings must be configured correctly. In addition to configuring precise fluid transfers for your experiments, these settings also helps prevent flooding of the assay plate.

The settings enable you to define volumes for up to three transfers. However, you must keep in mind the actual maximum volume allowed in the wells you use as you define the settings. The maximum cumulative volume depends on the assay plate type you select. See Plate Type Settings on page 220.

For the Endpoint and Kinetic read types, the transfers are done to all the selected wells before the first read.

For the Flex read type, the transfers are done for a column while that column is being read. All transfers and reads are done for that column before moving to the next column.

No. of Compound Transfers

You can enable up to three compound transfers in each column during a read.

From the **No. of Compound Transfers** drop-down list, select the number of transfers required for the assay.

- With **0** selected, no other options are displayed.
- With **1**, **2**, or **3** selected, color-coded images representing each of the transfers and the transfer settings are displayed.

Initial Volume

In the **Initial Volume** field, enter a value that equals the largest initial volume in the wells of the assay plate before the compound transfers.

- For a 96-well microplate, this value can be set from 0 µL to 269 µL, although typical values are about 10 µL to 200 µL.
- For a 384-well microplate, this value can be set from 0 µL to 120 µL, although typical values are about 5 µL to 80 µL.

If there is no fluid volume in the assay plate before the compound transfers, enter **0** in this field.

The software defines all wells as having the same initial volume. The software uses this value to compute the total volume in each well after all fluids have been dispensed. The software makes this calculation to warn you in the **Compound & Tips Columns** settings of the potential for overflow of fluid from the wells. See Compound and Tips Columns Settings on page 257.

The value you enter is saved with the file but the value displays only in the **Compound Transfer** settings and in the **Compound & Tips Columns** settings.

Transfer Settings

Click a transfer image to define the parameters for that transfer.

The color-coded transfer image you click displays with a light blue background.

> **Note:** There are no other indications in the transfer settings for which transfer you are defining, so pay close attention to which transfer settings you are modifying.

- In the **Pipette Height** field, enter a value of 1 µL to 300 µL for a 96-well microplate or 1 µL to 130 µL for a 384-well microplate. This setting determines the volume of fluid in microliters, measured from the bottom of the assay plate well, above which the tip of the pipette will remain during the dispensing portion of the transfer event. This setting helps make sure that the tip of the pipette is below the surface of the liquid at the end of

the transfer, minimizing the possibility that undispensed drops remain on the tips.

> **Note:** As you configure subsequent transfers, calculate the amount of fluid added and set the pipette height accordingly. For example, if the initial volume is 15 µL and the first transfer dispenses 20 µL, then you can set the pipette height for the first transfer to 25 µL. If the second transfer dispenses 15 µL, then you can set the pipette height for the second transfer to 45 µL.

- In the **Volume** field, enter a value to define the volume of liquid to be dispensed from the source to each individual well chosen to receive that transfer.
    - For a 96-well microplate, the range is 1 µL to 200 µL.
    - For a 384-well microplate, the range is 1 µL to 30 µL.

> **Note:** Keep in mind the maximum total volume each well can hold as you accumulate volumes with multiple transfers.

- In the **Rate** field, enter a value of 1 to 8. This setting determines the rate at which the fluid is dispensed into the well of the assay microplate.
    - In a 96-well microplate, a setting of 1 is equal to 26 µL per second and each subsequent number increases in increments of 26 µL per second, so that a setting of 2 is equal to 52 µL per second.
    - In a 384-well microplate, a setting of 1 is equal to 6 µL per second and each subsequent number increases in increments of 6 µL per second, so that a setting of 2 is equal to 12 µL per second.

  A setting of 3 or 4 can help minimize cell damage or dislodgment. For non-contact dispensing, use a rate of 8 to make sure that all liquid is dispensed from the pipette tip.

- In the **Time Point** field, enter a value that is equal to or greater than the value in the **Minimum Time** up to 9999 seconds. This setting determines the time after the start of the read when the fluid is scheduled to begin being dispensed. Your time point becomes your baseline time. This is not the time interval between transfers. In Endpoint and Kinetic reads, **Time Point** defines the time at which the first dispense occurs. The Endpoint or Kinetic read begins after all transfers are made.

> **Note:** The **Time Point** value cannot be smaller than the **Minimum Time** identified by the software for each transfer.

- The **Minimum Time** field displays the minimum time required before a pipetting event can occur. This minimum time value is cumulative, not an interval between pipetting events. The value is the minimum number of seconds of elapsed time from the beginning of the read. It takes into account the mechanical speed of the pipette head and the time needed to load and unload tips, aspirate and dispense fluids, triturate fluids, and shake the microplate.

  The minimum time for the second pipetting event depends on when the first pipetting event occurs. The calculation for the second event starts at the end of the first event and adds the total time necessary to load pipette tips, aspirate new fluids from the compound plate, and dispense them into the assay plate.

Leave Tips On Between Columns

For a Flex read type, the pipettor returns the tips to the tip rack after all the transfers for a column are complete, and then installs tips from the tip rack before the first transfer for the next column. If you are using tips from the same position in the tip rack for the first and last transfers in each column, then you can reduce the cycle time between columns by selecting the **Leave tips on between columns** check box. The tip assignments are defined in the **Compound & Tips Columns** settings.

Avoid Problems

Time point calculations are based on the number of wells read, and the tip column and the compound column used during the transfer. If you select time points that are not long enough to be compatible with the selected volumes and transfer speed, the software notifies you that the time point needs to be increased.

If you select transfer volumes that add up to more than the maximum that can fit in the assay plate, an overflow warning appears to indicate that you have exceeded the volume limits of the assay plate.

Pay attention to the minimum time value that displays under the time point field for each transfer, as the minimum time values are different depending on which transfer you are configuring.

The **Compound Transfer** settings work together with the **Compound & Tips Columns** settings. Until well assignments are made in the **Compound & Tips Columns** settings, the **Compound Transfer** settings display **No targets assigned**. See Compound and Tips Columns Settings on page 257.

Compound Source Settings

The **Compound Source** settings enable you select a compound source plate. Compound plates store fluids that are aspirated (or withdrawn) from the compound plate and then dispensed (or injected) into the assay plate during the run.

Make sure that you select a plate type that matches the type and well configuration of the actual compound plate to use. It must also be compatible with the number of wells you select in the plate type settings for the assay plate.

The well bottom height is different for different types of microplates. Select the plate type correctly to prevent jamming the pipette tips into the bottom of the well.

- For 96-well microplates, the instrument aspirates using a pipette height of 20 μL above the bottom of the well.
- For 384-well microplates, the instrument aspirates using a pipette height of 10 μL above the bottom of the well.

The pipette height for the compound source plate is set automatically. The pipette height for the assay plate is defined in the Compound Transfer settings.
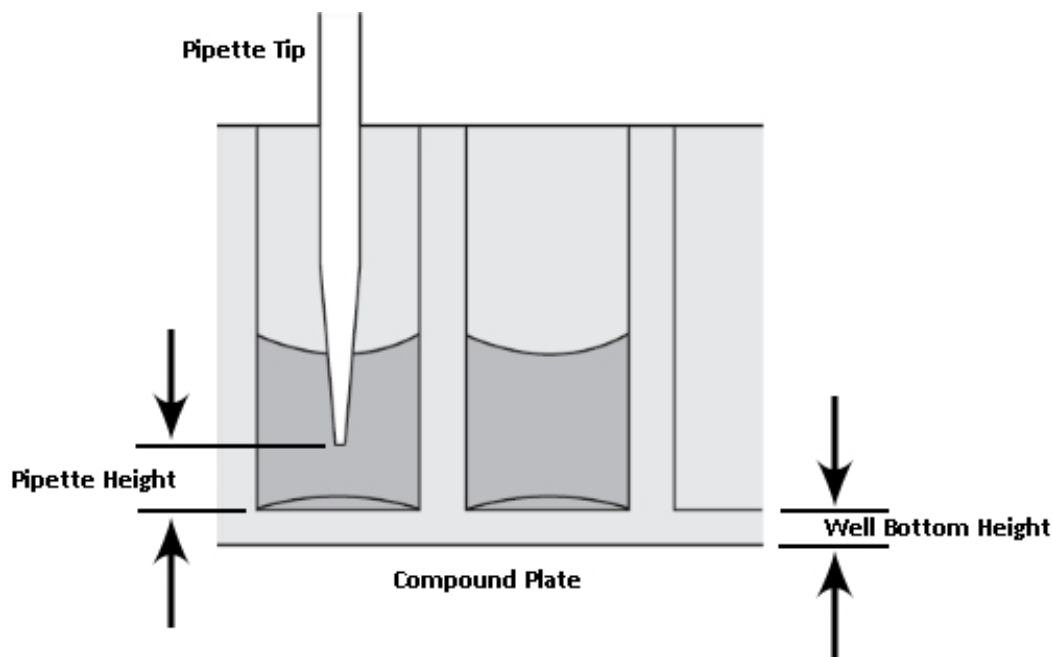
Figure 7-2: Well Bottom Height

⚠️ **CAUTION!** Selection of an incorrect compound plate type can result in the pipette tips jamming into the wells and damaging the microplate, the tips, and the instrument.

Pipette Tips Layout Settings

The **Pipette Tips Layout** settings display a column of tips in a 96-well (12 tips) or 384-well (24 tips) layout that matches the selection you made in the **Plate Type** settings for the assay plate.

If you use a partial rack of tips, make sure that the **Pipette Tips Layout** settings match the configuration of the partial rack you insert in the tip drawer. The selected tips must be contiguous.

To select a contiguous set of tips, use your mouse to drag the pointer and select the tips to use.

To use all the tips in the rack, select the **Select All** check box.

If you change the selection in the **Plate Type** settings for the assay plate to a microplate with a different number of wells (for example, from 96-wells to 384-wells), the selection in the **Pipette Tips Layout** settings defaults to the selection of the full rack of tips.

⚠️ **CAUTION!** Molecular Devices recommends that you use a full rack of tips each time you do a fluid transfer. If you enable a pipetting function from a tip that is not present, your samples do not receive the intended compound. There is potential of damage to the microplate, the tips, and the instrument.

Compound and Tips Columns Settings

The Compound & Tips Columns settings that are available depend on the selection you make in the Plate Type settings for the assay plate and the number of transfers you define in the Compound Transfer settings.

If you enable one or more transfers, these settings enable you to choose the tips and compounds to use for each transfer.
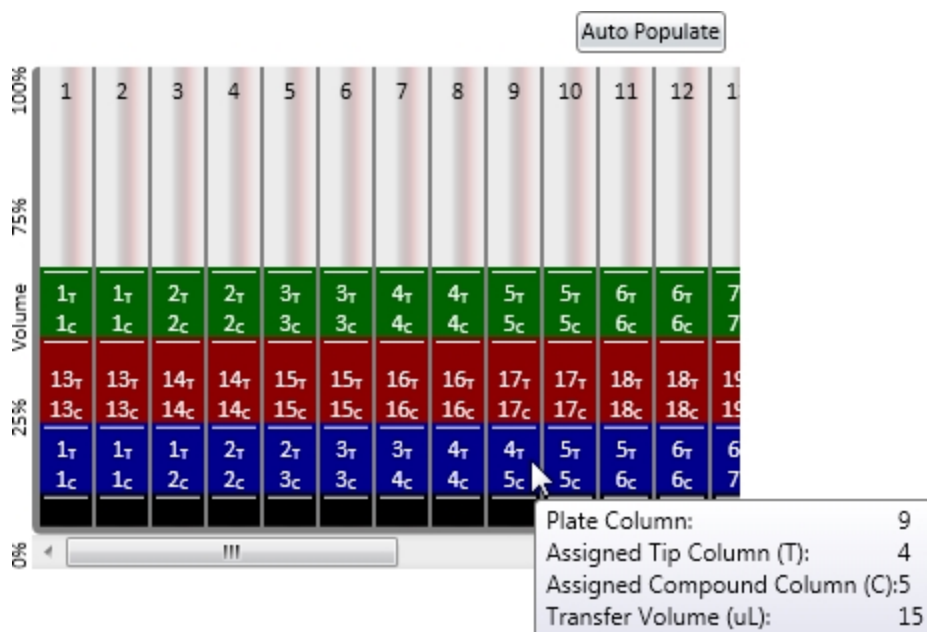
The black area at the bottom represents the initial volume in the wells. Each color-coded transfer is then stacked on top of the previous transfer.

- For a 96-well assay plate, all 12 columns display.
- For 384-well columns, the first 12 columns display. To view the other columns, use the scroll bar on the right.

You can restrict the number of columns that are available in the Read Area or Wells to Read settings, and restrict the number of tips that are available in the Pipette Tips Layout settings.

Each transfer is assigned a set of tips from the tip rack and a column from the source plate:

- To select a set of tips from the tip rack for a transfer, click the tip assignment indicated by the letter **t** and then select the number of the tip set from the list.
- To select a column from the source plate for a transfer, click the column assignment indicated by the letter **c** and then select the number of the source column from the list.

If the assay requires that no fluid is to be transferred to the assay microplate for a column during one or more transfer operations, click the red **X** at the top of the drop-down list. A color-coded drop icon is displayed for that column indicating that it will be skipped.

To enable a transfer for a skipped column, click the drop icon and then assign the tip and source columns for the transfer.

You can manually assign tips and source columns for each and every transfer.

✳ **Tip:** To leave the tips on between columns for a Flex read type, select the same set of tips in the tip rack for the first transfer of a column as was selected for the last transfer of the previous column.

Assign Compound and Tip Columns Automatically

To have the software automatically assign the tips and columns, click **Auto Populate**.

The SoftMax Pro Software automatically enters information in the **Compound and Tip Columns** settings taking into consideration the number of transfers and the **Read Area** or **Wells to Read** settings. The software assigns fluid to be aspirated from the compound plate starting with the first available column. Aspirated fluid is then be dispensed to the first available column in the assay plate as indicated in the **Read Area** or **Wells to Read** settings.

The software makes the assignments based on the following conditions:

- All columns you select in the **Read Area** or **Wells to Read** settings are to receive fluid.
- The fluids are transferred from left to right. The read-transfer-read sequence in each column is initiated only after the read-transfer-read event from the previous column is completed (the total read time for that column).
- The fluid transfer targets are cumulative from transfer to transfer. That is, the targets for the second transfer start with the next available clean tip and untargeted compound column rather than reusing tips and compound columns targeted by the preceding fluid transfer.
- Each fluid transfer uses a new tip.

For example, suppose you configure the instrument for three fluid transfers as follows:

- A selection in the **Read Area** or **Wells to Read** settings of columns 1 through 4.
- A Compound Source selection of a 12-column compound source plate, such as a 96-well microplate.
- A selection in the **Pipette Tips Layout** settings of a full rack of tips.

From this configuration, the SoftMax Pro Software selects tip and compound columns 1 through 4 for the first transfer. The second transfer uses the next four columns (5 through 8), and the third transfer uses the remaining four unused columns (9 through 12).

> **Note:** The SoftMax Pro Software does not assign targets beyond the limitations of the available tips and compound source columns. After all available tips and compound source columns have been assigned, the software stops assigning targets.

Triturate Settings

Trituration is mixing of the contents of the wells in a microplate by using the pipettor to alternately aspirate the fluid from a well and then dispense it back into the well. You can choose to triturate in either the compound source plate or the assay plate, or both.

Applications for which trituration is recommended include when the compound in the source plate needs to be resuspended before the addition, when transferring low volumes of fluid to the assay plate, or when transferring fluid before or during an absorbance read to make sure that the well contents are properly mixed.

The Triturate settings are based on the number of compound transfers you specify in the Compound Transfer settings. If no transfers are enabled, no triturate settings are available.

Select the transfer during which trituration is to be done, and then select either the **Compound Source** check box or the **Assay Plate** check box, or both.

Enter values for the **Volume** of fluid in microliters to withdraw from the well, and for the number of **Cycles**, or times, to aspirate and dispense the fluid in the well.

For the Assay Plate settings, you must enter a value for the **Height** at which the trituration occurs. The height setting must take into account the defined trituration volume and the total volume in the well, so that the tips remain below the liquid surface and do not aspirate air.

If you select dispense times that are incompatible with the Triturate settings, an error message appears. To modify the dispense time, return to the Compound Transfer settings.

Define and Run Workflows

The Workflow view provides a drag-and-drop workflow editor and tools to run and monitor a workflow. In Workflow view you can define a workflow that uses the defined Plate sections and instrument settings to run multitask kinetic reads that last hours or days.

To change to the Workflow view, click the **Workflow** button.

Drag tasks from the menu on the left to the timeline on the right.
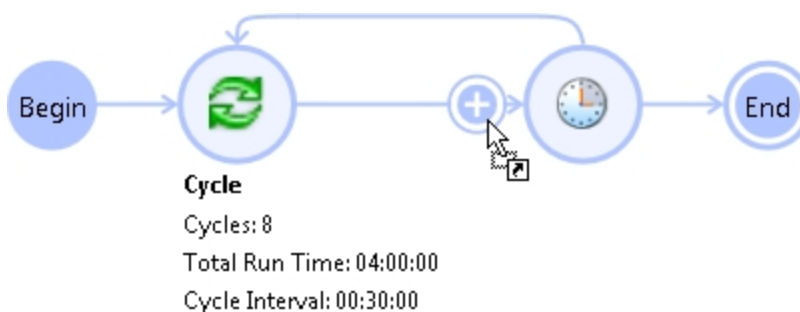
Define repeated tasks by placing the tasks in a defined Cycle task.

✳ **Tip:** You can gather data over long periods of time by using a Kinetic read type and then defining the Timing settings to acquire a single point with each read. See Timing Settings on page 228.

📓 **Note:** Place only Kinetic reads within a cycle. Using other read types causes the software to overwrite the previously acquired data in the Plate section. You can place an Endpoint, Well Scan, Spectrum, or Flex read before or after a cycle to preserve the acquired data.

When you click a task in the timeline, a pencil icon appears. Click the pencil to edit the settings for the task. See Edit Workflow Tasks on page 261.

To remove the task from the timeline, click the red X.

Click the options in the Execution group to start, pause, resume, and cancel the workflow. See .

Select the **Workflow** tab in the ribbon. Click the options in the **Designer** group to undo or redo actions, clear all tasks from the editor, and zoom in and out of the editor. Click **Mini Map** to open the Overview dialog that helps you navigate a long timeline. To export an image of the timeline in JPG, PNG, GIF or XPS format, click **Save as Image**.

The Status group displays the current status of the workflow. Click **Execution Log** to view the status of the tasks to run a workflow in real time.

Edit Workflow Tasks

In the Workflow editor, drag tasks from the menu on the left to the timeline on the right and then click the pencil icon to edit the settings for that task.

Select options from the drop-down lists or enter text in the fields. When you finish editing the task, press ENTER or click on a blank area of the workflow editor.

Each task has its own set of options:

Read

From the **Experiment** drop-down list, select the name of the experiment that contains the Plate section for the read.

From the **Plate Section** drop-down list, select the name of the Plate section for the read.

The read uses the instrument settings you define for the Plate section you select. To edit the instrument settings, click ![icon]. See Instrument Settings on page 216.

✳   **Tip:** If you place a Kinetic read in a Cycle for a SpectraMax iD3, SpectraMax i3x, SpectraMax Paradigm, or FilterMax reader, turn off **Show Pre-Read Optimization Options** for the read to prevent having the software wait for user interaction before each read. See Show Pre-Read Optimization Options on page 250.

Open Drawer

Select the **Wait while drawer is open** check box to pause the workflow until the drawer is closed. If you require the workflow to continue to the next task with the drawer open, clear this check box.

In the **Message to Show** field, enter the message to display when the drawer opens. You might want to enter instructions for the operator.

Set Temperature

To set the temperature in the read chamber, click **On** and then enter the temperature in degrees Celsius in the **Plate Chamber Set Point** field.

Select the **Wait while the temperature changes** check box to pause the workflow until the read chamber reaches the required set point. If you require the workflow to continue to the next task before the chamber reaches its set point, clear this check box.

To turn off temperature control, click **Off**.

For more information, see .

Shake

In the **Shake time** field, enter the number of seconds to shake the microplate.

Delay

Enter values for the delay in Days:Hours:Minutes:Seconds, or click one of the values and then click the arrow on the right to change the value.

Note: The Delay task continues even if the workflow is paused.

Cycle

Total Run Time defines the total time for all the cycles to complete. Enter values in Days:Hours:Minutes:Seconds, or click one of the values and then click the arrow on the right to change the value.

Cycle Interval defines the time from the start of one cycle to the start of the next cycle. Enter values in Days:Hours:Minutes:Seconds, or click one of the values and then click the arrow on the right to change the value.

The number of cycles is calculated based on how many Cycle Intervals can be completed within the Total Run Time.

To include a task in the cycle, drag the task between the start and end points of the Cycle task.

Cycle
Cycles: 8
Total Run Time: 04:00:00
Cycle Interval: 00:30:00

When you define the timing for a Cycle, make sure that the tasks in the Cycle can be completed within the allotted time. The workflow editor cannot calculate the time it takes to do a read. If the read takes longer than the time allotted for the Cycle, then the next Cycle begins immediately after the Cycle tasks have completed.

Note: The Cycle Interval continues even if the workflow is paused.

Run Workflows

After you define the workflow and insert your prepared microplate in the microplate reader, you can run the workflow.

Note: Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

- To run a workflow, click **Start** on the Workflow tab in the ribbon.

- To stop a running workflow, click **Cancel** .

- To temporarily stop a running workflow, click **Pause** . Pausing a workflow lets you work with other features in the software, such as define protocols or run short reads, while the software waits to do the next non-timing task in the workflow.

  When you pause a workflow all delays and cycle timings continue to run. When the workflow reaches a non-timing task, such as a read, the task starts after the workflow is resumed. If the workflow is resumed after the scheduled time for a task to start, then the task starts when the workflow is resumed and all subsequent tasks are rescheduled to a later time.

  For example, if a delay before a read is set for 30 minutes and the workflow is resumed after 40 minutes, then the read starts when the workflow is resumed and all subsequent tasks are rescheduled to start 10 minutes later than their original times.

- To restart a paused workflow, click **Resume** .

Configure Microplate Templates

The Template Editor is a representation of the microplate or set of cuvettes shown as a grid of wells that you can use 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 on the Home tab in the ribbon or in the toolbar at the top of the section to open the Template Editor dialog.

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 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 Define Groups on page 265.

   - 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 how to use blanks, see Define Blanks on page 270.

   - To remove wells from a group, select the wells to remove and click **Clear**.
   - To print the Template Editor dialog, click **Print**.

4. To assign wells to a series, select the wells and then click **Series**. See Create a Series on page 267.

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

To save the template in a protocol file for use in future experiments, see Save Protocol Files on page 68.

Modify Templates

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 the 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. See Configure Microplate Templates on page 264.

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

Define Groups

The Template Editor enables you to assign wells to group types. 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 the Template Tools on the Home tab in the ribbon or in the toolbar at the top of the active section to display the Template Editor.
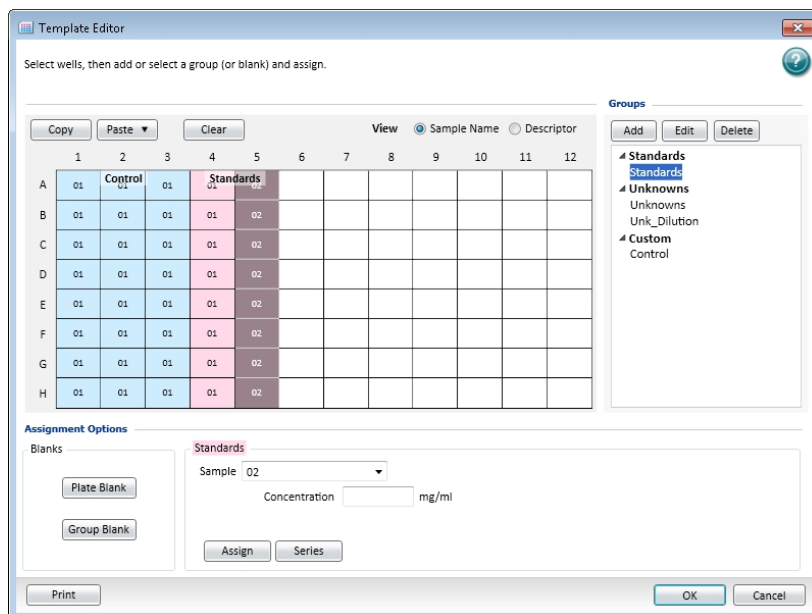
The Template Editor contains a representation of the rows and columns of the microplate in the active Plate section.

When you open the Template Editor with a Cuvette Set section active in the workspace, the Template Editor 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. Use your mouse to drag the pointer to select one or more wells to define as a group.

4. In the Groups area, select a group and click **Edit**.

   To create a new group, click **Add**. See Create Groups on page 124.

5. On the Group Settings dialog, if a sample descriptor is defined for the group you select, enter a value in the field for the descriptor.

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

7. Click **Assign** to assign the selected wells to the group.

   To define a series for the selected wells, click **Series**. See Create a Series on page 267.

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

Create a Series

You can define several samples as a series, which enables you to enter incremental sample descriptors (for example, dilutions or concentrations) and sample names to the template when the increment can be expressed as a mathematical operation. This enables 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 before you generate a standard curve. To create replicates, click **Assign** or use the Series dialog.

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

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

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

3. On 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 a group name.

> **Note:** A series is related to a group. If no groups are defined, you need to define a group and then define the series. To create a new group, click **Add** to open the Group Settings dialog. See Group Settings on page 123.

5. Click **Series**.

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**, enter the number of replicates in the Y-Direction and enter the number of replicates in the X-Direction.

     The maximum number of replicates depends on the well selection. For example, if you fill down from the top and you choose a block of wells that is 4 wells wide by 8 wells high, the maximum number of replicates is four. With the same block of wells selected, filling from the left would permit 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 are labeled as more 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** enter 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 is defined for the selected group, enter a 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 starts with 0.5 and divides 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.

Increment the Names Only

To increment the names of a series of samples without applying a concentration or dilution:

1. Create a series on the Series dialog.

2. If a sample descriptor is defined for the group you select, clear the check box next to the descriptor.

Increment with a Constant

To increment the names of a series of samples with a constant concentration or dilution:

1. Create a series on the Series dialog.

2. In the **Starting Value** field for the sample descriptor, enter 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, then the SoftMax Pro Software 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.

Copy and Paste Template Content

You can copy the content of a template and paste the content to a different section within the same experiment or between different experiments. You can copy only contiguous subsets 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 you make 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 you copy and paste 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 have no effect on the source section.
- Data from the two Plate sections are not analyzed together.

Regions within a Template

You can copy and paste only contiguous subsets in 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 **Copy** on the left above the template.
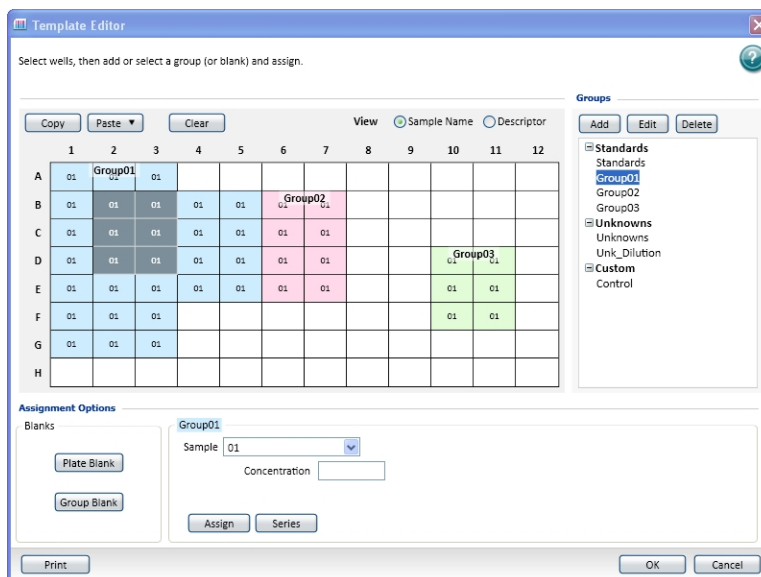
5. Click a different location in the same template and then click **Paste**.

Note: You can select the same footprint, or click the top-left well of the desired target.

6. From the menu click **Paste** or **Paste With New Group**.
   - **Paste** adds the copied wells to the current group.
   - **Paste With New Group** pastes the copied cells into a new group.

If more wells are selected to paste than were selected to copy, the extra wells are left empty.

The real value in this method is the means to copy a complicated template and then to analyze the data separately from the original plate.

Define Blanks

The Template Editor enables you to configure Plate Blanks and Group Blanks.

The different blank types have the following features:

- Use **Plate Blanks** to correct readings when all samples on a plate are 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.
- Use **Group Blanks** to correct readings when samples on a plate are prepared in a different buffer or matrix, and therefore need 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.

You can use blanks in combination and have cumulative effects.

Plate Blanks

You can assign Plate Blanks in Plate sections and in Cuvette Set sections.

Plate Sections

You can assign wells 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 to open the Template Editor dialog.
3. Select the wells to use for the Plate Blank.
4. Click **Plate Blank**.

Use Plate Blanks when all samples on the plate are prepared in the same way, and therefore can be corrected from a single blank reading. You can turn off Plate Blank subtraction on the Data Reduction dialog so that the data can be reviewed with or without Plate Blank subtraction. See Data Reduction on page 325.

For Kinetic and Spectrum data, the blank value is averaged and subtracted from each point in the read.

Cuvette Set Sections

To do a similar function to Plate Blanking for Cuvette Set sections, you can assign cuvettes to a Blank group in the Template Editor in the Cuvette Set section and create a Template Blank:

1. Select 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 to open the Template Editor dialog.
3. Select the cuvettes to use 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)

You can assign blank wells within a group (other than the "Blank" group) on 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 to open the Template Editor dialog.
3. Select the wells or cuvettes to use for the group blank. These should be wells or cuvettes that are 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 is assigned, it is automatically subtracted and you do not have the option to review the uncorrected data. To see the reduced number without the group blank subtracted, you must mask the group blank wells or cuvettes. See Mask Wells or Cuvettes on page 324.

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.

Export Templates

To export template information from a Plate section:

1. Click the **Plate** section with the template to export to make it active in the workspace.
2. Click **Export** 🔼 in the 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. Enter 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 saved 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 272.

Import Templates

You can import template information into a Plate section from a tab-delimited ASCII text file in Unicode (UTF-8) format. Template files can be prepared outside SoftMax Pro Software and then imported when ready to run samples.

For information about the format of an imported or exported template file, see Template File Formats on page 272.

✳ **Tip:** A good way to determine the correct format to import a template is to export a template from SoftMax Pro Software and examine the contents of the resulting file.

