



SoftMax Pro Data Acquisition and Analysis Software

User Guide

Software Version 7.3

SoftMax Pro Data Acquisition and Analysis Software User Guide

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The SoftMax® Pro Data Acquisition and Analysis Software controls Molecular Devices spectrophotometers and absorbance, luminescence, and fluorescence microplate readers and detection platforms.



Note: For the rest of this document, all references to the SoftMax Pro Software - Standard edition are also applicable to the SoftMax Pro Software - MiniMax Imaging edition and references to the SoftMax Pro Software are applicable to all editions of the SoftMax Pro Software unless otherwise specified.

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 Protocol Library contains over 160 protocols that contain instrument settings and formulas. You can customize the protocols in the Protocol Library to facilitate the data analysis and report creation.

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

For a complete list of the instruments this release of the SoftMax Pro Software supports, see [Supported Instruments on page 315](#).

Documentation

The SoftMax Pro Software installation places a copy of the microplate reader user guides (.pdf files) in the following location on the computer:

C:\ProgramData\Molecular Devices\User Guides

Use the following link for the most up-to-date microplate reader user guides on the Molecular Devices Knowledge Base:

[Spectranet](#)

To access the Knowledge Base from the software:

- Select the **Help** tab, click **Contact Us**, and then select **Knowledge Base**.

Important Installation Information

You should install the SoftMax Pro Software on the computer before you set up the microplate reader. Please be aware that some updates to the SoftMax Pro Software require a purchase.

See the following documents for computer system requirements and installation instructions:

- *SoftMax Pro Data Acquisition and Analysis Software - Standard Edition and MiniMax Imaging Edition - Installation Guide*
- *SoftMax Pro GxP Microplate Data Compliance Software System - Installation Guide for the Multi Computer Setup*
- *SoftMax Pro GxP Microplate Data Compliance Software System - Installation Guide for the Single Computer Setup*

Uninstalling the SoftMax Pro Software - Standard Edition



Note: To uninstall the SoftMax Pro Software - GxP edition, contact Technical Support. See [Obtaining Support](#) on page 18.

Before you uninstall the SoftMax Pro Software - Standard edition, back up your data and documents to a folder outside of the SoftMax Pro Software folder. This method removes related Windows registry information.

To uninstall the software:

1. Click **Start > Control Panel**.
2. Click **Programs and Features**.
3. In the program list, select **SoftMax Pro <n.n>**.
4. Click **Uninstall** or **Remove**.
5. Follow the prompts to uninstall the program.

Starting the Software

You can choose to run the software in Offline mode and/or Simulator mode with the computer not connected to an instrument. You must connect the computer to an instrument for plate reads and data collection. See [Selecting an Instrument on page 64](#).

Power on the instrument and wait for the instrument to complete its start-up sequence before you start the software.

To start the software:

- For the SoftMax Pro Software - Standard edition: Double-click the  **SoftMax Pro** icon on the desktop or from the Windows Start menu, click **Start > All Programs > Molecular Devices > SoftMax Pro <n.n> > SoftMax Pro <n.n>.exe**.
- For the SoftMax Pro Software - GxP edition: Double-click the  **SoftMax Pro GxP** icon on the desktop or from the Windows Start menu, click **Start > All Programs > Molecular Devices > SoftMax Pro <n.n> GxP > SoftMax Pro <n.n> GxP.exe**
 - Enter your username, password, and select a Project. Then click **Log On**. See [Logging In to the SoftMax Pro GxP Software on page 254](#).



Note: When you use the FilterMax™ F3 Multi-Mode Microplate Reader, FilterMax™ F5 Multi-Mode Microplate Reader, SpectraMax® i3 Multi-Mode Microplate Reader, SpectraMax® i3x Multi-Mode Microplate Reader, and SpectraMax® Paradigm® Multi-Mode Microplate Reader for the first time, the installation process installs the latest instrument firmware updates.

The SoftMax Pro Software saves instrument firmware updates on the computer hard drive. If you put a new image on the computer or you replace the computer, this information is lost. However, the software installation and updates automatically install the latest instrument firmware.

Instrument firmware information saves in the following file on the computer:
C:\ProgramData\Multimode\Detection Software\Templates\UpdateInfo.xml

Activating Software Licenses

The Software License Activation dialog displays when the SoftMax Pro Software license is inactive or is ready to expire. The SoftMax Pro Software package includes the software product key. For information about the features of your license or to obtain a new license key, contact Molecular Devices.

If the computer does not have access to the Internet, see [Activating Software Licenses Offline](#) on page 11.

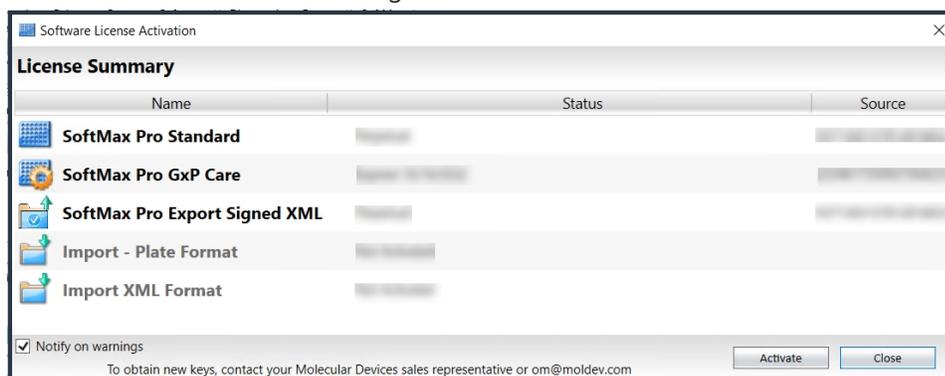
Activating Software Licenses Online



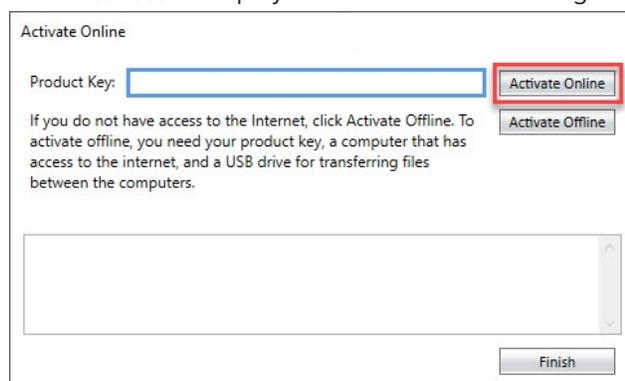
Note: Port 443 must be open and the computer must be able to access <https://smplicensingonline.moleculardevices.com/> to use the online activation feature.

To activate software licenses online:

1. In the Ribbon, select the **Help** tab, and then click  **Software License** to display the Software License Activation dialog.



2. Click **Activate** to display the Activate Online dialog.



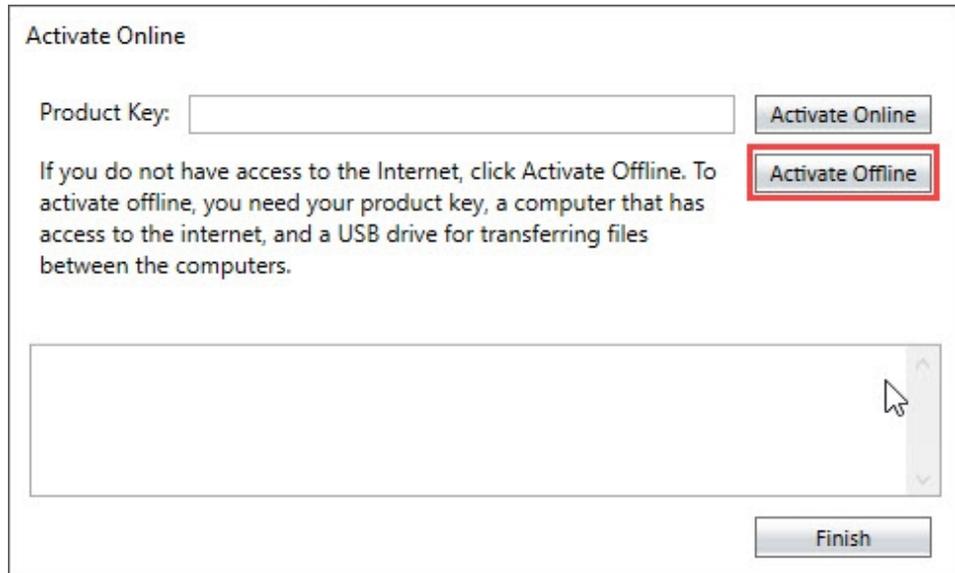
3. In the **Product Key** field, enter the product key.
4. Click **Activate Online**.
5. Wait for the *Activation Success* message and click **Finish**.

Activating Software Licenses Offline

When the computer onto which you install the SoftMax Pro Software cannot connect to the Internet, you need a USB drive and access to a computer that is connected to the Internet.

To activate software licenses when the computer cannot connect to the Internet:

1. In the Activate Online dialog:



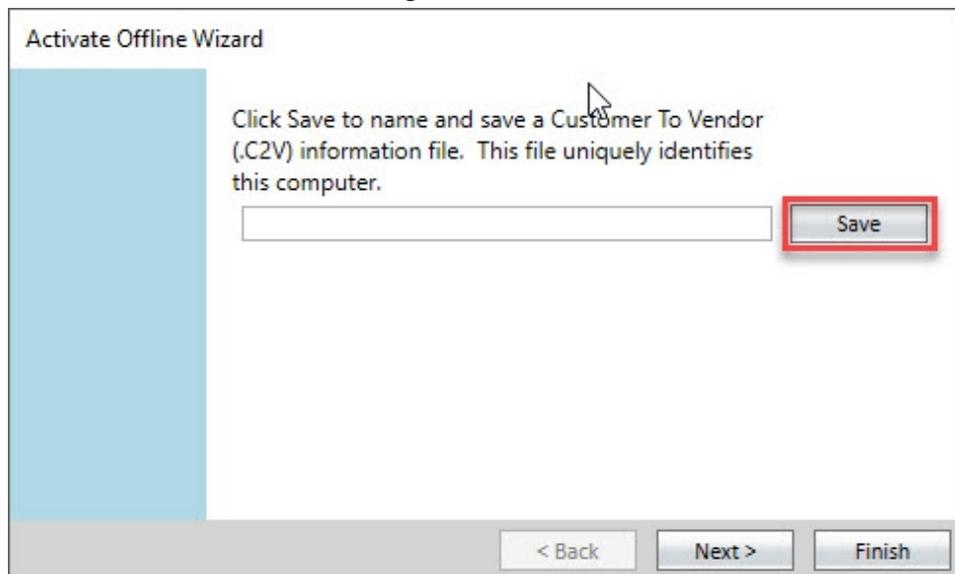
Activate Online

Product Key:

If you do not have access to the Internet, click **Activate Offline**. To activate offline, you need your product key, a computer that has access to the internet, and a USB drive for transferring files between the computers.

Click **Activate Offline**.

2. In the Activate Offline Wizard dialog:

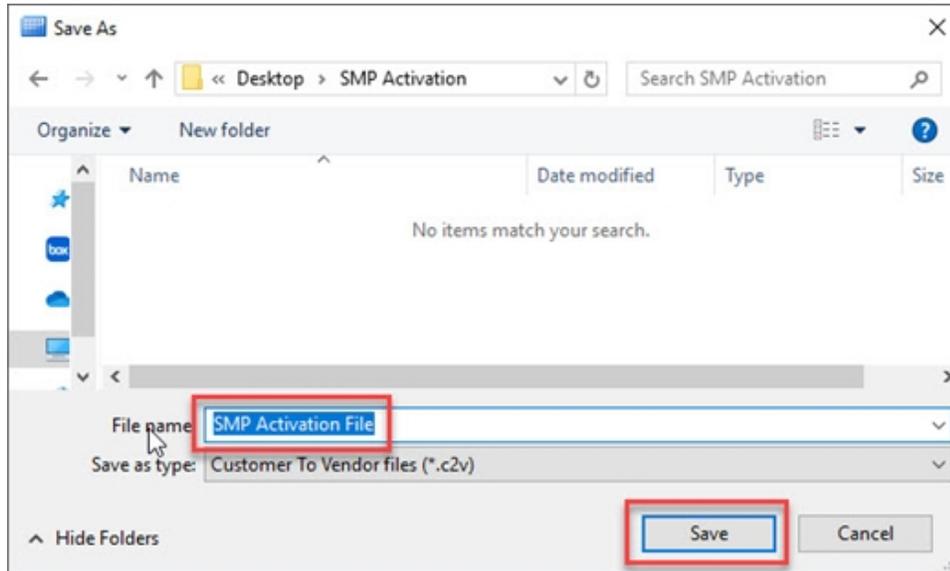


Activate Offline Wizard

Click Save to name and save a Customer To Vendor (.C2V) information file. This file uniquely identifies this computer.

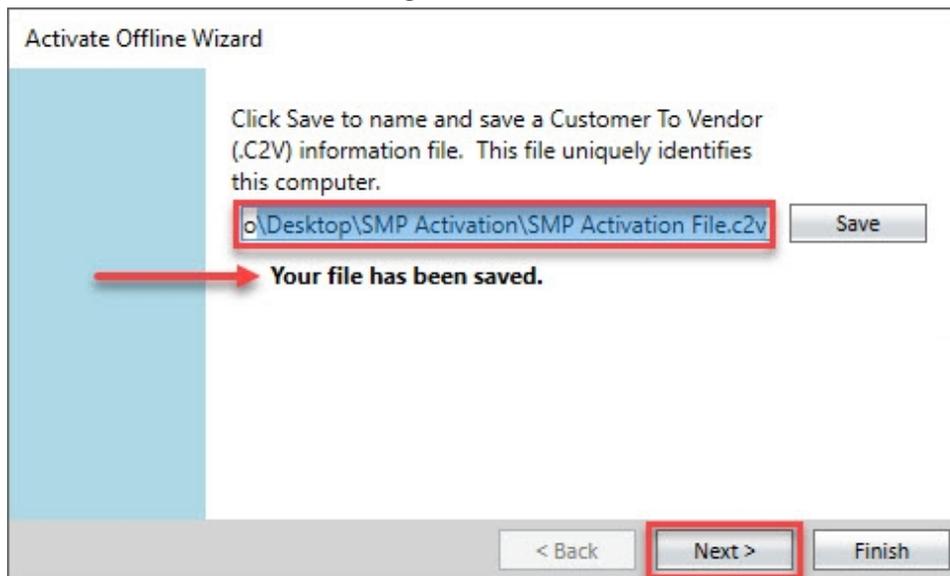
Click **Save**.

3. In the Save As dialog:



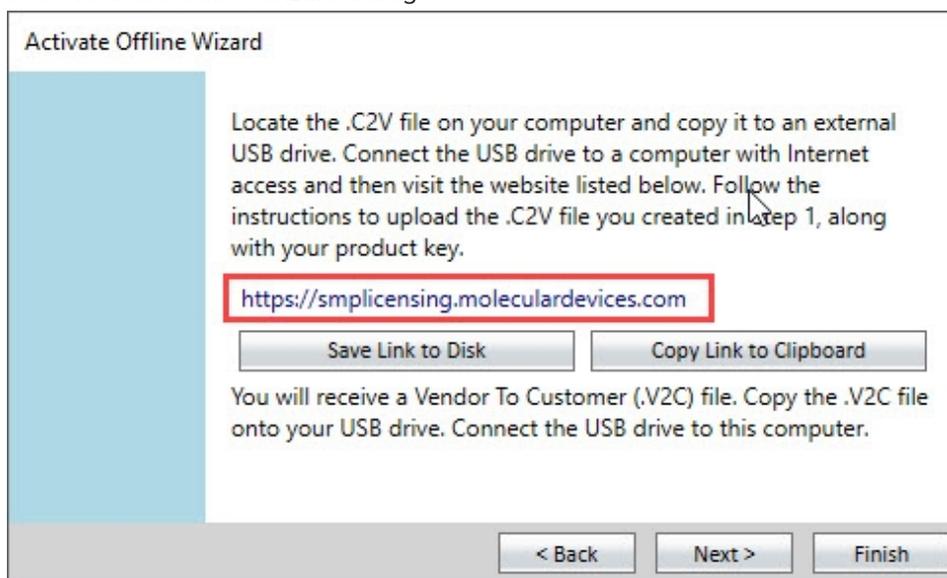
- a. In the **File Name** field, enter a name for the customer to vendor file (.c2v).
- b. Navigate to the folder on the USB in which to save the .c2v file.
- c. Click **Save**.

4. In the Activate Offline Wizard dialog:



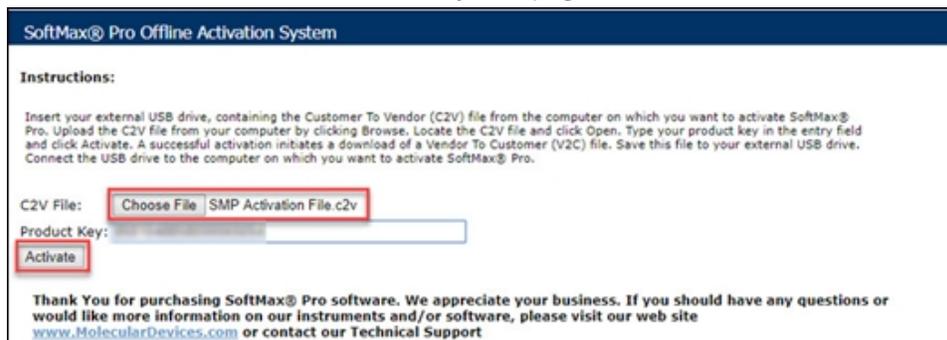
Note the path to the file and click **Next**.

- In the Activate Offline Wizard dialog:



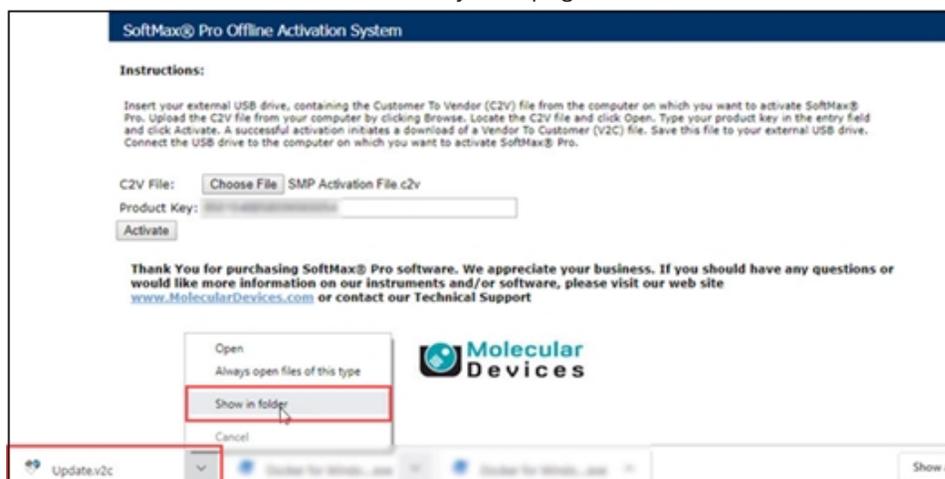
Click **Save Link to Disk**, **Copy Link to Clipboard** or make note of the link. You need this link for the next steps.

- Take the USB drive to a computer that has access to the Internet.
- Open an Internet browser and go to the link provided.
- On the SoftMax Pro Offline Activation System page:

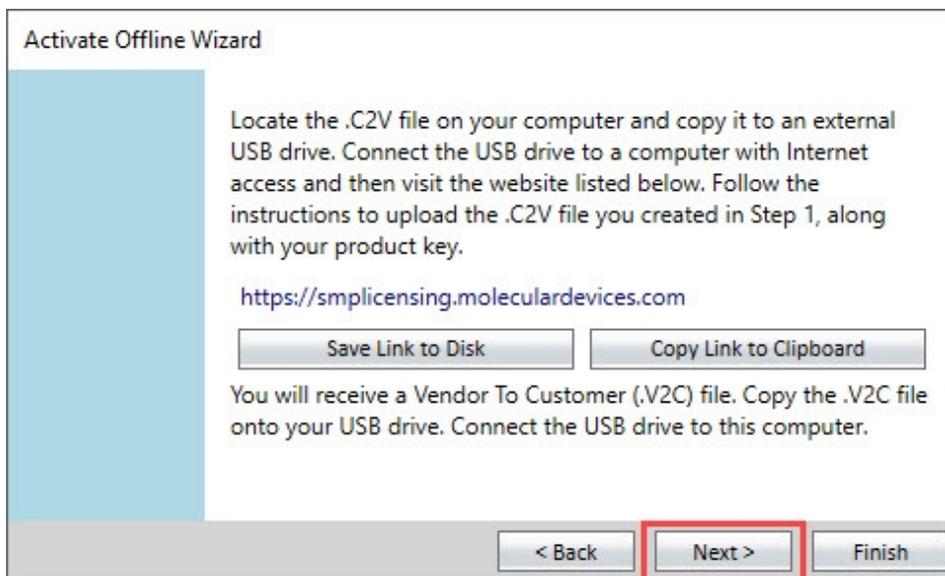


- Click **Choose File**.
- Navigate to and select the **.c2v** file on the USB drive.
- Click **Activate**.

9. On the SoftMax Pro Offline Activation System page:

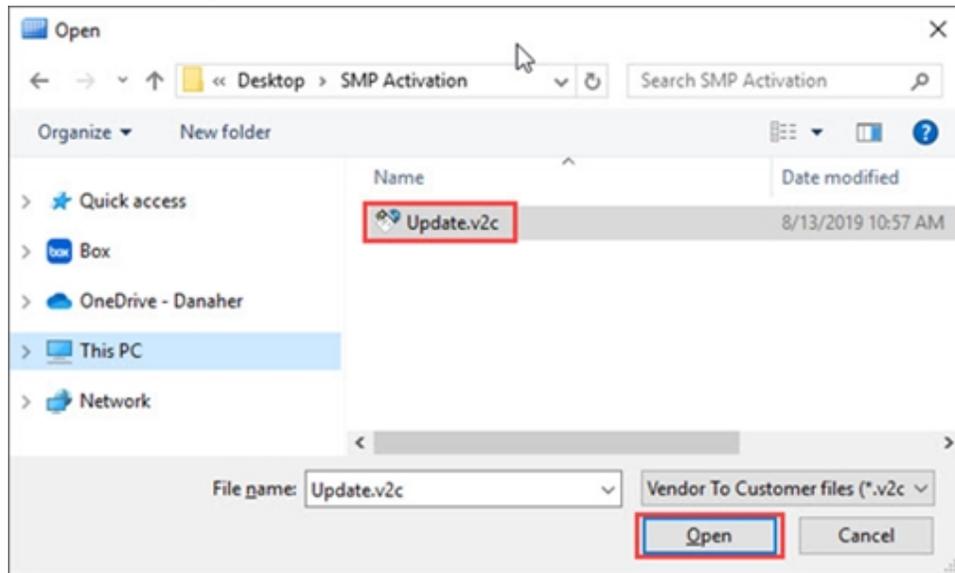


- a. Click **Show in Folder** to display the location of the **Update.v2c** file that the activation system downloads to your computer. Otherwise, locate the **Update.v2c**.
 - b. Copy the **Update.v2c** file to the USB drive.
10. Take the USB drive to the computer running the SoftMax Pro Software. In the Activate Offline Wizard:

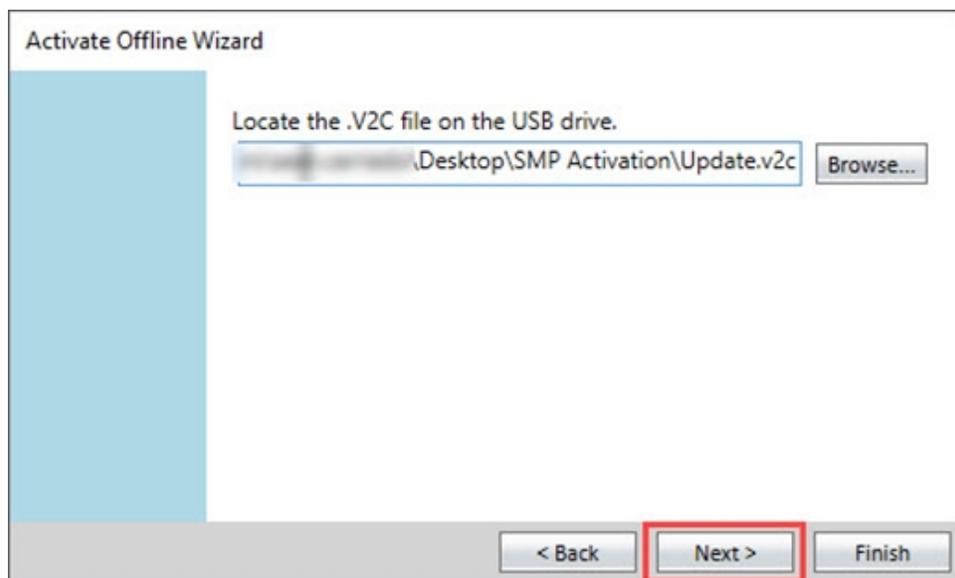


Click **Next**.

11. In the Open dialog:

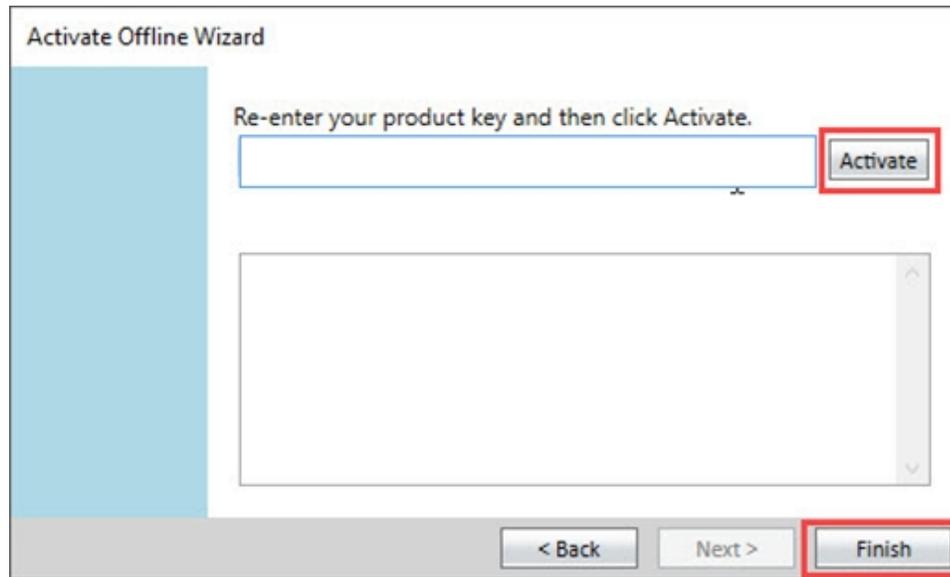


- a. Navigate to and select the **Update.v2c** file on the USB drive.
 - b. Click **Open**.
12. In the Activate Offline Wizard:



Note the name and location of the **Update.v2c** file and click **Next**.

13. In the Activate Offline Wizard:



- a. In the **Re-enter Your Product Key and then Click Activate** field re-enter the product key.
- b. Click **Activate**.
- c. Click **Finish**.

Barcode Reader

The software allows you to use a barcode reader as an input device. 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 software, you must install the correct barcode reader drivers. 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 functions correctly, the text embedded in the barcode appears in the text editor.

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

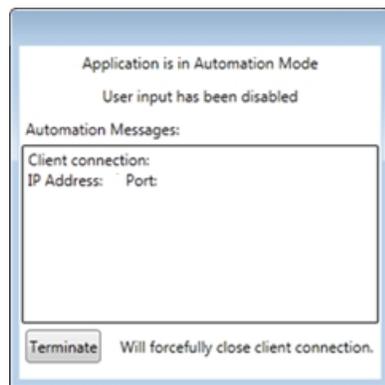
Automation Mode

You can use Application Programming Interface (API) command scripts to automate the SoftMax Pro Software. By default, Automation mode uses port 9000 and this port must be open in order to use the API. To change the Automation port number, see [SoftMax Pro Options on page 52](#).

To start Automation mode:

1. Select the **Operations** tab in the Ribbon.
2. Click  **Automation Mode**.

Automation mode prevents user interaction and the software displays the following dialog in front of the main window.



When the software acquires data, the Plate section displays in the main window behind the automation mode dialog and displays the data.

The **Automation Messages** area displays relevant information.

To stop the automation process, click **Terminate**.

The StakMax[®] Microplate Handling System is an integrated microplate handler for use with Molecular Devices microplate readers, to provide simple, powerful, walk-away benchtop automation. The StakMax Microplate Handling System uses the SoftMax Pro Software Automation mode and the StakMax Software has a scripting tool. See [StakMax Microplate Handling System on page 347](#).

Automation Scripts

You can script and run the automation commands in the SoftMax Pro Software through the automation API. For experienced automation programmers, the *SoftMax Pro Data Acquisition and Analysis Software Automation API Reference Guide* provides information about the available commands and parameters.

Legacy scripts must use the Windows message WM_COPYDATA command. The WM_SETTEXT command is not supported. Legacy scripts might not work correctly with the current automation interface. If your legacy script does not work properly, you should use the most recent version of the automation commands to rewrite the script.

To let the software accept legacy commands from scripts written for the SoftMax Pro Software automation interface version 5.x:

1. Select the **Operations** tab.
2. Click **Automation Mode**.

Getting Help

Help is available for dialogs that have a  help icon or a More Information link.

The Help tab in the Ribbon provides access to the help library.

- Click  **SoftMax Pro Help** to view the Application Help.
- Click  **Formula Reference** to view the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.
- Click  **Release Notes** to view the *SoftMax Pro Data Acquisition and Analysis Software Release Notes*.
- Click  **Contact Us** to access the following pages on Molecular Devices web site: What's New in the SoftMax Pro Software, the Molecular Devices Home page, the Molecular Devices Knowledge Base, and the Molecular Devices Support page.
- Click  **Software License** to manage the software license. See [Activating Software Licenses on page 10](#).
- Click  **Diagnostics** to turn on Diagnostics mode in order to record a read that generates an instrument diagnostics log file. The log file contains information from the connected instrument to help solve technical issues. (Available for the SpectraMax i3 Multi-Mode Microplate Reader, SpectraMax i3x Multi-Mode Microplate Reader, SpectraMax® iD3 Multi-Mode Microplate Reader, SpectraMax® iD5 Multi-Mode Microplate Reader, and SpectraMax Paradigm Multi-Mode Microplate Reader.) See [Instrument Diagnostics Mode on page 66](#).
- Click  **Export** to export the instrument diagnostics log file after you record a read in Diagnostics mode. This exports the log file to the computer hard drive or a USB drive so that you can send the file to Molecular Devices Support.
- Click  **SoftMax Pro** to view the software version number and release information.

Obtaining 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 level of technical service.

Our Support website, support.moleculardevices.com, has a link to the Knowledge Base, which contains technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, you can submit a request to Molecular Devices Technical Support.

You can contact your local representative or Molecular Devices Technical Support at 800-635-5577 x 1815 (North America only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

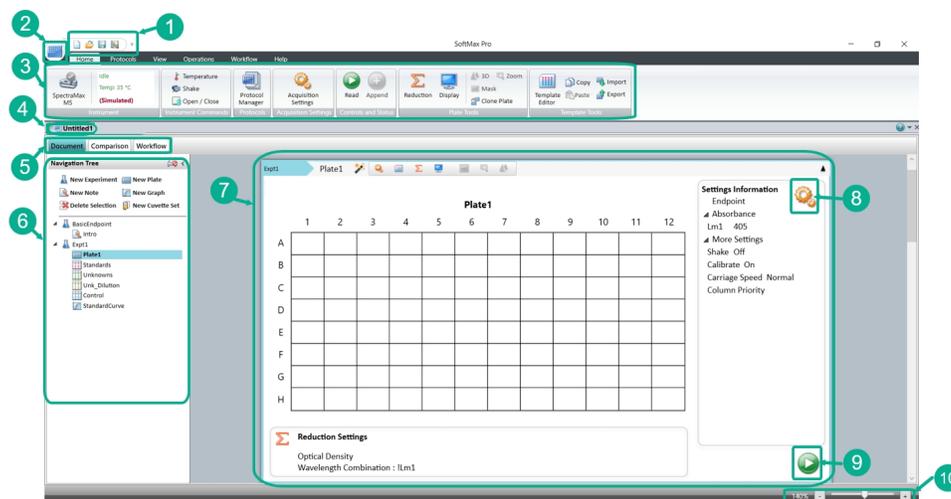
To find regional support contact information, visit www.moleculardevices.com/contact.

Use the SoftMax Pro Software to perform state-of-the-art analysis. Use the software to control most Molecular Devices microplate reader instrument functions including the ability to define the settings to have the instrument run complete experiments and to define the formulas for result analysis.

When you start the software, the Untitled1 document tab displays in the Document Tabs area (number 4 in the following image) and the Workspace (number 7) displays the contents of the default protocol.



Note: For the SoftMax Pro Software - GxP edition, no document tabs display and the Workspace area is empty. Your permissions determine which actions you can perform. See [SoftMax Pro Software - GxP Edition on page 251](#).



The numbers in the following table correspond to the image above.

Item	Description
1	Quick Access Toolbar: Use to create, open, and save protocols and data documents that contain the settings to run the instrument for data collection. You can customize this toolbar and you can change its location. See Quick Access Toolbar on page 54 .
2	Application Menu: Use to manage protocols and data documents and to define options for the SoftMax Pro Software. See Application Menu on page 21 .
3	Ribbon: Use to select protocols from the Protocol Manager and to define the settings for each document. The Ribbon also provides the ability to change how you view the Workspace, manage read workflows, and define instrument settings. See Ribbon on page 55 . Note: One of the first things you will do is to select an instrument on the Home tab in the Ribbon. See Selecting an Instrument on page 64 .
4	Document Tabs: Use to select the document to work with. The Document tabs display one tab for each protocol and data document you open. In the Ribbon, use the View tab to change the appearance and location of the Document tabs. See Document Tabs on page 67 .

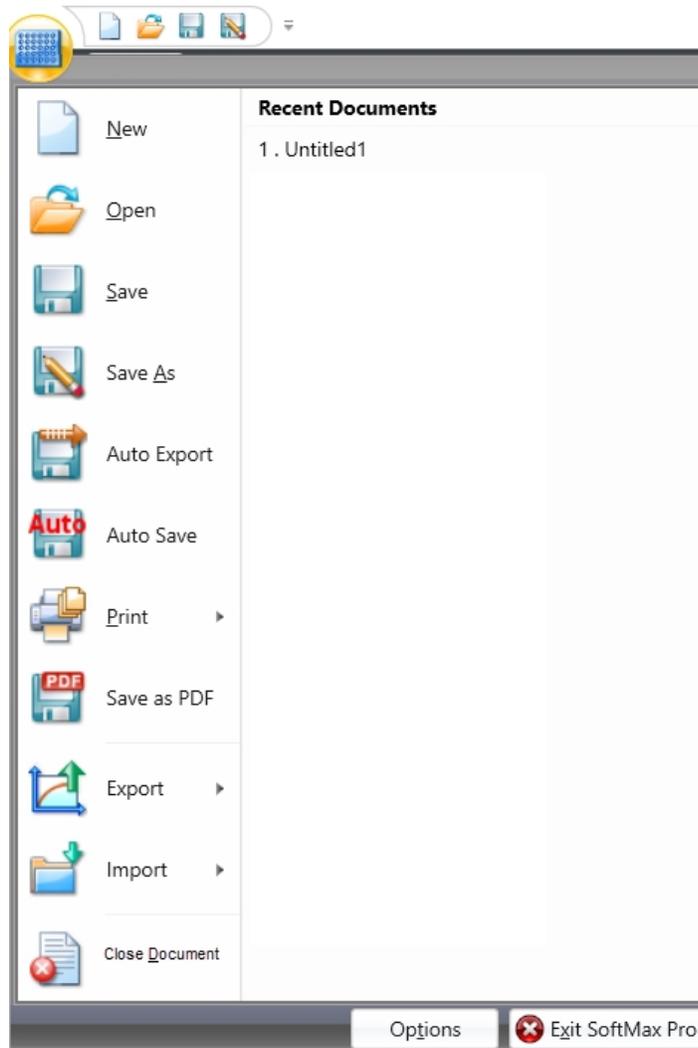
Item	Description
5	Workspace View Selectors: Use the three buttons to change how you work with the information in the Workspace. See Workspace on page 70 .
6	Navigation Tree: Use to manage and navigate the sections that make up the experiments in a document. See Navigation Tree on page 68 .
7	Workspace: Use to define and manage the protocols and data documents. Each document contains one or more experiments and each experiment contains multiple sections for notes, instrument settings, results, and graphs. See Workspace on page 70 .
8	Acquisition Settings: Use to define the settings for the instrument to use to read a Plate section or a Cuvette Set section in an experiment. See Acquisition Settings on page 112 .
9	Read: Use to run the experiment. The software collects and stores all raw data from the instrument. As information is read, the software uses the formulas you define to analyze the data and the Workspace display updates to the results. See Collecting Data on page 111 .
10	Workspace Zoom Slider: Use to zoom the focus of the Workspace in or out to view details of the experiment section content.

If the computer has access to the Internet, a browser window appears to display the Molecular Devices SoftMax Pro Insider page. To stop the appearance of the web page, see [SoftMax Pro Options on page 52](#).

Application Menu

The  Application menu icon in the upper-left corner of the window provides access to the Application menu. Use the Application menu to create, open, save, rename, print, export, and import documents. Use the Recent Documents section to access the documents you most recently used.

The Application menu also provides access to the SoftMax Pro Options dialog where you set options for Data Recovery, Web Browser, Plate Setup Helper, Plate Height Warning, Plate Eject After Read, Automation, Calculation, and SpectraMax iDx Control. See [SoftMax Pro Options on page 52](#).



Click  to open the Application menu.

- Select  **New** to create a new document. See [Creating Protocols and Data Documents on page 27](#).
- Select  **Open** to open a document or select a document from the **Recent Documents** list on the right. See [Opening Protocols and Data Documents on page 28](#).
- Select  **Save** to save updates to a document.
- Select  **Save As** to rename a document, to save a protocol as a data document, to save a data document as a protocol, to move a document to a different location, or to give a new document a name. See [Saving Protocols and Data Documents on page 29](#).
- Select  **Auto Export** to define settings to export documents to a new location, in a format that is compatible with other software applications. See [Auto Export on page 30](#).
- Select  **Auto Save** to define settings to save documents. See [Auto Save on page 33](#).
- Click  **Print** and select one of the following:
 - Select  **Print All** to print the information, settings, and results in a document.
 - Select  **Print Preview** to create a preview of the information, settings, and results before you choose to print. See [Previewing Print Jobs on page 35](#).
 - Select  **Print Selected** to select the experiment sections to print and to exclude experiment sections from the printout. See [Printing Select Sections on page 36](#).
 - Select  **Printing Options** to define the header and footer area for the printout, to add a logo image, and to set the print quality. See [Printing Options on page 37](#).
- Select  **Save As PDF** to save the printed output of a document to a portable document format (.pdf). See [Saving Data as a PDF on page 39](#).
- Click  **Export** and select one of the following:
 - Select  **Export As Data File (sdax)** to export a SoftMax Pro Software - GxP edition data document to the computer hard drive or other network storage device in a format that can then be imported into a different Project or to a different SoftMax Pro Software - GxP edition version 7.3 database. See [Exporting as Data File on page 268](#).
 - Select  **Export As Protocol File (sprx)** to export a SoftMax Pro Software - GxP edition protocol document to the computer hard drive or other network storage device in a format that can then be imported into a different Project or to a different SoftMax Pro Software - GxP edition version 7.3 database. See [Exporting as Protocol File on page 269](#).
 - Select  **Export to XML XLS TXT** to export the data in a data document to a .xml, .xls, or .txt file format for use in other applications. See [Exporting to XML, XLS, TXT on page 41](#).
 - Select  **Bulk Export** to export multiple documents. See [Bulk Export on page 43](#).

- Click  **Import** and select one of the following:
 - Select  **Plate Format** to import data into a Plate section in plate format. This requires a separate license, contact Molecular Devices. See [Importing Data in Plate Format on page 46](#).
 - Select  **XML Format** to import data into a Plate section in .xml format. This requires a separate license, contact Molecular Devices. See [Importing Data in XML Format on page 48](#).
 - Select  **Import Legacy Files** to import legacy SoftMax Pro Software protocols and data documents into the SoftMax Pro Software - GxP edition version 7.1.x+ database. See [Importing Legacy Files on page 49](#).
- Select  **Close Document** to close the document that is open in the Workspace.
- Click **Options** at the bottom of the menu to set application options. See [SoftMax Pro Options on page 52](#).
- Click **Exit SoftMax Pro** at the bottom of the menu to exit the SoftMax Pro Software.

Managing Documents

The SoftMax Pro Software uses two basic document types: protocols and data documents. You save both protocols and data documents just like any other file you save to a computer.

Use the  Folder Locations icon on the Protocols tab to add or remove the folders that display in the Protocol Library. See [Protocol Library Folder Locations on page 63](#).

- Protocols contain the settings that the instrument uses to collect data and the formulas to analyze data. Protocols are useful when you repeat experiments and when you create new documents.
- Data documents contain the data the software collects when you run the experiment. Data documents allow you to create reports and graphs from the data you collect.

SoftMax Pro Software - Standard edition version 7.3 can open documents from version 4.0 or later.

SoftMax Pro Software - GxP edition version 7.3 requires that you to import legacy documents into the SoftMax Pro Software - GxP edition database. See [Importing Legacy Files on page 49](#).



Note: Before you open a document, make sure the instrument you select uses the same settings or compatible settings as the instrument used to create the document.

Protocols

You can use predefined protocols and you can create your own protocols. Protocols contain plate well layout assignments, reduction parameters, and instrument settings, but no data. Protocols are useful when you want to repeat experiments because protocols save instrument experiment settings and formulas for reuse. See [Creating Protocols and Data Documents on page 27](#).

When you open a new document, the settings from the default protocol appear in the Workspace. The default protocol settings help you get started when you create a new document. When you install the software, the Basic Endpoint protocol is set as the default protocol with the file name *default.spr*.

Use the  Save As Default icon on the Protocols tab to change the default protocol settings to the settings you define in the document that displays in the Workspace. See [Protocols Tab on page 57](#).

Use the  Protocol Manager icon on the Home tab and on the Protocols tab to access the Protocol Library. The Protocol Library contains over 160 protocols that contain instrument settings and formulas. You can customize the protocols in the Protocol Library to facilitate the data analysis and report creation.

Use the  Folder Locations icon on the Protocols tab to add or remove the folders that display in the Protocol Library. This helps you find the protocols you use most often. See [Protocol Library Folder Locations on page 63](#).

Use the  Protocol Home Page icon on the Protocols tab to access the sample protocols and the protocols you use with the SpectraTest Validation Plates.



Note: When you save a data document as a protocol, the software creates a new protocol file that contains the experiment settings without the data. Any data you collect in the current experiment remains unchanged and is saved with the data document file.

Protocol File Extensions

You can open protocols that have the following file extensions. See [Opening Protocols and Data Documents on page 28](#):

- .ppr for SoftMax Pro Software - Standard edition version 4 and 5 protocols.
- .spr for SoftMax Pro Software - Standard edition version 6 and 7 protocols.
- .epf for SoftMax Pro Software - GxP edition version 4 and 5 protocols before you import them into the version 7.1.x and higher database. See [Importing Legacy Files on page 49](#).
- .sprx for SoftMax Pro Software - GxP edition version 6 and 7.0.x protocols before you import them into the version 7.1.x and higher database.
- SoftMax Pro Software - GxP edition versions 7.1.x and higher do not display file extensions for protocols.

Data Documents

Data documents contain the instrument settings, the raw data the instrument collects, and the completed data analysis.

Data Document File Extensions

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

- .pda for SoftMax Pro Software - Standard edition version 4 and 5 data documents.
- .sda for SoftMax Pro Software - Standard edition version 6 and 7 data documents.
- .eda for SoftMax Pro Software - GxP edition version 4 and 5 data documents before you import them into the version 7.1.x and higher database.
- .sdax for SoftMax Pro Software - GxP edition version 6 and 7.0.x data documents before you import them into the version 7.1.x and higher database.
- SoftMax Pro Software - GxP edition versions 7.1.x and higher do not display file extensions for data documents.

When you create a data document for an Imaging mode experiment or a Western Blot experiment, the software creates a folder with the same name as the data document.

Imaging Read Mode Data Documents

Before you start an image acquisition for an Imaging experiment or a Western Blot experiment, you must save the data document in a location with enough capacity for the image files. For best results, save Imaging experiment data documents on the secondary hard drive inside the computer. Use of an external hard drive can slow the data acquisition and is not recommended. Use of a USB drive or saving to a network location is not supported.

When you create a data document for an Imaging experiment or a Western Blot experiment, the software creates a folder with the same name as the data document.

For example, if you save the data document with the name *Imaging_Data*, the software creates a new folder named *Imaging_Data* in the same folder where you save the data document. This folder stores the images and results.



The software stores the images separately for each plate in the IMAGES folder in .tiff format. The software stores image analysis data in the RESULTS folder. Western Blot experiments do not create or use the RESULTS folder.



Note: Do not rename, move, or delete the related folders or files. If you change the structure of these files and folders, the software may not be able to open the data document or might display inaccurate results.

Use the  Application menu to copy or delete data documents, image files, and analysis data files.

To delete the images or the analysis data for an Imaging mode experiment:

1. In the Navigation Tree right-click the Plate section.
2. Select **Delete Images** or **Delete Cell Data**.

If you delete the .tiff file for a Western Blot experiment, the software recreates the .tiff image from the intensity data stored in the data document.



Note: When you save an Imaging experiment data document as a protocol, the software saves only the settings and does not create other folders or files.

Acquisition of images for Imaging experiments requires significant computer memory and resources. Each image file can be larger than 2 megabytes. When you acquire the image of a single site in each well of a 96-well plate you can generate 300 megabytes of image data. A 384-well plate can generate 1 gigabyte of image data. When you acquire images of multiple sites, you increase the data storage requirement.

Before you start an Imaging experiment, turn off all other programs to minimize the demands on computer memory and resources. When the 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. See [Optimizing the Computer for Image Acquisition on page 172](#).

For Imaging experiments, save the data to a .txt file format. If you use Auto Export to export multiple Imaging experiment data documents, the software exports each new data document without the folders required to save images and results. See [Auto Export on page 30](#).

Creating Protocols and Data Documents

Use the Quick Access toolbar and the  Application menu to create a new document that you can save as a protocol or data document. The Protocol Library contains protocols that you can use to create a new protocol or data document.

To create a document:

1. Click  in the Quick Access toolbar or click  to display the Application menu and select  **New**. A new tab displays in the Document tabs and the Workspace displays the settings from the default protocol.



Note: You should save and name the protocol before you collect data. For the SoftMax Pro Software - GxP edition, you must save the document before you can proceed. See [Saving Protocols and Data Documents on page 29](#).

2. Click  in the Quick Access toolbar or click  to display the Application menu and select  **Save As** to display the Save As dialog.
3. Navigate to where you want to save the document.
4. In the **File Name** field, enter the document name.
5. Click the **Save As Type** drop-down:
 - For the SoftMax Pro Software - Standard edition, select **Protocol Files (*.spr)** to save the settings with no data as a protocol.
 - For the SoftMax Pro Software - Standard edition, select **Data Files (*.sda)** to save the settings with no data as a data document.
 - For the SoftMax Pro Software - GxP edition, select **Protocol Documents** to save the settings with no data as a protocol.
 - For the SoftMax Pro Software - GxP edition, select **Data Documents** to save the settings with no data as a data document.
6. Set up the experiments for the document in the Navigation Tree. See [Workspace on page 70](#).
7. Configure the settings for the instrument to use to read the Plate sections and Cuvette Set sections. See [Acquisition Settings on page 112](#).
8. Create templates. See [Template Editor on page 91](#).*
9. Set Data Reduction parameters. See [Data Reduction on page 196](#).*
10. Set Display options. See [Data Display Options on page 206](#).*
11. Click  in the Quick Access toolbar or click  to display the Application menu and select  **Save** to save your changes.

*Can be done at any point in the process, before or after the initial read.

Opening Protocols and Data Documents

Use the Quick Access toolbar and the  Application menu to open documents. The Protocol Library contains protocols you can use to create a new protocol or a blank data document.

The SoftMax Pro Software - Standard edition can open documents created by version 4.0 or later. When you open a document from a version earlier than version 7.x, the software converts it to the SoftMax Pro Software version 7.x format. After you save a document, you cannot open the new document in earlier software versions.

The SoftMax Pro Software - GxP edition version 7.1.x and higher requires a few additional steps if you want to use documents that are from a version earlier than version 7.1.x. See [Importing Legacy Files on page 49](#).



Note: Each instrument has different settings. Before you open a document, verify that the instrument used to create the document is the same as the instrument you select on the Home tab. Some instruments have incompatible settings.

To open documents:

1. Click  in the Quick Access toolbar or click  to display the Application menu and select  **Open** to display the Open dialog.
2. Click the **Data Files (*.sda)** drop-down (SoftMax Pro Software - Standard edition) or the **Open Type** drop-down (SoftMax Pro Software - GxP edition). SoftMax Pro Software - GxP edition version 7.1.x and higher documents do not display the file extension.
 - For SoftMax Pro Software - Standard edition, select **Protocol Files (*.spr)** to open a protocol with an .spr file extension.
 - For SoftMax Pro Software - Standard edition, select **Data Files (*.sda)** to open a data document with an .sda file extension.
 - For SoftMax Pro Software - GxP edition, select **Protocol Documents** to open a version 7.1.x and higher protocol.
 - For SoftMax Pro Software - GxP edition, select **Data Documents** to open a version 7.1.x and higher data document.
3. Navigate to the document and select it.
4. Click **Open**. A new Document tab displays above the Workspace with the document name.
5. Before you use a protocol to collect data, you should save it as a data document.

Saving Protocols and Data Documents

Use the Quick Access toolbar and the  Application menu to save documents. Before you use a protocol, you should save the protocol as a data document. You can use the Auto Save feature to automatically save the document. See [Auto Save on page 33](#).

To save documents:

1. Select one of the following:
 - Click  in the Quick Access toolbar or click  to display the Applications menu and select  **Save** to save the open document with updated information in the same location with the same name.
 - Click  in the Quick Access toolbar or click  to display the Applications menu and select  **Save As** to rename a document, to save a protocol as a data document, to save a data document as a protocol, to move a document to a different location, or to give a new document a name. The Save As dialog displays.
 - Click  to display the Applications menu and select  **Save As PDF** to save the document in a .pdf format. See [Saving Data as a PDF on page 39](#).
2. For Save As, in the Save As dialog, navigate to where you want to save the document.
3. In the **File Name** field, enter the document name.
4. Click the **Save As Type** drop-down.
 - For the SoftMax Pro Software - Standard edition, select **Protocol Files (*.spr)** to save the document as a protocol.
 - For the SoftMax Pro Software - Standard edition, select **Data Files (*.sda)** to save the document as a data document.
 - For the SoftMax Pro Software - GxP edition, select **Protocol Documents** to save the document as a protocol.
 - For the SoftMax Pro Software - GxP edition, select **Data Documents** to save the document as a data document.
5. Click **Save**.

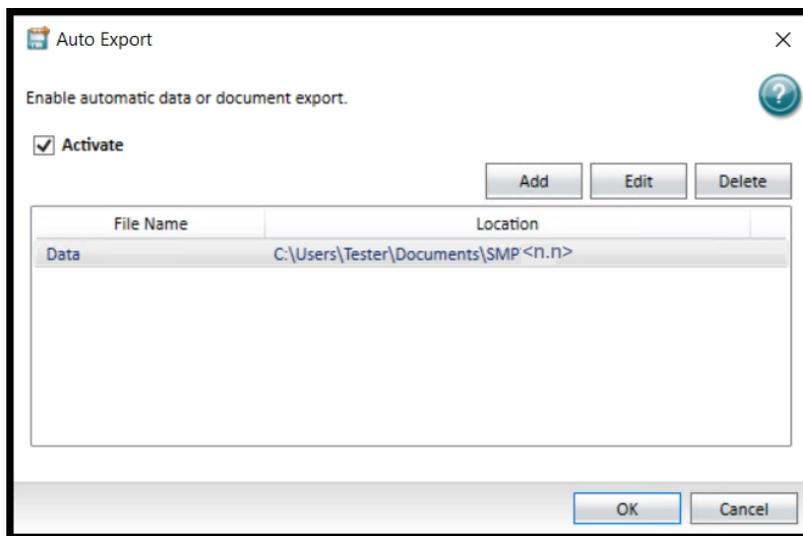
Auto Export

Use the Auto Export dialog to define how and where the software is to automatically export data immediately after each plate read completes. Auto export reduces the likelihood of lost data, particularly when you choose to export documents to corporate network file locations that are backed up on a regular basis. You can define multiple auto export instances. The software saves auto export settings with each document.

This feature helps prevent data loss when you do automated reads, such as with the StakMax Microplate Handling System. See [StakMax Microplate Handling System on page 347](#).

For Imaging mode experiments, export the data to a .txt file format. If you use auto export to export multiple Imaging mode data documents, the software exports each new data document without the folders required to save images and results.

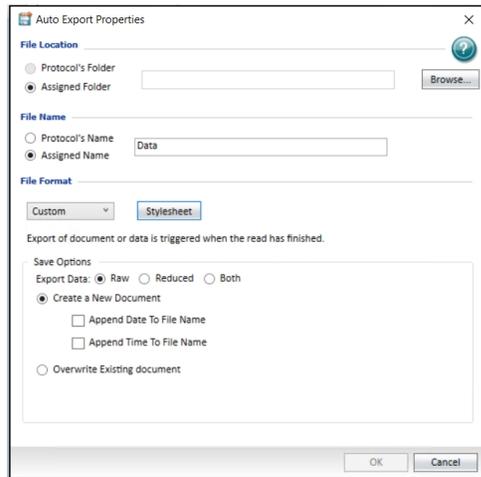
For the SoftMax Pro Software - GxP edition, Auto Export saves a copy of the document to the location you define. The original document remains in the SoftMax Pro Software database.



To automatically export a document:

1. Open the protocol or data document to export.
2. Click  to display the Application menu and select  **Auto Export** to display the Auto Export dialog.
3. Select the **Activate** check box to use auto export.
Clear the **Activate** check box to not use auto export and the following fields are not available.

- Click **Add** or select a document in the list and click **Edit** to display the Auto Export Properties dialog.



Note: Auto export properties settings are specific to the new export location or the export location you select to edit from the Auto Export dialog. Use this to customize the settings for each location to which you use auto export.

- Select a **File Location** option.
 - Select **Protocol's Folder** to export documents back to the folder that contains the originating document (SoftMax Pro Software - Standard edition only).
 - Select **Assigned Folder** to export documents to a folder to which the computer has access. Click **Browse** to display the Browse For Folder dialog. Navigate to the folder or click **Make New Folder** to create a new folder where you want to export the document.
- Select a **File Name** option.
 - Select **Protocol's Name** to name the document with the protocol name.
 - Select **Assigned Name** to assign a name to the document. In the text field, enter the name for the document. The software appends this text to the start of each document name.
- Click the **File Format** drop-down:
 - Select **.sda** or **.sdax** to export the file as a data document.
 - Select **.xml** to export the file in an .xml format using UTF-16 suitable for Excel. To export the file in an .xml format using UTF-8 suitable for LIMS (Laboratory Information Management System), select the **XML-V5** check box.
 - Select **.txt (list)** or **.txt (plate)** to export the document as a tab delimited text file that most word processor, spreadsheet, or database programs can open.
 - Select **.xls (list)** or **.xls (plate)** to export the document in a format that Microsoft Excel can open. The Excel output has the same format as a tab delimited file with an .xls file extension.
 - Select **Custom** to use an .xslt custom stylesheet that allows skilled users to custom define the format and content of the output file. Click **Stylesheet** to select the stylesheet you create from the Open dialog.

- For the SoftMax Pro Software - GxP edition, if you select .xml, select the **Sign** check box to increase the integrity of the .xml file in order to protect the file from being tampered with. Clear the **Sign** check box to not sign the export file.



Note: This feature requires separate license. To obtain a license contact Molecular Devices.

Save Options: The available save options depend on the file format you select.

- Select an **Export Data** option.
 - 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.
- Select one of the following:
 - Select **Create a New File** to create a new document after each read. When you select this option and do not select either of the following check boxes, the software numbers documents with the same name sequentially. For example, *Data 1*, *Data 2*, and so on.
 - Select the **Append Date to File Name** check box to add the date after the document name. If you select to append only the date to the document name, the software indexes the runs for that day starting with *1* and increments with each successive run. For example, the software names the first document named *Data* that you export on January 22nd, 2025 *Data-01-22-25 1* and names the second document you export *Data-01-22-25 2*, and so on.
Clear the **Append Date to File Name** check box to not append the date to the file name.
 - Select the **Append Time to File Name** check box to add the time after the document name.
Clear the **Append Time to File Name** check box to not append the time to the file name.
If you select both the date and the time, the time follows the date.
 - Select **Overwrite Existing File** to overwrite an existing document for each read. Use this carefully because this can cause data loss. A document can overwrite only itself. If a different document with the same name exists in the save file location, the software appends a number to the end of the document name.
- Select **Append to File** to append the data from multiple plates into one document. Enter the maximum number of plates to append per document. This is available when you select either the .txt or .xls format.



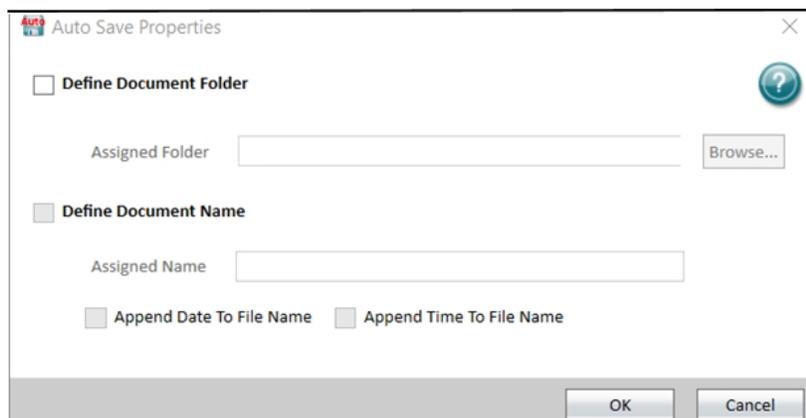
Tip: If you use the StakMax Microplate Handling System to read multiple plates (for example, 10), use this to export the data from all 10 plates in a single document.

- After you define each document name and location, click **OK**.

Auto Save

Use the Auto Save Properties dialog to define how and where the software is to automatically save a document. The software saves auto save settings with each document.

Auto Save creates a copy of the document in the location you define within the SoftMax Pro Software database and saves the original document in the original folder in the database.



To automatically save a document:

1. Open the protocol or data document to save.
2. Click  to display the Application menu and select  **Auto Save** to display the Auto Save Properties dialog.
3. Select the **Define Document Folder** check box to use auto save.
Clear the **Define Document Folder** check box to not use auto save and the following fields are not available.
4. Next to the **Assigned Folder** field, click **Browse** to display the Assigned Folders dialog.
5. Navigate to the folder where you want to save the document and then click **OK** to return to the Auto Save Properties dialog.
6. Select the **Define Document Name** check box to define the document name when the document saves.
Clear the **Define Document Name** check box to use the protocol name as the document name and the following fields are not available.
7. In the **Assigned Name** field, enter the name for the document when the document saves.
8. Select the **Append Date to File Name** check box to add the date after the document name.
If you select to append only the date to the document name, the software indexes the runs for that day starting with *1* and increments with each successive run. For example, the software saves the first document named *Data* that you save on January 22nd, 2025 *Data-01-22-25 1* and names the second document you save *Data-01-22-25 2*, and so on.
Clear the **Append Date to File Name** check box to not append the date to the file name.
9. Select the **Append Time to File Name** check box to add the time after the document name.
Clear the **Append Time to File Name** check box to not append the time to the file name.



Note: If you select both check boxes, the time follows the date.

Printing

Use the  Application menu to print the contents of the document that is open in the Workspace. You can print all sections or select the sections to print. You can preview the printout before you print and you can define the page header, footer, and print quality options.

Print Options

Before you print, you can add a logo image, and define the header, footer, and print quality in the Printing Options dialog. See [Printing Options on page 37](#).

Click  to open the Application menu, click  **Print**, and then select  **Printing Options** to display the Printing Options dialog.

Print

The Application menu provides several ways to print the document content. You can click a section in the Navigation Tree and click  to have a section not print or you can select the sections to print from the Application menu.

To print a document:

1. Open the document to print.
2. Click  to display the Application menu.
3. Click  **Print** and select an option:
 - Select  **Print All** to print all the included sections in the document. The Print dialog displays.
 - Select  **Print Preview** to display the Print Preview dialog where you preview the print job before you print.
 - Select  **Print Selected** to display the Print Selected dialog where you select the sections to print and the sections to exclude from the printout.
 - Select  **Printing Options** to display the Printing Options dialog where you define the header, footer, and logo for the pages and the print quality.
4. In the Print dialog, select the printer to use and set the print settings, including the number of copies to print. To print to a .pdf file, select the **Molecular Devices (PDF Writer)** or click  to display the Application menu and select  **Save As PDF**.

Previewing Print Jobs

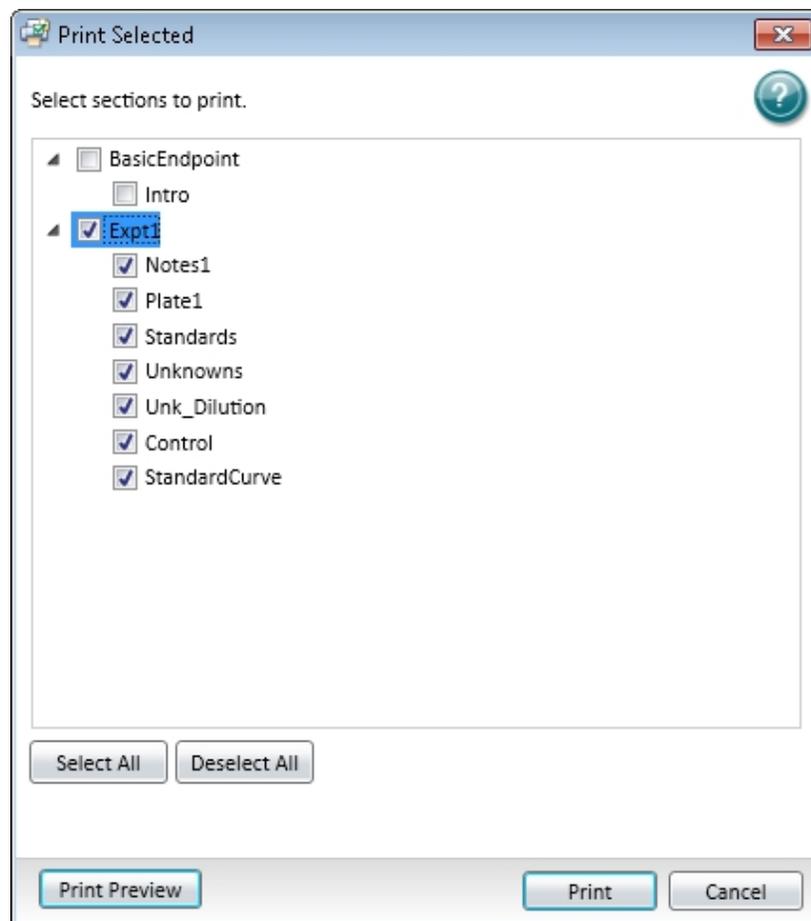
Use the Print Preview dialog to preview the print job before you print. Use the Print Options dialog to add a header, footer, and logo image. See [Printing Options on page 37](#).

To preview the printed output:

1. Open the document to preview.
2. Click  to display the Application menu, click  **Print**, and then select  **Print Preview** to display the Print Preview dialog.
3. The dialog provides the following tools.
 - Scroll through the pages
 -  - Zoom in
 -  - Zoom out
 -  - Scale to 100%
 -  - Scale to page width
 -  - Scale to the whole page
 -  - Scale to display two pages
4. Click  to display the Print dialog.
5. Select the printer to use and set the print settings, including the number of copies to print. To print to a .pdf file, select the **Molecular Devices (PDF Writer)**. See [Saving Data as a PDF on page 39](#).
6. Click **Print**.

Printing Select Sections

Use the Print Selected dialog to select the document sections to print and the document sections to exclude from the printout. Use the Print Options dialog to add a header, footer, and logo image.

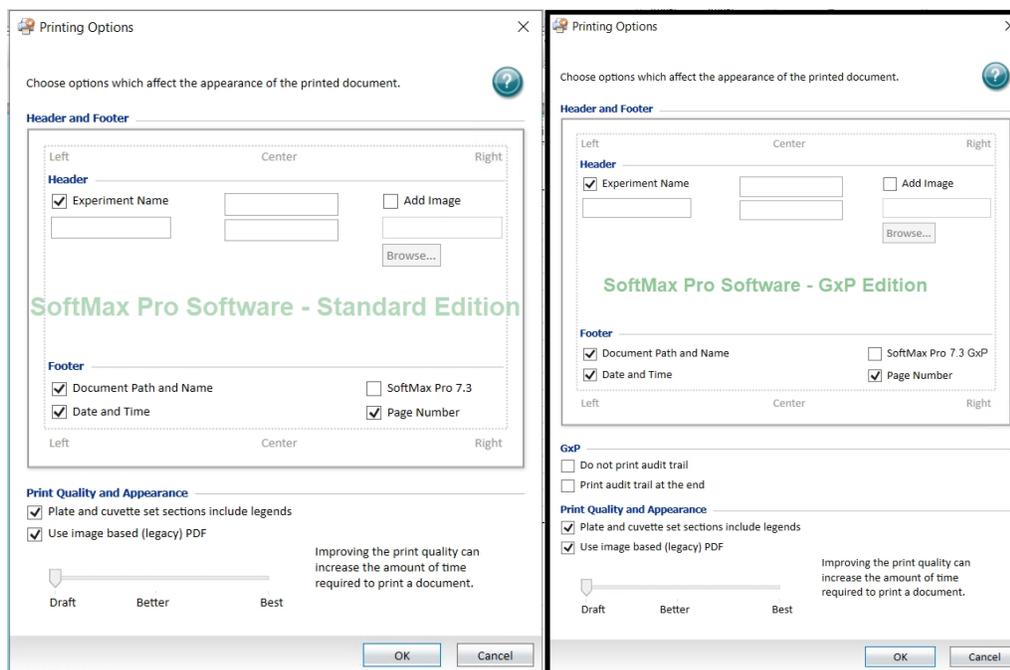


To print select sections of a document:

1. Open the document to print.
2. Click  to display the Application menu, click  **Print**, and then select  **Print Selected** to display the Print Selected dialog.
3. Select the check box for each section to print.
Clear the check box for each section to not print.
 - Click **Select All** to select all the check boxes.
 - Click **Deselect All** to clear all the check boxes.
 - Click **Print Preview** to preview the print job before you print.
4. Click **Print** to display the Print dialog.
5. Select the printer to use and set the print settings, including the number of copies to print.
To print to a .pdf file, select the **Molecular Devices (PDF Writer)**.
6. Click **Print**.