---

1. Click the Plate section where you want to import template information to make it active in the workspace.
2. Click **Import** 🗎 in the Template Tools on the Home tab in the ribbon.
3. In the Open dialog, navigate to and select the text file to import.
4. Click **Open**.

Template File Formats

Template information you save as a tab-delimited ASCII text file in Unicode (UTF-8) format can be imported to or exported from Plate sections. A template file consists of ten columns of data separated by tabs. Each line of the file provides information for a single well in the microplate.

When you export template information, 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 is 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 to import 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.

You can open a tab-delimited ASCII file 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 unless the layout of the cells match the correct format for import, and the file is saved as an ASCII text file in Unicode (UTF-8) format.

Table 7-3: 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. A text string can be entered in this field.

Table 7-3: ASCII Template File Columns and Descriptions (continued)

Column	Column Entry	Description
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 is assigned to the well, although the sample name is included in the group.
D	Sample Name	The sample name is set in the **Sample** field on the Template Editor dialog. If this field is left blank, no sample name is assigned to the well, although the sample name is 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. Supported strings are unit/ml, mg/ml, µg/ml*, ng/ml, mg, ng, and ml. If this column is left blank, unit/ml is assigned.
H	Descriptor2 Name	Assigned in the Group column name assigned to the descriptor 2 is shown here.
I	Descriptor2 Value	Assigned in the Group Settings dialog. A numeric value can be entered in this field or it can be left blank.
J	Descriptor2 Units	Assigned in the Group Settings dialog. Supported strings are unit/ml, mg/ml, µg/ml*, ng/ml, mg, ng, and ml. If this column is left blank, unit/ml is assigned.

* The µ symbol can be imported only if the ASCII text file is saved in Unicode (UTF-8) format.

Create Absorbance Mode Protocols

To create an Absorbance Mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select an instrument. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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. For a SpectraMax Paradigm Multi-Mode Microplate Reader, click the Absorbance Detection Cartridge from the **Cartridges** list. For the SpectraMax i3x Multi-Mode Detection Platform, use the built-in monochromator.

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 you define the protocol settings, click **OK** to close the Settings dialog.

9. Create a template, if applicable. See Configure Microplate Templates on page 264.

10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.

11. Set Display options, if applicable. See Data Display Settings on page 318.

12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Absorbance Mode Protocol Overview

Absorbance is the quantity of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. If there is no turbidity, then 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.

Optimize Absorbance Assays

You can adjust the wavelength of the incident light in 1 nm increments. SoftMax Pro Software permits reading up to six wavelengths per plate or cuvette for Endpoint and Kinetic read types, and four wavelengths for Well Scan reads.

It is good scientific practice to designate wells in the microplate to serve as plate blanks or group blanks. You can do this on the Template Editor dialog of the Plate section in the SoftMax Pro Software. See Configure Microplate Templates on page 264.

You can use the PathCheck Pathlength Measurement Technology feature to normalize the data to a 1 cm pathlength. See PathCheck Pathlength Measurement Technology on page 139.

Note: Assay optimization requires the use of a computer and SoftMax Pro Software.

Create Fluorescence Intensity Mode Protocols

To create a Fluorescence Intensity protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.
2. Select an instrument. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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 you use the SpectraMax Paradigm Multi-Mode Microplate Reader, click one of the Fluorescence Intensity (FI) cartridges in the **Cartridges** list. When you use the SpectraMax i3x Multi-Mode Detection Platform, use the built-in monochromator or click one of the Fluorescence Intensity (FI) cartridges in 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 you define the protocol settings, click **OK** to close the Settings dialog.
9. Create a template, if applicable. See Configure Microplate Templates on page 264.
10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.
11. Set Display options, if applicable. See Data Display Settings on page 318.
12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Fluorescence Intensity Mode Overview

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

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 7-3: Excitation and Emission Spectra

✳ **Tip:** If the Stokes shift is small, 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, permitting better selection and quantitation of the emitted light.

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 readers incorporate many features designed to restrict interference from reflected excitation light. These features include a set of long-pass emission cutoff filters that can be set automatically by the instrument or manually by the user.
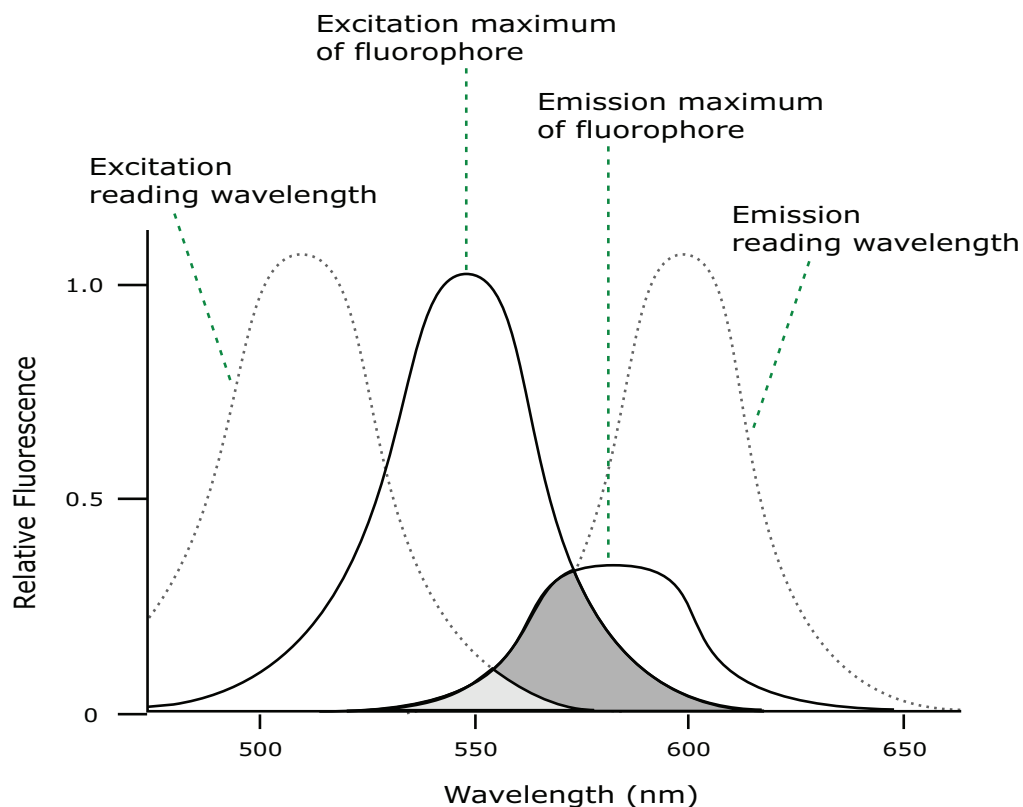
Figure 7-4: Optimized Excitation and Emission Reading Wavelengths

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 quantity 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 permits scanning of both excitation and emission wavelengths, using separate tunable monochromators. One benefit of scanning emission spectra is that you can determine 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. One more benefit is that you can find excitation and emission wavelengths that prevent 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 optimal setting is where the ratio of the sample emission to background emission is at the maximum.

Optimize Fluorescence Intensity Assays

The optimal 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 can affect 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 280.

- 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 lets the instrument and SoftMax Pro Software 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 a different 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 instrument is isothermal at ambient as well as at elevated temperatures.

  The temperature in the reading chamber can be adjusted from 2°C (3.6°F) above ambient to the maximum temperature for the instrument. See the user guide for your instrument.

Spectral Scanning

This procedure optimizes excitation and emission wavelengths for fluorescence assays using spectral scanning.

If you use the SpectraMax iD3 Multi-Mode Microplate Reader, the SpectraMax i3x Multi-Mode Detection Platform, or the SpectraMax Paradigm Multi-Mode Microplate Reader with a Tunable Wavelength (TUNE) Detection Cartridge, see Spectral Optimization on page 307.

Pipette 200 μL of sample that includes the fluorophore and 200 μL of a buffer control into separate wells of a microplate.

Do 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. Do 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.
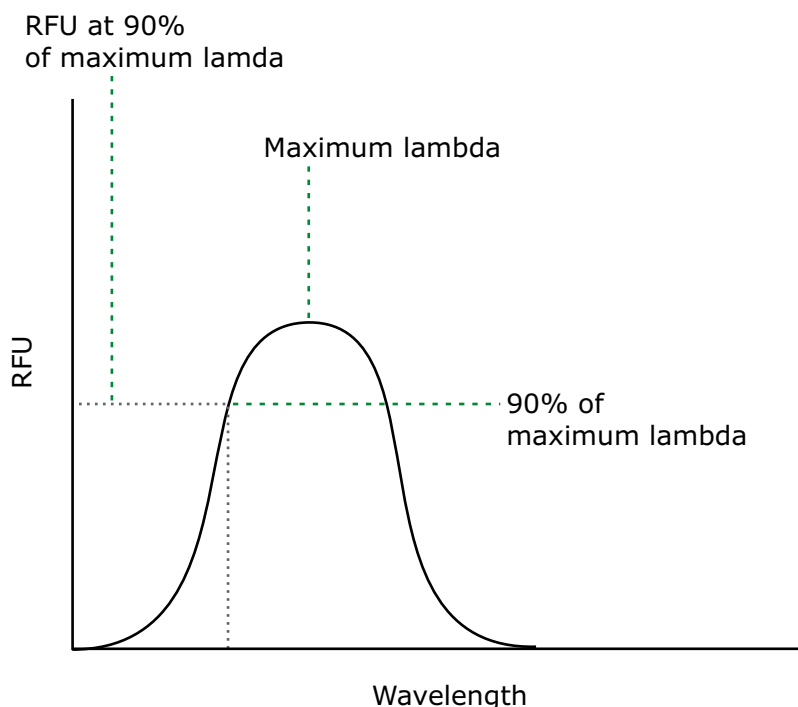
Figure 7-5: Plot of RFU and Wavelength

Do 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 Do the Excitation Scan on page 280.

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. Do 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 a minimum of 10 nm less than the emission wavelength. If you have questions about this procedure, contact Molecular Devices Technical Support and speak to an application scientist.

Do Emission Scan #2

1. In the SoftMax Pro Software, set up a third plate section for an emission scan as specified in Do Emission Scan #1 on page 281, except selecting Manual Cutoff Filter and setting the wavelength to that determined in Choose the Emission Filter on page 282.

2. Do 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 282 and Do Emission Scan #2 on page 282 with a different choice of cutoff filter.

Results

The optimal excitation and emission wavelengths are those determine in Do the Excitation Scan on page 280 and Do Emission Scan #2 on page 282.

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. Start 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 7-4: Emission cutoff filter default settings for SpectraMax M2, M2e, M3, M4, M5, M5e, and FlexStation 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

Create Luminescence Mode Protocols

To create a Luminescence Mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.
2. Select an instrument. See Select an Instrument on page 16.

   > **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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 you use the SpectraMax Paradigm Multi-Mode Microplate Reader, select a Luminescence (LUM) detection cartridge from the **Cartridges** list. When you use the SpectraMax i3x Multi-Mode Detection Platform, use the built-in monochromator or select a Luminescence (LUM) 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 you define the protocol settings, click **OK** to close the Settings dialog.
9. Create a template, if applicable. See Configure Microplate Templates on page 264.
10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.
11. Set Display options, if applicable. See Data Display Settings on page 318.
12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Luminescence Mode Protocol Overview

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.

In luminescence read mode, no excitation is necessary as the species being measured emit light naturally. For this reason, the lamp does not flash, so no background interference occurs. A dark estimate is done over a dark reference, and multiple readings are averaged together into one reading per well.

The default setting for luminescence is the "zero order" position where the grating monochromator acts as a mirror that reflects all light to the PMT detector. If wavelength selection is desired, you can choose the wavelength where peak emission is expected to occur. Also, multiple wavelength choices let species with multiple components be differentiated and measured easily. In luminescence read mode, no emission cutoff filter is used.

Optimize Luminescence Assays

Luminescence can be read from the top or the bottom of a microplate. Solid white microplates or white microplates 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 optimal emission wavelength is between 360 nm and 630 nm. On the Settings dialog the emission says **All**.

For wavelength-selectable luminescence, the emission monochromator is used to differentiate the wavelengths being emitted from the well. Multiple emission wavelengths within the specified reader range can be defined.

If reading only one luminescent event in the well, you can get the best sensitivity 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 1500 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 setting.

If it seems 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. To help eliminate background luminescence from a microplate that has been exposed to light, Molecular Devices recommends dark adaptation of the microplate by placing the sample-loaded microplate in the instrument for several minutes before starting the read.

Create Time-Resolved Fluorescence Mode Protocols

To create a Time-Resolved Fluorescence Mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.
2. Select an instrument. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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 the SpectraMax Paradigm Multi-Mode Microplate Reader or the SpectraMax i3x Multi-Mode Detection Platform, select a TRF-capable detection cartridge in 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 you define the protocol settings, click **OK** to close the Settings dialog.
9. Create a template, if applicable. See Configure Microplate Templates on page 264.
10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.
11. Set Display options, if applicable. See Data Display Settings on page 318.
12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Time-Resolved Fluorescence Mode Overview

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) is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cut off of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the microplate) vanishes while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period with the added benefit of reduced background.

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.

In normal Fluorescence Intensity mode, the 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.

Optimize Time-Resolved Fluorescence Assays

Time-resolved fluorescence is done by flashing the excitation lamp and, after it is off, collecting the delayed emission for a period of time before the lamp flashes again. Long-lifetime rare-earth lanthanide dyes are generally used to provide a long-lived fluorescent signal that persists after the lamp turns off. Background fluorescence usually fades, while lanthanide chelates and cryptates have fluorescent lifetimes between 100 µs and 2 ms.

Time-resolved fluorescence (TRF) assays can be read from the top or bottom of a microplate. Solid white plates are recommended for top time-resolved fluorescence reads, and white plates with clear bottoms are recommended for bottom reads.

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.

If the time-resolved fluorescence assay you are using has low signal or gives results with high %CV, use 100 readings per well. If a faster read speed is required, turn the settling time off and experiment with fewer flashes per well until acceptable precision and speed are achieved.

The important settings for getting the best results in TRF assays are integration delay and integration time:

- The integration delay is the amount of time that elapses between the flash of the lamp (excitation) and the beginning of data acquisition from the well (emission).
- The integration time is the amount of time that the well is read.

The integration delay and integration time are usually specified in the package insert of commercially available TRF reagent kits. If a kit is not used, start with a delay of 50 µs and try different delays up to 400 µs with a fixed integration time of 400 µs. After the optimal delay is chosen, based on the highest ratio of a well containing a fluorophore divided by wells containing only buffer, optimize the integration time, which is generally between 400 µs and 1000 µs.

Some examples of TRF assays are:

- IMAP® TR-FRET
- Cisbio HTRF

- LanthaScreen TR-FRET
- LANCE TR-FRET
- DELFIA TRF

Create FRET Mode Protocols

To create a Fluorescence Resonance Energy Transfer (FRET) protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select an instrument. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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 you use the SpectraMax Paradigm Multi-Mode Microplate Reader or the SpectraMax i3x Multi-Mode Detection Platform, select a FRET-capable detection cartridge 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 you define the protocol settings, click **OK** to close the Settings dialog.

9. Create a template, if applicable. See Configure Microplate Templates on page 264.

10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.

11. Set Display options, if applicable. See Data Display Settings on page 318.

12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

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 a molecular collision. The transfer of energy leads to a reduction in the fluorescence intensity and excited state lifetime of the donor, and an increase in the emission intensity of the acceptor. 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 each other.
- 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 must be approximately parallel.

Create Fluorescence Polarization Mode Protocols

To create a Fluorescence Polarization Mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.
2. Select an instrument. See Select an Instrument on page 16.

   > **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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 the SpectraMax Paradigm Multi-Mode Microplate Reader or the SpectraMax i3x 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 you define the protocol settings, click **OK** to close the Settings dialog.
9. Create a template, if applicable. See Configure Microplate Templates on page 264.
10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.
11. Set Display options, if applicable. See Data Display Settings on page 318.
12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Fluorescence Polarization Mode Protocol Overview

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 a different molecule of equal or greater size can be monitored through its speed of rotation.

Fluorescence polarization mode returns two sets of data: one for fluorescence intensity parallel (P) to the excitation plane, and the other for fluorescence intensity perpendicular (S) to the excitation plane. These S and P values are used to calculate the Polarization (mP) and Anisotropy (r) values in the SoftMax Pro Software.

Although the raw S and 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 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:

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

Optimize Fluorescence Polarization Assays

Fluorescence polarization for the SpectraMax i3x Multi-Mode Detection Platform, the SpectraMax Paradigm Multi-Mode Microplate Reader, the SpectraMax M5 and M5e Multi-Mode Microplate Readers, the FlexStation 3 Multi-Mode Microplate Reader, and the FilterMax F3 and F5 Multi-Mode Microplate Readers can be read only from the top of a microplate. The plastic from which a microplate is formed has an effect on the light polarization, precluding reading from the bottom and also reading a covered plate from the top.

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 quantity 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 done.

For best precision in assays using a low quantity of fluorophore (for example, <5 nm fluorescein), set the PMT sensitivity to High and the number of readings to 100. If a faster read speed is required, turn the settling time off and experiment with fewer flashes per well until acceptable precision and speed are achieved.

Note: Fewer flashes result in a higher speed, but with less precision.

Create AlphaScreen Mode Protocols

To create an AlphaScreen Mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select the SpectraMax i3x Multi-Mode Detection Platform or the SpectraMax Paradigm Multi-Mode Microplate Reader. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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 you define the protocol settings, click **OK** to close the Settings dialog.

9. Create a template, if applicable. See Configure Microplate Templates on page 264.

10. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.

11. Set Display options, if applicable. See Data Display Settings on page 318.

12. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

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 moves beads (coated with A and B, respectively) together, a cascade of chemical reactions 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) lets diffusion reach up to only around 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 beads are light sensitive. Beads are best handled under subdued (<100 lux) or green filtered (Roscolux filters #389 from Rosco, or equivalent) light conditions. Do incubation steps in the dark.

For more information, see AlphaScreen Read Mode on page 168.

Create Western Blot TRF Mode Protocols

To create a Western Blot TRF Mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select a SpectraMax Paradigm or SpectraMax i3x Instrument. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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. In the **Cartridges** list, click **SCANLATER WB** for the ScanLater Western Blot (WB) Detection Cartridge.

5. Click **TRF** to select the **Time-Resolved Fluorescence** read mode.

6. Click the **Membrane** read type.

7. Define the settings.

   For more information about the settings, see the SoftMax Pro Application Help.

8. After you define the protocol settings, click **OK** to close the Settings dialog.

9. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Western Blot TRF Mode Overview

Protein detection is an important task for pharmaceutical and clinical research, and Western Blots (WB), or protein immunoblots, are one of the most common methods employed for this purpose. Various techniques are used to detect proteins on Western Blot membranes including fluorescence, silver staining, and chemiluminescence. However, each technique has its limitations, and there is a continuous need to improve quantitation, accuracy, and dynamic range of Western Blots.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that is incorporated into a SpectraMax® Paradigm® or SpectraMax® i3x Multi-Mode Detection Platform. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. Europium has a long fluorescence lifetime, on the order of 1 msec, and detection is done in Time Resolved Fluorescence (TRF) mode which significantly reduces background from auto-fluorescence or other sources of short lifetime emissions. The membranes are placed into a microplate reader where they are scanned with the ScanLater Western Blot (WB) Detection Cartridge.

The method does not involve enzyme detection, and the Eu-chelates are resistant to photo-bleaching, so the signal remains stable for long periods of time (weeks to months). This permits repeat reading of membranes and the potential for comparison of band intensities to known standards for more accurate quantitation.

The TRF detection employs photon counting, so the theoretical dynamic range is >10^5. In practice, however, dynamic range is limited by saturation of binding sites on high-abundant bands and non-specific binding to background membrane. There is also no camera blooming, as can occur with chemiluminescence or fluorescence detection, so the system gives sharp bands and excellent image quality.

The SpectraMax Western Blot system is a simple, sensitive, and stable platform that provides excellent Western Blot capability in a SpectraMax Paradigm or SpectraMax i3x Multi-Mode Detection Platform extending the uses of these essential laboratory instruments.

Load the Membrane into the Membrane Holder

Note: Handle membranes only by the edges using clean forceps. Take care to not touch the membrane with gloved or bare hands.

To scan the membrane in a SpectraMax Paradigm or SpectraMax i3x Instrument, you must place the membrane in a Molecular Devices ScanLater™ membrane holder. The maximum size for a membrane is 109 mm x 77 mm to let it fit in the membrane holder.

1. Place the membrane on the membrane holder with one corner of the membrane aligned with the A1 corner of the membrane holder.
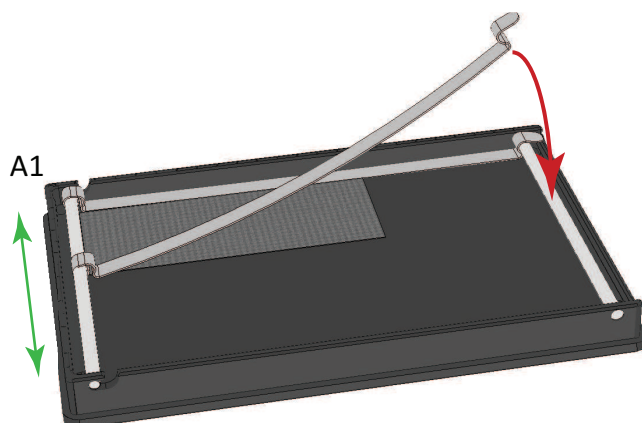
2. Slide the holder clips to place them over the membrane as close to the edges of the membrane as possible to expose the maximum scanning area of the blot.

3. Lower the holder clips into place and then gently press the end of each holder clip to snap it into place and secure the membrane to the holder.

CAUTION! To prevent damage to the instrument or the membrane holder, make sure that all the holder clips are securely snapped into place before you insert the membrane holder into the microplate reader.

4. Load the membrane holder into the microplate drawer with the A1 corner in the A1 position of the drawer. See the user guide for your instrument.

Create Imaging Mode Protocols

To create an Imaging mode protocol:

1. Start the SoftMax Pro Software. See Start the Software on page 14.

2. Select the SpectraMax i3x Multi-Mode Detection Platform using the **Instrument Connection** dialog. See Select an Instrument on page 16.

> **Note:** It is not necessary to physically connect the computer to an instrument to create a protocol. If the computer is 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. Click **MiniMax** in the **Optical Configuration** list.

5. Click **Imaging** in the **Read Modes** list.

   If you have previously acquired images and want to make changes to the analysis settings for those images, click **Re-analysis** in the **Read Modes** list.

4. Define the **Imaging** read settings or the **Re-analysis** settings.

   For more information about the **Imaging** read settings or **Re-analysis** settings, see the **SoftMax Pro Application Help**.

6. After you define the protocol settings, click **OK** to close the Settings dialog.

7. Create a template, if applicable. See Configure Microplate Templates on page 264.

8. Set Data Reduction parameters, if applicable. See Data Reduction on page 325.

9. Set Display options, if applicable. See Data Display Settings on page 318.

10. Save the settings to a protocol file. See Save Settings to Protocol Files on page 299.

Imaging Mode Protocol Overview

Imaging read mode does whole-cell imaging assays.

Whole-cell imaging assays are cell-based, or object-based, rather than the single-point measurements found in other types of microplate reads. These types of assays can yield more biologically meaningful results that can discriminate the fluorescence related to objects, such as cells or beads, from the bulk solution within a microplate well.

The measurement is primarily fluorescent with quantification of cell size, shape, area, and intensity. Label-free quantification is also supported through brightfield, transmitted light imaging. The camera resolution in the SpectraMax MiniMax 300 Imaging Cytometer is sufficient to determine the approximate shape of small 8 micron objects, such as blood cells.

For more information, see Imaging Read Mode on page 174.

Optimize Your Computer for Image Acquisition

Acquiring images requires a large portion of computer memory and resources.

Before starting an image acquisition, you must save the data file in a location with enough capacity for the image files. When you create a data file for an imaging experiment or a Western Blot experiment, the SoftMax Pro Software creates a folder with the same name as the data file. See Image Data Files and Folders on page 59.

Each acquired image file for an Imaging mode read can be larger than 2 megabytes. Acquiring the image of a single site in each well of a 96-well microplate can generate 300 megabytes of image data. A 384-well microplate can generate 1 gigabyte of image data. Acquiring images of multiple sites increases the data-storage requirement. Molecular Devices recommends that you employ a data management system.

For best results, save your imaging data file on a secondary internal hard drive. You can use an external hard drive, but this can slow the data acquisition and is not recommended.

Note: To prevent data loss, do not save your imaging data file on a USB flash drive or to a network location. These methods are not supported by Molecular Devices.

Before starting an image acquisition, minimize the demands on computer memory and resources by turning off all other programs. When the SoftMax Pro Software has limited access to computer memory and resources, image acquisition can take a long time. In some cases, images of some of the wells can be lost.

To prevent data loss, turn off all sleep and hibernation settings for the hard disk, the CPU, and the USB ports. Disable automatic Windows Updates. Update Windows manually when the instrument is not used by the software. You can set these options in Windows Control Panel. See Required Computer Settings on page 11.

Molecular Devices recommends that you use Auto Save with imaging data for saving to text files only. If you use Auto Save to save multiple imaging data files, each new data file is saved without the related folders required for saving images and analysis results. See Auto Save Settings on page 69.

Zoom Image in a Well

An image of the selected well displays in the **Image Acquisition**, **Image Selection**, and **Region of Interest** categories on the **Settings** dialog. To view a larger version of the image area, double-click the image to open the Zoom Well dialog.

In the **Zoom Well** dialog, the image area displays the acquired image from the selected well. To zoom the acquired image in and out, right-click the image and select **Zoom In** or **Zoom Out**. You can also hold the **CTRL** key down and press **+** or **-**, or use the mouse wheel to zoom in or out. Drag the scroll bars to move the zoomed image in the image area, or click the image and drag it into position.

To close the dialog, click **Close**.

Save Settings to Protocol Files

To save the settings for the experiment into a protocol file:

1. Click the **Application** button to open the Applications menu.
2. Click **Save As**.
3. In the **Save As** dialog, navigate to where you want to save the protocol file.
4. In the **File name** field, enter 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 the data in the file, leaving only the configuration information.

Collect Data from Microplates

You can start a read after you define the 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. The settings in the Template Editor have no effect on these raw values.

> **Note:** Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

To automatically read multiple **Plate** sections in the same experiment, see Enable Auto Read on page 301.

To read a microplate:

1. To open the microplate drawer of the instrument, click **Open/Close** on the **Operations** tab in the ribbon.

    The microplate carrier on the EMax Plus Microplate Reader moves in and out of the read chamber during the read, and then returns to the home position after the read ends. If the microplate carrier does not return to the home position, you can initialize the carrier to return it to its home position. Click **Initialize** on the **Operations** tab in the ribbon. See Operations Tab on page 43.

2. Insert the prepared microplate matching well A1 with position A1 in the microplate drawer. Make sure the microplate is flat against the drawer bottom or against the adapter.

3. Click **Open/Close** to close the microplate drawer.

4. For information about how to open and close other drawers on the instrument, see Open and Close a Drawer on page 47.

5. Open a SoftMax Pro Software data file or protocol file that contains the applicable experiment settings for the plate read. As an alternative, create new settings by selecting the **Plate** section in the SoftMax Pro Software program and configuring the instrument settings using the Settings dialog. See Select Instrument Settings on page 206.

6. Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

7. Click **Read** on the **Home** tab in the ribbon.

    The Read button changes to **Stop**, to enable you to terminate a read.

    You can also use the controls on the **Home** tab to interrupt and restart a read in progress. See Read Data on page 39.

8. The active **Plate** section is read.
   - If you use the SpectraMax i3x Multi-Mode Detection Platform, the SpectraMax Paradigm Multi-Mode Microplate Reader, the SpectraMax iD3 Multi-Mode Microplate Reader, or the FilterMax F3 and F5 Multi-Mode Microplate Readers and selected to do pre-read optimizations, the **Pre-Read Optimization Options** dialog appears. See Pre-Read Optimization on page 302.
   - If you use the SpectraMax i3x Multi-Mode Detection Platform or the SpectraMax Paradigm Multi-Mode Microplate Reader with a Tunable Wavelength (TUNE) Detection Cartridge and select to run spectral optimization, the **Spectral Optimization Wizard** runs before the microplate is read. See Spectral Optimization on page 307.

9. After the read is complete, the microplate drawer of the instrument opens to enable you to remove the microplate. If temperature control is on, the drawer closes again after approximately 10 seconds.

If you prefer to have the microplate remain inside the instrument after the read is complete, you can set this option in the **SoftMax Pro Options** dialog. See Application Settings on page 92.

Note: If you return to the instrument and find the microplate drawer closed after a read has finished, click **Open/Close** on the **Operations** tab in the ribbon or press the **Drawer** button on the instrument.

After the read completes, save the data. See Save Data Files on page 62.

For information on the data-analysis tools in the SoftMax Pro Software, see Analyze Data on page 315.

Enable Auto Read

Auto Read automatically advances to the next Plate section after a read in the sequence that the Plate sections display within a single Experiment. When you start a read for a Plate section that is enabled to automatically advance with AutoRead, the Auto Read feature advances to the next Plate section in the Experiment and then reads that plate. If multiple Plate sections are enabled to automatically advance with AutoRead, the Auto Read feature reads all enabled Plate sections until it reads a non-enabled Plate section or the last Plate section in the Experiment. Plate sections that come before the selected Plate section are not read.

For example, if you have five Plate sections (Plate 1, Plate 2, Plate 3, Plate 4, and Plate 5) in an Experiment, you can enable Plate 1, Plate 2, and Plate 3 to automatically advance with Auto Read. If you start a Read operation for Plate 2, then Plate 2, Plate 3, and Plate 4 are read in sequence. Plate 1 is not read, since it comes before Plate 2. Plate 5 is not read since Plate 4 is not enabled to automatically advance with AutoRead.

You can set an interval, or delay time, between plate reads.

The SoftMax Pro Software can collect data from one or more microplates and store it in a single data file, using the same or different instrument settings for different microplates. Assays that require a read in two or more read modes or read types can be combined in a single experiment and run with a single command in the software when you define separate microplate reads and enable Auto Read.

1. In the Navigation Tree or the workspace, select the Experiment that has the Plate sections that you want to enable to automatically advance with Auto Read.