Printing Options

Use the Printing Options dialog to add a logo to the printout, to define the contents of the header and footer, and to set the quality of the printed output.



To define printing options:

1. Open the document to print.
2. Click  to display the Application menu, click  **Print**, and then select  **Printing Options** to display the Printing Options dialog.
3. Define the page header:
 - a. Select the **Experiment Name** check box to include the experiment name in the printout header.
Clear the **Experiment Name** check box to not include the experiment name in the header.
 - b. In the upper center text field, enter a title to include in the upper section of the printout header.
 - c. Select the **Add Image** check box and click **Browse** to locate and select an image file (.jpg format). The path to the image file displays in the text field below the check box. The software sizes the image to fit in the upper-right section of the printout header. Clear the **Add Image** check box to not include an image in the printout.
 - d. In the lower left text field, enter the text to include in the lower left side of the printout header.
 - e. In the lower center text field, enter the text to include in the lower center of the printout header.

4. Define the page footer:
 - a. Select the **File Path and Name** check box to include the document name and path in the printout footer.
Clear the **File Name and Path** check box to not include the document name and path in the printout.
 - b. Select the **Date and Time** check box to include the date and time in the printout footer.
Clear the **Date and Time** check box to not include the date and time in the printout.
 - c. Select the **SoftMax Pro <n.n>** check box to include the version of the SoftMax Pro Software in the printout footer.
Clear the **SoftMax Pro <n.n>** check box to not include the version of the SoftMax Pro Software in the printout.
 - d. Select the **Page Number** check box to include page numbers in the printout footer.
Clear the **Page Number** check box to not include page numbers in the printout.
5. For the SoftMax Pro Software - GxP edition:
 - a. Select the **Do Not Print Audit Trail** check box to not include the document audit trail in the printout.
Clear the **Do Not Print Audit Trail** check box to include the document audit trail in the printout.
 - b. If you clear the Do Not Print Audit Trail check box, select the **Print Audit Trail At The End** check box to include the document audit trail at the end of the printout.
Clear the **Print Audit Trail At The End** check box to include the document audit trail at the beginning of the printout.
6. Select the **Plate And Cuvette Set Sections Include Legends** check box to include the settings details with the reduction information in the printout.
Clear the **Plate and Cuvette Set Sections Include Legends** check box to not include the settings details in the printout.
7. Select the **Use Image Based (Legacy) .pdf** check box to output the information in a in a standard .pdf format that cannot be edited, copied, or pasted.
Clear the **Use Image Based (Legacy) .pdf** check box to output the information format that can be copied and pasted into other software application similar to the behavior of the software in previous versions.
8. Slide the **Print Quality** slide to adjust the quality of the printout.
9. Click **OK**.

Saving Data as a PDF

You can save the contents of a document in .pdf format.

To define .pdf settings:

Click  to display the Application menu and select  **Save As PDF**. You can also select the **Molecular Devices (PDF Writer)** in the Print dialog.

The following tables define the .pdf settings:

General Tab

Option	Description
Option set	Select a set of .pdf settings from this list. To define a new set, select <Edit>.
Format	To create a .pdf file, select .pdf or 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 file.
Open destination folder after creation	Select to open the folder where the file is saved after the file saves.
Open the document after creation	Select to open the file after the file saves.
Append if output exists	Select to append this file to an existing file instead of overwriting an existing file.
Email	Select to email the .pdf after creation of the file.

Document Tab

Option	Description
Author	Enter the name of the author to display in the document properties.
Title	Enter the title of the document to display in the document properties.
Subject	Enter the subject of the document to display in the document properties.
Keywords	Enter keywords for the document to display in the document properties.
Quality	Select a quality to define the size of the .pdf file.
Initial zoom level	Select a zoom level to define how the document displays.
Compatibility level	Select a format to make the .pdf file compatible with older versions of PDF readers. Some options might not be supported in older version.
PDF/A-1b	Select this check box to use the .pdf/A-1b archiving standard.

Image Tab

Option	Description
DPI	Select the resolution for the image in dots per inch.
Text alpha bits	Select the number of bits to use for the alpha channel for text.

Watermark Tab

Option	Description
Text	Enter the text to display in the watermark.
Font	Select the typeface for the watermark.
Color	Click <input type="button" value="..."/> 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 display in outlines. To have the text display as a solid color, leave this field blank.
Layer	To have the watermark text display on top of the document text, select Stamp. To have the watermark text display 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.

Merge Tab

Option	Description
Append pdf	Enter the name of the file to which to append this file. To browse for the file, click <input type="button" value="..."/> .
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	To print the data on an existing file, enter the file name in this field. Use this to print on company letterhead. To browse for the file, click <input type="button" value="..."/> .
Layer	To have the new file display on top of the old file, select Stamp. To have the new file display behind the old file, select Watermark.

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 use for document encryption.
Set permissions	Select this check box to limit access to the document for the user level.
Print	Select whether to permit printing and the print quality to permit.
Copy to clipboard	Select to permit users to copy text from the file to the clipboard.

Exporting Data

Use the  Application menu to export data from the software.

Click  to display the Application menu, click  **Export**:

- Select  **Export as Data File (sdax)** to export a SoftMax Pro Software - GxP edition data document to the computer hard drive or other network storage device in a format that can then be imported into a different Project or into a different SoftMax Pro Software - GxP edition version 7.3 database. See [Exporting as Data File on page 268](#).
- Select  **Export as Protocol File (sprx)** to export a SoftMax Pro Software - GxP edition protocol document to the computer hard drive or other network storage device in a format that can then be imported into a different Project or into a different SoftMax Pro Software - GxP edition version 7.3 database. See [Exporting as Protocol File on page 269](#).
- Select  **Export to XML XLS TXT** to export a data document to a format that can be used in other software applications. See [Exporting to XML, XLS, TXT on page 41](#).
- Select  Bulk Export to export multiple data files. See [Bulk Export on page 43](#).

Exporting to XML, XLS, TXT

Use the Export dialog to export data to a .xml, .xls, or .txt format. The software exports data values with the maximum floating point arithmetic precision, regardless of the display settings you define.



Note: For instructions to export membrane data, see [Viewing Western Blot Membrane Data on page 244](#) and for instructions to export Imaging data to Microsoft Excel or PowerPoint see [Viewing Imaging Data in Wells on page 209](#).



To export data:

1. Open the document that contains the data to export.
2. Click  to display the Application menu, click  **Export**, and then select  **Export to XML XLS TXT** to display the Export dialog.

3. Select the following:
 - Select the **All Plates** check box to export all Plate section and Cuvette Set section data.
 - Select the **All Groups** check box to export all Group section data.
 - Select the **All Notes** check box to export all Note section data.
 - Select the check box for each section to export.
 - Clear the check box for each section to not export.
4. If you select to export Plate section data, select a **Plate Data** option:
 - 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.



Note: These options apply only to Plate sections.

5. Select an **Output Format** option:
 - Select **Columns** to export data in a single column of text for each well.
 - Select **Plate** to export data in a text matrix that corresponds to a plate grid.
 - Select **XML** to export data in an .xml format using UTF-16 suitable for Excel.
 - Select **XML-V5** to export Note section and Group section data to an .xml format using UTF-8 that was available in SoftMax Pro Software version 5.x and is compatible with LIMS.
 - Select **Custom** to use an .xslt custom stylesheet that allows skilled users to custom define the format and content of the output file. Click **Stylesheet** to select the stylesheet you create from the Open dialog.



Note: These options apply only to Plate sections.

6. For the SoftMax Pro Software - GxP edition, if you select XML, select the **Sign** check box to increase the integrity of the .xml file in order to protect the file from being tampered with. Clear the **Sign** check box to not sign the export file and the output file can be edited.

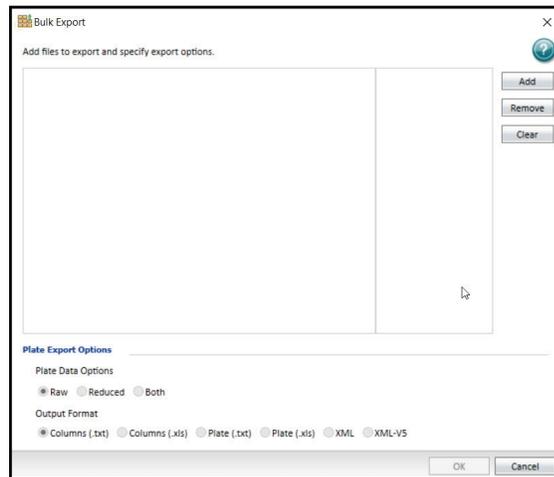


Note: This feature requires separate license. To obtain a license contact Molecular Devices.

7. Click **OK** to display the Save As dialog.
8. Navigate to the folder where you want to save the data.
9. In the **File Name** field, enter the export file name.
10. Click the **Save As Type** drop-down and select the file type for the export file.
 - If you select the XML Output Format, the only option is **.xml**. For a copy of the SoftMax Pro .xml schema, contact Molecular Devices.
 - For details about the format and content of the .txt and .xls format export file, see [Exporting File Details on page 385](#).
11. Click **Save**.

Bulk Export

Use the Bulk Export dialog to export multiple documents. The software exports data values with the maximum floating point arithmetic precision, regardless of the display settings you define.



To export data:

1. Click  to display the Application menu, click  **Export**, and then select  **Bulk Export** to display the Bulk Export dialog.
2. Click **Add** to display the Open dialog where you select the files to export.
 - a. Click the **File Type** drop-down and select the document type to export.
 - b. Navigate to the files to export and select them.
 - c. Click **Open** to return to the Bulk Export dialog.
3. If you select to export Plate section data, select a **Plate Data Option**:
 - 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.



Note: These options apply only to Plate sections.

4. Select an **Output Format** option:
 - Select **Columns (.txt)** to export data in a single column of text for each well.
 - Select **Columns (.xls)** to export data in a single column to a .xls format for each well.
 - Select **Plate (.txt)** to export data that corresponds to a plate grid to a .txt format.
 - Select **Plate (.xls)** to export data that corresponds to a plate grid to a .xls format.
 - Select **XML** to export data in an .xml format using UTF-16 suitable for Excel.
 - Select **XML-V5** to export Note section and Group section data to an .xml format using UTF-8 suitable for LIMS (Laboratory Information Management System).
 - Select **Custom** and then click **Stylesheet** to display the Open dialog where you select the customized style sheet format to which to export the document.



Note: These options apply only to Plate sections.

5. For the SoftMax Pro Software - GxP edition, if you select XML or XML-V5, select the **Sign** check box to increase the integrity of the .xml file in order to protect the file from being tampered with.
Clear the **Sign** check box to not sign the export file.



Note: This feature requires separate license. To obtain a license contact Molecular Devices.

6. Click **OK** to display the Save As dialog.
7. Navigate to the folder where you want to save the data.
8. In the **File Name** field, enter the export file name.
9. Click the **Save As Type** drop-down and select the file type for the export file.
 - If you select the XML or XML V5 output format, the only option is **.xml**. For a copy of the SoftMax Pro .xml schema, contact Molecular Devices.
 - For details about the format and content of the .txt and .xls format export file, see [Exporting File Details on page 385](#).
10. Click **Save**.

Importing Data

Use the  Application menu to import data into the software.

Click  to display the Application menu, click  **Import:**

- Select  **Plate Format** to import tab delimited text data into a Plate section. See [Importing Data in Plate Format on page 46](#).
- Select  **XML Format** to import .xml data into a Plate section. See [Importing Data in XML Format on page 48](#).
- Select  **Import Legacy Files** to import SoftMax Pro Software legacy documents into the SoftMax Pro Software - GxP edition version 7.1.x and higher database. See [Importing Legacy Files on page 49](#).



Note: The import data in Plate format feature and the import data in the .xml format feature require separate licenses. To obtain a license for the Plate format data import feature and the .xml format data import feature, contact Molecular Devices.

The software installation includes templates to import data in the plate format and a schema to import data in the .xml format.

When you import plate data, the software creates a new Plate section with the section name from the import file. If the Plate section name already exists, then the import data overwrites the existing data in the section. See [Plate Sections on page 80](#).

When you import plate data into an existing Plate section, the software preserves the template and reduction settings in the existing Plate section if the data you import is compatible with the settings in the existing section.

To preserve the template and reduction settings, the name of the Plate section in the source file must match the name of the Plate section in the destination file. If the names do not match, the software creates a new Plate section.

Another way to preserve template and reduction settings is to copy the import data from the new Plate section and then paste the data into an existing Plate section. The software uses the template and reduction settings of the section into which you paste the data.



Note: To maximize the number of data points the software uses for reduction, the end time reduction parameter changes to match the read time from the import file.

Importing Data in Plate Format

Use the  Application menu to import data in the plate format.

Before you can import plate data that you export in the plate format from SoftMax Pro Software version 5.x and 6.x or that you acquire from a different software program, you must first use that software program to export the data into a format that is compatible with the SoftMax Pro Software version 7.1.x and higher. The SoftMax Pro Software installation includes templates to import data in the plate format.

To import data in the plate format:

1. Open or create a new protocol.
2. Define a Plate section into which to import the plate data. Name the Plate section with the same name as the Plate section in the import file.
3. Click  to display the Application menu.
4. Click  **Import** and select  **Plate Format** to display the Open dialog.
5. Navigate to and select the file that contains the tab delimited text data to import.
6. Click **Open** to import the data. The Plate sections display the imported data.
7. Save the file.

After you import the data into the Plate sections, the Read Information area on the right indicates that the data was imported and shows the time and date of the import. For the SoftMax Pro Software - GxP edition, the Read Information includes the name of the user who imported the data.

Import Template Details

Import templates are in a sub-folder of the Program Files folder where you install the SoftMax Pro Software. The default installation path is:

C:\Program Files\Molecular Devices\SoftMax Pro <n.n>\Plugins\Import-PlateFormat\Templates

The templates have the .xlsx format, but you must save the file in the .txt format with tab-separated values before you import the data.

The cells in the template are color-coded.

Background Color	Description
White	Do not alter cell.
Green	Cell requires information entry.
Light Yellow	Cell information entry optional.

You can paste data directly into the template or use the template as a model for a script that converts 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 in the document.

- `##BLOCKS = 1`

Each Plate section has the following format:

- Header lines

The first header line contains the description of the titles for the data in the second header line. The second header line contains the type of section, Plate, and the title to give to the Plate section in the document.

The fields in the header lines define the read type, number of wells in the plate, wavelengths for the read, and other relevant information. The layout and format of the header lines depend on the read type. For more information, see the template for the read type of the 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 depend on the read mode and read type.

- The first column displays the time points for a kinetic read, the data points in a well scan read, or the wavelengths in a spectrum read. This column is blank for endpoint reads.
- The second column displays the temperature for the read, if you use the incubator for the read. The value is zero if the incubator is off.
- The third column is where the read data starts.

The data displays in a table to indicate the columns and rows of the plate you use 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 displays 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	...
	

- 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 name of the import file, and the date and time that the file was saved. This information follows the end line of the last Plate section in the file.

Importing Data in XML Format

Before you can import data that you acquire from a different software application, you must first use that application to export the data into a compatible format. The SoftMax Pro Software installation includes templates to import data in a schema for the .xml format. You must use the SoftMax Pro .xml schema from software version 6.4.1 or later.

The .xml schema file for data import (XMLImportSchema.xsd) is in a sub-folder of the Program Files folder where you install the SoftMax Pro Software. The default installation path is:

C:\Program Files\Molecular Devices\SoftMax Pro <n.n>\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 you define each Plate section between a set of <PlateSection> tags.

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

Use the  Application menu to import data in the .xml format.

To import data in .xml format:

1. Open or create a new protocol.
2. Define a Plate section into which to import the plate data. Name the Plate section with the same name as the Plate section in the import file.
3. Click  to display the Application menu.
4. Click  **Import** and select  **XML Format** to display the Open dialog.
5. Navigate to and select the file to import.
6. Click **Open** to import the data. The Plate sections display the data you import.
7. Save the file.

After the data imports into the Plate sections, the Read Information area on the right indicates that the data was imported and shows the time and date of the import. For the SoftMax Pro Software - GxP edition, the Read Information includes the name of the user who imported the data.

Importing Legacy Files

Import SoftMax Pro Software - Standard Edition Version 4 and 5 Documents

The SoftMax Pro Software - Standard edition version 7.3 can open documents created by version 4.0 or later. To open documents created in a version earlier than version 4.0, you must first open the document in the SoftMax Pro Software 4.0 or later, and then save it as a version 4.0 or later document. The document must also meet instrument compatibility requirements.

To use a document from an earlier version of the SoftMax Pro Software, save the document with a different name or in a different location after you open the document with the current version.

After you open a document from an earlier version, review the document to make sure the conversion went well. The document conversion might change instrument settings, curve fits, or other items that have been updated for version 7.3.

Legacy documents have the following file extensions.

- .ppr for SoftMax Pro Software - Standard edition version 4 and 5 protocols
- .pda for SoftMax Pro Software - Standard edition version 4 and 5 data documents
- .epr for SoftMax Pro Software - GxP edition version 4 and 5 protocols
- .eda for SoftMax Pro Software - GxP edition version 4 and 5 data documents
- .spr for SoftMax Pro Software - Standard edition version 6 and 7 protocols.
- .sda for SoftMax Pro Software - Standard edition version 6 and 7 data documents.
- .sprx for SoftMax Pro Software - GxP edition version 6 and 7.0.x protocol files.
- .sdax for SoftMax Pro Software - GxP edition version 6 and 7.0.x data documents.
- SoftMax Pro Software - GxP edition version 7.1.x and higher protocols and data documents do not display a file extension.

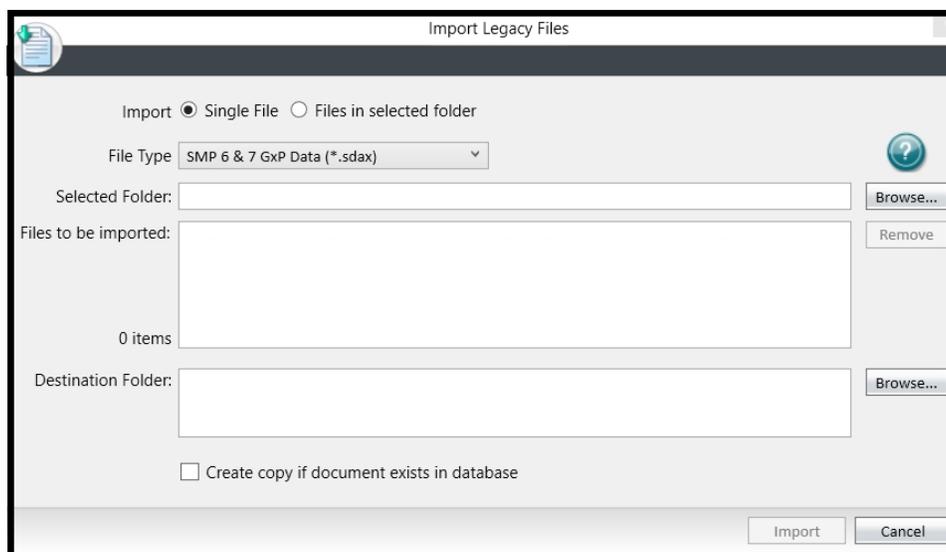
Import Documents Into the SoftMax Pro Software - GxP Edition Database

For SoftMax Pro Software - GxP edition version 7.1.x and higher additional steps are required to import earlier version documents into the database. Use the Import Legacy Files dialog to import legacy protocols and data documents into the SoftMax Pro Software - GxP edition version 7.3 database. Documents in the SoftMax Pro Software - GxP edition version 7.3 database do not display file extensions.

When you log on to the SoftMax Pro Software - GxP edition, you select a Project. The Import Legacy Files dialog displays only folders for that Project. To import documents into the folders for a different Project, you must log out and then select the different Project when you log back on.



Note: You cannot edit the documents you import into the SoftMax Pro Software - GxP edition version 7.3 database. If you choose to do anything with the document after you import it, you must open the document and use Save As to make a copy of the document. The copy has the status In Work.



To import legacy documents:

1. Click  to display the Application menu, click  **Import**, and then select  **Import Legacy Files** to display the Import Legacy Files dialog.
2. Select an **Import** option:
 - Select **Single File** to import one document.
 - Select **Files in Selected Folder** to import all the documents in the folder you select. If you select this option an additional check box displays. Select the **Include Subfolders and Import Whole Folder Structure** check box to import all documents in the folder you select plus all documents in any subordinate folders. This copies the folder structure and all documents to the folder hierarchy for the Project you selected when you logged in.
3. Click the **File Type** drop-down:
 - Select **SMP 4 & 5 Data (*.pda)** to import SoftMax Pro Software - Standard edition version 4 and 5 data documents.
 - Select **SMP 4 & 5 Protocol (*.ppr)** to import SoftMax Pro Software - Standard edition version 4 and 5 protocols.
 - Select **SMP 4 & 5 Data (*.eda)** to import SoftMax Pro Software - GxP edition version 4 and 5 data documents.
 - Select **SMP 4 & 5 Protocol (*.epr)** to import SoftMax Pro Software - GxP edition version 4 and 5 protocols.
 - Select **SMP 6 & 7 Data (*.sda)** to import SoftMax Pro Software - Standard edition version 6 and 7.0.x data documents.
 - Select **SMP 6 & 7 Protocol (*.spr)** to import SoftMax Pro Software - GxP edition version 6 and 7.0.x protocols.
 - Select **SMP 6 & 7 Data (*.sdax)** to import SoftMax Pro Software - GxP edition version 6 and 7.0.x data documents.
 - Select **SMP 6 & 7 Protocol Files (*.sprx)** to import SoftMax Pro Software - GxP edition version 6 and 7.0.x protocols.
4. Next to the **Selected Folder** field, click **Browse** to display either the Open dialog or the Select Folder dialog. The path displays in the Selected Folder field. The path and the file name can have a maximum of 256 characters.

5. Navigate to and select the document or folder to import and click **Open** or **Select Folder** to return to the Import Legacy Files dialog.
6. In the **Files To Be Imported** field, select the documents you do not want to import and click **Remove** to remove the documents from the list.
7. Next to the **Destination Folder** field, click **Browse** to display the Select Folder for Import dialog.
8. Select the folder into which to import the documents and click **OK** to return to the Import Legacy Files dialog. The path displays in the Destination Folder field. The path and the file name can have a maximum of 256 characters.

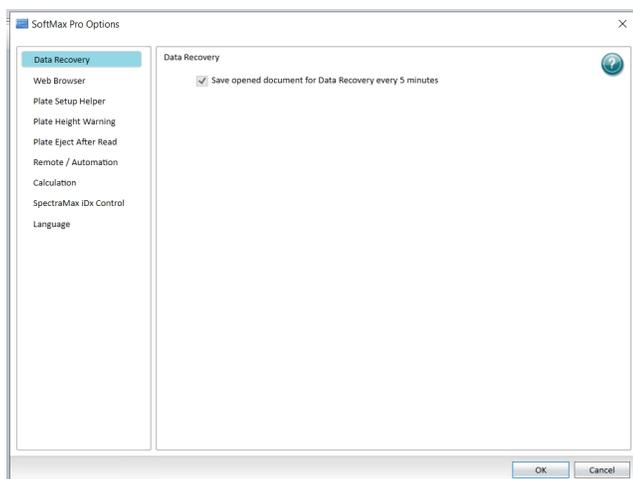


Note: The path (including the file name) can have a maximum of 256 characters. The file name (without the path) can have a maximum of 50 characters. The file name cannot contain the following characters: . < > | : * ? \ /

9. Select the **Create Copy if Document Exists in Database** check box to create a new document in the database if a document with the same name exists.
Clear the **Create Copy if Document Exists in Database** check box to prevent the software from importing duplicate documents. The import does not overwrite a document.
10. Click **Import** to display the List of Files dialog that indicates the status of the import and list of imported documents.
11. Click **Close** to finish the import.

SoftMax Pro Options

Use the SoftMax Pro Options dialog to define preference options that affect the application holistically.



Click  to display the Application menu, and then click the **Options** button at the bottom of the menu to display the SoftMax Pro Options dialog.

The SoftMax Pro Options dialog contains the following tabs along the left side:

- Data Recovery** - The **Save Opened Files For Data Recovery Every 5 Minutes** check box is selected by default. The software saves unsaved documents to a temporary file every five minutes to assist with data recovery. If the software experiences an unexpected interruption, when the software restarts, the Document Recovery dialog displays the list of the documents that you can recover. Recovered documents save to C:\Users\<<windows user>\Documents\SMP<n.n>. You cannot turn this option off.
- Web Browser** - Select the **No Web Browser with 'What's New' Page is Opened On Startup** check box to stop the display of the SoftMax Pro Insider page of the Molecular Devices web site every time you start the software. This used to be the What's New tab that appeared in previous versions of the software. When the computer has access to the Internet, if you select this check box, you can still view the SoftMax Pro Insider page. Select the **Help** tab, click  **Contact Us**, and then select **What's New**. See [Help Tab on page 62](#). Clear the **No Web Browser with 'What's New' Page is Opened On Startup** check box to display the SoftMax Pro Insider page every time you start the software.
- Plate Setup Helper** - Select the **Disable Auto Opening of Helper On New Protocol** check box to prevent the display of the Plate Setup Helper dialog when you open a new document. See [Plate Setup Helper on page 83](#). Clear the **Disable Auto Opening of Helper On New Protocol** check box to display the Plate Setup Helper dialog every time you open a new document.
- Plate Height Warning** - Select the **Disable Plate Height Warning On Protocol Run** check box to prevent the display of the plate height warning when you start a read. Clear the **Disable Plate Height Warning On Protocol Run** check box to have the plate height warning message display as a reminder to correctly set the plate height in order to prevent damage to the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm. See [Plate Editor on page 119](#).

- **Plate Eject After Read** - Select the **Plate Remains Inside Instrument After Completion of Read** check box to have the plate drawer remain closed after a read completes. Clear the **Plate Remains Inside Instrument After Completion of Read** check box to have the plate drawer open after a read completes.
- **Remote/Automation** - In the **Automation API Port** field, enter the port to use for the Automation API application. This can require assistance from your company Technical Support.
- **Calculation** -
 - Select the **Calculate During Acquisition** check box to have the software perform data calculations of the kinetic data while a kinetic acquisition is in progress. In the **Calculate Every ___ Cycles** field, enter the number of kinetic reads between successive calculations. This option allows for the fact that calculations may take any amount of time, depending on the complexity of the protocol and the speed of the computer. Clear the **Calculate During Acquisition** check box to not use this feature.
 - Select the **Use of the Computer Time Stamp in Kinetics for SpectraMax M-series Instruments** check box to use real time calculations based upon the computer time for kinetic reads. Clear the **Use of the Computer Time Stamp in Kinetics for SpectraMax M-series Instruments** to have the SoftMax Pro Software calculate the time for kinetic reads.

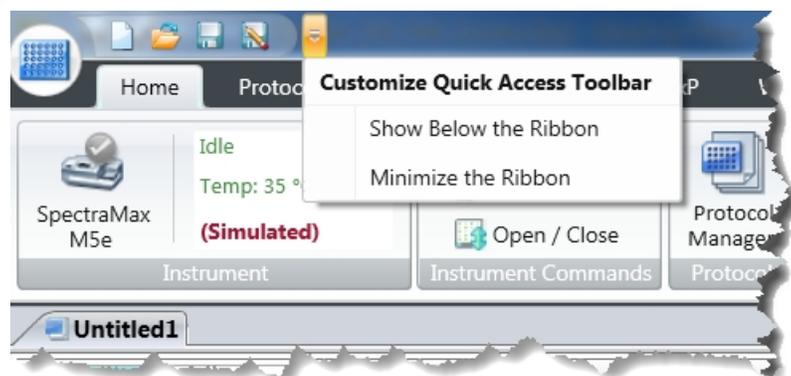


Note: This setting is applicable for the following microplate readers: EMax, EMax Plus, FlexStation 3, Gemini EM, Gemini XPS, SpectraMax 190, SpectraMax 340PC384, SpectraMax ABS, SpectraMax ABS Plus, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, VersaMax, and VMax.

- Select the **Legacy Rounding** check box to improve consistency with the SoftMax Pro Software version 5. This introduces a small factor to force rounding upward. Clear the **Legacy Rounding** check box to not use this feature.
- **SpectraMax iDx Control** - When you use the SpectraMax iD3 and SpectraMax iD5, select the **Enable Control of SpectraMax iDx Instruments** check box to use the software to connect to 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 to connect the computer to multiple instruments on your network. To find the instrument IP address, on the instrument touchscreen touch **Maintenance > System Information**. See [SpectraMax iD5 Multi-Mode Microplate Reader on page 333](#) and [SpectraMax iD3 Multi-Mode Microplate Reader on page 330](#). Clear the **Enable Control of SpectraMax iDx Instruments** check box to not use this feature.
- **Plate Handling GxP** - SoftMax Pro Software - GxP edition only.
 - Select the **Append New Plate Section on Read** check box to create a new plate section in the Navigation Tree each time you click Read so that the existing data is not overwritten. Clear the **Append New Plate Section on Read** check box to overwrite the plate data when you click Read a subsequent time for a Plate section.
 - Select the **Append New Experiment Section on Read** check box to create a new Experiment in the Navigation Tree each time you click Read. Clear the **Append New Experiment Section on Read** check box to not create a new Experiment in the Navigation Tree each time you click Read.
- **Language** - Click the drop-down and select a language. You must restart the software for this change to occur.

Quick Access Toolbar

The Quick Access toolbar provides a shortcut to the features in the Ribbon and in the Application menu. The toolbar displays to the right of the  icon in the upper-left corner of the main window. You can change the content of the Quick Access toolbar and its location.



The Quick Access toolbar has the following icons by default.

-  - Click to create a new document. A new document tab displays above the Workspace.
-  - Click to open a document. Use the Open dialog to navigate the folder hierarchy and select a document.
-  - Click to save changes to a document. For a new document, the Save As dialog displays to save the document.
-  - Click to rename a document, to save a protocol as a data document, to save a data document as a protocol, to move a document to a different location, or to give a new document a name.
-  - Click to change the location of the Quick Access toolbar.
 - Select **Show Below the Ribbon** to move the Quick Access toolbar below the Ribbon above the Workspace.
 - Select **Show Above the Ribbon** to move the Quick Access toolbar above the Ribbon.
 - Select **Minimize the Ribbon** to not display the icons in the Ribbon. The Ribbon tab names continue to display and you click the tab name to display the tab icons. Clear the check box next to this option to display the icons in the Ribbon.

Customizing the Quick Access Toolbar

To add Ribbon icons and Application menu items to the Quick Access toolbar:

1. In the Ribbon or the Application menu, right-click an icon and select **Add to Quick Access Toolbar**.
2. In the Quick Access toolbar, right-click an icon and select **Remove from Quick Access Toolbar**.
3. When you right-click an icon in the Quick Access toolbar, the Application menu, or in the Ribbon, you can move the location of the Quick Access toolbar and minimize/maximize the Ribbon.

Ribbon

The Ribbon contains tabs with icons that vary depending on the instrument you connect to the computer and your context within the software. You can add the tools from the Ribbon to the Quick Access Toolbar to expedite your workflows in the software.



Home Tab

The Home tab displays the following icons:

- Click  **Instrument** to connect the software to the instrument. Displays other instrument information such as connection status and chamber temperature. See [Selecting an Instrument on page 64](#).
- **Instrument Commands** - Icons depend on the instrument you select.
 - Click  **Temperature** to display the Temperature Control dialog where you set the temperature of the plate chamber or cuvette chamber. See [Temperature Control on page 114](#).
 - Click  **Shake** and hold the mouse button to shake the plate. Release the mouse button to stop the shake. This shake icon is independent of the shake settings in an experiment. When you create an experiment, a separate workflow allows you to define how to shake the plate as part of the experiment.
 - Click  **Open/Close** to open and close the instrument plate drawer. The software can open and close other drawers for some instruments such as the drawers for the FlexStation® 3 Multi-Mode Microplate Reader. Click  **Tips** for the tip rack drawer or click  **Reagent** for the compound plate drawer.



Note: The Info icon on the Operations tab provides access to the Instrument Information dialog. Use the Instrument Information dialog to open and close the cartridge drawers for the SpectraMax i3x and SpectraMax Paradigm. Use this dialog to open and close the filter drawers for the FilterMax F3 and FilterMax F5. See [Instrument Information on page 373](#).

- Click  **Protocol Manager** to display the list of protocols in the Protocol Library. The Protocol Library contains over 160 protocols that contain instrument settings and formulas. You can customize the protocols in the Protocol Library to facilitate the data analysis and report creation.
- Click  **Acquisition Settings** to display the Settings dialog where you define instrument settings for the read. See [Acquisition Settings on page 112](#).
 - Click  **ABS**,  **FL**, and  **LUM** to do a quick start absorbance, fluorescence, or luminescence read using default settings. (Available for SpectraMax iD3 and SpectraMax iD5.)

- **Controls and Status** - Icons to start and stop a read.

- Click  **Read** to read the Plate section or Cuvette Set section you select in the Navigation Tree.



Note: Use the  Auto Read icon on the Operations tab to read from multiple Plate sections or Cuvette Set sections. As information is read, the Workspace display the data.

- Click  **Stop** to stop the read. The read stops immediately. If you click  Stop while the plate is being read for a kinetic time point, the software completes the data collection for that time point before the read stops.



Note: If you stop a kinetic read before it completes, you cannot restart the read or append data to the read. You must either let the read finish or click  **Interrupt** to pause the read.

- For kinetic reads, 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 the read for all the wells you select in the plate and then waits for your input before continuing. This type of discontinuous kinetic read helps with acquisition development.
- After a kinetic read ends successfully, click  **Append** to read the same Plate section again and append the data to the data that already exists in the Plate section.
- When you select a section in the Navigation Tree, the Home tab displays section-specific icons:
 - **Notes Controls** and **Formatting Tools** display when you select a Note section. See [Note Sections on page 75](#).
 - **Plate Tools** and **Template Tools** display when you select a Plate section. See [Plate Sections on page 80](#).
 - **Cuvette Tools** and **Template Tool** display when you select a Cuvette Set section. See [Cuvette Set Sections on page 84](#).
 - **Column Tools**, **Summary Tools**, and **Formatting Tools** display when you select a Group section. See [Group Sections on page 87](#).
 - **Graph Type** and **Graph Tools** display when you select a Graph section. See [Graph Sections on page 90](#).

Protocols Tab

The Protocols tab displays the following icons:

- **Protocol Manager** - Icons to manage and access the Protocol Library.
 - Click  **Folder Locations** to manage the list of folders that display when you click  Protocol Manager. See [Protocol Library Folder Locations on page 63](#).
 - Click  **Save As Default** to overwrite the default protocol (default.spr) with the protocol settings you define for the document that is open in the Workspace. When you open a new document, the settings from the default protocol appear in the Workspace. The default protocol settings help you get started when you create a new document. When you install the software, the Basic Endpoint protocol is set as the default protocol.



Note: When you save a document as the default protocol, the data you collect in the current document remains unchanged. To keep the data, save the document as a data document.

- Click  **Protocol Manager** to display the list of protocols in the Protocol Library. The Protocol Library contains over 160 protocols that contain instrument settings and formulas. You can customize the protocols in the Protocol Library to facilitate the data analysis and report creation.
- **Community** - Access to the SoftMax Pro Software Community web site.
 - Click the  **Protocol Home Page** drop-down:
 - Select **Molecular Devices Protocol Library** to display the SoftMax Pro Protocol Home page website. Use this website to download sample protocols. The computer must have access to the Internet.
 - Select **SectraTest Validation Protocols** to display the SpectraTest® Validation Package User Manual Download page website. Use this website to download the protocols for the SpectraTest validation plates that are specific to the instrument to validate. The computer must have access to the Internet.

View Tab

The View tab displays the following icons:

- **Show/Hide Panel** - Select the **Navigation Tree** check box to display the Navigation Tree in the Workspace.
Clear the **Navigation Tree** check box to hide the Navigation Tree to provide more space to view the content of the Workspace. See [Navigation Tree on page 68](#).
- **Window** - Icons to change the layout of the Document tabs in the Workspace. See [Workspace on page 70](#).
 - Click  **Single Tab Group** to display the data for a single Document tab in the Workspace.
 - Click  **New Horizontal Tab Group** to tile the content of the Document tabs horizontally. The tab you select moves below inactive tabs in the Workspace. Content resizes to accommodate the display for all documents you open in the Workspace.
 - Click  **New Vertical Tab Group** to tile the content of the Document tabs vertically. The tab you select moves to the right of inactive tabs in the Workspace. Content resizes to accommodate the display for all documents you open in the Workspace.
- **Expand/Collapse Sections** - Icons to change the amount of data that displays in each section.
 - Click  **Collapse All Sections** to display only the experiment names in the Navigation Tree and only the section names in the Workspace.
 - Click  **Expand All Sections** to display all sections in each experiment in the Navigation Tree and to expand the display of the section content in the Workspace.

Operations Tab

The Operations tab displays the following icons:

- **Instrument Tools** - Icons that are specific to the instrument that you connect to the computer.
 - Click  **Calibration** to air-calibrate the instrument for the absorbance read mode. The instrument calibration values are stored in the instrument firmware. See [Instrument Calibration on page 371](#).
 - Click  **Info** to view instrument information such as the serial number and firmware version. Displays the list of detection cartridges you install in the SpectraMax i3, SpectraMax i3x, and SpectraMax Paradigm. Displays the filters you install in the FilterMax F3, FilterMax F5, SpectraMax iD5, and SpectraMax® Mini Multi-Mode Microplate Reader. Use this to configure the excitation and emission filter slides for the FilterMax F3 and FilterMax F5. See [Instrument Information on page 373](#).
 - Click  **Filters** to define the positions of the filters you install in the EMax® Endpoint Microplate Reader and VMax® Kinetic Microplate Reader. See [Configuring Filters for the EMax, EMax Plus, and VMax Microplate Readers on page 317](#).
 - Click  **Configure** to adjust the intensity of the lamp for each of the filters you install in the EMax® Plus Microplate Reader. You can view the positions and wavelengths of the filters you install and other instrument information like the serial number and firmware version. See [Adjusting the Lamp on page 316](#).
 - Click  **Initialize** to return the EMax Plus plate carrier to its home position.
 - Click  **Recover** to restore the FlexStation 3 fluidics module to operating conditions after a read error.
 - Click  **Air Gap** to enter the FlexStation 3 pipette air gap which is the volume between the end of the pipette tip and the bottom of the liquid in the tip.
 - Click **Refresh** to update the software for the detection cartridges you install in the SpectraMax i3, SpectraMax i3x, and SpectraMax Paradigm and the filter slides you install in the FilterMax F3 and FilterMax F5.
 - Click  **Wash Injectors** to wash the injectors for the SpectraMax® L Luminescence Microplate Reader.
 - Click  **Prime Injectors** to prime the injectors for the SpectraMax L.
 - Click  **Reverse Injectors** to reverse the injectors for the SpectraMax L.
 - Click  **Injector Tubing** to determine when to replace injector tubing for the SpectraMax L.
 - Click  **Change Aperture** to change the aperture from 96-well plates to 384-well plates or from 384-well plates to 96-well plates for the SpectraMax L with a manual aperture.
 - Click  **Shake Settings** to shake the plate for the SpectraMax L.

- **Calculations** - The software does continuous recalculation of the data when you read plates, create or change formulas, or change settings that influence data. You may want to disable recalculations to edit the protocol without waiting for recalculations to complete. See [Calculations and Numerical Precision on page 193](#).
 - Click  **Suspend Calculations** to disable automatic recalculation. While suspended, no recalculation occurs regardless of what you change, add, or delete from the experiment. Use this when you have a large, complicated protocol and you want to modify the formulas without waiting for recalculations to complete.
 - Click  **Resume Calculations** to use automatic recalculation.
 - Click  **Recalculate Now** to recalculate all data one time. Use this when recalculation is suspended to see the results of the changes you made without enabling continuous recalculation.
 - Click  **Function Editor** to display the Function Editor dialog where you create custom curve fits for graphs. See [Custom Curve Fit Settings on page 232](#).

Under certain circumstances, a calculation status message displays in the Workspace footer. See [Workspace on page 70](#).

- **Calculating:** Displays when the software calculates the formulas in an experiment and when you click **Recalculate Now**.
- **Calculating Suspended:** Displays when you click **Suspend Calculation**.
- No message displays under other conditions.
- **Automation** - Icons to do multiple plate reads and for automation of the software.
 - Click  **Auto Read** to automatically read multiple Plate sections in the sequence in which the Plate sections display in an experiment. See [Using Auto Read on page 182](#).
 - Click  **Plate Stacker** when you use the StakMax Microplate Handling System that uses the StakMax Software that is integrated in the SoftMax Pro Software. See [StakMax Microplate Handling System on page 347](#).
 - Click  **Automation Mode** to place the SoftMax Pro Software in automation mode. See [Automation Mode on page 17](#).

GxP Tab (SoftMax Pro Software - GxP Edition)

The GxP tab is available when you use the SoftMax Pro Software - GxP edition ([SoftMax Pro Software - GxP Edition on page 251](#)) and displays the following icons:

- **User Account** - Icons to manage user account information.
 - Click  **Account Information** to display the User Account Information dialog where you view information about the current user. See [Account Information on page 257](#).
 - Click  **User Log Off** to log the current user off without closing the software.
 - Click  **User Lock Session** to reactivate a user session after the session times out due to inactivity.
 - Click  **Change Password** to display the Change Password dialog where you change your password. See [Changing Passwords on page 258](#).
- **Data** - Icons to track software usage.
 - Click  **Statements** to display the Statements dialog where you view, edit, add, and sign statements. See [GxP Statements on page 270](#).
 - Click  **Audit Trail** to display the Audit Trail dialog where you view activity related to the document that is open in the Workspace. See [Document Audit Trail on page 276](#).
 - Click  **Document Status** to change the status of the document that is open in the Workspace. See [Document Status on page 267](#).
 - Click  **Release Protocol** to release a protocol for the next step in the document workflow. See [Releasing Protocols on page 267](#).
- **Instrument** - Icons to enable and disable the touchscreen for the SpectraMax iD3 or SpectraMax iD5. See [SpectraMax iD3 Multi-Mode Microplate Reader on page 330](#) and [SpectraMax iD5 Multi-Mode Microplate Reader on page 333](#).

Workflow Tab

The Workflow tab is for use with the Workflow view of the Workspace ([Defining and Running Workflows on page 106](#)) and displays the following icons:

- **Execution** - Icons to  **Start**,  **Pause**,  **Resume**, and  **Cancel** the workflow.
- **Designer** - Icons to manage the tasks in the workflow and to change the appearance of the Workspace when you use the Workflow view.
 - Click **Undo**, **Redo**, and **Clear** to undo, redo, or clear the tasks in the Workspace.
 - Click **Zoom In**, **Zoom Out**, and **Reset Zoom** to change the appearance of the tasks in Workspace.
 - Click **Fit to Screen** to size the tasks so that the tasks all fit on one screen.
 - Click **Mini Map** to display an Overview dialog that helps you navigate a long timeline.
 - Click **Save as Image** to export an image of the timeline to a .jpg, .png, .gif, or .xps format.
- **Status** - Displays the status of the workflow.
 - Click  **Execution Log** to display the Execution Log dialog where you view the status of the tasks as the workflow runs in real time.

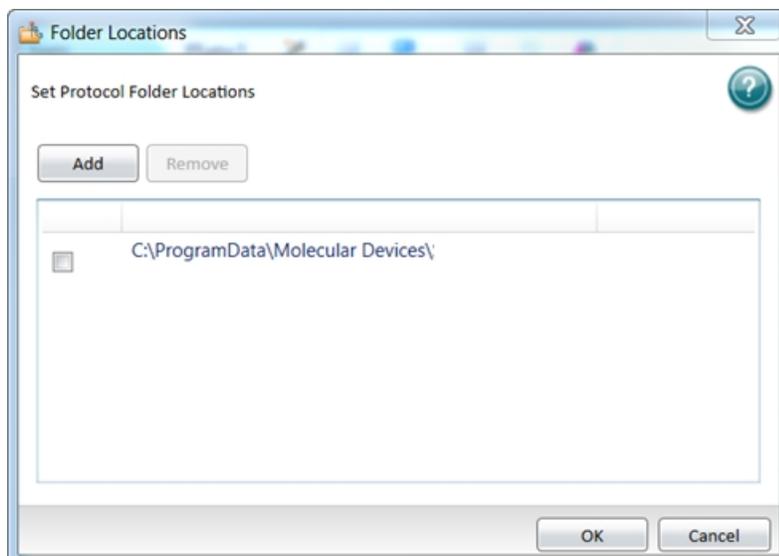
Help Tab

The Help tab displays the following icons:

- **Help**
 - Click  **SoftMax Pro Help** to view the application help.
 - Click  **Formula Reference** to view the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*. This document has four main sections:
 - **Introduction** - Provides a general introduction to formulas.
 - **Operators** - Describes the types of operators you use to assemble formulas.
 - **Functions** - Describes the types of functions you use to assemble formulas.
 - **Accessors** - Describes special functions and protocol settings that you include in formulas.
 - Click  **Release Notes** to view the *SoftMax Pro Data Acquisition and Analysis Software Release Notes* for this software release.
 - If the computer has access to the Internet, click  **Contact Us** to display a menu with the following options:
 - Select **What's New** to view information about what is new in the software. If you miss the What's New tab that displayed in the Document Tabs in previous versions of the software, you can view the information here.
 - Select **Home Page** to view the Molecular Devices web site home page.
 - Select **Knowledge Base** to view the Molecular Devices Knowledge Base.
 - Select **Request Support** to access the Molecular Devices Support Request web page.
 - Click  **Software License** to display the Software License Activation dialog where you activate the software license. See [Activating Software Licenses on page 10](#).
- Troubleshooting
 - Click  **Diagnostics** to turn on Diagnostics mode in order to record a read that generates an instrument diagnostics log file. The log file contains information from the connected instrument to help solve technical issues. Displays  when you turn the Diagnostics mode on and the Home tab displays *Diagnostics On* in the Instrument area. (Available for SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm.) See [Instrument Diagnostics Mode on page 66](#).
 - Click  **Export** to export the diagnostics log file after you record a read in Diagnostics mode. This exports the log file to the computer hard drive or to a USB drive so that you can send the file to Molecular Devices Support.
- **About** - Click  **SoftMax Pro** to view the software version number and release information.

Protocol Library Folder Locations

Use the Folder Locations dialog to manage the list of folders that appear in the Protocol Library. The Protocol Manager provides access to protocols included with the software and the protocols you store in the Protocol Library.



To manage the folders that display in the Protocol Library:

1. In the Ribbon, select the **Protocols** tab and click  **Folder Locations** to display the Folder Locations dialog.
2. Click **Add** to display the Select Folder dialog.
3. To add a folder to the Protocol Library, either:
 - Navigate to a folder and click **Select Folder**.
 - Select the folder into which to add a new folder, click **New Folder**, enter the folder name, and then click **OK**.
4. To delete a folder from the Protocol Library, select the check box for each folder to remove and click **Remove**.



Note: The folder no longer displays in the Protocol Library but the folder and the documents in the folder remain in the database.

5. Click **OK**.

Selecting an Instrument

Use the Instrument Connection dialog to connect the SoftMax Pro Software to the microplate reader. See the instrument user guide for instructions to connect the cables between the computer and the instrument. See [Supported Instruments on page 315](#).



Note: You can use the software without a physical connection between the computer and the instrument in Offline mode to do data analysis or in Simulator mode to create protocols.



To select an instrument:

1. Power on the instrument and wait for the instrument initialization process to complete.
2. In the Ribbon, select the **Home** tab and click **Instrument** to display the Instrument Connection dialog.
3. If the instrument does not appear in the Available Instruments list, click **Refresh**.
4. In the **Available Instruments** list:
 - Select the communication port to which you connect the instrument.
 - To work offline for data analysis, select **Offline**, then click the **Reader** drop-down and select an instrument.
 - To work in simulator mode to create protocols, select **Offline**, click the **Reader** drop-down and select an instrument, 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.

Instrument Connection Status

In the Ribbon, on the Home tab, the instrument icon indicates the status of the connection between the computer and the instrument. If the status indicates the instrument is disconnected, make sure that the instrument is powered on and that the cables between the computer and the instrument are secure.



- Instrument connected



- Instrument disconnected



- Instrument in Simulator mode

Instrument Calibration

You should calibrate some instruments before use. See [Instrument Calibration on page 371](#).

SpectraMax Paradigm Considerations

When you first setup the SpectraMax Paradigm, you must remove hardware transport locks as described in the *SpectraMax Paradigm Multi-Mode Microplate Reader User 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. See [Removing Software Locks on page 343](#).

SpectraMax iD3 and SpectraMax iD5 Considerations

When you use the SoftMax Pro Software to operate the SpectraMax iD3 or SpectraMax iD5, your company network configuration could prevent the connection 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 computer to the instrument via your company intranet. See [SpectraMax iD3 Multi-Mode Microplate Reader on page 330](#) and [SpectraMax iD5 Multi-Mode Microplate Reader on page 333](#).

You may have to do the following to select the SpectraMax iD3 and SpectraMax iD5:

1. On the SpectraMax iD3 and SpectraMax iD5 instrument touchscreen, navigate to the **Maintenance** page **System Information** tab and note the instrument IP address.
2. In the SoftMax Pro Software, click  to open the Application menu and then click the **Options** button to display the SoftMax Pro Options dialog.
3. Select the **SpectraMax iDx Control** tab on the left.
4. Select the **Enable Control of SpectraMax iDx Instruments** check box.
5. In one of the text fields, enter the IP address of the instrument. There are five fields to enter up to five IP addresses.
6. Click **OK**.
7. Select the **Home** tab and click **Instrument** to display the Instrument Connection dialog.
8. Click **Refresh** if needed and the SpectraMax iD3 and SpectraMax iD5 instruments 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.

For users that use the SoftMax Pro Software - GxP edition to operate the instrument, the user must have the Lock/Unlock Instrument permission to use the following icons to lock and unlock the instrument touchscreen:

- 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 you must operate the instrument from a computer running the SoftMax Pro Software - GxP edition.
- Click  **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to use the instrument touchscreen to run experiments.



Note: The instrument remains locked until the user with the appropriate permission

clicks  **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an instrument that is locked in GxP mode.

Instrument Diagnostics Mode

For the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm, the Help tab contains a  Diagnostics icon that you use to turn on the Diagnostics mode. In Diagnostics mode, you run a read that generates an instrument diagnostics log file. The log file contains information that helps you and Technical Support resolve instrument issues.

To generate an instrument diagnostics log file:

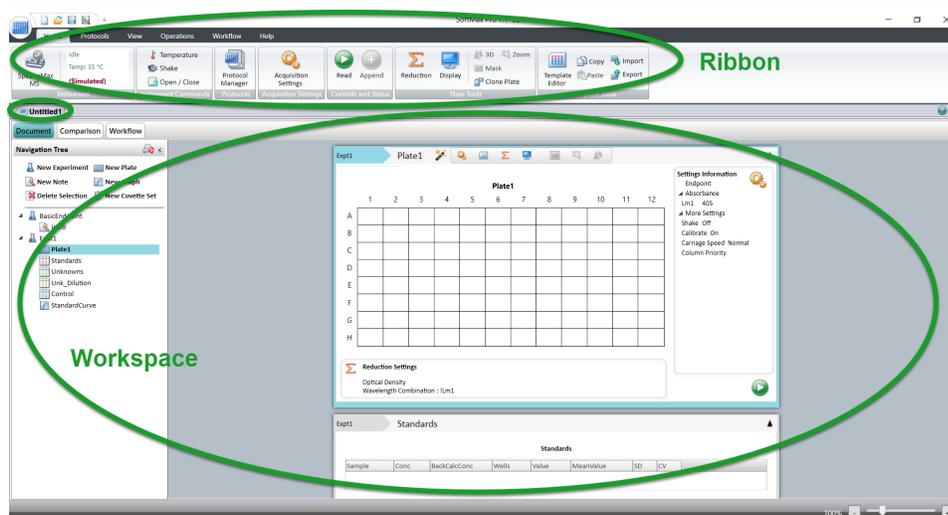
1. Connect the software to a SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, or SpectraMax Paradigm.
2. Select the **Help** tab and click  **Diagnostics**. The icon changes to  Diagnostics On.
3. Click **OK** in the confirmation message dialog.
4. Select the **Home** tab and note that *Diagnostics On* displays in the Instrument area.
5. Open a document and select a Plate section.
6. Click  **Read** to read the Plate section.
7. Select the **Help** tab and click  **Diagnostics On**. The icon changes to  Diagnostics.
8. Click **OK** on the confirmation message dialog.
9. Click  **Export** to export the instrument diagnostics log file that contains the diagnostic information to the computer hard drive or a USB drive.
10. In the Save As dialog, navigate to the location where you want to save the log file and in the **File Name** field, enter the name of the log file.
11. Click **Save**. You can then send the file to Molecular Devices Support.

Document Tabs

Each tab between the Ribbon and the Workspace is a protocol or data document. The Untitled1 tab displays the default protocol when you log on for the first time to help you create your first document. Depending on your View tab settings, as you use the software to define additional documents, each document appears as a tab above the Workspace to the left of the Untitled1 tab.



Note: For the SoftMax Pro Software - GxP edition, no document tabs display and the Workspace area is empty. Your permissions determine which actions you can perform. See [SoftMax Pro Software - GxP Edition on page 251](#).



Select a tab to display the contents of the document in the Workspace.



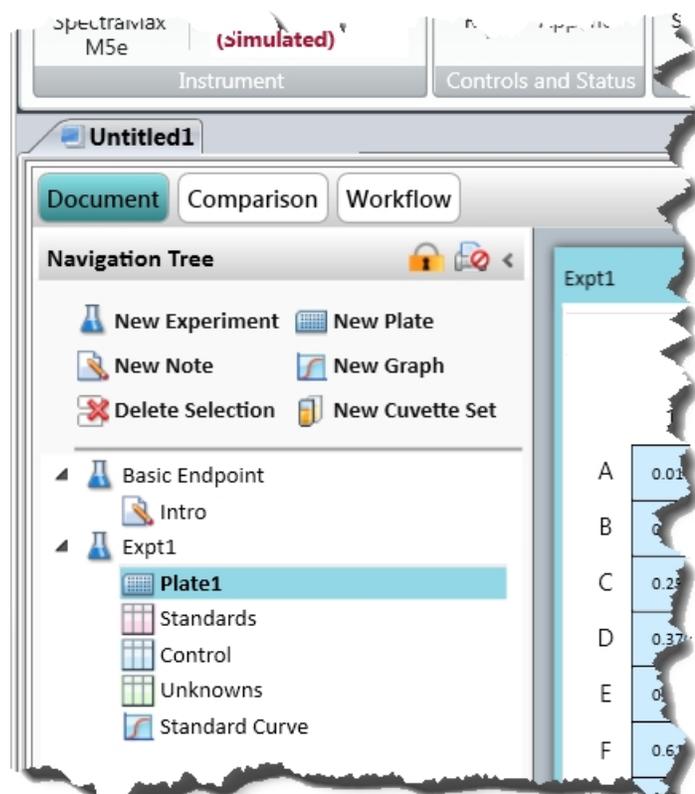
Tip: If you miss the What's New tab from previous versions of the software, the Help tab on the Ribbon provides access to the Molecular Devices What's New web page. See [Help Tab on page 62](#).



The Application menu provides access to the SoftMax Pro Options dialog where you can choose to display the What's New information every time you start the software if the computer has access to the Internet. See [SoftMax Pro Options on page 52](#).

Navigation Tree

The Navigation Tree displays section controls, the list of experiments in the open document and the sections in each experiment. The Navigation Tree displays to the left of the Workspace when you use the Document view and the Comparison view for a document. The Workflow view displays icons you can drag into the Workspace. See [Workspace on page 70](#).



The following icons appear above the Navigation Tree:

-  - For the SoftMax Pro Software - GxP edition, select a section and click to lock the section and prevent edits. Sections that are locked display . Click  again to unlock the section. See [SoftMax Pro Software - GxP Edition on page 251](#).
-  - Select a section and click to prevent the section from appearing in the printed version of the document. Sections that do not print display . Click  again to have the section appear in the printed versions of the document. To prevent or allow printing of all the sections in an experiment, select the experiment in the Navigation Tree and click . You can override this setting from the  Application menu when you print the contents of the document.
-  - Click to hide the Navigation Tree and increase the Workspace area display. In the Ribbon, use the View tab to hide the Navigation Tree.

Section Controls

Use the controls in the Navigation Tree to manage the experiments in the document and to manage the sections in the experiments. Sections include Note, Plate, Graph, and Cuvette Set. Experiments can also contain Group sections. A Group section contains a group of plate wells and related formulas. You create Group sections when you configure Plate sections and Cuvette Set sections from the Template Editor dialog. See [Template Editor on page 91](#).

After you add an experiment or section to the Navigation Tree, drag and drop experiments within the hierarchy and drag and drop sections within an experiment.

To manage experiments:

1. Above the Workspace, select a Document tab. The experiments and the sections in the document display in the Navigation Tree.
2. To add an experiment to the document, select the experiment that most closely resembles the experiment to create and click  **New Experiment**. A duplicate of the experiment you select displays below the experiment. The software duplicates the sections and settings but does not duplicate the data.
3. To add a Note section, Plate section, or Cuvette Set section to an experiment, select the section that most closely resembles the section to create. The software adds the new section below the section you select.
 - Click  **New Note** to add a Note section. See [Note Sections on page 75](#).
 - Click  **New Plate** to add a Plate section. See [Plate Sections on page 80](#).
 - Click  **New Cuvette Set** to add a Cuvette Set section. See [Cuvette Set Sections on page 84](#).
4. To add a Graph section, click  **New Graph** to display the New Graph dialog. Set the options for the graph. See [Graphing Data on page 214](#). Click **OK**. The Graph section displays below the section you select. See [Graph Sections on page 90](#).
5. To delete a section, select the section or sections to delete (Ctrl+click to multi-select) or select an experiment to delete an entire experiment, then click  **Delete Selection**. You cannot undo the deletion. When you delete a Plate section, the software also deletes the data from the Plate section.
6. To change the sequence of the sections in an experiment, drag the section name and drop it elsewhere in the Navigation Tree. You can move the sections within an experiment. You cannot move sections between experiments.
7. Select a section to display the section content in the Workspace.

Renaming, Duplicating, Deleting Experiments and Sections

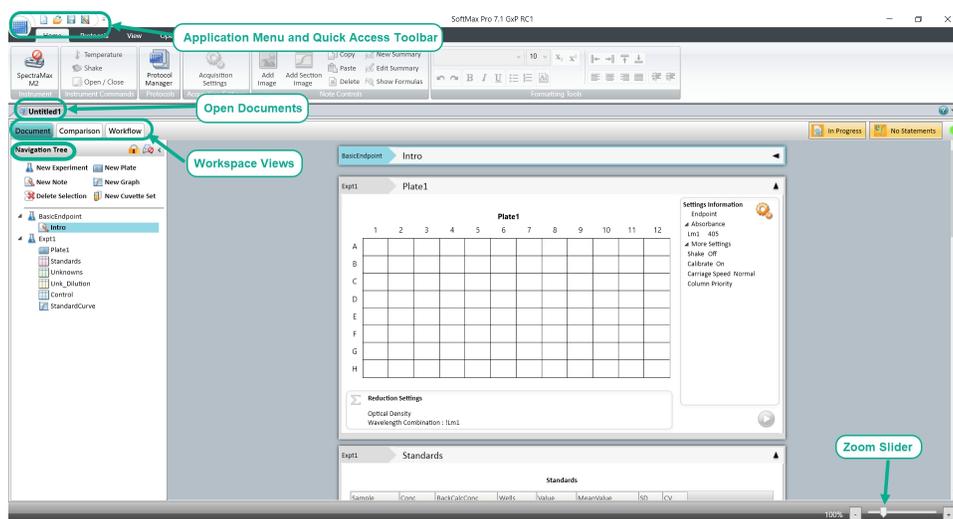
Right-click an experiment or a section to Rename, Duplicate, or Delete it.

- When you duplicate an experiment, the software duplicates all sections in the experiment to a new experiment, without data.
- When you duplicate a Note section or a Graph section, the software creates a new section with the contents of the source section.
- When you duplicate a Group section, the software creates a new Group section with the same properties as the source section.
- When you duplicate a Plate section, the software creates a new Plate section with the settings from the source Plate section, without data.

Workspace

The Workspace appears below the Ribbon. The Document tabs above the Workspace list the documents you have open. You open and save documents from the Quick Access toolbar and from the  Application Menu.

In the Ribbon, use the View tab to split the Workspace to view multiple documents at the same time. See [View Tab on page 58](#).



Use the three buttons below the Document tabs to change the Workspace view. Use each Workspace view to manage the open document and experiment results.

- Use the **Document** view to scroll through the experiment sections.
- Use the **Comparison** view to drag sections from the Navigation Tree and arrange them in the Workspace wherever you want.
- Use the **Workflow** view to manage complex experiments that include repeated tasks and multitask kinetic reads.

The left side of the Workspace is the Navigation Tree that displays the experiments in the open document. Each experiment can contain a virtually unlimited number of Note, Plate, Cuvette Set, Group, and Graph sections.

The Navigation Tree for the Document view and Comparison view displays section controls and a list of the experiment sections. See [Workspace Sections on page 73](#).

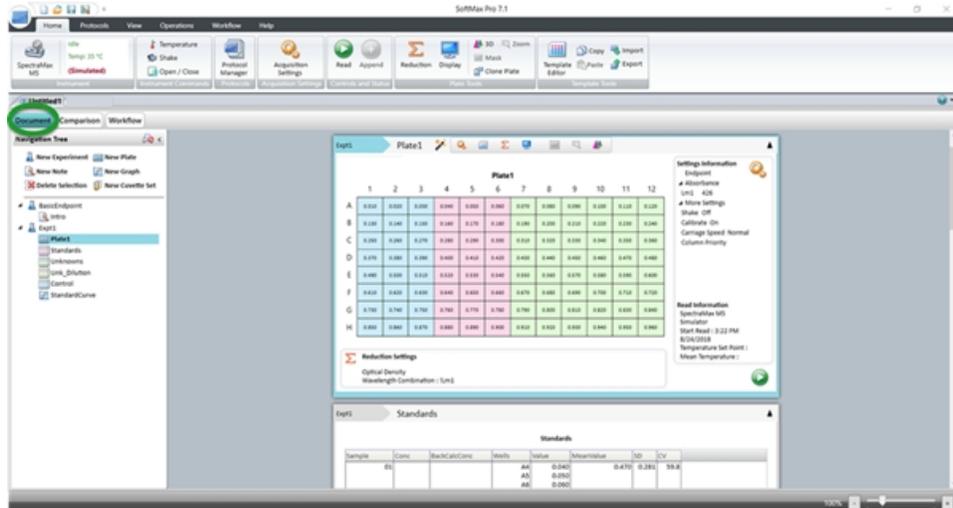
After you use the Document view or the Comparison view to define the experiments, the Workflow view contains icons that you drag into the Workspace to define an experiment workflow that can include delays and repeated cycles for more complex experiments.

Drag the slider on the bottom right to zoom the focus of the Workspace in or out.

Workspace Views

Document View

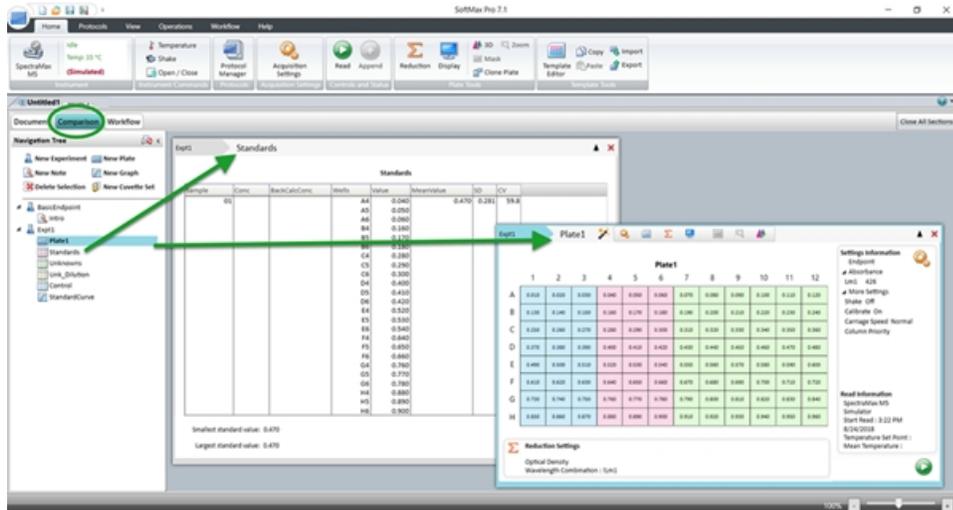
Use the Document view scrollable area to view the experiments and sections in the open document. The Document view displays by default. Click the **Document** button above the Navigation Tree, if needed.



To view a section either click the section in the Navigation Tree or use the scroll bar on the right until the section comes into view.

Comparison View

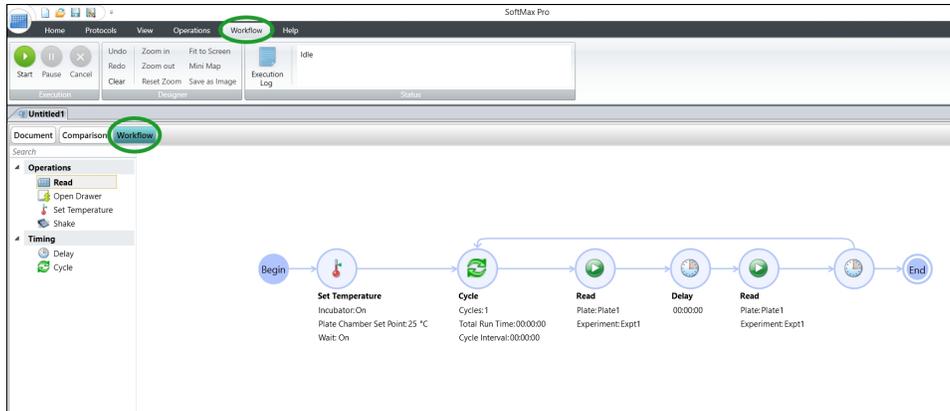
Use the Comparison view to move the sections to the positions where the sections are most useful. To use the Comparison view, click the **Comparison** button above the Navigation Tree.