2. Click **Auto Read** on the **Operations** tab in the ribbon.
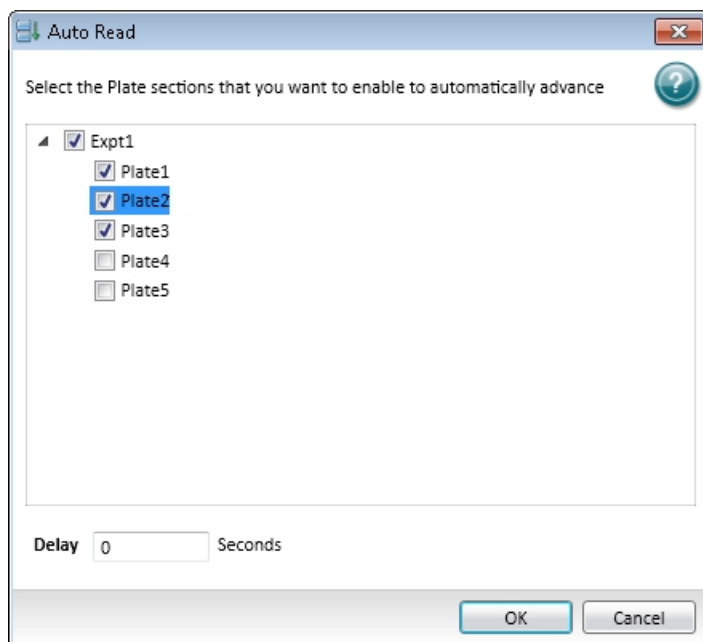
3. On the Auto Read dialog, select the Plate sections that you want to enable to automatically advance with Auto Read.

4. Optionally, enter the number of seconds to delay (the interval) between reading each Plate section.

5. Click **OK** to close the dialog.

> **Note:** If you have a Cuvette Set section following an enabled Plate section, Auto Read does not advance to or beyond the Cuvette Set section.

Pre-Read Optimization

For the SpectraMax i3x Multi-Mode Detection Platform, the SpectraMax Paradigm Multi-Mode Microplate Reader, the SpectraMax iD3 Multi-Mode Microplate Reader, or the FilterMax F3 and F5 Multi-Mode Microplate Readers in certain read modes, you can use pre-read optimization options. This function enables you to do microplate optimization and read height adjustment, when available.

The Pre-Read Optimization Options dialog appears when you select the **Show Pre-Read Optimization Options** check box on the **More Settings** tab on the Settings dialog. In the Acquisition View, you can add Plate Optimization to the beginning of the time line. The available optimization options are dependent on the instrument and the read mode.

> **Note:** For the SpectraMax i3x Multi-Mode Detection Platform, the SpectraMax iD3 Multi-Mode Microplate Reader, or the SpectraMax Paradigm Multi-Mode Microplate Reader with a 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 307.

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 303.
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 306.
3. Click **Run Optimization** to run the optimization options.
4. If the microplate has a lid, select the **Plate is Lidded** check box.
5. Optionally, for some instruments 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 can have a potential effect on measurement accuracy. SoftMax Pro Software lets microplate dimensions 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 displays if you select the **Show Pre-Read Optimization Options** check box on the **More Settings** tab on the **Settings** dialog. In the Acquisition View, you can add Plate Optimization to the beginning of the time line. See Select Instrument Settings on page 206.

When selected, the **Pre-Read Optimization Options** dialog runs after you click **Read**.

To do 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 304
- Run Optimization on page 304
- Select the Centers of the Four Corner Wells on page 305
- Verify Microplate Dimensions on page 305

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 correct 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 displays 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 Run Optimization on page 304. The optimization read starts automatically.

Run Optimization

Optimization in Progress displays the status of the optimization read and provides the means 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 **Cancel**.

When the optimization read is complete, you can select the centers of each of the four corner wells. See Select the Centers of the Four Corner Wells on page 305.

Select 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. Do 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 center of the well.

   Note: The cross hairs display after you place the cursor in the well image.

3. Click **Next** to define the centers of the remaining well reads.

After you define all four well centers, the Verify Microplate Dimensions step displays. See Verify Microplate Dimensions on page 305.

Verify Microplate Dimensions

Use the Verify Microplate Dimensions step to verify that the x and y offsets and distances between rows and columns are correct. You can edit the offsets, distances, and lot name 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. Define all offsets and well-spacing dimensions in millimeters.

3. If necessary, click the + next to **Microplate Name** to display the default name assigned to the new microplate definition.

4. Enter the microplate name.

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 more information, see Plate Type Settings on page 220.

If you select the **Run Read Height Adjustment** check box on the Pre-Read Optimization Options dialog, the Read Height Optimization Wizard runs before the plate is read. See Read Height Optimization on page 306.

Read Height Optimization

The SpectraMax iD3 Multi-Mode Microplate Reader, SpectraMax i3x Multi-Mode Detection Platform, and the SpectraMax Paradigm Multi-Mode Microplate Reader feature an objective lens that can be moved up and down to optimize the read height used in luminescence, fluorescence intensity, 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 is displayed if the **Show Pre-Read Optimization Options** check box is selected on the **More Settings** tab of the Settings dialog. In the Acquisition View, you can add Plate Optimization to the beginning of the time line. See .

When selected, the Pre-Read Optimization Options dialog appears after you click **Read**.

To do Read Height optimization:

1. Select the **Run Read Height Adjustments before reading the plate** check box.
2. Select **Microplate options**.
3. Click **Run Optimization**.

Run Read Height Optimization

Select the well to be measured to do 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 a minimum of 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 that contains the optimization sample.
3. Click **Next** to start the optimization.

   The Optimization in Progress step displays. 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 displays, displaying the Optimized Read Height. To specify a different read height, enter 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 most fluorophores or luminescence labels that are compatible with the wavelength ranges of the instrument or detection cartridge.

Spectral optimization for fluorescence intensity reads is available for the SpectraMax iD3 Multi-Mode Microplate Reader and for the SpectraMax i3x Multi-Mode Detection Platform using the built-in monochromator.

Spectral Optimization for both fluorescence intensity and luminescence reads is available for the SpectraMax Paradigm Multi-Mode Microplate Reader with the Tunable Wavelength (TUNE) Detection Cartridge.

To run the Spectral Optimization Wizard, select **Unknown** for the Wavelengths on the Settings dialog.

When you select **Unknown**, the Spectral Optimization Wizard dialog displays after you click **Read**.

Run 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 a minimum of 20 nm greater than the **Start Excitation Wavelength** value. Molecular Devices recommends using an emission value that is a minimum of 40 nm greater than the excitation value.

   To do Spectral Optimization, there must be a minimum of 10 data points in the range for both the excitation and emission wavelengths. Make sure that the Wavelength Increment permits a minimum of 10 data points in each range.

   For a luminescence read, only emission values can be specified.

2. To specify the **Reading Height** or **Integration Time**, click **Advanced parameters** and then enter 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 Blank Well step, select the well in the plate layout that corresponds with a well in the microplate that contains buffer or other liquid for background correction.

5. Click **Next** to continue.

6. In the Sample Well step, select the well in the plate layout that corresponds with the well in the microplate that contains your sample.

7. Click **Next** to continue.

8. Wait while the indicator in the Optimization in Progress step displays the progress.

9. In the Optimization Complete step, a 3-dimensional heat map image displays. 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 enter values in the fields.

The Emission value must be a minimum of 20 nm greater than the Excitation value. Molecular Devices recommends using an emission value that is a minimum of 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 displays, 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 **Yes** in the message that appears.

Right-click on the image to display the shortcut menu, and then select from the following options:

- **Copy Raw Data** copies the data in tab-delimited format that can be pasted into a spreadsheet or text editor. This option is available only for the Monochromator of the SpectraMax i3x Instrument.
- **Copy Image** copies the image into a bitmap file that can be pasted into an image editing program.
- **Save Raw Data to Disk** saves the data into a text file in tab-delimited format. This option is available only for the Monochromator of the SpectraMax i3x Instrument.
- **Save Image to Disk** saves the image into a .bmp file.

10. Click **Read** to save the protocol with the specified excitation and emission wavelengths and use these values to read the microplate.

If you select to do pre-read optimizations, the Pre-Read Optimization Options dialog appears before the microplate is read. See Pre-Read Optimization on page 302.

Collect Data from Cuvettes

Two types of data collection are possible using a cuvette: a reference and a sample reading.

You can start a reading after you define the 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 required number of cuvettes in the section. As an alternative, 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 a cuvette in the Cuvette Set section and choose to read it. Click **Read** ▶ on the **Home** tab in the ribbon.

The Read button changes to **Stop** ✕, to terminate a read.

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 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 correct reference in the cuvette port.
3. Click **Ref**.

The time and date stamp of the reference for the selected cuvettes changes.

Any new cuvettes you create 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 select specific cuvettes. The reference value does not display 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 Cuvette Set section and Ref on air (empty cuvette port).
2. Read a cuvette that contains 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 click **Read**.
6. Click **Read** again to read the remaining samples.

The optical density of the cuvette you designate 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 applicable 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 more 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 displays in the Values column. The reference value for each sample displays in the custom column you created.

Collect Data from Membrane Holders

You can start a read after you define the instrument settings.

> 📓 **Note:** Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

To automatically read multiple Plate sections in the same experiment, see Enable Auto Read on page 301.

If you use the SoftMax Pro GxP Software, you must enable the following permissions to complete a Western Blot membrane read:

- **Read empty plates/cuvettes** to start a read.
- **Save data files** to save the data file before the read.
- **Overwrite plate/cuvette data** to do a high-resolution ROI read.

See SoftMax Pro GxP Software on page 417.

To read a membrane:

1. Open the drawer of the instrument by clicking **Open/Close** 📷 on the **Home** tab in the ribbon.

2. Insert the prepared membrane holder matching the A1 position on the membrane holder with the A1 position in the drawer in landscape orientation. Make sure the membrane holder is flat against the drawer bottom.

   > ⚠️ **CAUTION!** To prevent damage to the instrument or the membrane holder, make sure that all the holder clips are securely snapped into place before you insert the membrane holder into the microplate reader.

3. Click **Open/Close** 📷 to close the drawer.

4. Open a SoftMax Pro Software data file or protocol file that contains the applicable experiment settings for the membrane read. As an alternative, create new settings by selecting the **Plate** section in the SoftMax Pro Software program and configuring the instrument settings using the **Settings** dialog. See Select Instrument Settings on page 206.

5. Before you start a read, it is good practice to save your data file. For Imaging reads and Western Blot reads, you must save your data file before you start the read. See Save Data Files on page 62.

6. Click **Read** ▶ on the **Home** tab in the ribbon.

   The Read button changes to **Stop** ✖, to terminate a read.

7. The active **Plate** section is read.

8. To select and scan a region of interest (ROI), click ▦ on the left side of the Plate section and then drag across the image to define the region. Click **Read** ▶ on the **Home** tab in the ribbon to scan the region using the ROI Scan Resolution you define on the Settings dialog. The data from the ROI scan replaces the data from the original scan. See View Western Blot Membrane Data on page 395.

9. When the read is complete, click **Open/Close** ▦ to open the drawer and remove the membrane holder.

After the read is complete, save the data. See Save Data Files on page 62.

For information on the data-analysis tools for Western Blot images, see View Western Blot Membrane Data on page 395.

Chapter 8: Analyze Data

8
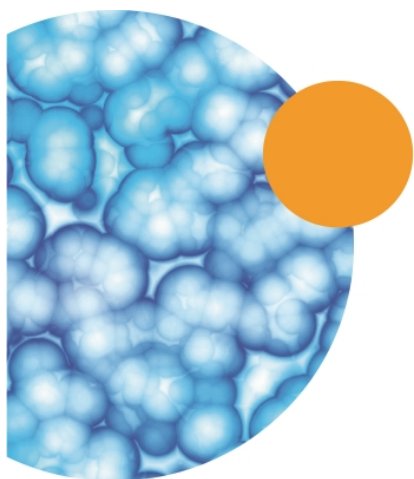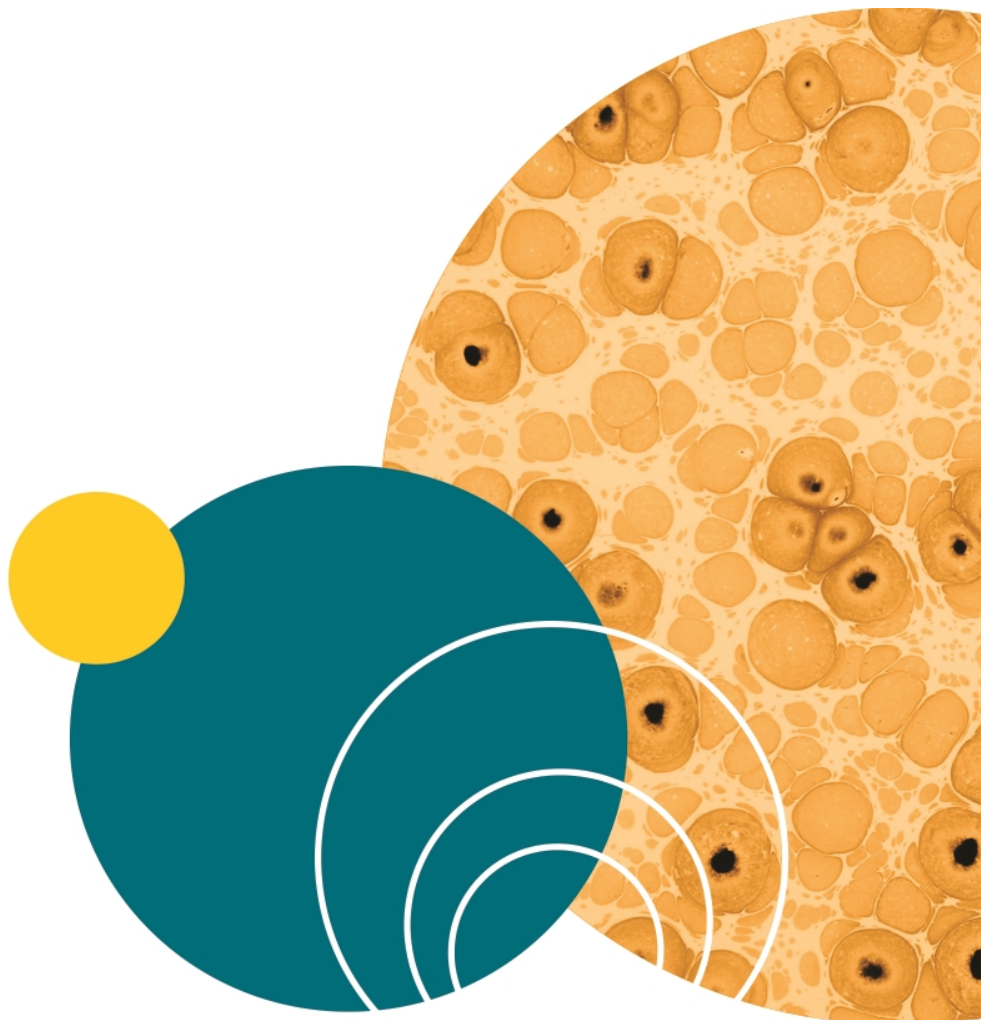
Each SoftMax Pro Software data file has a minimum of one Plate section or one Cuvette Set section. Most files also have an assigned plate template and related Group sections, Graph sections, and Note sections. However, depending upon the protocol and how you want to report the data, only some of these 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, let you 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 316
- Data Display Settings on page 318
- Data Reduction on page 325
- View Data in Three-Dimensional Graphs on page 337
- Graph Data on page 343
- Analyze Western Blot Images on page 400

Calculations and Numerical Precision

The calculations done by the SoftMax Pro Software on real numbers use 64-bit double-precision (15+ digits of precision) as defined by the *Institute of Electrical and Electronics Engineers standard for floating point arithmetic (IEEE 754)*. Computations always use the maximum available precision. Rounding occurs only as required for display formatting purposes, or in the evaluation of a formula in which the Round operator is used.

See "Technical Details of Numerical Calculations in the SoftMax Pro Software" in the application help.

Numbers from Plate sections, Cuvette Set sections, Group sections, and Summaries are displayed, printed, and exported with as many 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 double-precision.

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

You can customize the data display to **Numeric notation** or **Scientific notation** format, and specify the number of **Significant Figures** or **Decimal Places**. These options can be set separately for raw data and reduced data.

When the number of digits is increased, the font size of the values in the Plate section is decreased. If the value becomes too long, then the numbers are cut off at the edge of the wells.

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 microplate reader ultimately determines the precision of the calculations. For more information about the numerical resolution of a reader, see the documentation for that reader.

Note: In versions of the SoftMax Pro Software older than version 6.0, the software used a text-conversion algorithm with a slight upward bias. This can result in a mismatch between the rounded values in the newer version and the older version. For example, when rounding the raw value 14.849999… to one decimal place, versions 6.0 and newer display 14.8, while older versions display 14.9. This does not affect the accuracy of the calculations, since rounding occurs only as required for display formatting purposes, or in the evaluation of a formula in which the Round operator is used. Computations always use the maximum available precision.

For information on suspending and resuming calculations, see Operations Tab on page 43.

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 Absorbance Read Mode on page 137.
- For information on pathlength calculations, see PathCheck Pathlength Measurement Technology on page 139.
- For information on Fluorescence Polarization calculations, see Fluorescence Polarization Read Mode on page 164.
- For information on kinetic and spectrum reductions, see Kinetic Data Reduction Options on page 333 and Spectrum Data Reduction Options on page 335.
- For information on curve fit calculations, see Curve Fit Functions on page 356.
- For a technical discussion of calculations and numerical precision, see "Technical Details of Numerical Calculations in the SoftMax Pro Software" in the application help.
- For information on statistical functions, see the *SoftMax Pro Software Formula Reference Guide*.

Calculation Hierarchy

When reducing the data collected from the instrument, the SoftMax Pro Software does calculations hierarchically. If an option in the hierarchy 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 done, or has not been defined in the Template dialog or the Reductions dialog, then the SoftMax Pro Software continues with the next listed calculation in the hierarchy.

The following is the general hierarchy of reduction calculations:

1. Apply Well Masking
2. Subtract Plate Background constant
3. Apply PathCheck Technology normalization
4. Subtract Plate Blank
5. Apply Smoothing
6. Time Alignment (Interpolate Raw Data)
7. Subtract Group Blank (pre-reduction option)
8. Calculate mP or Anisotropy
9. Set first data point to zero
10. Convert OD to %T
11. Apply Reduction Limits
12. Apply Wavelength Combination
13. Apply Baseline Options
14. Apply Kinetic or Spectrum Reduction
15. Subtract Group Blank (post-reduction option)

Data Display Settings

In an active Plate section or Cuvette Set section, you can change how the data is presented.

Click **Display** on the **Home** tab in the ribbon or in the toolbar at the top of the section.

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

For Imaging read mode **Imaging Data** replaces the **Raw Data** option.

You can customize the data display to **Numeric notation** or **Scientific notation** format, and specify the number of **Significant Figures** or **Decimal Places**. These options can be set separately for raw data and reduced data.

The **Default** format determines the number of decimal places to display based on the space available. For example, for a plate with 96 wells or fewer, each well is limited to five digits, plus one character for the decimal point, if applicable. For a 384-well plate, each well is limited to four characters, including the decimal point. If the value requires greater precision than can fit in the available space, the format changes to scientific notation.

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.

You can view both raw and reduced data or multiple reductions of the same data by cloning a plate. See .

View 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

Select **Raw Data** to display 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, select **with Reduced Number**.

View 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 Data Reduction on page 325.

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, enter 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, enter values in the fields. To also view the reduced number, select the **Reduced Number** check box.

View Imaging 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 **Imaging Data**.

   To display reduced imaging data, see View Reduced Data on page 319.

3. Select a **Data Type**. The options available in the measurement selections from the **Image Analysis Settings** in the Settings dialog. See View and Select Measurement Statistics on page 239.

4. Optionally, click **with Reduced Number** to include the reduced data number on the display.

5. Click **OK**.

Imaging Data Options

The **Data Type** list contains the measurement selections from the Image Analysis Settings on the Settings dialog. See View and Select Measurement Statistics on page 239.

Select from one of the **Data Types** that follow. Separate data types are available for each measured wavelength and classification, if applicable.

- **Object Count** gives the total number of objects detected in the image. This measurement is not used for field analysis of confluent areas.

- **Field Count** gives the total number of confluent areas detected in the image. This measurement is not used for discrete object analysis.

- **Object Percentage** gives the percentage the objects detected in the image by classification. This measurement is not used for field analysis of confluent areas.

- **Covered Area** gives the combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.

- **Object Area** gives the average area of the objects detected in the image expressed in μm^2. This measurement is not used for field analysis of confluent areas.

- **Field Area** gives the average area of the confluent areas detected in the image expressed in μm^2. This measurement is not used for discrete object analysis.

- **Object Roundness** gives the average roundness of each object detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for field analysis of confluent areas.

- **Field Roundness** gives the average roundness of each confluent area detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for discrete object analysis.

- **Object Average Intensity** gives the average fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).

- **Field Average Intensity** gives the average fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).

- **Object Intensity** gives the average total fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).

- **Field Intensity** gives the average total fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).

- **Total Intensity** gives the combined total fluorescent signal intensity of the objects or confluent areas detected in the image expressed in million intensity counts. This measurement is not used for transmitted light (TL).

Zoom 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 magnify the display of imaging data in a well, see View Imaging Data in Wells on page 322.

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. You can also double-click the well to open the Zoom Well dialog.

   The data from the selected well or cuvette is displayed 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 displays. See Data Reduction on page 325.

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

- ● **Graph Type Settings** lets you enable or disable connected points or plotted symbols on the graph.
- ● **Characteristics** lets you 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**. These changes have an effect on only the well graph being viewed.

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

View Imaging Data in Wells

You can view a combination of the raw image and the analysis data in up to four wells from a **Plate** section or the two wells from the Image Analysis Settings. See Plate Sections on page 105 or Image Analysis Settings on page 233.

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

To display the **SoftMax Pro MiniMax Well Viewer** dialog from a Plate section, double-click the well that you want to zoom. You can also click **Zoom** 🔍 on the **Home** tab in the ribbon or **Zoom** 🔍 in the toolbar at the top of the section. In the Image Analysis Settings Measurement Selection, click **Show Details**.

To view more than one well in a Plate section, drag your pointer to select up to four wells before opening the Zoom Well dialog.

It can take some time before the **SoftMax Pro MiniMax Well Viewer** dialog displays. It can also display behind the SoftMax Pro Software window. To bring the well viewer to the front, click the **SoftMax Pro MiniMax Well Viewer** icon in the Windows task bar.

The **SoftMax Pro MiniMax Well Viewer** dialog displays a zoomed image of the well, a table of data, and chart of the data. To choose to display one or more of these areas, select or clear the **Show Images**, **Show Tables**, or **Show Charts** check boxes in the lower-left area of the dialog.

The three areas in the dialog are interactive.

- Click the cells in the image to highlight the data in the table and the data in the chart.
- Click the data in the table to highlight the cells in the image and the data in the chart.
- Click the data in the chart to highlight the cells in the image and the data in the table.

To use an image of the **SoftMax Pro MiniMax Well Viewer** dialog in a Microsoft PowerPoint presentation, click **Send to PowerPoint**. A new document opens in PowerPoint with an image of the **Zoom Well** dialog in a slide.

Image Area Options

The image area displays the acquired image from the selected well. To zoom the acquired image in and out, right-click the image and select **Zoom In** or **Zoom Out**. You can also hold the **CTRL** key down and press **+** or **-**, or use the mouse wheel to zoom in or out. Drag the scroll bars to move the zoomed image in the image area, or click the image and drag it into position.

Click the buttons above the image to switch the view of the image between wavelengths, and to display or hide the found objects or confluent areas.

- When a button is filled in, the related wavelength, objects, or areas are displayed in the image.
- When a button is outlined, the related wavelength, objects, or areas are not displayed in the image.

When you click a button above the images, it affects the display of all the images.

Use the controls below the image to separately adjust the intensity of each wavelength.

From the list on the left, select the wavelength that you want to adjust.

Drag the sliders to adjust the intensity range of the image.

- The bar on the left controls the low level of intensity.
- The bar on the right controls the high level of intensity.

When you drag a bar the intensity level displays above the bar.

To move the sliders to the outside edges, click the double-arrow button on the left.

From the list on the right, select the color for the view.

To automatically scale the intensity of all the wavelengths in the image, click **Auto Scale All Wavelengths**.

Table Area Options

In the table area, you can change the size the columns by dragging the separator lines. Drag the scroll bars to view the data in the table.

To use the table data in Microsoft Excel, click **Send to Excel**. A new spreadsheet opens in Excel with the data from the table in the cells.

Chart Area Options

To change the type of chart that displays in the chart area, click **Histogram** or **Scatter Plot**.

- For a histogram, you can select a data type for the **X Axis Data**.
- For a scatter plot, you can select data types for the **X Axis Data** and the **Y Axis Data**.

Mask 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**.

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 does calculations hierarchically. Only the data reduction calculations that apply to the data based on the instrument read mode, read type, and settings are done. See Calculation Hierarchy on page 317.

Click **Reduction** on the **Home** tab in the ribbon or in the toolbar at the top of the section 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 does 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 139.
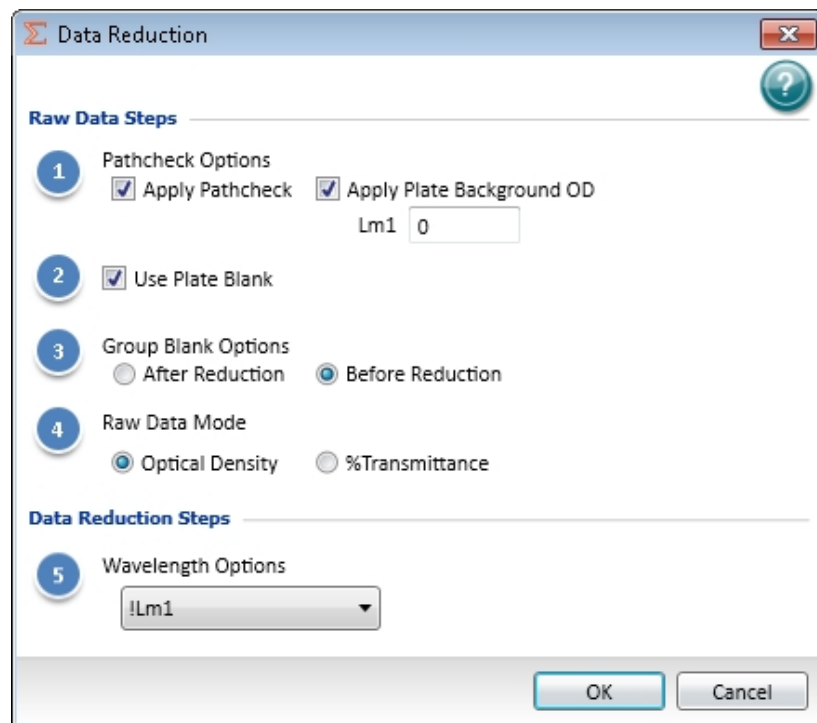
Apply Plate Background OD (Absorbance Endpoint only)

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 Define Blanks on page 270.

Smoothing

Smoothing is used to reduce noise in kinetic reads for FlexStation Instrument data. This setting takes the moving average of the raw data and displays the plot using the averaged values.

The default is no smoothing, or a setting of 1 "averaged" point.

- A setting of 3 provides the least amount of smoothing, where the value of the center point is added to the values of the points on either side. The sum of these points is then divided by three and the resulting value is substituted for the center point.
- A setting of 9 averages the center point with the four points on each side.

When multiple wavelengths are read, smoothing is done before doing wavelength combination calculations such as ratios or multiplication. For example, if you read at two wavelengths and choose to take a ratio of those wavelengths, smoothing is performed on each individual wavelength and the wavelength ratio is calculated using smoothed data.

Time Alignment

For FlexStation Instrument data, each data value is logged with its own read time. The SoftMax Pro Software can align the time points by **Wavelength** or by **Well** and interpolate the data against a single time point.

- Select the **Time Alignment** check box and then click **Wavelength** so that you can do wavelength combination calculations correctly. For example, you can take a ratio of two different wavelengths (Lm1/Lm2) in a single well that have been read at two different time points. The **Wavelength** option is available only when two or more wavelengths are present in the data.

  With **Wavelength** selected, the SoftMax Pro Software uses quadratic interpolation to calculate the interpolated values. This results in the loss of one point at each end of the plot. Before starting the calculation, the software determines the starting point by averaging the initial data points of the separate wavelengths. For example, if the first data points of the two wavelengths have time values of 0.3 and 0.5 seconds, the software averages these data points and uses 0.4 seconds as its initial point for interpolation.

- Select the **Time Alignment** check box and then click **Well** to normalize the kinetic read times of the wells in the column against the read time of the first well in the column.

  Injection occurs at the same time in all the wells of a column, but each of the wells is read sequentially. So, each well is read at a different time point during the reaction from the injection. With **Well** selected, the data in the wells are interpolated against the time point of the read of the first well.

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 process or a post-reduction process.

- To enable the group blank value subtraction calculation as a pre-reduction process, click **Before Reduction**.
- To enable the group blank value subtraction calculation as a post-reduction process, 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 Define Blanks on page 270.

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 the SoftMax Pro Software. Click the data type to use as the raw data for the reduction.

G Factor

The 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**, enter 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 quantity of light passing through a sample to a detector relative to the total quantity 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 done on a linear scale, while %T calculations are done on a logarithmic scale. However, SoftMax Pro Software does not do 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 the readings that apply to other calculations since the data might not be calculated correctly.

Data Reduction Steps

Data Reduction display includes one or more of the following calculations:

Baseline Options

Baseline options affect the way that the data is graphed for FlexStation Instrument data.

- **Absolute** displays the raw data without any change in the way it is graphed. Raw data is used in subsequent reduction calculations.

- **Zero Baseline** offsets the graph slightly. For example, if you enter **5** for the number of points, then the average of the first 5 points is used as the baseline value, and all raw data are subtracted from this value.

  Baseline subtraction is applied only to the reduced data. The raw data plots do not change. To view the plot with baseline subtraction, select **Reduced Plot** in the Display dialog or select **Show Reduced** in a Zoom Well dialog.

  If two or more wavelengths are present in a reading, and you combine these wavelengths while **Zero Baseline** is selected, the zero baseline applies only after the wavelength combination.

- **% Baseline** makes data from a FlexStation Instrument look similar to data from a FLIPR System. Data from a FLIPR System can be imported into the SoftMax Pro Software, and this option modifies the plot and reduction calculations to make data from a FlexStation Instrument comparable to data from a FLIPR System.

  This method averages the first number of points you specify (the upper box), divides the raw data by this average value, and then multiplies by 100 (%). Enter a value from 0.001 to 99999 to use to multiply the % Baseline value.