Drag sections from the Navigation Tree into the Workspace. Sections can overlap. Double-click a section to add the section to the bottom of the list of sections in the Workspace.

Workflow View

After you define the experiment in the Document view or the Comparison view, use the Workflow view to drag-and-drop tasks into the Workspace to create a timeline for a multi-task document that can involve several experiments with multiple reads. Define the experiments with all applicable sections from the Document view and then use the Workflow view to create the workflow that uses the experiments and Plate sections to run more complicated assays such as multitask kinetic reads that last hours or days. When you click the Workflow button above the Navigation Tree, the Ribbon automatically displays the Workflow tab. See [Defining and Running Workflows on page 106](#).

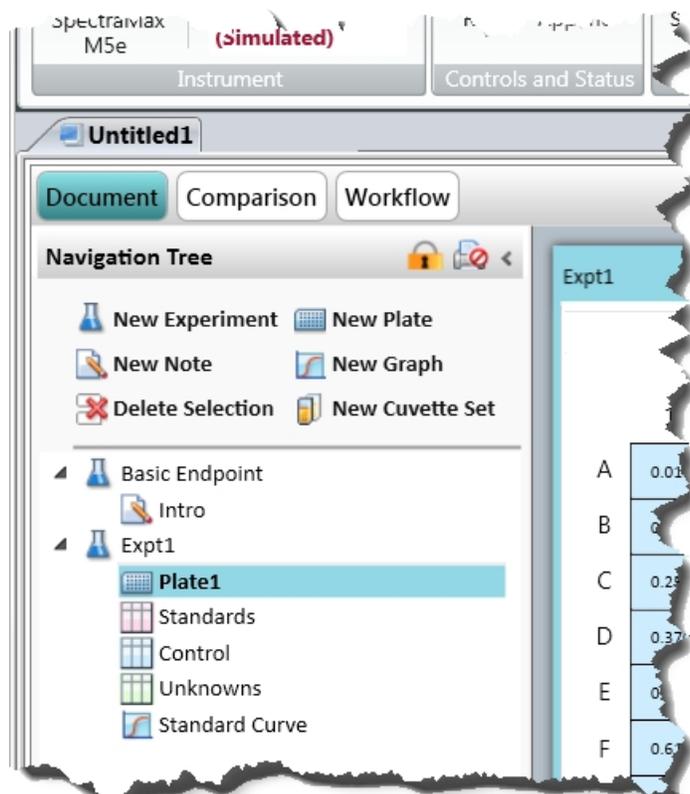


To create a workflow:

- 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.
- In the Ribbon, use the Workflow tab to start, pause, resume, and cancel the workflow.

Workspace Sections

Use the Workspace to manage documents. Each document can contain a virtually unlimited number of experiments. The Navigation Tree displays the experiments and experiment sections in the document.



Experiments

 Experiments are an organizational tool within the document to maintain separate data sets. Each experiment can contain a virtually unlimited number of sections. Use sections to document what the experiment is designed to accomplish, define instrument settings, define analytical formulas, and define the presentation of the data that the software collects.

Sections in Experiments

Each experiment is made up of Note sections, Plate sections, Cuvette Set sections, Group sections, and Graph sections. You can create and edit sections at any point in the data collection process: before you collect data, to adjust how you collect data, and to manage the presentation of the data you collect. Links in the following list take you to the description of the settings for each section. The SoftMax Pro Software - GxP edition allows you to lock each section to restrict changes. See [Locking and Unlocking Sections on page 256](#).

-  **Note** - Use Note sections to enter text and report summary information to the experiment. Note sections can contain text, Summary formulas, images such as your company logo, and images of the other sections in the document. See [Note Sections on page 75](#).

-  **Plate** - Use Plate sections to define plate settings and instrument settings to collect data from the instrument. Use plate settings to define the data display and data reduction. Each Plate section can have unique instrument settings to run different types of reads within the experiment. See [Plate Sections on page 80](#).
-  **Cuvette Set** - Use Cuvette Set sections to define instrument settings to collect data from a cuvette port. Each Cuvette Set section can have unique instrument settings to run different types of reads. See [Cuvette Set Sections on page 84](#).
-  **Group** - Use Group sections to combine the data from wells in a plate or several cuvettes into groups. You create Group sections in the Template Editor dialog when you work with Plate sections and Cuvette Set sections. See [Group Sections on page 87](#).
-  **Graph** - Use Graph sections to define the graphical presentation of the data the software collects as scatter graphs or bar graphs. See [Graph Sections on page 90](#).

Managing Experiments

Use the  New Experiment icon in the Navigation Tree to add an experiment to a document.

To add an experiment:

1. In the Navigation Tree, select an experiment that is similar to the one you want to add.
2. Click  **New Experiment**.

A duplicate of the experiment displays below the experiment you select. The software duplicates the sections and settings, but not the data. Drag it to a different location in the hierarchy, if needed.

Right-click the experiment:

- Select **Rename** to change the name of the experiment. Use the Rename Section dialog to enter a new name for the experiment.
- Select **Duplicate** to copy the experiment.
- Select **Delete** to delete the experiment. The software deletes all sections and all data in the experiment.

Note Sections

Use Note sections to enter text and data analysis formulas for an experiment. A Note section can contain text, images, and Summary formulas. Summary formulas can be part of a Note section or Group section to generate analysis data, text, or user-entered constants such as certificate values. See [Formulas on page 102](#).

Managing Note Sections

Use the  New Note icon in the Navigation Tree to add a Note section to an experiment.

To add a Note section:

1. In the Navigation Tree, select the section above where you want to add a Note section.
2. Click  **New Note**.

A new blank Note section displays below the section you select. Drag it to a different location in the hierarchy, if needed.

Right-click the Note section:

- Select **Rename** to change the name of the Note section. The Rename Section dialog displays where you to enter a new name for the Note section.
- Select **Duplicate** to copy the Note section.
- Select **Delete** to delete the Note section.

When you select a Note section in the Navigation Tree, the Note section content displays in the Workspace and the section toolbar contains the following icons:

-  - Click to add a formula from the Formula Editor dialog. See [Formula Editor on page 104](#).
-  - Click to add an image from the computer file system. See [Managing Images on page 78](#).
-  - Click to add an image of a section in the experiment. See [Managing Section Images on page 78](#).
-  - Click to show or hide the formula in a Summary formula frame. [Summary Formula Tools on page 77](#).
-  - Click to refresh the data in a section image.

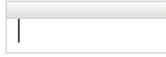
The Home tab provides:

- **Note Controls** - Contains the icons described above plus icons to  Copy,  Paste,  Delete, and  Edit Summary formulas.
- **Formatting Tools** - Contains icons to manage text in the frames and the placement of frames in the section. See [Formatting Text in Section Frames on page 79](#).

Frames

Everything in a Note section is contained in frames. When you click in the blank area of a Note section in the Workspace, a new frame appears. You can add as many frames as you need. A frame has a header bar and an outline. When you click into a frame to make it active, the frame header bar and outline display. Use Ctrl+click to make multiple frames active within a Note section.

1. Click in a blank area of the Note section to create a new frame.



Note: You must place each image in its own frame and the frame cannot contain text.

- To enter text, click into a frame and start typing. Use the Home tab Formatting Tools to format the text within the frame.
- To add an image, click into an empty frame and use the icons in the Note section toolbar or on the Home tab.
- To resize a frame, hover the cursor over the frame side border to display a two arrow head cursor, then drag the border to change the size.
- To move a frame, hover the cursor over the frame header bar to display a four arrow head cursor, then drag the frame to the new location.
- To align frames, the Home tab Formatting Tools section and the frame header right-click menu provide the following icons/options (Ctrl+click to multi-select):
 - Click  to align a single frame to the left edge of the Note section or align the left edges of multiple frames to the left-most frame.
 - Click  to align a single frame to the right edge of the Note section or align the right edges of multiple frames to the right-most frame.
 - Click  to align a single frame to the top edge of the Note section or align the top edges of multiple frames to the top-most frame.
 - Click  to align a single frame to the bottom edge of the Note section or align the bottom edges of multiple frames to the bottom-most frame.
- To copy a frame:
 - Right-click a frame and select **Copy** then right-click in a Note section and select **Paste**.
 - Select the frame in the Note section, select the **Home** tab and click **Copy** in the Note Controls then click in a Note section and click **Paste** on the Home tab.
- To delete a frame:
 - Right-click on the frame header and select **Delete**.
 - Select the frame in the Note section, select the **Home** tab and click **Delete** in the Note Controls.

Managing Text

You manage the text in each frame the same way most text processing software applications allow you to manage text. The Home tab provides Formatting Tools to format text.

Within a frame, select the text to manage:

- Right-click and select **Cut** to move the text to the clipboard.
- Right-click and select **Copy** to copy the text to the clipboard.
- Right-click and select **Paste** to paste the cut or copied text to the new location.
- Press the **Delete** key to delete the text.

Summary Formula Tools

Use formulas to automate data analysis for results reports. Summary formulas typically reduce data to a single value or array. Use the Formula Editor dialog to manage the Summary formulas in Note sections.

For a complete guide to formulas and their use, select the **Help** tab and click  **Formula Reference**.

- To create a new Summary formula, click  in the Note section toolbar or click  **New Summary** on the Home tab.
- To edit a Summary formula, double-click in a frame that contains a formula or click a frame and click  **Edit Summary** on the Home tab.
- To copy a Summary formula to the clipboard, click a frame that contains a formula and click  **Copy** on the Home tab.
- To paste the Summary formula to a new location, click where you want to paste the formula and click  **Paste** on the Home tab.
- To delete a Summary formula, click a frame that contains a formula (Ctrl+click to multi-select) and click  **Delete** on the Home tab.

By default, Summary formulas in the Note section display the result of the formula without displaying the actual formula. You can show or hide the formula with the result.

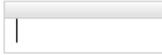
- Click  in the section toolbar or click  **Show Formulas** on the Home tab to display all formulas in the Note section.
- Click  in the section toolbar or click  **Hide Formulas** on the Home tab to hide all formulas in the section.

Managing Images

There are two types of images you can insert into a Note section. You can insert an image from your computer file system such as a company logo and you can insert an image of another section in the experiment.

To insert an image from your computer file system:

1. In the Navigation Tree, select a Note section.
2. Click in a blank area of the Note section to create a new frame.



Note: You must place each image in its own frame and the frame cannot contain text.

3. Click  in the Note section toolbar or click  **Add Image** on the Home tab to display the Open dialog. You can also right-click in a Note section and select **Add Image**.
4. Navigate to and select the image to insert.
5. Click **Open** to display the image in the Note section.

To delete an image:

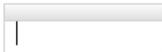
- Right-click on the frame header and select **Delete**.
- In the Note section, select the frame. In the Ribbon, select the Home tab. In the Note Controls, click  **Delete**.

Managing Section Images

A section image is a dynamic copy of an entire section you add to a Note section. As you make changes to the section, the image of that section also updates in the Note section.

To manage section images:

1. In the Navigation Tree, select a Note section.
2. Click in a blank area of the Note section to create a new frame.



Note: You must place each image in its own frame and the frame cannot contain text.

3. Click  in the Note section toolbar or click  **Add Section Image** on the Home tab to display the Add Section Image dialog. You can also right-click in the Note section and select **Add Section Image**.
4. In the list of sections, select the option next to the section to display as an image in the Note section.
5. Click **Add Section Image** to display the section image in the Note section.

To delete a section image, select the section image to delete, right-click and select **Delete**.



Note: When you delete a section from the document, the software also deletes images of the section from the Note sections. When you delete an experiment from the document, the software deletes the images of the sections in the experiment from the remaining Note sections.

Refreshing Section Images

To refresh the section images in a Note section, click  in the Note section toolbar.

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, the Graph section image in the Note section refreshes.



Note: Section images automatically refresh when you open the document, scroll up and down, switch between Document and Comparison views, or start to print or print preview the document.

Formatting Text in Section Frames

The Home tab provides Formatting Tools to format the text you select in a text frame in a Note section or a Group section. The Formatting Tools are similar to the format tools in most word processing software applications.

To format text:

1. In the Navigation Tree, select a Note section or a Group section.
2. Select the text in the frame that you want to format.
3. Use the following, as needed:

- Select a font type
- Select a font size
- Click  to make text subscript.
- Click  to make text superscript.
- Click  to undo the previous action.
- Click  to redo the previous action.
- Click  to make text bold.
- Click  to make text italic.
- Click  to underline text.
- Click  to create an unordered bulleted list.
To add a blank line between lines of bulleted text, click at the end of a line and then press **Shift+Enter**.
- Click  to create an ordered numbered list.
To add a blank line between lines of numbered text, click at the end of a line and then press **Shift+Enter**.
- Click  to change the font color.
- Click  to align the text to the left edge.
- Click  to center align the text.
- Click  to align the text to the right edge.
- Click  to left and right justify the text.
- Click  to out dent indented text.
- Click  to indent the text.
- Click , , , and  to align the frames within the section. See [Note Sections on page 75](#).

Plate Sections

Use Plate sections to define plate related instrument settings, data reduction settings, and data display settings. When you want to read the same physical plate twice with different instrument settings you create two Plate sections in the experiment. You can create as many Plate sections as you need. Use Auto Read to read multiple plate sections. See [Using Auto Read on page 182](#).

Plate sections contain a grid that represents the wells in the plate you select, instrument Settings Information to the right of the grid, and Reduction Settings below the grid. If colors appear in the plate grid, the Plate section contains templates. You assign each group in the template a different color and the Group section table displays the corresponding color. After a read, Read Information displays below the instrument Settings Information on the right.

Managing Plate Sections

Use the  New Plate icon in the Navigation Tree to add a Plate section to an experiment.

To add a Plate section:

1. In the Navigation Tree, select the section in the experiment above where you want to add a Plate section.
2. Click  **New Plate**.

A new blank Plate section displays below the section you select. Drag it to a different location in the hierarchy, if needed.

Right-click the Plate section:

- Select **Rename** to change the name of the Plate section. Use the Rename Section dialog to enter a new name for the section.
- Select **Duplicate** to copy the Plate section. When you duplicate a Plate section, the new Plate section contains all the settings from the original Plate section, without data.
- Select **Delete** to delete the Plate section.

When you select a Plate section in the Navigation Tree, the Plate section settings display in the Workspace and the section toolbar displays the following icons:

-  - Click to display the Plate Setup Helper dialog that contains most of the following icons plus the ability to select a different instrument to do the read. See [Plate Setup Helper on page 83](#).
-  - Click to display the Settings dialog where you configure the acquisition settings such as read mode, read type, wavelengths, plate type, read area, etc. Also displays in the Settings Information area to the right of the grid. See [Acquisition Settings on page 112](#).
-  - Click to display the Template Editor dialog where you assign wells in the plate to groups and create templates and Group sections. See [Template Editor on page 91](#).
-  - Click to display the Data Reduction dialog where you choose data reduction settings for analysis. Also displays in the Reduction Settings area below the grid. See [Data Reduction on page 196](#).
-  - Click to display the Display Options dialog where you choose options for how to display results. See [Data Display Options on page 206](#).
-  - Click to mask wells. See [Masking Wells or Cuvettes on page 211](#).
-  - Click to zoom. See [Zooming Well and Cuvette Display on page 212](#).

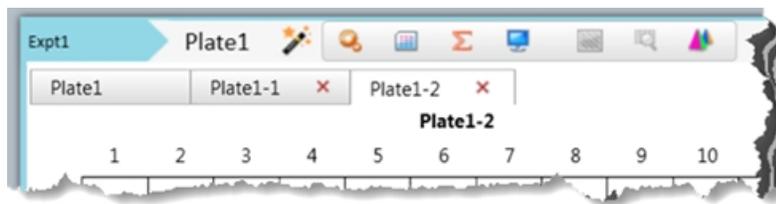
-  - Click to manage historical raw data generated when you overwrite plate data. See [Managing Historical Raw Data on page 84](#).
-  - Click to display reduced data in a three dimensional graph. See [Viewing Data in 3D Graphs on page 239](#).
-  - Click to start the read after you define all settings for the plate. Icon is located in the Settings Information area to the right of the grid, bottom right corner. See [Collecting Data on page 111](#).

The Home tab provides:

- **Plate Tools** - Contains the icons described above plus a  Clone Plate icon to clone a plate in the Plate section.
- **Template Tools** - Contains the  Template Editor icon described above plus icons to  Copy,  Paste,  Import, and  Export template data. See [Template Tools on page 93](#).

Cloning Plates

Use plate clones to apply multiple templates and reductions to the same set of raw data. You can add as many plate clones as needed to the Plate section. Plate clones display in tabs at the top of the Plate section. The first tab on the left is the original plate. Each plate clone tab displays the original plate name followed by the number of the clone.



To clone a plate:

1. In the Navigation Tree, select the Plate section that contains the plate to clone.
2. Select the **Home** tab and click  **Clone Plate** in the Plate Tools. The Plate section displays the plate clone as a tab next to the original plate name.
3. Click the tab for the plate clone to make the plate clone active in the section.
4. To delete a plate clone, click the **X** on the clone tab.

Copying Plate Data

You can copy plate data from one Plate section to a different Plate section within the same document or between different documents. The format of the source plate data must match the format of the target Plate section. The Clone Plate feature is the most efficient way to copy plate data between Plate sections in the same document.

You can paste endpoint plate data that you copy from an external program, such as a spreadsheet or text editor when the format of the data from the external program exactly matches the format of the target Plate section.



Note: You can paste endpoint data for multiple wavelengths and kinetic data for one wavelength.

To copy plate data:

1. In the Navigation Tree, select the source Plate section.
2. To copy all data from the entire plate, hover the cursor over the plate grid in the Workspace, right-click and select **Copy**.
3. In the Navigation Tree, select the destination Plate section.
4. Hover the cursor over the plate grid in the Workspace, right-click and select **Paste Plate Data**.

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

5. When you paste over existing data, click **Yes** on the confirmation message.
If the format of the data does not match the format of the target plate, a message displays and data does not paste.

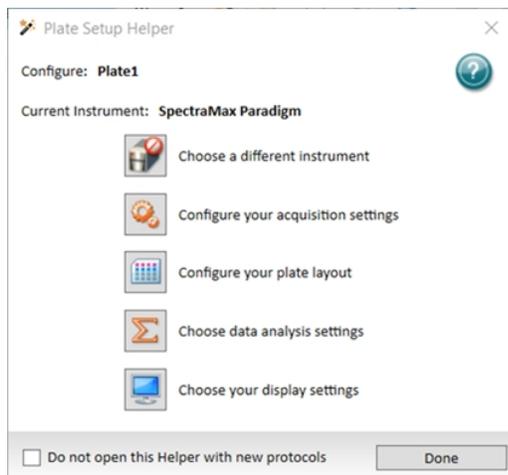
The Read Information on the right indicates that the data was pasted and shows the time and date of the paste.

Plate Section Data Display

Plate section settings depend on the plate type you select and the actual collection of data. After you define the Plate section settings and the instrument reads the plate data, the section displays the results of the read. The precision of the data display depends on the available screen space. The values that display are a representation of the data. The software rounds the last digit that displays based on the actual data value. You can customize the data display to Numeric notation or Scientific notation format and specify the number of significant figures or decimal places. You can set these options separately for raw data and reduced data. See [Collecting Data on page 111](#).

Plate Setup Helper

Use the Plate Setup Helper dialog to setup a plate to acquire data. This dialog is useful when you first learn the software because it walks you through each step to create a Plate section in sequence. However, each icon is duplicated in the Plate section toolbar and/or on the Home tab.



When you first start using the software and you select a Document tab above the Workspace, the Plate Setup Helper appears. Click  in the Plate section toolbar to display the Plate Setup Helper.

The **Configure** caption displays the name of the plate.

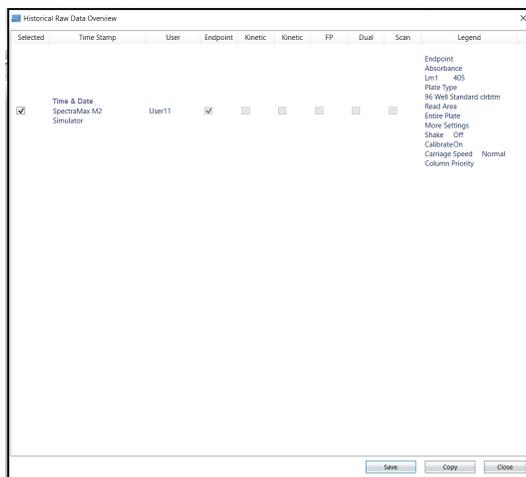
The **Current Instrument** caption displays the instrument to do the read.

-  - Click to display the Instrument Connection dialog where you select the instrument to do the read. The icon image changes to reflect the instrument you select and the icon displays the connection status between the software and the instrument. After you select the instrument, you can click any of the following icons in any sequence. The following are instrument specific and are reset if you change the instrument. See [Selecting an Instrument on page 64](#).
-  - Click to display the Settings dialog where you define acquisition settings such as read mode, read type, wavelengths, etc. See [Acquisition Settings on page 112](#).
-  - Click to display the Template Editor dialog where you assign wells in the plate to Group sections and to create templates. See [Template Editor on page 91](#).
-  - Click to display the Data Reduction dialog where you define data reduction settings for analysis. See [Data Reduction on page 196](#).
-  - Click to display the Display Options dialog where you define how to display results. See [Data Display Options on page 206](#).

Select the **Do Not Open This Helper With New Protocols** check box to prevent the display of the Plate Setup Helper dialog each time you open a new document. The SoftMax Pro Options dialog has a setting to make the dialog display each time if you change your mind. See [SoftMax Pro Options on page 52](#).

Managing Historical Raw Data

Use the Historical Raw Data Overview dialog to manage data that was generated from a previous read and was then overwritten by a subsequent read.



To manage historical raw data:

1. In a Plate section that has had data overwritten, in the section toolbar click .
2. In the **Selected** column, select the check box for each set of historical data to save and/or copy.
 - Click **Save** to save the data to a file on the computer hard drive.
 - Click **Copy** to copy the data to the computer clip board from where you can paste the data into a different software application.

Cuvette Set Sections

Use Cuvette Set sections to define cuvette related instrument settings, data reduction settings, and data display settings from instruments that have a cuvette port. You can create as many Cuvette Set sections as you need and each Cuvette Set section can contain up to 96 cuvettes. Cuvettes are read one at a time.

Cuvette Set sections contain a cuvette data display, instrument Settings Information on the right, and Reduction Settings below the data display. The software numbers the cuvettes in a Cuvette Set section as if the cuvettes were wells in a plate. The first 12 cuvettes are numbers A1 through A12, and then the next cuvette is number B1. This representation matches the software data analysis capabilities. If colors appear in the cuvette data display, the Cuvette Set section contains templates. You assign each group in the template a different color and the Group section table displays the corresponding color. After a read, Read Information displays below the instrument Settings Information on the right.

Managing Cuvette Set Sections

Use the  New Cuvette Set icon in the Navigation Tree to add a Cuvette Set section to an experiment.

To add a Cuvette Set section:

1. In the Navigation Tree, select the section in the experiment above where you want to add a Cuvette Set section.
2. Click  **New Cuvette Set**.

A new blank Cuvette Set section displays below the section you select. Drag it to a different location in the hierarchy, if needed.



Note: When you create a Cuvette Set section with an existing Cuvette Set section active in the workspace, the 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.

Right-click the Cuvette Set section:

- Select **Rename** to change the name of the Cuvette Set section. Use the Rename Section dialog to enter a new name for the section.
- Select **Duplicate** to copy the Cuvette Set section.
- Select **Delete** to delete the Cuvette Set section.

When you select a Cuvette Set section in the Navigation Tree, the Cuvette Set section settings display in the Workspace and the section toolbar contains the following icons:

-  - Click to display the Settings dialog where you configure the acquisition settings such as read mode, read type, wavelengths, etc. Also displays in the Settings Information area to the right of the display. See [Acquisition Settings on page 112](#).
-  - Click to display the Template Editor dialog where you assign cuvettes in the section to groups and to create templates and Group sections. See [Template Editor on page 91](#).
-  - Click to display the Data Reduction dialog where you choose data reduction settings for analysis. Also displays in the Reduction Settings area below the display. See [Data Reduction on page 196](#).
-  - Click to display the Display Options dialog where you choose options for how to display results. See [Data Display Options on page 206](#).
-  - Click to mask cuvettes. See [Masking Wells or Cuvettes on page 211](#).
-  - Click to zoom. See [Zooming Well and Cuvette Display on page 212](#).

The Home tab provides:

- **Controls and Status** - Contains icons to  Read,  Append, and  Reference read the cuvette you select.
- **Cuvette Tools** - Contains the icons described above plus icons to  New and  Delete cuvettes in the Cuvette Set section.
- **Template Tools** - Contains the  Template Editor icon described above.

Managing Cuvettes in a Cuvette Set Section

Each Cuvette Set section can contain up to 96 cuvettes. You can also use the Template Editor dialog to add cuvettes to a Cuvette Set section.

To manage cuvettes:

1. In the Navigation Tree, select a Cuvette Set section.
2. Select the **Home** tab and click  **New** in the Cuvette Tools to add a cuvette to the section.
3. Click to select a cuvette or Ctrl+click to multi-select cuvettes.
4. Click  **Delete** in the Cuvette Tools on the Home tab.

Copying 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 document or between documents. Right-click and select **Duplicate** to copy all the cuvette data from one Cuvette Set section to all the cuvettes in a new Cuvette Set section in the same document.

To copy cuvette data:

1. In the Navigation Tree, select a Cuvette Set section.
2. Click a cuvette or Ctrl+click to multi-select cuvettes.
3. Right-click and select **Copy Cuvette Data** to copy the data to the clipboard.
4. Click the cuvette into which to paste the data. If you copy data from multiple cuvettes, click the first cuvette where you want to paste the data. The software pastes cuvette data into consecutive cuvettes.
Example: If you copy data from three cuvettes and then click cuvette A6 to paste the data, the data pastes into cuvettes A6, A7, and A8.
5. Right-click and select **Paste Cuvette Data**.

The Read Information on the right indicates that the data was pasted and shows the time and date of the paste.

Cuvette Set Section Data Display

Cuvette Set section settings depend on to the actual collection of data. After you define the Cuvette Set section settings and the instrument reads the cuvette data, the Cuvette Set section displays the results of the read. See [Collecting Data on page 111](#).

Group Sections

Use Group sections to group the wells in a plate or the cuvettes in a Cuvette Set section for data analysis. When you click a Plate section or a Cuvette Set section in the Navigation Tree, use the Template Editor dialog to create Group sections. See [Template Editor on page 91](#).

Group sections contain a table and an area to add frames. Frames can contain text or Summary formulas. See [Formulas on page 102](#).

Each column in the table contains a formula to apply to the data in the column. The table displays reduced numbers from the Plate section or Cuvette Set section in the Values column. You can use the columns in the table as plots in the graphs contained in Graph sections. See [Graph Sections on page 90](#).

For a complete guide to formulas and their use, select the **Help** tab and click  **Formula Reference**.

Managing Group Sections

You add Group sections from the Template Editor dialog. You access the Template Editor dialog from a Plate Section or from a Cuvette Set section.

To add a Group section:

1. In the Navigation Tree, select either a Plate section or a Cuvette Set section.
2. Click  in the section toolbar or on the Home tab to display the Template Editor dialog. See [Template Editor on page 91](#).
3. Select the wells or the cuvettes to group.
4. On the right side of the Template Editor, click **Add** in the Groups area to display the Group Settings dialog. See [Group Settings on page 96](#).
5. Complete the fields in the Group Settings dialog and click **OK**.
6. Click **OK** in the Template Editor dialog to display a new Group section in the Navigation Tree.

Drag the section to a different location in the hierarchy, if needed.

Right-click the Group section:

- Select **Rename** to change the name of the Group section. Use the Rename Section dialog to enter a new name for the Group section.
- Select **Duplicate** to copy the Group section.
- Select **Delete** to delete the Group section.

When you select a Group section in the Navigation Tree, the Group section settings display in the Workspace and the section toolbar contains the following icons:

-  - Click to add a column.
-  - Click to delete a column.
-  - Click to display the Formula Editor dialog. See [Formula Editor on page 104](#).
-  - Click to show or hide the formula in a Summary formula frame. See [Summary Formula Tools on page 89](#).
-  - Click to show or hide columns in the table.

The Home tab provides:

- **Column Tools** - Contains icons to manage columns in the table. See below.
- **Summary Tools** - Contains the icons described above plus icons to  Copy,  Paste,  Delete, and  Edit Summary formulas.
- **Formatting Tools** - Contains icons to manage text in the frames and the placement of frames in the section. See [Formatting Text in Section Frames on page 79](#).

Column Tools

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

For example, to subtract the mean values in one group from those in a different group, create a new column (in either group). Two groups named “Group 1” and “Group 2” contain a column entitled “Mean.” Create a column within “Group 1” that subtracts the mean values in “Group 2” from those in “Group 1.” The column formula is:

Mean – (Mean@Group2)

Drag and drop columns in the table to change the column sequence. Drag the column header borders to make columns wider or narrower.

Use the Column Tools on the Home tab to manage the column you select in the Group section. Click a column in the table. Some icons allow you to Ctrl+click to multi-select columns. Each icon has a corresponding right-click menu option.

-  **New** - Click to add a column to the right of the column you select. The Formula Editor dialog displays.



Note: Do not use reserved characters or names of operators, functions, or accessors in the formula name if you plan to reference the formula for this column in a different formula. For example, if you plan to reference the formula in a column of a group table. See [Formula Rules on page 103](#).

-  **Copy** - Click to copy the column you select to the clipboard.
-  **Paste** - Select the column to the left of where you want to paste the column and click  **Paste** to paste the column to the right of the column you select. If the Paste icon is disabled, there is no applicable content on the clipboard. You must first copy content to the clipboard.
-  **Delete** - Click to delete the column you select.
-  **Edit** - Click to edit the column you select. The Formula Editor dialog displays.
-  **Cut** - Click to remove the column you select to the clipboard.
-  **Auto Size** - Click to size the column you select to fit the column content.
-  **Show/Hide** - Click to display the Show/Hide Group Table Columns dialog. Select the check box for each column to display and clear the check box for each column to hide. Click **Show All** to select all check boxes. Click **Hide All** to clear all check boxes.
-  **Hide Replicates** - Click to hide replicates in the table.

Summary Formula Tools

Use formulas to create data analysis templates that automate the analysis and results for experiments. Use the Formula Editor dialog to add and edit Summary formulas in Group sections. Summary formulas typically reduce data to a single value or array.

For a complete guide to formulas and their use, select the **Help** tab and click  **Formula Reference**.

- To create a new Summary formula, click  in the section toolbar or click  **New Summary** on the Home tab.
- To edit a Summary formula, double-click in a frame that contains a formula or click a frame and click  **Edit Summary** on the Home tab.
- To copy a Summary formula to the clipboard, click a frame that contains a formula and click  **Copy** on the Home tab.
- To paste the Summary formula to a new location, click where you want to paste the formula and click  **Paste** on the Home tab.
- To delete a Summary formula, click a frame that contains a formula (Ctrl+click to multi-select) and click  **Delete** on the Home tab.

By default, Summary formulas in the Group section display the result of the formula without displaying the actual formula. You can show or hide the formula with the result.

- Click  in the section toolbar or click  **Show Formulas** on the Home tab to display all formulas in the active Group section.
- Click  in the section toolbar or click  **Hide Formulas** on the Home tab to hide all formulas in the section.

You can copy a formula or column from one area of the program to a different area or from one experiment to a different experiment. After you paste a formula you can edit the copy. You can copy and paste formulas in contiguous column formulas as a unit.

Frames

Group sections can contain frames. Group section frames are identical to Note section frames except you cannot enter images in a Group section. See [Note Sections on page 75](#).

Formatting Tools

The Formatting Tools for a Group section are the same as the Formatting Tools for a Notes section.

Graph Sections

Use Graph sections to plot information as a scatter graph or a bar graph. You can create custom graphs and you can graph the data from Group section tables. You can use columns in a Group section table as the X or Y input for plots on a graph in a Graph section. The Graph section can use data from any Group section in the document and you can create as many Graph sections as you need.

Graph sections contain either a scatter graph or a bar graph. Scatter graphs have a legend area and curve fit results. You can define Graph sections at any point in the data collection process but graphs tend to be more relevant for data analysis.

Managing Graph Sections

Use the  New Graph icon in the Navigation Tree to add a Graph section to an experiment.

To add a Graph section:

1. In the Navigation Tree, select the section in the experiment above where you want to add a Graph section.
2. Click  **New Graph**.
3. Use the Plot Editor dialog to create the graph. See [Graphing Data on page 214](#).

After you click OK in the Plot Editor dialog, a new Graph section displays below the section you select in the Navigation Tree. Drag it to a different location in the hierarchy, if needed.

Right-click the Graph section:

- Select **Rename** to change the name of the Graph section.
- Select **Duplicate** to copy the Graph section.
- Select **Delete** to delete the Graph section.

When you select a Graph section in the Navigation Tree, the Graph section settings display in the Workspace and the section toolbar contains the following icons:

-  - Click to display the Plot Editor dialog to edit the graph.
-  - Click to display the Graph Appearance dialog to label the axes and other display options. See [Graph Appearance on page 222](#).
-  - Click to add annotation text to the graph. See [Annotation Editor on page 220](#).
- **Category** - Click the drop-down and select a category (scatter graphs only).
- **Fit** - Click the drop-down and select a fit (scatter graphs only).
-  - Click to display the Curve Fit Settings dialog to define curve fits. See [Curve Fit Settings on page 223](#).
- Right-click a data point in a scatter graph and select **Highlight Data Source** to display the data source in the corresponding Template section. See [Templates on page 91](#).

The Home tab provides:

- **Graph Types** - Contains icons to switch between a  Scatter graph and a  Bar graph.
- **Graph Tools** - Contains the icons described above plus an  Export Graph icon to export graph data to an image format file.

Templates

Templates provide the link between raw data and analysis groups. A template uses a map of the wells in the plate you assign to a Plate section or the cuvettes in a Cuvette Set section to allow you to identify the wells that are blanks, replicates, samples, and groups. Groups appear as Group sections in the Navigation Tree. Template tools provide access to the Template Editor dialog where you create groups and templates. See [Template Editor on page 91](#).

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.

You apply each sample to a column or some other subset of wells on a plate or to one or more cuvettes. Each well you designate to be part of a group has a group name, a sample name (or replicate ID), a sample descriptor (optional), and a column format for the formula calculations and data reports you define in the corresponding Group section. Samples and groups can exist across multiple Plate sections and Cuvette Set sections.

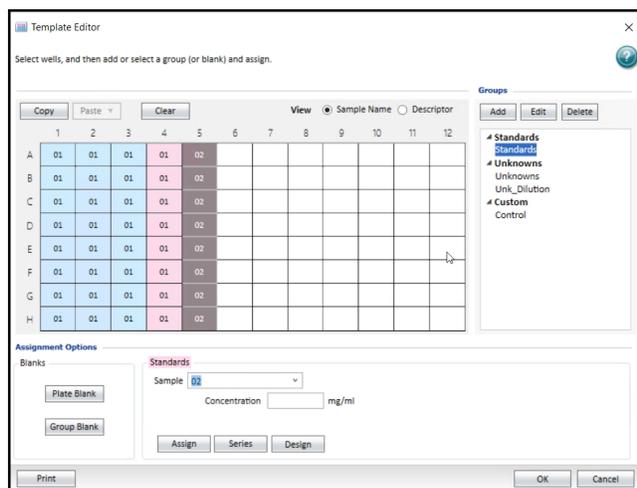
Template Editor

The Template Editor displays a representation of the plate type or the set of cuvettes as a grid. Use the grid to designate the location of blanks, standards, controls, unknowns, empty wells, or to assign wells/cuvettes to groups that become Group sections in an experiment.

The Template Editor for a Cuvette Set section displays a grid with 96 cells in the column and row format of a 96-well plate. The software numbers the cuvettes in the template as if the cuvettes were wells in a plate. This representation corresponds with the data analysis capabilities of the software. When you add a group to a Cuvette Set section template, you add cuvettes to the Cuvette Set section.

To import a template file or to export a template to a document for use in future experiments, see [Template Tools on page 93](#).

Each Plate section and Cuvette Set section can have one template. If the section contains a template, the Template Editor displays the existing template when you click .



To manage templates:

1. In the Navigation Tree, select a Plate section or Cuvette Set section.
2. Click  in the section toolbar, on the Home tab, or in the Plate Setup Helper dialog to display the Template Editor dialog.
3. Select a **View** option:
 - Select **Sample Name** to display the sample name in the wells in the grid.
 - Select **Descriptor** to display the descriptor information only.
4. Drag the cursor across the grid to select wells. When you select multiple wells, the selection must be a rectangular group of contiguous wells.
5. Click one of the following:
 - In the Assignment Options area, click **Plate Blank** (Plate sections) or **Blank** (Cuvette Set sections) to specify wells/cuvettes as blanks. The software does not add a group in the Groups area. For kinetic and spectrum data, the software averages and subtracts the blank value from each point in the read.
 - In the Groups area, click **Add** to create a Group section. The Group Settings dialog displays. See [Group Settings on page 96](#).
6. After you complete the fields in the Group Settings dialog, use the Template Editor to do the following for a group.
 - Select a group and click **Edit** to display the Group Settings dialog where you edit the group settings.
 - Select a group and click **Delete** to delete the group from the template and to delete the Group section from the Navigation Tree.
 - Select a group and click **Plate Blank** or click **Group Blank**.
 - To specify wells as group blank, select the wells, click **Group Blank**, and then click **Assign**. Select the wells or cuvettes to use for the group blank. These should be wells or cuvettes you want to assign to a group, but not the “Blank” group. Select a group from the **Group Types** list. Click **Group Blank** and then click **Assign**.
 - Select a group, select additional wells, and then click **Assign** to add the wells to the group.
 - Select a group, select wells, and then click **Series** to display the Series dialog where you define a series. See [Creating a Series on page 99](#).
 - Select a group (not a control group), select wells, and then click **Design** to display the Design dialog where you define sample concentrations based on the design of experiment theory. See [Design of Experiment on page 101](#).
 - Select wells in one or more groups and click **Copy** to copy the well assignments to the clipboard. Use this to duplicate well assignments within the current template. You can also paste the same sample names but create new groups. You can copy only contiguous wells in the template. Then click in the well at the corner of the paste area or highlight the paste area and click **Paste** to display a menu. To copy and paste an entire template to a different experiment section see [Template Tools on page 93](#).
 - Select **Paste** to add the new wells to the current group.
 - Select **Paste With New Group** to have the wells create a new group in the Groups list.
 - Select wells that are in a group and click **Clear** to remove the wells from the group. If you clear all the wells you assign to a group, the group continues to display in the Groups area and the Group section continues to display in the Navigation Tree.
7. Select a group then click the **Sample** drop-down and select a sample. In the **Concentration** field, enter the concentration.

8. Click **Print** to print the Template Editor dialog. You must click **OK** to save the template before you can print.
9. Click **OK**.

Blanks

Use the Template Editor to configure plate blanks (Plate sections), blanks (Cuvette Set sections), and group blanks. You can use blanks in combination and have cumulative effects.

Use plate blanks and blanks when you prepare all samples on the plate in the same way. You can turn off plate blank subtraction in the Data Reduction dialog to review data with or without plate blank subtraction. See [Data Reduction on page 196](#). Use plate blanks to correct reads when you prepare all samples on a plate in the same buffer or matrix, and therefore can use the same correction value. The software subtracts plate blanks from the raw data or after pathlength correction if you use PathCheck® technology. For kinetic and spectrum data, the software averages and subtracts the blank value from each point in the read. For Cuvettes Sets blanks, the software subtracts the average value of all cuvettes in a blank group from individual cuvette reads in the same Cuvette Set section. Template blanks are subtracted after the cuvette reference read is subtracted.

In a plate blank group the software subtracts the average value of all the wells in the blank group from each individual well value in the plate. If you have an instrument that supports PathCheck technology, the software subtracts the Plate Blank after pathlength correction.

Group Blanks (Plates and Cuvettes)

Use group blanks to correct reads when you prepare samples on a plate 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 assign blank wells within a group, other than the “Blank” group.

The software subtracts the group blank when you assign a group blank 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 [Masking Wells or Cuvettes on page 211](#).

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.

Template Tools

For a Plate section, use the Home tab Template Tools to copy a template and then paste the template to another Plate section in the document or even to a comparable section in a different document. Both Plate sections must have the same plate type. If the destination Plate section contains a template, Paste overwrites the existing template with the template copy.

To copy a template:

-  **Copy** - Select the source Plate section in the Navigation Tree and click  **Copy** to copy the template from the active Plate section to the clipboard.
-  **Paste** - Select the destination Plate section in the Navigation Tree and click  **Paste**.
 - Select **Paste** to paste only the template settings without the groups.
 - Select **Paste With New Groups** to paste the template and its groups.

When you copy a template from one experiment and paste it into a different experiment, the group names and sample names of the destination template are identical to those of the source template. The full name is different because the templates are in different experiments.

For example, group@experiment#2 instead of group@experiment#1.

- Changes made to the template in the destination Plate section have no effect on the source Plate section.
- Data from the two Plate sections are not analyzed together.

When you copy a template from a Plate section in an experiment to a different Plate section in the same experiment, the destination template is an extension of the source template. The software considers all wells in the destination section to be replicates of the wells in the source section, with the same group and sample names.

- Changes you make to the template in the destination Plate section are also made to the template in the source Plate section.
- The software analyzes data from the two Plate sections together.

Import and Export

On the Home tab, use the Template Tools to import template information into a Plate section from a tab-delimited ASCII text file in Unicode (UTF-8) format. You can prepare template files outside the SoftMax Pro Software and then import the file when you are ready to run samples.

 **Tip:** To determine the correct format to import a template, export a template from the SoftMax Pro Software and examine the contents of the export file.

To import a template:

1. In the Navigation Tree, select the destination Plate section.
2. Select the **Home** tab and click  **Import** to display the Open dialog.
3. Navigate to and select the <template>.txt file to import.
4. Click **Open**.

To export a template:

1. In the Navigation Tree, select the source Plate section.
2. Select the **Home** tab and click  **Export** to display the Save As dialog.
3. Navigate to the location to save the template file.
4. In the **File Name** field, enter a name for the file.
5. Click the **Save As Type** drop-down and select **Text files (*.txt)** or **Excel files (*.xls)**. Text files save in tab delimited format.
6. Click **Save**.

Template File Format

When you save template data as a tab delimited ASCII text file in Unicode (UTF-8) format, the template file consists of ten columns of data separated by tabs. Each line of the file provides information for a single well in the plate.

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 the export file contains a line for each well in the plate. If no template information exists for a well in a plate, the corresponding line in the file is left blank.

The order of the import template information must match the export order. Blank lines in the file indicate a well with no template setting.

ASCII Template File Columns and Descriptions

Col	Column Entry	Description
A	Well Location	In this column, the wells do not need to be in order.
B	Group Name	Assign in the Group Settings dialog. If this field is missing, the rest of the line is ignored. You can enter a text string in this field.
C	Group Type	Assign in the Template Editor dialog. The software supports three text strings: Standards, Unknowns, Custom. Blank groups have an Empty column format. If this field is 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 in the Template Editor. If this field is blank, no sample name is assigned to the well, although the sample name is included in the group.
E	Descriptor1 Name	Assign in the Group Settings dialog.
F	Descriptor1 Value	Assign in the Group Settings dialog. You can enter a numeric value in this field or it can be blank.
G	Descriptor1 Units	Assign in the Group Settings dialog. Supported strings are unit/ml, mg/ml, $\mu\text{g/ml}^*$, ng/ml, mg, ng, and ml. If this column is blank, unit/ml is assigned.
H	Descriptor2 Name	Assign in the Group Settings dialog.
I	Descriptor2 Value	Assign in the Group Settings dialog. You can enter a numeric value in this field or it can be blank.
J	Descriptor2 Units	Assign in the Group Settings dialog. Supported strings are unit/ml, mg/ml, $\mu\text{g/ml}^*$, ng/ml, mg, ng, and ml. If this column is blank, unit/ml is assigned.

* The μ symbol can be imported only if the ASCII text file is saved in Unicode (UTF-8) format.

You can open a tab delimited ASCII file with a spreadsheet program, such as Microsoft Excel.



Note: Do not edit the file in Excel because the SoftMax Pro Software import only supports the format for files you save as an ASCII text file in Unicode (UTF-8) format.

Group Settings

Use the Group Settings dialog to define the name for a group of related samples, a descriptor related to the samples, and the initial column format for the data the software calculates and reports in a Group section.

Use the Template Editor to assign wells to groups. Some protocols automatically create groups such as Standard or Unknown. When you create a group in the Template Editor dialog, (even if you do not select wells in the template), the software adds a Group section in the Navigation Tree for the experiment. To delete a group, you must delete the Group section. When you clear a group from the Template Editor, you only remove the assignment of wells from that group name. Clearing a group does not delete the group.

Define a group of related samples.

Group Name

Group01 Set Color...

Group Table Layout

Standards Unknowns Custom

Group Table Column Preview

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%

Add Columns

Sample Descriptor 1 Sample Descriptor 2

Column Name: Concentration Column Name:

Units: None Units: None

OK Cancel

To define group settings:

1. In the Navigation Tree, select a Plate section or Cuvette Set section.
2. Click  in the section toolbar, on the Home tab, or in the Plate Setup Helper dialog to display the Template Editor dialog.
3. Drag the cursor to select one or more wells. In the Groups area, click **Add** or select a group and click **Edit** to display the Group Setting dialog.
4. In the **Group Name** field, enter the name to assign to the group.
5. Click **Set Color** and select the color to display for the wells in the group.

6. Select a **Group Table Layout** option. The Group Table Column Preview shows how the columns will display in the table in the Group section. The Columns and Formulas table on the following page summarizes the formulas associated with each column.
 - Select **Standards** to create a group with the following group table columns: Sample, Well, Concentration, Values, Mean Value, Standard Deviation, and CV%. The Concentration column becomes the **Sample Descriptor1** by default under the assumption that the Group section will contain concentration information. Use this column for numerical data such as dilution factor or fraction number.
 - Select **Unknowns** to create a group with the following group table columns: Sample, Well, Values, Result, Mean Result, Standard Deviation, and CV%.



Note: The Unknowns group does not have a sample descriptor by default. Select the respective check boxes to add one or two sample descriptors and then enter the descriptive information.

- Select **Custom** to create a group with the following group table columns: Sample, Well, Values, Mean Value, Standard Deviation, and CV%.



Note: The Custom group does not have a sample descriptor by default. You can select the respective check boxes to add one or two sample descriptors and then enter the descriptive information.

7. In the Add Columns area, select the **Sample Descriptor1** check box to use the descriptor. When you select the Standards Group Table Layout the software selects this by default and sets the column Name to Concentration.
8. In the **Column Name** field, enter the name of the sample descriptor.



Note: Do not use reserved characters or names of operators, functions, or accessors in the formula name if you plan to reference the name of this column in a formula. See [Formula Rules on page 103](#).

9. Click the **Units** drop-down and select the unit of measure.
10. Select the **Sample Descriptor2** check box to add a second sample descriptor column to the table.



Note: The Sample Descriptor you assign in the Group settings dialog yields group table columns that you cannot edit. To create editable sample descriptor columns, add new columns and enter a formula such as: !Sampledescriptor, !factor, !concentration.

11. Click **OK** to close the Group Settings dialog. The software places the wells you select in the new group.
12. Click **Series** to display the Series dialog where you define a series for the wells you select.
13. Click **OK** to close the Template Editor and apply the template.

Group Table Layout - Default Columns

The Group Table Layout you select defines which columns are in the Group section table. The following lists the default column names and each column's formula for the Standard, Unknown, or Custom Group Table Layouts.

Columns and Formulas

Column Name	Formula	Standard	Unknown	Custom
Sample	!SampleNames	✓	✓	✓
Wells	!WellIDs	✓	✓	✓
Concentration	!Concentration	✓		
Values	!WellValues	✓	✓	✓
MeanValue	Average(Values)	✓		✓
Std.Dev.	Stdev(!WellValues)	✓		✓
CV%	Cv(!WellValues)	✓		✓
Result	InterpX(STD@ StandardCurve, Values)		✓	
MeanResult	Average(Result)		✓	
Std.Dev.	Stdev(Result)		✓	
CV%	Cv(Result)		✓	

Creating a Series

Use the Series dialog to define several samples as a series. Use a series to enter incremental sample descriptors (e.g., dilutions or concentrations) and sample names to a template when the increment is expressed as a mathematical operation. When you work with groups of wells in the template, the standard value or dilution factor increases or decreases in specific steps. The series name can also increment.

You define a series in one direction (starting from left, right, top, or bottom) and you must configure samples sequentially in either ascending or descending order.

To create a series:

1. In the Navigation Tree, select a Plate section or Cuvette Set section.
2. Click  in the section toolbar, on the Home tab, or in the Plate Setup Helper dialog to display the Template Editor dialog.
3. In the Template Editor dialog, click and drag the mouse to select wells to be part of the series.
4. In the Groups area, select a group name.
5. Click **Series** to display the Series dialog.
6. In the Series Layout area select a **Start From** option for how the series fills the wells.

7. In the Patterns of Replicates area, enter the number of replicates in the **X-Direction** field and enter the number of replicates in the **Y-Direction** field.

The maximum number of replicates depends on the well selection and the fill direction. For example, if you select a block of wells that is 4 wells wide by 8 wells high and you select to fill down from the top, the maximum number of replicates is four. With the same block of wells, if you select to fill from the left, the number of replicates is eight. If you choose a number of replicates that does not divide evenly into number of wells and the fill direction you select, the software labels the wells that cannot contain replicates as individual wells in the series. To create a series that includes all the wells in the plate, select all the wells, select the fill direction, and then set the replicates to 1.

8. Select the **Reverse Series Order** check box to reverse the order of the series.
Clear the **Reverse Series Order** to not reverse the order of the series.
9. In the Sample Information area, in the **Starting Sample Name** field, enter the name of the first sample in the series. Subsequent replicates use this name as a base and either increment the number within the name or append a number to it.
10. Select the **Concentration** check box to increment the sample name and the concentration. The following two fields are available:
Clear the **Concentration** check box to increment only the names of a series of samples without incrementing the concentration or dilution.
 - a. In the **Starting Value** field, enter a value for the descriptor if you define a sample descriptor for the group.
 - b. Click the **Step By** drop-down and select the operator for the series (+, -, *, or /) and then enter a value for the increment. For example, enter 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.



Note: Click the **Step By** drop-down, select the multiplication operator (*), and then enter a value of 1 for the increment to increment the names of a series of samples with a constant concentration or dilution.

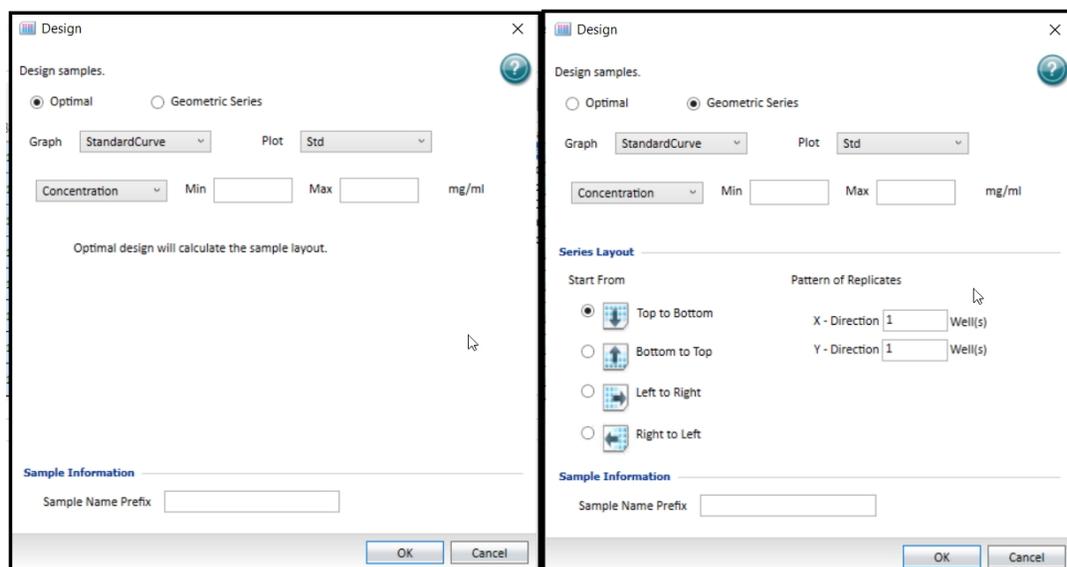
11. Click **OK**.

Design of Experiment

Use the Design dialog to assign sample concentrations based on design of experiment theory. You specify whether the design is optimal or an optimized geometric (dilution) series. You specify the graph and plot (chosen from the current experiment section) to use as the basis for the design. Configure the calculation on the Design tab in the Curve Fit Settings dialog for the graph you select. See [Curve Fit Settings on page 223](#).

The Design dialog allows you to specify the minimum and maximum sample concentration, and a prefix to use for the sample names.

If you define the design as an optimized series, choose the sample layout for a series assignment.



To create a design:

1. In the Navigation Tree, select a Plate section or Cuvette Set section.
2. Click  in the section toolbar, on the Home tab, or in the Plate Setup Helper dialog to display the Template Editor dialog.
3. In the Template Editor dialog, click and drag the mouse to select wells to be part of the design.
4. In the Groups area, select a group name.
5. Click **Design** to display the Design dialog.
6. Select a **Design Sample** option:
 - Select **Optimal** to have the software calculate the optimal design that you cannot further configure.
 - Select **Geometric Series** to optimize a geometric series such as a dilution series.
7. Click the **Graph** drop-down and select a sample descriptor.
8. Click the **Plot** drop-down and select a plot option.
9. Click the drop-down and select an option.
 - In the **Min** field, enter the minimum value.
 - In the **Max** field, enter the maximum value.

10. If you select the Geometric Series Design Sample option, in the Series Layout area:
 - a. Select a **Start From** option for how the series fills the wells.
 - b. In the Patterns of Replicates area, enter the number of replicates in the **X-Direction** field and enter the number of replicates in the **Y-Direction** field.

The maximum number of replicates depends on the well selection and the fill direction. For example, if you select a block of wells that is 4 wells wide by 8 wells high and you select to fill down from the top, the maximum number of replicates is four. With the same block of wells, if you select to fill from the left, the number of replicates is eight. If you choose a number of replicates that does not divide evenly into number of wells and the fill direction you select, the software labels the wells that cannot contain replicates as individual wells in the series. To create a series that includes all the wells in the plate, select all the wells, select the fill direction, and then set the replicates to 1.
11. In the **Sample Name Prefix** field, enter the name of the first sample in the series. Subsequent replicates use this name as a base and either increment the number within the name or append a number to it.
12. Click **OK**.

Formulas

Formulas are data analysis tools that automate the analysis and results reporting for plate reads. Formulas are mathematical functions you create with operators and accessors to convert and analyze raw data for presentation in the appropriate output format.

The software includes many formulas to expedite data analysis. The formulas in the software use a hierarchical naming structure, much like the directories and sub directories in computer file systems. All objects (for example, columns, Summaries, wells, graphs, plots) in the SoftMax Pro Software have names. Use these names to refer to the objects when you write formulas.

The *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide* identifies and enumerates the details about the operators, functions, and accessors in formulas. Select

the **Help** tab and click  **Formula Reference**.

- Use Summary formulas in Group sections and Note sections to reduce data to a single value or array.
- Use Reduction formulas in Plate sections and Cuvette Set sections to apply a formula to the entire section.
- Use Column formulas in Group sections to apply a formula to each column in the section.
- You can assign formulas to the X and Y axes in a Graph section.

Operators

Use operators to combine other formula building blocks together. Operators let you combine and compare numbers, functions, accessors, and text strings in a variety of ways within formulas. Most operators are mathematical symbols, e.g. +, -, *, /, <, > etc. Boolean operators have text, e.g. If, And, Or, Not.

Functions

In contrast to operators, functions have names and accept up to four parameters. Functions do operations such as standard deviation or slope. You can input zero to four parameters into functions. You can use lists of numbers, arrays of numbers, and lists of text strings as input parameters, and the function can return either lists or arrays of numbers or text. Functions return numbers, text, or Booleans.

The general format for a function is:

FunctionName(Parameter1, Parameter2, Parameter3, Parameter4)

If a function has more than one parameter, the order in which you list the parameters is extremely important. If you enter the parameters in the incorrect order, the function returns erroneous results.

Accessors

Accessors are special functions that provide access to other specific information in the software. For example, the number of Vmax Points used to calculate the Vmax Rate. Accessors are frequently used by themselves as column formulas. Use accessors as parameters within several functions, most notably kinetic and spectrum reduction functions. Combine accessors with functions and mathematical operators to create complex formulas.

Unlike functions, accessors do not have related parameters. Accessors are always preceded by an exclamation point (!, also known as a “bang”).

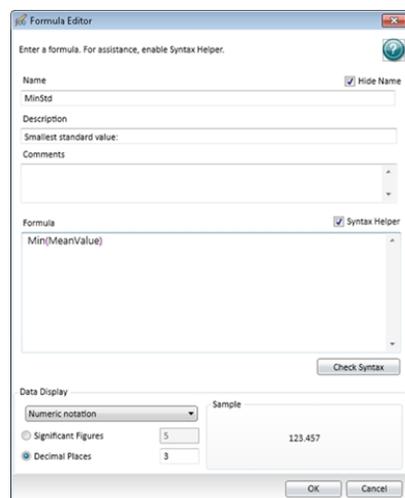
Formula Rules

The following is a very high level summary of formula rules:

- Formulas are not case sensitive.
- If an object’s name contains a space, starts with a number, or includes one or more of the following special characters: # \$ % / * ?, you must enclose the name in single quotation marks. For example, ‘OD Values’. Do not use ^ @ ~ or & in object names because these characters have special meanings in the software.
- When you create a formula that references columns in more than one Group section, the Group sections must have the same number of rows (samples).
- Column formulas must evaluate either to text, to numbers, or to a Boolean (for example, to True or False) because a column cannot contain text (“Pass”) and numeric (“1.002”) results.
- When a formula includes a text result that you want to display, you must enclose the result in double quotation marks, for example, “Out of Range”.

Formula Editor

Use the Formula Editor dialog to create formulas that convert and analyze raw data for presentation in the appropriate output format. Use formulas to create powerful data analysis templates that can automate the analysis and results reporting for experiments.



The Formula Editor dialog varies based on how you access the dialog.

To create formulas:

- There are several ways to access the Formula Editor.
 - In the Navigation Tree, select a Note Section or a Group section. In the section toolbar click , on the Home tab click  **New Summary**, or select a formula in a section and click **Edit Summary**. This is a Summary formula.
 - In the Navigation Tree, select a Plate section or a Cuvette Set section. Click  in the section toolbar, the Reduction Settings area, or on the Home tab. In the Data Reduction dialog select **Custom** from a drop-down and click . This is a Reduction formula. See [Data Reduction on page 196](#).
 - In the Navigation Tree, select a Group section. In the section toolbar click , double-click a column header, or on the Home tab click  **New** or click  **Edit** in the Column Tools. This is a Column formula.
 - In the Navigation Tree, select a Graph section or in the Navigation Tree click  **New Graph**. In the section toolbar click  or on the Home tab click  **Plot Editor**. In the Plot Editor dialog in the Data Input area, click the **X-Axis** or **Y-Axis** drop-down and select **Custom** and then click .



Note: The following steps describe the flow for Summary formulas. The Formula Editor has fewer fields for other formula types but the workflow for the Formula field is the same for all formulas.

- In the **Name** field, enter the formula name.

3. Select the **Hide Name** check box to not display the formula name in the Note/Group section.
Clear the **Hide Name** check box to display the name and/or description in the section.



Note: If you plan to reference this formula in a different formula, for example referencing the formula in a column of a group table, do not use reserved characters or names of operators, functions, or accessors in the formula name. See [Formula Rules on page 103](#).

4. In the **Description** field, enter a description of the formula. This displays in the section and can be left blank.
 5. In the **Comments** field, enter a comment to annotate the formula. This displays only in the Formula Editor dialog.
 6. Select the **Syntax Helper** check box to display the Syntax Helper formula completion tool. The Syntax Helper displays the formulas that are available and the parameters each formula expects to receive.
Clear the **Syntax Helper** check box to not use the Syntax Helper feature.
-

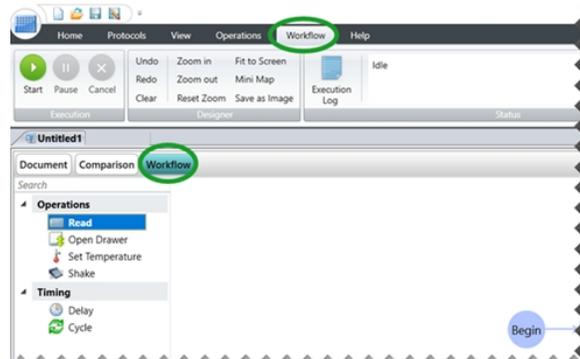


Tip: When you start to enter a formula, a list displays below the text as you type. The Syntax Helper analyzes text as you type and compares it to formulas that are available for use in the software.

7. In the **Formula** field, enter the formula.
8. Click **Check Syntax** to check the validity of the formula. A message displays to help you troubleshoot the formula syntax.
9. Click the **Data Display** drop-down.
 - Select **Numeric Notation** to display the full numeric value in the result.
 - Select **Scientific Notation** to display a concatenated numeric value in the results.
10. A preview of the Data Display displays in the Sample area on the right when you select one of the following:
 - Select **Significant Figures** and enter the number of figures to display.
 - Select **Decimal Places** and enter the number of decimal places to display.
11. Click **OK**.

Defining and Running Workflows

Use workflows to define complex reads such as multitask kinetic reads that last hours or days. Use the Workflow view of the Workspace to drag-and-drop tasks into a timeline. The Workflow tab in the Ribbon contains the icons to execute and monitor the workflow.

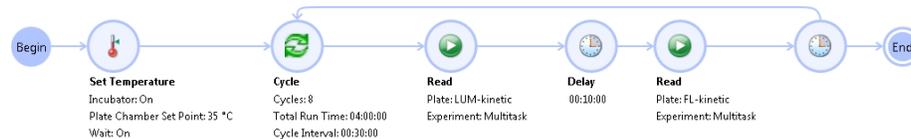


Pause a workflow to work with other features in the software, such as define protocols or run short reads, while the software waits to do the next non-timing task in the workflow. When you pause a workflow, all delays and cycle timings continue to run. When the workflow reaches a non-timing task, such as a read, the task starts after you resume the workflow. If you resume the workflow after the scheduled time for a task to start, then the task starts when you resume the workflow and all subsequent tasks are rescheduled to a later time.

For example, if you set a delay before a read to 30 minutes and you resume the workflow after 40 minutes, then the read starts when you resume the workflow and the software reschedules all subsequent tasks to start 10 minutes later than their original times.

To define and run a workflow:

1. Open a document in the Workspace.
2. Select the **Document** view of the Workspace and create the experiments with the applicable sections. You can define the acquisition settings for each Plate section from the Workflow view.
3. Select the **Workflow** view of the Workspace. The Ribbon automatically displays the Workflow tab.
4. Drag tasks from the Navigation Tree on the left into the timeline on the right.



- To repeat kinetic tasks, place the tasks to repeat in a Cycle task.
- To gather data over long periods of time, use the kinetic read type and then define the Timing settings to acquire a single point with each read. See [Timing Settings on page 125](#).



Note: Place only kinetic reads within a cycle. If you place other read types in a cycle, the software overwrites 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 data you acquire.

5. Click  to edit the task settings. See [Workflow Task Settings on page 108](#).

6. Click  to remove a task from the timeline.
7. In the Ribbon, select the **Workflow** tab and use the Designer buttons to manage the creation of the workflow.
 - Click **Undo** to undo the most recent change you made to the timeline.
 - Click **Redo** to redo an action.
 - Click **Clear** to clear the timeline and start over.
 - Click **Zoom In** to zoom in to the Workflow view similar to using the slider on the bottom right.
 - Click **Zoom Out** to zoom out of the Workflow view.
 - Click **Reset Zoom** to set the zoom to the default setting.
 - Click **Fit To Screen** to set the view so that the workflow fits on the screen without scroll bars.
 - Click **Mini Map** to display the Overview dialog that helps you navigate a long timeline.
 - Click **Save As Image** to export an image of the timeline to an image file with the .jpg, .png, .gif, or .xps format.
8. Save the document before you start a read.
9. Insert the plate in the instrument.
10. In the Ribbon, select the **Workflow** tab and use the Execution icons to execute the workflow.
 - Click  **Start** to run the workflow.
 - Click  **Pause** to temporarily stop the workflow.
 - Click  **Resume** to restart the paused workflow.
 - Click  **Cancel** to cancel the workflow.
11. Click  **Execution Log** to view the status of the tasks the software executes including the time, step name, and related messages.

Workflow Task Settings

Each task in the workflow allows you to define settings that are applicable for that task.

1. Select a task in the timeline.
2. Click  to edit the settings for that task.

Read Task Settings

Use the read task settings to select the experiment and Plate section you define from the Document view of the Workspace.



To define read task settings:

1. Click the **Experiment** drop-down and select the experiment that contains the Plate section to read.
2. Click the **Plate Section** drop-down and select a Plate section.
3. The read uses the acquisition settings you define for the Plate section. To edit the acquisition settings, click . See [Acquisition Settings on page 112](#).

 **Tip:** If you place a kinetic read task in a Cycle for the FilterMax F3, FilterMax F5, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm clear the **Show Pre-Read Optimization Options** check box. Pre-Read optimization requires user interaction. If you select this check box, the software waits for user interaction before each read. See [Show Pre-Read Optimization Options on page 145](#).

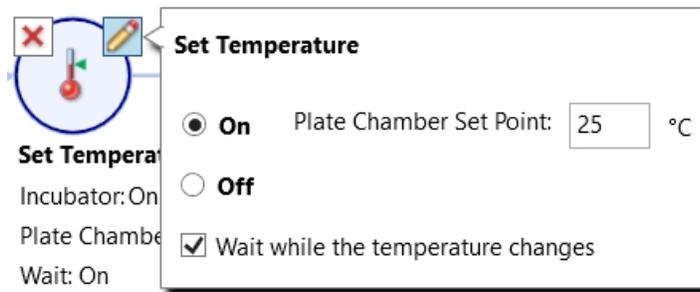
Open Drawer Task Settings



To define drawer task settings:

1. Select the **Wait While Drawer is Open** check box to pause the workflow until the drawer is closed.
Clear the **Wait While Drawer is Open** check box to have the workflow continue to the next task with the drawer open.
2. In the **Message to Show** field, enter the message to display when the drawer opens or enter instructions for the operator.