  This calculation is done only on the reduced data and only after any wavelength combinations are calculated.

  To view the modified plot, select **Reduced Plot** on the Display dialog or select **Show Reduced** on the Zoom Well dialog.

Limits

Apply the specified reduction limits to the reduced data after this step. To specify the limits, enter values in the fields.

Limits define the data that are viewed and included in data reduction. If you change a limit to show less data, you can change the limit to display the excluded data again.

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. 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. 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 before 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. 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. 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. Values from the reading that are above this limit do not display 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 𝑓 to open the Formula Editor. See Data Reduction Formulas on page 331.

Kinetic Reduction

Select a Kinetic reduction mode from the list. To use a custom reduction, select **Custom** from the list and then, click 𝑓 to open the Formula Editor. See Data Reduction Formulas on page 331.

For more information about kinetic reductions, see Kinetic Data Reduction Options on page 333.

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 [f(x) icon] to open the Formula Editor. See Data Reduction Formulas on page 331.

For more information about spectrum reductions, see Spectrum Data Reduction Options on page 335.

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 [f(x) icon] to open the Formula Editor. See Data Reduction Formulas on page 331.

Group Blank Subtracted

When you select **After Reduction** 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 Group Sections on page 123 and Graph Data on page 343.

Custom Reduction Formulas

If the predefined reduction formulas do not meet your needs, you can create custom reduction formulas. Select **Custom** from the menus or dialogs to display a Formula button that you can click to open a Formula dialog.

Examples of some formulas you can use when combining multiple wavelengths in a Formula dialog are in the following tables.

Table 8-1: 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 8-2: 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 Pathlength Measurement 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 a well with an OD/RFU/RLU less than A, "high" for an OD/ RFU/RLU greater than B, and the OD/ RFU/RLU of a well 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 8-3: 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 8-4: Spectrum or Kinetic Reduction Formula Example

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.

The accessor names for custom reduction formulas for imaging data are enclosed in square brackets to prevent conflicts with other formula elements.

Table 8-5: Imaging Reduction Formula Example

Formula	Description
![TypeAObjectCount] - ![TypeBObjectCount]	Reports the difference between the number of Type A and Type B cells detected in the image.

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 displays 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 done 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 that starts at the first reading as defined by **Lag Time** and ends at a total number of readings equal to the **Vmax Points** setting. The second and subsequent slopes are calculated to start at the second time point and end at a total number of readings equal to the **Vmax Points** setting. 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 have an effect on 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 331.

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 331.

Imaging Data Reduction Options

The **Imaging Data** list contains the measurement selections from the **Image Analysis Settings** on the Settings dialog. See View and Select Measurement Statistics on page 239.

Select from one of the reduction data types that follow. Separate data types are available for each measured wavelength and classification, if applicable.

- **Object Count** gives the total number of objects detected in the image. This measurement is not used for field analysis of confluent areas.
- **Field Count** gives the total number of confluent areas detected in the image. This measurement is not used for discrete object analysis.

- **Object Percentage** gives the percentage the objects detected in the image by classification. This measurement is not used for field analysis of confluent areas.
- **Covered Area** gives the combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area** gives the average area of the objects detected in the image expressed in μm^2. This measurement is not used for field analysis of confluent areas.
- **Field Area** gives the average area of the confluent areas detected in the image expressed in μm^2. This measurement is not used for discrete object analysis.
- **Object Roundness** gives the average roundness of each object detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for field analysis of confluent areas.
- **Field Roundness** gives the average roundness of each confluent area detected in the image. A shape factor of 1.00 is perfectly round, while a shape factor of 0.00 is not round at all. This measurement is not used for discrete object analysis.
- **Object Average Intensity** gives the average fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).
- **Field Average Intensity** gives the average fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).
- **Object Intensity** gives the average total fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. This measurement is not used for transmitted light (TL).
- **Field Intensity** gives the average total fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis. This measurement is not used for transmitted light (TL).
- **Total Intensity** gives the combined total fluorescent signal intensity of the objects or confluent areas detected in the image expressed in million intensity counts. This measurement is not used for transmitted light (TL).

To use a custom reduction, select **Custom** from the list and then, click to open the Formula Editor. See Data Reduction Formulas on page 331.

View Data in Three-Dimensional Graphs

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 on 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.
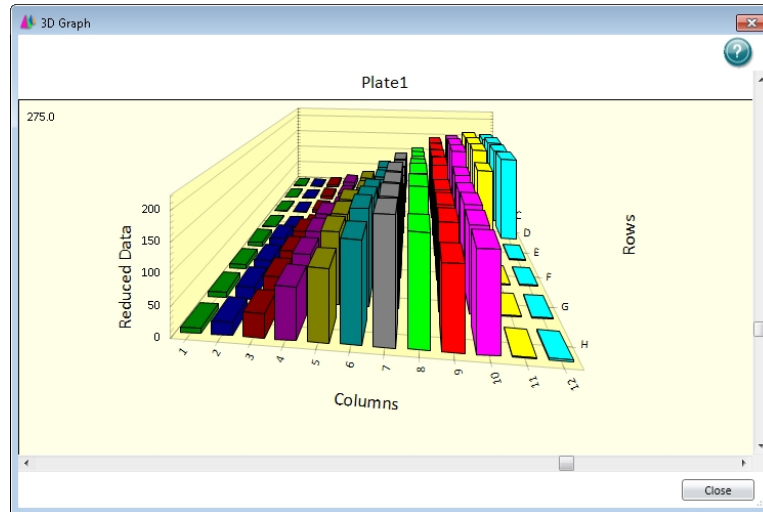
To rotate the 3D graph:

- 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 on the 3D graph.

You can choose a customization option from the menu, or click **Customization Dialog** to change several options at the same time. See Customize Three-Dimensional Graphs on page 338.

Other options in the 3D graph dialog include:

- **Plotting Method** lets you 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 is displayed** the Export Control dialog lets you export the graphical image to your choice of formats, or export the data to a text format. See Export 3D Graphs on page 340.

To close the 3D Graph dialog, click **Close**.

Customize Three-Dimensional Graphs

The customization dialog has five tabs that contain customization options for the current 3D graph.
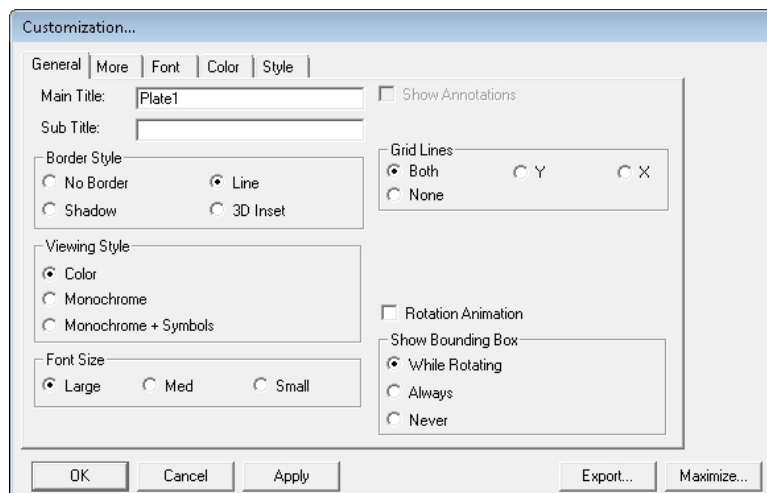
Click a tab to work with the options under that tab.

- For the **General** tab, see 3D Graph General Customization Settings on page 338.
- For the **More** tab, see More 3D Graph Customization Settings on page 339.
- For the **Font** tab, see 3D Graph Font Customization Settings on page 339.
- For the **Color** tab, see 3D Graph Color Customization Settings on page 339.
- For the **Style** tab, see 3D Graph Style Customization Settings on page 340.

After you make changes, click **Apply** to view how the changes are applied to the graph.

To close the Customization dialog and save unapplied changes, click **OK**.

Click **Export** to close the Customization dialog and open the Export Control dialog that enables you to export the graphical image, or to export the data to a text format. See Export 3D Graphs on page 340.

Click **Maximize** to close the Customization dialog and expand the graph to a full screen view. To return to the normal view, click the title bar or press **Esc**.

3D Graph General Customization Settings

Select the **General** tab to customize the 3D graph with the following options:

- **Main Title**: Enter the text you want for the title of the graph.
- **Sub Title**: Enter the text you want below the title of the graph.
- **Show Annotations**: To show notes, select this check box. This option is not available if there are no notes 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 graph, select this check box.
- **Show Bounding Box**: To display a wire-frame box around the graph under specific conditions, click **While Rotating**, **Always**, or **Never**.

More 3D Graph Customization Settings

Select the **More** tab to customize the 3D graph with the following options:

- **Rotation Detail**: To change the display style when the 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 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 graph.
- **Plotting Method**: To change the display of the data plot, click **Wire Frame**, **Surface**, or **Surface with Shading**.

3D Graph Font Customization Settings

Select the **Font** tab to customize the text in the 3D graph:
- **Main Title** includes only the title of the graph.
- **Sub-Title** includes only the text directly below the title of the graph, if applicable.
- **Subset/Point/Axis Labels** includes all of the other text in the 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** displays at the bottom.

3D Graph Color Customization Settings

Select the **Color** tab to customize the colors used in the 3D graph.

Changes in the **Color** tab are applied to the 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 graph.
- **Desk Background** includes the area behind the title and subtitle of the graph.

- **Shadow Color** includes the border shadowing when you select Shadow for the Border Style on the General tab.
- **Graph Foreground** includes all the text other than the title and subtitle, and the border around the graph.
- **Graph Background** includes the area within the border and behind the graph.
- **X and Z Axis Plane** includes the floor of the graph.
- **Y Axis Plane** includes the walls of the graph.

Quick Styles group lets you select from pre-defined styles for the graph:

- Select the **Bitmap/Gradient Styles** check box to add texture to the graph.
- Click the **Inset**, **Shadow**, **Line**, and **No Border** options in the **Light**, **Medium**, and **Dark** columns to view what effect the selected styles have on the graph.

3D Graph Style Customization Settings

Select the **Style** tab to customize colors used in the 3D graph.

Changes in the **Style** tab are applied to the graph as they are selected.

The customizable items in the list on the left are dependent on the some of the selections on the other tabs, and on the type of data.

Click an item in the list, and then click a color to apply to it.

Export 3D Graphs

Before you export an image of a 3D graph, make sure that you rotate the graph to the view that you want and that you set your customization options. See Customize Three-Dimensional Graphs on page 338.

For export options, right-click on the graph and then click **Export Dialog** to open the Export Control dialog.
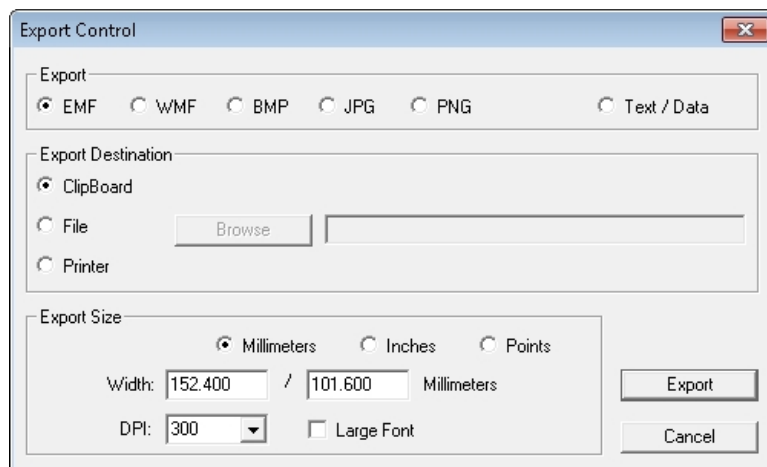
Click the graphic format from the Export area. If you select **Text/Data**, only the data is exported. For more information about how to export the data, see Export Data from 3D Graphs on page 341.

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 to paste it into a different 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 to export to a Printer.
- **No Specific Size** is available for the WMF format export to the clipboard or a file. Click this option to let the export operation select the size for the image.
- **Pixels** is available for BMP, JPG, and PNG formats export to the clipboard or a file.
- **Millimeters**, **Inches**, and **Points** are available for EMF and WMF formats export to the clipboard or a file, and for all graphic formats export to a printer.
- If you select measurement units, enter the width and height for the export image.
- For all graphic formats export to the clipboard or a file, select the **DPI** (dots per inch) for the export image.
- For all graphic formats export to the clipboard or a file, select the **Large Font** check box to increase the size of the text in the export image.

When you finish your export options, click **Export** or **Print**.

If you export the data as text, then the Export dialog displays with more options to format the export data. See Export Data from 3D Graphs on page 341.

Export Data from 3D Graphs

To export just the data from a 3D graph:

1. Right-click on the graph and select **Export Dialog**.

2. On 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 to paste it into a different 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** section, select to export **All Data** or **Selected Data**.

   If you select **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 select to export just the **Data** or the **Data and Labels**.

7. If you have multiple values available in your data, select an option in the **Data to Export** section.

8. In the **Export Style** section, 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 export data.

   - When you export a **Table**, 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.

9. In the **Numeric Precision** area, click **Current Precision** to export the data using the rounding defined in the graph, or click **Maximum Precision** to export the rounding available in the data file.

10. Click **Export** to export the data to the format you define in Step 3.

> **Note:** If you export 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.

Graph 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 you create a graph, you can add and delete plots, customize the axes, and change the size and type of graph. You can enable and disable the grid lines for the graph. Grid lines are enabled by default.

Other methods to visualize data include viewing a zoomed well plot of the data in a well in a Plate section or a cuvette in a Cuvette Set section, and viewing a three-dimensional graphical display of the data. See Zoom Display of a Well or Cuvette on page 321 and View Data in Three-Dimensional Graphs on page 337.

Graph Tools

Graph sections enable you to plot information from groups as scatter plots or bar graphs.

You can create more than one Graph section within a data file, and you can create plots in the Graph section from any Experiment in the file.
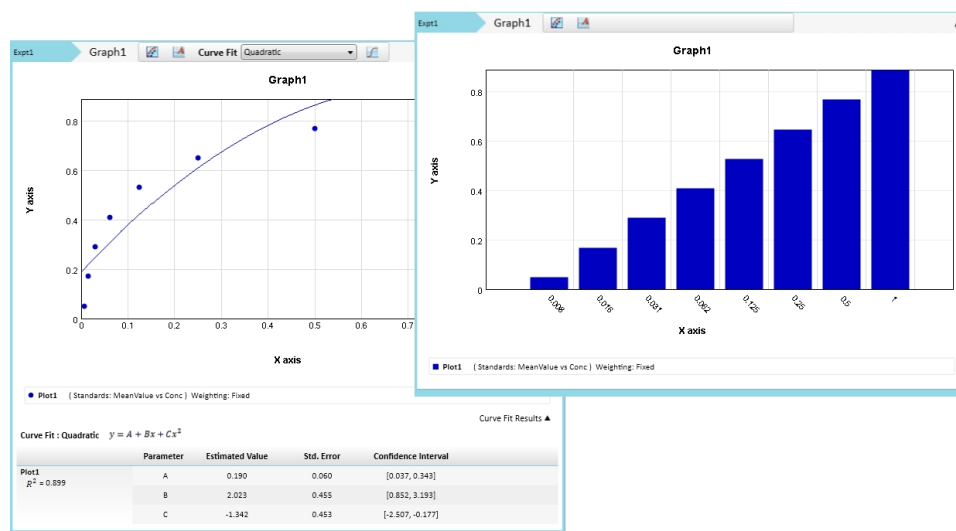
Graph sections are divided into the following areas:

- The body of the Graph section.
- The Legend. See Graph Legend on page 344.

To change the name of the Graph section, right-click the Graph section in the **Navigation Tree** and select **Rename**. See Rename Experiments or Sections on page 31.

For more information about graphs, see the following topics:

- Create Graphs on page 345
- Edit Graphs on page 347
- Curve Fit Settings on page 348
- Change Graph Section Name on page 391

- [Change Graph Appearance on page 391](#)
- [Zoom Graph Display on page 394](#)
- [Export an Image of a Graph Section on page 394](#)

Graph Legend

The graph legend for a scatter plot 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. A bar graph does not have a legend.

Independent Fits

For each curve fit function:

- The formulas for the selected curve fits
- For each plot:
  - R^2 value
  - EC_{50} value (where applicable)
  - For each parameter:
    - Estimated Value
    - Standard Error (Std. Error) of the estimate
    - Confidence Interval 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 based on 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 354](#).
    - 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 a good fit for the data set.

      For example, if the data set is sigmoidal with clear lower and upper asymptotes, 4-parameter fits should be applicable 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 344)
  - Independence (see explanation under Independent Fits on page 344)
  - EC_{50} value (where applicable)
- The list of weighting formulas
- R^2 value
- For each parameter:
  - Estimated Value
  - Standard Error (Std. Error) of the estimate
  - Confidence Interval of the estimate
  - Independence

Create Graphs

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

The Plot Editor dialog enables you to name the graph, assign specific information to plot on the X and Y axes, and choose which icon and color to use.

All groups created or assigned within the file display in the **Available Groups** list.

To create a new graph:

1. Click **New Graph** in the Plate section on the **Home** tab to open the Plot Editor dialog.
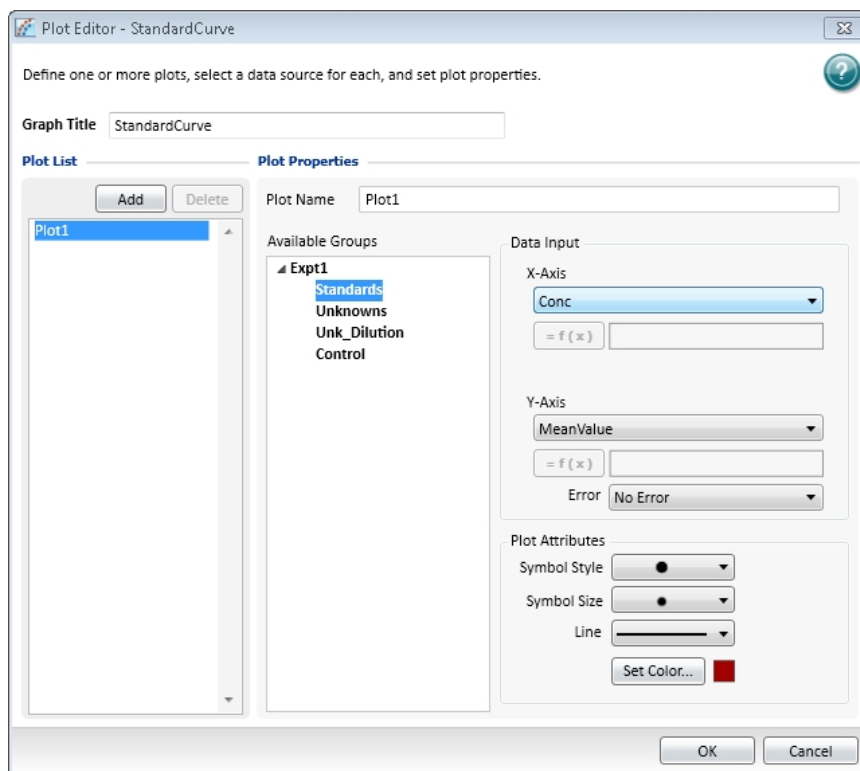
2. In the **Graph Title** field, enter a name for the graph.

3. Select a plot.

   The **Plot List** displays the 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 click **Delete**.

   When you select a plot from the list, the parameters for that plot display in the **Plot Properties** section.

4. Optionally, enter a name for 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 is plotted in the graph.

   > **Note:** Groups are defined using the template editor. If no groups display in the list, go to the Template Editor dialog and define one or more groups. See Define Groups on page 265.

6. In the **Data Input** section, select column types from the list to assign to the plot in the X-Axis and Y-Axis. The list contains the group column names available for the group that you select in **Available Groups**. When you select the **Custom** type, the Formula Editor opens to enable you to define the custom formula.

7. Select a column or **No Error** in the **Error** field to define the display of error bars.

> **Note:** When data displays as a scatter plot, 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 outside of a plotted value in either or both directions and graphically represent some error in plotted data.

8. Use the **Plot Attributes** area to change the appearance of the graph:
   - Select the **Symbol Style** to define the symbol to use to mark each data point in a scatter plot.
   - Select the **Symbol Size** to define the size of the symbol in a scatter plot.
   - Select the **Line Style** to define the line to use between data points in a scatter plot.
   - Click **Set Color** and select the color from the color picker to define the color of the symbol and line in a scatter plot or the color of the bar in a bar graph.
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.

Edit Graphs

You can change a graph by changing the type of 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.

You can display the data in a scatter plot or a bar graph. To change the graph type, click **Scatter** or **Bar Graph** in the **Graph Type** section on the **Home** tab in the ribbon.

To edit the appearance of the graph, see Change Graph Appearance on page 391.

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 changes to the graph definitions. See Create Graphs on page 345.
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 more plots click **Add**. To delete a plot from the graph select the plot and click **Delete**.

Curve Fit Settings

Note: Curve fits are used only in scatter plots.

When you create a graph of the data, it does not have a related fit. You can fit a plot to one of the curve fit selections. These selections display in the **Fit** list in the toolbar at the top of the **Graph** section. You can filter the list of curve fits by selecting a category from the **Category** list. 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 compared to the mean value for the Standard group.

After you select 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 done, 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).

Judge a Good Curve Fit

Visual inspection is always useful to evaluate a curve fit. The legend at the bottom of the graph contains information that summarizes 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. You should not use this to determine the statistical significance of the fit, or to compare the suitability of different curve fit functions. For such purposes, you can use statistics such as chi-squared or Fisher-F. 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 Settings** 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 to define the curve fit:

- **Curve Fit**, see Curve Fit Tab on page 349.
- **Weighting**, see Curve Fit Weighting Tab on page 352.
- **Statistics**, see Curve Fit Statistics Tab on page 354.

After you finish, click **OK**.

Curve Fit Tab

When you first create a graph of the data, it does not have a related fit. You can fit a plot to one of the predefined curve-fit functions.

These selections display in the **Fit** list in the Graph section toolbar. You can filter the list of curve fits by selecting a category from the **Category** list.

For information about the available curve fit functions, see Curve Fit Functions on page 356.

You can select a plot from the graph and a curve fit to apply to that plot from the list on the **Curve Fit** tab on the Curve Fit Settings dialog.
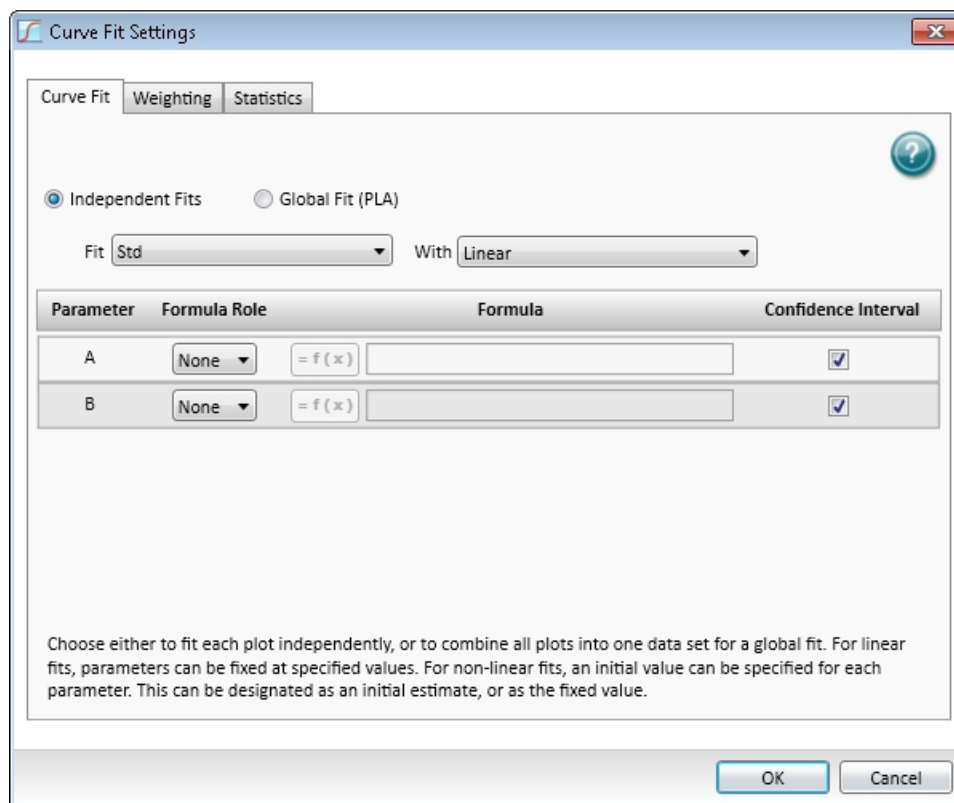
All plots on a graph can have the same type of fit or each plot can have a different fit applied individually. The **Curve Fit** tab of the Curve Fit Settings dialog provides the means to set a different fit to individual plots.

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 Independent Fits on page 350.
- To combine all plots into one data set, see Global Fit (PLA) on page 351.

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

After you select 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.

> **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 based on 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 Settings** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section.
3. On the Curve Fit Settings dialog, on the **Curve Fit** tab, select **Independent Fits**.
4. Select a plot from the **Fit** list.
   You can filter the list of curve fits by selecting a category from the **Category** list.
5. Select the curve fit function to apply to the plot from the **With** list.
6. Continue to select plots and apply curve fit functions as needed.
7. If applicable, select the **Weighting** tab. See Curve Fit Weighting Tab on page 352.
8. If applicable, select the **Statistics** tab. See Curve Fit Statistics Tab on page 354.
9. Click **OK**.

> **Note:** For independent fits (and global to a limited extent), you can fix values. Curve fit parameter values can either be fixed, or "manually seeded" to start the curve fit algorithm search.

Global Fit (PLA)

When all the plots in a graph are to have the same curve fit functions applied:

1. Open a Graph section with multiple plots in the workspace.

2. Click **Curve Fit Settings** [icon] in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the **Graph** section.

3. On the Curve Fit Settings dialog, select **Global Fit (PLA)**.
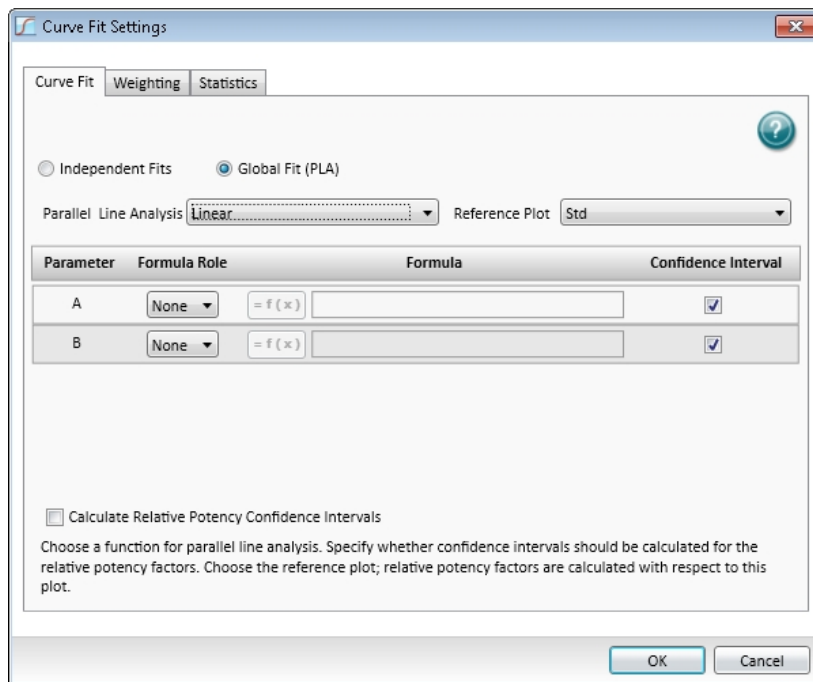
The available global fit is parallel line analysis. See Parallel Line Analysis on page 352.

4. Select the plot from the **Fit** list.

   You can filter the list of curve fits by selecting a category from the **Category** list.

5. Select an option, except point-to-point or cubic spline, from the **With** list.

6. If applicable, select **Relative Potency Confidence Interval**.

7. Select a plot for the **Reference Plot** list.

8. If applicable, select the **Weighting** tab. See Curve Fit Weighting Tab on page 352.

9. If applicable, select the **Statistics** tab. See Curve Fit Statistics Tab on page 354.

10. 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. Below 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 more details, see the *SoftMax Pro Formula Reference Guide*.

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 weighting is not provided, then all points have equal weight.

The weighting feature permits independent weighting of individual plots within a Graph section.

To apply weighting to a plot:

1. On the graph, select **Curve Fit Settings**.

2. On the Curve Fit Settings dialog, select the **Weighting** tab.
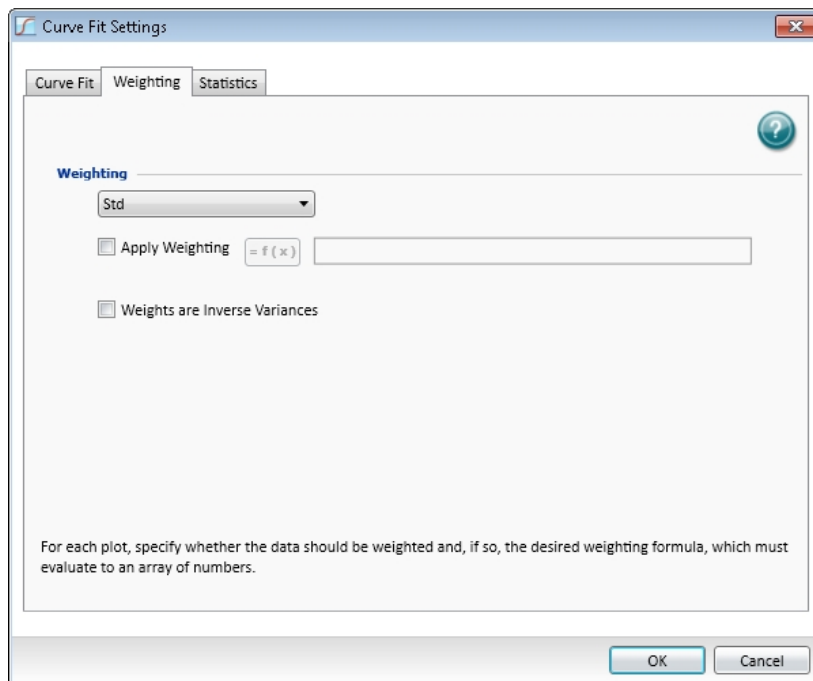
3. Select a plot from the **Weighting** list.

4. Select **Apply Weighting**.

5. Click the **Formula** button.

6. In the **Formula** field, enter the formula to apply as the weighting.

   If a formula for the weighting is not provided, then all points have equal weight.

7. If the data are weighted with the inverse of their variances, then select the **Weights are Inverse Variances** check box.

   If you select the **Apply Weighting** and the **Weights are Inverse Variances** check boxes on the **Weighting** tab for each plot, then the **Profile Method (Chi-Squared distribution)** option is available in the **Confidence Interval Method** list on the **Statistics** tab. See Curve Fit Statistics Tab on page 354.

8. Click **OK**.

Curve Fit Statistics Tab

On the **Statistics** tab, you can specify the statistics display.
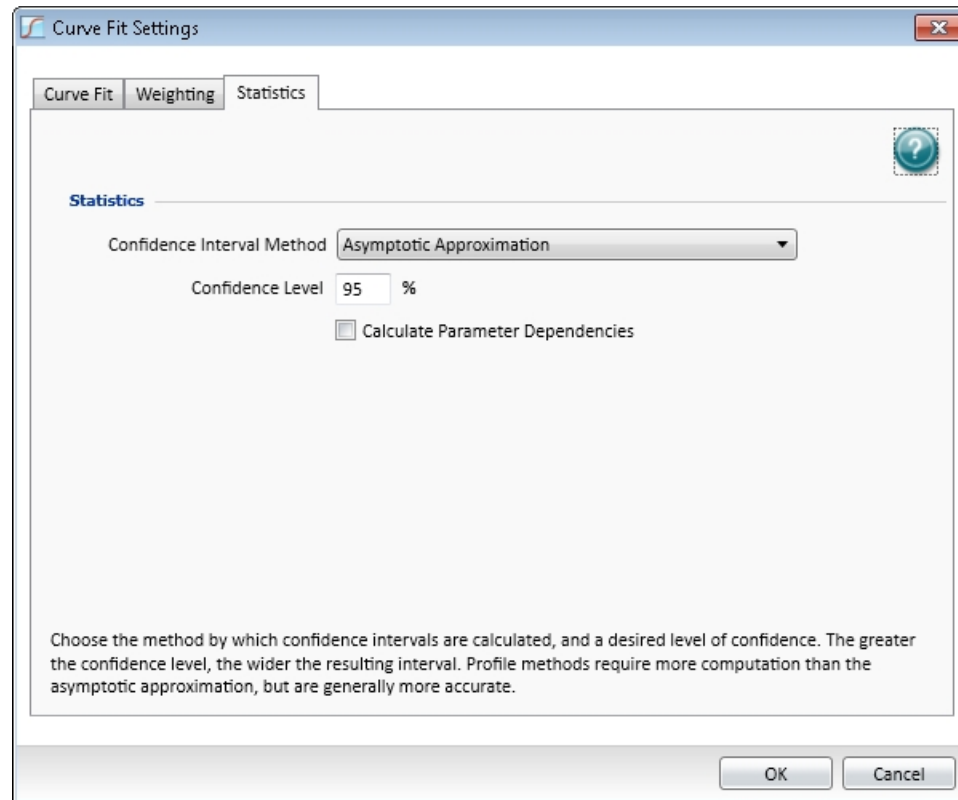
Choose the method by which confidence intervals are calculated, and a desired level of confidence. See Table 8-6: Confidence Interval Method on page 355.

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 based on 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 8-6: 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. If you select the **Apply Weighting** and the **Weights are Inverse Variances** check boxes on the **Weighting** tab for each plot, then the **Profile Method (Chi-Squared distribution)** option is available in the **Confidence Interval Method** list on the **Statistics** tab. See Curve Fit Weighting Tab on page 352.
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 Graph Legend on page 344.

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 a good fit for the data set.

For example, if the data set is sigmoidal with clear lower and upper asymptotes, 4-parameter fits should be applicable 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.

Curve Fit Functions

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

After you select 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 done, the parameter values display 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).

When you first create a graph of the data, it does not have a fit related to it. You can fit a plot to one of the following curve-fit functions.

These selections display in the **Fit** list in the toolbar at the top of the Graph section. You can filter the list of curve fits by selecting a category from the **Category** list.

No Fit

Select **No Fit** to apply no curve fit, and to plot the scatter of the data points.

2-Parameter Exponential

The **2-Parameter Exponential** curve fit is also known as:

- 2P Exponential
- Exponential Growth on page 358
- Exponential Decay on page 359
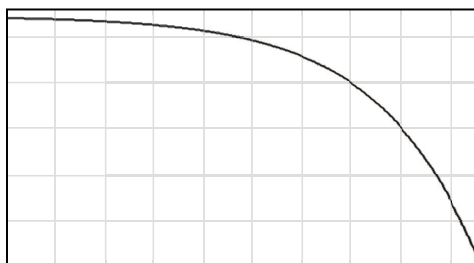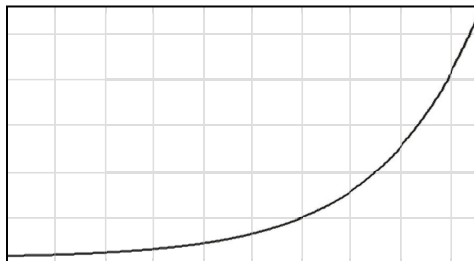
$$y = A \times e^{Bx}$$

y = Concentration
x = Time

$A = Y_o$
$B = k$

For exponential growth, B > 0
For exponential decay, B < 0

A 2-parameter exponential curve fit is commonly used for radioactive decay, first order kinetics, and unimolecular decay. It is also used for exponential growth:

$$\text{Doubling time} = B \times \ln(2) = B \times 0.693$$

Mathematically, a 2-parameter exponential curve fit is a special case of a 3-parameter exponential curve fit. See 3-Parameter Exponential on page 360.

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 2-parameter exponential curve fit algorithm is 2.

Exponential Growth

The **Exponential Growth** curve fit is also known as:

-

$$y = A \times e^{Bx}$$

y = Concentration
x = Time

$A = Y_o$
$B = k$

For exponential growth, B > 0

An exponential growth curve fit is commonly used for radioactive decay, first order kinetics, and unimolecular decay.

Mathematically, this is a special case of a 3-parameter exponential curve fit. See .

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 growth curve fit algorithm is 2.

Exponential Decay

The **Exponential Decay** curve fit is also known as:

- 2-Parameter Exponential on page 357
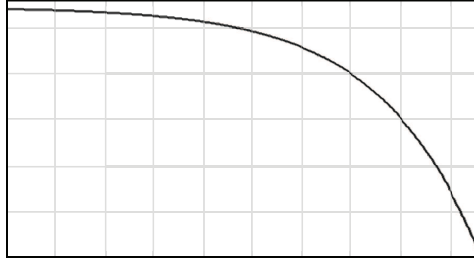
$$y = A \times e^{Bx}$$

y = Concentration
x = Time

$A = Y_o$
$B = k$

For exponential growth, $B > 0$
For exponential decay, $B < 0$

A 2-parameter exponential curve fit is commonly used for exponential growth:

$$\text{Doubling time} = B \times \ln(2) = B \times 0.693$$

Mathematically, this is a special case of a 3-parameter exponential curve fit. See 3-Parameter Exponential on page 360.

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 2-parameter exponential curve fit algorithm is 2.

3-Parameter Exponential

The **3-Parameter Exponential** curve fit is also known as:

- Exponential (in previous versions of the SoftMax Pro Software)
- 3P Exponential
- One-Phase Exponential Decay, see page 361
- One-Phase Exponential Association, see page 363
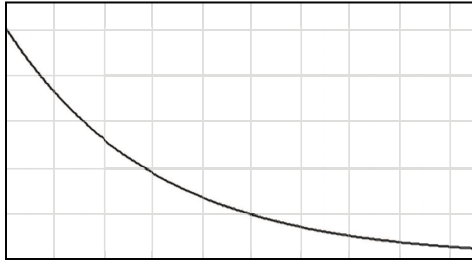
$$y = A + B \times (1 - e^{-x/C})$$

x = Time (usually)
y = Specific + Nonspecific Binding or Response

One-Phase Exponential Decay (B negative)

A = Y_0 = Y_{max} = Plateau + Span
B = −Span
C = 1/k = 1/decay constant

One-Phase Exponential Association (B positive)

A = Y_0 = Y_{min}
B = Span = Y_{max} − Y_0
C = 1/k = 1/association constant

An exponential curve fit is commonly used for drug elimination, dissociation of ligand from a receptor, and radioisotope decay. It is also used for pseudo-first order kinetics of ligand/receptor interaction or substrate/enzyme interaction.

The SoftMax Pro Software expresses C as the reciprocal of k, so it has same units as X.

$$t_{1/2} = C \times \ln2 = C \times 0.693$$

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.

Note: 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.

One-Phase Exponential Decay

The **One-Phase Exponential Decay** curve fit is also known as:

- 3-Parameter Exponential, see page 360
- 3P Exponential
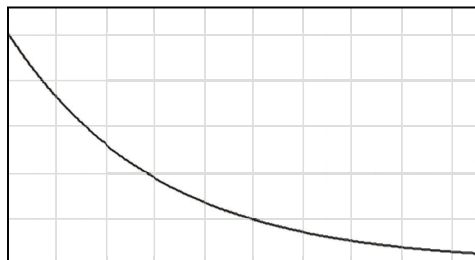
$$y = A + B \times (1 - e^{-x/C})$$

x = Time (usually)
y = Specific + Nonspecific Binding or Response

A = Y_0 = Y_{max} = Plateau + Span
B = −Span
C = 1/k = 1/decay constant

The SoftMax Pro Software expresses C as the reciprocal of k, so it has same units as X.

$$t_{1/2} = C \times \ln2 = C \times 0.693$$

A one-phase exponential decay curve fit is commonly used for drug elimination, dissociation of ligand from a receptor, and radioisotope decay.

The curve is the same as one-phase exponential association, except B is negative instead of positive. See One-Phase Exponential Association on page 363.

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 one-phase exponential decay curve fit algorithm is 3.

One-Phase Exponential Association

The **One-Phase Exponential Association** curve fit is also known as:
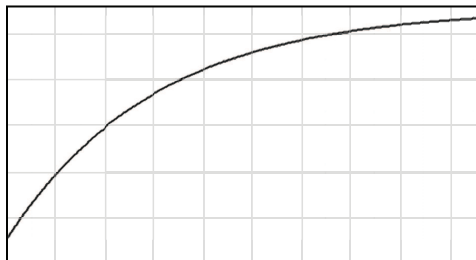
- 3-Parameter Exponential, see page 360
- 3P Exponential

$$y = A + B \times \left(1 - e^{-x/C}\right)$$

x = Time (usually)
y = Specific + Nonspecific Binding or Response

A = Y_0 = Y_{min}
B = Span = $Y_{max} - Y_0$
C = 1/k = 1/association constant

A one-phase exponential association curve fit is commonly used for pseudo-first order kinetics of ligand/receptor interaction or substrate/enzyme interaction.

The curve is the same as one-phase exponential decay, except B is positive instead of negative. See One-Phase Exponential Decay on page 361.

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 one-phase exponential association curve fit algorithm is 3.

4-Parameter Logisitic

A **4-Parameter Logistic** equation is also known as:

- Sigmoidal Dose-Response (variable slope)
- Four-Parameter Logistic Equation
- Four-Parameter Dose-Response Curve
- Hill Equation
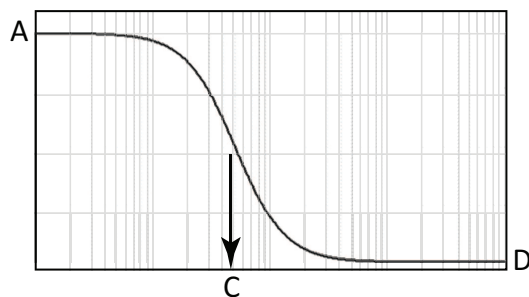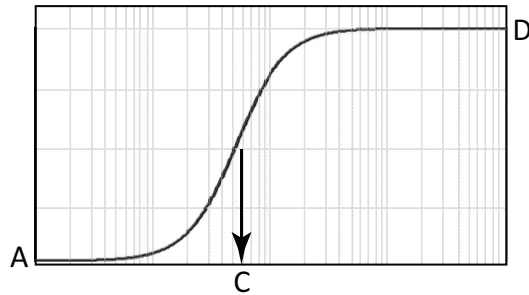
$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

A = First Asymptote
B = Slope Factor/Hill Coefficient
C = EC_{50}/IC_{50}
D = Second Asymptote

Note: In the SoftMax Pro Software, B is always positive, assuming neither A nor D is fixed. A is always the first asymptote, and D is always the last asymptote. So A and D can swap between top and bottom according to the shape of the curve, whether rising or falling with increasing concentration. However, if either A or D has been fixed in the software, the B parameter is no longer required to be positive. In such cases, A and D might or might not be the first and last asymptotes respectively.

A 4-parameter curve fit is commonly used in dose-response studies and estimation of EC_{50}/IC_{50} values. It can also be used as standard curves.

A 4-parameter curve fit assumes a sigmoidal data set with clear lower and upper asymptotes. If either asymptote is not clearly defined or is missing (as in a marginal data set), it is nearly impossible to estimate where the asymptote lies, or if exists at all. Likewise the EC_{50} cannot be estimated.

The 4-parameter curve possesses rotational symmetry about the point:

$$\{C, (A+D)/2\}$$

This point is sometimes referred to erroneously as an inflection point. In reality, for B > 1, the inflection point is:

$$\left\{C\left(\frac{(B-1)}{(B+1)}\right)^{\left(\frac{1}{B}\right)}, \frac{((A+D)+(A-D)/B)}{2}\right\}$$

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 is 4.

Note: For 3-parameter sigmoidal dose-response curves, use the 4-parameter curve fit and fix B or one of the asymptotes to a constant. See 3-Parameter Dose-Response Curves on page 366.

The minimization method for the 4-parameter equation 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.

3-Parameter Dose-Response Curves

3-Parameter Dose-Response Curves are special cases of a 4-Parameter Logistic equation with one parameter fixed. See 4-Parameter Logisitic on page 364.

3-Parameter Dose-Response Curves with a fixed slope are also known as:

- Sigmoidal Dose-Response (Fixed Slope)
- One-Site Competition
- Three-Parameter Logistic Equation
- Hill Equation (Hill Slope = −1)

3-Parameter Dose-Response Curves with a fixed asymptote are also known as:

- Sigmoidal Dose-Response (Fixed Asymptote)
- Three-Parameter Logistic Equation
- Hill Equation (Bottom Asymptote is Fixed to 0)
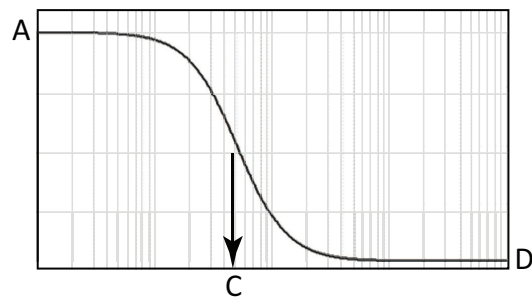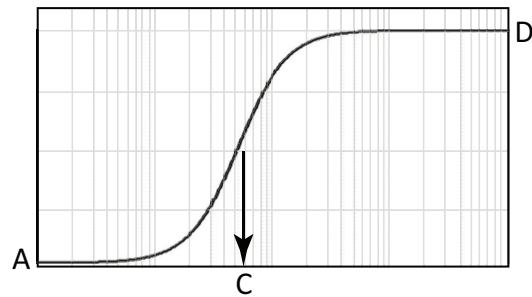
$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^{B}}$$

Fixed Slope

If B is positive:

A = First Asymptote
B = Fixed (generally to 1)
C = EC_{50}/IC_{50}
D = Second Asymptote

If B is negative:

A = Second Asymptote
B = Fixed (generally to -1)
C = EC_{50}/IC_{50}
D = First Asymptote

Fixed Asymptote

A = First Asymptote (A or D fixed)
B = Slope Factor/Hill Coefficient
C = EC_{50}/IC_{50}
D = Second Asymptote (A or D fixed)

3-parameter dose-response curves are commonly used in dose-response studies and estimation of EC_{50}/IC_{50} values. They can also be used as standard curves.

3-parameter dose-response curves assume a sigmoidal data set with clear lower and upper asymptotes. If either asymptote is not clearly defined or is missing (as in a marginal data set), it is nearly impossible to estimate where the asymptote lies, or if exists at all. Likewise the EC_{50} cannot be estimated.

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 is 4.

The minimization method for the 3-parameter dose-response curves equation 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.

5-Parameter Logistic

A **5-Parameter Logisitic** equation is also known as:

- Five-Parameter Logistic Equation
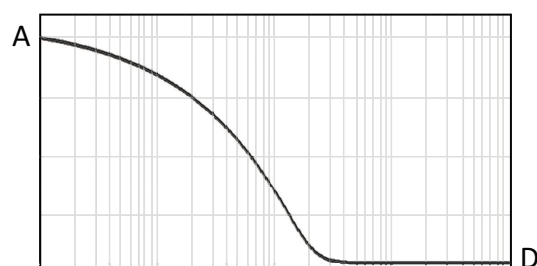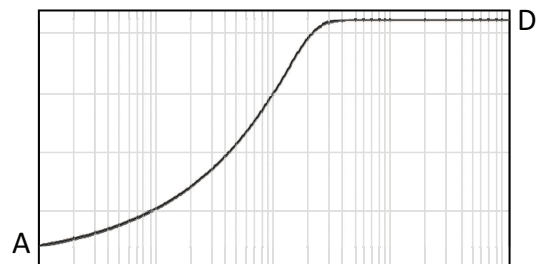
$$y = D + \frac{A - D}{\left\{ 1 + \left(\frac{X}{C}\right)^{B} \right\}^{G}}$$

A = First Asymptote
B = Slope Factor
C = [no physical meaning]
D = Second Asymptote
G = Asymmetry Factor

Note: In the SoftMax Pro Software, B is always positive, assuming neither A nor D is fixed. A is always the first asymptote, and D is always the last asymptote. So A and D can swap between top and bottom according to the shape of the curve, whether rising or falling with increasing concentration. However, if either A or D has been fixed in the software, the B parameter is no longer required to be positive. In such cases, A and D might or might not be the first and last asymptotes respectively.

A 5-parameter curve fit is commonly used in dose-response studies and estimation of EC_{50}/IC_{50} values. It can also be used as standard curves.

A 5-parameter curve fit assumes an asymmetric sigmoidal data set with clear lower and upper asymptotes. If either asymptote is not clearly defined or is missing (as in a marginal data set), it is nearly impossible to estimate where the asymptote lies, or if exists at all. Likewise the EC_{50} cannot be estimated.

Unlike the 4-parameter logistic equation, C is not the IC_{50} value. The IC_{50} value needs to be calculated separately. This is described in the *SoftMax Pro Software Formula Reference Guide*.

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 5-parameter curve fit algorithm is 5.

The minimization method for the 5-parameter equation 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.

5-Parameter Logistic Alternate

This is a re-parameterization of the standard five-parameter logistic, such that parameters B and C have the same interpretation as in the case of the four-parameter logistic. In particular, the EC_{50} value is given by C.

A **5-Parameter Logistic Alternate** equation is also known as:

- Five-Parameter Alternate Logistic Equation
- 5P Alternate
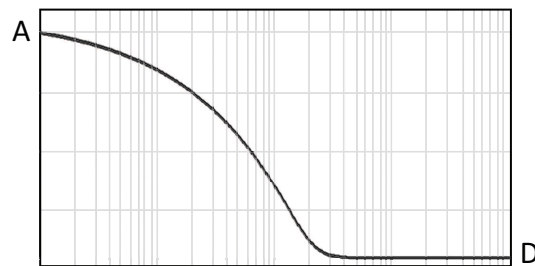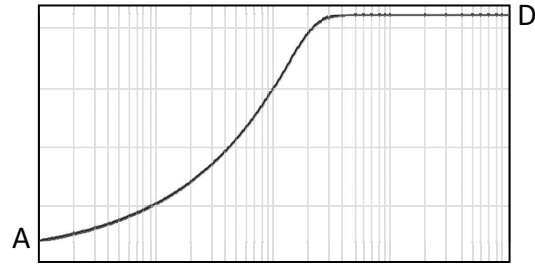
$$y = D + (A - D)\left\{1 + \left(2^{\frac{1}{G}} - 1\right)\left(\frac{X}{C}\right)^{B/2G\left(1 - 2^{-\frac{1}{C}}\right)}\right\}^{-G}$$

A = First Asymptote
B = Slope Factor
C = EC_{50}/IC_{50}
D = Second Asymptote
G = Asymmetry Factor

Note: In the SoftMax Pro Software, B is always positive, assuming neither A nor D is fixed. A is always the first asymptote, and D is always the last asymptote. So A and D can swap between top and bottom according to the shape of the curve, whether rising or falling with increasing concentration. However, if either A or D has been fixed in the software, the B parameter is no longer required to be positive. In such cases, A and D might or might not be the first and last asymptotes respectively.

A 5-parameter logistic alternate equation is commonly used in dose-response studies and estimation of EC_{50}/IC_{50} values. It can also be used as standard curves.

The 5-parameter logistic alternate equation is identical to the 5-parameter logistic equation, except that it has been re-parameterized such that the C parameter is the estimated EC_{50}/IC_{50} value.

A 5-parameter logistic alternate equation assumes a asymmetric sigmoidal data set with clear lower and upper asymptotes. If either asymptote is not clearly defined or is missing (as in a marginal data set), it is nearly impossible to estimate where the asymptote lies, or if exists at all. Likewise the EC_{50} cannot be estimated.

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 5-parameter logistic alternate equation algorithm is 6.

Bi-Exponential

The **Bi-Exponential** curve fit is also known as:

- Double Exponential Decay
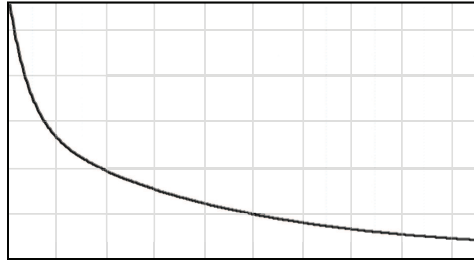
$$y = Ae^{Bx} + Ce^{Dx} + G$$

A = Span1
B = k1
C = Span2
D = k2
G = Plateau

When the curve is decaying, B and D are both negative.

The bi-exponential curve fit is commonly used when the decay is the result is the sum of a fast and a slow exponential decay.

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 bi-exponential curve fit algorithm is 5.

Two-Phase Exponential Decay

The **Two-Phase Exponential Decay** curve fit is also known as:

-
- Double Exponential Decay
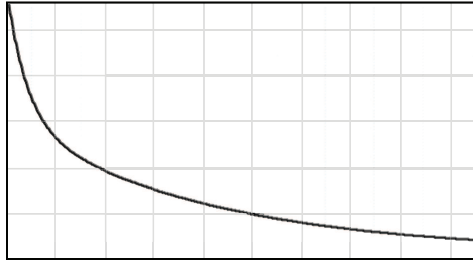
$$y = Ae^{Bx} + Ce^{Dx} + G$$

A = Span1
B = k1
C = Span2
D = k2
G = Plateau

When the curve is decaying, B and D are both negative.

The two-phase exponential decay curve fit is commonly used when the decay is the result is the sum of a fast and a slow exponential decay.

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 two-phase exponential decay curve fit algorithm is 5.

Bi-Rectangular Hyperbola

The **Bi-Rectangular Hyperbola** curve fit is also known as:
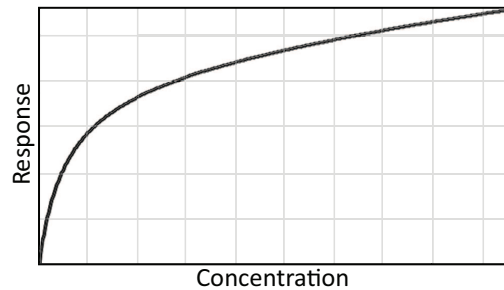
$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$

Two-Site Equilibrium Binding (specific binding)

y = Specific binding
A = Maximum binding (high affinity)
B = Kd(high)
C = Maximum binding (low affinity)
B = Kd(low)

Michaelis-Menten with Two Isozymes

Y = V_i
A = $V_i$max (1)
B = Km (1)
C = $V_i$max (2)
B = Km (2)

A bi-rectangular curve fit is commonly used for estimation of maximum (specific) binding and Kd values in the presence of two binding sites: high-affinity and low-affinity. It is also used for evaluation of enzyme activity, as function of [S] when two isozymes are present.

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 bi-rectangular hyperbola curve fit algorithm is 4.

Two-Site Equilibrium Binding (specific binding)

The **Two-Site Equilibrium Binding (specific binding)** curve fit is also known as:

- Bi-Rectangular Hyperbola on page 374
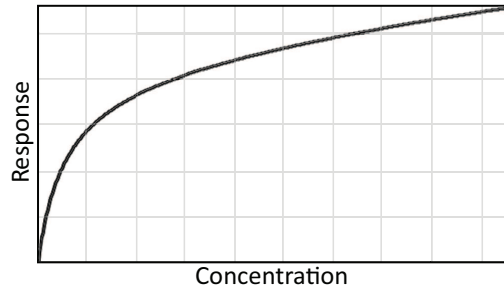
$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$

y = Specific binding
A = Maximum binding (high affinity)
B = Kd(high)
C = Maximum binding (low affinity)
B = Kd(low)

A two-site equilibrium binding (specific binding) curve fit is commonly used for estimation of maximum (specific) binding and Kd values in the presence of two binding sites: high-affinity and low-affinity.

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 two-site equilibrium binding (specific binding) curve fit algorithm is 4.

Michaelis-Menten with Two Isozymes

The **Michaelis-Menten with Two Isozymes** curve fit is also known as:

- Bi-Rectangular Hyperbola, see page 374

Concentration
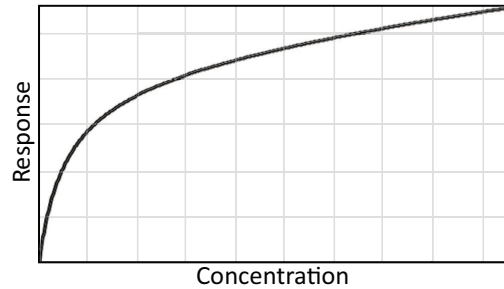
$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$

Y = V_i
A = $V_i$max (1)
B = Km (1)
C = $V_i$max (2)
B = Km (2)

A Michaelis-Menten with two isozymes curve fit is commonly used for evaluation of enzyme activity, as function of [S] when two isozymes are present.

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 Michaelis-Menten with two isozymes curve fit algorithm is 4.

Brain Cousens

The Brain Cousens curve fit is also known as:

- Sigmoidal Dose-Response with Hormesis
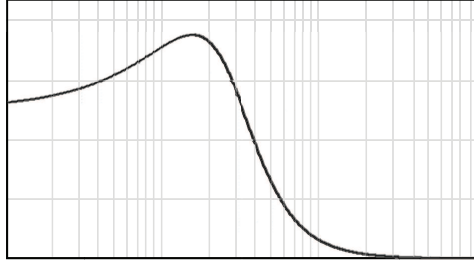
$$y = D + \frac{A - D + Gx}{1 + \left(1 + \frac{2CG}{A - D}\right)\left(\frac{x}{C}\right)^{B}}$$

A = First Asymptote
B = Slope Factor
C = EC_{50}/IC_{50}
D = Second Asymptote
G = Hormesis Factor

A brain cousens curve fit is commonly used for studies of test systems that show hormesis (opposite responses at low and high doses). An example is when an inhibitor shows an enhanced response at low doses and an inhibitory response at high doses.

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 brain cousens curve fit algorithm is 4.

Cubic

A **Cubic** curve fit is also known as:

- Polynomial Third Order
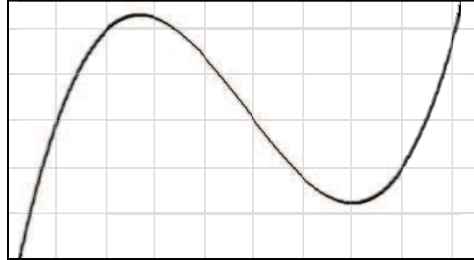
$$y = A + Bx + Cx^2 + Dx^3$$

A cubic curve fit can be used for standard curves, but there is risk of multiple inflection points, and so ambiguity in InterpX results.

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.

Cubic Spline

A **Cubic Spline** curve fit is a series of cubic segments joining adjacent pairs in a set of points such that the first and second derivatives match at the joints. There is no simple formula for the entire function.
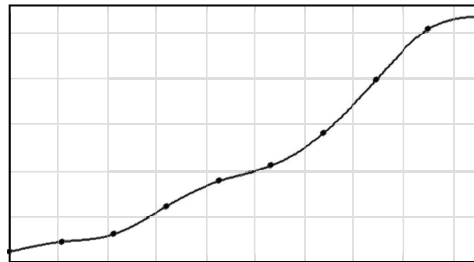
A cubic spline curve fit is commonly used for cosmetic curve smoothing.

Gaussian

The **Gaussian** function consists of a single peak.

$$y = A * e^{-\left(\frac{x - B}{C}\right)^2} + D$$

A = Height – D
B = Peak Center
C is related to the peak width
D = Baseline

Peak width at half-height = ParmC(Plot@Graph)*2*Sqrt(Ln(2))

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 gaussian curve fit algorithm is 4.

Linear

A **Linear** curve fit is also known as:

- Polynomial First Order

$$y = A + Bx$$

A = Intercept
B = Slope

Use a linear fit when the values seem 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.

Log-Log

The **Log-Log** curve fit fits 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.
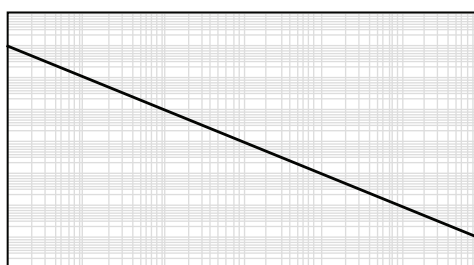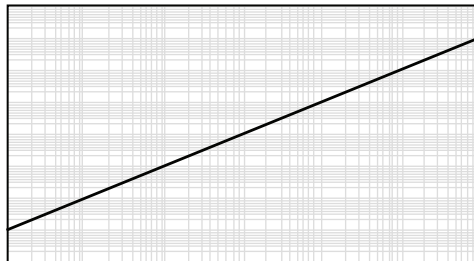
$$Log(y) = A + B \times Log(x)$$

A = Intercept
B = 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.