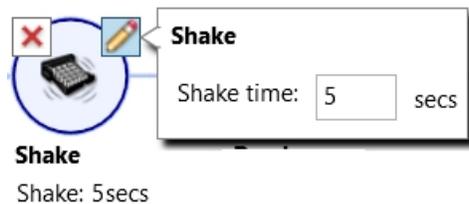
Set Temperature Task Settings



To define set temperature settings:

- Select **On** to turn on the read chamber incubator.
 - In the **Plate Chamber Set Point** field, enter the temperature.
 - Select the **Wait While the Temperature Changes** check box to pause the workflow until the read chamber reaches the set point.
Clear the **Wait While the Temperature Changes** check box to have the workflow continue to the next task before the chamber reaches the set point.
- Select **Off** to turn off temperature control.

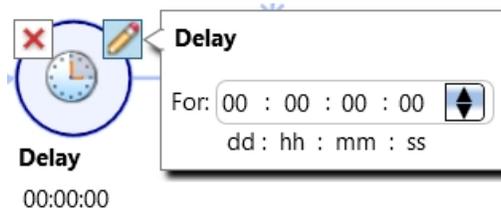
Shake Task Settings



To define shake task settings:

- In the **Shake time** field, enter the number of seconds to shake the plate.

Delay Task Settings



To define delay task settings:

- Enter values for the delay in Days:Hours:Minutes:Seconds, or click a value and use the arrows on the right.

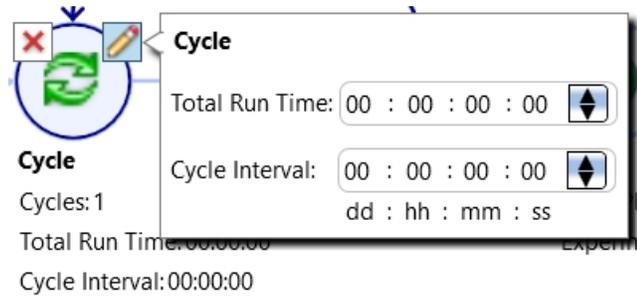


Note: The Delay task continues even when you pause the workflow.

Cycle Task Settings

The software calculates the number of cycles based on how many Cycle Intervals can be completed within the Total Run Time.

When you define the timing for a cycle, make sure that the tasks in the cycle finish within the time you allot. The workflow editor does not calculate the time it takes to do a read. If the read takes longer than the time you allot for the cycle, then the next cycle begins immediately after the cycle tasks complete.



To define cycle task settings:

1. To include a task in the cycle, drag the task between the start and end points of the cycle task.
2. In the **Total Run Time** field, enter values in Days:Hours:Minutes:Seconds to define the total time for all the cycles to complete.
3. In the **Cycle Interval** field, enter values in Days:Hours:Minutes:Seconds to define the time from the start of one cycle to the start of the next cycle.



Note: The cycle interval continues even when you pause the workflow.

Data collection depends upon the settings you define for each Plate section and each Cuvette Set section in the document.



Note: When you do not physically connect the computer to an instrument, you can select Offline or Simulator On to create documents for later use. Document settings are specific to the instrument that you select. If you change instruments, some settings may be reset to the default protocol settings.

Instrument Settings

Use the Settings dialog to configure the settings for the instrument to use to read each Plate section and Cuvette Set section. Settings are dependent upon the instrument, read type, and read mode.

To access the Settings dialog:

1. In the Navigation Tree, select a Plate section or Cuvette Set section.
2. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.

Standard View

Use the Settings dialog, for all instruments, to select a read mode and a read type. The settings that determine how the instrument is to read the plate or cuvette are then grouped into categories along the left side of the dialog. This is known as the Standard view of the Settings dialog. See [Acquisition Settings on page 112](#).

You must use the Standard view to define the settings for Imaging reads and ScanLater Western Blot reads.



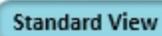
Note: If you define an Imaging read or a ScanLater Western Blot read in the Standard view and then switch to the Acquisition view, your settings are not saved.

Acquisition View

The Settings dialog for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 provides an Acquisition view to configure plate reads.

The Acquisition view uses an acquisition plan with tools that allow you to define settings in a workflow design when you need to do multi-step plate reads. See [Acquisition View and Reads With Injectors on page 156](#).

To switch between the Standard view and the Acquisition view:

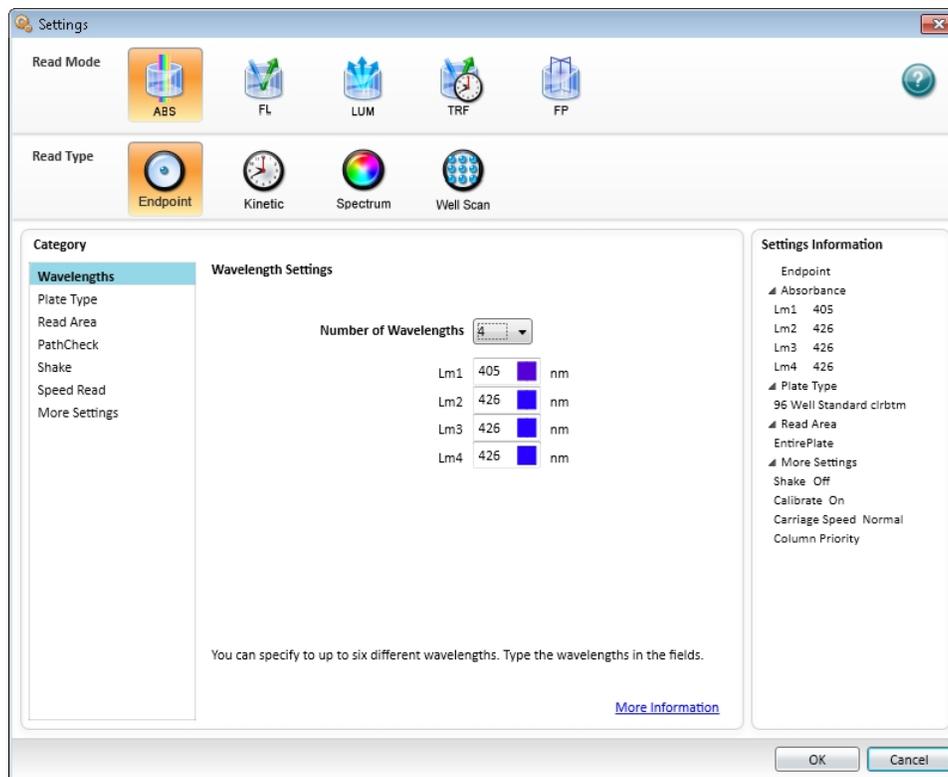
- Click   in the lower-left corner in the Settings dialog.



Note: You must use the Acquisition view to define reads with injection. If you define a read with injection and then switch to the Standard view, your settings are not saved.

Acquisition Settings

Use the Settings dialog to configure the parameters for a Plate section or Cuvette Set section. The settings are dependent upon the instrument, read mode, and read type.



Each Plate section or Cuvette Set section in a document must use the same instrument but can have different parameters for the acquisition settings.

To define acquisition settings:

1. In the Navigation Tree, select a Plate section or Cuvette Set section.
2. Click  in the section toolbar, in the Settings Information area, or on the Home tab to display the Settings dialog.
3. For the SpectraMax i3, SpectraMax i3x, and SpectraMax Paradigm select the detection cartridge or optical configuration to use. This determines the available read modes. See [Selecting a Detection Cartridge or Optical Configuration on page 114](#).

4. For all instruments, select a read mode. This determines the available read types.
 - [Absorbance Read Mode](#), see page 280
 - [AlphaScreen Read Mode](#), see page 285
 - [Fluorescence Intensity Read Mode](#), see page 287 Fluorescence Intensity Dual Wavelength is available for the SpectraMax Paradigm.
 - [Fluorescence Polarization Read Mode](#), see page 292
 - [FRET Read Mode](#), see page 295
 - [Imaging Read Mode](#), see page 298
 - [Luminescence Read Mode](#), see page 301 Luminescence Dual Wavelength is available for the FilterMax F3, FilterMax F5, SpectraMax i3x, SpectraMax iD5, SpectraMax L, and SpectraMax Paradigm.
 - [Time-Resolved Fluorescence Read Mode](#), see page 304 Time Resolved Fluorescence Dual Wavelength is available for the SpectraMax i3x and SpectraMax Paradigm.
 - [TR-FRET Read Mode](#), see page 308
 - [Western Blot Read Mode](#) on page 311
5. Select a read type. This determines which setting categories appear in the Category list.
 - [Dual Read Type](#), see page 277
 - [Endpoint Read Type](#), see page 277
 - [Fast Kinetic Read Type](#), see page 277
 - [Flex Read Type](#), see page 278
 - [Kinetic Read Type](#), see page 278
 - [Membrane Read Type](#), see page 278
 - [Spectrum Read Type](#), see page 279
 - [Well Scan Read Type](#), see page 279
6. Select each category in the list on the left to display the corresponding settings.
 - [Wavelength Settings](#), see page 115
 - [Plate Type Settings](#), see page 118
 - [Read Area Settings](#), see page 121
 - [PathCheck Settings](#), see page 122
 - [TRF Settings](#), see page 123
 - [PMT and Optics Settings](#) on page 123
 - [Timing Settings](#) on page 125
 - [Well Scan Settings](#), see page 126
 - [Shake Settings](#), see page 127
 - [Speed Read Settings](#), see page 127
 - [Well Area Settings](#), see page 128
 - [Image Acquisition Settings](#), see page 129
 - [Image Analysis Settings](#), see page 131
 - [Region of Interest Settings](#), see page 143
 - [More Settings](#), see page 144
 - [Compound Transfer Settings](#), see page 147
 - [Compound Source Settings](#) on page 150
 - [Pipette Tips Layout Settings](#), see page 151
 - [Compound and Tips Columns Settings](#), see page 152
 - [Triturate Settings](#), see page 155
 - [Acquisition View and Reads With Injectors](#), see page 156
7. After you define the instrument settings, click **OK** to close the Settings dialog.

Selecting a Detection Cartridge or Optical Configuration

When the instrument uses detection cartridges or another optional optical configuration, the Settings dialog displays the additional options above the read modes. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes for some applications. See [Supported Detection Cartridges on page 375](#).

- The SpectraMax i3x supports user-installable detection cartridges to expand its read capabilities. Cartridges are for top reads only.
- The SpectraMax Paradigm requires detection cartridges to do reads.



Note: When you select to work offline in Simulator mode, the Settings dialog displays all detection cartridges that the instrument supports.

When you connect the computer to an instrument, the Settings dialog displays the detection cartridges that you install in the instrument.

For the SpectraMax i3x:

- Select **Monochromator** to use the instrument monochromator.
- Select **MiniMax** for imaging reads that use the SpectraMax® MiniMax™ 300 Imaging Cytometer. For transmitted light imaging, you must install the Transmitted Light (TL) detection cartridge, but you do not need to select it in the Settings dialog.

After you select the detection cartridge or optical configuration, select the read mode and read type for the protocol.

Temperature Control

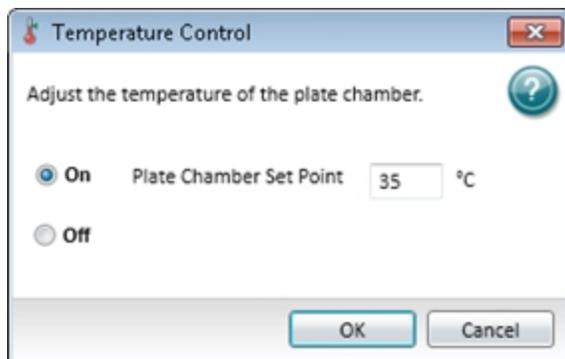
Use the Temperature Control dialog to set the temperature in the plate chamber when you use an instrument that provides temperature control. The instrument monitors the temperature of the air inside the plate chamber and the software displays the air temperature of the chamber on the Home tab. The temperature display continuously updates with the current air temperature in the chamber.

For instruments with a cuvette chamber, the instrument control panel displays the temperature within the cuvette chamber. The plate chamber temperature can be different from what displays on the instrument control panel. The software plate chamber temperature display should be similar to the control panel temperature display after both chambers reach equilibrium. During warm-up, you might notice a discrepancy in temperatures, which is normal.

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.



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



To set the temperature in the plate chamber:

1. Select the **Home** tab and click  **Temperature** to display the Temperature Control dialog.
2. Select the **On** option.
3. In the **Plate Chamber Set Point** field, enter the temperature in degrees Celsius.
4. Click **OK**.
5. After you turn off the temperature control, open the plate drawer to help the plate chamber cool to the ambient temperature.

Wavelength Settings

Use the Wavelengths category in the Settings dialog to define wavelength settings. These settings are dependent on the instrument, read mode, and read type you select.

Some instruments allow you to choose to use all wavelengths for the read or to specify specific bandwidths. Select the spectral bandwidth of the wavelength. The instrument uses the bandwidth you select for all the wavelengths in a multiple wavelength read.

The user guide for each instrument describes the instrument's bandwidth range and how to optimize excitation and emission wavelengths for a protocol. See [Documentation on page 7](#).

Fluorescence Intensity Read Mode

To define wavelengths for the Fluorescence Intensity read mode:

1. Click the **Number of Wavelength Pairs** drop-down and select the number of wavelength pairs.
2. In the **Excitation** fields, enter the excitation wavelengths.
3. In the **Emission** fields, enter the emission wavelengths.

Endpoint, Kinetic, Well Scan, and Flex Read Types

To define wavelengths for the endpoint, kinetic, well scan, and flex read types:

1. Click the **Number of Wavelengths** drop-down and select the number of wavelengths to read.
2. For each wavelength, enter or select the wavelength value for the read.

Spectrum Read Type

To define wavelengths for the spectrum read type:

1. In the **Start** field, enter the starting wavelength.
2. In the **Stop** field, enter the ending wavelength.
3. In the **Step** field, enter the value for how much the wavelength should increment between reads. The minimum step increment is 1 nm.

Cutoffs

The Gemini™ EM Dual Scanning Microplate Spectrofluorometer, Gemini™ XPS Dual Scanning Microplate Spectrofluorometer, SpectraMax® M2 Multi-Mode Microplate Reader, SpectraMax® M2e Multi-Mode Microplate Reader, SpectraMax® M3 Multi-Mode Microplate Reader, SpectraMax® M4 Multi-Mode Microplate Reader, SpectraMax® M5 Multi-Mode Microplate Reader, and SpectraMax® M5e Multi-Mode Microplate Reader support cutoff filters. The term cutoff refers to the longpass filters the instrument uses to block unwanted residual excitation light and minimize background interference.

The cutoff settings depend on the read mode (Fluorescence, Fluorescence Polarization, or Time Resolved Fluorescence) and the read type (endpoint, kinetic, spectrum, or well scan). The Luminescence read mode does not use emission cutoff filters.

To define the cutoff filters:

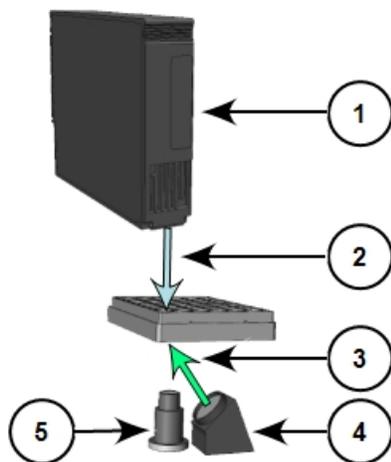
- Select the **Auto Cutoff** check box to have the software choose the longpass emission filter based on the excitation wavelength you enter.
- Clear the **Auto Cutoff** check box, then click the drop-down and select the cutoff filter for each wavelength. For the spectrum read type you must select a cutoff filter (or None).

When you clear the Auto Cutoff check box, to determine the manual setting for a cutoff filter, you need to know the 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 capable of exciting the fluorophore. This causes less of the excited light to overlap the emission spectrum which permits better selection and quantitation of the emitted light.

Imaging Read Mode

When you install the SpectraMax MiniMax 300 Imaging Cytometer with the SpectraMax i3x, you can select the wavelengths or channels to use. The software acquires images separately for each wavelength or channel and you can analyze each image separately.

The SpectraMax MiniMax 300 Imaging Cytometer captures images from the bottom of each plate well. You can illuminate the sample with white transmitted light from the top of the plate when you use the Transmitted Light (TL) detection cartridge, or you can use fluorescent excitation from the bottom of the plate. See [Imaging - Transmitted Light Detection Cartridge on page 379](#).



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

For best results with transmitted-light reads, use a plate with no cover. You can use a clear cover, if required. For Fluorescent reads, you can use a plate with a solid cover.

Plate Type Settings

Use the Plate Type category in the Settings dialog to select the type of plate to read. The software includes a plate library that contains plates of various sizes with standard dimensions. If you are uncertain which plate to use, select the Standard plate definition.

If you change the Plate Type setting, the software discards the well assignments for the template you assign to that Plate section. Group sections remain and you can select new wells to assign to the existing Group sections.

Your plate selection determines the display of the wells in the Plate section in the Workspace.

To define the plate setting:

1. Click the **Plate Format** drop-down and select the number of wells in the plate.
2. Select the **Is Lidded** check box if the plate has a lid.



Note: If the plate has a lid, you must verify that the plate height with a lid is set accurately in the Plate Editor dialog before you start a read.

3. In the **Select Specific** list, select the plate to read.

For the FilterMax F3, FilterMax F5, and SpectraMax Paradigm you can define the plate orientation to match the orientation of the plate in the drawer.

Click the **Orientation** drop-down:

- 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.

Edit Plate

Some instruments allow you to edit the dimensions of a plate or add custom plates to the plate library.



Note: You can use the custom plate definitions you create or edit for Imaging reads when you select the Imaging read mode. Plate definitions you create, edit, or optimize for other read modes do not appear in the list of available plates for the Imaging read mode.

To edit the dimensions of a plate or to add new plates to the library:

1. Select a plate from the list.
 2. Click **Edit Plate** to display the Plate Editor dialog. See [Plate Editor on page 119](#).
 3. For a new plate, in the **Plate Name** field, enter the name for the plate.
 4. Define the plate dimensions and click **Save**.
 5. Select a plate and click **Remove** to remove a plate from the plate library.
-



CAUTION! The FilterMax F5, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm have an automatic read height (Z-height) adjustment for Fluorescence read mode and Luminescence read mode. For top reads, the lens or the plate can be damaged if you do not properly set the plate height. If the plate has a lid, you must select the Is Lidded check box in the Settings dialog and verify that the plate height with a lid is set accurately in the Plate Editor dialog before you start a read.

Import Plate Definitions

You can import plates for the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm.

To import plate definitions:

1. Click **Import Plate** to display the Open dialog.
2. Navigate to and select the plate definition file: Plate File .plt file extension or Multimode File .xml file extension.
3. Click **Open**.

Plate Editor

Use the Plate Editor dialog to modify the definition of a plate or to add plate definitions to the plate library. The software saves plate definitions with a .plt file extension in a sub-folder of the Program Data folder. Custom plate definitions save to the computer. When you open a protocol that uses a custom plate definition that you save on a different computer, the software allows you to save the custom plate definition on the new computer.



CAUTION! To prevent damage to the instrument, set the plate height and read height accurately before you start a read.

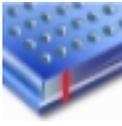
To edit a plate definition:

1. In the Navigation Tree, select a Plate section.
2. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
3. Select the **Plate Type** category.
4. Click the **Plate Format** drop-down and select the number of wells in the plate.
5. In the **Select Specific** list, select the plate to edit or copy.
6. Click **Edit Plate** to display the Plate Editor dialog. In the Acquisition view, click  to display the Plate Editor dialog.
7. To create a new plate, in the **Plate Name** field, enter the new plate name.
8. If you access the Plate Editor from the Acquisition view, you can click **Delete** to remove a custom plate definition. You cannot remove the plates installed with the software.
9. After you define the plate settings described in the table on the following page, click **Save**.



Note: For the Left Edge to Left Well Center measurement in the table on the following page, the distance from the left edge of the plate 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 plate in the lab.

Plate Editor Settings (all measurements in millimeters)

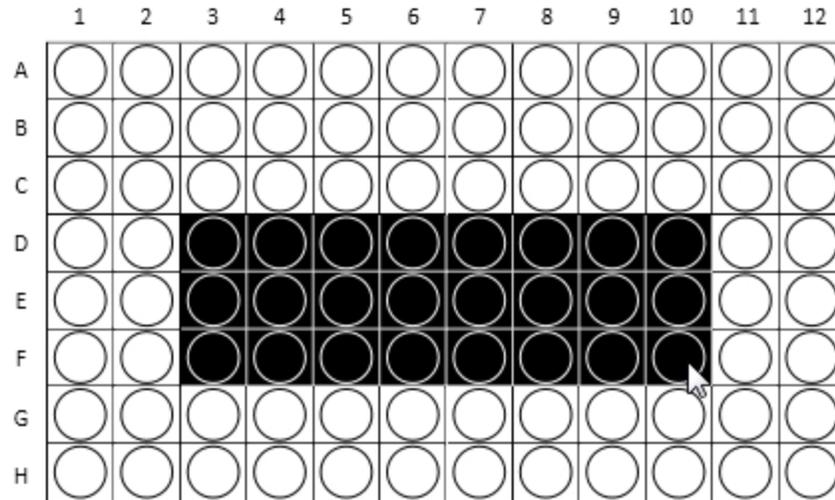
Field Name	Image	Description
Left Edge to Left Well Center		Enter the distance from the left edge of the plate to the center of well A1.
Horizontal Center to Center		Enter the horizontal distance between well centers.
Top Edge to Top Left Well Center		Enter the distance from the top edge of the plate to the center of well A1.
Vertical Center to Center		Enter the vertical distance between well centers.
Well Diameter and Well Shape		Enter the diameter of a round well or the width of a square well. For the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm, select the Well Shape Square check box for square wells.
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 with the SpectraMax MiniMax 300 Imaging Cytometer, enter the thickness of the bottom of the well of the plate. This determines the initial focus distance for Imaging reads.
Well Volume / Depth		For the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm, enter the depth of the well. For the SpectraMax i3x with the SpectraMax Injector cartridge, enter the working volume of the well for the plate in microliters. This determines the maximum allowable dispense volume for reads with injection.
Plate Height		For the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm, enter the height of the plate in millimeters and also the height of the plate with a lid. This determines the read height for top reads.
Plate Length		For the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm, enter the length of the plate.
Plate Width		For the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm, enter the width of the plate.

Read Area Settings

Use the Read Area category in the Settings dialog to select the wells to read. Partial plate reads are available for all read types. This is useful for the fast kinetic read type and the Imaging read mode to significantly reduce the time required for the kinetic read or to acquire images.

To select a contiguous, rectangular region on the plate:

- Drag the mouse pointer to select the wells to read.



Columns do not need to start with “1” but must be contiguous.

To read all the wells in the plate:

- Select the **Select All** check box.

To select non-contiguous wells for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Mini:

1. Select the **More Settings** category.
2. Select the **Well** read order.
3. Select the **Read Area** category.
4. Press the **Ctrl** key as you click each well.

When you do not select to read all wells in the plate, the Workspace displays only the wells you select to read, to indicate that the instrument will not collect data for the other wells. All wells display in the Template Editor dialog.

Western Blot Membrane Read Area Settings

You must place the membrane in a Molecular Devices ScanLater™ membrane holder to scan a membrane. See [Loading the Membrane Holder on page 180](#).

A high resolution scan of the entire surface can take approximately 15 minutes. Define a smaller area for the scan to reduce the scan time. The Read Area category displays the approximate area to scan.

To 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.

PathCheck Settings

Use the PathCheck category in the Settings dialog to use PathCheck technology for Absorbance read mode endpoint read type protocols. See [PathCheck Technology on page 282](#).

To define PathCheck settings:

- Select the **PathCheck** check box to use the PathCheck technology Water Constant correction.
- Clear the **PathCheck** check box to not use the PathCheck technology Water Constant correction.

The temperature-independent PathCheck technology normalizes 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:

$$A = \epsilon cL$$

where A is the absorbance, ϵ is the molar absorptivity of the sample, c is the concentration of the sample, and L is the pathlength. The longer the pathlength, the higher the absorbance.

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 makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis to quantify 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 you use 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.

TRF Settings

Use the TRF Settings category in the Settings dialog to define the integration delay before data is collected and the integration time of the fluorescence signal. The Time-Resolved Fluorescence read mode measures fluorescence as a function of time after excitation. See [Time-Resolved Fluorescence Read Mode on page 304](#).

1. Click the **Integration Delay** drop-down and select the amount of time to elapse between the flash of the lamp (excitation) and the beginning of data acquisition from the well (emission).
2. Click the **Integration Time** drop-down and select the amount of time for the instrument to read the well.

PMT and Optics Settings

Use the PMT and Optics category in the Settings dialog to define sensitivity settings for the Photomultiplier Tubes and optics for all read modes except Absorbance.

Click the **PMT Gain** drop-down:

- Select **Automatic** to have the instrument automatically adjust the PMT voltage for varying concentrations of sample in the plate. Available for the Fluorescence read mode with the Endpoint, Spectrum, and Well Scan read types. The Luminescence and Time-Resolved Fluorescence read modes use the Automatic setting and the following options are not available.
- Select **High** for samples that have a lower concentration (dim samples).
- Select **Medium** for samples that have medium concentration.
- Select **Low** for samples that have a higher concentration (bright samples).
- Select **Manual** to enter a specific voltage.

The SpectraMax i3x 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. The instrument uses automatic LED power adjustment 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 set to Automatic for the Luminescence and Time Resolved Fluorescence read modes. For these instruments, the PMT Gain category is not available for these read modes.

To set the resolution for the full scan and for a secondary scan of a region of interest for Western Blot membrane scans:

1. Click the **Default Scan Resolution** and select the resolution to apply to the entire read area of the membrane you define in the Read Area category settings.
2. Click the **ROI Scan Resolution** and select the resolution to apply to the region of interest (ROI) you define in the Plate section.

A High resolution scan provides the best results. A Low resolution scan provides the fastest read.

The general workflow to acquire Western Blot data is to scan the read area of the membrane you define with a low resolution and then scan the region of interest (ROI) you define at a high resolution.

For some instruments in certain read modes, you can enter the **Integration Time** or the number of **Flashes Per Read**.

On-the-Fly Detection

Some detection cartridges for the SpectraMax i3x and SpectraMax Paradigm provide the On the Fly Detection options.

On-the-Fly Detection yields faster read times because the plate moves continuously as the instrument measures each well.

To disable On-the-Fly Detection and have the plate stop moving for each read:

- Select **Off-Stop and Go**.

To use On-the-Fly Detection:

- 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 plate. However, there is a tradeoff between the data quality and read speed because the instrument samples each well for shorter integration times.

Integration Time

To set the integration time for the FilterMax F5, SpectraMax i3x, and SpectraMax Paradigm with some detection cartridges:

- In the **Integration Time** field, enter the number of milliseconds to validate the measurement time per well.

Bottom Read

To have an instrument with bottom read capability read up through the bottom of the plate:

- Select the **Read From Bottom** check box.

Read Height

For the FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm you can enter the read height.



CAUTION! To prevent damage to the instrument, set the plate height and read height accurately before you start a read.

To set the read height:

- In the **Read Height** field, enter the distance in millimeters between the objective lens and the plate.

Pre-Read Optimization

Use the Read Height Optimization feature to find the optimized read height before the read.

1. Select the **More Settings** category.
2. Select the **Show Pre-Read Optimization Options** check box.

Timing Settings

Use the Timing category in the Settings dialog to define the total run time and the time interval between reads for the Kinetic read type and Flex read type.

Each instrument has a unique minimum read interval that depends on factors that include the number of wavelengths, the number of wells to read, and the distance the instrument filter wheel must move. The maximum read interval also varies by instrument, for example, the SpectraMax M2 has a maximum read interval of 2 hours and 45 minutes, and the SpectraMax Paradigm has a maximum read interval of 18 hours and 12 minutes. If you enter an invalid read interval, a message indicates the minimum or maximum value for the read interval.

 **Tip:** In some cases, choosing wavelengths in ascending order can yield the shortest possible interval.

The software calculates the minimum interval, maximum interval, and number of reads based on the values you enter in the Total Run Time field and the Interval field. The minimum permitted interval values display below the Interval field.

To define Timing settings when you select the Row and Column read order in the More Settings category:

1. In the **Total Run Time** field, enter the total run time.
2. In the **Interval** field, enter the time to wait between reads.

To define Timing settings when you select the Well read order in the More Settings category:

1. In the **Well Run Time** field, enter the well run time.
2. In the **Interval** field, enter the time to wait between reads. You can define the Interval down to the millisecond.

Single Point Read

Single Point Read sets the Total Run Time and the Interval to the same value and is useful for a multitask kinetic read with alternating read modes within a cycle. See [Defining and Running Workflows on page 106](#).

To read only one time point:

- Click **Single Point Read**.

Well Scan Settings

Use the Well Scan Settings category in the Settings dialog to define the pattern and density for the reads within each well.

The well scan read type takes reads at more than one location within a well. The well scan read type takes multiple reads of a single well of a plate on an evenly spaced pattern inside of each well at single or multiple wavelengths.

For applications that involve the detection of cells in large area tissue culture plates, the well scan read type permits maximum surface area detection in cell-based protocols. Since many cell lines tend to grow as clumps or in the corners of plate wells, you can choose the well scan pattern and define the number of points to scan in order achieve the best results.

The image in the Well Scan settings displays the shape of the well based on the definition of the plate you select. The fill pattern is either round or square to match the well shape. The number of available points depends on the well size.

Density determines the number of points to read in the line patterns and the maximum number of horizontal and vertical points to include in the cross or fill pattern.

To define Well Scan settings:

1. Select a **Pattern**. Up to four scan patterns are available, depending on the instrument.



2. Click the **Density** drop-down and select the density.

Shake Settings

Use the Shake category in the Settings dialog to set options to shake the plate in the chamber. Shake options are dependent on the instrument and the read type you select.

To define shake settings:

- Select the **Before First Read** check box to shake the plate for the duration you enter into the text field before the first wavelength read.
- Clear the **Before First Read** check box to not shake the plate.

For the Kinetic read type, you can shake the plate before the first read and you can shake the plate between reads. For single wavelength reads, this shakes the plate before each read at that wavelength. For multiple wavelength reads, this shakes the plate before each read at the first wavelength only.

To define shake settings for the Kinetic read type:

- Select the **Between Reads** check box to shake the plate for the duration you enter into the text fields between reads.
- Clear the **Between Reads** check box to not shake the plate between reads.



Note: The Before First Read and Between Reads options are available in the Standard View. To shake the plate from the Acquisition view, place a Shake node in the timeline where you want the shake to occur.

For the FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, and SpectraMax Paradigm, define the following shake settings:

1. Select the **Before First Read** check box.
2. In the text field, enter the length of time to shake the plate in seconds.
3. Click the **Shake Intensity** drop-down and select **Low**, **Medium**, or **High**. The actual shake speed is based on the plate format.
4. Click the **Shake Mode** drop-down and select **Linear** or **Orbital**.

For the SpectraMax Mini, SpectraMax iD3, and SpectraMax iD5, define the following shake settings:

1. Select the **Before First Read** check box.
2. In the text field, enter the length of time to shake the plate in seconds.
3. Click the **Shake Intensity** drop-down and select **Low**, **Medium**, or **High**. The actual shake speed is based on the plate format.
4. Click the **Shake Mode** drop-down and select **Orbital**, **Linear**, or **Double Orbital**.

Speed Read Settings

Use the Speed Read category in the Settings dialog to read the plate faster for the Absorbance read mode by decreasing the number of lamp flashes. The Speed Read option is useful for the spectrum read type and can greatly reduce the protocol run time.

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.

To define Speed Read settings:

- Select the **Speed Read** check box to use the speed read.
- Clear the **Speed Read** check box to not use speed read.

Well Area Settings

Use the Well Area Settings category in the Settings dialog to acquire images at more than one location, or site, within a well to form a single seamless tiled image for the Imaging read mode. If you select to acquire images of more than one site in the well, the software tiles the images together into a single image for analysis. Image acquisition time increases with each site you select. For faster image acquisition, select fewer sites.

To define Well Area settings, select the number of sites to acquire:

Well Area Settings

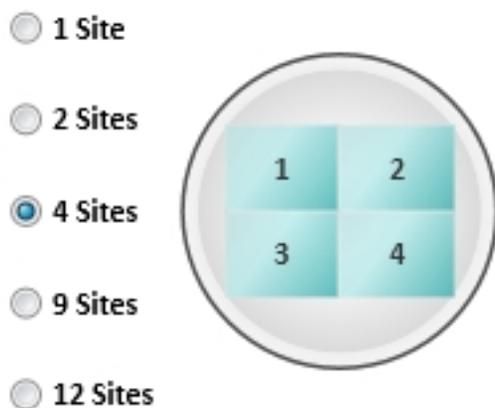


Image Acquisition Settings

Use the Image Acquisition Settings category in the Settings dialog to define the exposure time and focus adjustment for two sample wells. The instrument uses these settings for every well in the plate during acquisition. You can set the exposure and focus adjustment separately for each wavelength or channel.

The instrument acquires one site in each well. If you select to acquire more than one site, the instrument acquires the first site.

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 well you expect to have the brightest image. The well displays red.
3. Click **Acquire Max Image** and wait for the instrument to acquire the image.

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 well you expect to have the darkest image. The well displays blue.
3. Click **Acquire Min Image** and wait for the instrument to acquire the image.

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 image in and out:

- Right-click the image and select **Zoom In** or **Zoom Out**,
- Hold the **CTRL** key and press + or -.
- Use the mouse wheel to zoom in or out.

To move the image in the image area:

- Use the scroll bars or click the image and drag it into position.

To view a larger version of the image area:

- Double-click the image to display the Zoom Well dialog.

See [Zooming Image in a Well on page 173](#).

Set Exposure Time and Focus Adjustment

You can adjust the exposure and focus 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 you designate for the specific channel.

Exposure

Use Auto Exposure to have the software determine 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 have the software determine the best exposure:

- Click **Auto Expose**.

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 the intensity.

To manually adjust the intensity of the images,

- In the **Exposure** field, enter the number of milliseconds to hold the camera shutter open.

Focus

The system optics use a laser automatic focus system and software algorithms to find the initial focus distance for the cells in the plate wells. 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. The instrument uses this setting for every well in the plate during acquisition.

For transmitted light images, make sure that enough contrast exists between the objects and the background. Less contrast exists as you make the focus sharper. For best results when you use a preset drawing analysis, make sure that the objects are brighter than the background.

To adjust the focus:

- In the **Focus Adjustment** field, enter the number of microns to move the objective lens closer to or farther away from the sample.

After each adjustment, acquire the images again to view what effect the change has on the focus.

Image Analysis Settings

Use the Image Analysis Settings category in the Settings dialog to define how to analyze images for the Imaging read mode.

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. You can use transmitted light or a fluorescent wavelength to find nuclei or easily separable objects in the image. The StainFree Cell Detection Algorithm uses proprietary transmitted light analysis technology to eliminate cell staining for cell counting and confluency measurements.

To define discrete object analysis settings:

1. Click **Discrete Object Analysis** to display the Wavelength for Finding Objects list.
2. Select the wavelength to use to identify the objects in the well images.
3. Select the **Image Analysis Settings** category.
4. Click **Find Objects** and enter size and intensity values or use proprietary algorithms with the drawing tools to graphically define the objects and the background areas for the analysis. See [Finding Discrete Objects or Confluent Areas on page 132](#).
5. Click **Classify Objects** and define separate measurements for different types of found objects, or cells. See [Classifying Discrete Objects on page 136](#).
6. Click **Select Measurements** and view the results of the analysis for the wells you select. See [Viewing and Selecting Measurement Statistics on page 137](#).

Field Analysis

Field Analysis uses proprietary algorithms to analyze confluent areas based on parameters including the signal intensity over the background and the size of the areas.

To define field analysis settings:

1. Click **Field Analysis** to display the Wavelength for Finding Confluent Areas list.
2. Select the wavelength 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.
3. Click **Find Confluent Areas** and use proprietary algorithms with the provided drawing tools to graphically define the confluent areas and the background areas for the analysis. See [Finding Discrete Objects or Confluent Areas on page 132](#).
4. Click **Select Measurements** and view the results of the analysis for the wells you select. See [Viewing and Selecting Measurement Statistics on page 137](#).

The SoftMax Pro Software proprietary algorithms with the drawing tools in the Find Objects and Find Confluent Areas settings help the software learn the patterns of the objects you want to find. Depending on the complexity of the object patterns, this teaching process can take several iterations. See [Resolving Issues with Object Drawing and Finding on page 138](#).

Finding Discrete Objects or Confluent Areas

The Find Objects or Find Confluent Areas settings display the minimum and maximum images from the Image Acquisition Settings.

- Click  to view the page in full screen.
- Click  to restore the page to its original size.

In full screen view, select the tabs at the top of the page to switch between the different analysis settings.

Use 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 button is filled, 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 adjust the intensity of each wavelength.



To adjust the wavelength intensity:

1. Select the wavelength to adjust.



2. Drag the slider bar on the left to adjust the low level of intensity or click  to move the sliders to the outside edges. The intensity level displays above the bar.
3. Drag the slider bar on the right to adjust the high level of intensity or click  to move the sliders to the outside edges.
4. 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 apply separately to each object in the image that meets the defined size range. Use this analysis 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 you want to find.

Set Size and Intensity

The Set Size and Intensity settings are available for fluorescent acquisition and discrete object analysis.

To define set size and intensity settings:



1. Click .
2. Click four or more representative objects on the images that you want to detect as cells.
3. In the **Min Width** field, enter the minimum width of the object to find, in microns.
4. In the **Max Width** field, enter the maximum width of the object to find, in microns.
5. In the **Intensity Above Background** field, enter 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.
6. Click **Apply**.

Grow

Use the Grow setting to define the number of pixels to increase the diameter of the found objects in one wavelength, so the objects 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

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. Each preset drawing analysis works with specific cell types. Select the preset analysis that works best with your images.

To use a preset drawing analysis:

1. In the **Settings** list, select a preset drawing.
2. Click **Apply** to find objects or confluent areas that use the preset.
3. After you acquire data, select **Stored Setting** in the Settings list to use the previously defined analysis settings.
4. To start from scratch, select **Create New Setting** in the Settings list.

To select the objects for fluorescent acquisition and discrete object analysis:

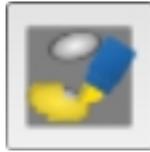


Note: This method is not available for transmitted light acquisition or for confluent area analysis.

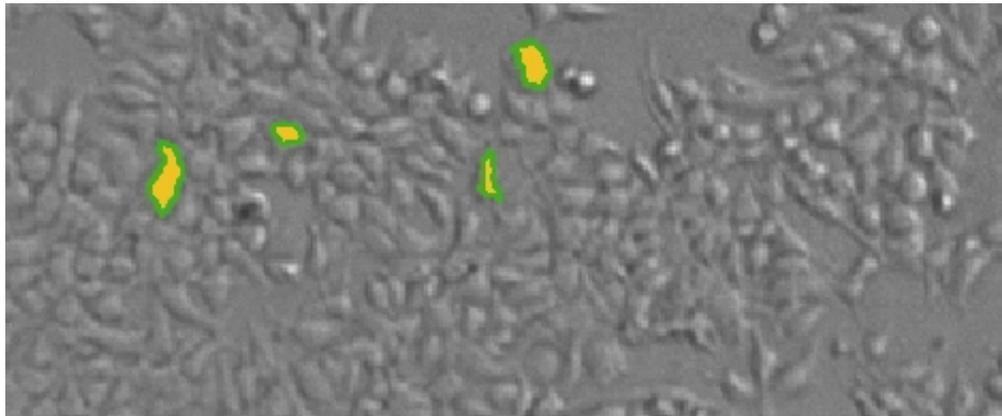


1. Click .
2. Click a line width.
3. Click four or more representative objects on the images.

To define the objects or confluent areas:



1. Click the object or area pen .
2. Select a line width.
3. 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.
4. For field analysis, draw across confluent areas, avoiding background. For best results, draw across four or more representative areas.



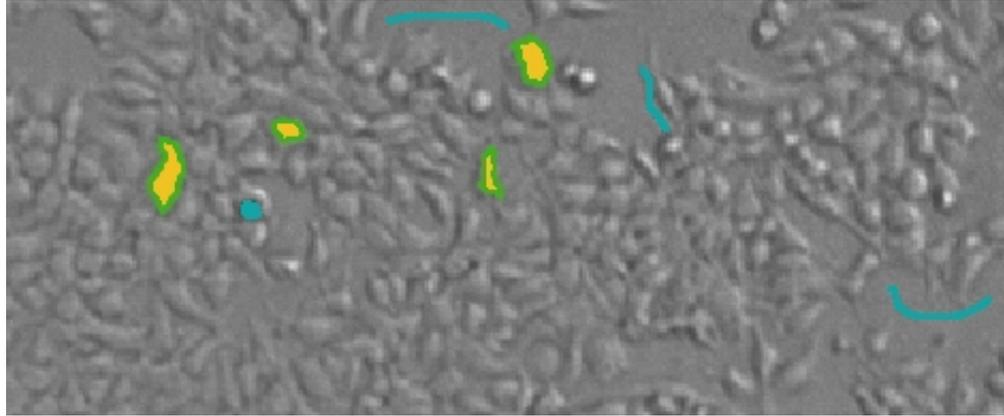
To define the background:



1. Click the background pen .
2. Select a line width.
3. Draw on the image to define artifacts and background areas in blue.



Tip: For best results, define four or more representative background areas.



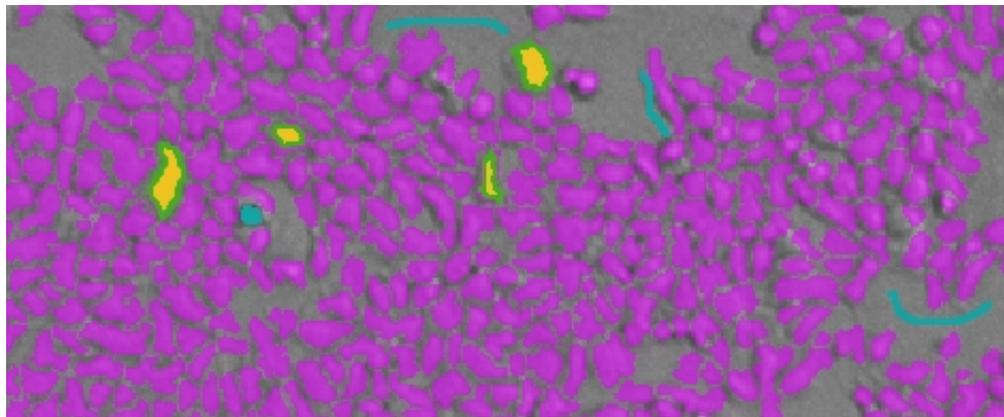
To correct a drawing of an object or area:

- Click **Undo** to undo the most recent action.
- Click **Redo** to restore the most recent action.
- Click **Start Over** to clear the drawings from the image.

To erase parts of drawings:



1. Click **Erase**.
2. Select a line width.
3. Drag the eraser across the drawings to remove.
4. After you define the objects and areas, click **Apply** to find the objects or confluent areas in the image.
5. Continue to draw and to apply until you get satisfactory results. See [Resolving Issues with Object Drawing and Finding](#) on page 138.



You can then classify objects or view the measurement statistics.

Classifying 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 [Finding Discrete Objects or Confluent Areas on page 132](#).

Use the bottom of the page to classify the found objects in the images into two separate classifications.

To classify the found objects:

- Select **Use Existing Classification** to use the previously defined classification settings when in Re-Analysis mode.
- Select **Skip Classification** to not use classification in the analysis.
- Select **Create New Classification** to replace the Type A and Type B titles with your own titles for the classifications. See below.

Create New Classification

When you select Create New 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. Titles must start and end with a letter. The titles must be unique and you cannot use "Exclude" as a title, since this is a reserved word.

To create a new classification:

1. Click to select objects for the first classification then click the objects in the images.
2. Click to select objects for the second classification then click the objects in the images.
3. To exclude objects from being classified, click 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.
4. To deselect a selected object click it again.
5. To remove all the selections from the images, click **Start Over**.
6. Click **Apply** to find and classify the objects in the image.
7. Continue to select and to apply until you get satisfactory results.
8. View the measurement statistics.

Viewing and Selecting Measurement Statistics

The Measurement Selections settings table displays the analysis results for the minimum intensity and maximum intensity images. The possible included measurements are as follows:

- **Object Count:** The total number of objects detected in the image. Do not use for field analysis of confluent areas.
- **Field Count:** The total number of confluent areas detected in the image. Do not use for discrete object analysis.
- **Object Percentage:** The percentage the objects detected in the image by classification. Do not use for field analysis of confluent areas.
- **Covered Area:** The combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area:** The average area of the objects detected in the image expressed in μm^2 . Do not use for field analysis of confluent areas.
- **Field Area:** The average area of the confluent areas detected in the image expressed in μm^2 . Do not use for discrete object analysis.
- **Object Roundness:** 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. Do not use for field analysis of confluent areas.
- **Field Roundness:** 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. Do not use for discrete object analysis.
- **Object Average Intensity:** The average fluorescent signal intensity of the objects detected in the image. Do not use for field analysis of confluent areas or for transmitted light.
- **Field Average Intensity:** The average fluorescent signal intensity of the confluent areas detected in the image. This is not used for discrete object analysis or for transmitted light.
- **Object Intensity:** The average total fluorescent signal intensity of the objects detected in the image. Do not use for field analysis of confluent areas or for transmitted light.
- **Field Intensity:** The average total fluorescent signal intensity of the confluent areas in the image. Do not use for discrete object analysis or for transmitted light.
- **Total Intensity:** The combined total fluorescent signal intensity of the objects or confluent areas in the image expressed in million intensity counts. Do not use for transmitted light.

To include the measurements in the analysis:

1. Click **Select Measurements** in the lower-right area of the page.
 - Click **Remove** to exclude a measurement.
 - Click **Add** to include a measurement.
 - Click **Remove All** to exclude all measurements.
 - Click **Add All** to include all measurements.
2. Click **Done** to add the measurements 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 80](#) or [Group Sections on page 87](#).
3. Click **Show Details** to view the measurements in an interactive image, data table, and chart. See [Viewing Imaging Data in Wells on page 209](#).
4. If no measurements display and you did not make 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.

Resolving 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.

The 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.
- Over splitting finds portions of cells or confluent areas and can include several found objects within a single cell or confluent area.
- Artifacts can be found and counted as cells or confluent areas.
- Ghosting finds background areas next to cells or confluent areas.

Each preset drawing analysis is designed to work with specific cell types. Select the preset analysis that works best with your images.

To resolve issues:

1. In the **Settings** list, select a preset drawing analysis.
2. Click **Apply** to find objects or confluent areas using the preset.

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 plate.

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, move the lens closer to make the objects darker than the background, move the lens farther away to make 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.

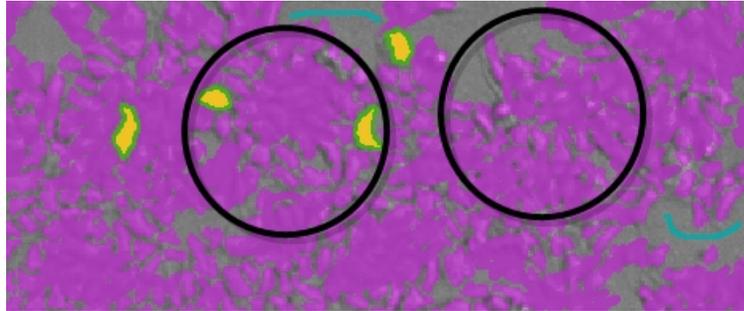
To create an iterative training set for complex images:

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 look worse as the algorithm emphasizes the fixes. Continue to adjust 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.

Resolve Under Splitting Issues

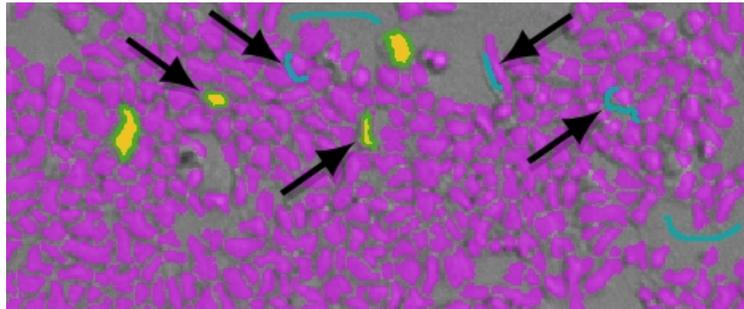
Under splitting finds multiple cells or confluent areas that can also include background.



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.

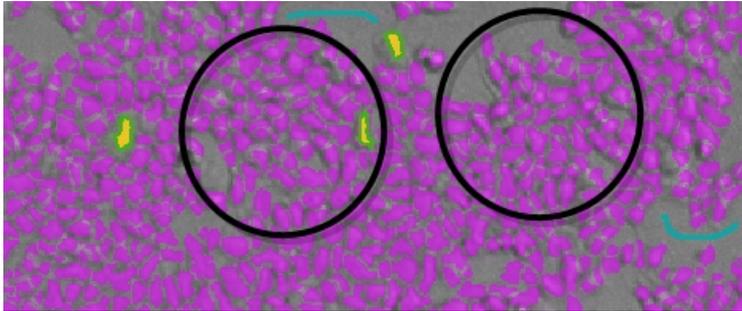
To resolve under splitting issues:

- 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

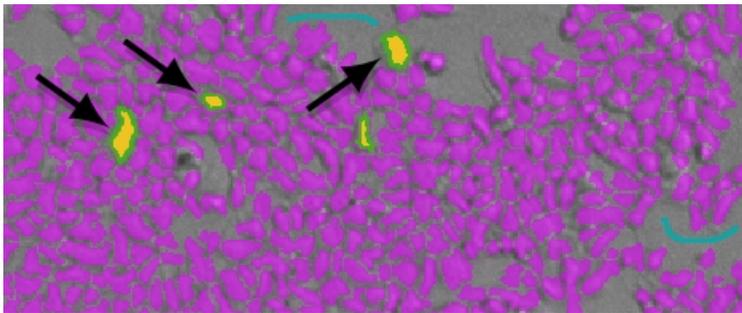
Over splitting finds portions of cells or confluent areas and can include several found objects within a single cell or confluent area.



Over splitting can be caused by drawing too small within the cells or confluent areas. Sometimes over splitting can happen after you use 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.

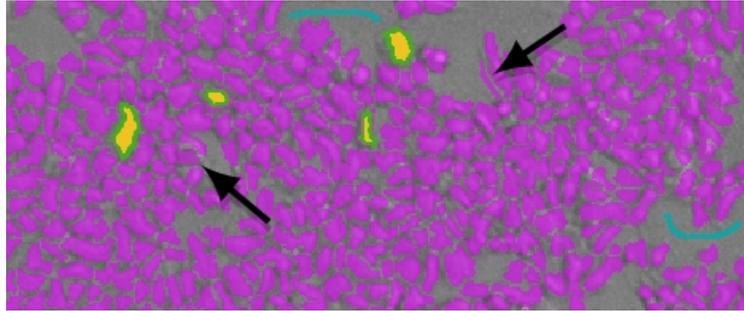


If over splitting is the result of artifact removal:

- Erase the blue from the artifact you defined with the background pen.

Resolve Artifact Issues

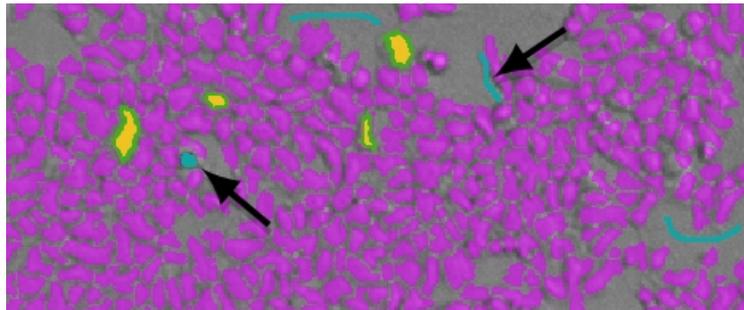
Artifacts can be found and counted as cells or confluent areas.



Artifact issues can be caused by debris or other artifacts in the image where the edges and surrounding pixels confuse the software.

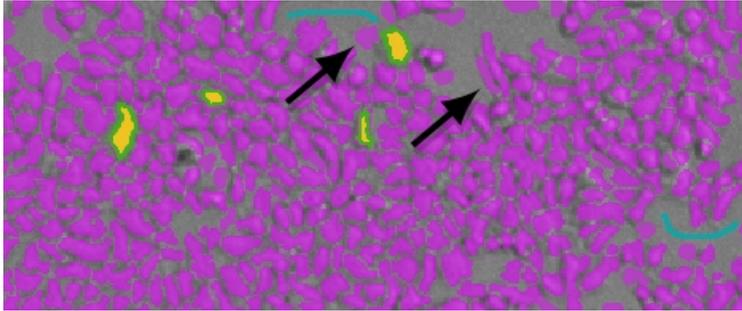
To resolve artifact issues:

- Use the background pen to define artifacts as background and not cells or confluent areas.



Resolve Ghosting Issues

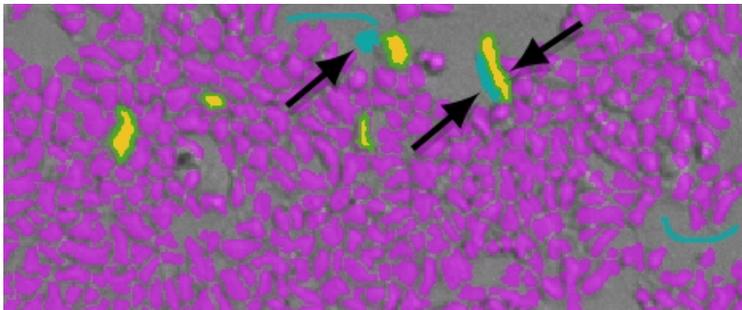
Ghosting finds background areas next to cells or confluent areas.



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 interest for the images in all wells to analyze for your application. The software uses only the region of interest you select for 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:

1. Click  to the right of the **Well** field above the image area. The well fills with red and is used to determine the region of interest.
2. 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 image in and out:

- Right-click the image and select **Zoom In** or **Zoom Out**,
- Hold the **CTRL** key and press **+** or **-**.
- Use the mouse wheel to zoom in or out.

To move the image in the image area:

- Use the scroll bars or click the image and drag it into position.

To define a region of interest:

1. Select the **Enable Region Selection** check box to display a rectangle on the image. Click **Resize** and then drag the corners of the rectangle to redefine the size of the region. Clear the **Enable Region Selection** check box to use the entire image for the analysis.
2. To start over, click **Draw** and then drag a new rectangle on the image to define the region.

More Settings

The More Settings category in the Settings dialog provides more settings options. The options vary depending on the instrument, read mode, and read type.

Calibrate

The More Settings category in the Settings dialog provides a Calibrate option for some instruments.

For the Absorbance read mode, the instrument makes an air-calibration measurement at each wavelength before the read. If you turn calibration off, the software uses the air-calibration values stored in the instrument firmware or the values from the previous calibration.

For Fluorescence and Luminescence read modes, the instrument makes a reference read at each wavelength before the read. If you turn calibration off, the software uses the default values stored in the instrument firmware or the values from the previous calibration.

For the kinetic read type, the instrument makes the calibration measurement between reads during a run, unless the read interval is too short to allow calibration.

New calibrations overwrite the calibration setting stored in firmware for the current data acquisition read, and for any subsequent reads you do with the Calibration check box clear. When you power the instrument off and then back on, calibrations reset to those stored in the firmware.

- Select the **Calibrate** check box to have the instrument run calibration for the wavelengths you specify before each read. You should calibrate at each assay wavelength for at least one plate before you turn off calibration.
- Clear the **Calibration** check box to have the instrument begin reads or complete reads more quickly.

To update air-calibration values stored in the firmware:

1. Select the **Operations** tab.
2. Click **Calibration**.
3. See [Instrument Calibration on page 371](#).

Carriage Speed

The More Settings category in the Settings dialog provides a Carriage Speed option for some instruments. You can slow down the carriage speed for samples (such as, fragile clots) that rapid carriage movement can disrupt.

Click the **Carriage Speed** drop-down:

- Select **Normal** to use the normal carriage speed.
- Select **Slow** to use a slower carriage speed.

Read Order

The More Settings category in the Settings dialog provides a Read Order option that varies depending on the instrument, read mode, and read type.

Some instruments offer two options.

Click the **Read Order** drop-down:

- Select **Column** to have the instrument read the entire plate (or a number of strips) at the first wavelength. The instrument then goes back and reads the plate at the second and subsequent wavelengths.
- Select **Wavelength** to have the instrument read all wavelengths for the first column of wells in the plate first and then read all wavelengths for the second column, third column, and so on.

Some instruments offer three options

Click the **Read Order** drop-down:

- Select **Row** to have the instrument read the entire plate row-by-row at the first wavelength. The instrument then goes back and reads the plate at the second and subsequent wavelengths.
- Select **Column** to have the instrument read the entire plate column-by-column at the first wavelength. The instrument then goes back and reads the plate at the second and subsequent wavelengths.
- Select **Well** to read each well individually with all wavelengths and intervals you define for the read before the software reads the next well.

Settling Time

The More Settings category in the Settings dialog provides a Settling Time option for some instruments.

Settling Time is the delay between the motion of the instrument and the start of the read. This delay allows time for the meniscus motion to cease, which can improve precision, especially in the low density, high volume plates with fewer than 96 wells.

- Select the **Settling Time** check box to add a delay between the read of each column. In the **Duration** field, enter the delay time between 100 and 1000 milliseconds. There is no delay between each well in a column.
- Clear the **Settling Time** check box to not have a delay.

Show Pre-Read Optimization Options

The More Settings category in the Settings dialog provides a Show Pre-Read Optimization Options check box for some instruments. From the Acquisition view, you can add Plate Optimization to the beginning of the timeline. Plate dimensions can vary slightly between production lots, which can have a potential effect on measurement accuracy.

To define pre-read optimization settings:

- Select the **Show Pre-Read Optimization Options** check box to display the Pre-Read Optimization Options dialog before the instrument reads the plate. Use this dialog to optimize the plate dimensions after you click Read and before the instrument reads the plate. See [Pre-Read Optimization Options on page 185](#).
- Clear the **Show Pre-Read Optimization Options** check box to use the plate dimensions saved in the plate library.

Use the Pre-Read Optimization Options dialog helps you optimize plate dimensions by determining the centers of the four corner wells on the plate. The software adds a new plate definition to the plate library each time you optimize a plate. If you change the plate orientations for measurements, you must optimize each orientation separately.

If the instrument has an adjustable objective lens, the Pre-Read Optimization Options dialog allows you to move the lens up and down to optimize the read height for several read modes. You optimize the read height to match the focus of the optics with the sample volume. This maximizes the raw signal, which yields the highest precision and maximum sensitivity.

Attenuation

The More Settings category in the Settings dialog provides an Attenuation option for some instruments. Attenuation filters reduce the intensity of very bright samples that can exceed the linear dynamic range of the detection system.

To define Attenuation settings:

- Select the **Attenuation** check box to use attenuation filters for very bright samples.
- Clear the **Attenuation** check box to not use attenuation filters.

Normalization

The More Settings category in the Settings dialog provides a Normalization option for instruments that support the AlphaScreen read mode. The conversion rate of photons to counts is individual for each detector. Raw data from the same plate can seem to be different from one instrument to the next. The data format each instrument manufacturer uses can be counts normalized-per-second or not-normalized counts, therefore raw data can be different by several orders of magnitude.



Note: The number of counts and the size of figures is in no way an indication of sensitivity.

To define normalization settings:

- Select the **Normalization** check box to normalize raw data to counts per second.
- Clear the **Normalization** check box to not normalize raw data to counts per second.

Interlaced Reading

The More Settings category in the Settings dialog provides an Interlaced Reading option for the SpectraMax i3x and SpectraMax Paradigm when you install the AlphaScreen detection cartridge and do an Endpoint read type.

When you read a sample with a small signal, an interference can occur from the afterglow of a very strong emitting adjacent sample that the instrument just measured. Such cross talk can occur through the wall of a white 384-well plate. Use Interlaced Reading to have the instrument read every other well in a checkerboard pattern, and then do another plate run to read the omitted wells.

Although background is significantly lower with the AlphaScreen read mode than with the Fluorescence Intensity read mode measurements, you should use blank or assay controls for background correction. Use blank replicates to effectively measure the background. For improved results, use replicates for all blanks, controls, and samples.

To define interlaced reading settings:

- Select the **Interlaced Reading** check box to prevent interference.
- Clear the **Interlaced Reading** check box to not use the feature.

Compound Transfer Settings

The Compound Transfer category in the Settings dialog displays for the FlexStation 3.

You must configure all compound transfer settings correctly. When you select to do compound transfers, four additional categories appear in the Settings dialog. Use these settings to define precise fluid transfers for the protocol and help prevent flooding of the assay plate. Keep in mind the actual maximum volume the wells allow as you define the settings. The maximum cumulative volume depends on the assay plate type you select.

For the Endpoint and Kinetic read types, the transfers are done to all the wells you select before the first read.

For the Flex read type, the transfers are done for a column while that column is read. All transfers and reads are done for that column before the instrument moves to the next column.

To set the number of compound transfers in each column during a read:

Click the **No. of Compound Transfers** drop-down.

- Select **0** to not do compound transfers for the read. No other options display and no other settings are required.
- Select **1** to do one compound transfer.  displays with additional settings.
- Select **2** to do two compound transfers.  displays with additional settings.
- Select **3** to do three compound transfers.  displays with additional settings.

Initial Volume

The software assigns the initial volume to all wells and uses this value to compute the total volume in each well after all fluids dispense. As you define the volume for each transfer in the Compound & Tips Column category, the software uses the Initial Volume value to warn you of the potential for overflow of fluid from the wells. See [Compound and Tips Columns Settings on page 152](#).

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. If there is no fluid volume in the assay plate before the compound transfers, enter **0**.

- For a 96-well plate, this value can be 0 μL to 269 μL . Typical values are about 10 μL to 200 μL .
- For a 384-well plate, this value can be 0 μL to 120 μL . Typical values are about 5 μL to 80 μL .

Transfer Settings

You define the following settings for each compound transfer.

1. Select the compound transfer to define.



Note: The compound transfer you select displays with a light blue background and this is the only indication for which transfer you are defining.

2. In the **Pipette Height** field, enter a value between 1 μL to 300 μL for a 96-well plate or 1 μL to 130 μL for a 384-well plate. 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 is to remain during the dispense portion of the transfer event. Use this setting to place the tip of the pipette below the surface of the liquid at the end of the transfer to minimize 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. Example: If the initial volume is 15 μL and the first transfer dispenses 20 μL , set the pipette height for the first transfer to 25 μL . If the second transfer dispenses 15 μL , set the pipette height for the second transfer to 45 μL .

3. In the **Volume** field, enter the volume of liquid to dispense to each well of the assay plate that you select to receive the transfer.
 - For a 96-well plate, the range is 1 μL to 200 μL .
 - For a 384-well plate, 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.

4. In the **Rate** field, enter a value of 1 to 8. This setting determines the rate at which the instrument dispenses fluid into the well of the assay plate.
 - In a 96-well plate, a setting of 1 is equal to 16 μL per second and each subsequent number increases in increments of ~ 16 μL per second, so that a setting of 2 is equal to 31 μL per second.
 - In a 384-well plate, a setting of 1 is equal to 4 μL per second and each subsequent number increases in increments of 4 μL per second, so that a setting of 2 is equal to 8 μL per second.

A setting of 1 or 2 can help minimize cell damage or dislodgment. For non-contact dispensing, use a rate of 8 to make sure that all liquid dispenses from the pipette tip.

5. In the **Time Point** field, enter the point in time after the read starts when the fluid is to begin being dispensed. This value must be equal to or greater than the value the instrument displays for the **Minimum Time** up to 9999 seconds. This time point becomes the baseline time. This is not the time interval between transfers. For the Endpoint and Kinetic read types, Time Point is 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 less than the Minimum Time the software identifies 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 considers 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 plate.
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 the 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.

- Select the **Leave Tips On Between Columns** check box to use tips from the same position in the tip rack for the first and last transfers in each column. This can reduce the cycle time between columns.
- Clear the **Leave Tips On Between Columns** check box to have the instrument install tips from the tip rack before the first transfer for the next column.

You define tip assignments in the **Compound & Tips Columns** category. See [Compound and Tips Columns Settings on page 152](#).

Avoiding Problems

Time point calculations are based on the number of wells you select in the Read Area category and the Pipette Tips & Columns category settings you define for the transfer. If your Time Point entry is not long enough to be compatible with the volumes and transfer speed you define, a message instructs you to increase the Time Point entry.

If you enter transfer volumes that add up to more than the maximum that can fit in the assay plate, an overflow message indicates that you have exceeded the volume limit of the assay plate.



Note: The Minimum Time Value that displays below the Time Point field is different for each transfer you define.

Compound Source Settings

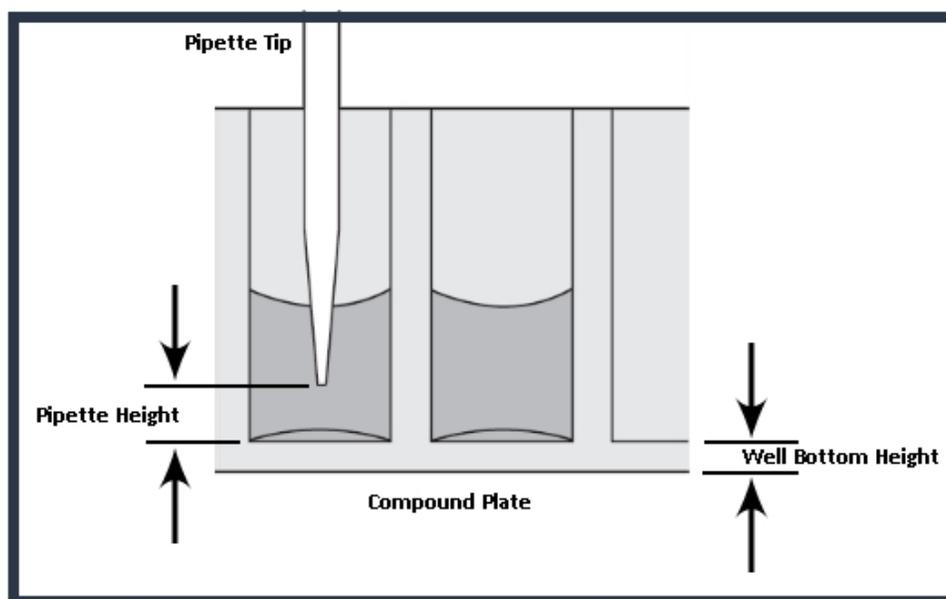
Use the Compound Source category in the Settings dialog to select the plate type for the compound plate. The instrument aspirates (withdraws) fluids from the compound plate and then dispenses (injects) the fluids into the assay plate during the run.