Log-Logit

The **Log-Logit** curve fit routine uses the same equation as the **4-Parameter** curve fit, but uses a different curve fitting algorithm. It is an older method and might be considered outdated. See 4-Parameter Logisitic on page 364.
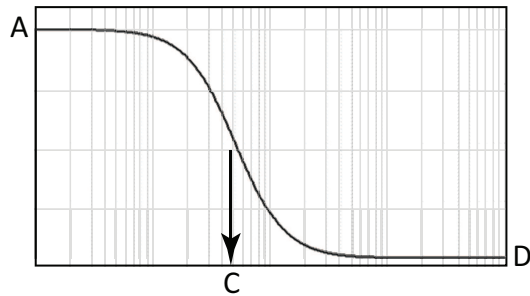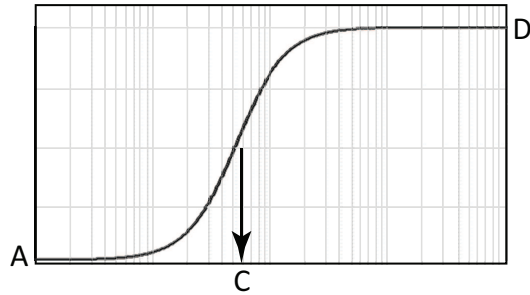
$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

A = First Asymptote
B = Slope Factor/Hill Coefficient
C = EC_{50}/IC_{50}
D = Second Asymptote

Point-to-Point

The **Point-to-Point** curve fit uses a linear equation to fit each pair of data points.
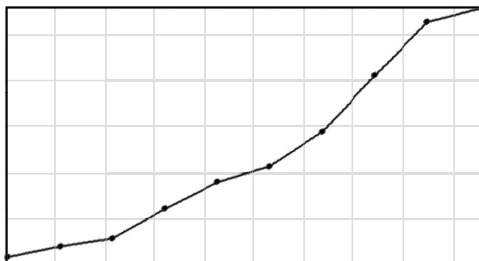
This curve fit is composed of multiple linear pieces joining the points. The line segment defined by each pair is used to interpolate data between those points.

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.

Quadratic

A **Quadratic** curve fit is also known as:

* Polynomial Second Order
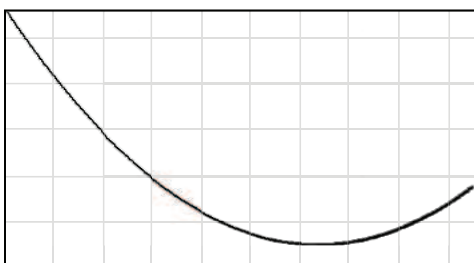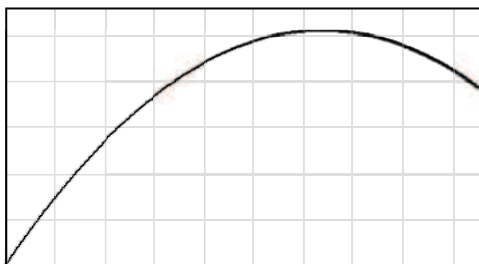
$$y = A + Bx + Cx^2$$

A = Intercept
B = Slope of the Curve at the Intercept
C = a measure of the curvature of the parabola

The quadratic fit is useful for standard curves.

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.

Quartic

A **Quartic** curve fit is also known as:

* Polynomial Fourth Order
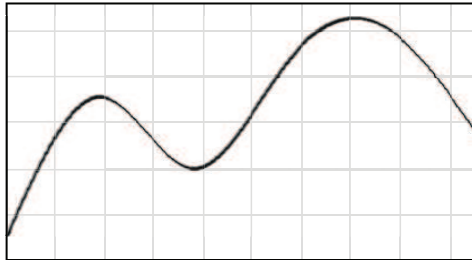
$$y = A + Bx + Cx^2 + Dx^3 + Gx^4$$

A quartic curve fit can be used for standard curves, but there is risk of multiple inflection points, and so ambiguity in InterpX results.

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 quartic curve fit algorithm is 5.

Rectangular Hyperbola

A **Rectangular Hyperbola** curve fit is also known as:
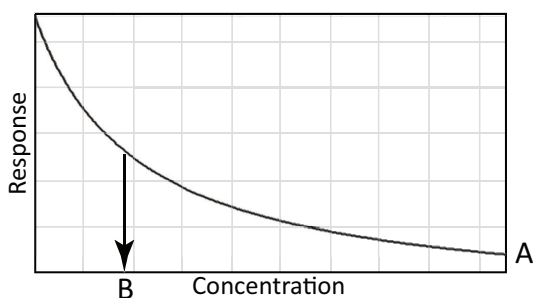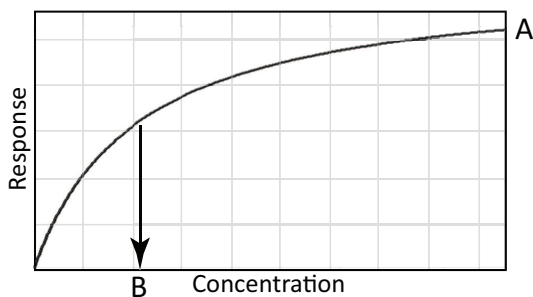
$$y = \frac{Ax}{x + B}$$

For Michaelis-Menten:

y = V$_i$
A = V$_i$max
B = Km

For One-Site Equilibrium Binding (specific binding):

y = Specific Binding
A = Maximum Binding
B = Kd

A rectangular hyperbola curve fit is commonly used estimation of V$_i$max, Km, maximum binding, and Kd.

A rectangular hyperbola curve fit is a special case of the 4-parameter curve fit where B = 1, D = 0, and x is 1/concentration.

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 rectangular hyperbola curve fit algorithm is 2.

Michaelis-Menten

A **Michaelis-Menten** curve fit is also known as:

- Rectangular Hyperbola, see page 385
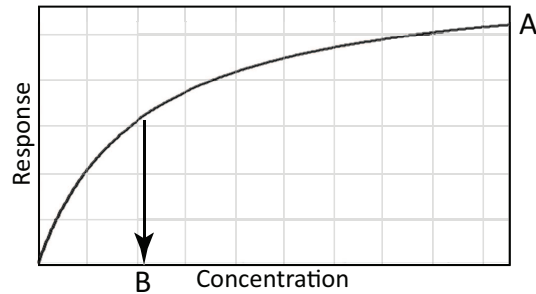
$$y = \frac{Ax}{x + B}$$

$y = V_i$
$A = V_i max$
$B = Km$

A Michaelis-Menten curve fit is commonly used estimation of $V_i max$ and Km.

A Michaelis-Menten curve fit is a special case of the 4-parameter curve fit where B = 1, D = 0, and x is 1/concentration.

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 Michaelis-Menten curve fit algorithm is 2.

One-Site Equilibrium Binding (specific binding)

A **One-Site Equilibrium Binding (specific binding)** curve fit is also known as:

- Rectangular Hyperbola, see page 385
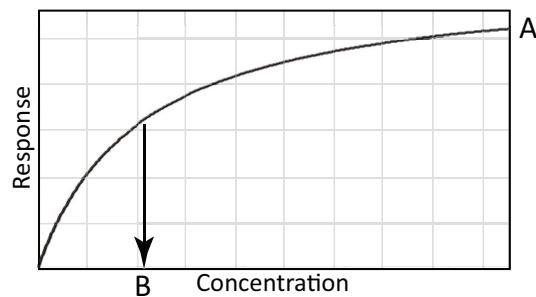
$$y = \frac{Ax}{x + B}$$

y = Specific Binding
A = Maximum Binding
B = Kd

A one-site equilibrium binding (specific binding) curve fit is commonly used estimation of maximum binding and Kd.

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 one-site equilibrium binding (specific binding) curve fit algorithm is 2.

Rect. Hyper. Plus Linear

A **Rect. Hyper. Plus Linear** (rectangular hyperbola plus linear) curve fit is also known as:

- RHPlusLinear (in previous versions of the SoftMax Pro Software)
- One-Site Equilibrium Binding (total and nonspecific), see page 388
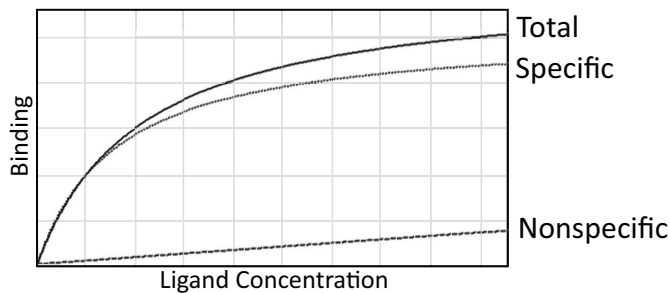
$$y = \frac{Ax}{x + B} + Cx$$

y = Total binding
A = Specific Binding B_{max}
B = Kd (specific binding)
C = nonspecific binding coefficient

A rectangular hyperbola plus linear curve fit is commonly used for estimation of maximum binding and Kd (of specific binding) in the presence of nonspecific binding.

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 rectangular hyperbola plus linear curve fit algorithm is 3.

One-Site Equilibrium Binding (total and nonspecific)

A **One-Site Equilibrium Binding (total and nonspecific)** curve fit is also known as:

- Rectangular Hyperbola Plus Linear
- Rect. Hyper. Plus Linear, see page 387
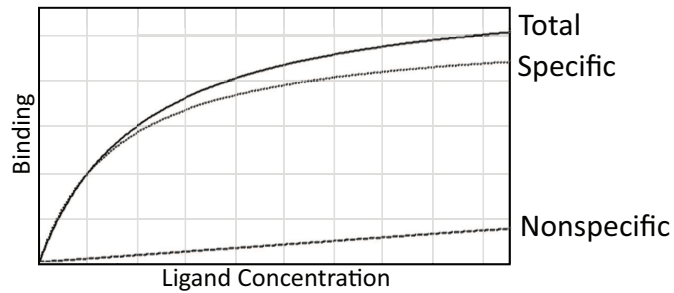
$$y = \frac{Ax}{x + B} + Cx$$

y = Total binding
A = Specific Binding B_{max}
B = Kd (specific binding)
C = nonspecific binding coefficient

A one-site equilibrium binding (total and nonspecific) curve fit is commonly used for estimation of maximum binding and Kd (of specific binding) in the presence of nonspecific binding.

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 one-site equilibrium binding (total and nonspecific) curve fit algorithm is 3.

Semi-Log

The **Semi-Log** curve fit fits the best straight line to a set of data for log(x) plotted against y.
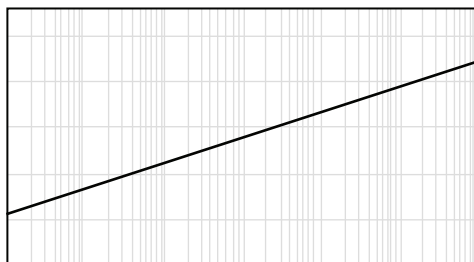
$$y = A + B \times Log(x)$$

A = y-intercept at x=1
B = 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.

Two-Site Competition

The **Two-Site Competition** curve fit is applicable to competition studies involving two receptor binding sites.

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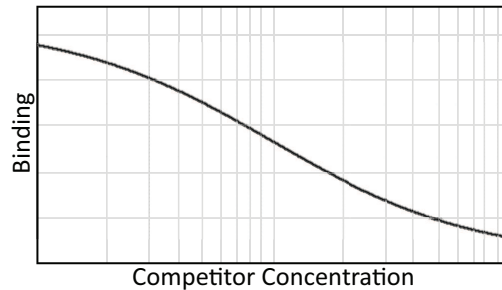
$$y = D + (A - D) \left(\frac{B}{1 + \left(\frac{x}{C}\right)} + \frac{1 - B}{1 + \left(\frac{x}{G}\right)} \right)$$

A = Y_0 = First Asymptote

B = Fraction of Sites with Affinity Associated with C

C = EC_{50} for One Site

G = EC_{50} for the Other Site

D = Second Asymptote

1 − B = Fraction of Sites with Affinity Associated with G

Assumptions include:

- Ligand has identical affinity for both receptors
- Competitor had different affinities
- Fraction1 = Fraction of Receptors with (1)
- Span = Top − Bottom

Note: To obtain a one-site competition fit, use the 4-parameter logistic and fix B to −1. See 3-Parameter Dose-Response Curves on page 366.

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 two-site competition curve fit algorithm is 4.

Change Graph Section Name

To change the name of a graph.

1. Click the Graph section to make it active in the workspace.

1. Double-click the name of Graph section at the top of the section to display the Rename Section dialog.

   You can also right-click the graph in the Navigation Tree and then select **Rename**.
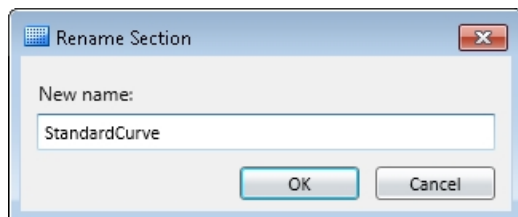
2. In the **New name** field, enter the name.

3. 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 Edit Graphs on page 347.

Change Graph Appearance

With a Graph section active in the workspace, click **Graph Appearance** in the **Graph Tools** section on the **Home** tab in the ribbon or in the toolbar at the top of the section.

The Graph Appearance dialog enables you to specify labels, styles, and graphic-display options.
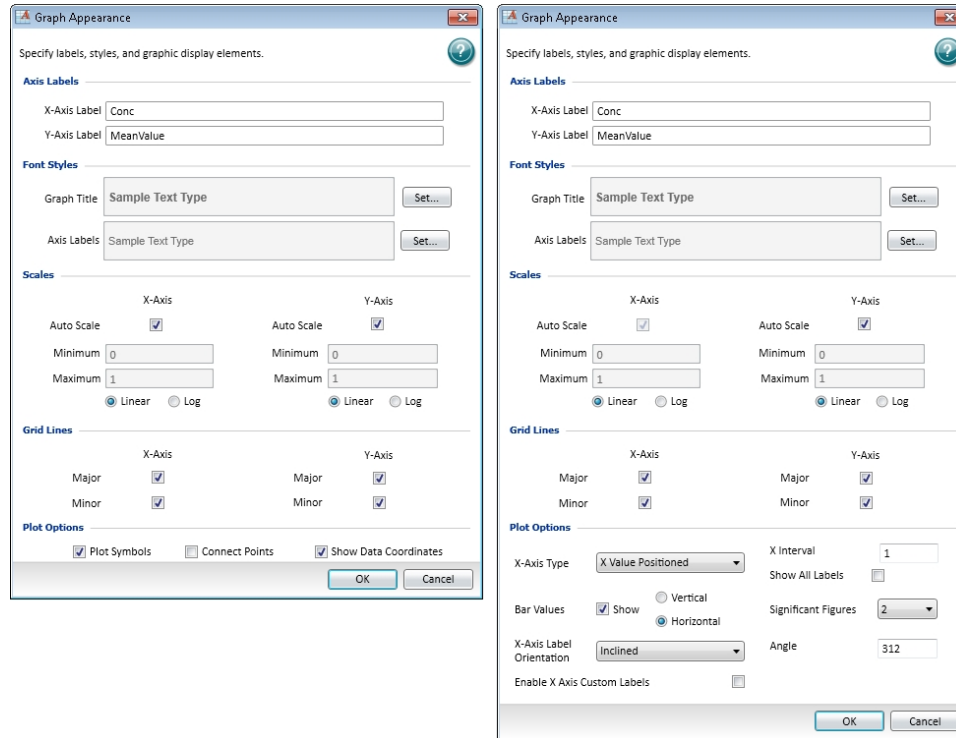
Axis Labels

Define labels for the X-Axis and Y-Axis by entering the text of the labels in the fields. These labels display in the font you specify in the **Font Styles** section.

Font Styles

Font Styles provides separate control of the fonts to use for the **Graph Title** and **Axis Labels**. Click **Set** to display the Font Style dialog.
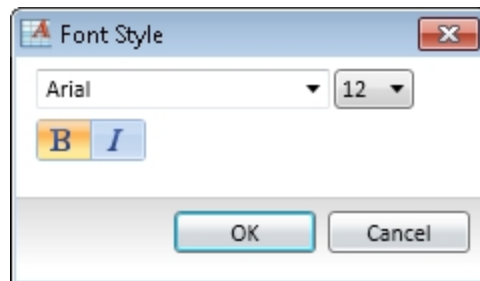
After you define the font style, click **OK**. The font style displays in the **Sample Text Type** example text.

Scales

To automatically scale one or both axes, select the **Auto Scale** check box for the axis.

As an alternative, you can define **Minimum** and **Maximum** scaling values.

You can 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

For a scatter plot, you can select the following options:

- Select **Plot Symbols** to display the defined symbol for each data point.

  You can define the size and style of the symbol, and the color of the symbol and the line, in the Plot Editor dialog. See Create Graphs on page 345.

- 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 over the data point.

For a bar graph, you can select the following display options:

- From the **X-Axis Type** drop-down list, select **Evenly Spaced Axis**, **Separated Axis**, or **X Value Positioned**.

  For the **X Value Positioned** option, enter a value in the **X Interval** field. To display all the labels for the defined interval, select the **Show All Labels** check box. Clear this check box to display labels only for the bars that have data.

- To display the data values above each bar, select the **Show Values** check box and then click **Vertical** or **Horizontal**. Select a number from the **Significant Figures** drop-down list.

- From the **X-Axis Label Orientation** drop-down list, select **Horizontal**, **Vertical**, or **Inclined**.

  For the **Inclined** option, enter a value in the **Angle** field.

- To edit the labels directly in the Graph section, select the **Enable X-Axis Custom Labels** check box.

  When you select this option, you can double-click the label in the Graph section to edit the label. Clear this check box to return the labels to their default values.

> **Note:** When you select the **Enable X-Axis Custom Labels** check box, the **X-Axis Label Orientation** is set to **Horizontal**. After you edit the labels, you can change the **X-Axis Label Orientation** to **Vertical** or **Inclined**.

Zoom 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 region you select.

To return to the default view, double-click the graph body.

Zooming is supported for scatter plots only.

Export an Image of a Graph Section

You can export a 300 DPI image of a Graph section so that you can import the image into another program. You can also copy the Graph section and then paste the 300 DPI image directly into a word processor or spreadsheet program.

To copy a Graph section, right-click anywhere on the section and then click **Copy**.

Note: The 300 DPI image might appear very large in the program where it is imported or pasted. You might need to scale or resize the image in that program after you import or paste the Graph section image.

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 is displayed the way you want, including whether or not the graph legend is displayed. See Graph Legend on page 344.
3. Click **Export Graph** in the **Graph Tools** section on the **Home** tab in the ribbon.
4. On the Save As dialog, browse to the file location where you want to save the .png file.
5. In the **File name** field, enter a name for the file.
6. Click **Save**.

An image of the active Graph section is saved as a .png file at 300 DPI resolution.

View Western Blot Membrane Data

After you scan a membrane for Western Blot data, the data displays in the SoftMax Pro Software as an image. Use the image tools in the **Plate** section to zoom, crop, colorize, and adjust the intensity of the image. You can select a region of interest (ROI) and rescan the membrane at a higher resolution.

- To zoom the image in or out, drag the slider on the side of the **Plate** section.
- To view the image at its actual size, click 1:1 on the side of the **Plate** section.
- To fit the image to the **Plate** section, click Fit on the side of the **Plate** section.
- To zoom in on a specific area of the image, click 🔍 on the side of the **Plate** section and then drag across the image to define the area to zoom.
- To move the image within the **Plate** section, click ✋ on the side of the **Plate** section and then drag the image to move it.
- To crop the image, click ⛶ on the side of the **Plate** section and then drag across the image to define the cropped area.
- To colorize the image from a look-up table (LUT), from the **LUT** list at the top of the **Plate** section, select a color option.
- To adjust the intensity of the image, in the **Subtract** field at the top of the **Plate** section, enter a value to subtract from the image intensity.
- To scale the image intensity, select the **Log** check box.
- To invert the colors of the image, similar to a photographic negative, select the **Invert** check box.
- To select and scan a region of interest (ROI), click 🖼 on the left side of the Plate section and then drag across the image to define the region. Click **Read** ▶ on the **Home** tab in the ribbon to scan the region using the ROI Scan Resolution you define on the Settings dialog. The data from the ROI scan replaces the data from the original scan.
- To adjust the resolution of the scanned image, click 🖼 to view the image at the scanned resolution, click 🖼 to view the image at a fine resolution suitable for external analysis, or click 🖼 to view the image at a very fine resolution suitable for publishing.
- To restore the image using the data from the last scan, click ⤢ on the top of the **Plate** section.

The **Plate** section contains options to export the image for further analysis or to publish. These tools are on the right side of the top of the **Plate** section

- To open the raw image data in a Microsoft Excel template for analysis, click and then click **Excel Analysis**. See Use Excel to Analyze Western Blot Data on page 396.
- To open the raw image in the ImageJ software, click and then click **ImageJ Editor**. See Analyze Western Blot Images on page 400.
- To open the raw image in a pre-defined image-analysis tool, click and then click **External Image Editor**. This option is available only if you have .tif files associated with a program other than ImageJ software. See Analyze Western Blot Images on page 400.

> **Note:** If you use the ImageJ software or an external image-analysis tool to make and save changes to the original image file, the edited image displays in the **Plate** section and you cannot edit the image from within the SoftMax Pro Software indicated by 🔒 at the top of the section. To restore the image using the data from the last scan, click 🖼.

- To export the raw image data to a text file, click , click **Export as Text**, and then select a name for the exported text file.
- To export the edited image as it displays in the **Plate** section, click , click **Export as Image**, and then select a name and format for the image export file.

For information on how to locate the generated image, see Image Data Files and Folders on page 59.

Use Excel to Analyze Western Blot Data

You can export the Western Blot image data to a Microsoft Excel template to analyze the image data.

To export the raw image data to an Excel template, click and then click **Excel Analysis**.

An Excel file opens displaying the exported data. There are three worksheets in the Excel file.

- The **Analysis** worksheet displays the data using automatic background subtraction. You can select the bands to measure and display a bar graph showing an analysis of the selected bands in the **Analysis** worksheet. See Measure Selected Bands on page 397.
- The **Segmentation** worksheet displays the segmented bands with background subtraction. The segmented bands are displayed on a white background. You can adjust the background noise level and the minimum and maximum band size in the **Segmentation** worksheet. See Define Segmentation Criteria on page 398.

- The **Data** worksheet displays the TIFF image and a color rendering of the raw data. No further analysis is shown in the **Data** worksheet. If the automatic background subtraction in the **Analysis** worksheet is not sufficient for your data, you can manually define the background for subtraction in the **Data** worksheet. See Manually Select Background for Subtraction on page 400.

To save the data and analysis for future reference, save the Excel file.

Measure Selected Bands

You can select the bands to measure and display a bar graph showing an analysis of the selected bands in the **Analysis** worksheet. To view the **Analysis** worksheet, click the **Analysis** tab at the bottom of the Excel window.
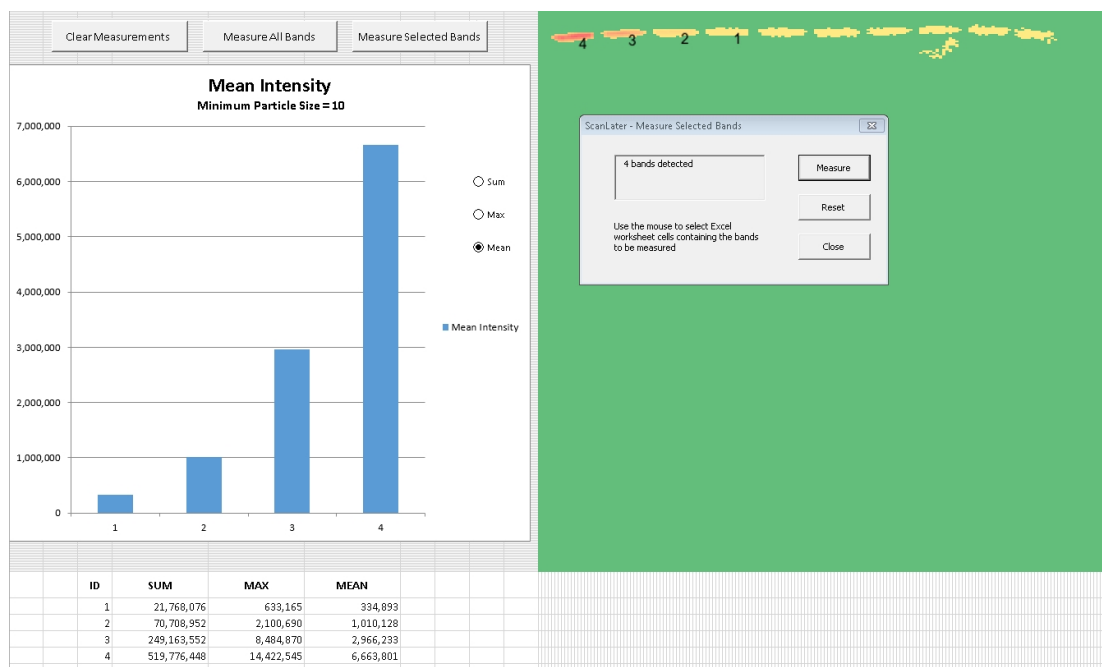
The **Analysis** worksheet displays the data using automatic background subtraction.

- To measure all the displayed bands, click **Measure All Bands**.
- To select a group of bands to measure, click **Measure Selected Bands**, and then use your mouse to draw a rectangle around the bands that you want to measure. In the **ScanLater - Measure Selected Bands** dialog, click **Measure**.
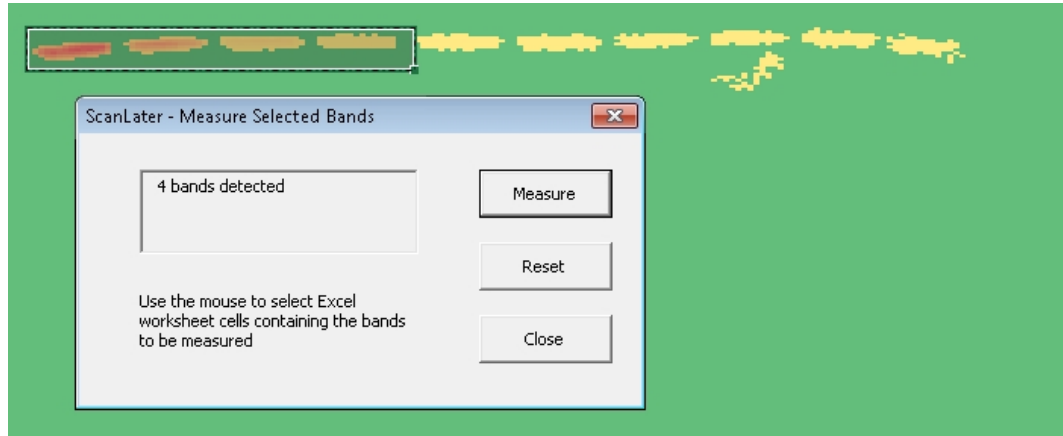
- To remove the measurement analysis from the worksheet, click **Clear Measurements**.

After you select the bands to measure, a bar graph is displayed with a table below it to show an analysis of the bands based on the analysis criteria. Labels on the blot show the locations of the bands that are referenced in the graph and the table.

You can adjust the background noise level and the minimum and maximum band size in the **Segmentation** worksheet. See Define Segmentation Criteria on page 398.

If the automatic background subtraction is not sufficient for your data, you can manually define the background for subtraction in the **Data** worksheet. See Manually Select Background for Subtraction on page 400.

Define Segmentation Criteria

The **Segmentation** worksheet displays the segmented bands with background subtraction. The segmented bands are displayed on a white background. You can adjust the background noise level and the minimum and maximum band size in the **Segmentation** worksheet. You can also remove artifacts that you do not want to include in your measurements.
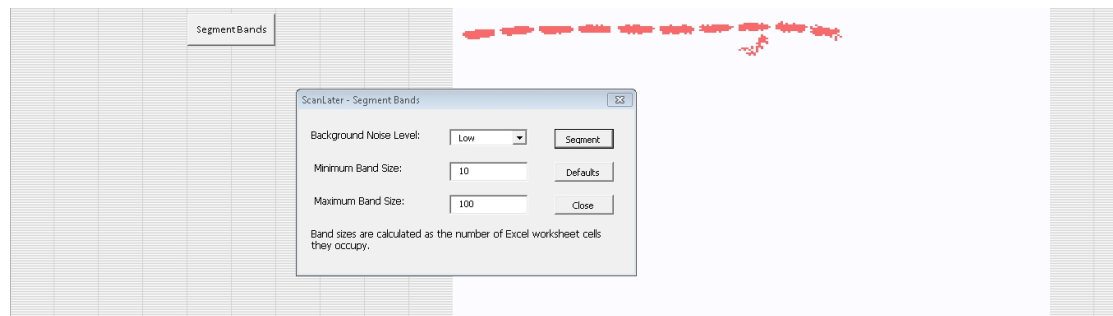
To adjust the segmentation of the bands, click **Segment Bands**. In the **ScanLater - Segment Bands** dialog, select either a **Low** or **High** background noise level to subtract, and define the minimum and a maximum band size for the segmentation. The band size is the total number of cells in the Excel worksheet that the band occupies. To view the result of your settings, click **Segment**.

To remove artifacts from the displayed data, use your mouse to drag a rectangle around the artifact, and then right-click the rectangle and select **Clear Contents**.
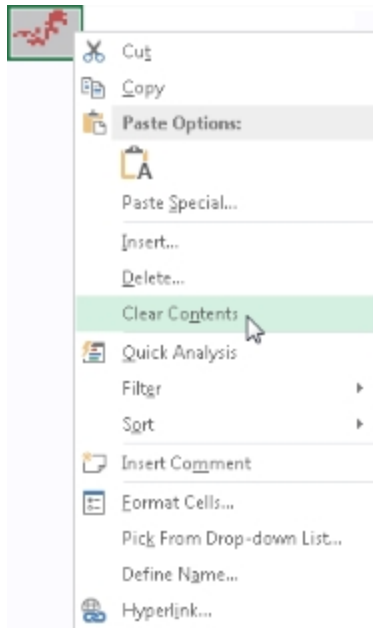
You can select the bands to measure and display a bar graph showing an analysis of the selected bands in the **Analysis** worksheet. See Measure Selected Bands on page 397.

If the automatic background subtraction is not sufficient for your data, you can manually define the background for subtraction in the **Data** worksheet. See Manually Select Background for Subtraction on page 400.

Manually Select Background for Subtraction

The **Data** worksheet displays the TIFF image and a color rendering of the raw data. No further analysis is shown in the **Data** worksheet. If the automatic background subtraction is not sufficient for your data, you can manually define the background for subtraction in the **Data** worksheet.