The plate type you select in the Compound Source category must match the type and well configuration of the actual compound plate to use and it must be compatible with the number of wells in the plate type you select for the assay plate in the Plate Type category. See [Plate Type Settings on page 118](#).

The instrument sets the pipette height for the compound plate. The well bottom height is different for different types of plates. Select the plate type correctly to prevent jamming the pipette tips into the bottom of the well.

- For 96-well plates, the instrument aspirates using a pipette height of 20 μL above the bottom of the well.
- For 384-well plates, the instrument aspirates using a pipette height of 10 μL above the bottom of the well.

You define the pipette height for the assay plate in the Compound Transfer category. See [Compound Transfer Settings on page 147](#).

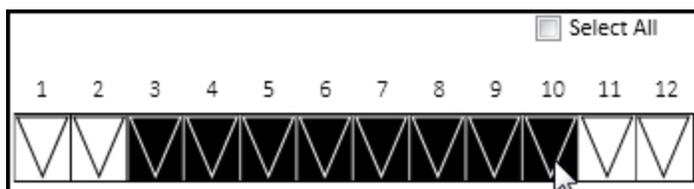


CAUTION! Selection of an incorrect compound plate type can result in the pipette tips jamming into the wells and damaging the plate, the tips, and the instrument.

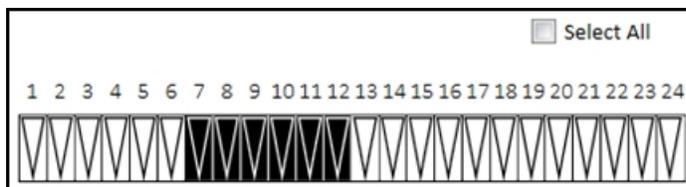
Pipette Tips Layout Settings

Use the Pipette Tips Layout category in the Settings dialog to define which pipette tips to use. If you use a partial rack of tips, use this category to have the software instruct the instrument to pull tips from the location of the tips you insert in the tip drawer.

When you select a 96-well assay plate in the Plate Type category, the Plate Tips Layout category displays a column of 12 tips. See [Plate Type Settings on page 118](#).



When you select a 384-well assay plate in the Plate Type category, the Plate Tips Layout category displays a column of 24 tips.



To use all the tips in the rack:

- Select the **Select All** check box.

The tips selection must be contiguous. To select a contiguous set of tips:

- Drag the mouse to select the tips to use.

If you change the assay plate type in the Plate Type category to a plate with a different number of wells (for example, from 96-wells to 384-wells), the Pipette Tips Layout category defaults to the selection of the full rack of tips.



CAUTION! You should use a full rack of tips each time you do a fluid transfer. If you configure 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 plate, the tips, and the instrument.

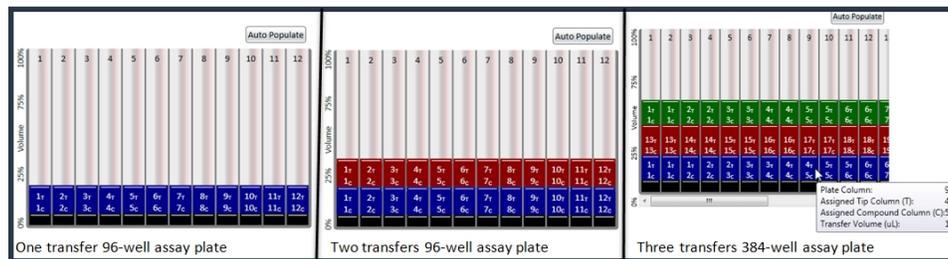
Compound and Tips Columns Settings

Use the Compound & Tips Columns category in the Settings dialog to define the tips and compounds to use for each transfer.

These settings are dependent on the selection you make in the Plate Type category for the assay plate and the number of transfers you define in the Compound Transfer category. See [Plate Type Settings on page 118](#) and [Compound Transfer Settings on page 147](#).

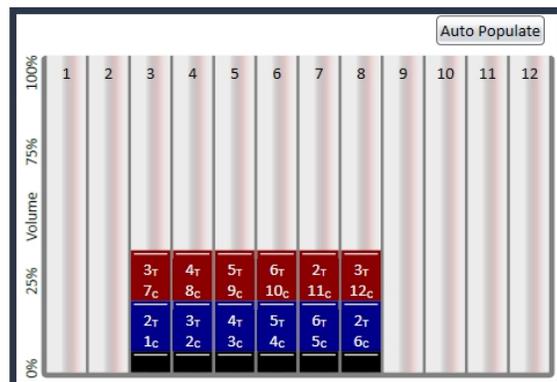
- For a 96-well assay plate, 12 columns display.
- For 384-well assay plate, use the scroll bar to view the additional columns.

Each transfer appears as a color coded row.



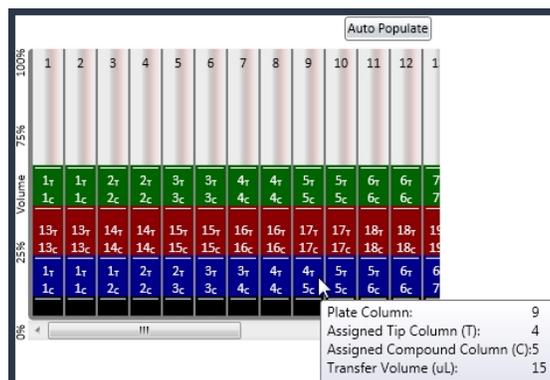
The black row at the bottom represents the initial volume in the wells based on your Initial Volume entry in the Compound Transfer category.

Your Read Area category setting also affects these settings. See [Read Area Settings on page 121](#).



Display for a read area that includes columns 3 - 8 on a 96-well assay plate

The left axis displays the dispensed compound volume as a percentage of the total well volume on the assay plate.



Each assay plate column has two indicators for each transfer:

- #T = Tips: Displays the column in the tip rack drawer from which the instrument will pull the tip for the transfer. You define available tips in the Pipette Tips Layout category. See [Pipette Tips Layout Settings on page 151](#).
- #C = Compound: Displays the column in the compound plate from which the instrument will aspirate the fluid to transfer to the assay plate for each pipetting event.
- Hover the mouse over a cell to display details including the transfer volume.



Tip: To leave the tips on between columns for the Flex read type, select the Leave Tips On between Columns check box in the Compound Transfer category.

Auto Populate

Use the Auto Populate feature to have the software assign the fluid to aspirate from the compound plate starting with the first available column. The instrument then dispenses the aspirated fluid to the first available column in the assay plate based on your Read Area category settings.

The software makes the assignments based on the following conditions:

- All columns you select in the Read Area category are to receive fluid.
- The fluids transfer 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 completes (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.

Example: Suppose you define the following settings for three fluid transfers:

- Read Area category: Columns 1 through 4
- Compound Source category: 12-column compound plate, such as a 96-well plate
- Pipette Tips Layout category: Full rack of tips

The software instructs the instrument to select tips from columns 1 - 4 in the tip rack drawer, to aspirate compound from columns 1 - 4 in the compound plate drawer, and to dispense the compound into the wells in columns 1 - 4 on the assay plate in the read chamber drawer. 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 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.

To have the software automatically assign the tips and columns:

- Click **Auto Populate**.

Manually Populate

For more complex assays, you can manually define the tips to use and the columns from which to aspirate compound.

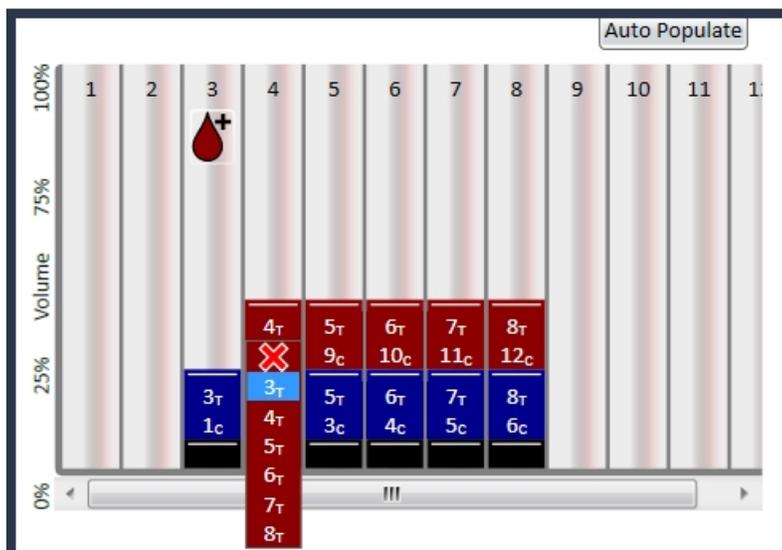


Image example displays:

Read Area: columns 3 - 8 on a 96-well assay plate

Compound Transfers: 2

Selecting a tip with Pipette Tips Layout: columns 3 - 8

No fluid transfer for compound 2 into column 3

You can manually assign tips and source columns for each and every transfer.

To assign each transfer a set of tips from the tip rack and a column from the source plate:

1. Click **T** in a column to display a drop-down list of the pipette tip columns you made available in the Pipette Tips Layout category. Select the column from which to have the instrument pull the tip from the instrument tip drawer.
2. Click **C** in a column to display a drop-down list of the number of columns in the compound plate from which the instrument can aspirate the compound. Select the column on the source plate from which to aspirate the compound.
3. If the assay requires that no fluid is to transfer to the assay plate for a column during one or more transfer operations, click **X** at the top of the drop-down list.  displays for the columns to skip.
4. To allow a transfer for a skipped column, click , then assign the tip and source columns for the transfer.

 **Tip:** To leave the tips on between columns for the Flex read type, select the Leave Tips On between Columns check box in the Compound Transfer category.

Triturate Settings

Use the Triturate category in the Settings dialog to define how to mix the contents of the wells in a plate by using the pipettor to alternately aspirate fluid from a well and then dispense it back into the well.

Trituration is recommended when you need to resuspend the compound in the source plate before it is added, when you transfer low volumes of fluid to the assay plate, or when you transfer fluid before or during an absorbance read to make sure that the well contents are properly mixed.

To define triturate settings:

1.  - Select the compound transfer to define and repeat the following steps for each applicable transfer.
2. Select the **Compound Source** check box to do trituration in the compound plate.
3. Select the **Assay Plate** check box to do trituration in the assay plate.



Note: Select both check boxes to do trituration in both plates.

4. In the **Volume** field, enter the volume of fluid in microliters to withdraw from the well.
5. In the **Cycles** field, enter the number of times to aspirate and dispense the fluid in the well.
6. For the Assay Plate, enter a value for the **Height** at which the trituration occurs. The height setting must consider the trituration volume you enter and the total volume in the well to have the tips remain below the liquid surface and not aspirate air.



Note: If you enter a Time Point that is incompatible with the Triturate settings, a message appears. You modify the Time Point in the Compound Transfer category. See [Compound Transfer Settings on page 147](#).

Acquisition View and Reads With Injectors

The Settings dialog for the SpectraMax i3, SpectraMax i3x, SpectraMax iD3, and SpectraMax iD5 provides two methods to configure a plate read.

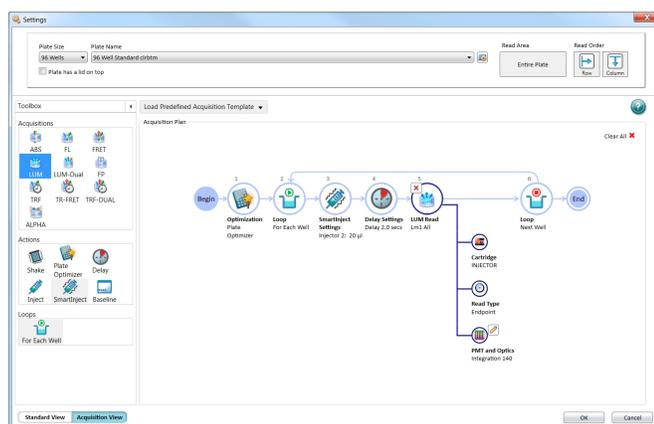
The Acquisition view uses an acquisition plan with tools that allow you to define settings in a workflow design when you need to do multi-step plate reads.

To switch between the views:

- Click **Standard View** **Acquisition View** in the lower-left corner in the Settings dialog.

You must use the Acquisition view to define reads with injection. If you define a read with injection and then switch to the Standard view, your settings are not saved.

The following image shows a read for a 96-well Standard plate of an entire plate with an Acquisition Plan for a Luminescence kinetic read with SmartInject™ technology. The Acquisition Plan starts with plate optimization and does 10 baseline reads before the injection step. It then delays one second after the injection ends before the Luminescence read starts. All the acquisition steps are in a Loop between the For Each Well node and the Next Well node.



To define plate settings:

1. Click the **Plate Size** drop-down and select the number of wells in the plate.
2. Click the **Plate Name** drop-down and select a plate.
3. Click  to display the Plate Editor dialog where you edit and create plates. See [Plate Editor on page 119](#).
4. Select the **Plate has a Lid on Top** check box if the plate has a lid.



Note: If the plate has a lid, you must verify that the plate height with a lid is set accurately in the Plate Editor dialog before you start a read.

5. Click **Read Area:**
 - Select the **Select All** check box to read all the wells in the plate.
 - To select a contiguous, rectangular region on the plate, drag the cursor and select the wells to read.
6. Select a **Read Order:**
 - Select **Row** to read the plate row-by-row.
 - Select **Column** to read the plate column-by-column.
 - To do a Well read order, add a For Each Well loop in the Acquisition view.

Toolbox and Acquisition Plan

The lower area of the dialog provides a toolbox that contains the available steps and the Acquisition Plan that displays the timeline of steps for the protocol.

-  **Shake** - Add a Shake step to have the instrument shake the plate in the chamber. Click  to display Shake settings where you define the shake duration and shake method. To shake the plate at the same time as an injection, add a SmartInject step not an Inject step or a Shake step.
-  **Plate Optimizer** - Add a Plate Optimizer node to have the software display the Pre-Read Optimization Options dialog before the plate is read. You can place this step only at the beginning of the Acquisition Plan. See [Show Pre-Read Optimization Options on page 145](#).
-  **Delay** - Add a Delay step after an Inject step or a SmartInject step to have the instrument wait after the injection before the read begins. Click  to display Delay settings where you define the number of seconds to wait between steps (from 0.001 seconds to 100 seconds). The Delay node compensates for the hardware delay. See below for details.
-  **Inject** - Add an Inject step for a simple injection. Click  to display Inject settings. 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 plate type you select. Define the dispense volume based on the requirements of your assay and the net volume available in the well. To shake the plate after an injection, add a Shake step after the Inject step. To shake the plate during an injection, use a SmartInject step not an Inject step.
-  **Smart Inject** - Add a SmartInject step to shake the plate during an injection. The plate continues to shake through the following delay step, if you add a Delay step and stops shaking before the instrument reads the well. Click  to display SmartInject settings. 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 plate type you select. Define the dispense volume based on the requirements of your assay and the net volume available in the well.
-  **Baseline** - For the kinetic read type, add a Baseline step before an Inject step or a SmartInject step to have the instrument take baseline reads. Click  and enter the number of baseline reads (from 1 to 999). You can then subtract the baseline data from the reads that follow the injection. You can use the Formula Editor dialog to automate baseline subtraction.
-  **For Each Well** - Reads with injection are generally done well-by-well. Use the For Each Well loop to have the instrument run all the steps in the Acquisition Plan 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 a Next Well node to the end of the plan. Add the steps for the assay between the For Each Well node and the Next Well node.

Creating Acquisition Plans

The steps in the Acquisition Plan occur in sequence from left to right between the Begin node and the End node. When a step has multiple settings, the settings display below the step in the timeline.

To create an acquisition plan:

1. To start from scratch, click **Clear All** or click the **Load Predefined Acquisition Template** drop-down and select a predefined Acquisition Plan.
2. In the Toolbox area on the left, either double-click or drag **Acquisitions, Actions, and Loops** to the Acquisition Plan. The software allows you to place each step in an appropriate place in the plan. Example: You can place the Plate Optimizer node only at the beginning of the plan.
3. When you add a step to the Acquisition Plan, a prompt appears:
 - Some Acquisitions require you to select hardware for the optical configuration. This can be the monochromator or a detection cartridge for the SpectraMax i3x. See [Supported Detection Cartridges on page 375](#).
 - All Acquisitions require you to select a read type. See [Endpoint Read Type on page 277](#), [Kinetic Read Type on page 278](#), [Spectrum Read Type on page 279](#), and [Well Scan Read Type on page 279](#).
4. Add Actions and Loops.
5. Add an injector step or a SmartInject step.
6. Click a node to display  .
 - Click  to delete the node.
 - Click  to display settings for the node.
 - [Wavelength Settings, see page 115](#)
 - [PathCheck Settings, see page 122](#)
 - [PMT and Optics Settings, see page 123](#)
 - [Timing Settings, see page 125](#)
 - [Well Scan Settings, see page 126](#)
 - [Shake Settings, see page 127](#)
 - [Speed Read Settings, see page 127](#)
 - [Normalization, see page 146](#) (for SpectraMax i3x)
 - [Interlaced Reading, see page 146](#)
7. After you define the settings for a step in the Acquisition Plan, click the Workspace outside of the settings.
8. After you define the instrument settings, click **OK** to close the Settings dialog.
9. Before you start a read, you should save the document. See [Saving Protocols and Data Documents on page 29](#).

SpectraMax i3x Injector Reads

To define injector reads for the SpectraMax i3x:

1. Install the SpectraMax Injector cartridge into the cartridge drawer.
2. When you add a Luminescence read to the Acquisition Plan, select **INJECTOR** from the Select Hardware list to use the SpectraMax Injector cartridge for the read. A Luminescence read with injection is taken from the top of the plate.
3. When you add a Fluorescence Intensity read to the Acquisition Plan, select **MONO** from the Select Hardware list to use the instrument's built-in monochromator for the read.

Delay Details

SpectraMax 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.

SpectraMax iD3 and SpectraMax iD5 Delay

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, 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.

- 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 injection ends.

Creating Absorbance Mode Protocols

To create an Absorbance Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or a Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x, select **Monochromator**. For the SpectraMax Paradigm, select the **Absorbance detection cartridge**. See [Absorbance Detection Cartridge on page 375](#).
7. Select the  **ABS** read mode.
8. Select a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

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 intensity of the incident light before it enters the sample divided by the light after it passes through the sample, and A is the measured absorbance.

Applications of Absorbance

Absorbance measurements are used to quantify the concentration of an absorbing species. It must be known at what wavelength a species absorbs light. For example, the concentration of nucleic acids can be quantified by measuring the absorbance at 260 nm.

Optimizing Absorbance Assays

You can adjust the wavelength of the incident light in 1 nm increments. The software allows you to read up to six wavelengths per plate or cuvette for Endpoint and Kinetic read types and up to four wavelengths for Well Scan reads.

It is good scientific practice to designate wells in the plate to serve as plate blanks or group blanks in the Template Editor dialog from a Plate section. See [Template Editor on page 91](#).

You can use the PathCheck technology feature to normalize the data to a 1 cm pathlength. See [PathCheck Technology on page 282](#).

Creating AlphaScreen Mode Protocols

To create an AlphaScreen Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select the SpectraMax i3x or SpectraMax Paradigm. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. Select an AlphaScreen detection cartridge. See [AlphaScreen Detection Cartridges on page 376](#).
7. Select the  **Screen** read mode.
8. Select a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

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 result signal is triggered by laser excitation (680 nm), that makes a photosensitizer on the A-beads convert oxygen to an excited (singlet) state. The 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 to generate 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.

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.

Creating Fluorescence Intensity Mode Protocols

To create a Fluorescence Intensity protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x, select either **Monochromator** or select a Fluorescence Intensity cartridge. For the SpectraMax Paradigm, select a Fluorescence Intensity cartridge. See [Fluorescence Intensity Detection Cartridges on page 376](#)
7. Select the  **FL** read mode.
8. Select a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

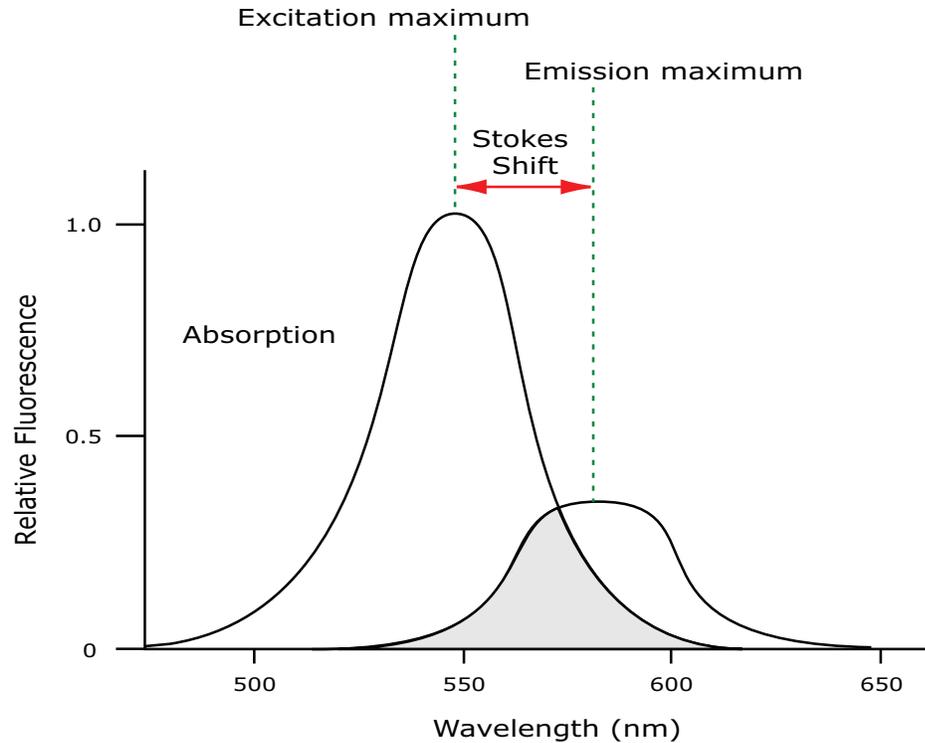
Fluorescence Intensity Mode Overview

Fluorescence occurs when absorbed light is re-radiated at a longer wavelength. In the Fluorescence Intensity 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:

$$\text{Fluorescence} = \text{extinction coefficient} \times \text{concentration} \times \text{quantum yield} \times \text{excitation intensity} \times \text{pathlength} \times \text{emission collection efficiency}$$

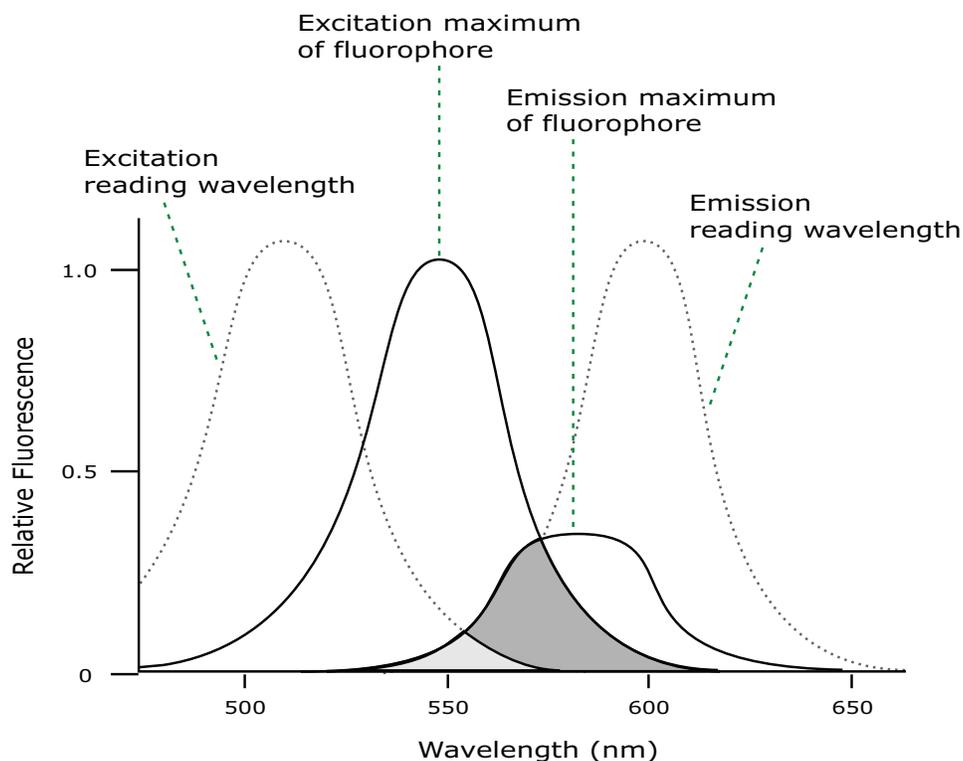
Fluorescent materials absorb light energy of a characteristic wavelength (excitation), undergo an electronic state change, and instantaneously emit light of a longer wavelength (emission). Most common fluorescent materials have well-characterized excitation and emission spectra. The following figure 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.



*** 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.



The previous figure shows that the best results are often obtained when the excitation and emission wavelengths used for reads are not the same as the peak wavelengths or maxima of the excitation and emission spectra of the fluorophore. When the 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 permit 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.

Optimizing Fluorescence Intensity Assays

The optimal instrument settings for detection of a particular fluorophore depend on a number of different factors. Settings you can adjust for assay optimization include the excitation and emission wavelengths, emission cutoff filter, reads per well, the PMT voltage, and the temperature of the read 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 165](#).
- **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 the software determine which cutoff filter to use for the endpoint and kinetic read types. The spectrum read type default uses no cutoff filter.
- **Reads Per Well**

The number of reads per well can vary between 1 (for a quick estimate) and 100 (for very precise measurements). The default number of reads per well varies with the read mode: for Fluorescence, the default is 6, and for Luminescence the instrument does 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) for all read modes. For the endpoint and spectrum read types, you can select Automatic to have the instrument automatically adjust 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 read chamber can be adjusted from 2°C (3.6°F) above ambient to the maximum temperature for the instrument. See the instrument user guide.

Spectral Scanning

This procedure optimizes excitation and emission wavelengths for Fluorescence assays using spectral scanning.

For the SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm with a Tunable Wavelength (TUNE) detection cartridge, see [Spectral Optimization on page 183](#).

Pipette 200 µL of sample that includes the fluorophore and 200 µL of a buffer control into separate wells of the plate.

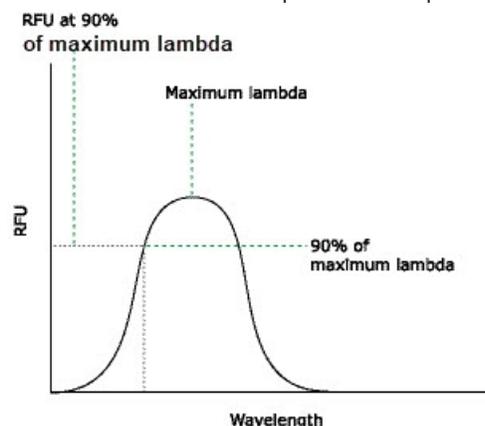
Excitation Scan

1. Set up a Plate section for the Fluorescence read mode, spectrum read type, 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 the 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 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 a Missing Data Points message occurs, it can be due to possible saturation reported by the software 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 you scan with the PMT set to Low, you may need 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.



Plot of RFU and Wavelength

Emission Scan #1

1. Set up a second Plate section for the Fluorescence read mode, spectrum read type, Ex Fixed/Em Scan, with no cutoff filter (default), and Medium PMT.
2. Set the excitation wavelength to the value you determine in the Excitation Scan section above.
3. Set the emission scan to start/stop approximately 50 nm below or above the tentative emission value 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, contact Molecular Devices Technical Support and speak to an application scientist.

Emission Scan #2

1. Set up a third Plate section for an emission scan as specified in Emission Scan #1, except select the Manual Cutoff Filter and set the wavelength to the value you determine in the Choose the Emission Filter section above.
2. Do the scan and view the results as a plot of fluorescence vs. emission wavelength. Note the wavelength that gives the maximum emission (the optimal emission wavelength).
3. Compare the spectra of the sample that contains 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 the Choose the Emission Filter steps and the steps in this section with a different choice of cutoff filter.

Results

The optimal excitation and emission wavelengths are those you determine in the Excitation Scan section and the Emission Scan #2 section.

Comments

- For the Fluorescence read mode with the endpoint or kinetic read type, the **AutoCutoff** feature generally selects the same cutoff filter wavelength as the previously described optimization method. However, you can specify the cutoff filters manually.
- For emission wavelengths less than 325 nanometers, experimental iteration is usually the best method to determine 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. 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.

Emission Cutoff Filter Default Settings For FlexStation 3, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, and SpectraMax M5e

#	Automatic Cutoff Selection Wavelength (nm)	Endpoint and Kinetic Modes Emission Wavelength (nm)
1	None	< 415
2	420	415–434
3	435	435–454
4	455	455–474
5	475	475–494
6	495	495–514
7	515	515–529
8	530	530–549
9	550	550–569
10	570	570–589
11	590	590–609
12	610	610–629
13	630	630–664
14	665	665–694
15	695	695–850

Creating Fluorescence Polarization Mode Protocols

To create a Fluorescence Polarization Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x and SpectraMax Paradigm, select a Fluorescence Polarization (FP) detection cartridge. See [Fluorescence Polarization Detection Cartridges on page 378](#).
7. Select the  FP read mode.
8. Select a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

Fluorescence Polarization Mode Protocol Overview

Fluorescence Polarization detection is based on Fluorescence Intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Microplate readers measure the Fluorescence Polarization 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, the binding of a small molecule to an interaction partner of equal or greater size can be monitored through its speed of rotation.

The Fluorescence Polarization read 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. The software uses the S and P values to calculate the Polarization (mP) and Anisotropy (r) values.

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 raw values that display are exported, whether mP, r, or Raw (S&P).

Polarization (mP) is calculated as follows:

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

Anisotropy (r) is calculated as follows:

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

Optimizing Fluorescence Polarization Assays

Fluorescence Polarization can be read from the top of a plate. The plastic from which a plate is formed has an effect on the light polarization, precluding bottom reads and reads of a covered plate from the top.

You should use solid black plates for reads. If the assay components seem to bind to the plate, as evidenced by poor mP dynamic range (small difference between bound and unbound tracer), you should use plates treated to minimize binding, or polypropylene plates, and add a very small quantity of detergent to the assay buffer.

Background wells, which contain all assay components minus the fluorophore, should be run alongside samples. If the signal in the background wells is more than 1/10 the signal in the wells that contain fluorophore, then background wells should be run on each assay plate. The average raw signal from the background's parallel and perpendicular reads should be subtracted from the raw parallel and perpendicular reads of each sample well before the mP calculation is done.

For best precision in assays that uses a low quantity of fluorophore (for example, <5 nm fluorescein), set the PMT sensitivity to High and the number of reads to 100. If a faster read speed is required, experiment with fewer flashes per well until acceptable precision and speed are achieved. Fewer flashes result in a higher speed, but with less precision.

Creating FRET Mode Protocols

To create a Fluorescence Resonance Energy Transfer (FRET) protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x and SpectraMax Paradigm select a FRET-capable detection cartridge. See [FRET Detection Cartridges on page 378](#).
7. Select the  FRET read mode.
8. Select a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

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, you can use FRET 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.

Creating Imaging Mode Protocols

To create an Imaging mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select the SpectraMax i3x. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. Select  **MiniMax** in the Optical Configuration list.
7. Select the **Imaging** read mode.
If you have previously acquired images and want to make changes to the analysis settings for those images, select the **Re-analysis** read mode.
8. 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 Software application help.
9. After you define the instrument settings, click **OK** to close the Settings dialog.
10. Create a template, if applicable. See [Template Editor on page 91](#).
11. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
12. Set Display options, if applicable. See [Data Display Options on page 206](#).
13. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

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 plate 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 plate well.

The measurement is primarily fluorescent with quantification of cell size, shape, area, and intensity. Brightfield, transmitted light imaging supports label-free quantification. 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.

Optimizing the Computer for Image Acquisition

Acquiring images requires a large portion of computer memory and resources. When the 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. Turn off all other programs to minimize the demands on computer memory and resources.

Before you start an image acquisition, save the protocol to a .txt file format, in a location with enough capacity for the image files. For best results, save Imaging mode files on the secondary hard drive inside the computer. If you use Auto Save to save multiple Imaging mode documents, the software saves each new document without the folders required to save images and analysis results.



Note: Use of an external hard drive can slow the data acquisition and is not recommended. Use of a USB flash drive or saving to a network location is not supported.

When you create a document for an Imaging mode experiment or a Western Blot experiment, the software creates a folder with the same name as the document. Each image file can be larger than 2 megabytes. The software can generate 300 megabytes of image data when you acquire the image of a single site in each well of a 96-well plate. A 384-well plate can generate 1 gigabyte of image data. When you acquire images of multiple sites, you increase the data storage requirement.

Zooming Image in a Well

An image of the selected well displays in the Image Acquisition, Image Selection, and Region of Interest categories in the Settings dialog.

To view a larger version of the image area:

- Double-click the image to display the Zoom Well dialog.

In the Zoom Well dialog, the image area displays the acquired image from the selected well.

To zoom the image in and out:

- Right-click the image and select **Zoom In** or **Zoom Out**,
- Hold the **CTRL** key and press + or -.
- Use the mouse wheel to zoom in or out.

To move the image in the image area:

- Use the scroll bars or click the image and drag it into position.

Creating Luminescence Mode Protocols

The SpectraMax L has a unique set of Luminescence Mode settings. For details see the *SpectraMax L Luminescence Microplate Reader User Guide*.

To create a Luminescence Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x, select either **Monochromator** or select a Luminescence cartridge. For the SpectraMax Paradigm, select a Luminescence cartridge. See [Luminescence Detection Cartridges on page 379](#).
7. Select the  **LUM** read mode.
8. Select a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

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 reads are averaged together into one read 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. 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.

Optimizing Luminescence Assays

Luminescence can be read from the top or the bottom of a plate. You should use solid white plates or white plates with clear bottoms 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. In the Settings dialog the emission says **All**.

For wavelength-selectable luminescence, the instrument uses the emission monochromator to differentiate the wavelengths being emitted from the well. You can define multiple emission wavelengths.

To read 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 the total integration time you enter (between 1 ms and 1500 ms). Typical luminescence assays require between 500 ms and 1000 ms integration.

If wells are incubated for a long period of time, you should mix the plate before the read. 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), you should dark adapt the plate to reduce the auto-luminescence of the white plastic. To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

Creating Time-Resolved Fluorescence Mode Protocols

To create a Time-Resolved Fluorescence Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x and SpectraMax Paradigm, select a TRF-capable detection cartridge. See [Time Resolved Fluorescence Detection Cartridges on page 382](#).
7. Click  **TRF** to select the **Time-Resolved Fluorescence** read mode.
8. Click a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

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 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 cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) 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 reads are taken while the lamp is on. The most common limitation to sensitivity in normal fluorescence is excitation energy or background fluorescence that cannot be eliminated from the emission signal. Since the lamp is the source of excitation energy, turning it off provides the best means of eliminating background excitation. The elimination of background excitation is the critical difference between fluorescence intensity measurements and TRF measurements.

Optimizing Time-Resolved Fluorescence Assays

For the Time-Resolved Fluorescence read mode, the instrument flashes the excitation lamp, then, after the lamp is off, collects 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 fades, while lanthanide chelates and cryptates have fluorescent lifetimes between 100 μs and 2 ms.

Time-Resolved Fluorescence assays can be read from the top or bottom of a plate. You should use solid white plates for top reads, and white plates with clear bottoms for bottom reads.

To optimize data collection for a particular assay, you define when to start and end data acquisition. The minimum is 50 μs after the lamp turns off and the maximum is 1450 μs , in 50 μs or 200 μs steps.

If the assay you use has low signal or gives results with high %CV, use 100 reads per well. If you require a faster read speed, experiment with fewer flashes per well until you achieve acceptable precision and speed.

The important settings to get the best results in Time-Resolved Fluorescence assays are integration delay and integration time.

1. Click the **Integration Delay** drop-down and select the amount of time to elapse between the flash of the lamp (excitation) and the beginning of data acquisition from the well (emission).
2. Click the **Integration Time** drop-down and select the amount of time for the instrument to read the well.

The integration delay and integration time are usually specified in the package insert of commercially available Time-Resolved Fluorescence reagent kits. If you do not use a kit, start with a delay of 50 μs and try different delays up to 400 μs with a fixed integration time of 400 μs . After you choose the optimal delay, based on the highest ratio of a well that contains a fluorophore divided by wells that contain 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

Creating TR-FRET Mode Protocols

To create a TR-FRET Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select an instrument. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation Tree, select a Plate section or Cuvette Set section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. For the SpectraMax i3x and SpectraMax Paradigm, select the Cisbio HTRF Detection Cartridge. See [TR-FRET Detection Cartridge on page 383](#).
7. Click  **TR-FRET** to select the **TR-FRET** read mode.
8. Click a Read Type.
9. Define the settings.
For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Create a template, if applicable. See [Template Editor on page 91](#).
12. Set Data Reduction parameters, if applicable. See [Data Reduction on page 196](#).
13. Set Display options, if applicable. See [Data Display Options on page 206](#).
14. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

TR-FRET Mode Overview

TR-FRET is a measurement technique based on fluorescence resonance energy transfer (FRET) using the advantages of time-resolved fluorescence (TRF) read. TR-FRET read mode requires the installation of the Enhanced TRF module in the SpectraMax iD5 or the Cisbio HTRF detection cartridge in the SpectraMax i3x or SpectraMax Paradigm.

TR-FRET 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 plate 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.

Creating Western Blot TRF Mode Protocols

To create a Western Blot TRF Mode protocol:

1. Start the software. See [Starting the Software on page 9](#).
2. Select the SpectraMax i3x, SpectraMax iD5, or SpectraMax Paradigm. See [Selecting an Instrument on page 64](#).
3. Open a document.
4. In the Navigation tree, select a Plate section.
5. Click  in the section toolbar, the Settings Information area, or on the Home tab to display the Settings dialog.
6. Select the **SCANLATER WB** cartridge for the ScanLater Western Blot (WB) detection cartridge. (Skip this step for the SpectraMax iD5.) See [ScanLater Western Blot Detection Cartridge on page 380](#).
7. Select the  TRF read mode.
8. Select the  **Membrane** read type.
9. Define the settings. For more information about the settings, see the SoftMax Pro Software Application Help.
10. After you define the instrument settings, click **OK** to close the Settings dialog.
11. Save the settings to a protocol. See [Saving Protocols and Data Documents on page 29](#).

Western Blot TRF Mode Overview

Western Blots (WB), or protein immunoblots, are one of the most common methods for protein detection in pharmaceutical and clinical research. You can use various techniques to detect proteins on Western Blot membranes including fluorescence, silver staining, and chemiluminescence. Each technique has its limitations. 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. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that you can use with the SpectraMax i3x, SpectraMax iD5, and SpectraMax Paradigm. 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 the instrument where the membranes are scanned with the ScanLater Western Blot (WB) detection cartridge (SpectraMax iD5 uses the Enhanced TRF module).

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 allows you repeat the read of membranes and the potential for comparison of band intensities to known standards for more accurate quantitation. 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 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.

The SpectraMax Western Blot system is a simple, sensitive, and stable platform that provides excellent Western Blot capability for the SpectraMax i3x, SpectraMax iD5, and SpectraMax Paradigm to extend the uses of these essential laboratory instruments.

Loading the Membrane Holder

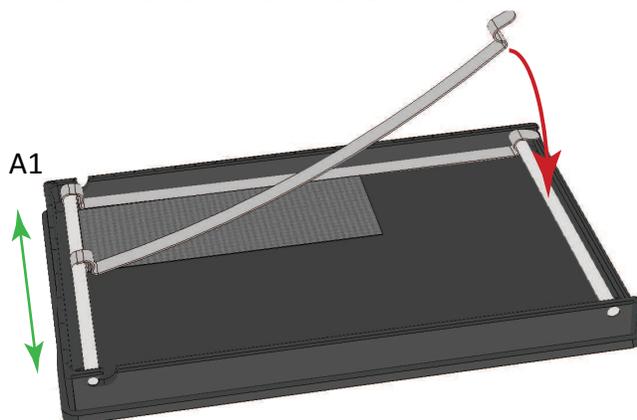


Note: Handle membranes only by the edges using clean forceps. Take care to not touch the membrane with gloved or bare hands.

You must place the membrane in a Molecular Devices ScanLater™ membrane holder to scan a membrane. The maximum size of a membrane that fits in the membrane holder is 109 mm x 77 mm.

To load 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 instrument.

4. Load the membrane holder into the plate drawer with the A1 corner in the A1 position of the drawer. See the user guide for your instrument.

Collecting Data From Plates

You can start a read after you define the acquisition settings. You do not need to define groups and assign wells in the Template Editor dialog before the read. The values that the software receives from the instrument are raw values. The settings in the Template Editor have no effect on these raw values.

The SoftMax Pro Options dialog has an option to have the plate remain inside the instrument after the read completes. See [SoftMax Pro Options on page 52](#).

Use auto read to read multiple Plate sections in the same experiment. See [Using Auto Read on page 182](#).

To collect data from plates:

1. To open the plate drawer of the instrument, select the **Home** tab and click  **Open/Close**. See [Home Tab on page 55](#).

The plate carrier on the EMax Plus moves in and out of the read chamber during the read, and then returns to the home position after the read ends. If the plate carrier does not return to the home position, select the Operations tab and click **Initialize**.

2. Insert the prepared plate matching well A1 with position A1 in the plate drawer. Make sure the plate is flat against the drawer bottom or against the adapter.
3. Click  **Open/Close** to close the plate drawer.
4. Open the document that contains the acquisition settings for the plate read. As an alternative, create new acquisition settings for the Plate section in the Settings dialog. See [Acquisition Settings on page 112](#).
5. Save the document before you start a read. For Imaging reads and Western Blot reads, you must save the document before you start the read.
6. Click  **Read** below the Settings Information area or on the Home tab.

The Read button changes to  **Stop** so you can terminate a read.

7. The software reads the Plate section you select.
 - For the FilterMax F3, FilterMax F5, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm, when you select to do pre-read optimizations, the Pre-Read Optimization Options dialog displays. See [Pre-Read Optimization Options on page 185](#).
 - For the SpectraMax i3x and SpectraMax Paradigm with a Tunable Wavelength (TUNE) detection cartridge, when you select to run spectral optimization, the Spectral Optimization Wizard runs before the instrument reads the plate. See [Spectral Optimization on page 183](#).
8. After the read completes, the plate drawer opens so you can remove the plate. If temperature control is on, the drawer closes again after approximately 10 seconds.
9. After the read completes, save the document and do any additional data analysis.

Using Auto Read

Use auto read to have the software automatically advance to the next Plate section after a read. The reads progress in the sequence that the Plate sections display within an experiment in the Navigation Tree. When you start a read for a Plate section that you configure to automatically advance with Auto Read, the software advances to the next Plate section in the experiment and then reads that plate. If you configure multiple Plate sections to automatically advance with Auto Read, 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. The software does not read the Plate sections that appear above the Plate section you select in the Navigation Tree.

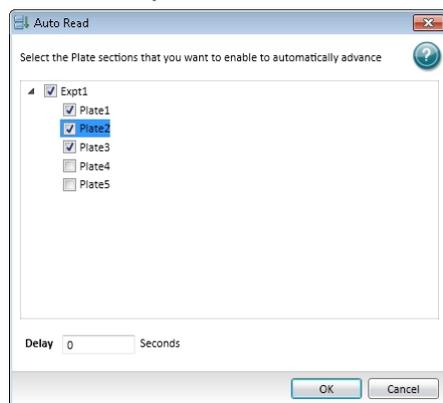
For example, if you have five Plate sections (Plate 1, Plate 2, Plate 3, Plate 4, and Plate 5) in an experiment and you configure Plate 1, Plate 2, and Plate 3 to automatically advance with Auto Read, if you select Plate 2 when you start the read, the software reads Plate 2, Plate 3, and Plate 4 in sequence. Plate 1 is not read, since it comes before Plate 2. Plate 5 is not read since Plate 4 is not configured to automatically advance with Auto Read.

The software collects the data using the instrument settings that are specific for each Plate section and stores the data in a single document.

You can set an interval, or delay time, between plate reads.

To enable Auto Read:

1. In the Navigation Tree, select the experiment that has the Plate sections you want to automatically advance with Auto Read.
2. Select the **Operations** tab and click  **Auto Read** to display the Auto Read dialog.



3. Select the check box for each Plate section you want to automatically advance with Auto Read.
4. In the **Delay** field, enter the number of seconds to delay (the interval) between each Plate section read.
5. Click **OK**.



Note: If you have a Cuvette Set section that follows an enabled Plate section, Auto Read does not advance to or beyond the Cuvette Set section.

Spectral Optimization

Spectral optimization is available for Fluorescence Intensity reads on the SpectraMax i3x (using the instrument's built-in monochromator), SpectraMax iD3, SpectraMax iD5, and SpectraMax Paradigm when you install the Tunable Wavelength (TUNE) detection cartridge.

Spectral optimization in spectral assays improves accuracy while reducing data acquisition and computational burden. Spectral optimization helps 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.

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.

The start emission wavelength value must be a minimum of 20 nm greater than the start excitation wavelength value. You should use an emission value that is a minimum of 40 nm greater than the excitation value.

To add spectral optimization to a protocol:

1. In the Navigation Tree, select a Plate section.
2. Click  in the section toolbar, in the Settings Information area, or on the Home tab to display the Settings dialog.
3. Select the **Fluorescence Intensity** read mode and any read type other than spectrum. For the SpectraMax Paradigm you can select the **Luminescence** read mode.
4. On the left, select the **Wavelengths** category.
5. Select the **Unknown** option.
6. Define all acquisition settings and click **OK** to close the Settings dialog.

Spectral Optimization Wizard

To run spectral optimization:

1. In the Navigation Tree, select the Plate section.
2. Click  **Read** below the Settings Information area or on the Home tab to display the Spectral Optimization Wizard.
3. On the Read Settings page:
 - a. Enter the wavelength ranges and other optimization parameters. You can enter only emission values for Luminescence reads.
 - b. Click **Advanced Parameters** to display advanced parameters.
 - c. In the **Reading Height** field, enter the read height.
 - d. In the **Integration Time** field, enter the integration time.
 - e. For Time-Resolved Fluorescence reads, enter **Pulse Length**, **Number of Pulses**, and **Measurement Delay**.
 - f. Click **Next** to display the Blank Well page.
4. On the Blank Well page:
 - a. Select the well that corresponds with a well that contains buffer or other liquid for background correction.
 - b. Click **Next** to display the Sample Well page.
5. On the Sample Well page:
 - a. Select the well that corresponds with a well that contains your sample.
 - b. Click **Next** and wait while the indicator displays the progress.

6. On the Optimization Complete page:
 - a. View the 3-dimensional heat map image. The software uses the formula $(S - B) / B$ to generate, where S = signal and B = background.



Note: For the Luminescence read mode, this is a 2-dimensional graph.

- b. In the image, locate the cross hair that indicates the optimized peak wavelengths.
7. To change the wavelengths for the read, either:
 - Drag the cross hair to a new location
 - Enter values in the **Excitation** field and the **Emission** field. The Emission value must be a minimum of 20 nm greater than the Excitation value. Use an emission value that is a minimum of 40 nm greater than the excitation value. You can specify only emission values for Luminescence reads.
8. Select the **Smoothen** check box to average the neighboring values along the excitation and emission axis (total of nine values) to smooth ghost peaks and help find the best wavelength more efficiently.
Clear the **Smoothen** check box to display the actual peak values.
9. Select the **Use Log Scale** check box to use log scale for the display of the heat map image.
Clear the **Use Log Scale** check box to not use log scale.
10. If you edit the Wavelength (Custom) values, click **Reset to Optimized Peak** to reset the values to the software defined optimized peak.
11. Right-click on the image:
 - Select **Copy Raw Data** to copy the data in a tab-delimited format that you can paste into a spreadsheet or text editor (not available for the SpectraMax Paradigm).
 - Select **Copy Image** to copy the image into a bitmap file that you can paste into an image editing program.
 - Select **Save Raw Data to Disk** to save the data into a text file in tab delimited format (not available for the SpectraMax Paradigm).
 - Select **Save Image to Disk** to save the image into a .bmp file.
12. Click **Restart Wizard** to redefine the settings and run the wizard again.
13. To save the wavelength values without reading the plate, click **Cancel** and then click **Yes** in the message.
14. Click **Read** to save the protocol with the specified excitation and emission wavelengths and use these values to read the plate.

Pre-Read Optimization Options

Use the Pre-Read Optimization Options dialog to run the Microplate Optimization wizard and the Read Height Optimization wizard before the instrument reads the plate.

Plate dimensions can vary slightly between production lots, which can have a potential effect on measurement accuracy. Use the software to optimize plate dimensions by determining the centers of the four corner wells on the plate. Each time you optimize a plate, the software adds a new plate definition with dimensions specific to that lot.

For the FilterMax F3, FilterMax F5, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm, in certain read modes, you can use pre-read optimization to do plate optimization and read height adjustment. The settings are dependent on the instrument and the read mode.

To have the Pre-Read Optimization Options dialog display before a plate read:

1. In the Navigation Tree, select a Plate section.
2. Click  in the section toolbar, in the Settings Information area, or on the Home tab to display the Settings dialog.
3. On the left, select the **More Settings** category.
4. Select the **Show Pre-Read Optimization Options** check box to display the Pre-Read Optimization Options dialog before each plate read.
Clear the **Show Pre-Read Optimization Options** check box to not display the Pre-Read Optimization Options dialog before each plate read.
From the Acquisition view, add a **Plate Optimizer** node to the beginning of the Acquisition Plan.
5. Define all acquisition settings and click **OK** to close the Settings dialog.

Run Pre-Read Optimization Options

To run a protocol with pre-read optimization options:

1. In the Navigation Tree, select the Plate section.
2. Click  **Read** below the Settings Information area or on the Home tab to display the Pre-Read Optimization Options dialog.
3. Select the **Run Microplate Optimization...** check box to run the Microplate Optimization wizard before the plate read.
Clear the **Run Microplate Optimization...** check box to not run the Microplate Optimization wizard before the plate read.
4. Select the **Run Read Height Adjustment...** check box to run the Read Height Optimization wizard before the plate read.
Clear the **Run Read Height Adjustment...** check box to not run the Read Height Optimization wizard before the plate read.
5. Select the **Plate is Lidded** check box if the plate has a lid.
Clear the **Plate is Lidded** check box if the plate does not have a lid.

6. Some instruments allow you to select the plate orientation. If you change the plate orientation for a read, you must do plate optimization for each plate orientation separately. Select a Microplate orientation option:
 - 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.
7. Click **Run Optimization** to run the optimization options.
 - If you select the Run Microplate Optimization... check box, the Microplate Optimization wizard displays.
 - If you select the Run Read Height Adjustment... check box, the Read Height Optimization wizard displays. If you select both check boxes the Read Height Optimization wizard displays after you finish the Microplate Optimization wizard.
8. After the wizards run, the Pre-Read Optimization Options dialog displays. Click **Read Plate** to start the read.

Microplate Optimization Wizard

Use the Microplate Optimization wizard to optimize the plate dimensions to determine the centers of the four corner wells on the plate. Each time you optimize a plate, the software adds a new plate to the plate library.

To do plate optimization:

1. In the Pre-Read Optimization Options dialog, select the **Run Microplate Optimization Before Reading the Plate** check box.
2. Select the **Plate Is Lidded** check box if the plate has a lid.
Clear the **Plate Is Lidded** check box if the plate does not have a lid.
3. Some instruments allow you to select the plate orientation. If you change the plate orientation for a read, you must do plate optimization for each plate orientation separately. Select a Microplate orientation option:
 - 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.
4. Click **Run Optimization** to display the Microplate Optimization wizard.

5. On the Insert the Microplate page:
 - a. Click  **Open the Microplate Drawer** to move the plate drawer outside the instrument.
 - b. Fill the corner wells of the plate with identical samples. To ensure accuracy, samples must be applicable for the read mode. Sample concentration and volume must be identical in each well.
 - c. Place the prepared plate on the plate drawer.
 - d. Click  **Close the Microplate Drawer** to load the plate into the instrument.
 - e. Select the **Select the Microplate Orientation** option that matches the orientation of the plate in the plate drawer and is the same as the orientation you select in the Pre-Read Optimization Options dialog. The orientation displays on the right, with well A1 marked.
 - f. Click **Next** to start the optimization.
6. On the Optimize page:
 - Wait for the software to optimize the plate.
 - If needed, click **Stop Optimization** to stop the plate optimization.
7. On the Select the Center of the Up... page:
 - a. Drag the cross hair to the center of the upper left well.
 - b. Click **Next**.
8. On the Select the Center of the Lo... page:
 - a. Drag the cross hair to the center of the lower left well.
 - b. Click **Next**.
9. On the Select the Center of the Up... page:
 - a. Drag the cross hair to the center of the upper right well.
 - b. Click **Next**.
10. On the Select the Center of the Lo... page:
 - a. Drag the cross hair to the center of the lower right well.
 - b. Click **Next**.
11. On the Verify Well Centers page:
 - a. Verify that the x and y offset and distances between rows and columns are correct. If needed, click in each **Microplate Dimensions** field and enter the dimension in millimeters.
 - b. In the **Microplate Name** field, enter the name of the plate.
12. Click **Save** to add the plate definition to the plate library and to either return to the Pre-Read Optimization dialog or to start the Read Height Optimization wizard.

Read Height Optimization Wizard

When the instrument features an objective lens, use the Read Height Optimization Wizard to adjust the read height for the Luminescence, Fluorescence Intensity, Fluorescence Polarization, and Time-Resolved Fluorescence read modes. Read height is the distance between the top (for top reads) or bottom (for bottom read) of the plate and the objective lens. You optimize the read height to match the focus of the optics with the sample volume. This maximizes the raw signal, which yields the highest precision and maximum sensitivity.

Use a standard or sample position with a good signal or pipette liquid with a known maximum signal into a well. 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 you use a layout with standards, the software selects the standard well closest to the center of the plate. If you do not use a layout with standards, the software selects the first sample.



Note: When you optimize read height for the Fluorescence read mode, make sure the optimization sample is the same fluorescent substance that the detection method is configured to detect.

To use the Read Height Optimization wizard:

1. In a plate of the same type for the read, place a single sample that has a known maximum signal and volume.
2. Place the plate into the instrument plate chamber.
3. In the Pre-Read Optimization Options dialog, select the **Run Read Height Adjustment...** check box.
4. Select the **Plate Is Lidded** check box if the plate has a lid.
Clear the **Plate Is Lidded** check box if the plate does not have a lid.
5. Some instruments allow you to select the plate orientation. If you change the plate orientation for a read, you must do read height optimization for each plate orientation separately. Select a Microplate orientation option:
 - 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.
6. Click **Run Optimization** to display the Read Height Optimization wizard.
7. On the Select Well page:
 - a. Select the well in the plate image that contains the sample.
 - b. Click **Next**.
8. On the Optimize page:
 - Wait for the software to optimize. Optimization can take from several seconds to several minutes depending on the detection methods you use.
9. On the Optimization Complete page:
 - a. To specify a different read height, enter a value in the **Custom Read Height** field, in millimeters.
 - b. Click **Save** to save the read height with the protocol and to display the Pre-Read Optimization dialog. The software uses this read height for all subsequent runs of the protocol until you either perform a new optimization or you manually enter a read height.
10. Click **Read Plate** to start the plate read.

Collecting Data From Cuvettes

Two types of data collection are possible when you use a cuvette: a sample read and a reference read.

You can start a read after you define the acquisition settings. You do not need to define groups and assign cuvettes in the Template Editor dialog before the read, 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 the  New Cuvette Set icon in the Navigation Tree.

Instrument settings must be the same for all cuvettes in a Cuvette Set section.

Cuvette Set sections have a limit of 96 cuvettes. If you reach this limit, create a new Cuvette Set section.

Sample Read

If you select more than one cuvette, the software starts with the left-most selected cuvette that does not contain sample read data.

To do a sample read:

1. In the Navigation Tree, select a cuvette in the Cuvette Set section.
2. Click  **Read** on the Home tab or below the Settings Information area. The Read button changes to  **Stop**, to terminate a read.

Reference Read

You can take a reference read either on air or you can use a cuvette that contains the buffer of your sample.

The reference can be read before or after you read samples.

If you do not take a reference read in a Cuvette Set section before samples were read and you then read a reference, the following occurs:

- The software applies the reference read to all cuvettes in the Cuvette Set section if it is the first reference read.
- In subsequent reference reads, if you select one or more cuvettes, the software selectively replaces the reference read for the cuvettes you select.
- The time and date of the read displays under each cuvette in the section.
- The software applies the reference to new cuvettes you subsequently create in the section.

You can have more than one reference per Cuvette Set section.

To do a reference read:

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 cuvettes you select changes.

Any new cuvettes you create in the Cuvette Set section after you read a second reference use the new reference read.

The software applies the reference read to all cuvettes in the Cuvette Set section, unless you select specific cuvettes. The reference value does not display. To see the reference value, 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 section 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 software subtracts the optical density of the cuvette you designate as the template blank from the optical density of all the other cuvettes in the Cuvette Set section.

Procedure 2:

1. Create a Cuvette Set section 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 read and you 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 create.

Collecting Data From Membrane Holders

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

- **Read Empty Plates/Cuvettes** to start a read.
- **Create/Save Data Documents** to save the data document before the read.
- **Overwrite Plate/Cuvette Data** to do a high-resolution ROI read.

See [SoftMax Pro Software - GxP Edition on page 251](#).

For information on the data-analysis tools for Western Blot images, see [Viewing Western Blot Membrane Data on page 244](#).

To collect data from a membrane holder:

1. Select the **Home** tab and click  **Open/Close** to open the instrument drawer.
2. Insert the prepared membrane holder matching the A1 position on the membrane holder with the A1 position in the plate 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 instrument.

3. Click  **Open/Close** to close the drawer.
4. Open the document for the membrane read. To define membrane holder acquisition settings, select a Plate section in the Navigation Tree and configure the acquisition settings in the Settings dialog. See [Acquisition Settings on page 112](#).
5. Save the document before you start a read. For Imaging reads and Western Blot reads, you must save the document before you start the read.
6. Click  **Read** on the Home tab or below the Settings Information area. The Read button changes to  **Stop**, to terminate a read.
7. After the software reads the Plate section, click  on the left side of the Plate section and then drag across the image to define the region of interest (ROI) to scan.
8. Click  to scan the region using the ROI Scan Resolution you define in the Settings dialog. The data from the ROI scan replaces the data from the original scan. See [Creating Western Blot TRF Mode Protocols on page 179](#).
9. When the read completes, click  **Open/Close** to open the drawer and remove the membrane holder.
10. Save the document.



Each document has a minimum of one Plate section or one Cuvette Set section. Most documents have a plate template and related Group sections, Graph sections, and Note sections. Depending upon the protocol and how you want to report the data, some documents may not have all sections.

Some simple protocols, such as a read of the absorbance of proteins at 280 nm in a cuvette or in a plate with PathCheck technology, let you calculate concentration in the Plate section or Cuvette Set section which makes Group sections and Graph sections necessary only if you want to display the data in tables or graphically.

Many protocols consist of data acquired in a Plate section or Cuvette Set section. You define standards and unknowns in a template. The software then interpolates the unknowns from a standard curve.

Calculations and Numerical Precision

The calculations done by the 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 on page 194](#).

Numbers from Plate sections, Cuvette Set sections, Group sections, and Summaries display, print, and export with as many digits after the decimal point as you specify. All numbers the software saves for the document are stored with full 64-bit double-precision.

The precision of the Plate section data display varies based on the space available to view the characters. When you increase the number of digits, the font size of the values in the Plate section decreases. If the value becomes too long, the numbers are cut off at the edge of the wells. When you export plate data, the values are not limited by space and can have more digits for each well. The values that display in a Plate section are only a representation of the data. In both the Plate section and in the exported data, the last digit that displays or exports is rounded based on the actual data value.

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

Although the software can display reads and the numbers derived from those reads with more than four decimal places, the resolution of the instrument ultimately determines the precision of the calculations. For more information see the instrument user guide.



Note: In SoftMax Pro Software versions 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. 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 you use the Round operator. Computations always use the maximum available precision.

Calculation Hierarchy

When reducing the data the instrument collects, the software does calculations hierarchically. If you do not select an option in the hierarchy in the Settings dialog, an option is not available for the instrument you use, an option is not applicable to the type of read being done, or an option is not defined in the Template Editor dialog or the Data Reductions dialog, then the 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)

For more information on calculations, see:

- [Kinetic Data Reduction Options on page 203](#)
- [Spectrum Data Reduction Options on page 205](#)
- [Absorbance Read Mode on page 280](#) Optical Density and %Transmittance calculations
- [PathCheck Technology on page 282](#)
- [Fluorescence Polarization Read Mode on page 292](#)
- [Curve Fit Functions on page 395](#)
- For information on statistical functions, see the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.

Technical Details of Numerical Calculations

The accuracy and precision of numerical calculations in the SoftMax Pro Software are naturally a matter of concern to any user. To address this concern requires some understanding of how computers perform arithmetic. See [Calculations and Numerical Precision on page 193](#).

Computation with Integers and Real Numbers

A fundamental problem in numerical computation is how numbers should be represented in a finite amount of memory.

In the case of *integers* (whole numbers), which constitute a countable infinity, the solution is relatively straightforward. Any integer in a specified range can be represented exactly in a finite amount of memory. For example, integers in the range -32768 to $+32767$ can be encoded in two bytes (16 bits). If a wider range is required, four or eight bytes can be used, for example. Arithmetic with such a representation, provided that it stays within the specified range, is mathematically exact.

In contrast, *real numbers* (numbers that have a fractional part) present a problem because they constitute an uncountable infinity. Between any two real numbers, no matter how close, there is an infinite number of other real numbers. This implies that, in general, real numbers cannot be represented exactly, and similarly real arithmetic cannot be performed exactly. The generally adopted solution is to represent real numbers approximately by floating-point numbers. This allows real numbers in a specified range to be represented with acceptable precision.

To represent a real number, the nearest floating-point number is used. An infinite number of real numbers maps to the same floating point number. Arithmetic with floating-point numbers is an approximate emulation of real arithmetic. Results are, in general, not mathematically exact. Even such basic laws as associativity of addition, for example $(x+y)+z = x+(y+z)$, do not hold. So, expressions that are mathematically equivalent might not necessarily have the same value when evaluated using floating-point arithmetic.

Floating Point Calculations

Most numerical software, including the SoftMax Pro Software, adopts the *Institute of Electrical and Electronics Engineers standard for floating point arithmetic (IEEE 754)*.

This standard defines several binary number formats, together with elementary arithmetic operations. In particular, a format termed *binary64*, commonly called *double precision*, is specified. This provides a precision of 15 to 17 significant decimal digits and is used in the SoftMax Pro Software for all numerical computations. The standard requires that the results of addition, subtraction, multiplication, division, and square root agree with those of a reference algorithm that produces correctly rounded results, in other words, results that are the nearest floating-point number to the exact result.

Although a more recent revision of the standard (*IEEE 754-2008*) is more restrictive than the original (*IEEE 754-1985*), some variation between conformant floating-point implementations is still allowed. There might not be exact agreement on the value of transcendental (non-algebraic) functions such as *log*.

As is the case with most software, the SoftMax Pro Software does not itself implement IEEE 754. Rather, it relies on the implementation provided by external libraries or the computer hardware. Most computational microprocessors implement IEEE 754.

Although the software uses double precision for all calculations, there are cases where the approximate nature of floating point arithmetic can manifest itself. The following sections describe common issues.

Numerical Comparison

In some applications, the software must compare numbers to produce a binary result. In theory the imprecise nature of floating-point arithmetic can lead to an incorrect conclusion. However, the precision of the *binary64* format far exceeds that of the data being processed by the SoftMax Pro Software, so this is much less likely than an incorrect conclusion due to measurement error.

Rounding

As mentioned previously, a real number is represented by the nearest floating-point number. That number can be less than, equal to, or greater than the specified real number. Although any difference will of course be very small, it can impact the rounding direction of a real number with the last decimal digit of 5. Since rounding can discard significant digits, its use on intermediate values in calculations should be avoided.

Formatting the Display

In some cases, the SoftMax Pro Software formats numbers for display to fit in the available space. In other cases, the user can specify the format. It is important to understand that this choice has no impact on numerical calculations. All such calculations use the full precision afforded by the *binary64* format. Note that this implies that numbers should not be transcribed from the user interface for further analysis. The formula system should be used to retain maximum precision throughout any calculation.

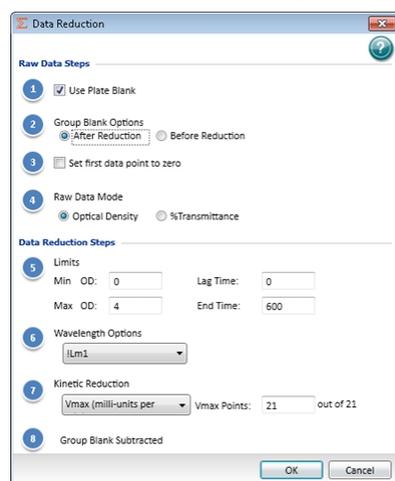
Curve Fitting

Some curve fits require the evaluation of transcendental functions, the implementation of which can vary among different processor types, as described previously. It is possible for the same software running on different computers to give different results. This is particularly the case for nonlinear fits that require many iterations where floating point differences can accumulate. However, bearing in mind that curve fit results are statistical estimates, in such cases the differences are not statistically significant.

Data Reduction

Use the Data Reduction dialog to define how the software is to reduce data. The reduction process 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. When the software reduces the raw information the instrument collects, calculations are done hierarchically. Only the data reduction calculations that apply to the data based on the instrument, read mode, read type, and settings are done.

Click  in the section toolbar, in the Reduction Settings area, or on the Home tab to display the Data Reduction dialog.



This dialog displays only the reduction options that are available for the instrument you use, the options you select in the Settings dialog, and the options you define in the template.

The software does the applicable calculations in the Plate section or Cuvette Set section in the order that displays in the Data Reduction dialog. If you do not select an option in the Settings dialog, an option is not available for the instrument you use, or an option is not defined in the template, the software continues with the next applicable calculation.

Raw Data Steps

The software uses the following steps to calculate the raw data displays:

Apply PathCheck (Absorbance Endpoint only)

PathCheck technology normalization calculations apply to the data only when you use PathCheck technology in the PathCheck category for an Absorbance Endpoint read. When you use PathCheck technology, you can disable this calculation in the Data Reduction dialog by clearing the **Apply PathCheck** check box. See [PathCheck Technology on page 282](#).