To define the background for subtraction, use your mouse to drag a rectangle around the area to use as the background. In the **ScanLater - Select Background** dialog, click **OK**.
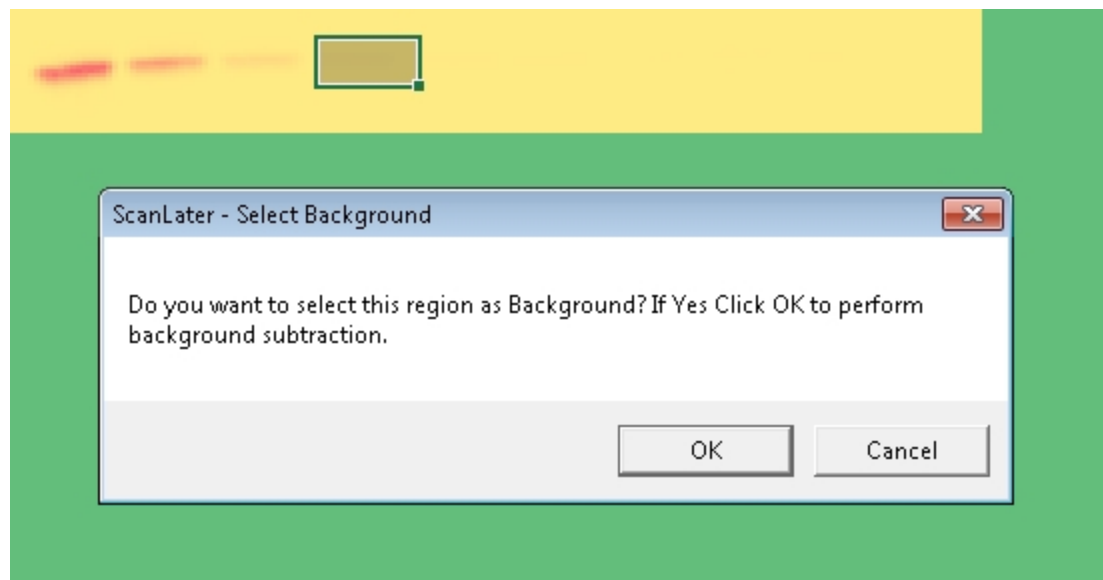
You can select the bands to measure and display a bar graph showing an analysis of the selected bands in the **Analysis** worksheet. See Measure Selected Bands on page 397.

You can adjust the background noise level and the minimum and maximum band size in the **Segmentation** worksheet. See Define Segmentation Criteria on page 398.

Analyze Western Blot Images

Western Blot membrane data are saved as a TIFF image that can be analyzed by the image-analysis tool of your choice. The SoftMax Pro Software comes with an installed version of the ImageJ software from U.S. National Institute of Health (NIH).

To view the membrane image in the ImageJ software, click in the toolbar at the top of the section and then click **ImageJ Editor**.

To view the membrane image in your selected image-analysis tool, click in the toolbar at the top of the section and then click **External Image Editor**. This option is available only if you have .tif files associated with a program other than ImageJ software. The icon used for this tool changes depending on the selected image-analysis tool. See Define the Membrane Image-Analysis Tool on page 401.

> **Note:** If you use the ImageJ software or an external image-analysis tool to make and save changes to the original image file, the edited image displays in the **Plate** section and you cannot edit the image from within the SoftMax Pro Software indicated by 🔒 at the top of the section. To restore the image using the data from the last scan, click 🔁.

To retain the editing capabilities within the SoftMax Pro Software and also edit the image with an external editor, export the image or make a copy of the generated TIFF image to open in the editor. For information on how to locate the generated TIFF image, see Image Data Files and Folders on page 59.

Define the Membrane Image-Analysis Tool

1. In Windows Explorer, right-click a file that has a **.tif** extension.
2. Click **Open with > Choose default program**.
3. In the **Open with** dialog, select the program you want to use for membrane image-analysis.
   - If the program you want is listed in the **Recommended Programs** list, then select it.
   - If the program you want is not listed in the **Recommended Programs** list, then click **Browse**. Locate and select the **.exe** file for the program that you want to use, and then click **Open**.

     The ImageJ software **.exe** file installed with the SoftMax Pro Software can be found in the **ImageJ** folder within the SoftMax Pro Software installation folder.
4. Make sure that the **Always use the selected program to open this kind of file** check box is selected.
5. Click **OK**.

The next time you open a Western Blot data file, the **Image** icon changes to match the selected program.

Save Data as a PDF

You can save the contents of the selected file in PDF format. Before you save 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 Print Settings on page 75 and Disable Sections from Print Jobs on page 76.

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 select the **Molecular Devices (PDF Writer)** on the **Print** dialog.

3. On the **Molecular Devices (PDF Writer) - Create File** dialog, name the file and define the PDF options. See PDF File Settings on page 403.

4. Click **Save**.

PDF File Settings

The following tables define the settings for each of the tabs in the **Molecular Devices (PDF Writer) - Create File** dialog:

Table 8-7: PDF File Settings in the General Tab

Option	Description
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	Enter 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.

Table 8-8: PDF File Settings in the Document Tab

Option	Description
Author	Enter the name of the author in this field for displaying in the document properties.
Title	Enter the title of the document in this field for displaying in the document properties.
Subject	Enter the subject of the document in this field for displaying in the document properties.
Keywords	Enter 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 is displayed 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.

Table 8-9: PDF File Settings in the Image Tab

Option	Description
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.

Table 8-10: PDF File Settings in the Watermark Tab

Option	Description
Text	Enter the text that you want to be displayed in the watermark.
Font	Select the typeface for the watermark.
Color	Click ⌐…¬ to select the color for the text.
Size	Enter the size of the text in points.
Outline width	Enter the width of the text outline in pixels to have the text be displayed in outlines. To have the text be displayed as a solid color, leave this field blank.
Layer	To have the watermark text be displayed on top of the document text, select **Stamp**. To have the watermark text be displayed 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, or bottom of the document.
Vertical adjustment	Enter 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	Enter a percentage offset for the horizontal watermark position.

Table 8-11: PDF File Settings in the Merge Tab

Option	Description
Append PDF	Enter 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 start of the old file, select **Top**.
Background PDF	If you want to print the data on an existing file, enter 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 display on top of the old file, select **Stamp**. To have the new file be displayed behind the old file, select **Watermark**.

Table 8-12: PDF File Settings in the Security Tab

Option	Description
Owner password	Enter the password that gives owner permissions for the file.
User password	Enter 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 permit printing and what print quality is permitted.
Copy to clipboard	Select this check box to permit users to copy text from the file to the clipboard.
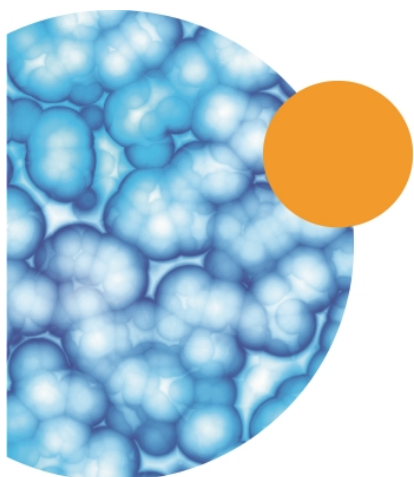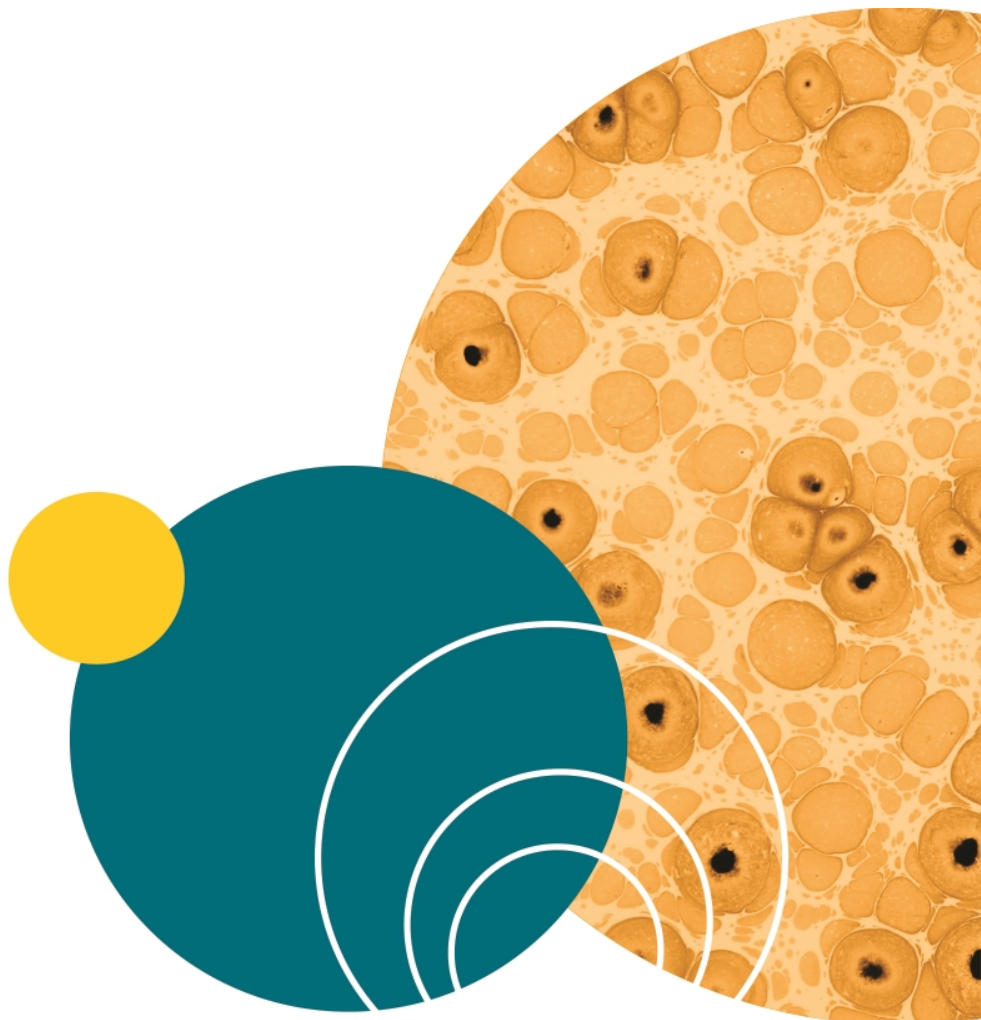
Chapter 9: Maintain and Calibrate Instruments

The user guide for each instrument describes instrument maintenance. See the user guide for your instrument.

Information on configuration, maintenance, and calibration tasks that can be done using the SoftMax Pro Software can be found in the following topics:

- Calibrate Microplate Readers on page 407
- Configure Filters for EMax and Vmax Microplate Readers on page 408
- Adjust the Lamp for EMax Plus Microplate Reader on page 408
- Instrument Information on page 409
- View Instrument Software Support on page 415

Calibrate Microplate Readers

If your instrument supports the SoftMax Pro Software air-calibration process, the **Calibration** button appears on the **Operation** tab in the ribbon.

Some instruments do not require or do not support the air-calibration process. If the Calibration button does not appear or is not active, then either the computer is not connected to the instrument or the instrument does not support the air-calibration process.

> **Note:** Air-calibration applies to absorbance reads. You do not need a microplate or cuvette for instrument calibration. If you do a cuvette calibration, the cuvette chamber must be empty.

During calibration, the instrument steps through all available wavelengths one-by-one to measure the signal that reaches the detector when there is no sample in the optical path. The software calculates air-calibration values and stores these values in the firmware. The instrument uses the stored air-calibration values when you enable Speed Read and when the Calibrate icon is disabled on the Settings dialog. See Speed Read on page 231 and Calibrate on page 249.

To calibrate the microplate reader:

1. Select the **Operations** tab in the ribbon and click **Calibration** to display the Calibration dialog.
2. Select **Plate** to calibrate for microplates and/or select **Cuvette** to calibrate for cuvettes.
3. Click **Calibrate Now**.
4. When the calibration indicator shows that the calibration is complete, click **Close**.

Configure Filters for EMax and Vmax Microplate Readers

EMax and Vmax 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 on the **Filter Configuration** dialog are incorrect, the microplate reader generates incorrect data. For information about how to change the filters, see the user guide for the EMax and Vmax Microplate Readers.

To define the filter positions:

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

   You can define up to six filters. The permitted wavelength range is 400 nm to 750 nm.
3. Click **OK**.

The filters you define are available for selection on the **Settings** dialog. See Create Absorbance Mode Protocols on page 274.

Adjust the Lamp for EMax Plus Microplate Reader

To adjust the halogen lamp, click **Configure** on the **Operations** tab in the ribbon.

On the **Configuration** dialog, you can adjust the intensity of the lamp for each of the installed filters in the EMax Plus Microplate Reader. You can view the positions and wavelengths of the installed filters. This dialog lists other instrument information including the serial number and firmware version.

The installed filters are available for selection on the **Settings** dialog. See Create Absorbance Mode Protocols on page 274.

To adjust the intensity of the lamp for each of the filters, click **Adjust Lamp**. The software and instrument step through each filter one at a time to adjust the lamp intensity for the best implementation of the filter wavelength.

> **Note:** The **Configuration** dialog must remain open until the lamp adjustment is complete.

Instrument Information

To view the **Instrument Information** dialog, on the **Operations** tab in the ribbon 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 SpectraMax iD3 Multi-Mode Microplate Reader, SpectraMax i3x or i3 Multi-Mode Detection Platform, SpectraMax Paradigm Multi-Mode Microplate Reader, FilterMax F3 Multi-Mode Microplate Reader, or FilterMax F5 Multi-Mode Microplate Reader. To update the PIC or instrument firmware, click the **Update** button. For more information about how to update firmware, contact Molecular Devices support.

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

SpectraMax i3x or i3 Multi-Mode Detection Platform Instrument Information

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

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

SpectraMax Paradigm Multi-Mode Microplate Reader Instrument Information

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

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

You can lock or unlock the detection cartridge drawers when you use the StakMax Microplate Handling System:

- When the microplate handler 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 microplate handler 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 drawers closed, click **Lock Instrument for Shipment** and follow the on-screen instructions. For information about how to prepare an instrument for shipment, contact Molecular Devices support.

For information about how to unlock the drawers, see Unlock SpectraMax Paradigm Instruments on page 20.

To align the transfer position of the StakMax Microplate Handling System with the plate drawer of the SpectraMax Paradigm Multi-Mode Microplate Reader, click **StakMax Alignment Wizard**. For information about how to use 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 discovers the installed excitation and emission filter slides and displays their configurations on the **Instrument Information** dialog. The identification number for the slide displays on the right. Each filter displays its wavelength, bandwidth, and read mode under its position in the slide.

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

To edit the filters in a slide, or to define more slides, click **Edit Slide Configuration**. See Configure Filter Slides on page 410.

Configure 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 microplate reader generates incorrect data. For information about how to change the filter slides, see the user guide for the FilterMax F3 and F5 Multi-Mode Microplate Readers.

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

The filters used to do 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 you exchange slides, an identification code built into the slide lets the SoftMax Pro Software 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 on 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 Add Filter Slides on page 412.

- Click **Remove Slide** to delete the selected filter slide. See Remove Filter Slides on page 413.

- Click **Export selected Slide** to export the filter definitions for the selected filter slide. See Export Filter Slides on page 413.

- Click **Import Slides** to import a filter slide definition file that defines one or more filter slides. See Import Filter Slides on page 414.

- Click **Load Slides** to replace all the current filter slide definitions with the imported definitions. See Replace Filter Slide Definitions on page 414.

- Click **Export Slides** to export all the defined filter slides. See Export Filter Slide Definitions on page 415.

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

Add Filter Slides

The filters used to do 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. On the **Operations** tab in the ribbon click **Info**.
2. On the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. On 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, enter 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, enter 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 Multi-Mode Microplate Reader only, you can select **Polarization** for filter positions that have a polarization filter installed.
    - After you select a read mode, the **Installed** field displays **Yes**.
    - If you do not select a read mode, the **Installed** field displays **No**, and the filter position cannot be used for measurements.

    Note: You cannot edit the **Installed** and **Position** fields.

11. In the **Bandwidth** field, enter the bandwidth of the filter.
12. In the **Order#** field, enter the manufacturer's part number for the filter for your reference.
13. Continue configuring the rest of filters on the slide.
14. After you configure the filters on the slide, click **OK**.

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

Remove Filter Slides

If a filter slide is no longer used with an instrument, you can remove it 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 you remove it from the list. See Export Filter Slides on page 413.

To remove a filter slide from the list:

1. On the **Operations** tab in the ribbon click **Info**.
2. On the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. On the **Filter Settings** dialog, click **Excitation** or **Emission** to display a list of the selected type of filter slides.
4. In the slide list, select the slide 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.

Export Filter Slides

Exported slide definitions are saved in XML files that you can import again later or import into the software on a different computer.

The **Export selected Slide** feature exports the definition for a single filter slide. To export all the current filter slide definitions, see Export Filter Slide Definitions on page 415.

To export a filter slide in the list:

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

   The default location for filter slide definitions is:
   C:\ProgramData\Molecular Devices\SMP651\MultiMode\Detection Software\Filters
7. Click **Save**.
8. On the **Filter Settings** dialog, click **OK**.

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

Import 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 the filter slide that to import exists in the list, rename the **Slide ID** or remove the slide from the list before you import.

To import filter slide definitions into the list:

1. On the **Operations** tab in the ribbon click **Info**.
2. On the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. On the **Filter Settings** dialog, click **Excitation** or **Emission** to display a list of the selected type of filter slides.
4. Click **Import Slides**.
5. On 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\SMP651\MultiMode\Detection Software\Filters
6. Click **Open**.
7. On the **Filter Settings** dialog, click **OK**.

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

Replace 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 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 excitation and emission filters. If you plan to use one or more of the current filter slide definitions again, export the slide definitions before you replace them in the list. See Export Filter Slides on page 413 or Export Filter Slide Definitions on page 415.

To replace the current filter slide definitions:

1. On the **Operations** tab in the ribbon click **Info**.
2. On the **Instrument Information** dialog, click **Edit Slide Configuration**.
3. On the **Filter Settings** dialog, click **Load Slides**.

4. In the message that displays, click **Yes**.

5. On 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\SMP651\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.

Export Filter Slide Definitions

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

The **Export Slides** feature exports all the filter slide definitions in the excitation and emission lists. To export a single filter slide definition, see Export Filter Slides on page 413.

To export all the filter slide definitions in the lists:

1. On the **Operations** tab in the ribbon, click **Info**.

2. On the **Instrument Information** dialog, click **Edit Slide Configuration**.

3. On the **Filter Settings** dialog, click **Export Slides**.

4. On the **Save As** dialog, navigate to the folder where you want to save the exported XML file and enter a name for the file in the **File name** field.

   The default location for filter slide definitions is:
   C:\ProgramData\Molecular Devices\SMP651\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.

View Instrument Software Support

Each instrument 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 these plug-ins for troubleshooting purposes. Generally, you view this list after you contact Molecular Devices technical support.
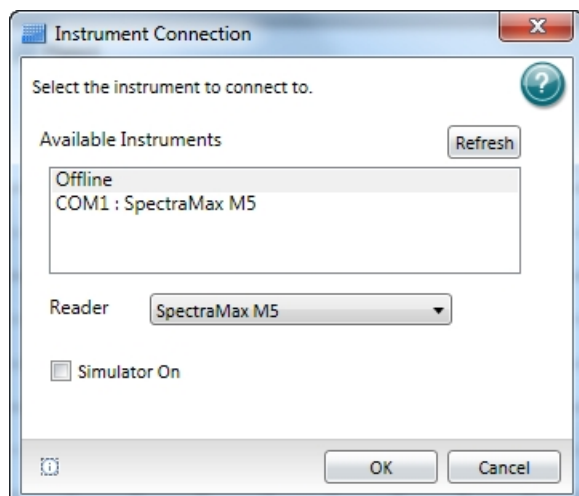
Click the **Information** button on the lower-left of the **Instrument Connection** dialog to open a list of the plug-ins installed with the SoftMax Pro Software.

For a list of the instruments that are supported by this version of the SoftMax Pro Software, see Supported Instruments on page 179.

Chapter 10: SoftMax Pro GxP Software

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. The SoftMax® Pro GxP Microplate Data Compliance Software extends the SoftMax Pro Software into regulated laboratories that work under GMP, GLP, and 21 CFR Part 11 guidelines for secure electronic records. SoftMax Pro GxP Software provides a method to electronically sign data files, and 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 Accounts 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 you can link to the SoftMax Pro GxP Software through a Network File Server or the optional MDC File Server. See SoftMax Pro GxP Software User Accounts on page 418.

You install the SoftMax Pro GxP Software on individual computers that you use for data collection or data analysis. You must link each user account to a User Accounts file before the user can log onto the SoftMax Pro GxP Software and use the software to collect and analyze microplate data.

SoftMax Pro GxP Software User Accounts

Use GxP Admin Software to create and maintain User Accounts (.edb) files. SoftMax Pro GxP Software uses these User Accounts files to regulate user access to the features of SoftMax Pro GxP Software and to provide traceability and accountability in electronic records. The User Accounts file records the activities of all approved users and their related information, including the following:

- User names, user logon IDs, and passwords.
- Assigned user permissions to SoftMax Pro GxP Software features.
- Global settings that pertain to all listed users, such as password aging, non-network use, guest access, and so on.
- Audit histories of all administrator actions in 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 network failure or use of SoftMax Pro GxP Software while traveling.

SoftMax Pro GxP Software users can connect 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 user uses the system browser to link each SoftMax Pro GxP Software installation directly to the User Account file.
- MDC File Server (TCP/IP Server connection) where the User Account file is placed on a secure network location and you link each SoftMax Pro GxP Software installation indirectly to the defined User Account file using the TCP/IP address of the MDC File Server.

For information about how to set up User Accounts files, see the *GxP Admin Software User Guide*.

Connect a User Accounts File

The SoftMax Pro GxP Software uses an associated User Accounts file to authenticate users. The Logon dialog enables you to select the User Accounts file to use.

To define the User Accounts file connection:

1. Start the SoftMax Pro GxP Software.

2. On the Log On dialog, click **Change**.

    If the software is running with a user logged on, select the **GxP** tab in the ribbon. In the

    User Account area, click **User Log Off** and then click **Select Account Database**

    .

3. On the User Accounts File Connection dialog, select a **Connection Type**:

    *   Select **File Path** and do the following. Click **Browse** button to display the Open dialog. Navigate the file structure to locate and select the User Accounts (.edb) file then click **Open** to return to the User Accounts File Connection dialog where the file path displays.

    *   Select **TCP/IP Server** and do the following. Enter the server computer **Name or IP Address** and the **Port Number** in the fields.

    For information about how to set up User Accounts files, see the *GxP Admin Software User Guide*.
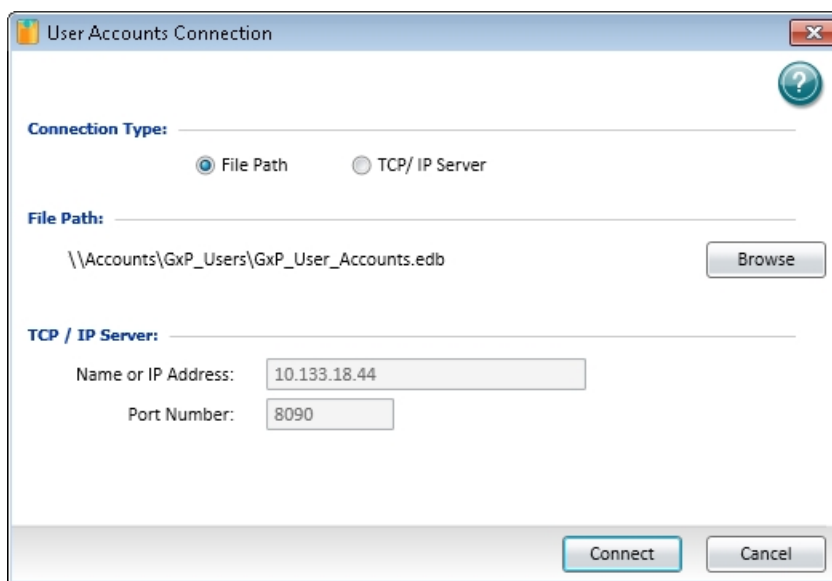
4. Click **Connect**.

5. On the **Log On** dialog, enter your user name and password.

6. Click **Log On**.

After you establish the User Accounts file connection, authorized users can log on and start to acquire and analyze data with the SoftMax Pro GxP Software.

When you use SoftMax® Pro GxP Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader and you log on as a user with the Sign Statements permission, two additional icons appear on the GxP tab in the ribbon.

- Users with the Sign Statements permission click **GxP Mode On** to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and the instrument must be operated from a computer running SoftMax Pro GxP Software.

- Users with the Sign Statements permission click **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to perform reads from the instrument.

Note: The instrument remains locked until the Sign Statements user clicks **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an SpectraMax iD3 instrument that is locked in GxP mode.

Grant TCP/IP Access to MDC File Server

When you use the SoftMax Pro GxP Software with MDC File Server in a Windows 7, 64-bit operating system, the Windows Firewall sometimes blocks user access to MDC File Server through TCP/IP.

To grant user access through TCP/IP, create a new inbound rule and a new outbound rule in the Windows Firewall Advanced Settings.

Access Windows Firewall Advanced Settings

1. Open **Control Panel**.
2. Click **System and Security**.
3. Click **Windows Firewall**.
4. In the left pane, click **Advanced settings** to display the Windows Firewall with Advanced Security dialog.

Create a New Inbound Rule

1. In left pane of the Windows Firewall with Advanced Security dialog, click **Inbound Rules**.
2. In the right pane, click **New Rule**.
3. In the New Inbound Rule Wizard, click **Port**.
4. Click **Next**.
5. Click **TCP**.
6. Click **Specify local ports**.
7. Enter **9001** in the field.
8. Click **Next**.
9. Complete the rest of the wizard based on your network configuration and access rules your system administrator defines.

Creating a New Outbound Rule

1. In left pane of the Windows Firewall with Advanced Security dialog, click **Outbound Rules**.

2. In the right pane, click **New Rule**.

3. In the New Outbound Rule Wizard, click **Port**.

4. Click **Next**.

5. Click **TCP**.

6. Click **Specify local ports**.

7. Enter **9001** in the field.

8. Click **Next**.

9. Complete the rest of the wizard based on your network configuration and access rules your system administrator defines.

Log onto SoftMax Pro GxP Software

Each time you start the SoftMax Pro GxP Software, you must log on before you can continue. If the software is running and the user has logged off, select the **GxP** tab in the ribbon. In the **User Accounts** section, click **User Log On** .

On the **Log On** dialog, enter your user ID and your password and click **Log On**.

> **Note:** The user ID is not case sensitive. The password is case sensitive.

To associate the SoftMax Pro GxP Software with a User Accounts file, or to change the related User Accounts file, click **Change**. See Connect a User Accounts File on page 419.

If the GxP Admin Software administrator permits guest authentication, users without an account can log on as a guest. Guests can open, view, and print existing data files or protocols. They cannot modify or save changes to data files or protocols. The Log On dialog explicitly states the guest user permissions and a **Guest** button is available. For more information, see the *GxP Admin Software User Guide*.

When you use SoftMax® Pro GxP Software to operate the SpectraMax iD3 Multi-Mode Microplate Reader and you log on as a user with the Sign Statements permission, two additional icons appear on the GxP tab in the ribbon.

- Users with the Sign Statements permission click **GxP Mode On** to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and the instrument must be operated from a computer running SoftMax Pro GxP Software.

- Users with the Sign Statements permission click **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to perform reads from the instrument.

> **Note:** The instrument remains locked until the Sign Statements user clicks **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an SpectraMax iD3 instrument that is locked in GxP mode.

View User Information

To view information about the current user, select the **GxP** tab in the ribbon. In the User Accounts section, click **Account Information** .

The User Account Information dialog provides information about the current user.
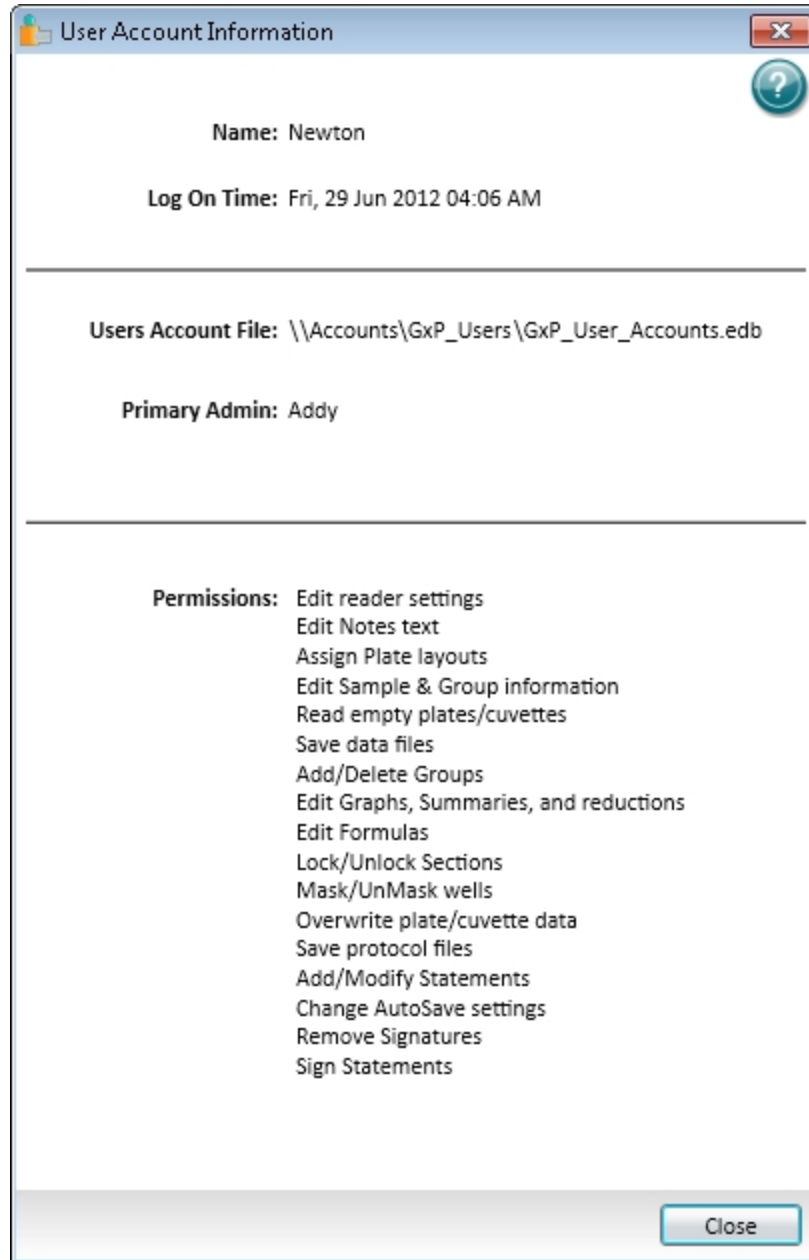
- The top section displays the user name and the time the user logged on.
- The middle section displays the path to the User Accounts file and the name of the GxP Admin Software administrator.
- The lower section displays the permissions assigned to the user by the GxP Admin Software administrator.