The screenshot shows the 'Data Reduction' dialog box with the following settings:

- Raw Data Steps:**
 - 1 Pathcheck Options: Apply Pathcheck, Apply Plate Background OD. Lm1: 0
 - 2 Use Plate Blank
 - 3 Group Blank Options: After Reduction, Before Reduction
 - 4 Raw Data Mode: Optical Density, %Transmittance
- Data Reduction Steps:**
 - 5 Wavelength Options: !Lm1

Buttons: OK, Cancel

Apply Plate Background OD (Absorbance Endpoint only)

The Plate Background OD is the OD of the plate material. It is independent of liquid volume in the well. Subtraction of the Plate Background constant applies to the data only when you use PathCheck technology in the PathCheck category for an Absorbance Endpoint read. To define the constant, enter a value in the field. When you use PathCheck technology, you can disable this calculation 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 you specify a plate blank in the Template Editor for the plate. To allow subtraction of the plate blank value, select the **Use Plate Blank** check box. See [Template Editor on page 91](#).

Smoothing

Use smoothing to reduce noise in Kinetic reads for the FlexStation 3 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 software adds the value of the center point to the values of the points on either side. The software then divides the sum of these points by three and substitutes the resulting value for the center point.
- A setting of 9 averages the center point with the four points on each side.

When you read multiple wavelengths, the software does smoothing 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, the software performs smoothing on each individual wavelength and the wavelength ratio is calculated using smoothed data.

Time Alignment

For the FlexStation 3, the software logs each data value with its own read time and can align the time points by Wavelength or by Well to interpolate the data against a single time point.

- Select the **Time Alignment** check box and then click **Wavelength** to 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 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 you specify a group blank in the Template Editor for the plate. The subtraction of the group blank value can be selected as a pre-reduction process or a post-reduction process.

- To use the group blank value subtraction calculation as a pre-reduction process, click **Before Reduction**.
- To use the group blank value subtraction calculation as a post-reduction process, click **After Reduction**. When you select After Reduction in Group Blank Options, the last step in the Data Reduction dialog shows Group Blank Subtracted.

Polarization or Anisotropy (Fluorescence Polarization read mode only)

The Fluorescence Polarization read 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. The software uses the S and P values to calculate the Polarization (mP) and Anisotropy (r) values. Click the data type to use as the raw data for the reduction.

G Factor

Use the G factor, or grating factor, in Fluorescence Polarization to correct polarization data for optical artifacts which converts relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. G factor corrects for this instrument-based bias. To define the **G Factor**, enter a value in the field.

Set First Data Point to Zero (Kinetic read type only)

Select this check box to offset the first data point to (0,) and shift all other data accordingly. If you clear this check box, some data points that do not fall within the reduction limits can 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 read mode only)

For Absorbance reads, 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:** The ratio of transmitted light to the incident light (for the Absorbance read mode).

Click the data type to use as the raw data for the reduction.



Note: The software uses separate mathematical calculations to handle Optical Density (OD) and %Transmittance (%T) calculations for Pre-read plate blanking, PathCheck technology, and Reference, because OD calculations are done on a linear scale, while %T calculations are done on a logarithmic scale. However, the software does not do other calculations differently for OD and %T modes. Because of this, you should use %T only for raw OD, raw OD with Pre-read plate blank subtraction, or raw OD reads which are normalized by the PathCheck technology. Use caution when you use %T on reduced numbers or the reads that apply to other calculations since the software might not calculate data correctly.

Data Reduction Steps

Baseline Options

Baseline options affect the way that the data is graphed for the FlexStation 3.

- **Absolute** displays the raw data without any change in the way it is graphed. The software uses raw data in subsequent reduction calculations.
- **Zero Baseline** offsets the graph slightly. For example, if you enter 5 for the number of points, then the software uses the average of the first 5 points as the baseline value, and all raw data is subtracted from this value.

Baseline subtraction applies 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 the Zoom Well dialog.

If a read has two or more wavelengths and you select **Zero Baseline** to combine the wavelengths, the zero baseline applies only after the wavelength combination.

- **% Baseline** makes data from the FlexStation 3 look similar to data from the FLIPR System. You can import data from the FLIPR System into the software, and this option modifies the plot and reduction calculations to make data from the FlexStation 3 comparable to data from the 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** in the Display dialog or select **Show Reduced** in the Zoom Well dialog.

Limits

Apply the reduction limits to the reduced data. To specify the limits, enter values in the fields. Limits define the data that displays and is included in data reduction. If you change a limit to show less data, you can change the limit to display the excluded data. 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 read that are under this limit do not display and are excluded from data reduction. The default is 0 OD or RFU/RLU. To display negative Kinetics, set the value below 0 (zero).
- **Max OD, Max RFU, or Max RLU** defines the limit for the maximum value to report. Values from the read that are above this limit do not display and are excluded from data reduction.
- **Lag Time** for the Kinetic read type defines the period of time to be excluded from data reduction. It can be used to ignore the initial lag phase of reactions.
- **End Time** for the Kinetic read type specifies the time after which data points are excluded from data reduction. The default setting is the total assay time for the Kinetic read type. Select the **End of Data** check box to included data that is generated after the end time. Clear the **End of Data** check box to exclude data generated after the end time.
- **Start** for the Spectrum read type specifies the limit in nm for the minimum wavelength setting to report. Values from the read that are under this limit do not appear in the reduced display and are excluded from data reduction.
- **End** for the Spectrum read type specifies the limit for the maximum wavelength setting to report. Values from reads that are above this limit do not appear in the reduced 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  to display the Formula Editor dialog. See [Data Reduction Formulas on page 202](#).

Kinetic Reduction

Select a Kinetic reduction mode from the list. To use a custom reduction, select **Custom** from the list and then, click  to display the Formula Editor dialog. See [Data Reduction Formulas on page 202](#).

Set the number of Vmax Points as needed to ensure that the Vmax line segment passes through the most linear region of the reaction.

Spectrum Reduction

Select a Spectrum reduction mode from the list. To use a custom reduction, select **Custom** from the list and then, click  to display the Formula Editor dialog. See [Data Reduction Formulas on page 202](#).

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  to display the Formula Editor dialog. See [Data Reduction Formulas on page 202](#).

Group Blank Subtracted

When you select **After Reduction** under Group Blank Options, the last step in the Data Reduction dialog displays Group Blank Subtracted.

After you set all applicable data reduction options, click **OK**.

Data Reduction Formulas

The software reduction process is based on formulas that reduce the raw data to show a single number for each well or cuvette. Further analysis of the reduced number is done in Group sections and Graph sections. See [Group Sections on page 87](#) and [Graphing Data on page 214](#).

For a full discussion of custom formulas, see the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.

Custom Reduction Formulas

If the predefined Reduction formulas do not meet your needs, you can select Custom from the menus or dialogs to display a Formula button. The Formula button provides access to the Formula Editor dialog where you can create custom formulas.

The following tables list examples of formulas you use to combine multiple wavelengths.

Wavelength Reduction Formula Examples for Specific Wavelength Combinations

2 Wavelengths	3 Wavelengths	4 to 6 Wavelengths
$!Lm1 + !Lm2$	$Lm1 + !Lm2 + !Lm3$	$!Lm1 + !Lm2 + \dots + !Lmn$
$!Lm1 - !Lm2$	$(!Lm1 - !Lm3) / (!Lm2 - !Lm3)$	$(!Lm1 - !Lm6) / (!Lm2 - !Lm5) / (!Lm3 - !Lm4)$
$!Lm1 / !Lm2$	$!Lm1 / !Lm3$	$!Lm1 / !Lmn$
$!Lm1 * !Lm2$	$!Lm1 * !Lm3$	$!Lm1 * !Lmn$
$\text{Log10}(!Lm1 / !Lm2)$	$\text{Log10}(!Lm1 / !Lm3)$	$\text{Log10}(!Lm1 / !Lmn)$
$!Pathlength$	$!Pathlength$	$!Pathlength$

Wavelength Reduction Formula Examples for Wavelength Combinations

Formula	Description
$!Lmx / \text{constant}$	For example, $Lm1 / 1.44$ for quantitating a polyclonal antibody by measuring the absorbance at A280 with PathCheck technology on.
$\text{Average}(!Lm1\&!Lm2\&!Lm3)$	Averages together the optical densities for multiple reads at the same wavelength (for example, if you read the well three times at 280 nm).
$\text{Min}(!Lm1\&!Lm2\&!Lm3)$	Reports the minimum OD/RFU/RLU recorded for multiple wavelength reads in each well.
$\text{Max}(!Lm1\&!Lm2\&!Lm3)$	Reports the maximum OD/RFU/RLU recorded for multiple wavelength reads in each well.
$\text{If}(!Lmz < A, \text{makeerr}(118), (\text{if}(!Lmx > B, \text{makeerr}(117), !Lm1)))$	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 that lies between A and B.

Use custom Reduction formulas that contain mathematical operators or terms to obtain specific types of data. The following table provides examples of formulas for the kinetic read type and the spectrum read type.

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.

Spectrum or Kinetic Reduction Formula Example

Formula	Description
Nthitem (!Lm1,X)	Reports the optical density at item X in the list of reads. 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.

Enclose the accessor names for custom Reduction formulas for imaging data in square brackets to prevent conflicts with other formula elements.

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.

Kinetic Data Reduction Options

The software applies kinetic reductions 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. The software calculates Vmax 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.

An alternative method to analyze non-linear kinetic reactions is to report the elapsed time until the maximum reaction rate is reached, rather than to report the maximum rate itself. When you use this 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 the kinetic read type. 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 read modes).

Vmax (milli-units per min) and **Vmax (units per sec)** reductions are available for all instruments that support the kinetic read type. The default Absorbance kinetic reduction is **Vmax (milli-units per min)**.

Time to Vmax is useful for applications that include 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 software determines the number of available Vmax Points from the Timing settings. Enter a value in the **Vmax Points** field to define the maximum size of the line segment used to determine the slope of the line used to calculate the rate of the reaction. The default is the total number of points taken in the read.

The first slope is calculated for a line drawn that starts at the first read as defined by Lag Time and ends at a total number of reads 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 reads 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, the software uses all the time points to determine the slope of the data.

Onset Time

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

Reports the time at the minimum OD, RFU/RLU, or %T that falls within the reduction limits.

Time at Maximum

Reports the time at the maximum OD, RFU/RLU, or %T that falls within the reduction limits.

Time at 1/2 Maximum

Reports the time at the half of the maximum OD, RFU/RLU, or %T that falls within the reduction limits.

To calculate this reduction, the 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.

Spectrum Data Reduction Options

The software applies the Spectrum Reduction formula to the list of numbers in each well (values at each wavelength) after the wavelength combination formula is applied. The default reduction for the spectrum read type is Lambda at Maximum.

- **Maximum:** Reports the maximum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.
- **Minimum:** Reports the minimum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.
- **Lambda at Maximum:** Reports the wavelength at which maximum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.
- **Lambda at Minimum:** Reports the wavelength of minimum absorbance (optical density) or percent transmittance (%T), RFU, or RLU within the reduction limits.
- **Area Under Curve:** Estimates the area under the curve as defined by the data plots, within the reduction limits. The data plots are treated as a series of trapezoids with vertices at successive data points and at the X-axis coordinates of the data points. The software computes and sums the areas defined by each of the trapezoids.

Imaging Data Reduction Options

The Imaging Data list contains the measurement selections from the Image Analysis Settings in the Settings dialog. See [Viewing and Selecting Measurement Statistics on page 137](#).

Separate data types are available for each measured wavelength and classification.

- **Object Count:** The total number of objects detected in the image. Do not use for field analysis of confluent areas.
- **Field Count:** The total number of confluent areas detected in the image. Do not use for discrete object analysis.
- **Object Percentage:** The percentage the objects detected in the image by classification. Do not use for field analysis of confluent areas.
- **Covered Area:** The combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area:** The average area of the objects detected in the image expressed in μm^2 . Do not use for field analysis of confluent areas.
- **Field Area:** The average area of the confluent areas detected in the image expressed in μm^2 . Do not use for discrete object analysis.
- **Object Roundness:** 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. Do not use for field analysis of confluent areas.
- **Field Roundness:** 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. Do not use for discrete object analysis.
- **Object Average Intensity:** The average fluorescent signal intensity of the objects detected in the image. Do not use for field analysis of confluent areas or for transmitted light.
- **Field Average Intensity:** The average fluorescent signal intensity of the confluent areas detected in the image. This is not used for discrete object analysis or for transmitted light.
- **Object Intensity:** The average total fluorescent signal intensity of the objects detected in the image. Do not use for field analysis of confluent areas or for transmitted light.
- **Field Intensity:** The average total fluorescent signal intensity of the confluent areas in the image. Do not use for discrete object analysis or for transmitted light.

- **Total Intensity:** The combined total fluorescent signal intensity of the objects or confluent areas in the image expressed in million intensity counts. Do not use for transmitted light.

Data Display Options

Use the Display Options dialog to specify how to display data for Plate sections and Cuvette Set sections. The values that display in a Plate section are only a representation of the data. In the Plate section, the last digit that displays is rounded based on the actual data value. The values are not limited by space and can have more digits for each well when you export plate data.

You can clone a plate to view both raw and reduced data or multiple reductions of the same data. See [Plate Sections on page 80](#).



Note: If you change the display setting for one cuvette, you change the display settings for all the cuvettes in that Cuvette Set section.

To define display options:

1. Click  in the section toolbar or on the Home tab to display the Display Options dialog.
2. Select a **Specify How Data Is Displayed** option:
 - Select **Raw Data** to display the default data type for the read type you select. See the Raw Data Settings section below.
 - Select **Reduced Data** to display reduced data. See the Reduced Data Settings section below.
 - For the Imaging read mode **Imaging Data** replaces the **Raw Data** option.
3. In the **Raw/Reduced Data Format** area, click the drop-down:
 - Select **Default** to have the software determine 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.
 - Select **Numeric Notation** to display data in numeric format. Select to display either **Significant Figures** or **Decimal Places** and then enter the number of figures or places to display.
 - Select **Scientific Notation** to display data in scientific notation format. Select to display either **Significant Figures** or **Decimal Places** and then enter the number of figures or places to display.
4. Click **OK**.

Raw Data Settings

Select **Raw Data** to display the default data type for the read type you select.

- The Endpoint read type displays raw absorbance, fluorescence, or luminescence values.
- The Kinetic read type displays the change in raw OD/RFU/RLU values over time as a plot.
- The Spectrum read type displays raw OD/RFU/RLU values for the range of wavelengths as a plot.
- The Well Scan read type displays raw OD/RFU/RLU values as shades of blue to red.
- To display a reduced number, select the **With Reduced Number** check box.

Reduced Data Settings

Select **Reduced Data** to display reduced data only. The software reports the reduced number in the Group section when you define a template. The reduced data display is based on the selections you make in the Data Reduction dialog. See [Data Reduction on page 196](#).

- Click the **Options** drop-down:
 - Select **Number** to view only the reduced number.
 - Select **Plot** for the Kinetic read type to display a plot of the raw 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, that range from light for values less than or equal to the low limit, to dark for values greater than or equal to the high limit. Enter the **High Limit** and the **Low Limit** for the grayscale map. To also view the reduced number, select the **Reduced Number** check box.
 - Select **Color Map** to display the raw data in eight colors, that range 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.

Imaging Data Settings

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

- Click the **Data Type** drop-down. The options available are the measurement selections you set in the Image Analysis Settings category in the Settings dialog. Separate data types are available for each measured wavelength and classification, if applicable. See [Viewing and Selecting Measurement Statistics on page 137](#).
 - Select **Object Count** to display the total number of objects detected in the image. Not used for field analysis of confluent areas.
 - Select **Field Count** to display the total number of confluent areas detected in the image. Not used for discrete object analysis.
 - Select **Object Percentage** to display the percentage the objects detected in the image by classification. Not used for field analysis of confluent areas.
 - Select **Covered Area** to display the combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
 - Select **Object Area** to display the average area of the objects detected in the image expressed in μm^2 . Not used for field analysis of confluent areas.
 - Select **Field Area** to display the average area of the confluent areas detected in the image expressed in μm^2 . Not used for discrete object analysis.
 - Select **Object Roundness** to display 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. Not used for field analysis of confluent areas.
 - Select **Field Roundness** to display 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. Not used for discrete object analysis.
 - Select **Object Average Intensity** to display the average fluorescent signal intensity of the objects detected in the image. This measurement is not used for field analysis of confluent areas. Not used for transmitted light.
 - Select **Field Average Intensity** to display the average fluorescent signal intensity of the confluent areas detected in the image. This measurement is not used for discrete object analysis and not used for transmitted light.
 - Select **Object Intensity** to display the average total fluorescent signal intensity of the objects detected in the image. Not used for field analysis of confluent areas and not used for transmitted light.
 - Select **Field Intensity** to display the average total fluorescent signal intensity of the confluent areas detected in the image. Not used for discrete object analysis and not used for transmitted light.
 - Select **Total Intensity** to display the combined total fluorescent signal intensity of the objects or confluent areas detected in the image expressed in million intensity counts. Not used for transmitted light.
- Optionally, select the **With Reduced Number** check box to include the reduced data number in the display.

Viewing Membrane Images

After you scan a membrane for Western Blot data, the data displays in the 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 [Viewing Western Blot Membrane Data on page 244](#).

Western Blot membrane data is saved as a TIFF image to allow you to use the image analysis tool of your choice for analysis. The SoftMax Pro Software includes a version of the ImageJ software from U.S. National Institute of Health (NIH). See [Analyzing Western Blot Images on page 249](#).

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

To view the membrane image with another image-analysis tool, click  in the section toolbar and then click **External Image Editor**. This option is available if you have .tif files associated with a program other than the ImageJ software. The icon for this tool changes depending on the image-analysis tool you select. See [Defining the Membrane Image-Analysis Tool on page 249](#).



Note: The image tools are available when you view a Plate section that contains membrane data.

Viewing 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 80](#) or [Image Analysis Settings on page 131](#).

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 or click  in the section toolbar or the Home tab. In the Image Analysis Settings Measurement Selection, click **Show Details**.

To view more than one well in a Plate section, drag the cursor to select up to four wells before you access 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.

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 well you select.

To zoom the image in and out:

- Right-click the image and select **Zoom In** or **Zoom Out**,
- Hold the **CTRL** key and press **+** or **-**.
- Use the mouse wheel to zoom in or out.

To move the image in the image area:

- Use the scroll bars 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 display in the image.
- When a button is outlined, the related wavelength, objects, or areas do not display 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 adjust the intensity of each wavelength.



To adjust the wavelength intensity:

1. Select the wavelength to adjust.



2. Drag the slider bar on the left to adjust the low level of intensity or click  to move the sliders to the outside edges. The intensity level displays above the bar.
3. Drag the slider bar on the right to adjust the high level of intensity or click  to move the sliders to the outside edges.

4. 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, drag the separator lines to change the size the columns. 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, select a data type for the **X Axis Data**.
- For a scatter plot, select data types for the **X Axis Data** and the **Y Axis Data**.

Masking Wells or Cuvettes

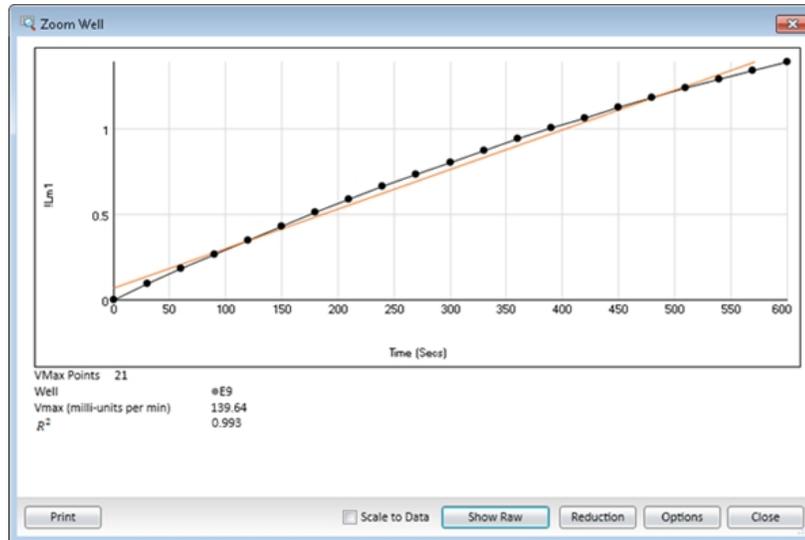
Use the Mask feature to hide data so that the data is not used for calculations and is not reported. You mask wells to suppress outliers from data reduction calculations. You can use masking as a “what if?” tool. For example, if you include a group blank in a template and want to see the data both with and without the blank, mask the group blanks to suppress the blanking function and unmask the group blanks to use the blanking function.

To mask wells and cuvettes:

1. In a Plate section or a Cuvette Set section, select the wells or cuvettes to mask.
 - Click and drag the cursor across the wells to select multiple wells in a plate.
 - Press CTRL and click to select multiple cuvettes.
2. Click  in the section toolbar or on the Home tab or right-click a well or cuvette and select **Mask**.
3. Click  a second time to unmask masked wells or cuvettes.

Zooming Well and Cuvette Display

Use the Zoom Well and Zoom Well Options dialogs to magnify the well display. This feature is available when the data in a well or cuvette can be enlarged.

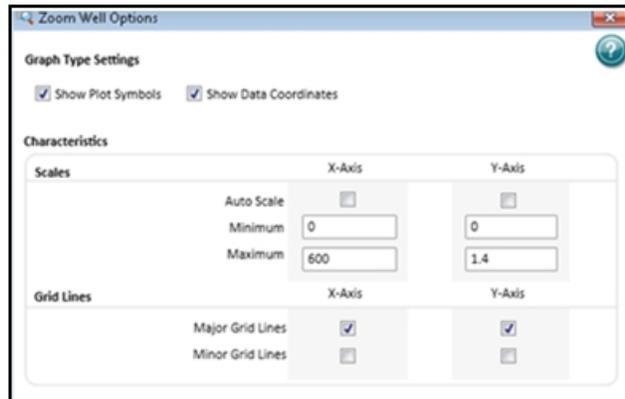


To zoom a well or cuvette:

1. Click the well or cuvette into which to zoom.
2. Click  in the section toolbar or on the Home tab or double-click the well to display the Zoom Well dialog where the data displays in a zoomed graph.
 - Click **Print** to print the graph dialog.
 - Select the **Scale to Data** check box to scale the display to the data.
 - Click **Show Reduced** or **Show Raw** to toggle between reduced and raw data.
 - Click **Reduction** to display the Data Reduction dialog where you modify the reduction settings. See [Data Reduction on page 196](#).
3. Click **Close** to close the Zoom Well dialog.

Zoom Well Options

Use the Zoom Well Options dialog to change the display of the well graph that displays in the Zoom Well dialog.



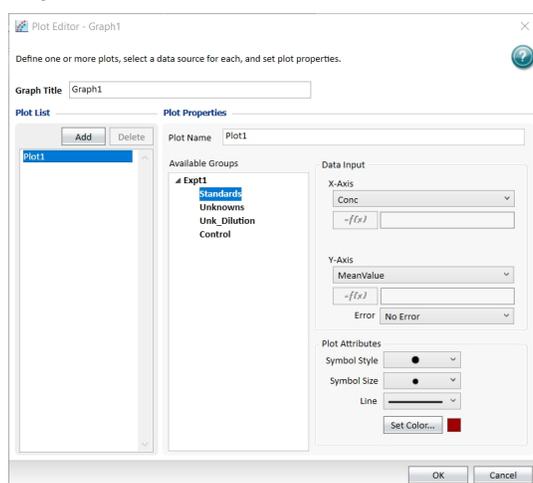
To define zoom well options:

1. In the Zoom Well dialog, click **Options** to display the Zoom Well Options dialog.
2. In the **Graph Type Settings** area:
 - Select the **Show Plot Settings** check box to display the plot symbols you define in the Plot Editor dialog. Clear the **Show Plot Settings** check box to display the graph line without plot symbols.
 - Select the **Show Data Coordinates** check box to display the data coordinates upon cursor hover. Clear the **Show Data Coordinates** check box to not have the coordinates display.
3. In the **Characteristics** area, do the following for both the X-Axis and the Y-Axis:
 - Select the **Auto Scale** check box to have the software scale the data. Clear the **Auto Scale** check box and enter the **Minimum** and **Maximum** scale values to define the scale.
 - Select each **Grid Lines** check box for the grid lines to display.
4. Click **OK**.

Graphing Data

Use the Plot Editor dialog to create a graph from the data the software collects.

Use Graph sections to plot information as a scatter graph or a bar graph. You can create custom graphs and you can graph the data from Group section tables. You can use columns in a Group section table as the X or Y input for plots on a graph in a Graph section. The Graph section can use data from any Group section in the document and you can create as many Graph sections as you need.



To create a new graph:

1. Click  **New Graph** above the Navigation Tree to display the Plot Editor dialog.
2. In the **Graph Title** field, enter the graph name.
3. Use Plot1 to get started. In the **Plot Name** field, enter the plot name. The Plot List displays the name change.
4. In the **Available Groups** list, select the Group section that contains the data to graph. The columns in the group you select appear in the X-Axis drop-down and the Y-Axis drop-down.
5. In the Data Input area, click the **X-Axis** drop-down and select a Group section column or select **Custom** to assign data to the graph's X-axis. If you select Custom, the Formula Editor dialog displays where you define the formula for the data to appear in the X-axis. See [Formula Editor on page 104](#).
6. Click the **Y-Axis** drop-down and select the Group section column or select **Custom** to assign data to the Y axis. If you select Custom, the Formula Editor dialog displays where you define the formula for the data to appear in the Y-axis.
7. Click the **Error** drop-down and select a column or select **No Error** to define the display of error bars.



Note: For scatter graphs, you can 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. In the **Plot Attributes** area:
 - Click the **Symbol Style** drop-down and select the symbol to use to mark each data point in a scatter graph.
 - Click the **Symbol Size** drop-down and select the size of the symbol in a scatter graph.
 - Click the **Line** drop-down and select the line style to use between data points in a scatter graph.
 - Click **Set Color** and select the color of the symbol and the line in a scatter graph or the color of the bar in a bar graph.
9. Click **OK**.

The Graph section displays in the Navigation Tree and the data plots display in the Workspace. See [Workspace on page 70](#).

Editing Graphs

Use Graph sections to plot information as a scatter graph or a bar graph. You can create custom graphs and you can graph the data from Group section tables. You can use columns in a Group section table as the X or Y input for plots on a graph in a Graph section. The Graph section can use data from any Group section in the document and you can create as many Graph sections as you need.



To edit a graph:

1. In the Navigation Tree, select a Graph section.
2. To change the name of the Graph section, right-click the Graph section in the Navigation Tree and select **Rename**. The Rename Section dialog displays.
3. Select the Home tab:
 - Click  to display data as a scatter graph.
 - Click  to display data as a bar graph.
4. Click  in the Graph section toolbar or on the Home tab to display the Plot Editor dialog where you change the plots and the data or plot attributes. See [Plot Editor on page 218](#).
5. Click  in the Graph section toolbar or on the Home tab to display the Graph Appearance dialog where you define the graph appearance including axis labels. See [Graph Appearance on page 222](#).
6. For scatter graphs, use the Category drop-down, Fit drop-down, and  to define curve fits. See [Curve Fit Settings on page 223](#).

7. Right-click in a graph and select **Add Annotation** to add an annotation to the graph. See [Annotation Editor on page 220](#).
8. Right-click on a data point in a scatter graph and select **Highlight Data Source** to highlight the sample in the Group section that corresponds to the data point. See [Templates on page 91](#).
9. Click  to export the graph data. See [Exporting Images of Graph Sections on page 238](#).

Graph Legend

A bar graph does not have a legend. For a scatter graph, click **Curve Fit Results** on the right below the graph to view or hide the 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 the confidence intervals that contain the true value is the specified confidence level. Select the Confidence Interval Method on the Statistics tab in the Curve Fit Settings dialog. See [Curve Fit Statistics Tab on page 229](#).
 - 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 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 (PLA)

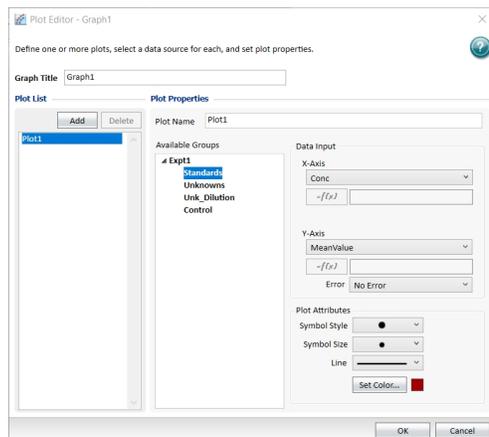
- The formula for the selected curve fit
- For each plot:
 - Estimated Relative Potency (Estimated Rel. Pot.)
 - Standard Error (Std. Error) of the estimate
 - Confidence Interval of the estimate (see above)
 - Independence (see above)
 - EC₅₀ value (where applicable)
- The list of weighting formulas
- R² value
 - Chi-Squared (sum of squared errors)
 - F-Stat (the F-statistic comparing the parallel and unconstrained models)
 - F-Prob (the probability associated with F-Stat)
- For each parameter:
 - Estimated Value
 - Standard Error (Std. Error) of the estimate
 - Confidence Interval of the estimate
 - Independence

Global Fits (Shared)

- The formula for the selected curve fit
 - R² value
- For each shared parameter:
 - Standard Error (Std. Error) of the estimate
 - Confidence Interval of the estimate (see above)
 - Independence (see above)
- For each plot:
 - R² value
- For each parameter:
 - Standard Error (Std. Error) of the estimate
 - Confidence Interval of the estimate (see above)
 - Independence (see above)

Plot Editor

Use the Plot Editor dialog to change the plots and the data or plot attributes.



To change plots and plot attributes:

1. In the Navigation Tree, select a Graph section.
2. Click  in the Graph section toolbar or on the Home tab to display the Plot Editor dialog.
3. In the **Graph Title** field, enter the graph name.
4. Above the Plot List, click **Add** to add a plot to the graph. Use Plot1 to get started. Select a plot and click **Delete** to remove a plot from the graph.
5. In the **Plot List**, select a plot to define its properties on the right side of the dialog. You do the following for each plot in the Plot List.
6. In the **Plot Name** field, enter the plot name. The Plot List displays the name change.
7. In the **Available Groups** list, select the Group section to graph. The columns in the group you select appear in the X-Axis drop-down and the Y-Axis drop-down.
8. In the Data Input area, click the **X-Axis** drop-down and select a Group section column or select **Custom** to assign data to the graph's X-axis. If you select Custom, the Formula Editor dialog displays where you define the formula for the data to appear in the X-axis.
9. Click the **Y-Axis** drop-down and select the Group section column or select **Custom** to assign data to the Y axis. If you select Custom, the Formula Editor dialog displays where you define the formula for the data to appear in the Y-axis.
10. Click the **Error** drop-down and select the data to display error bars or select **No Error** to not display error bars. Error bars are lines that extend outside of a plotted value in either or both directions and graphically represent some error in plotted data.
Scatter graphs can display error bars for the plot of data for the X-axis and Y-axis.
11. In the **Plot Attributes** area:
 - Click the **Symbol Style** drop-down and select the symbol to use to mark each data point in a scatter graph.
 - Click the **Symbol Size** drop-down and select the size of the symbol in a scatter graph.
 - Click the **Line** drop-down and select the line style to use between data points in a scatter graph.
 - Click **Set Color** and select the color of the symbol and the line in a scatter graph or the color of the bar in a bar graph.
12. Click **OK**.

Graph Legend

A bar graph does not have a legend. For a scatter graph, click **Curve Fit Results** on the right below the graph to view or hide the 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 the confidence intervals that contain the true value is the specified confidence level. Select the Confidence Interval Method on the Statistics tab in the Curve Fit Settings dialog. See [Curve Fit Statistics Tab on page 229](#).
 - 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 asymptotes is missing, the A or the D parameter will have few bars, indicating that reliable values cannot be deduced from the data set.

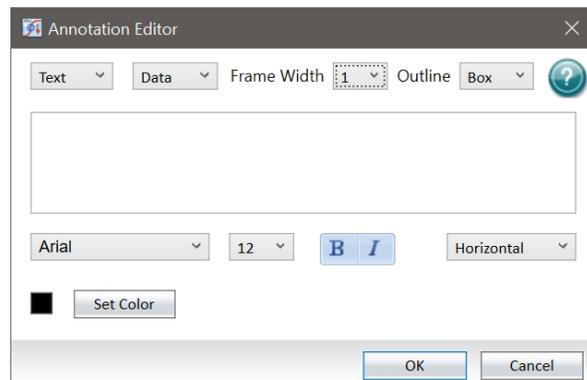
Global Fits

- 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 above)
 - Independence (see above)
 - EC_{50} value (where applicable)
- The list of weighting formulas
- R^2 value
 - Chi-Squared (sum of squared errors)
 - F-Stat (the F-statistic comparing the parallel and unconstrained models)
 - F-Prob (the probability associated with F-Stat)

- For each parameter:
 - Estimated Value
 - Standard Error (Std. Error) of the estimate
 - Confidence Interval of the estimate
 - Independence

Annotation Editor

Use the Annotation Editor dialog to add text, images, and shapes to a graph.



To add annotations to a graph:

1. In the Navigation Tree, select a Graph section.
2. Click  or right-click in the graph and select **Add Annotation** to display the Annotation Editor dialog.
3. Click the annotation type (first) drop-down (see below for selection-specific steps):
 - Select **Text** to add text.
 - Select **Image** to add an image.
 - Select **Shape** to add a shape.
4. The annotation displays either at the default location or at the location of the right-click. Drag and drop the annotation on the graph. See the following steps.
 - Data annotations are associated with a coordinate pair.
 - Screen annotations are associated with a pixel location.
5. Right click the annotation:
 - Select **Edit Annotation** to edit the annotation.
 - Select **Delete Annotation** to delete the annotation.

Text

If you select Text:

1. Click the first drop-down and select **Text**.
2. Click the second drop-down:
 - Select **Screen** to have the annotation remain in the same place on the graph if you rescale the graph.
 - Select **Data** to have the annotation move with a data point if you rescale the graph (scatter graphs only).
3. Click the **Frame Width** drop-down and select the frame width. If you select anything other than 0, click the **Outline** drop-down and select **Box** for a square or rectangular frame or select **Oval** for a round or oval frame.
4. In the text field, enter the text to display on the graph.

5. To format the text:
 - Click the font (first) drop-down below the text field and select the font style.
 - Click the size (second) drop-down and select the font size.
 - Click **B** to bold the text.
 - Click *I* to italicize the text.
6. Click the orientation (right) drop-down:
 - Select **Horizontal** to display the text horizontally.
 - Select **Vertical Left** to display the text vertically facing left.
 - Select **Vertical Right** to display the text vertically facing right.
7. Click **Set Color** and select the font color.

Image

If you select Image:

1. Click the first drop-down and select **Image**.
2. Click the second drop-down:
 - Select **Screen** to have the annotation remain in the same place on the graph if you rescale the graph.
 - Select **Data** to have the annotation move with a data point if you rescale the graph (scatter graphs only).
3. Click the **Frame Width** drop-down and select the frame width. If you select anything other than 0, click the **Outline** drop-down and select **Box** for a square or rectangular frame or select **Oval** for a round or oval frame.
4. Click **Select Image**. Navigate to and select the image to add to the graph.
5. Click the **Scale Factor** drop-down and select the image scale to size the image.
6. Click the orientation (right) drop-down:
 - Select **Horizontal** to display the text horizontally.
 - Select **Vertical Left** to display the text vertically on the left.
 - Select **Vertical Right** to display text vertically on the right.
7. Click **Set Color** and select a font color.

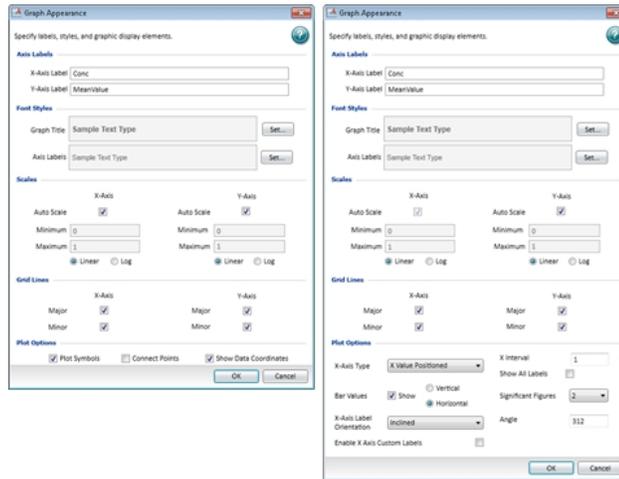
Shape

If you select Shape:

1. Click the first drop-down and select **Shape**.
2. Click the second drop-down:
 - Select **Screen** to have the annotation remain in the same place on the graph if you rescale the graph.
 - Select **Data** to have the annotation move with a data point if you rescale the graph (scatter graphs only).
3. Click the shape (first) drop-down and select the shape.
4. Click the size (second) drop-down and select the shape size.
5. Click **Set Color** and select the shape color.

Graph Appearance

Use the Graph Appearance dialog to specify labels, styles, and graphic-display options.



To change a graph appearance:

1. In the Navigation Tree, select a Graph section.
2. Click  in the section toolbar or on the Home tab to display the Graph Appearance dialog.
3. In the Axis Labels area, **X-Axis Label** field, enter the label to display for the x-axis. Axis labels use the font you specify in the Font Styles area.
4. In the **Y-Axis Label** field, enter label to display for the y-axis.
5. In the Font Styles area, click **Set** next to **Graph Title** to display the Font Style dialog where you select the font, font size, bold, and italics for the graph title display.
6. Click **Set** next to **Axis Labels** to display the Font Style dialog where you select the font, font size, bold, and italics for the axis label display.
7. In the Scales area, either:
 - Select the **Auto Scale** check box for the **X-Axis** and/ or **Y-Axis** to automatically scale one or both axes.
 - OR
 - Enter the **Minimum** and **Maximum** scaling values and then select either **Linear** or **Log** for the display of each axis.
8. In the Grid Lines area, select the **Major** and/or **Minor** check boxes to display the major and/or minor grid lines for the **X-Axis** and the **Y-Axis**.
9. The Plot Options area for a scatter graph provides the following options:
 - Select the **Plot Symbols** check box to display the symbol you define for each data point in the Plot Editor dialog. See [Editing Graphs on page 215](#).
 - Select the **Connect Points** check box to display a line that connects the data points.
 - Select the **Show Data Coordinates** check box to display the coordinates when you hover the cursor over a data point.

10. The Plot Options area for a bar graph provides the following options:
- Click the **X-Axis Type** drop-down:
 - Select **Evenly Spaced Axis** to evenly space the axis.
 - Select **Separated Axis** to separate the axis.
 - Select **X Value Positioned** and then select the **Show All Labels** check box to display all the labels for the defined interval. Clear the **Show All Labels** check box to display labels only for the bars that have data.
 - Select the **Bar Values Show** check box to display the data values above each bar and then select either **Vertical** or **Horizontal**. Click the **Significant Figures** drop-down and select the number of significant figures to display.
 - Click the **X-Axis Label Orientation** drop-down and select **Horizontal**, **Vertical**, or **Inclined**. For **Inclined**, enter a value in the **Angle** field.
 - Select the **Enable X-Axis Custom Labels** check box allow users to double-click the label in the Graph section to edit the label. Clear this check box to lock the labels to the labels you define in the Graph Appearance dialog.



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.

Curve Fit Settings

For scatter graphs, use the Curve Fit Settings dialog to define the graph's curve fit. When you create a graph, the data does not have a related fit. Use the Graph section tool bar Category drop-down to filter the list of fits that appear in the Fit drop-down list. After you select a fit type, the software determines the parameter values that best fit the data. The function with these parameters is drawn on the graph. See [Judging Good Curve Fits on page 231](#).



Note: Ideally, the type of fit you use is determined by the underlying chemistry of the assay and can be set before data is read.

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

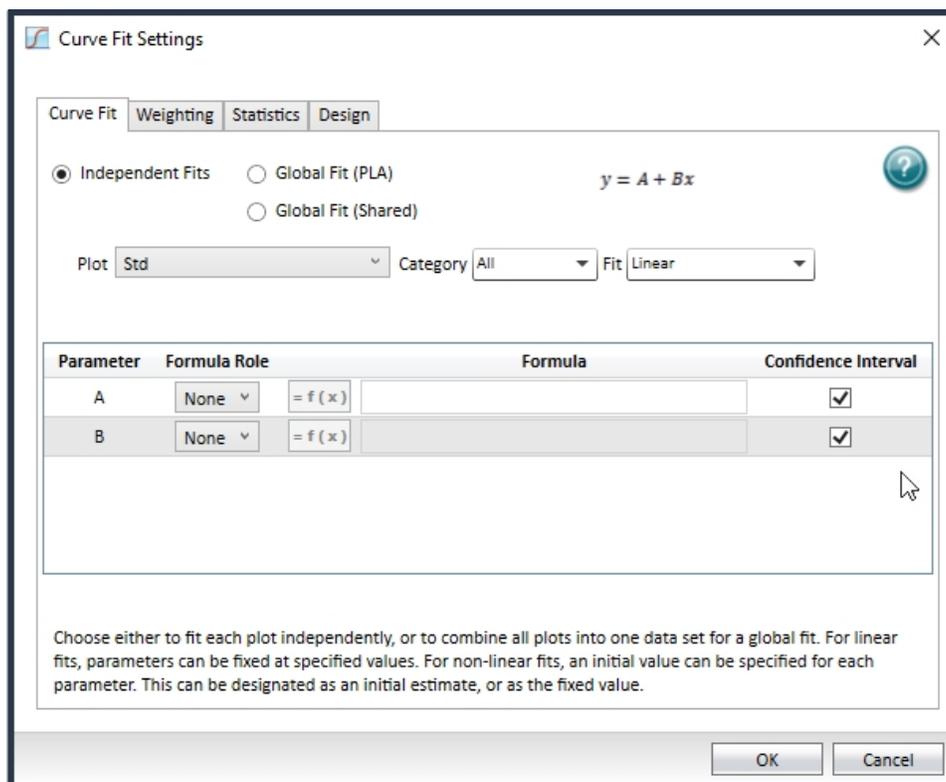
The software includes many curve fits and allows you to create custom curve fits, if needed. See [Custom Curve Fit Settings on page 232](#).

To define a curve fit for the plots in a graph, click  in the Graph section toolbar or on the Home tab to display the Curve Fit Settings dialog.

- Select the **Curve Fit** tab to apply a curve fit to each graph plot.
- Select the **Weighting** tab to specify whether the data should be weighted and the weighting formula. See [Curve Fit Weighting Tab on page 228](#).
- Select the **Statistics** tab to specify the statistics display, the method of confidence, and the level of confidence. See [Curve Fit Statistics Tab on page 229](#).
- Select the **Design** tab to specify the optimization settings to associate with either the graph (global fit) or each plot (independent fits) for the calculations you use in the design of experiment theory. See [Curve Fit Design Tab on page 230](#).

Curve Fit Tab

Use the Curve Fit tab to select a plot from the graph and a curve fit to apply to that plot.



You can independently define the fit for each plot or you can combine all plots into one data set and globally define the fit for all plots on a graph.

- Select **Independent Fits** to fit each plot independently.
- Select **Global Fit (PLA)** to globally fit the curve fit function to all plots in a graph using parallel line analysis.
- Select **Global Fit (Shared)** to globally fit the curve fit function to all plots in a graph, sharing some or all of the parameters.

Independent Fits

To independently fit a curve fit function to each plot in a graph:

1. Select the **Independent Fits** option.
2. Click the **Plot** drop-down and select a plot.
3. Click the **Category** drop-down and select a category to filter the list of curve fits.
4. Click the **Fit** drop-down and select the curve fit function to apply to the plot.

5. For each parameter, click the **Formula Role** drop-down:
 - Select **None** to not assign a formula role.
 - Select **Fixed** to fix the parameter value. Use the Formula Editor dialog to define the fixed curve fit formula for the parameter. Click  to display the Formula Editor dialog where you edit the formula. See [Formula Editor on page 104](#).
 - Select **Seed** to start the curve fit algorithm search. Use the Formula Editor dialog to define the curve fit formula for the parameter. Click  to display the Formula Editor dialog where you edit the formula.
6. For each parameter, select the **Confidence Interval** check box to calculate a confidence interval for the parameter. Leave clear to not calculate a confidence interval.
7. Repeat for each plot.



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.

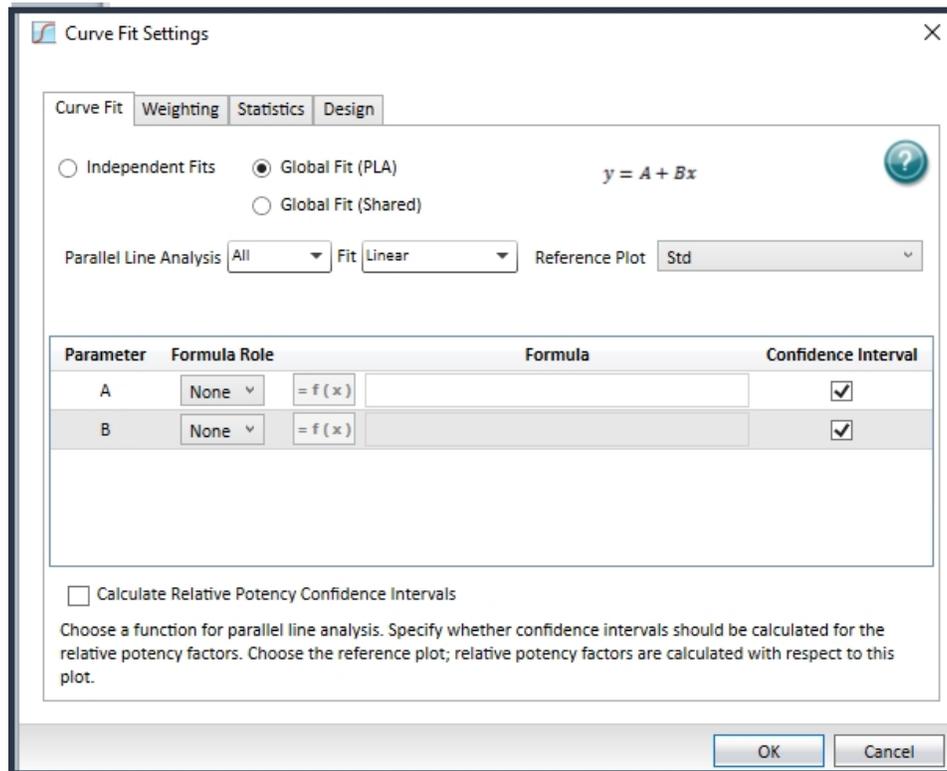
After you select a fit type, the software determines the parameter values that best fit the data. The function with these parameters is drawn on the graph.

- 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 the confidence intervals that contain the true value is the specified confidence level.

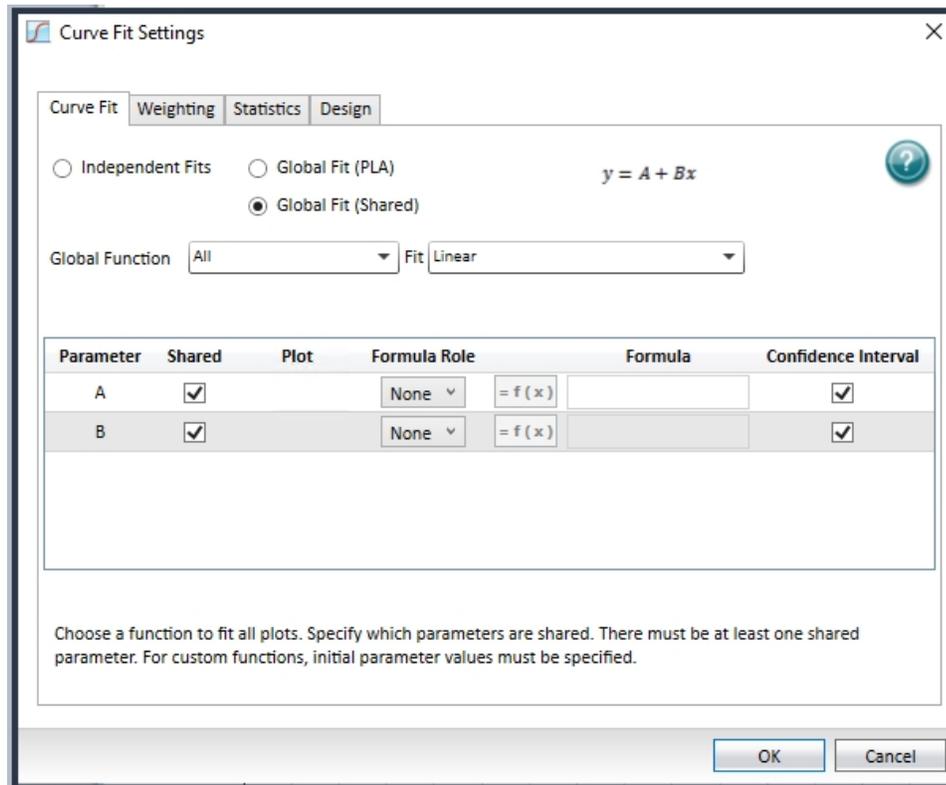
Global Fit (PLA)



To globally fit the curve fit function to all plots in a graph using parallel line analysis:

1. Select the **Global Fit (PLA)** option.
2. Click the **Parallel Line Analysis** drop-down and select an option to filter the list that appears in the Fit drop-down. See [Parallel Line Analysis on page 231](#).
3. Click the **Fit** drop-down and select the curve fit function to use.
4. Click the **Reference Plot** drop-down and select the plot to use as the reference.
5. For each parameter, click the **Formula Role** drop-down:
 - Select **None** to not assign a formula role.
 - Select **Fixed** to fix the parameter value. Use the Formula Editor dialog to define the fixed curve fit formula for the parameter. Click to display the Formula Editor dialog to edit the formula.
 - Select **Seed** to start the curve fit algorithm search. Use the Formula Editor dialog to define the curve fit formula for the parameter. Click to display the Formula Editor dialog to edit the formula.
6. For each parameter, select the **Confidence Interval** check box to include the parameter in confidence interval calculations. Leave clear to not include the parameter in the confidence interval calculations.
7. Select the **Relative Potency Confidence Interval** check box to calculate the relative potency confidence interval. Leave clear to not calculate the relative potency confidence interval.

Global Fit (Shared)



To globally fit the curve fit function to all plots in a graph, sharing some or all of the parameters:

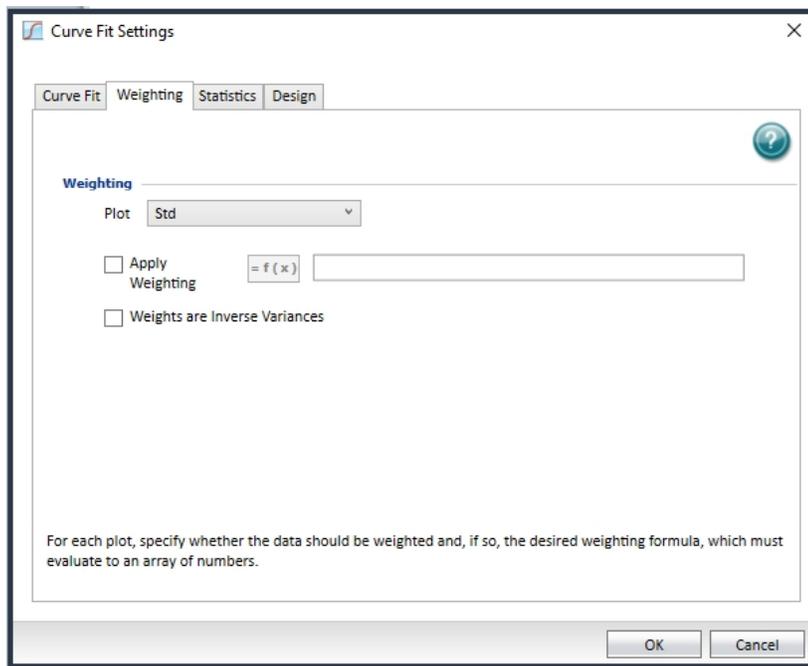
1. Select the **Global Fit (Shared)** option.
2. Click the **Global Function** drop-down:
 - Select **All** to combine all data in an independent fit.
 - Select a fit for the standard curve. See [Curve Fit Functions on page 395](#).
3. Click the **Fit** drop-down and select the curve fit function to use.
4. Select the **Shared** check box for each parameter that is common to all plots. You must select the check box for at least one parameter. Select all check boxes to combine all data in an independent fit.

Clear the **Shared** check box for each parameter that is not common to all plots. When clear, click the **Plot** drop-down and select the setting for that plot.
5. For each parameter, click the **Formula Role** drop-down:
 - Select **None** to not assign a formula role.
 - Select **Fixed** to fix the parameter value. Use the Formula Editor dialog to define the fixed curve fit formula for the parameter. Click to display the Formula Editor dialog to edit the formula.
 - Select **Seed** to start the curve fit algorithm search. Use the Formula Editor dialog to define the curve fit formula for the parameter. Click to display the Formula Editor dialog to edit the formula.
6. Select the **Confidence Interval** check box for each parameter to calculate a confidence interval for the parameter.

Clear the **Confidence Interval** check box for each parameter to not calculate the confidence interval for the parameter.

Curve Fit Weighting Tab

Use the Weighting tab in the Curve Fit Settings dialog to define the independent weighting of individual plots within a Graph section. Least-squares fitting assumes 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.



For each plot, specify whether the data should be weighted and, if so, the applicable weighting formula, which must evaluate to an array of numbers.

To define curve fit weighting:

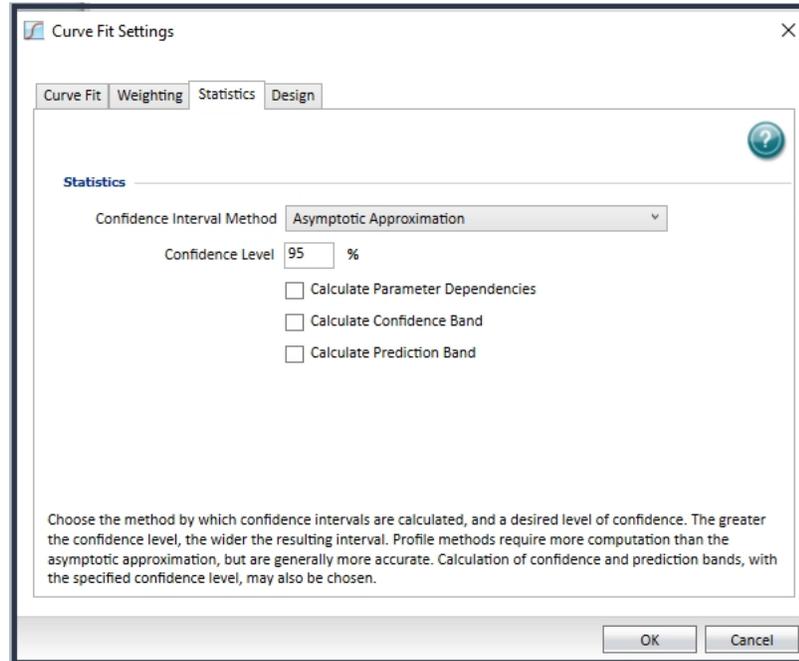
1. Click  in the Graph section toolbar or on the Home tab to display the Curve Fit Settings dialog.
2. Select the **Weighting** tab.
3. Click the **Weighting** drop-down and select a plot.
4. Select the **Apply Weighting** check box and then click  to display the Formula Editor dialog where you define the weighting formula. See [Formula Editor on page 104](#). Clear the check box to make all points have equal weight.
5. Select the **Weights are Inverse Variances** check box if the defined weighting is the inverse of the data variance. When you select this check box, and apply weighting, the confidence interval method based on the chi-squared distribution becomes available.
6. Repeat for each plot to weight.



Note: If you select both check boxes for each plot, the Profile Method (Chi-Squared Distribution) option is available on the Statistics tab. See [Curve Fit Statistics Tab on page 229](#).

Curve Fit Statistics Tab

Use the Statistics tab in the Curve Fit Settings dialog to specify the statistics display.



Choose the method by which confidence intervals are calculated and a level of confidence.

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 the confidence intervals that contain the true value is the specified confidence level.

To define curve fit statistics:

1. Click the **Confidence Interval Method** drop-down:
 - Select **Asymptotic Approximation** to calculate Wald confidence interval, which is simply related to the standard error.
 - Select **Profile Method (F Distribution)** to calculate based on model comparison with F-statistic.
 - Select **Profile Method (Chi-Squared Distribution)** to calculate based on model comparison with chi-squared-statistic. This option is available when you select the Apply Weighting check box and the Weights are Inverse Variances check box on the Weighting tab on the Weighting tab for each plot.
 - Select **Profile Method (t Distribution)** to calculate based on T-profile function.



Note: Profile methods require more computation than the asymptotic approximation but are generally more accurate.

2. In the Confidence Level field, enter the percentage for how likely the interval is to contain the parameter is determined. Increase the confidence level to widen the confidence interval.

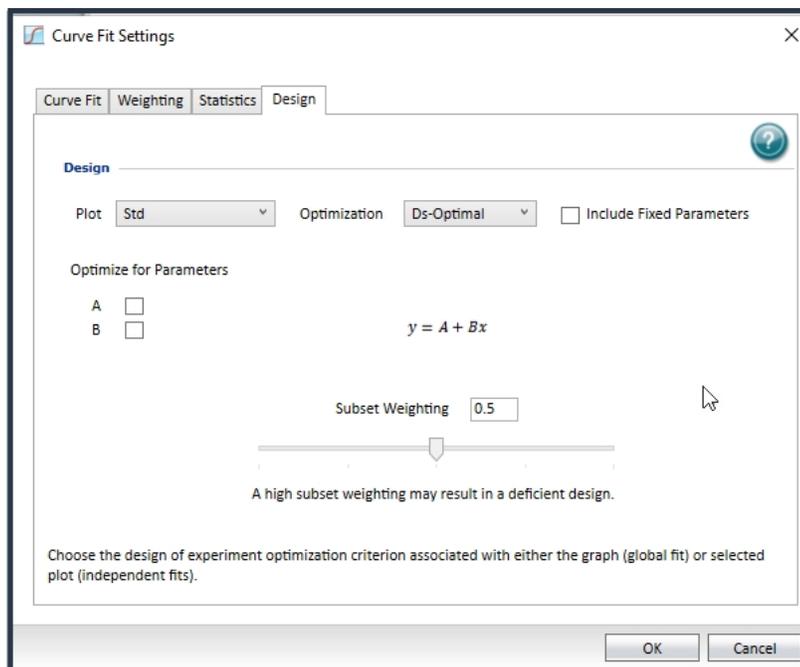
3. Select the **Calculate Parameter Dependencies** check box to calculate dependencies between parameters. With this option selected, the graphical representation in the graph legend represents the parameter independence of the curve fit.
4. Select the **Calculate Confidence Band** check box to allow calculation and display of the confidence band for each curve fit. The confidence band is a region bounded by lower and upper curves and is constructed such that the probability that it contains the true curve is approximately the specified confidence level. This calculation is available for all curve fits except Point-to-Point, Cubic Spline, and Log-Logit.
5. Select the **Calculate Prediction Band** check box to allow calculation and display of the prediction band for each curve fit. The prediction band is a region bounded by lower and upper curves and is constructed such that the probability that it contains all data points is approximately the specified confidence level. This calculation is available for all curve fits except Point-to-Point, Cubic Spline, and Log-Logit.

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 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 Design Tab

Use the Design tab in the Curve Fit Settings dialog to specify the optimization settings to associate with either the graph (global fit) or each plot (independent fits) for the calculations you use in the design of experiment theory. See [Design of Experiment on page 101](#).



To define the design settings:

1. Click  in the Graph section toolbar or on the Home tab to display the Curve Fit Settings dialog.
2. Select the **Design** tab (available options depend on the selections you make on the Curve Fits tab).
3. For independent fits, click the **Plot** drop-down and select a plot.
4. Click the **Optimization** drop-down:
 - Select **D-Optimal** to specify a D-optimal design.
 - Select **Ds-Optimal** to specify a D-subset-optimal design (not available for global fits with shared parameters).
5. Select the **Include Fixed Parameters** to include any fixed parameters in the optimization.
6. If you select Ds-Optimal:
 - a. In the **Optimize for Parameters** section, select the check box for each parameter to optimize.
 - b. In the **Subset Weighing** field, either enter the subset weight or use the slider to adjust the subset weight.
 - c. For a global fit with parallel line analysis (PLA), select the check box to optimize the relative potency parameters.

Parallel Line Analysis

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. The values, standard errors, and (optionally) confidence intervals display below the graph.

The software includes function operators that summarize this information in a Notes section. For more details, see the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.

Judging Good Curve Fits

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.

See “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).

Custom Curve Fit Settings

Use the Function Editor dialog to specify functions for custom curve fits. These functions are functions of a single independent variable, x , and a set of parameters denoted A, B, C, D, G, H, I, J. These functions supplement the curve fits that are included with the software and you can use custom curve fits anywhere within the protocols in which you define these functions.



Note: The equation images that display for the curve fits that are included in the software do not appear for custom curve fit functions.

A custom function is available to fit data in any graph section in the protocol in which it is defined. In the list of curve fits options, the curve fits you create in the Function Editor display in the Custom category. You must assign initial parameter values in the Curve Fit Settings dialog. This is in contrast to the built-in functions for which initial values may be specified but are not required. Initial parameter values are formulas and may contain document references. A parameter may be fixed at its initial value. After you assign the initial parameter value, the software stores the initial value formulas in the protocol.



Note: Parameter names display in upper case in the curve fit legend (below the graph), even if you define the name in lower case in the Function Editor dialog.

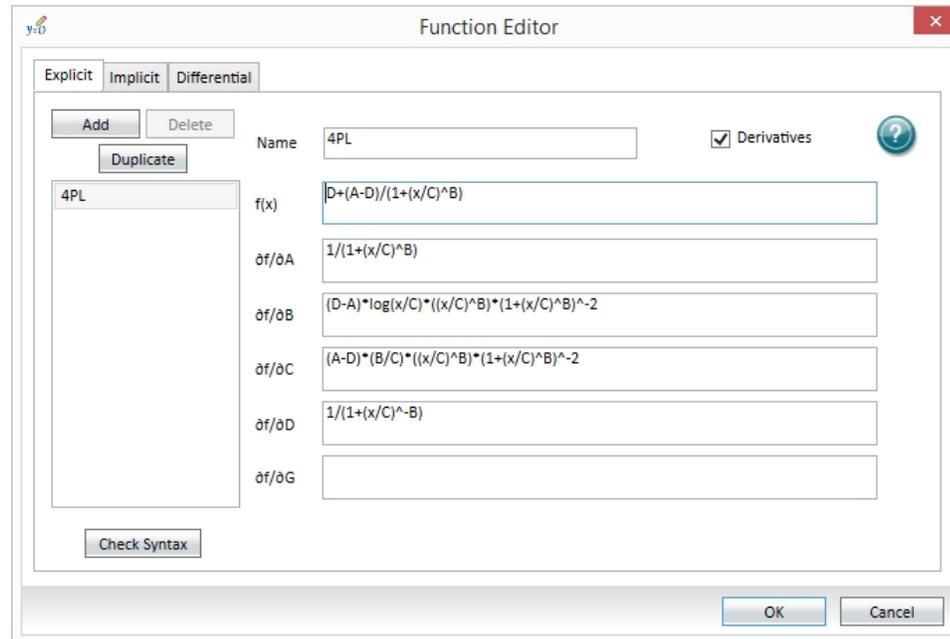
Use the three tabs in the Function Editor dialog to define custom curve fits:

- **Explicit** - Use to create an explicit function that is defined by an explicit formula $f(x,p)$, where p represents any subset of the parameters.
- **Implicit** - Use to create an implicit function that is defined by an implicit equation $f(x,y,p) = 0$. The value of the function is the value of y which satisfies this equation for a given value of x and set of parameters p . See [Custom Implicit Curve Fits on page 234](#).
- **Differential** - Use to create a differential function that is defined as a component in a system of first-order differential equations. Up to eight components are allowed and are denoted f_i ($i = 1$ to 8), with some particular f_i being the reference component that defines the function value. See [Custom Differential Curve Fits on page 235](#).

For information about the curve fit functions that are included in the software, see [Curve Fit Functions on page 395](#).

Explicit Tab

Use the Explicit Fit tab to create an explicit function that is defined by an explicit formula $f(x,p)$, where p represents any subset of the parameters.



To define a custom curve fit for the plots in a graph:

1. Select the Operations tab and click **Function Editor** to display the Function Editor dialog.
2. Click **Add** to get started. Function1 appears in the function list and in the Name field.
 - Click **Delete** to remove the function you select from the list.
 - Click **Duplicate** to make a copy of the function you select in the list.
3. In the **Name** field, update the function name. As you change the Name field the list displays the updates you make.
4. Select the **Derivatives** check box to specify the derivative of the function with respect to any parameter. The software numerically estimates any derivative that you do not specify. You should provide derivative formulas to improve the accuracy of curve fitting results. The derivative of each component may be a function of the parameters and of the components.
5. In the **f(x)** field, enter the formula. The syntax of the formulas is the same as that of the formula system, except that document references such as accessors, Summary formulas, and operators referencing plots are not allowed. Parameter names are case insensitive.
6. Click **Check Syntax** to check the syntax of the formula. This checks only the formula syntax not the mathematical correctness of the formula.
7. Click **Add** to add additional functions to the curve fit.
8. Click **OK**. The custom curve fit appears in the list of curve fits available from Graph sections.

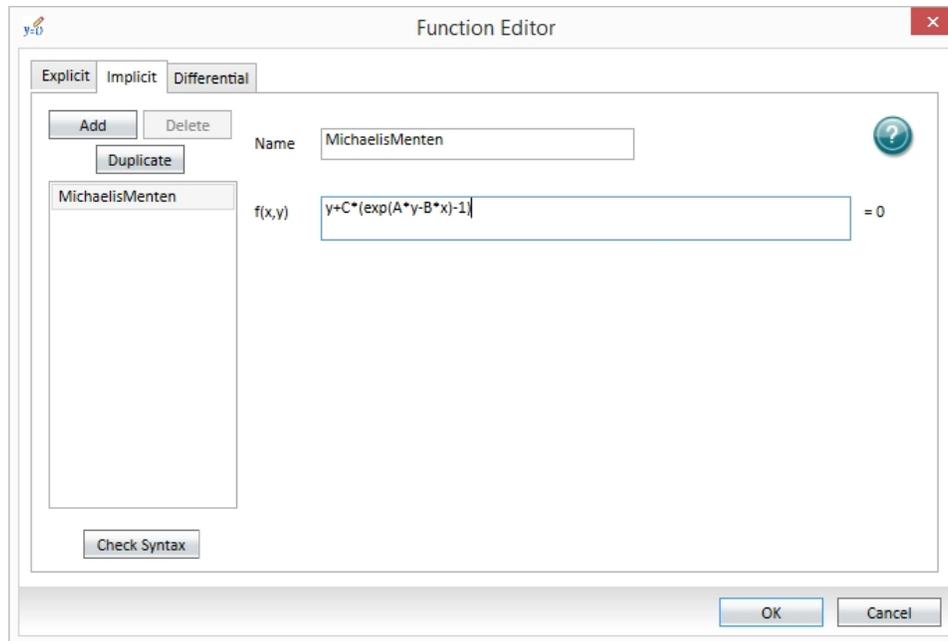
Custom Implicit Curve Fits

Use the Implicit tab in the Function Editor dialog to create an implicit function that is defined by an implicit equation $f(x,y,p) = 0$. The value of the function is the value of y which satisfies the equation for a given value of x and set of parameters p .



Note: Parameter names display in upper case in the curve fit legend (below the graph), even if you define the name in lower case in the Function Editor dialog.

The equation images that display for the curve fits that are included in the software do not appear for custom curve fit functions.



To independently fit a curve fit function to each plot in a graph:

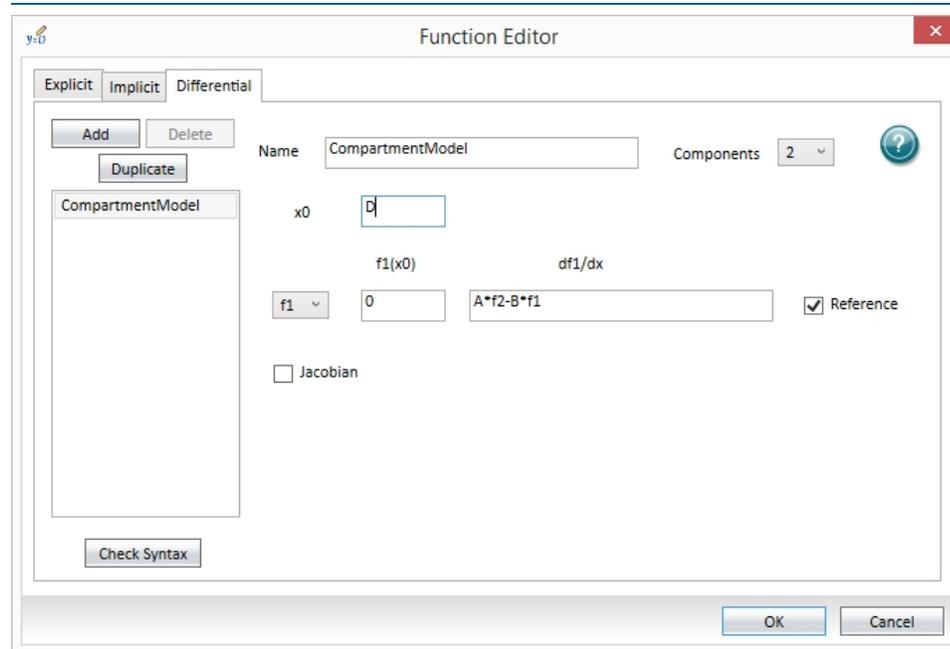
1. Select the Operations tab and click **Function Editor** to display the Function Editor dialog.
2. Select the **Implicit** tab.
3. Click **Add** to get started. Function1 appears in the function list and in the Name field.
 - Click **Delete** to remove the function you select from the list.
 - Click **Duplicate** to make a copy of the function you select in the list.
4. In the **f(x)** field, enter the formula. The syntax of the formulas is the same as that of the formula system, except that document references such as accessors, Summary formulas, and operators referencing plots are not allowed. Parameter names are case insensitive.
5. Click **Check Syntax** to check the syntax of the formula. This checks only the formula syntax not the mathematical correctness of the formula.
6. Click **Add** to add additional functions to the curve fit.
7. Click **OK**. The custom curve fit appears in the list of curve fits available from Graph sections.

Custom Differential Curve Fits

Use the Differential tab in the Function Editor dialog to create a differential function. A differential function is defined as a component in a system of first-order differential equations. Up to eight components are allowed and are denoted f_i ($i = 1$ to 8), with some particular f_i being the reference component that defines the function value.



Note: The equation images that display for the curve fits that are included in the software do not appear for custom curve fit functions. Parameter names display in upper case in the curve fit legend (below the graph), even if you define the name in lower case in the Function Editor dialog.



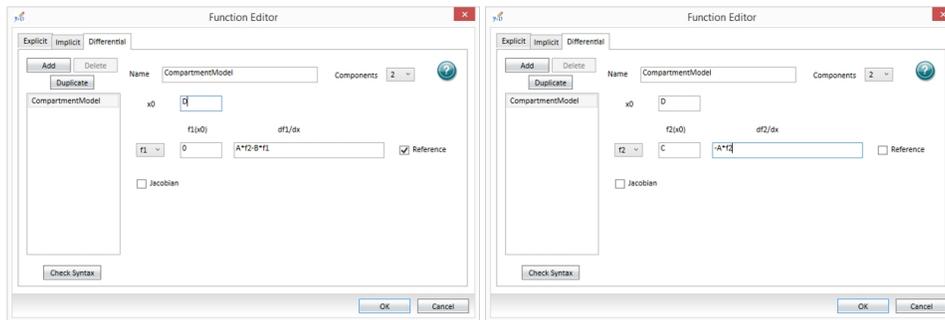
Note: For systems requiring stiff integration, the Jacobian matrix must be specified.

To define a Differential custom curve fit:

1. Select the Operations tab and click **Function Editor** to display the Function Editor dialog.
2. Select the **Differential** tab.
3. Click **Add** to get started. Function1 appears in the function list and in the Name field.
 - Click **Delete** to remove the function you select from the list.
 - Click **Duplicate** to make a copy of the function you select in the list.
4. Click the **Components** drop-down and select the number of components to include. Each component is denoted f_i ($i = 1$ to 8). You will select the **Reference** check box for the f_i that you want to be the reference component that defines the function value.
5. In the **x0** field, enter the initial value for X.
6. Click the **f<i>** drop-down and select the component to define.
7. In the **f<i>(x0)** field, enter the value for component f_i at x_0 .
8. In the **df<i>/dx** field, enter the formula. The syntax of the formulas is the same as that of the formula system, except that document references such as accessors, Summary formulas, and operators referencing plots are not allowed. Parameter names are case insensitive.
9. Click **Check Syntax** to check the syntax of the formula. This checks only the formula syntax not the mathematical correctness of the formula.

10. Repeat for each Component and remember to select the Reference check box for the fi that you want to be the reference component that defines the function value.
11. Click **Add** to add additional functions to the curve fit.
12. Click **OK**. The custom curve fit appears in the list of curve fits available from Graph sections.

The initial value of x and each component must be specified and may be a function of the parameters. The derivative of each component may be a function of the parameters and of the components. For example, this two component system defines a compartment model as used in pharmacokinetics (Bates, D.M., and Watts, D.G. (1988), Nonlinear Regression Analysis and Its Applications, John. Wiley & Sons, New York).



Confidence Interval Calculations

The following is a description of the calculation of confidence intervals for curve fit parameter estimates. Assuming normally distributed error in the data, a maximum likelihood parameter estimate, p , is obtained by least-squares regression. Given a required confidence level (typically but not necessarily 95%, corresponding to significance $\alpha = 0.05$), a confidence interval for the estimate may be constructed.

Two important points:

- Such an interval should not be construed to mean that there is a 95% probability that the true parameter value falls in the interval, which would be meaningless because the true value is not a statistic. Rather it implies that the interval has been calculated by a method such that it has a probability of 95% of containing the true value.
- For a function with P parameters, least squares regression implies a P -dimensional confidence region, not a set of P confidence intervals. Therefore, reported confidence intervals are actually the limits of the confidence region along each dimension.

The software implements various methods for the construction of confidence intervals, which are discussed in the following sections.

Asymptotic Approximation

This method is based on the formula relating confidence interval to the standard error of the mean of a normally distributed variable. The standard error is given by

$$SE = \sqrt{\frac{\text{Variance} * SSE}{N - P}}$$

where Variance is the variance of the parameter as determined by the regression calculation, SSE is the minimum sum-of-squared errors, N the number of data points and P the number of variable parameters. The end points of the confidence interval are then

$$p = p_{best} \pm SE * t(N - P, \alpha)$$

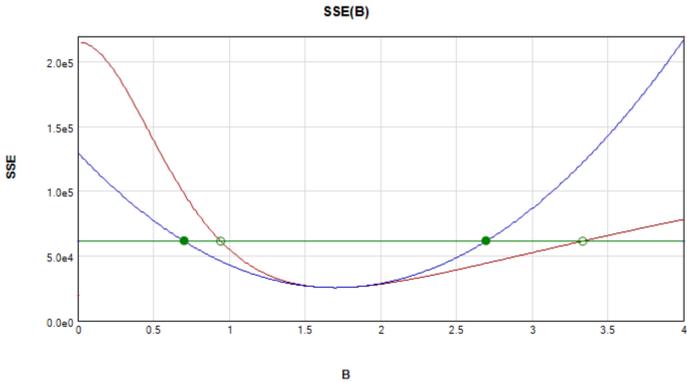
where t(N-P,α) is the value of the inverse Student's t distribution for degrees of freedom N - P and specified significance α. Note that the interval is symmetrical about the best fit value, p_{best}.

Profile t Method

This method is a generalization of the asymptotic approximation which is more appropriate for nonlinear regression [1]. The confidence interval is defined by the two solutions for p to the equation:

$$SSE(p) = \left(1 + \left(\frac{t(N - P, \alpha)^2}{N - P} \right) \right) * SSE$$

where SSE(p) is the sum-of-squared errors for parameter value p with all other parameters varied to minimize SSE(p). For general linear regression this equation may be solved analytically, the result being equal to the asymptotic approximation described above. However, for nonlinear regression numerical solution is required. The resulting interval is not in general symmetrical about the parameter estimate, and there is no guarantee that a solution exists. This method is illustrated below for parameter B in a four-parameter logistic fit. The function SSE(B) is shown in red, and the right-hand-side of the above equation is shown in green. The points of intersection, hollow green circles, define the confidence interval. For comparison, the asymptotic confidence interval is defined by the filled green circles, obtained by the asymptotic approximation to SSE(B), shown in blue.



Profile F Method

This method is based on model comparison between a set of parameters and the best fit parameters, using extra sum-of-squares ANOVA [1,2]. The confidence interval is defined by the two solutions for p to the equation:

$$SSE(p) = \left(1 + \left(\frac{P * F(P, N - P, \alpha)}{N - P} \right) \right) * SSE$$

where $SSE(p)$ is the sum-of-squared errors for parameter value p with all other parameters varied to minimize $SSE(p)$, and $F(P, N-P, \alpha)$ is the inverse of the Fisher F distribution with the specified degrees of freedom factors P , $N-P$ and significance α . As with the profile t method, numerical solution is required, and the interval is asymmetric and not guaranteed to exist.

Profile Chi-Squared Method

This method [3] is similar to the other profile methods but is applicable only if the sum-of-squared errors has the chi-squared distribution, which follows if data points are weighted with their inverse variances in forming the sum. The end points of the interval are defined by the equation:

$$SSE(p) = SSE + \chi^2(1, \alpha)$$

where $\chi^2(1, \alpha)$ is the critical value of the chi-squared distribution with one degree of freedom and significance α . As with the other profile methods numerical solution is required.

References:

1. Douglas M. Bates and Donald G. Watts, Nonlinear Regression Analysis and Its Applications, Wiley, 1988.
2. H.J. Motulsky and A Christopoulos, Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. 2003, GraphPad. Software Inc.
3. W.H. Press, S.A. Teukolsky, W.T. Vetterling, and B.P. Flannery, Numerical Recipes: The Art of Scientific Computing, Third Edition, Cambridge University Press, 2007.

Exporting Images of Graph Sections

Use the Home tab to export a 300 DPI image of a Graph section for use in another program.

You can also right-click anywhere on the Graph section and select **Copy** to then paste an image of the section directly into a word processor or spreadsheet program.



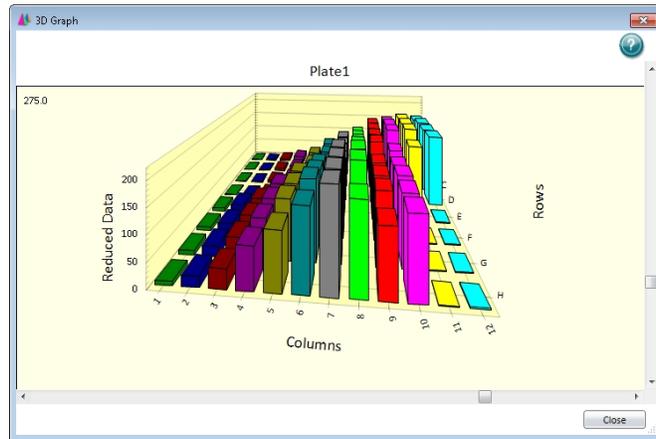
Note: The 300 DPI image might appear very large in the program into which you import or paste. You might need to scale or resize the image in that program after you import or paste the Graph section image.

To export a graph image:

1. In the Navigation Tree, select a Graph section.
2. Select the Home tab and click  to display the Save As dialog.
3. Navigate the computer file hierarchy to the file location where you want to save the .png file.
4. In the **File Name** field, enter a name for the file.
5. Click **Save**.

Viewing Data in 3D Graphs

Use the 3D Graph dialog to view a three-dimensional graph of reduced plate data.



In the Navigation Tree, select a Plate section and click  in the section toolbar or on the Home tab to display the 3D Graph dialog.

To rotate the 3D graph:

- To rotate the graph vertically, drag the vertical scroll bar located to the right of the graph.
- To rotate the graph horizontally, drag the horizontal scroll bar located below the graph.
- To start or stop a continuous animated horizontal graph rotation, double-click the graph.

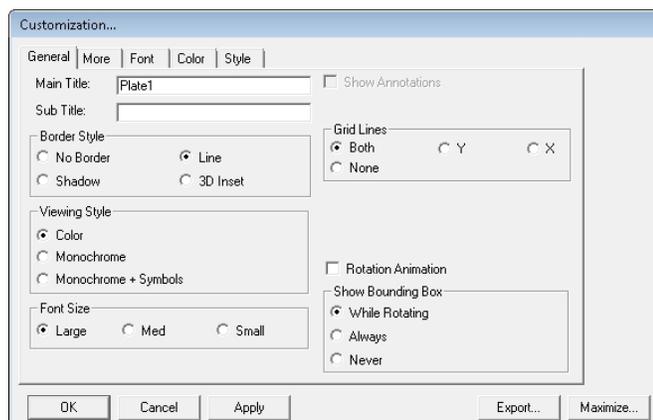
Drag a side or a corner of the dialog to resize the 3D Graph dialog.

Right-click on the 3D graph for additional options:

- Select **Viewing Style** and then select the viewing styles. The options you select display a check mark in the list.
- Select **Border Style** and then select: **No Border**, **Thin Line**, **Shadow**, or **Inset**.
- Select **Font Size** and then select: **Large**, **Medium**, or **Small**.
- Select **Grid Options** and then select: **Show X and Y Axis Grid Lines**, **Show Y-Axis Grid Lines**, **Show X-Axis Grid Lines**, or **Hide Grid Lines**.
- Select **Bounding Box** and then select: **While Rotating**, **Always**, or **Never**.
- Select **Rotation Animation** to have the image rotate continuously. Double-click the image to stop the rotation.
- Select **Rotation Increment** and then select the speed and direction of rotation. Values closer to zero rotate slower, while values farther from zero rotate faster. To rotate counter-clockwise, select a negative value.
- Select **Rotation Detail** and then select: **Wire Frame**, **Plotting Style**, or **Full Detail**.
- Select **Plotting Method** and then select: **Wire Frame**, **Surface**, or **Surface With Shading**.
- Select **Shading Style** and then select: **White** or **Color**.
- Select **Maximize** to display the graph in full screen mode. Pres **Esc** to shrink the image back.
- Select **Customization Dialog** to display the Customization dialog where you define the above settings and more. See [Customizing 3D Graphs on page 240](#).
- Select **Export Dialog** to display the Export Control dialog where you define settings to export the 3D graph. See [Exporting 3D Graphs on page 242](#).

Customizing 3D Graphs

Use the five tabs in the Customization dialog to customize a 3D graph.



Most of the following options are available when you right-click the 3D graph. Right-click a 3D graph and select Customization Dialog to display the Customization dialog.

General Tab

The following options appear on the General tab:

- **Main Title:** Enter the text for the graph title.
- **Sub Title:** Enter the text to display below the graph title.
- **Show Annotations:** Select this check box to display notes. Not available if there are no notes available.
- **Border Style:** Select **Line**, **Shadow**, or **3D Inset** to define the outside image border or select **No Border** to not display a border.
- **Viewing Style:** Select **Color**, **Monochrome**, or **Monochrome + Symbols** to define the image background.
- **Font Size:** Select **Large**, **Med**, or **Small** to define the text font size.
- **Grid Lines:** Select **Both**, **X**, or **Y** to display the x-axis and/or y-axis grid lines or select **None** to not display grid lines.
- **Rotation Animation:** Select this check box to continuously rotate the graph.
- **Show Bounding Box:** Select **While Rotating** or **Always** to display a wire-frame box around the graph or select **Never** to not display a wire frame.

More Tab

The following options appear on the More tab:

- **Rotation Detail:** To change the display style when the graph rotates, select **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:** Select an increment to control the speed and direction of the animated rotation. Values closer to zero rotate slower, while values farther from zero rotate faster. To rotate counterclockwise, select a negative value.
- **Shading Style:** Select **White** or **Color** to change the shading. This option has no effect on the display if there is no shading.
- **Plotting Method:** Select **Wire Frame**, **Surface**, or **Surface with Shading** to change the data plot display.

Font Tab

The following options appear on the Font tab:

- **Main Title:** Select the font for the graph title and then select to add **Bold**, **Italic**, and **Underline** styles.
- **Sub-Title:** Select the font for the text directly below the graph title and then select to add **Bold**, **Italic**, and **Underline** styles.
- **Subset/Point/Axis Labels:** Select the font for all other text in the graph and then select to add **Bold**, **Italic**, and **Underline** styles.

Color Tab

The following options appear on the Color tab.

Select a **Graph Attribute** option on the left to define color settings for that attribute and then repeat for all other attributes.

- **Desk Foreground:** Select to define the color of the text of the graph title and subtitle.
- **Desk Background:** Select to define the color of the area behind the graph title and subtitle.
- **Shadow Color:** Select to define the color of the border shadowing when you select Shadow for the Border Style on the General tab.
- **Graph Foreground:** Select to define the color of all text except the title and subtitle, and the border around the graph.
- **Graph Background:** Select to define the color of the area within the border and behind the graph.
- **X and Z Axis Plane:** Select to define the color of the graph floor.
- **Y Axis Plane:** Select to define the color of the graph walls.

Select a color from the color pallet.

In the **Quick Styles** area:

- Select the **Bitmap/Gradient Styles** check box to add texture to the graph.
- Select one option for the **Inset**, **Shadow**, **Line**, and **No Border** from the **Light**, **Medium**, or **Dark** columns.

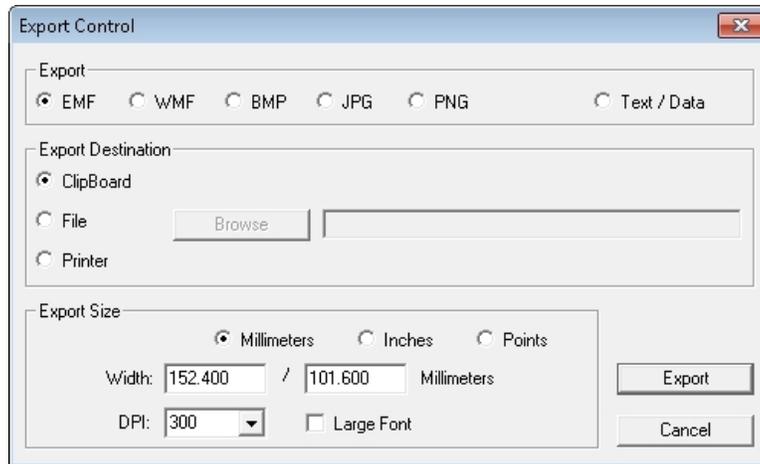
Style Tab

The options that appear on the Style tab are dependent on the selections on the other tabs, and on the type of data.

Select an item in the list and then click the color for your selection.

Exporting 3D Graphs

Use the Export Control dialog to export an image of a 3D graph and to export the data from a 3D graph. Before you export an image of a 3D graph, make sure to define all customization options including the rotation increment. See [Customizing 3D Graphs on page 240](#).



To export 3D graphs:

1. From the 3D Graph dialog, right-click the graph and select **Export Dialog** to display the Export Control dialog.
2. In the **Export** area, select the image file format or select **Text/Data** to export the data without the image.
3. In the Export Destination area, select the location to where you want to export the image or data:
 - Select **Clip Board** to export the image or data to the computer clipboard to paste into a word processor program or a spreadsheet.
 - Select **File** to save the image or data as a file on your computer or network. Click **Browse** to choose the destination and name the file.
 - Select **Printer** to export the image or data directly to a printer. After you click **Print** you can select the printer and printer options.
4. In the Export Size area, options are dependent on your previous selections:
 - Select **Full Page** to print to a full page. (Export Destination = Printer)
 - Select **No Specific Size** to have the export operation set the image size. (Export = WMF and Export Destination = Clipboard or File)
 - Select **Pixels** to define the number of pixels. (Export = BMP, JPG, or PNG and Export Destination = Clipboard or File)
 - Select **Millimeters, Inches, or Points** and then enter the **Width** and **Height** of the image. (Export = EMF or WMF all Export Destination and all Export options when Export Destination = Printer)
 - Click the **DPI** drop-down and select the number of dots per inch (Export = EMF, BMP, JPG, or PNG and Export Destination = Clipboard or File).
 - Select the **Large Font** check box to increase the size of the text. (Export Destination = Clipboard or File).
5. When you finish your export options, click **Export** or **Print**.
6. If you export the data as text, then the Export dialog displays with more options to format the export data.

Exporting Text/Data

Use the Export dialog to define the format for data you export from a 3D graph.

1. In the Export Control dialog, select the **Text/Data** Export option , complete the other options described above and then click **Export** to display the Export dialog.
2. In the **Select Subsets and Points** area:
 - Select **All Data** to export all data.
 - Select **Selected Data** and then use the Subsets to Export fields to select the number that corresponds to each column of data and the letter that corresponds to each data point. To multi-select **Shift** click or **CTRL**.
3. In the **Export What** area select to export just the **Data** or the **Data and Labels**.
4. When multiple values are available, select an option in the **Data to Export** area.
5. In the **Export Style** area:
 - Select **List** and then select **Tab** or **Comma** as the delimiter. The delimiter must match the requirements of the program to where the data will export.
 - Select **Table** and then select **Subsets/Points** to place the data from the plate column numbers into the rows of the table and the data from the plate row letter into the columns of the table, or select **Point/Subsets** to place the data from the plate row letters into the rows of the table and the data from the plate column numbers into the columns of the table.
6. In the **Numeric Precision** area:
 - Select **Current Precision** to export the data using the rounding defined in the graph.
 - Select **Maximum Precision** to export the rounding available in the document.
7. Click **Export**. If you export the data to the computer clipboard, immediately open the program where you want to use the data and paste the data into that program.

Viewing Western Blot Membrane Data

After you scan a membrane for Western Blot data, the data displays in the 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.

- Drag the slider on the side of the Plate section to zoom the image in or out.
- Click **1:1** on the side of the Plate section to view the image at its actual size.
- Click **Fit** on the side of the Plate section to fit the image to the Plate section.
- Click  on the side of the Plate section and then drag across the image to define the area to zoom to zoom in on a specific area of the image.
- Click  on the side of the Plate section and then drag the image to move it to move the image within the Plate section.
- Click  on the side of the Plate section and then drag across the image to define the cropped area to crop the image.
- Select a color option from the **LUT** list at the top of the Plate section to colorize the image from a look-up table.
- In the **Subtract** field, enter the value to subtract from the image intensity to adjust the intensity of the image.
- Select the **Log** check box to scale the image intensity.
- Select the **Invert** check box to invert the colors of the image similar to a photographic negative.
- Click  on the left side of the Plate section and then drag across the image to define the region of interest (ROI) to scan. Click  to scan the region using the ROI Scan Resolution you define in the Settings dialog. The data from the ROI scan replaces the data from the original scan.
- Click the following 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.
 - Click  to view the image at a very fine resolution suitable for publishing.
- Click  to restore the image and use the data from the last scan.

Use the Plate section to export the image for further analysis or to publish. These tools are on the right side of the top in the Plate section

- Click  and then **Excel Analysis** to open the raw image data in a Microsoft Excel template for analysis. See [Using Excel to Analyze Western Blot Data on page 245](#).
- Click  and then **ImageJ Editor** to open the raw image in the ImageJ software. See [Analyzing Western Blot Images on page 249](#).

- Click  and then **External Image Editor** to open the raw image in a pre-defined image-analysis tool. This option is available when you have .tif files associated with a program other than ImageJ software.



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  appears at the top of the section to indicate that you cannot edit the image. Click  to restore the image and use the data from the last scan.

- Click , click **Export as Text**, and then select a name for the exported text file to export the raw image data to a text file.
- Click , click **Export as Image**, and then select a name and format for the image export file to export the edited image as it displays in the Plate section.

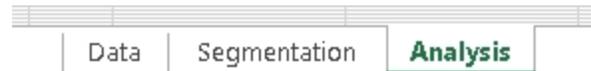
For information on how to locate the generated image, see [Managing Documents on page 23](#).

Using Excel to Analyze Western Blot Data

You can export the Western Blot image data to a Microsoft Excel template to analyze the image data.

Click  and then click **Excel Analysis** to export the raw image data to an Excel template.

An Excel file opens to display the data you export. The Excel file has three worksheets.

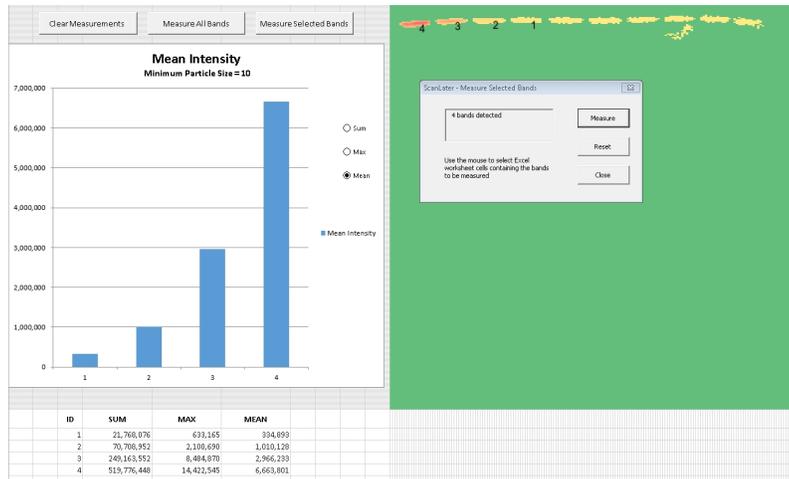


- The **Analysis** worksheet displays the data using automatic background subtraction. You can select the bands to measure and display a bar graph showing an analysis of the selected bands in the **Analysis** worksheet. See [Measuring Select Bands on page 246](#).
- The **Segmentation** worksheet displays 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 [Defining Segmentation Criteria on page 247](#).
- 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 [Selecting the Background for Subtraction on page 248](#).

Save the Excel file to save the data and analysis for future reference.

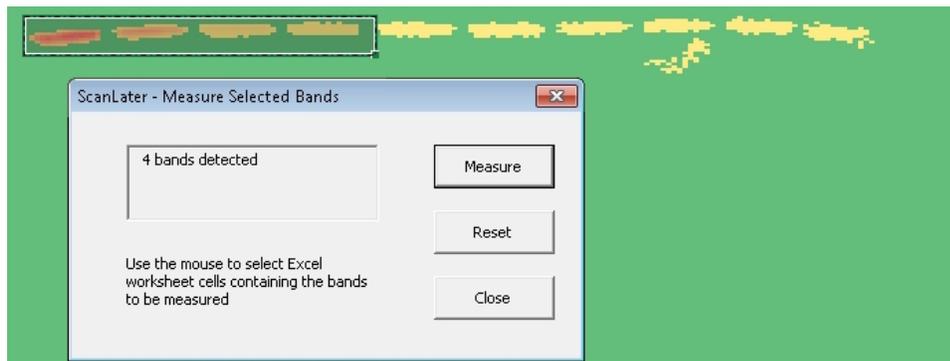
Measuring Select Bands

Use the Analysis tab at the bottom of the Excel window to select the bands to measure and displays a bar graph of the selected bands.



The Analysis worksheet uses automatic background subtraction for the data display.

- Click **Measure All Bands** to measure all the bands that display.
- Click **Measure Selected Bands**, and then drag the cursor to draw a rectangle around the bands to measure. In the ScanLater - Measure Selected Bands dialog, click **Measure**.



- Click **Clear Measurements** to remove the measurement analysis from the worksheet.

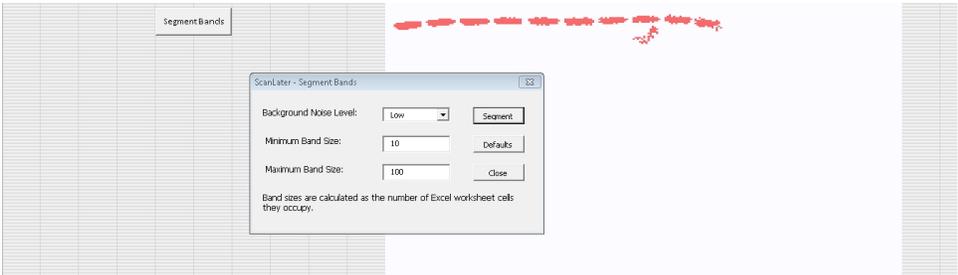
After you select the bands to measure, a bar graph displays with a table below it to show an analysis of the bands based on the analysis criteria. Labels on the blot show the locations of the bands that you reference in the graph and the table.

You can adjust the background noise level and the minimum and maximum band size in the Segmentation worksheet. See [Defining Segmentation Criteria on page 247](#).

If the automatic background subtraction is not sufficient for your data, you can define the background for subtraction in the Data worksheet. See [Selecting the Background for Subtraction on page 248](#).

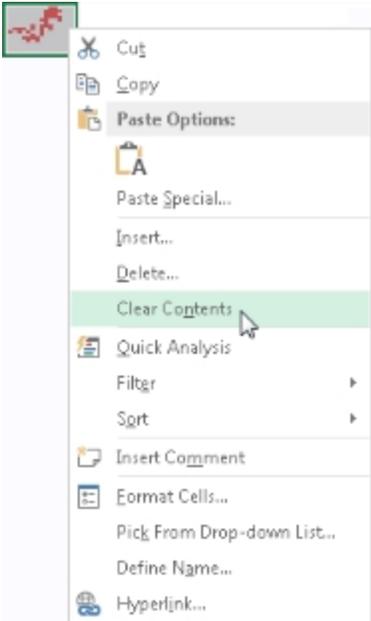
Defining Segmentation Criteria

Use the Segmentation worksheet to adjust the background noise level and the minimum and maximum band size. You can also remove artifacts that you do not want to include in your measurements. The segmented bands display on a white background.



To adjust the segmentation of the bands, click **Segment Bands** to display 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 data display, drag the cursor in a rectangle around the artifact, right-click the rectangle, and select **Clear Contents**.



You can select the bands to measure and display a bar graph that displays an analysis of the bands you select in the Analysis worksheet. See [Measuring Select Bands on page 246](#).

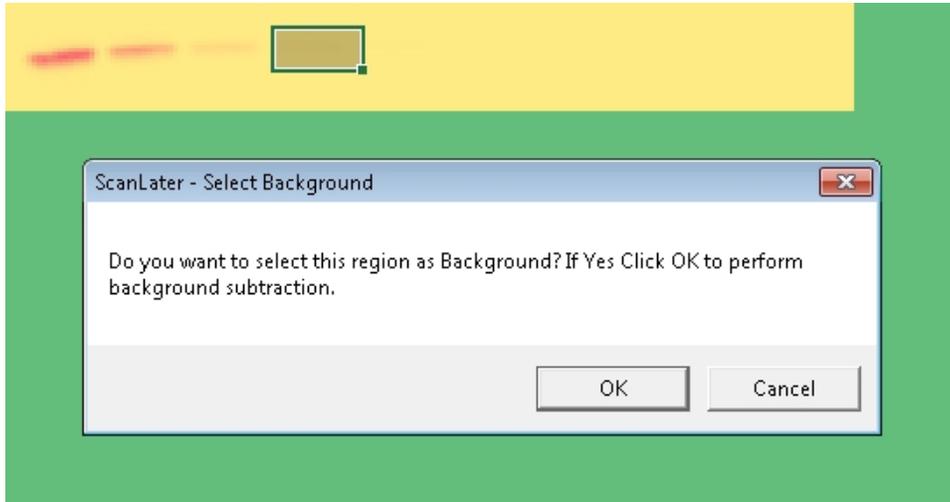
If the automatic background subtraction is not sufficient for your data, you can define the background for subtraction in the Data worksheet. See [Selecting the Background for Subtraction on page 248](#).

Selecting the Background for Subtraction

Use the Data worksheet to define the background for subtraction in the Data worksheet. The worksheet displays the TIFF image and a color rendering of the raw data with no further analysis.



To define the background for subtraction, drag the cursor in a rectangle around the area to use as the background to display the ScanLater - Select Background dialog. Click **OK**.



You can select the bands to measure and display a bar graph to display an analysis of the bands you select bands in the Analysis worksheet. See [Measuring Select Bands on page 246](#).

You can adjust the background noise level and the minimum and maximum band size in the Segmentation worksheet. See [Defining Segmentation Criteria on page 247](#).

Analyzing Western Blot Images

Western Blot membrane data is saved as a TIFF image to allow you to use the image analysis tool of your choice for analysis. The SoftMax Pro Software includes a 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 section toolbar and then click **ImageJ Editor**.

To view the membrane image with another image-analysis tool, click  in the section toolbar and then click **External Image Editor**. This option is available if you have .tif files associated with a program other than the ImageJ software. The icon for this tool changes depending on the image-analysis tool you select. See [Defining the Membrane Image-Analysis Tool on page 249](#).



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  appears at the top of the section to indicate that you cannot edit the image. Click  to restore the image and use the data from the last scan.

To retain the editing capabilities within the software and edit the image with an external editor, export the image or make a copy of the generated .tif image to open in the editor. For information on how to locate the generated .tif image, see [Managing Documents on page 23](#).

Defining the Membrane Image-Analysis Tool

1. In Windows Explorer, right-click a file with a .tif extension.
2. Click **Open With > Choose Default Program** to display the Open With dialog.
3. Select the program to use for membrane image analysis.
 - If the program displays in the **Recommended Programs** list, select it.
 - If the program does not display in the **Recommended Programs** list, click **Browse**. Locate and select the .exe file for the program 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. Select the **Always Use the Selected Program to Open This Kind of File** check box.
5. Click **OK**.

The next time you open a Western Blot document, the Image icon changes to match the program you select.

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 Software Suite 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 Software - GxP edition functions similarly to the SoftMax Pro Software - Standard edition but adds a user logon requirement, electronic signature capability, and auditing features that maintain audit trails for electronic records.

The typical regulated laboratory environment has several check points to confirm the integrity of an assay. The SoftMax Pro Software - GxP edition allows users with appropriate permissions to assign a corresponding status to documents to track and restrict the document through each check point. See [Document Workflow on page 259](#).

There are three software applications that make up this integrated solution:

- GxP Admin Software - (has no user interface)
- GxP Admin Portal Software
- SoftMax Pro Software - GxP edition

GxP Admin Portal Software

The GxP Admin Portal Software is the interface for the GxP Admin Software. Use the GxP Admin Portal Software to create users. You grant users Access permissions to allow use of specific software applications. You assign users with the SoftMax Pro Access permission to Projects that limit their access to documents. For each Project, you assign users to a Role that allows users to perform the tasks for which the users are responsible. See [Default Roles on page 260](#).

Each user action triggers an event and all events are added to the GxP Admin Portal Software System Audit Trail for compliance purposes. See [Audit Trail on page 275](#).

See the *GxP Admin Portal Software User Guide* for how to manage users, Projects, and Roles.

SoftMax Pro Software - GxP Edition

The SoftMax Pro Software - GxP edition is very similar to the SoftMax Pro Software - Standard edition for data collection and analysis of the data from a Molecular Devices microplate reader. Exceptions include a requirement that the user must enter a username and password and then select a Project to use the SoftMax Pro Software - GxP edition. Users with the SoftMax Pro Access permission are assigned to at least one Project and the user's Role in each Project restricts what the user can do in the software.

The SoftMax Pro Software - GxP edition creates an audit trail for each document. The document-specific audit trail records the events that users trigger as the users interact with the document. See [Document Audit Trail on page 276](#).

Administratively Controlled Access

Projects - Document Access Restrictions

Each user with the SoftMax Pro Access permission must be a member of at least one Project. Projects limit which documents the user can access. Each user can be a member of multiple Projects.

Roles - Workflow Permissions

Each user with the SoftMax Pro Access permission can have one Role in each Project. Each user can be a member of multiple Projects and can have a different Role in each Project. Roles restrict the user's responsibilities in the SoftMax Pro Software - GxP edition.

Document Access Permissions

Use Document Access permissions to manage what users can do with documents.

- **Open Imported** - Allows users to open documents with the status: Imported.
- **Open In Work** - Allows users to open documents with the status: In Work.
- **Open Review Pending** - Allows users to open documents with the status: Review Pending.
- **Open Reviewed** - Allows users to open documents with the status: Reviewed.
- **Open Released** - Allows users to open documents with the status: Released.
- **Open Outdated** - Allows users to open documents that have the status: Outdated.
- **Open In Progress** - Allows users to open documents with the status: In Progress.
- **Open Approval Pending** - Allows users to open documents with the status: Approval Pending.
- **Open Approved** - Allows users to open documents with the status: Approved.
- **Open Canceled** - Allows users to open documents with the status: Canceled.

Document Workflow Permissions

Use Document Workflow permissions to manage what users can do in the document workflow.

- **Set Review Pending** - Allows users to set documents to the status: Review Pending.
- **Set Reviewed** - Allows users to set documents to the status: Reviewed.
- **Release Protocol** - Allows users to release a document as a protocol which sets the document status to Released.
- **Set Outdated** - Allows users to set documents to the status: Outdated.
- **Generate Compliance Data** - Allows users to generate compliance data. For example, (using the system default Roles) this user would be the Lab Technician who can open a protocol document with the status Released. The software prompts the user to save the document as a data document and assigns the document the status In Progress. The user then runs the assay to generate compliance data.
- **Set Approval Pending** - Allows users to set documents to the status: Approval Pending.
- **Set Approved** - Allows users to set documents to the status: Approved.
- **Set Canceled** - Allows users to set documents to the status: Canceled.
- **Set In Progress** - Allows users to set an Approved document back to the status: In Progress

Document Editing Permissions

Use Document Editing permissions to manage what users can do with documents.

- **Edit Reader Settings** - Allows users to define microplate reader acquisition settings.
- **Edit Notes Text** - Allows users to edit Note sections in the experiments in documents.
- **Read Empty Plates/Cuvettes** - Allows users to read empty Plate sections and empty Cuvette Set sections.

- **Function Editor** - Allows users to create custom curve fit functions.
- **Overwrite Plate/Cuvette Data** - Allows users to overwrite the data in Plate sections and Cuvette Set sections.
- **Edit Graphs, Summaries, and Reductions** - Allows users to edit Graph sections and the corresponding Summary formulas, Reduction formulas, and curve fits.
- **Assign Plate Layouts** - Allows users to assign plate layouts.
- **Edit Sample and Group Information** - Allows users to edit sample and Group section information.
- **Add/Delete Groups** - Allows users to add and delete Group sections from the experiments in documents.
- **Edit Formulas** - Allows users to edit formulas in documents.
- **Edit Print Options** - Allows users to edit the print options.
- **Lock/Unlock Sections** - Allows users to lock and unlock sections in the experiments in documents.
- **Mask/Unmask Wells** - Allows users to mask and unmask wells.
- **Create/Save As Data Document** - Allows users to save data documents to the database.
- **Create/Save As Protocol** - Allows users to save protocol documents to the database.
- **Change Auto Save Settings** - Allows users to change Auto Save settings.
- **Change Auto Export Settings** - Allows users to change Auto Export settings.

Statements & Signatures Permissions

Use Statement and Signatures permissions to manage what users can do with statements.

- **Add/Modify Statements** - Allows users to edit statements.
- **Sign Statements** - Allows users to sign statements.
- **Revoke Own Signature** - Allows users to remove only their own signatures from statements.
- **Revoke Any Signature** - Allows users to remove any signature from statements.

Document Management Permissions

Use Document Management permissions to manage what users can do with the documents.

- **Rename Document** - Allows users to rename documents.
- **Move Document** - Allows users to move documents to different folders in the database.
- **Delete Document** - Allows users to delete documents from the database.
- **Unlock Document** - Allows users to unlock documents.
- **Import Documents** - Allows users to import documents into the database.
- **Export Documents** - Allows users to export documents out of the database.

Folder Management Permissions

Use Folder Access permissions to manage what users can do with document storage folders.

- **Add Folder** - Allows users to create new folders in the database.
- **Rename Folder** - Allows users to rename folders in the database.
- **Move Folder** - Allows users to move folders in the database.
- **Delete Empty Folder** - Allows users to delete empty folders from the database.
- **Hide/Unhide Folder** - Allows users to hide and unhide folders and to use hidden folders in the database.

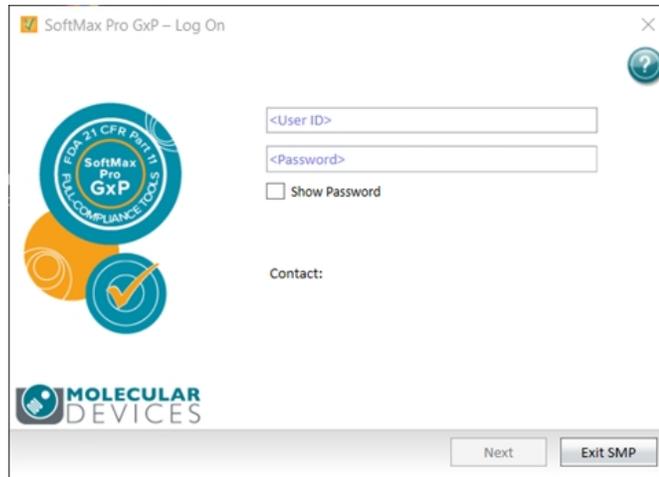
Instrument Permissions

Use Instrument permissions to manage what users can do with the microplate reader.

- **Lock/Unlock Instrument** - Allows users of the SpectraMax® iD3 Multi-Mode Microplate Reader and the SpectraMax® iD5 Multi-Mode Microplate Reader to lock and unlock the instrument touchscreen.
- **Instrument Simulator** - Allows users to simulate the connection between the SoftMax Pro GxP Software and an instrument.

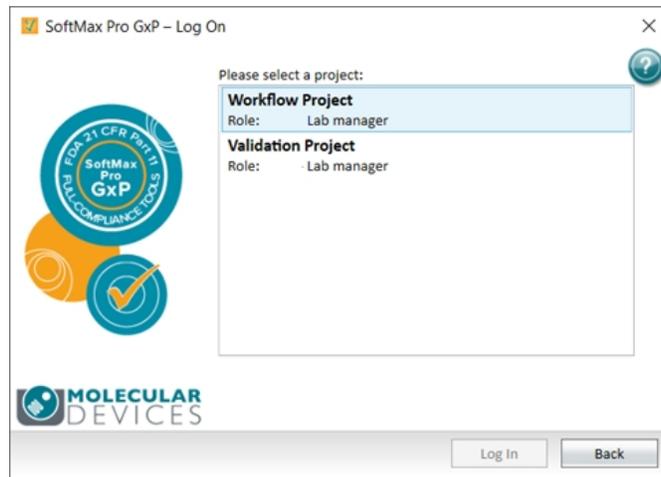
Logging In to the SoftMax Pro GxP Software

The SoftMax Pro GxP Log On dialog displays when you start the SoftMax Pro GxP Software.



To log on to the SoftMax Pro GxP Software:

1. In the **<User ID>** field, enter your username.
2. In the **<Password>** field, enter your password (case sensitive).
3. Click **Next** to display the Please Select a Project dialog.



4. Select a Project and click **Log In**.

Logging In From Within the Software

In the Ribbon, on the GxP tab, use the icons to log out of the software and then log on to a different Project or as a different user.

1. In the Ribbon, select the **GxP** tab.
2. Click  **User Log Off** to log off. The SoftMax Pro GxP Log On dialog displays.
3. Follow the steps above to log on.

SpectraMax iD3 and SpectraMax iD5 Considerations

For users that use the SoftMax Pro Software - GxP edition to operate the instrument, the user must have the Lock/Unlock Instrument permission to lock and unlock the instrument touchscreen.

In the Ribbon, on the GxP tab, users with the Lock/Unlock Instrument permission can use the following icons to lock and unlock the instrument touchscreen:

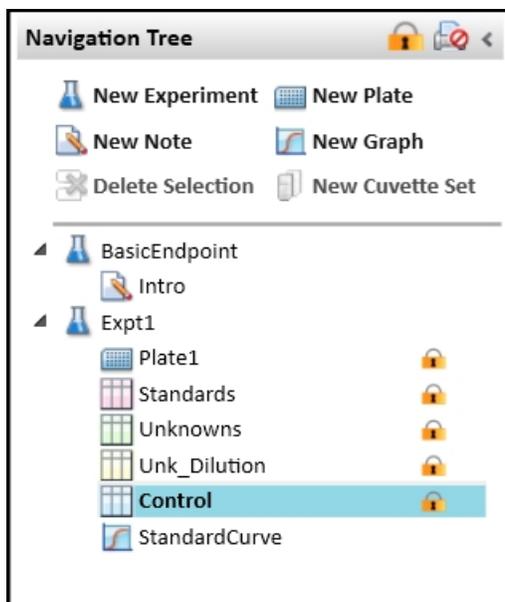
- Click  **GxP Mode On** to lock the instrument touchscreen and operate the instrument from the computer running the SoftMax Pro Software in GxP mode. This locks the instrument touchscreen for all users and you must operate the instrument from a computer running the SoftMax Pro Software - GxP edition.
- Click  **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to use the instrument touchscreen to run experiments.



Note: The instrument remains locked until the user with the Lock/Unlock Instrument permission clicks  **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an instrument that is locked in GxP mode.

Locking and Unlocking Sections

The SoftMax Pro Software - GxP edition allows users with the Lock/Unlock Sections permission to lock sections in an experiment to prevent changes. Users who do not have this permission cannot delete or edit a locked section. You can print locked sections with the document.



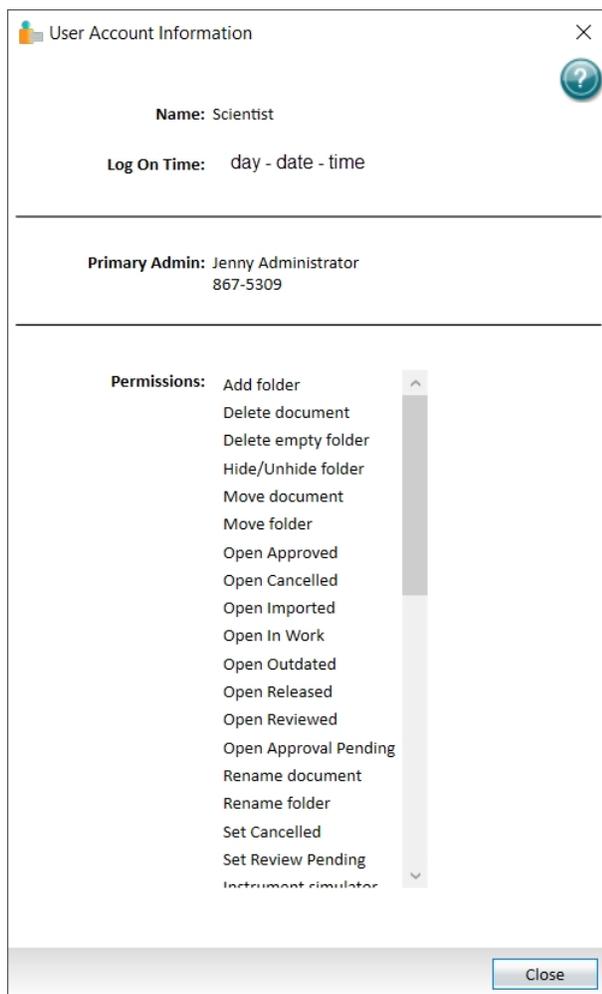
Select a section in the Navigation Tree and click  above the Navigation Tree to lock the section you select. Click  again to unlock the section.

Select an experiment in the Navigation Tree and click  above the Navigation Tree to lock all the sections in the experiment.

You cannot overwrite the data in a locked Plate section, but if a Plate section has no data, data can be acquired and added to the section.

Account Information

Use the User Account Information dialog to view information about the user who is logged on.



To view user account information:

1. In the Ribbon, select the **GxP** tab.
2. Click  **Account Information** to display the User Account Information dialog.
 - The top section displays the username and the time the user logged on.
 - The middle section displays the contact information for the GxP Admin Software administrator.
 - The lower section displays the permissions assigned to the user based on the user's Role in the Project selected upon log on.
3. Click **Close**.

User Lock Session

Use the Session Is Currently Locked dialog to unlock your SoftMax Pro Software - GxP edition session after the software locks you out due to inactivity. The GxP Admin Software administrator defines the inactivity before the software lock duration.

The software continues to collect data even when the user is locked out due to inactivity.



If your session is locked due to inactivity, do the following to unlock your session.

1. The Session Is Currently Locked dialog displays when your session is locked due to inactivity. You can also select the **GxP** tab and click  **User Lock Session** to display the Session Is Currently Locked dialog.
2. In the **Password** field, enter your password.
3. Click one of the following:
 - Click **Verify** to verify your credentials and unlock the software.
 - Click **Stop Read** to stop a read in progress.
 - Click **Close SMP** to close the SoftMax Pro Software - GxP edition and end the session (password not required).

Changing Passwords

Use the Change Password dialog to change your password. The GxP Admin Software administrator can define rules to require password changes and password strength.



To change your password:

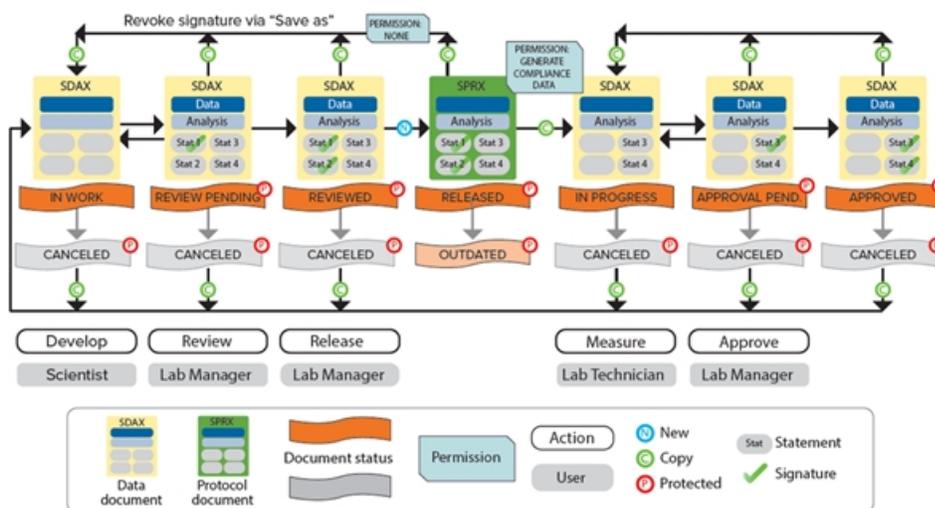
1. In the Ribbon, select the **GxP** tab.
2. Click  **Change Password** to display the Change Password dialog.
3. In the **<Password>** field, enter your current password.
4. In the **<New Password>** field, enter your new password.
5. In the **<Confirm Password>** field, enter your new password again to confirm it.
6. Click **OK**.

Document Workflow

The document life cycle in a compliant lab consists of multiple different development and review steps and requires a system to indicate the document state. The status system allows a Project team to track and approve documents while the document moves through the pipeline of development, review, release, and usage in a controlled environment.

The SoftMax Pro GxP Software Suite installation includes three default Roles: Scientist, Lab Technician, and Lab Manager. The default permissions for these Roles correspond to the document statuses that are present in the SoftMax Pro GxP Software. You can modify the default Role permissions, if needed. See [Default Roles on page 260](#).

The following document workflow corresponds to the default Roles. Your laboratory can define Roles and document workflows that meet your needs.



In Work Document Status - Scientist Role

Scientists open and save new data documents that contain the instrument settings in Plate sections and define data analysis settings. The software assigns a new document the status In Work. Scientists add statements to the documents to provide communication to the other users responsible for the document. Scientists sign the first statement to prevent further edits and set the document status to Review Pending.

Review Pending Document Status - Lab Manager Role

Lab managers review the data documents with the status Review Pending, sign the second statement, and set the document status to Reviewed.

Reviewed Document Status - Release Protocol - Lab Manager Role

If the data document with the status Reviewed is correct, the lab managers then release the document as a protocol. The software saves the protocols with the status Released for reuse. If a document has a newer iteration or is incorrect, the lab managers can set documents that have the status Released to the Outdated status.

In Progress Document Status - Lab Technician Role

Lab technicians open protocols with the Released status and the software prompts the lab technicians to save the protocols as data documents before the lab technicians can run the experiments. Because the Lab Technician Role has the Generate Compliance Data permission, the software sets the data document status to In Progress and removes the two signed statements. This allows lab technicians to run experiments and generate the compliance data.

When lab technicians finish their work, they sign a statement to prevent further changes and set the document status to Approval Pending.

Approval Pending Document Status - Lab Manager Role

Lab managers confirm that documents with the Approval Pending status meet regulatory requirements, sign a statement, and set the document status to Approved. If the lab manager determines the document is incorrect, they can set an Approved document back to the In Progress status.

Canceled Document Status

Users with appropriate permissions can set the document status to Canceled at the applicable points in the document workflow.

Default Roles

Use the GxP Admin Portal Software default Roles to assign users permissions to perform the tasks that correspond to each document status. You can create your own Roles and you can edit the default Roles.

Default Role Permissions

Permission	Lab Manager	Lab Technician	Scientist
Add/Delete Groups			✓
Add Folder	✓		✓
Add/Modify Statements			✓
Assign Plate Layouts		✓	✓
Change Auto Export Settings			✓
Change Auto Save Settings			✓
Create/Save As Data Document	✓		✓
Create/Save As Protocol			✓
Delete Document	✓		✓
Delete Empty Folder	✓		✓
Edit Formulas			✓
Edit Graphs, Summaries, and Reductions			✓
Edit Notes Text		✓	✓

Default Role Permissions (continued)

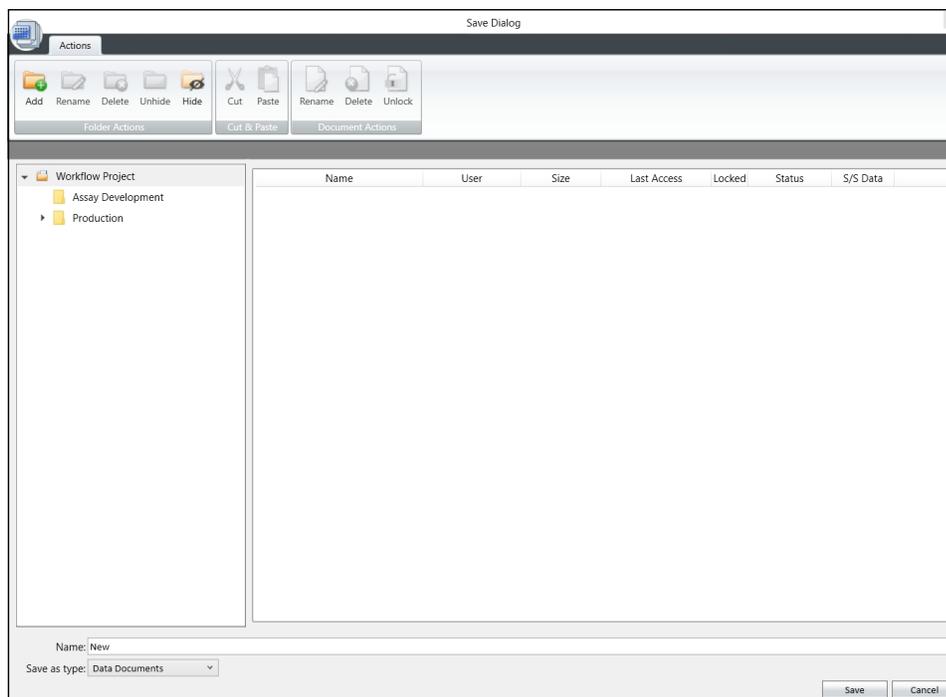
Permission	Lab Manager	Lab Technician	Scientist
Edit Print Options			✓
Edit Reader Settings			✓
Edit Sample and Group Information		✓	✓
Export Documents	✓	✓	✓
Function Editor			✓
Generate Compliance Data		✓	
Hide/Unhide Folder	✓		✓
Import Documents			✓
Instrument Simulator			✓
Lock/Unlock Instrument	✓		✓
Lock/Unlock Sections			✓
Mask/Unmask Wells		✓	✓
Move Document	✓		✓
Move Folder	✓		✓
Open Approval Pending	✓		✓
Open Approved	✓		✓
Open Canceled	✓		✓
Open Imported			✓
Open In Progress		✓	
Open In Work			✓
Open Outdated	✓		✓
Open Released	✓	✓	✓
Open Review Pending	✓		
Open Reviewed	✓		✓
Overwrite Plate/Cuvette Data			✓
Read Empty Plates/Cuvettes		✓	✓
Release Protocol	✓		
Rename Document	✓		✓

Default Role Permissions (continued)

Permission	Lab Manager	Lab Technician	Scientist
Rename Folder	✓		✓
Revoke Any Signature			
Revoke Own Signature	✓	✓	✓
Set Approval Pending		✓	
Set Approved	✓		
Set Canceled	✓	✓	✓
Set In Progress	✓		
Set Outdated	✓		
Set Review Pending			✓
Set Reviewed	✓		
Sign Statements	✓	✓	✓
Unlock Document	✓		

Saving Documents

Use the Save dialog to save a document to the database. The Save dialog displays the folders and documents that are included in the Project you select when you log on.



When you open a new document, the software prompts you to save the document before you do any additional work.

To save a document:

1. Save vs. Save As

- Click  in the Quick Access toolbar or click  to open the Applications menu and select  **Save** to update the currently open document with updated information in the same location with the same name. The document saves with no further steps required.
- Click  in the Quick Access toolbar or click  to open the Applications menu and select  **Save As** to rename a document, to save a protocol as a data document, to save a data document as a protocol, to move a document to a different location in the database, or to give a new document a name. The Save As dialog displays.

2. Navigate to where you want to save the document.
3. In the **Name** field, enter the document name.
4. Click the **Save As Type** drop-down.
 - Select **Protocol Documents** to save the document as a protocol.
 - Select **Data Documents** to save the document as a data document.
5. Click **Save**.

Save Icons

The left side contains the folder hierarchy similar to the folder hierarchy in any other computer application. The top level folder is the name of the Project you select when you log on and you have access only to the documents for that project.

The toolbar contains the following icons:

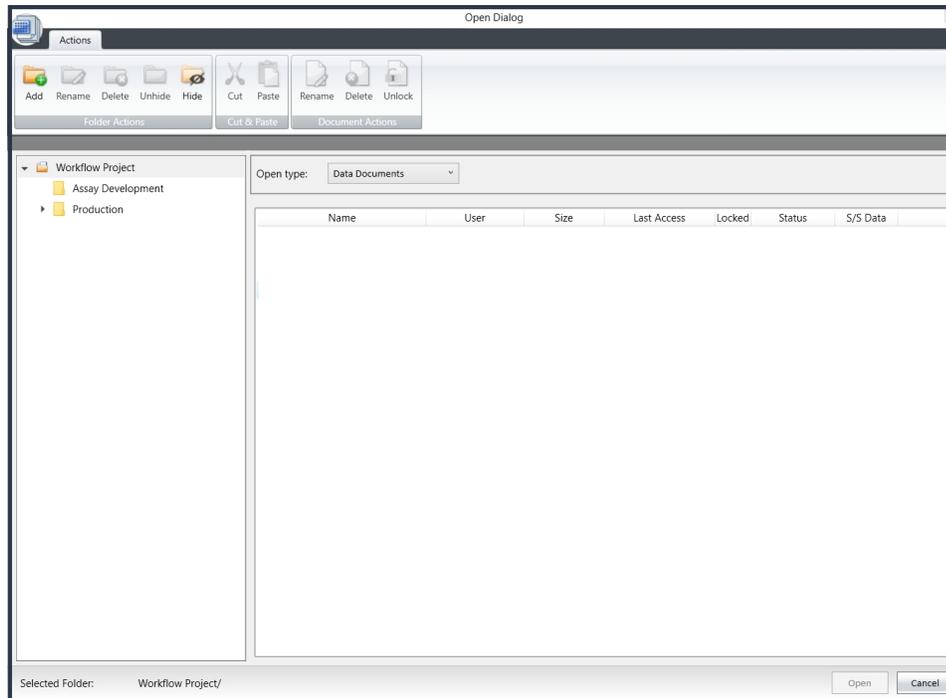
-  **Add** - Click to add a folder subordinate to the folder you select in the hierarchy.
-  **Rename** - Click to rename the folder you select in the hierarchy.
-  **Delete** - Click to delete the folder you select in the hierarchy.
-  **Unhide** - Click to unhide the hidden folder you select in the hierarchy.
-  **Hide** - Click to hide the folder you select in the hierarchy.
-  **Cut** - Click to copy the folder or document you select to the computer clipboard for the first step in moving the folder or document to a different location in the database.
-  **Paste** - Click to paste the folder or document from the computer clipboard to the location you select to move the folder or document in the database.
-  **Rename** - Click to rename the document you select in the document list.
-  **Delete** - Click to delete the document you select in the document list.
-  **Unlock** - Click to unlock a locked document.

The right side displays the documents in the folder you select along with the following information:

- **Name** - Displays the document name.
- **User** - Displays the full name of the user who last touched the document.
- **Size** - Displays the size of the document.
- **Last Access** - Displays the date and time the document was most recently accessed.
- **Locked** - Displays a check mark if the document is open by another user and is therefore locked to users who do not have the Unlock Files permission.
- **Status** - Displays the document status.
- **S/S Data** - Displays the number of statements and the number of signed statements.

Opening Documents

Use the Open dialog to open a document from the database. The Open dialog displays the folders and documents that are included in the Project you select when you log on.



To open an existing document:

1. Click  in the Quick Access toolbar or click  to open the Application menu and select  **Open** to display the Open dialog.
2. Click the **Open Type** drop-down:
 - Select **Data Documents** to open a data document.
 - Select **Protocol Documents** to open a protocol.
3. Navigate to the document and select it.
4. Click **Open**.

Open Icons

The left side contains the folder hierarchy similar to the folder hierarchy in any other computer application. The top level folder is the name of the Project you selected when you logged on and you have access only to the documents for that project.

The dialog toolbar contains the following icons:

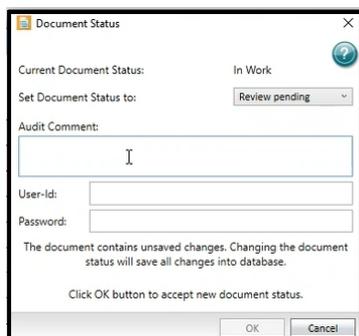
-  **Add** - Click to add a folder subordinate to the folder you select in the hierarchy.
-  **Rename** - Click to rename the folder you select in the hierarchy.
-  **Delete** - Click to delete the folder you select in the hierarchy.
-  **Unhide** - Click to unhide the hidden folder you select in the hierarchy.
-  **Hide** - Click to hide the folder you select in the hierarchy.
-  **Cut** - Click to copy the folder or document you select to the computer clipboard for the first step in moving the folder or document to a different location in the database.
-  **Paste** - Click to paste the folder or document from the computer clipboard to the location you select to move the folder or document in the database.
-  **Rename** - Click to rename the document you select in the document list.
-  **Delete** - Click to delete the document you select in the document list.
-  **Unlock** - Click to unlock a locked document.

The right side displays the documents in the folder you select along with the following information:

- **Name** - Displays the document name.
- **User** - Displays the full name of the user who last touched the document.
- **Size** - Displays the size of the document.
- **Last Access** - Displays the date and time the document was most recently accessed.
- **Locked** - Displays a check mark if the document is open by another user and is therefore locked to users who do not have the Unlock Files permission.
- **Status** - Displays the document status.
- **S/S Data** - Displays the number of statements and the number of signed statements.

Document Status

Use the Document Status dialog to change the status of a document to move the document through the document workflow.



The permissions granted to your Role determine what document statuses are available to you.

To change a document status:

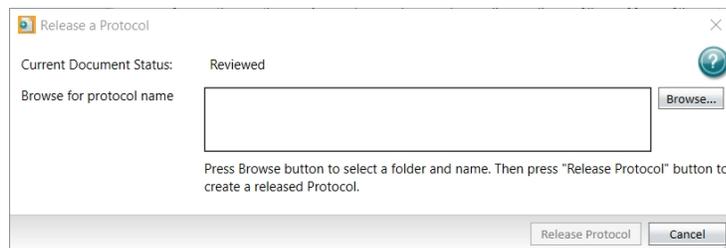
1. Select the **GxP** tab and click  **Document Status** or click the status indicator above the

right side of the Workspace  to display the Document Status dialog.

2. Click the **Set Document Status To** drop-down and select a document status.
3. In the **Audit Comment** field, enter the text to include in the audit trails. See [Document Audit Trail on page 276](#).
4. In the **User ID** field, enter your username.
5. In the **Password** field, enter your password.
6. Click **OK**.

Releasing Protocols

Use the Release a Protocol dialog to take a data document with the Reviewed status and release the document as a protocol with the Released status.