For more information about user accounts, see the *GxP Admin Software User Guide*.

Click **Close**.

Change User Password

Users cannot change their user names, but they can change their password. The GxP Admin Software administrator can also define rules to require password changes.

1. Select the **GxP** tab in the ribbon, in the User Accounts section, click **Change Password** .

2. On the Change Password dialog, enter your old password in the first field.
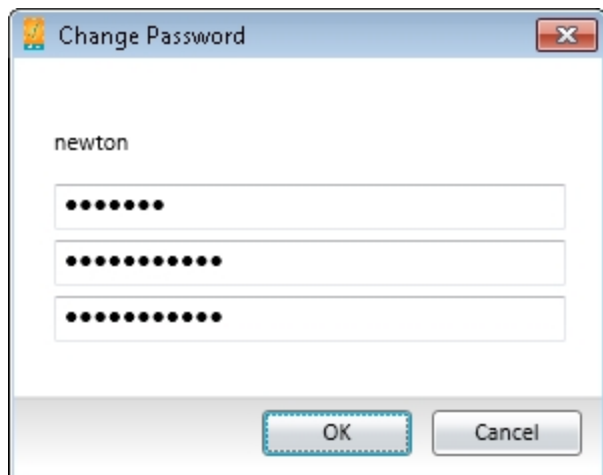
3. Enter your new password in the second field, and then enter it again in the third field to confirm it.

   The software compares the second and third field to confirm they match before the new password is saved.

   > **Note:** The password must be between 6 and 32 characters with a minimum of one alpha character and a minimum of one numeric character. The password is case sensitive.

4. Click **OK**.

The next time you log onto the software, you need to use the new password.

Work 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 you have reviewed, verified, or given approval to an experiment.

A user with the correct permission can apply an electronic signature to a statement. See Sign GxP Statements on page 429. After you sign a GxP statement, the SoftMax Pro GxP Software locks the data file to prevent further changes to the file. To unlock the file, remove all the statement signatures.

To work with GxP statements, select the **GxP** tab in the ribbon. In the Data section, click

Statements to open the Statements dialog.
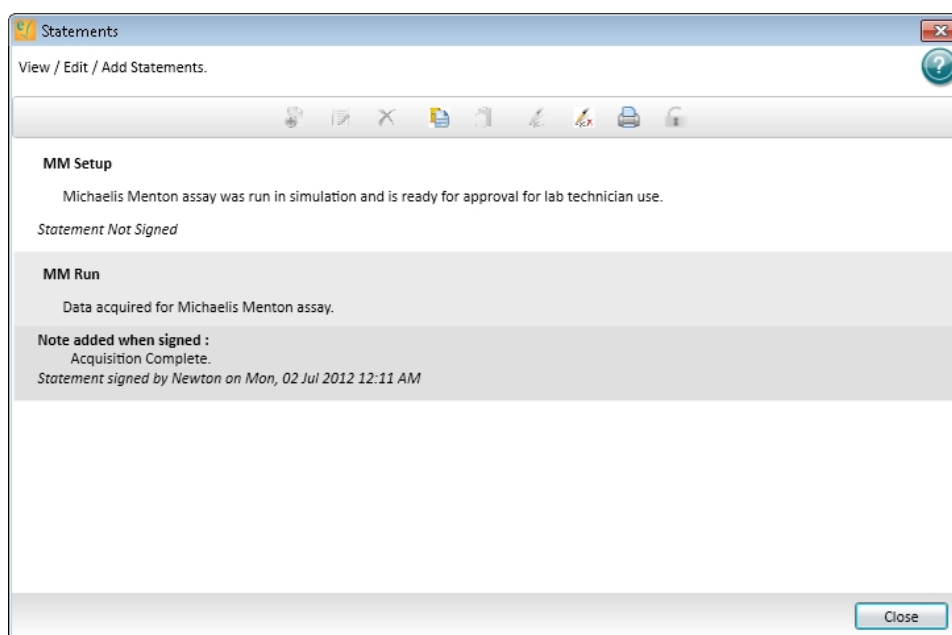
The following icons appear in the toolbar at the top of the Statements dialog.

Table 10-1: The Statements Toolbar

Tool	Description
	Add Statement (see Add GxP Statements on page 427)
	Edit Statement (see Edit GxP Statements on page 427)
	Delete Statements (see Delete All GxP Statements on page 428)
	Copy Statement (see Duplicate GxP Statements on page 428)
	Paste Statement (see Duplicate GxP Statements on page 428)
	Sign Statement (see Sign GxP Statements on page 429)
	Remove All Signatures (see Remove Signatures from All GxP Statements on page 430)

Table 10-1: The Statements Toolbar (continued)

Tool	Description
🖨	Disable printing of Statements (see Print GxP Statements on page 431)
🖨⊘	Enable printing of Statements (see Print GxP Statements on page 431)
🔓	Lock Statements (see Lock and Unlock GxP Statements on page 431)
🔒	Unlock Statements (see Lock and Unlock GxP Statements on page 431)

To close the Statements dialog, click **Close**.

The statements status button in the upper-right corner displays the number of statements in the file and how many statements are signed.

To open the Statements dialog, click the **(n) Statements ((n) Signed)** button.

Add GxP Statements

1. Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the Statements dialog.
2. If more than one statement displays, select the statement above where you want to add the new statement.
3. Click **Add Statement** in the dialog toolbar to open the Add Statement dialog.
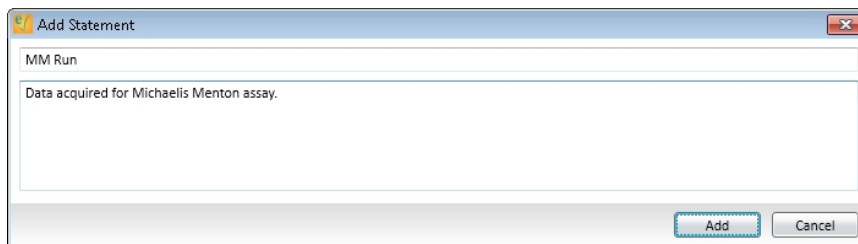
4. In the upper field, enter the statement title.

   Each statement requires a title.
5. In the lower field, enter the statement content.
6. Click **Add**.

Edit GxP Statements

1. Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the Statements dialog.
2. Select the statement to edit.
3. Click **Edit Statement** in the dialog toolbar to open the Edit Statement dialog.
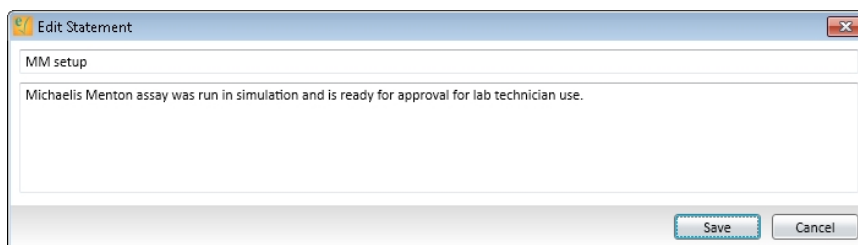
4. In the upper field, edit the statement title.
5. In the lower field, edit the statement content.
6. Click **Save**.

Delete All GxP Statements

1. Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the Statements dialog.
2. Click **Delete Statements** ✗ in the dialog toolbar.

> ⚠️ **CAUTION!** This deletes all the GxP statements in the SoftMax Pro GxP Software data file. There is no undo for this. After you delete the statements, you cannot retrieve them.
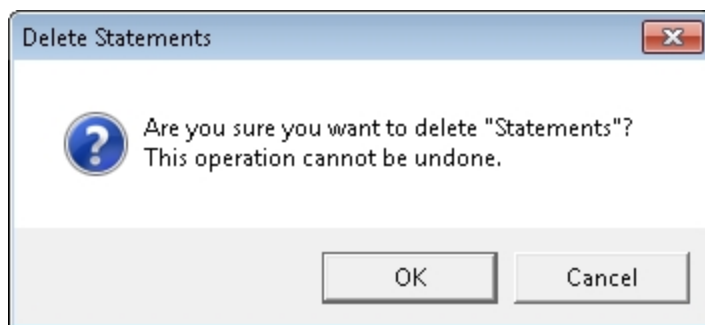
3. In the Delete Statements message, click **OK** to delete all the GxP statements in the SoftMax Pro GxP Software data file.

Duplicate GxP Statements

1. Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the Statements dialog.
2. Select the statement to duplicate.
3. Click **Copy Statement** in the dialog toolbar.
4. Click the statement directly above where you want to paste the copied statement.
5. Click **Paste Statement** in the dialog toolbar.

To edit the pasted statement, select the statement and click **Edit Statement** in the dialog toolbar. See Edit GxP Statements on page 427.
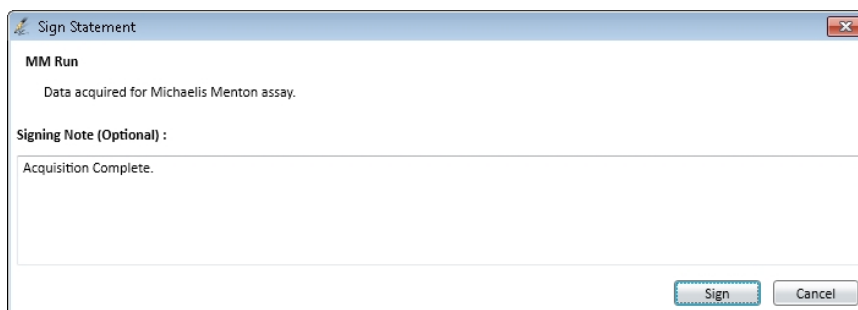
Sign GxP Statements

1. Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the Statements dialog.

2. Select the statement to sign.

   The statement you select has a light gray background.

   > **Note:** You cannot sign a statement if it is locked. To unlock statements, click **Unlock Statements** in the dialog toolbar.
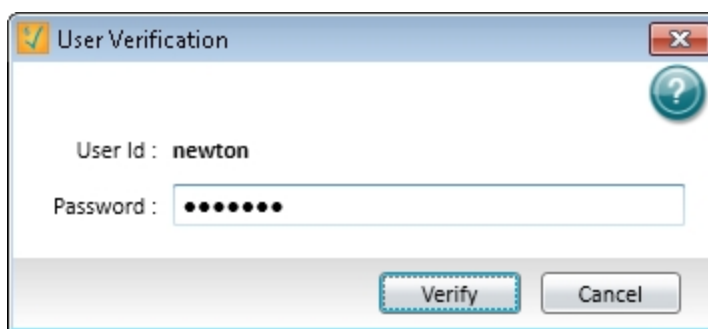
3. Click **Sign Statement** in the dialog toolbar to open the Sign Statement dialog.

   Sign Statement is not available until you select a statement.

4. Verify that the text at the top is the statement to sign.

5. In the lower field, enter the signing note, if applicable.

6. Click **Sign** to display the User Verification dialog.

7. Enter your password.

8. Click **Verify**.

   > **Note:** After you sign a GxP statement, the SoftMax Pro GxP Software locks the data file to prevent further changes to the file. To unlock the file, remove all the statement signatures.

Remove Signatures from All GxP Statements

1. Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the Statements dialog.

2. Click **Remove all signatures** in the dialog toolbar to open the Confirm E-Signature Removal dialog.

> ⚠️ **CAUTION!** This removes the signatures from all GxP statements in the SoftMax Pro GxP Software data file. There is no undo for this. After you remove the signatures, the data file is unlocked and changes can be made to the file.
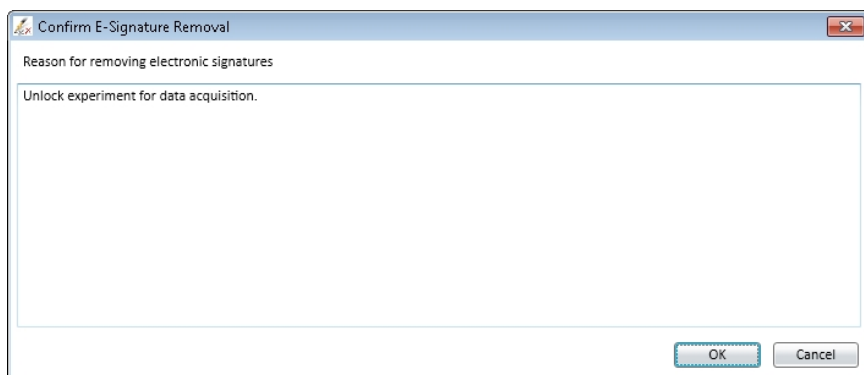
3. Enter the reason you removed the signatures.

4. Click **OK** to open the User Verification dialog.
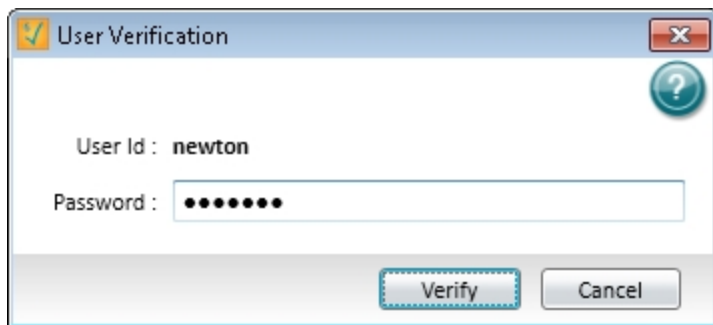
5. Enter your password.

6. Click **Verify**.

Print GxP Statements

The Statements dialog enables you to let the statements print or block the statements from printing.

Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the **Statements** dialog.

- To let the statements print with the data file, click **Enable printing of Statements** in the dialog toolbar.
- To prevent the statements from printing with the data file, click **Disable printing of Statements** in the dialog toolbar.

The button switches between and to indicate whether or not statements will print.

You can select to print or not to print statements with the data file on the Print Selected dialog. For information about how to print the contents of a data file, see Printing on page 72.

Lock and Unlock GxP Statements

The Statements dialog enables you to lock statements to prevent changes.

You cannot lock signed statements. After you sign a GxP statement, the SoftMax Pro GxP Software locks the data file to prevent further changes to the file. To unlock the file, remove all the statement signatures.

Select the **GxP** tab in the ribbon. In the Data section, click **Statements** to open the **Statements** dialog.

- To prevent changes to the statements, click **Lock Statements** in the dialog toolbar.
- To permit changes to the statements, click **Unlock Statements** in the dialog toolbar.

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 print options. See Print GxP Statements on page 431.

View GxP Audit Trail

The Audit Trail dialog provides read-only access to the audit trail for the open data file and enables you to attach a note to the audit trail.

Select the **GxP** tab in the ribbon. In the Data section, click **Audit Trail** to open the Audit Trail dialog.
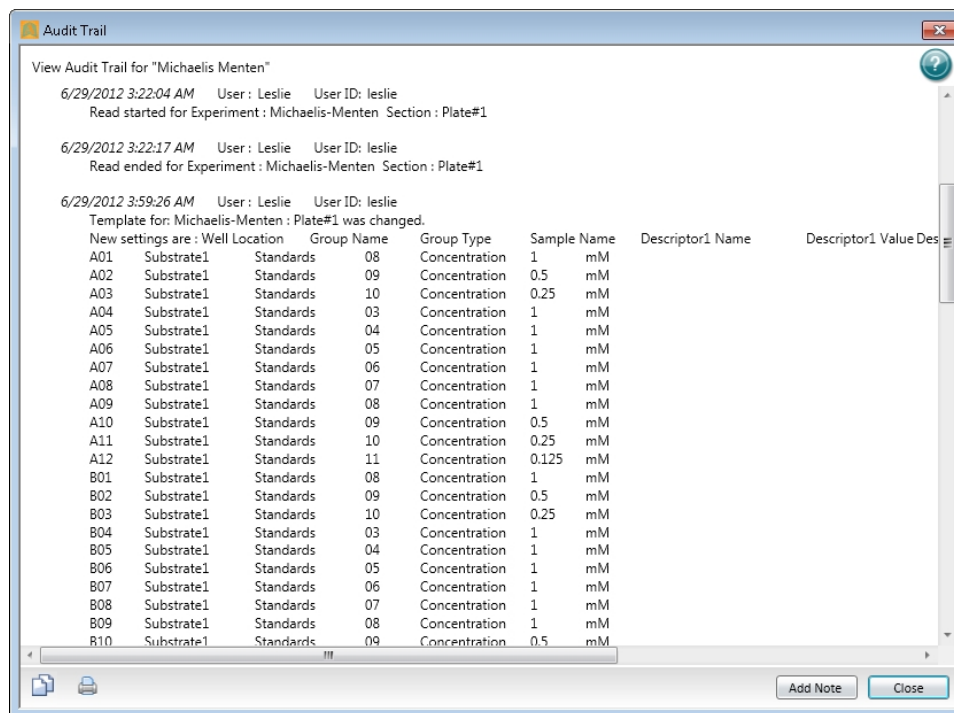
Each entry in the audit trail has a time-and-date stamp, and identifies the user who performed the task.

To add a note to the audit trail, see Add Notes to GxP Audit Trail on page 433.

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 to save or print.

To let the audit trail print or block the audit trail from printing, see Print GxP Audit Trail on page 433.

Click **Close**.

Add Notes to GxP Audit Trail

1. Select the **GxP** tab in the ribbon. In the Data section, click **Audit Trail** to open the Audit Trail dialog.
2. Click **Add Note** to open the Add a Note dialog.
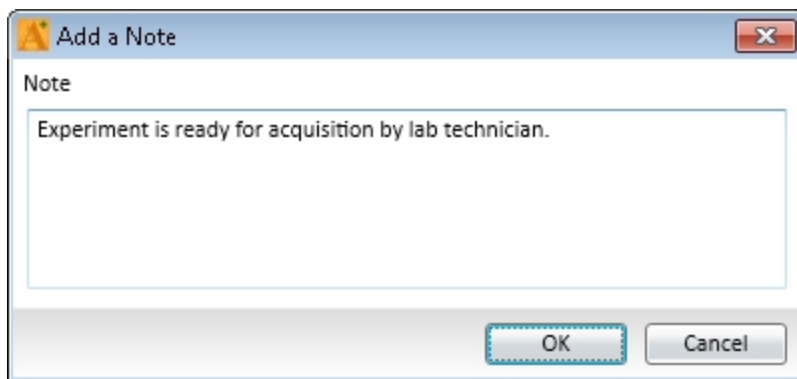
3. Enter the note content.
4. Click **OK**.

The note is added to the end of the audit trail and becomes part of the permanent record for the open data file.

Print GxP Audit Trail

The Audit Trail dialog enables you to let the audit trail print or block the audit trail from printing.

Select the **GxP** tab in the ribbon. In the Data section, click **Audit Trail** to open the **Audit Trail** dialog.

- To let the audit trail print with the data file, click **Enable printing of the audit trail** in the dialog toolbar.
- To prevent the audit trail from printing with the data file, click **Disable printing of the audit trail** in the dialog toolbar.

The button switches between and to indicate whether or not the audit trail will print.

You can select to print or not to print the audit trail with the data file in the Print Selected dialog. For information about printing the contents of a data file, see Printing on page 72.

Lock and Unlock Sections

In the SoftMax Pro GxP Software, you can lock individual sections in an experiment to prevent changes to them. A locked section cannot be deleted nor have its data changed. 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, select a section in the navigation tree and click **Lock Selection** 🔒 at the top of the Navigation Tree.
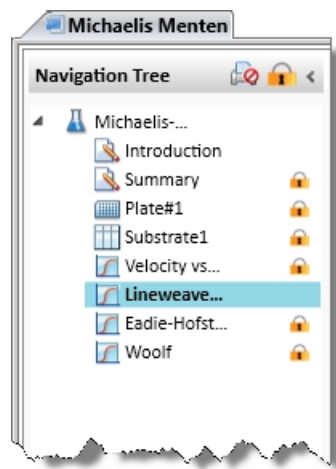
To lock or unlock all the sections in an Experiment, select the experiment and then click **Lock Selection** 🔒 at the top of the Navigation Tree.

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 on 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 applicable 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, along with 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. This provides features that let users demonstrate compliance with these regulations.

See SoftMax Pro GxP Software on page 417.

A

Absorbance

Absorbance is the quantity of light absorbed by a solution. To measure absorbance accurately, it is necessary to eliminate light scatter. If there is no turbidity, then 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 Absorbance Read Mode on page 137.

AlphaScreen

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 moves beads (coated with A and B, respectively) together, a cascade of chemical reactions 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) lets diffusion reach up to only around 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 beads are light sensitive. Beads are best handled under subdued (<100 lux) or green filtered (Roscolux filters #389 from Rosco, or equivalent) light conditions. Do incubation steps in the dark.

See .

AutoRead

Auto Read automatically advances to the next Plate section after a read in the sequence that the Plate sections display within a single Experiment. When you start a read for a Plate section that is enabled to automatically advance with AutoRead, the Auto Read feature advances to the next Plate section in the Experiment and then reads that plate. If multiple Plate sections are enabled to automatically advance with AutoRead, the Auto Read feature reads all enabled Plate sections until it reads a non-enabled Plate section or the last Plate section in the Experiment. Plate sections that come before the selected Plate section are not read.

For example, if you have five Plate sections (Plate 1, Plate 2, Plate 3, Plate 4, and Plate 5) in an Experiment, you can enable Plate 1, Plate 2, and Plate 3 to automatically advance with Auto Read. If you start a Read operation for Plate 2, then Plate 2, Plate 3, and Plate 4 are read in sequence. Plate 1 is not read, since it comes before Plate 2. Plate 5 is not read since Plate 4 is not enabled to automatically advance with AutoRead.

You can set an interval, or delay time, between plate reads.

See .

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 Auto Save Settings on page 69.

B

Baseline

Baseline reads are available for kinetic reads with injection.

The instrument collects baseline data before the injection, so that the baseline data can be subtracted from the reads that follow the injection.

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 227.

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. If you turn calibration off, SoftMax Pro Software uses the air-calibration values stored in the instrument firmware or the values from the previous calibration.

For Fluorescence and Luminescence reads, the instrument makes a reference read on the internal reference chip at each wavelength before reading the microplate. If you turn calibration off, SoftMax Pro Software uses the default values stored in the instrument firmware or the values from the previous calibration.

For kinetic readings the calibration measurement is made between readings during a run, unless the read interval is too short to allow calibration.

See More Settings on page 249.

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 you select the Luminescence read mode. 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 you enable Auto Cutoff, the instrument sets the cutoffs based on the wavelengths you choose to read. When you select the Spectrum read type, a manual setting for the emission monochromator is required, with the default being no cutoff filter.

See Wavelength Settings on page 218.

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 Cuvette Set Sections on page 115.

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 Save a Protocol as the Default Protocol on page 64.

E

Emission Spectrum Scan

An emission spectrum scan measures fluorescence or luminescence across a spectrum of wavelengths. Fluorescent reads use 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 439.

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. 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 Data Reduction on page 325.

Endpoint

In an endpoint read, a reading of each microplate well is taken 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 (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

See Endpoint Read Type on page 134.

Excitation Spectrum Scan

An excitation 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 439.

F

Fluorescence

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

The governing equation for fluorescence is:

Fluorescence = extinction coefficient × concentration × quantum yield × excitation intensity × pathlength × emission collection efficiency

See Fluorescence Intensity Read Mode on page 143.

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 a different molecule of equal or greater size can be monitored through its speed of rotation.

See Fluorescence Polarization Read Mode on page 164.

Fluorescence Resonance Energy Transfer (FRET)

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 a molecular collision. The transfer of energy leads to a reduction in the fluorescence intensity and excited state lifetime of the donor, and an increase in the emission intensity of the acceptor. 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 each other.
- 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 must be approximately parallel.

See FRET Read Mode on page 159.

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 Fluorescence Intensity Read Mode on page 143.

G

G Factor

The 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 Data Reduction on page 166.

Gain

Gain is the quantity 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 227.

Group

The Template Editor enables you to assign wells to group types. Depending on the protocol, certain group types, such as Standard or Unknown, can be created automatically. You can create other groups as required.

See Define Groups on page 265.

I

Imaging

Imaging read mode does whole-cell imaging assays.

Whole-cell imaging assays are cell-based, or object-based, rather than the single-point measurements found in other types of microplate reads. These types of assays can yield more biologically meaningful results that can discriminate the fluorescence related to objects, such as cells or beads, from the bulk solution within a microplate well.

See Imaging Read Mode on page 174.

K

Kinetic

In a kinetic read, the instrument collects data over time with multiple readings taken in the center of each well at regular intervals. To get the shortest possible interval for multiple-wavelength Kinetic readings, choose wavelengths in ascending order.

The values calculated based on raw kinetic data include 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 134.

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 before the lag time.

See Data Reduction on page 325.

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 Luminescence Read Mode on page 149.

LUT

A look-up table (LUT) determines the colors to use for the intensity values when displaying an image.

See View Western Blot Membrane Data on page 395.

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 Mask Wells or Cuvettes on page 324.

Membrane

The Membrane read type is used for a time-resolved fluorescence reading of a Western Blot membrane.

See Membrane Read Type on page 135 and Western Blot TRF Read Mode on page 171.

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 333.

Optical Density (OD)

Optical density (OD) is the quantity of light passing through a sample to a detector relative to the total quantity of light available. Optical Density includes absorbance of the sample plus light scatter from turbidity and background. You can compensate for background using blanks.

A blank well contains everything used with the sample wells except the chromophore and sample-specific compounds. Do not use an empty well for a blank.

Some applications are designed for turbid samples, such as algae or other micro-organisms in suspension. The reported OD values for turbid samples are likely to be different when read by different instruments.

See Absorbance Read Mode on page 137.

P

PathCheck Technology

The temperature-independent PathCheck® Pathlength Measurement Technology normalizes your absorbance values to a 1 cm path length based on the near-infrared absorbance of water.

The Beer–Lambert law states that absorbance is proportional to the distance that light travels through the sample:

$a - b = c\, d\, e + f$

where a is absorbance, b is blank, c is concentration, d is the depth of sample layer, e is extinction (coefficient of...), and f is further terms, e.g., non-linearity caused from turbidity.

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 do extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis for quantifying the unique absorptivity properties of compounds in solution. Quantitative analysis can be done 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 directly proportional to concentration when there is no background interference.

See PathCheck Pathlength Measurement Technology on page 139.

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 need to read the same physical plate twice with different instrument settings, then use two Plate sections. You can create as many Plate sections as you need.

See Plate Sections on page 105.

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 Manage Protocol Files on page 63.

R

Raw Data

Raw data is the signal reported from the instrument with no changes made. This is reported as optical density (OD), relative fluorescence units (RFU), or relative luminescence units (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 the SoftMax Pro Software.

See Data Reduction on page 325.

Read Mode

For more information on read modes, see the following topics:

- Absorbance Read Mode on page 137
- Fluorescence Intensity Read Mode on page 143
- Luminescence Read Mode on page 149
- Time-Resolved Fluorescence Read Mode on page 154
- HTRF Read Mode on page 160
- FRET Read Mode on page 159
- Fluorescence Polarization Read Mode on page 164
- AlphaScreen Read Mode on page 168
- Western Blot TRF Read Mode on page 171
- Imaging Read Mode on page 174

Read Type

For more information on read types, see the following topics:

- Endpoint Read Type on page 134
- Kinetic Read Type on page 134
- Well Scan Read Type on page 134
- Spectrum Read Type on page 134
- Flex Read Type on page 135
- Membrane Read Type on page 135

Reduced Data

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

Reference

A reference reading can be taken either on air or using a cuvette containing the buffer of your sample.

See Collect Data from Cuvettes on page 310.

ROI

A region of interest (ROI) is a small area or region of an image where items of interest can be found.

- For Western Blot Membrane reads, the region of interest is defined in the **Plate** section. See View Western Blot Membrane Data on page 395.
- For Imaging reads, the region of interest is defined in the **Settings** dialog. See Region of Interest Settings on page 245.

S

Series

You can define several samples as a series, which enables you to enter incremental sample descriptors (for example, dilutions or concentrations) and sample names to the template when the increment can be expressed as a mathematical operation. This enables 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 Create a Series on page 267.

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 333.

SmartInject

SmartInject™ technology is available for reads with injection.

The SmartInject™ technology shakes the microplate during the injection and continues to shake through the following delay step, if a **Delay** node follows the **SmartInject** node. The microplate stops shaking before the well is read.

Spectrum

Depending on the read mode selected, a spectrum read measures optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU) across a spectrum of wavelengths.

See Spectrum Read Type on page 134, Emission Spectrum Scan on page 439, and Excitation Spectrum Scan on page 439.

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 439 and Excitation Spectrum Scan on page 439.

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 you can use to designate the location of blanks, standards, controls, unknowns, empty wells, or to assign wells to other groups you create.

See Configure Microplate Templates on page 264.

Time-Resolved Fluorescence (TRF)

Time-resolved fluorescence (TRF) is a measurement technique that depends on three characteristics that lead to better discrimination between the specific signal, proportional to the quantity of label, and the unspecific fluorescence resulting from background and compound interference:

- Pulsed excitation light sources
- Time-gated electronics faster than the fluorescence lifetime
- Labels with prolonged fluorescence lifetime

The time-gating electronics introduce a delay between the cut off of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the microplate) vanishes while only a small portion of the specific fluorescence from the label is sacrificed. Enough of the specific signal remains during the decay period with the added benefit of reduced background.

See Time-Resolved Fluorescence Read Mode on page 154.

%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 333.

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 done 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 333.

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 permit 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 134.

Z

Z′

Z′ is the standard statistical parameter in the high-throughput screening community for measuring the quality of a screening assay independent of test compounds. It is used as a measure of the signal separation between the positive controls and the negative controls in an assay.

The value of Z′ can be determined using the following formula:

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|\, Mean_{c+} - Mean_{c-}\,|}$$

where **SD** is the standard deviation, **c+** is the positive control, and **c−** is the negative control.

A Z′ value greater than or equal to 0.4 is the generally acceptable minimum for an assay. Higher values might be desired when results are more critical.

Z′ is not linear and can be made unrealistically small by outliers that skew the standard deviations in either population. To improve the Z′ value, you can increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

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