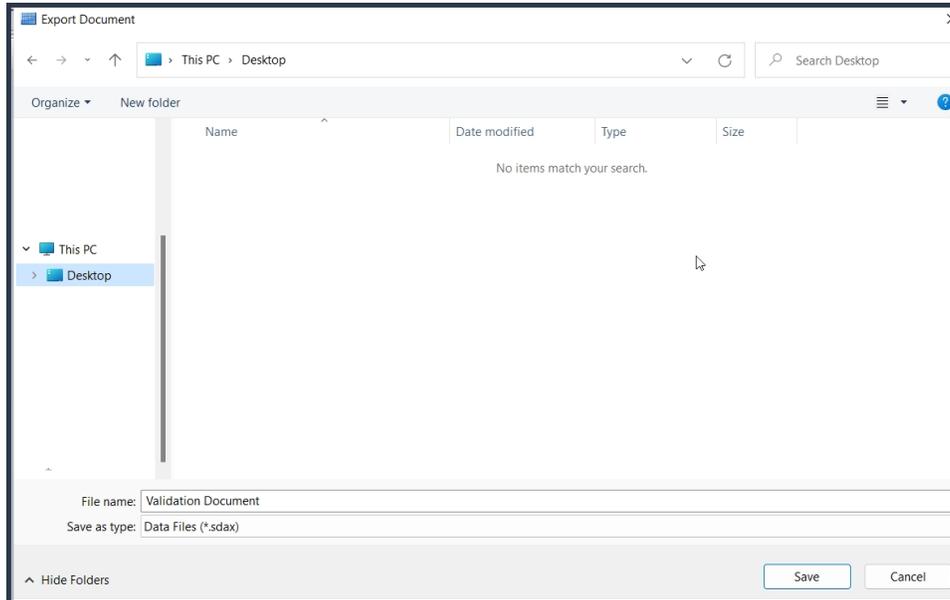


To release a protocol:

1. Open a document with the status Reviewed.
2. Select the **GxP** tab and click  **Release Protocol** to display the Release a Protocol dialog.
3. Click **Browse** to display the Save dialog. See [Saving Documents on page 263](#).
4. Navigate to the folder into which to save the document.
5. In the **Name** field, enter the name of the protocol.
6. Click **Save** to display the Release a Protocol dialog. The Browse For Protocol Name field displays the path and protocol name.
7. Click **Release Protocol**.

Exporting as Data File

Use the Export Document dialog to export a SoftMax Pro Software - GxP edition data document to the computer hard drive or other network storage device in a format that can then be imported into a different Project or into a different SoftMax Pro Software - GxP edition version 7.3 database.



To export a data document:

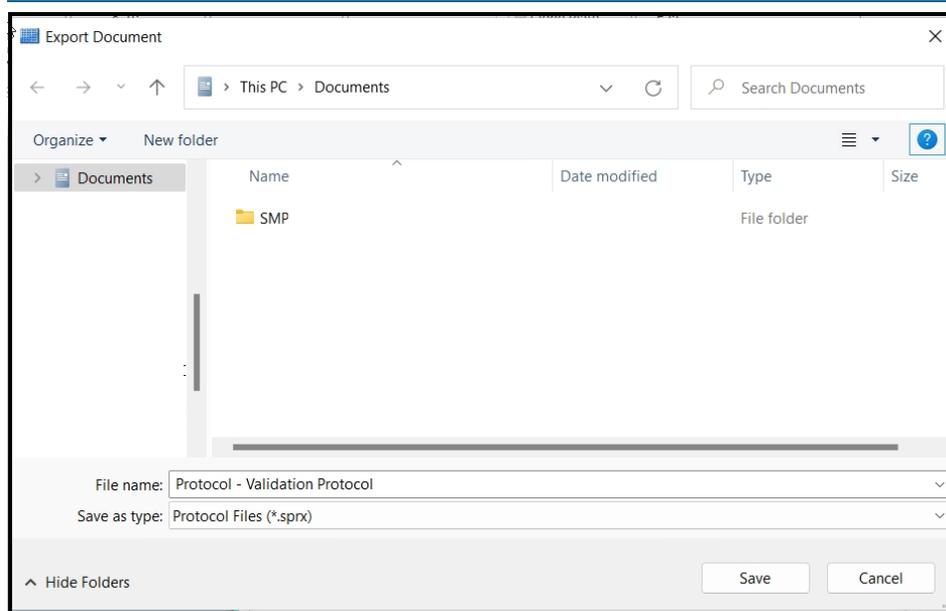
1. Open the data document.
2. Click  to display the Application menu, click  **Export**, and then select  **Export as Data File (sdax)** to display the Export Document dialog.
3. Navigate to the folder where you want to save the data document.
4. In the **File Name** field, enter the export file name.
5. Click **Save**.

Exporting as Protocol File

Use the Export Document dialog to export a SoftMax Pro Software - GxP edition protocol document, with the status Released, to the computer hard drive or other network storage device in a format that can then be imported into a different Project or into a different SoftMax Pro Software - GxP edition version 7.3 database.



Note: It is important to understand that Role permissions and document status are critical considerations.



To export a protocol document:

1. Log into the software with a **Lab Manager** level Role permission.
2. Open a protocol document that has the status **Review Pending**.
3. Set the document status to **Reviewed**.
4. Click the **Release Protocol** button and release the document to set the document status to Released. See [Releasing Protocols on page 267](#).
5. Click  to display the Application menu, click  **Export**, and then select  **Export as Protocol File (sprx)** to display the Export Document dialog.
6. Navigate to the folder where you want to save the protocol document.
7. In the **File Name** field, enter the export file name.
8. Click **Save**.

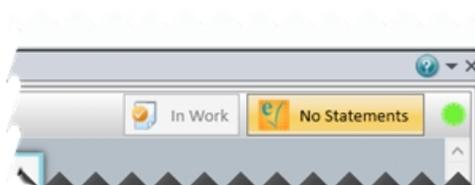
GxP Statements

Use the Statements dialog to manage statements. Use statements to communicate and get sign off for a document. You use statements in conjunction with the document status to indicate who created the document, reviewed the document, ran the assay, and approved the results.

Each document can have multiple statements. Each statement can have one signature so if the document requires multiple signatures, you need to add the corresponding number of statements. Each user can sign only one statement. After a statement is signed you cannot edit the document or add new statements so you should add all the required statements before you start getting signatures.

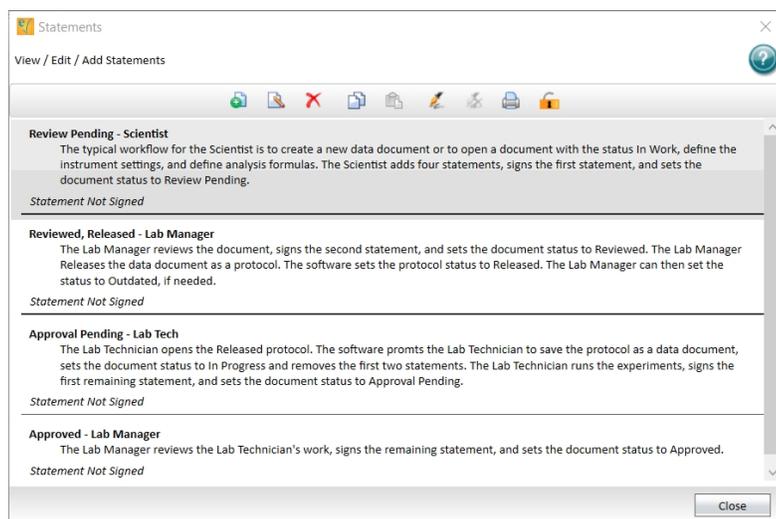
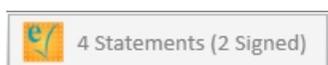
The following user permissions pertain to statements:

- **Add/Modify Statements** - Grants users permission to add or modify statements. See [Adding Statements on page 272](#).
- **Sign Statements** - Grants users permission to sign statements. See [Signing Statements on page 273](#).
- **Revoke Any Signature** - Grants users permission to revoke any signature from statements. See [Revoking Signatures from Statements on page 274](#).
- **Revoke Own Signature** - Grants users permission to revoke only their own signatures from statements.



Click  above the right side of the Workspace or select the **GxP** tab and click **Statements** to display the Statements dialog.

After you add statements and after you sign statements, the button reflects the changes made to the statements for the document.



The first time you access the Statements dialog you need to add a statement before the icons in the dialog toolbar are available.

Use the following icons in the Statements dialog toolbar to manage statements.

-  - Click to display the Add Statement dialog where you add additional statements to the document.
-  - Select a statement and click to display the Edit Statement dialog where you edit the statement. See [Editing Statements on page 272](#).
-  - Click to display the Delete Statements dialog. This deletes all statements in the list. You cannot delete an individual statement.
-  - Select a statement and click to copy the statement to the computer clipboard. You can then paste the statement into the list of statements for the current document or you can paste the statement to another document.
-  - Click to paste the statement from the computer clipboard into the list of statements. This can be for the original document or for a different document.
-  - Select a statement and click to display the Sign Statement dialog. After any statement associated with a document is signed, the document is locked. You cannot add additional statements or edit the document.
-  - Click to display the Revoke Signature dialog where you confirm that you want to revoke the signature from the statement you select.
-  and  - Click to include or exclude the statement from the printed version of the document. Statements also print when you print the document audit trail. See [Printing on page 34](#).
-  and  - Click to lock or unlock statements to permit or prevent the ability to edit and manage the statement. You cannot lock signed statements.

Click **Close** to close the Statements dialog.

Adding Statements

Use the Add Statement dialog to add a statement to a document. Each statement can have one signature so if the document requires multiple signatures, you need to add the corresponding number of statements. After a statement is signed you cannot edit the document or add new statements so you should add all the required statements before you start getting signatures.

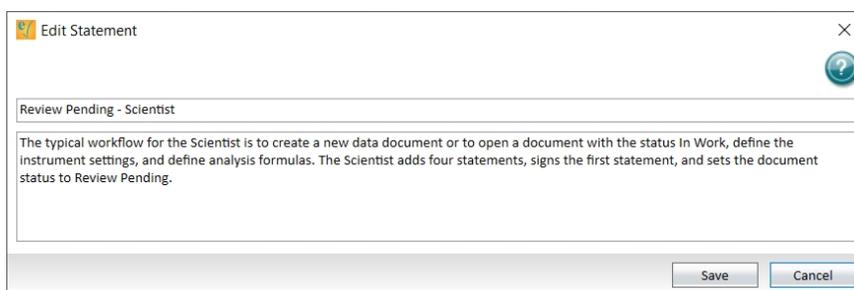


To add a statement:

1. Select the **GxP** tab and click **Statements** to display the Statements dialog.
2. If the document has no statements, complete the following two fields and then click **Add**. If the document has statements, select the statement above where you want to add the new statement and then click  in the Statements dialog toolbar to display the Add Statement dialog.
3. In the **New Statement Title** field, enter the statement title.
4. In the **New Statement Description** field, enter the statement content.
5. Click **Add**.

Editing Statements

Use the Edit Statement dialog to edit a statement. After any statement for a document is signed, you cannot edit the document or the statements associated with the document.

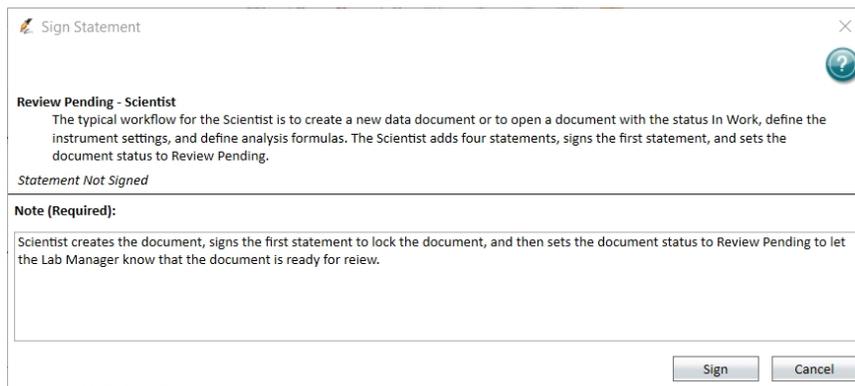


To edit a statement:

1. Select the **GxP** tab and click **Statements** to display the Statements dialog.
2. Select the statement to edit.
3. Click  in the Statements dialog toolbar to display 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**.

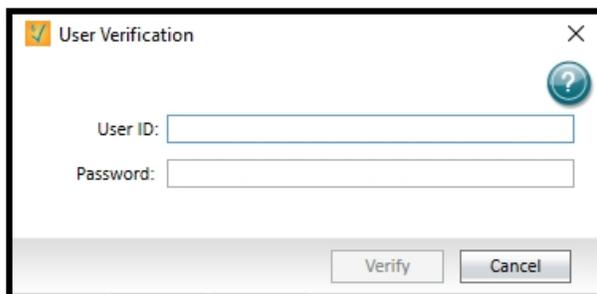
Signing Statements

Use the Sign Statement dialog to apply an electronic signature to a statement. Each statement can have one signature. After you sign a statement, the document is locked to prevent further changes and you cannot add additional statements. Remember to add as many statements as the document requires before you get any statements signed. You must revoke all signatures from all statements to unlock the document.



To sign a statement:

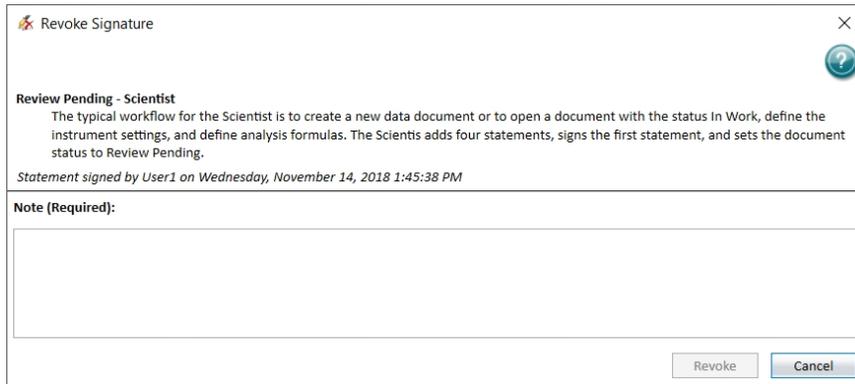
1. Select the **GxP** tab and click **Statements** to display the Statements dialog.
2. Select the statement to sign.
3. Click  in the Statements dialog toolbar to display the Sign Statement dialog.
4. In the **Note** field, enter a note.
5. Click **Sign** to display the User Verification dialog.



6. In the **User ID** field, enter your username.
7. In the **Password** field, enter your password.
8. Click **Verify**.

Revoking Signatures from Statements

Use the Revoke Signature dialog to revoke the signature from the statement you select.



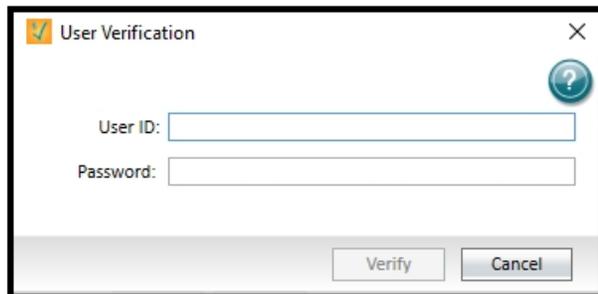
To revoke the signature from a statement:

1. Select the **GxP** tab and click **Statements** to display the Statements dialog.
2. Select the statement from which to revoke the signature.
3. Click  in the dialog toolbar to display a confirmation message.
4. Click **OK** to display the Revoke Signature dialog.



CAUTION! There is no undo for this. After you revoke all signatures, the document is unlocked and can be changed.

5. In the **Note** field, enter the reason for the signature removal.
6. Click **Revoke** to display the User Verification dialog.



7. In the **User ID** field, enter your username.
8. In the **Password** field, enter your password.
9. Click **Verify**.

Audit Trail

The GxP Admin Software and the SoftMax Pro Software - GxP edition generate audit trails that record the events that user actions trigger. For each event instance, the audit trail records the date and time, the username of the person who was logged on, and the event action. Users cannot alter the audit trail, nor can they edit or delete audit trail entries.

GxP Admin Software Events

The GxP Admin Software saves the audit trail to the database and you view the System Audit Trail in the GxP Admin Portal Software. For a list of events that are saved to the system audit trail, see the *GxP Admin Portal Software User Guide*.

SoftMax Pro Software - GxP Edition Events

In addition to the GxP Admin Software System Audit Trail, the SoftMax Pro Software - GxP edition records document-specific events to an audit trail for the document. You view the document-specific audit trail in the SoftMax Pro Software - GxP edition.

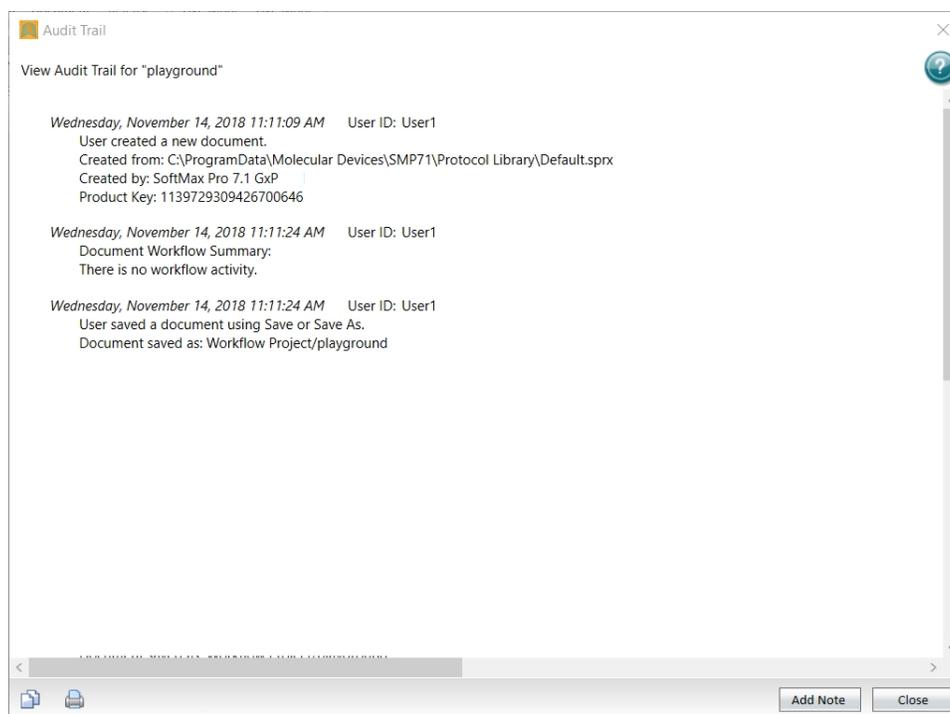
The document audit trail records events that pertain to the document that is active in the Workspace and saves the document-specific events with the document. Additionally, the document audit trail includes general activities such as log in and log out when a document is open during those events.

The audit trail for each Plate section in a document includes the following information:

- The name of the user who logged in when the read began.
- The instrument type used to do the read and the instrument ROM version number. When available, the audit trail also lists the instrument serial number and the serial number for any detection cartridges.

Document Audit Trail

Use the Audit Trail dialog to view the audit trail for the open document. You can add a note to the audit trail but you cannot edit the content of the audit trail.



For a list of the events that are included in the document audit trail, see the *GxP Admin Portal Software User Guide*.

Select the **GxP** tab and click  **Audit Trail** to display the Audit Trail dialog.

Each entry in the audit trail has a time-and-date stamp and identifies the user who performed the task.

-  - Click to copy the content of the entire audit trail to the computer clipboard. You can then paste the audit trail into a text editor software application.
-  and  - Click to include or exclude the audit trail in the printed version of the document. See [Printing on page 34](#).
- **Add Note** - Click to display the Add a Note dialog where you add a note to the audit trail.
- Click **Close**.

Read Modes

Use the software to define the parameters for the read mode and read type of your assay.

- Absorbance Read Mode, see page 280
- AlphaScreen Read Mode, see page 285
- Fluorescence Intensity Read Mode, see page 287 Fluorescence Intensity Dual Wavelength is available for the SpectraMax Paradigm
- Fluorescence Polarization Read Mode, see page 292
- FRET Read Mode, see page 295
- Imaging Read Mode, see page 298
- Luminescence Read Mode, see page 301 Luminescence Dual Wavelength is available for the FilterMax F3, FilterMax F5, SpectraMax i3x, SpectraMax iD5, and SpectraMax L
- Time-Resolved Fluorescence Read Mode, see page 304 Time Resolved Fluorescence Dual Wavelength is available for the SpectraMax i3x and SpectraMax Paradigm
- TR-FRET Read Mode, see page 308
- Western Blot Read Mode, see page 311

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

Read Types

For most read modes, you can use the endpoint, kinetic, spectrum, and well scan read types. The FlexStation 3 supports the flex read type that allow you to integrate fluid transfer with a kinetic read. The SpectraMax L supports the fast kinetic read type and the dual read type that allow the instrument to perform reads with injection.

Dual Read Type

For the SpectraMax L dual read type, each well receives two injections and two reads. The first injection is the P-injection with a subsequent read. The second injection is the M-injection with a subsequent read. You can set a delay time after each injection, from 0.1 to 3,600 seconds for the P-injection, and from 0 to 3,600 seconds for the M-injection.

Endpoint Read Type

For the endpoint read type, a read of each plate well is taken in the center of each well, at a single wavelength or at multiple wavelengths. Depending on the read mode, the software reports raw data values as optical density (OD), %Transmittance (%T), relative fluorescence units (RFU), or relative luminescence units (RLU).

Fast Kinetic Read Type

For the SpectraMax L fast kinetic read type, one or more wells are read repeatedly up to a 200 point maximum integration. All reads of a single well are made before the next well is read. You cannot pre-read a plate with the fast kinetic read type. Each well can receive multiple injections and reads. You can specify multiple injections and multiple reads, for multiple times. You can set the integration time from 0.01 to 3600 seconds.

Flex Read Type

The FlexStation 3 has a fluidics module that aspirates fluids from a compound source plate and dispenses 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 the total experimental time. At a certain point or points, the pipettor transfers up to three reagents from the compound plate to the assay plate. The instrument continues to read at the time intervals before and after each fluid transfer. After completion of a column read, the instrument repeats this cycle with other columns. All data the software collects in one document is represented as a 96-well or 384-well plate.

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

$$\text{Run time} \times \text{Number of columns} = \text{Plate time}$$

$$2 \text{ minutes} \times 12 \text{ columns} = 24 \text{ minutes}$$

Kinetic Read Type

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

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

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 plate assays, including ELISAs and the purification and characterization of enzymes and enzyme conjugates. Kinetic analysis can provide 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 Data Acquisition and Analysis Software Formula Reference Guide*.

Membrane Read Type

The SpectraMax i3x, SpectraMax iD5, and SpectraMax Paradigm use the membrane read type for Time-Resolved Fluorescence reads of a Western Blot membrane. The software reads the area you select and generates a TIFF image with the results of the read.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis. 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 support.moleculardevices.com.

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

- **Read Empty Plates/Cuvettes** to start a read.
- **Create/Save Data Documents** to save the data document before the read.
- **Overwrite Plate/Cuvette Data** to do a high-resolution ROI read.

See [SoftMax Pro Software - GxP Edition on page 251](#).

Spectrum Read Type

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

Well Scan Read Type

The well scan read type takes reads at more than one location within a well. The well scan read type takes multiple reads of a single well of a plate on an evenly spaced pattern inside of each well at single or multiple wavelengths.

For applications that involve the detection of cells in large area tissue culture plates, the well scan read type permits maximum surface area detection in cell-based protocols. Since many cell lines tend to grow as clumps or in the corners of plate wells, you can choose the well scan pattern and define the number of points to scan in order achieve the best results.

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).

Absorbance Read Mode

The instrument uses the Absorbance (ABS) read mode to measure 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 intensity of the incident light before it enters the sample divided by the light after it passes through the sample, and A is the measured absorbance.

The instrument allows you to choose whether to display absorbance data as Optical Density (OD) or %Transmittance (%T).

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, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will 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 is 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 Low Volume Microplate and SpectraDrop 64-well Low Volume Microplate.

For information about how to set up absorbance mode protocols, see [Creating Absorbance Mode Protocols](#) on page 160.

Absorbance Instruments

The following instruments have absorbance read mode capability:

- EMax Endpoint Microplate Reader, see page 316
- EMax Plus Microplate Reader, see page 317
- FilterMax F3 Multi-Mode Microplate Reader, see page 318
- FilterMax F5 Multi-Mode Microplate Reader, see page 321
- FlexStation 3 Multi-Mode Microplate Reader, see page 322
- SpectraMax 190 Microplate Spectrophotometer, see page 324
- SpectraMax 340PC384 Microplate Spectrophotometer, see page 324
- SpectraMax ABS Microplate Spectrophotometer, see page 325
- SpectraMax ABS Plus Microplate Spectrophotometer, see page 325
- SpectraMax i3 Multi-Mode Microplate Reader, see page 326
- SpectraMax i3x Multi-Mode Microplate Reader, see page 328
- SpectraMax iD3 Multi-Mode Microplate Reader, see page 330
- SpectraMax iD5 Multi-Mode Microplate Reader, see page 333
- SpectraMax M2 Multi-Mode Microplate Reader, see page 338
- SpectraMax M2e Multi-Mode Microplate Reader, see page 338
- SpectraMax M3 Multi-Mode Microplate Reader, see page 339
- SpectraMax M4 Multi-Mode Microplate Reader, see page 339
- SpectraMax M5 Multi-Mode Microplate Reader, see page 340
- SpectraMax M5e Multi-Mode Microplate Reader, see page 340
- SpectraMax Mini Multi-Mode Microplate Reader, see page 341
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342 Requires the Absorbance Detection Cartridge, see page 375.
- SpectraMax Plus 384 Microplate Spectrophotometer, see page 345
- VersaMax Microplate Spectrophotometer, see page 345
- VMax Kinetic Microplate Reader, see page 346

PathCheck Technology

To use PathCheck technology, select the PathCheck check box in the PathCheck Settings category in the Settings dialog for absorbance endpoint reads.

The temperature-independent PathCheck technology normalizes 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:

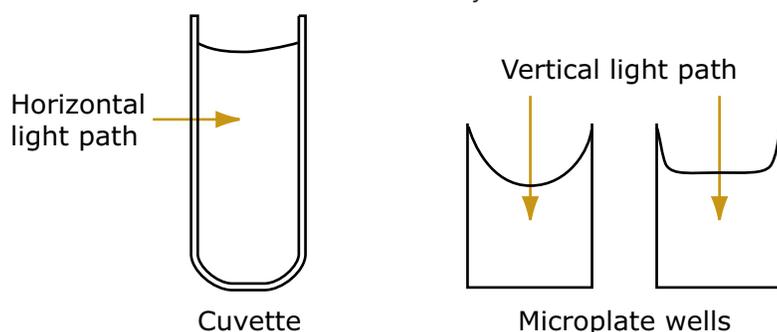
$$A = \epsilon cL$$

where A is the absorbance, ϵ is the molar absorptivity of the sample, c is the concentration of the sample, and L is the pathlength. The longer the pathlength, the higher the absorbance.

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 makes it confusing to compare results between microplate readers and spectrophotometers.

The standard pathlength of a 1 cm cuvette is the conventional basis to quantify 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 you use 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 plate, 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 determines the pathlength of aqueous samples in the plate and normalizes the absorbance in each well to a pathlength of 1 cm. This way of correcting the microwell absorbance values can be accurate to within $\pm 4\%$ of the values obtained directly in a 1 cm cuvette.



PathCheck technology normalizes the data acquired from an absorbance read mode endpoint read type to a 1 cm pathlength, correcting the OD for each well to the value expected if the sample were read in a 1 cm cuvette. The instrument uses the factory installed water constant to obtain the 1 cm values.

For the SpectraMax ABS Plus, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, and SpectraMax Plus 384 you can read a cuvette that contains deionized water or buffer to use the cuvette reference correction method (typically not necessary when you use aqueous solutions with minimal alcohol, salt, or organic solvent content).



Note: You must select the PathCheck check box before a read because you cannot apply the PathCheck technology after the read. After you read a plate with PathCheck technology turned on, the software stores PathCheck information permanently within the document.

Water Constant

The PathCheck technology is based on the absorbance of water in the near infrared spectral region (between 900 nm and 1000 nm). If the sample is completely aqueous, has no turbidity and has a low salt concentration (less than 0.5 M), the water constant correction method 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 ABS Plus, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, and SpectraMax Plus 384. This method is not applicable if the solvent contains a substance that absorbs in the 900 nm to 1000 nm range. See [Interfering Substances on page 284](#).

The cuvette reference that the software uses with the PathCheck technology is different from the reference read of a cuvette that occurs when you click the Ref button in the Cuvette Set section tool bar. The software that uses the cuvette reference data for PathCheck calculations does not produce data that displays in a Cuvette Set section. The software uses the cuvette reference data for the PathCheck calculations in plates, not cuvettes. However, you can use the accessors in the Formula Editor dialog to obtain these values. In the software, click  **Formula Reference** to view the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide* and reference the !PathCheckLm1000 and !PathCheckLm900 accessor.

Eliminating the Pathlength-Independent Component

Raw OD measurements of plate samples include both pathlength-dependent components (sample and solvent) and a pathlength-independent component (OD of plate 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 Plate Blanks

You can use this method if all samples in the plate are the same volume, and the read does not depend on the PathCheck technology to correct for variability in volumes.

To use a plate blank:

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 the 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 plate material is subtracted as part of the blank.
3. Select the Use Plate Blank check box in the Data Reduction dialog.

Using Plate Background OD

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

To determine the Plate Background OD:

1. Fill a clean plate with water.
2. Read at the wavelengths you will use for the samples.

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



Note: It is important that you put water in the wells and do not read a dry plate for the Plate Background OD. A dry plate has a slightly higher OD value than a water filled plate because of differences in refractive indices. Use of a dry plate 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. Use of the Cuvette Reference does not reliably correct for turbidity.

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

To determine possible color interference:

- 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 you should not use the PathCheck technology.

Organic solvents could interfere with the PathCheck technology if the solvents have absorbance in the region of the NIR water peak. Solvents such as ethanol and methanol do not absorb in the NIR region, so the solvents do not interfere, except to cause a decrease in the water absorbance to the extent of their presence in the solution. If the solvent absorbs between 900 nm and 1000 nm, the interference would be similar to the interference of highly colored samples. If you add 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.

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 result signal is triggered by laser excitation (680 nm), that makes a photosensitizer on the A-beads convert oxygen to an excited (singlet) state. The 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 to generate 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.

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.

You can normalize the raw data to counts per second.

Applications of AlphaScreen

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 capture the Europium emission line of AlphaLISA®.

For information about how to set up AlphaScreen mode protocols, see [Creating AlphaScreen Mode Protocols on page 161](#).

AlphaScreen Instruments

The following instruments have AlphaScreen read mode capability:

- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328](#) Requires [AlphaScreen Detection Cartridges, see page 376](#).
- [SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342](#) Requires [AlphaScreen Detection Cartridges, see page 376](#).

For more information, go to www.perkinelmer.com.

ALPHASCREEN and ALPHALISA are registered trademarks of PerkinElmer, Inc.

Analyzing 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 plate 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 are not indicators of sensitivity.

The raw data can be normalized to counts per second by selecting the Normalization option in the Settings dialog. See [Acquisition Settings on page 112](#).

Background Correction

Although background is significantly lower than with fluorescence intensity measurements, you should use blanks or assay controls for background correction. The background can be effectively measured using blank replicates. When you read 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 Read option in the Settings dialog. This option reads only every other well in a checkerboard pattern, and then reads the plate 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, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will be subtracted is the average value of all blanks.

Data Qualification and Validation

Z' is the standard statistical parameter in the high-throughput screening community to measure the quality of a screening assay independent of test compounds. Use this as a measure of the signal separation between the positive controls and the negative controls in an assay.

Use the following formula to determine the value of Z' :

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|Mean_{c+} - Mean_{c-}|}$$

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. You can use higher values 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, increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.



Note: The assay plate and the instrument should be kept at room temperature, since temperature variations cause fluctuations in signal.

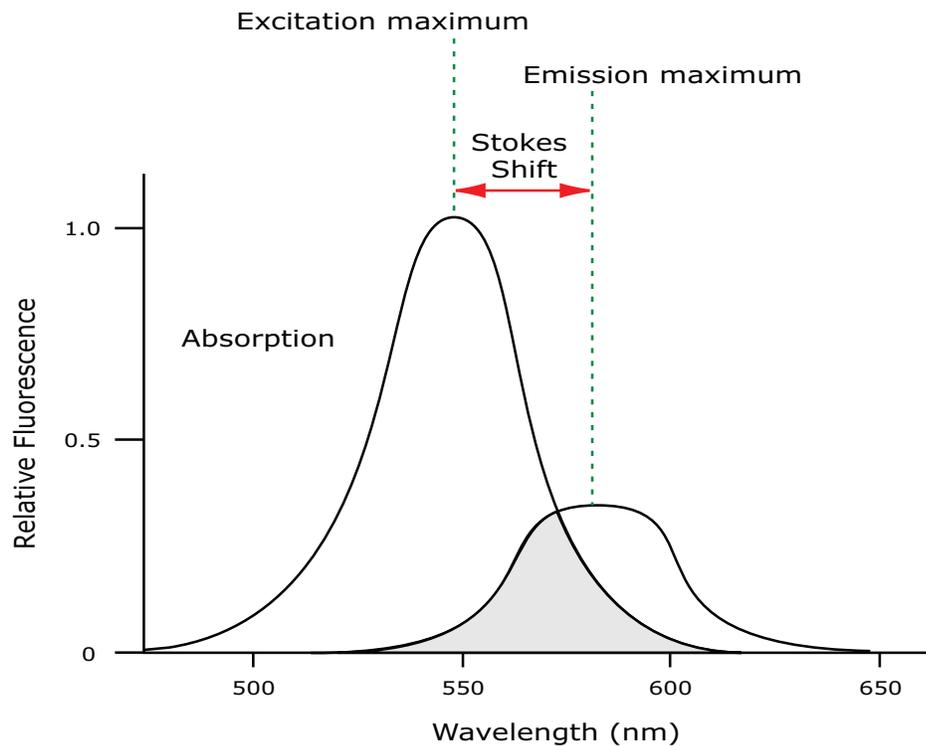
Fluorescence Intensity Read Mode

Fluorescence occurs when absorbed light is re-radiated at a longer wavelength. In the Fluorescence Intensity 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:

$$\text{Fluorescence} = \text{extinction coefficient} \times \text{concentration} \times \text{quantum yield} \times \text{excitation intensity} \times \text{pathlength} \times \text{emission collection efficiency}$$

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.

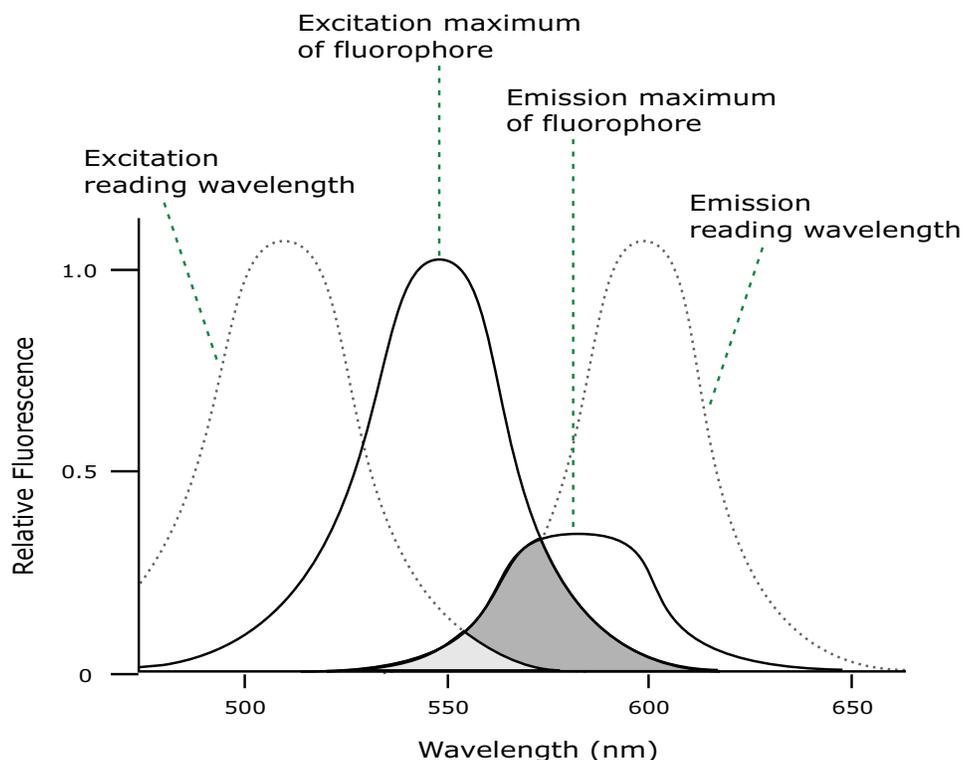


Excitation and Emission Spectra

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 able to stimulate the fluorophore so that less of the excited light overlaps the emission spectrum, which permits better selection and quantitation of the emitted light.

The Spectral Optimization wizard provides the best method to maximize the signal to background window (S-B)/B while minimizing the optimization time. The Spectral Optimization wizard is available for the SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, and the SpectraMax Paradigm with a Tunable Wavelength (TUNE) detection cartridge installed. See [Spectral Optimization on page 183](#).



Optimized Excitation and Emission Read Wavelengths

The previous figure shows that the best results are often obtained when the excitation and emission wavelengths you use for the read are not the same as the peak wavelengths of the excitation and emission spectra of the fluorophore. When the read 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, which results in a purer emission signal and more accurate data.

The instrument allows you to scan 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 several variables.

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 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.

For information about how to set up fluorescence intensity mode protocols, see [Creating Fluorescence Intensity Mode Protocols on page 162](#).

Fluorescence Intensity Instruments

The following instruments have fluorescence intensity read mode capability:

- [FilterMax F3 Multi-Mode Microplate Reader, see page 318](#)
- [FilterMax F5 Multi-Mode Microplate Reader, see page 321](#)
- [FlexStation 3 Multi-Mode Microplate Reader, see page 322](#)
- [Gemini EM Dual Scanning Microplate Spectrofluorometer, see page 323](#)
- [Gemini XPS Dual Scanning Microplate Spectrofluorometer, see page 323](#)
- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328 Supports Fluorescence Intensity Detection Cartridges, see page 376](#)
- [SpectraMax iD3 Multi-Mode Microplate Reader, see page 330](#)
- [SpectraMax iD5 Multi-Mode Microplate Reader, see page 333](#)
- [SpectraMax M2 Multi-Mode Microplate Reader, see page 338](#)
- [SpectraMax M2e Multi-Mode Microplate Reader, see page 338](#)
- [SpectraMax M3 Multi-Mode Microplate Reader, see page 339](#)
- [SpectraMax M4 Multi-Mode Microplate Reader, see page 339](#)
- [SpectraMax M5 Multi-Mode Microplate Reader, see page 340](#)
- [SpectraMax M5e Multi-Mode Microplate Reader, see page 340](#)
- [SpectraMax Mini Multi-Mode Microplate Reader, see page 341 \(Requires a Fluorescence filter cube\)](#)
- [SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342 Requires Fluorescence Intensity Detection Cartridges, see page 376](#)

Fluorescence Intensity Reads with Injectors

Injectors deliver a specified volume of a reagent to the wells of a plate. You generally use injectors when delivery of the reagent initiates a reaction that occurs rapidly and results in a luminescent or fluorescent signal that you must quickly detect.

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

When you install the SpectraMax Injector cartridge in the SpectraMax i3x, you can create a protocol to inject and read well by well to reduce signal loss.

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.

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

where conc_{std} is the concentration of the *standard*, and *sample*, *blank*, and *std* 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, $(\text{std} - \text{blank}) / \text{conc}_{\text{std}}$ is equivalent to the slope of the standard curve, and so the concentration of the label is determined by $(\text{sample} - \text{blank}) / (\text{slope of standard curve})$.

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

Detection Limit

The detection limit is the smallest sample concentration that can be detected 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:

$$\text{Det Limit} = \frac{3 \text{ StDev}_{\text{blank}}}{\left(\frac{\text{std-blank}}{\text{conc}_{\text{std}}}\right)}$$

where conc_{std} is the concentration of the standard, $\text{StDev}_{\text{Blank}}$ 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 detection limits for assays 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.

Fluorescence Polarization Read Mode

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

Fluorescence Polarization detection is based on Fluorescence Intensity, with the important difference that it uses plane-polarized light, rather than non-polarized light. Microplate readers measure the Fluorescence Polarization 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, the binding of a small molecule to an interaction partner of equal or greater size can be monitored through its speed of rotation.

When molecules are excited with polarized light, the change in the polarization of the emitted light depends on the size of the molecule to which the fluorophore is bound (the emitted light quickly depolarizes if the fluorescent molecule is unbound). Larger molecules yield a stronger polarization of the emitted light, while smaller molecules cause less polarization because of their rapid molecular movement. Use Fluorescence Polarization for molecular binding assays in high-throughput screening (HTS).

Applications of Fluorescence Polarization

Fluorescence Polarization measurements provide information on molecular mobility and are generally used to quantify the success of a binding reaction between a smaller labeled ligand and a binding site at a much larger or immobilized molecule. Use Fluorescence Polarization to quantify the dissociation or cleavage of the labeled ligand from a binding site.

Fluorescence Polarization is a homogeneous plate assay technique and requires only mixing and measuring—no wash steps are required as in an ELISA. It can also be miniaturized, which makes it useful for high-throughput screening applications.

For information about how to set up Fluorescence Polarization mode protocols, see [Creating Fluorescence Polarization Mode Protocols on page 169](#).

Fluorescence Polarization Instruments

The following instruments have Fluorescence Polarization read mode capability:

- [FilterMax F5 Multi-Mode Microplate Reader, see page 321](#)
- [FlexStation 3 Multi-Mode Microplate Reader, see page 322](#)
- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328](#) Requires Fluorescence Polarization Detection Cartridges, see page 378.
- [SpectraMax iD5 Multi-Mode Microplate Reader, see page 333](#)
- [SpectraMax M5 Multi-Mode Microplate Reader, see page 340](#)
- [SpectraMax M5e Multi-Mode Microplate Reader, see page 340](#)
- [SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342](#) Requires Fluorescence Polarization Detection Cartridges, see page 378.

Analyzing Fluorescence Polarization Data

The Fluorescence Polarization read 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. The software uses the S and P values to calculate the Polarization (mP) and Anisotropy (r) values.

Fluorescence Polarization assays in plates 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 Fluorescence Polarization 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.

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, which contain 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 that contain fluorophore, then background wells should be run on each assay plate. The average raw signal from the background's parallel and perpendicular reads must be subtracted from the raw parallel and perpendicular reads of each sample well before the mP calculation is done.

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

Data Reduction

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:

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

Anisotropy (r) is calculated as follows:

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

Use the G factor, or grating factor, in Fluorescence Polarization to correct polarization data for optical artifacts which converts relative mP data to theoretical mP data. Optical systems, particularly with reflective components, pass light of different polarization with different efficiency. G factor corrects for this instrument-based bias.

Data Qualification and Validation

When you validate the data of a Fluorescence Polarization measurement and the assay, the two factors to look at are the precision value and the Z' factor.

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 to measure the quality of a screening assay independent of test compounds. Use this as a measure of the signal separation between the positive controls and the negative controls in an assay.

Use the following formula to determine the value of Z' :

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|Mean_{c+} - Mean_{c-}|}$$

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. You can use higher values 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, 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.

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, you can use FRET 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.

For information about how to set up FRET mode protocols, see [Creating FRET Mode Protocols on page 171](#).

FRET Instruments

The following instruments have FRET read mode capability:

- [FilterMax F3 Multi-Mode Microplate Reader, see page 318](#)
- [FilterMax F5 Multi-Mode Microplate Reader, see page 321](#)
- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328](#) Requires the Cisbio HTRF detection cartridge. See [Time Resolved Fluorescence Detection Cartridges on page 382](#).
- [SpectraMax iD5 Multi-Mode Microplate Reader, see page 333](#) Requires the Enhanced TRF module.
- [SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342](#) Requires the Cisbio HTRF detection cartridge. See [Time Resolved Fluorescence Detection Cartridges on page 382](#).

BRET Luminescence

Bioluminescence Resonance Energy Transfer (BRET) is a measurement technique that shares the key feature of Fluorescence Resonance Energy Transfer (FRET) while using the advantages of luminescence reads.

BRET employs a luminescence substrate that can undergo cleavage due to an enzymatic group (donor label) resulting in luminescence emission (channel 1). Unlike other generic luminescence assays, the BRET luminescence can be quenched by a radiationless Energy Transfer to an acceptor label (a fluorophore) resulting in fluorescence emission at a longer wavelength (red shifted, channel 2). The acceptor must be chosen such that its excitation spectrum overlaps the emission spectrum of the luminescence (Resonance condition). In the presence of substrate the energy transfer can occur only when the enzymatic group and the acceptor are in close proximity. Because of this fact, when labels are applied to study protein-protein interactions for example, signal measured at the emission wavelength of the acceptor fluorophore indicates binding.

The SpectraMax i3x and SpectraMax Paradigm use the Dual Color Luminescence (LUM) (BRET²) detection cartridge to measure the light intensity from BRET² reagents. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence, and the detection of the acceptor fluorophore (channel 2) can be regarded as a luminescence also. Emission filters differentiate emissions from the primary luminescence and the acceptor fluorophore. So, a dual color luminescence read mode is applied.

Use BRET² to study various kinds of protein-protein interactions. It employs a luciferase label (where "label" generally means fused by the use of expression vectors) for the one protein and a Green Fluorescent Protein (GFP) variant label for the other. The type of luciferase (*Renilla*) metabolizes the luminescent substrate (a coelenterazine derivative) resulting in blue luminescence (emission filter 1). Given the substrate, when the other protein with the GFP label is in close proximity to the luciferase, it quenches the blue luminescence and emits a green luminescence (emission filter 2). A high green to blue ratio indicates binding. The energy transfer is possible by the selection of the labels, that is, the overlap of the blue luminescence spectrum with the excitation spectrum of the GFP (resonance condition). As a result, BRET² combines the advantage of being a homogeneous (no wash steps required) assay with the generic sensitivity of luminescence detection.

Energy Transfer data are generally normalized by taking the red shifted emission and dividing it by the blue shifted emission. In terms of the BRET² reagents, the normalization of the green signal with the blue signal mainly compensates for the decay of the signal due to the digestion of the substrate. The software supports data reduction in taking ratios of the two emission channels.

Because of the signal decay, you must measure the assay immediately after you add the luminescence substrate. The spectral separation of the green from the blue signal eliminates the need to pre-process the raw data.

When you do a binding assay, controls are involved that represent a maximum (high) and a minimum (low) G:B (green to blue) ratio, and you can apply the Z'. More than merely taking the ratio of high and low G:B values, the Z' includes replicate variability to validate the actual data quality.

Z' is the standard statistical parameter in the high-throughput screening community to measure the quality of a screening assay independent of test compounds. Use this as a measure of the signal separation between the positive controls and the negative controls in an assay.

Use the following formula to determine the value of Z':

$$Z' = 1 - \frac{3(SD_{c^+}) + 3(SD_{c^-})}{| \text{Mean}_{c^+} - \text{Mean}_{c^-} |}$$

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. You can use higher values 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, increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

You can apply the BRET² reagents in various ways to study other types of molecular interactions in cell based assays. For a list of applications, go to www.perkinelmer.com.

Imaging Read Mode

To use the Imaging read mode, you must install the SpectraMax i3x with the SpectraMax MiniMax 300 Imaging Cytometer. Imaging read mode does whole-cell imaging assays. You can obtain imaging data through visual inspection of the image or use the analysis tools in the SoftMax Pro Software.

Whole-cell imaging assays are cell-based, or object-based, rather than the single-point measurements found in other types of plate 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 plate well.

The measurement is primarily fluorescent with quantification of cell size, shape, area, and intensity. Brightfield, transmitted light imaging supports label-free quantification. 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.

These assays include the direct interrogation of individual cells instead of whole well or cell lysates which allows you to control cell numbers and heterogeneity in cell-based assays. Imaging provides increased sensitivity by detecting a few fluorescent cells per well and detecting cells that are, on average, less fluorescent.

The SpectraMax MiniMax 300 Imaging Cytometer can use transmitted light and two fluorescent channels during the same plate read to acquire image data. Use the Settings dialog to select the channels for the acquisition. The StainFree Cell Detection Algorithm uses proprietary transmitted light analysis technology to eliminate cell staining for cell counting and confluency measurements.

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.

The following dyes are supported:

- EarlyTox™ Cell Integrity Kit
- Fluorescein isothiocyanate (FITC)
- Calcein AM
- Alexa Fluor 488 or Alexa Fluor 647
- Cy5
- DRAQ5

For information about how to set up Imaging read mode protocols, see [Creating Imaging Mode Protocols on page 172](#).

Imaging Instruments

You install the [SpectraMax i3x Multi-Mode Microplate Reader](#), see [page 328](#) with the SpectraMax MiniMax 300 Imaging Cytometer to add imaging capability to the SpectraMax i3x so that you can visually inspect a sample and run cell-based assays at cellular or whole-cell resolution.

Install the SpectraMax Transmitted Light (TL) detection cartridge in the detection cartridge drawer to do brightfield, transmitted-light imaging. See [Imaging - Transmitted Light Detection Cartridge on page 379](#).

Analyzing Imaging Data

You can obtain imaging data through visual inspection of the image or you can use the analysis tools 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 objects, or cells, based on multiple parameters including the signal intensity over the background and the object size.

Click **Discrete Object Analysis** to display the Wavelength for Finding Objects list below the analysis types. Select the wavelength 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.

Select the **Image Analysis Settings** category to display the following:

- 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 [Finding Discrete Objects or Confluent Areas on page 132](#).
- Click **Classify Objects** to define separate measurements for different types of found objects, or cells. See [Classifying Discrete Objects on page 136](#).
- Click **Select Measurements** to view the results of the analysis for the wells you select. See [Viewing and Selecting Measurement Statistics on page 137](#).

Field Analysis

Field Analysis uses proprietary algorithms to analyze confluent areas based on parameters including the signal intensity over the background and the size of the areas.

Click **Field Analysis** to display the Wavelength for Finding Confluent Areas list below the analysis types. Select the wavelength to use to identify the confluent areas in the well images. The StainFree Cell Detection Algorithm uses proprietary transmitted light analysis technology to eliminate cell staining for cell counting and confluency measurements.

The Image Analysis Settings category expands to display the following:

- 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 [Finding Discrete Objects or Confluent Areas on page 132](#).
- Click **Select Measurements** to view the results of the analysis for the wells you select. See [Viewing and Selecting Measurement Statistics on page 137](#).

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 the expected results. See [Resolving Issues with Object Drawing and Finding on page 138](#).

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:** The total number of objects detected in the image. Do not use for field analysis of confluent areas.
- **Field Count:** The total number of confluent areas detected in the image. Do not use for discrete object analysis.
- **Object Percentage:** The percentage the objects detected in the image by classification. Do not use for field analysis of confluent areas.
- **Covered Area:** The combined area of all the objects or confluent areas detected in the image as a percentage of the entire image area.
- **Object Area:** The average area of the objects detected in the image expressed in μm^2 . Do not use for field analysis of confluent areas.
- **Field Area:** The average area of the confluent areas detected in the image expressed in μm^2 . Do not use for discrete object analysis.
- **Object Roundness:** 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. Do not use for field analysis of confluent areas.
- **Field Roundness:** 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. Do not use for discrete object analysis.
- **Object Average Intensity:** The average fluorescent signal intensity of the objects detected in the image. Do not use for field analysis of confluent areas or for transmitted light.
- **Field Average Intensity:** The average fluorescent signal intensity of the confluent areas detected in the image. This is not used for discrete object analysis or for transmitted light.
- **Object Intensity:** The average total fluorescent signal intensity of the objects detected in the image. Do not use for field analysis of confluent areas or for transmitted light.
- **Field Intensity:** The average total fluorescent signal intensity of the confluent areas in the image. Do not use for discrete object analysis or for transmitted light.
- **Total Intensity:** The combined total fluorescent signal intensity of the objects or confluent areas in the image expressed in million intensity counts. Do not use for transmitted light.

Luminescence Read Mode

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 (LUM) read mode, no excitation is necessary as the measured species emit light naturally. For this reason, the lamp does not flash, so no background excitation interference occurs.

For the luminescence read mode, the instrument provides measurements in Relative Light Units (RLUs).

To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start 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.

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.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls.

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

For information about how to set up luminescence mode protocols, see [Creating Luminescence Mode Protocols on page 174](#).

Luminescence Instruments

The following instruments have luminescence read mode capability:

- [FilterMax F3 Multi-Mode Microplate Reader, see page 318](#)
- [FilterMax F5 Multi-Mode Microplate Reader, see page 321](#)
- [FlexStation 3 Multi-Mode Microplate Reader, see page 322](#)
- [Gemini EM Dual Scanning Microplate Spectrofluorometer, see page 323](#)
- [Gemini XPS Dual Scanning Microplate Spectrofluorometer, see page 323](#)
- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328](#) Supports Luminescence Detection Cartridges, see page 379
- [SpectraMax iD3 Multi-Mode Microplate Reader, see page 330](#)
- [SpectraMax iD5 Multi-Mode Microplate Reader, see page 333](#)
- [SpectraMax L Luminescence Microplate Reader, see page 335](#)

- SpectraMax M2 Multi-Mode Microplate Reader, see page 338
- SpectraMax M2e Multi-Mode Microplate Reader, see page 338
- SpectraMax M3 Multi-Mode Microplate Reader, see page 339
- SpectraMax M4 Multi-Mode Microplate Reader, see page 339
- SpectraMax M5 Multi-Mode Microplate Reader, see page 340
- SpectraMax M5e Multi-Mode Microplate Reader, see page 340
- SpectraMax Mini Multi-Mode Microplate Reader, see page 341 (Requires the Luminescence filter cube)
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342 Requires Luminescence Detection Cartridges, see page 379

Luminescence Reads with Injectors

Injectors deliver a specified volume of a reagent to the wells of a plate. You generally use injectors when delivery of the reagent initiates a reaction that occurs rapidly and results in a luminescent or fluorescent signal that you must quickly detect.

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

When you install the SpectraMax Injector cartridge in the SpectraMax i3x, you can create a protocol to inject and read well by well to reduce signal loss.

Common inject-and-read assays include luciferase reporter assays.

The SpectraMax 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.

Analyzing 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 not a benchmark of sensitivity.

Concentrations or qualitative results are derived from raw data with a standard curve or by comparison with reference controls. 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.

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, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will be subtracted is the average value of all blanks.

To help eliminate background luminescence from a plate that has been exposed to light, you should dark adapt the plate by placing the sample-loaded plate inside the instrument for several minutes before you start the read.

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. Even if the reaction begins with a high concentration of ATP, as it gets used up it can become rate-limiting. 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, the read can be done with an attenuation filter.

Data Optimization

Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when you increase the read time. It is important to enter the read time when you compare 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 to measure the quality of a screening assay independent of test compounds. Use this as a measure of the signal separation between the positive controls and the negative controls in an assay.

Use the following formula to determine the value of Z':

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{| \text{Mean}_{c+} - \text{Mean}_{c-} |}$$

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. You can use higher values 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, 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 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 cutoff of each light pulse and the start of signal collection. During the delay, the unspecific fluorescence (caused by test compounds, assay reagents, and the plate) 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 μ s), samarium (lifetime of about 70 μ s), or terbium (lifetime of about 1000 μ s).

Applications of Time-Resolved Fluorescence

Time-Resolved Fluorescence 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 plate 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 [Homogeneous Time-Resolved Fluorescence on page 308](#).

For information about how to set up Time-Resolved Fluorescence mode protocols, see [Creating Time-Resolved Fluorescence Mode Protocols on page 176](#).

Time-Resolved Fluorescence Instruments

The following instruments have time-resolved fluorescence read mode capability:

- [FilterMax F5 Multi-Mode Microplate Reader](#), see page 321
- [FlexStation 3 Multi-Mode Microplate Reader](#), see page 322
- [Gemini EM Dual Scanning Microplate Spectrofluorometer](#), see page 323
- [Gemini XPS Dual Scanning Microplate Spectrofluorometer](#), see page 323
- [SpectraMax i3x Multi-Mode Microplate Reader](#), see page 328 Requires Time Resolved Fluorescence Detection Cartridges, see page 382.
- [SpectraMax iD5 Multi-Mode Microplate Reader](#), see page 333 Supports the Enhanced TRF module.
- [SpectraMax M2 Multi-Mode Microplate Reader](#), see page 338
- [SpectraMax M2e Multi-Mode Microplate Reader](#), see page 338
- [SpectraMax M4 Multi-Mode Microplate Reader](#), see page 339
- [SpectraMax M5 Multi-Mode Microplate Reader](#), see page 340
- [SpectraMax M5e Multi-Mode Microplate Reader](#), see page 340
- [SpectraMax Paradigm Multi-Mode Microplate Reader](#), see page 342 Requires Time Resolved Fluorescence Detection Cartridges, see page 382.

Analyzing Time-Resolved Fluorescence Data

A time-resolved fluorescence (TRF) measurement includes a number of pulses. Each pulse consists of turning the light source on, then 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). The pulses are repeated several times, as specified in the protocol parameters.

Blank Correction

Although background is significantly lower than with fluorescence intensity measurements, you should 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, you should run replicates for all blanks, controls, and samples. In this case, the blank value that will 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.

Data Optimization

There are two timing parameters you can optimize to adjust the performance of the measurement: time per well and integration time per cycle.

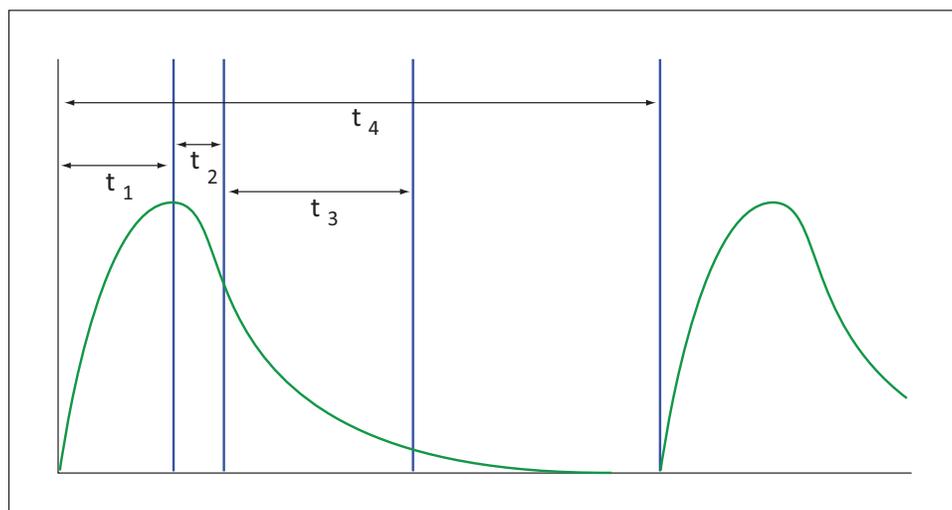
Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when you increase the read time. It is important to enter the read time when you compare measurements. For TRF, the read time per well increases with the selected number of pulses. The time between pulses and the intensity of each pulse 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. Use the following table and figure as guidelines for the timing parameters.

Time-Resolved Fluorescence Timing Parameters Example

Parameter	Value	Comment
Pulse length	0.100 ms	Period for excitation of sample, t_1 in the following figure. Suggested value for TUNE, MULTI, and TRF detection cartridges.
Measurement delay	0.010 ms	Delay to ensure excitation pulse is no longer detectable, t_2 in the following figure. Suggested value for TUNE, MULTI and TRF detection cartridges.
Integration time per cycle (pulse)	0.890 ms	Period to accumulate signal, t_3 in the following figure. Suggested value for TUNE and MULTI detection cartridges.
Integration time per cycle (pulse)	1.890 ms	Period to accumulate signal, t_3 in the following figure. Suggested value for TRF detection cartridge.
Total cycle time		Total cycle time is t_4 in the following figure.



Timing Parameters For Time-Resolved Fluorescence

When you neglect the time delay t_2 compared to the integration time window t_3 , you can approximate the accumulated signal A with the following equation:

$$A / A_{\max} = (1 - \exp(-M)) \times 100\%$$

In this 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), $M = 1890 / 700 = 2.7$. Insert this value of M into the first equation to yield $A / A_{\max} = 93\%$.

To optimize the integration time per cycle (pulse), you should set the integration time 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, you can set the integration time to $M(2.0)$ times the fluorescence lifetime (700 μs), or 1400 μs (1.4 ms).

Achievable Accumulated Signal Percentage Compared to M

M	0.25	0.50	0.75	1.00	1.25	1.50	2.00	3.00
$A / A_{\text{max}}[\%]$	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 you do 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. 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. You can use the known Europium concentration to remove the Europium cross-contamination in the Samarium channel.

TRF Suggested Timing Parameters: Dual Label Europium-Samarium

Parameter	Value	Comment
Pulse length	0.100 ms	Time interval for flash monitoring. Suggested value for TRF detection cartridge.
Measurement delay (first window)	0.010 ms	Delay to ensure excitation pulse is no longer detectable. Suggested value for TRF detection cartridge.
Integration time (first window)	0.100 ms	Period to accumulate Samarium signal. Suggested value for TRF detection cartridge.
Measurement delay (second window)	0.140 ms	Read out of Samarium signal. Suggested value for TRF detection cartridge.
Integration time (second window)	0.750 ms	Period to accumulate Europium signal. Suggested value for TRF detection cartridge.

TR-FRET Read Mode

Time-resolved fluorescence is a measurement technique based on fluorescence resonance energy transfer (FRET) using the advantages of time-resolved fluorescence (TRF) read.

TR-FRET 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 plate 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 TR-FRET

TR-FRET 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. TR-FRET is an 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.

TR-FRET Instruments

The following instruments have TR-FRET read mode capability:

- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328](#) Requires the Cisbio HTRF detection cartridge. See [TR-FRET Detection Cartridge on page 383](#).
- [SpectraMax iD5 Multi-Mode Microplate Reader, see page 333](#) Requires the Enhanced TRF module.
- [SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342](#) Requires the Cisbio HTRF detection cartridge. See [TR-FRET Detection Cartridge on page 383](#).

Homogeneous Time-Resolved Fluorescence

Homogeneous time-resolved fluorescence (HTRF) is a measurement technique based on fluorescence resonance energy transfer (FRET) using the advantages of time-resolved fluorescence (TRF) read.

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 plate 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.

HTRF is a registered trademark of Cisbio Bioassays.

Analyzing 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.

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

Measurement noise is dependent on the read time per sample (time per plate or time per well). The detection limit improves when you increase the read time. It is important to enter the read time when you compare measurements. For TRF, the read time per well increases with the number of pulses you enter. The time between pulses can be different on various systems.

HTRF Timing Parameters Example

Parameter	Value	Comment
Number of pulses	30	Number of flashes per read.
Measurement delay	30 μ s	Delay to ensure excitation pulse is no longer detectable.
Integration time per cycle (pulse)	400 μ s	Period to accumulate 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.

You can optimize two timing parameters to adjust the performance of the measurement: time per plate or time per well and integration time per cycle.

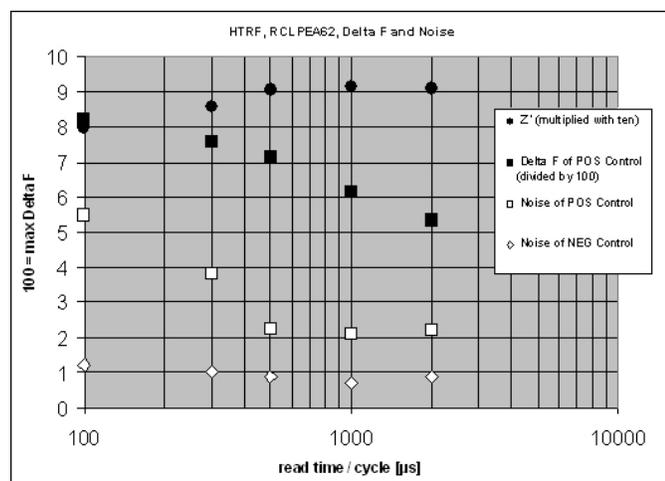
All low-light-level detection devices have some measurement noise in common. To average out the measurement noise, optimization of the time per well involves accumulating as many counts as possible. Within some range, you can reduce noise (CVs, detection limit) by increasing the read time per well, as 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 you can optimize is the Integration time per cycle. Take care when you optimize 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) displays to be in the 500 μ s to 1000 μ s range, as noise is minimized and Delta F is still relatively high. Going greater than 1000 μ s shows a sharp decline in Delta F without apparent improvement in noise.



Relationship Between Integration Time, Noise, and Delta F

Z' is the standard statistical parameter in the high-throughput screening community to measure the quality of a screening assay independent of test compounds. Use this as a measure of the signal separation between the positive controls and the negative controls in an assay.

Use the following formula to determine the value of Z':

$$Z' = 1 - \frac{3(SD_{c+}) + 3(SD_{c-})}{|Mean_{c+} - Mean_{c-}|}$$

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. You can use higher values 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, increase the quantity of label in the sample, if acceptable for the assay, or increase the read time per well.

Western Blot Read Mode

Western Blots (WB), or protein immunoblots, are one of the most common methods for protein detection in pharmaceutical and clinical research. You can use various techniques to detect proteins on Western Blot membranes including fluorescence, silver staining, and chemiluminescence. Each technique has its limitations. 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. Membranes are incubated with Eu-chelate labeled secondary antibodies or streptavidin that bind specifically to the target protein-specific primary antibody. The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis that you can use with the SpectraMax i3x, SpectraMax iD5, and SpectraMax Paradigm. 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 the instrument where the membranes are scanned with the ScanLater Western Blot (WB) detection cartridge (SpectraMax iD5 uses the Enhanced TRF module).

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 allows you repeat the read of membranes and the potential for comparison of band intensities to known standards for more accurate quantitation. 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 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.

The SpectraMax Western Blot system is a simple, sensitive, and stable platform that provides excellent Western Blot capability for the SpectraMax i3x, SpectraMax iD5, and SpectraMax Paradigm to extend 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 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.

For information about how to set up Western Blot TRF mode protocols, see [Creating Western Blot TRF Mode Protocols on page 179](#).

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

- **Read Empty Plates/Cuvettes** to start a read.
- **Create/Save Data Documents** to save the data document before the read.
- **Overwrite Plate/Cuvette Data** to do a high-resolution ROI read.

See [SoftMax Pro Software - GxP Edition on page 251](#).

Western Blot TRF Instruments

The following instruments have Western Blot TRF read mode capability:

- [SpectraMax i3x Multi-Mode Microplate Reader, see page 328](#) Requires the [ScanLater Western Blot Detection Cartridge, see page 380](#).
- [SpectraMax iD5 Multi-Mode Microplate Reader, see page 333](#) Requires the Enhanced TRF module.
- [SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342](#) Requires the [ScanLater Western Blot Detection Cartridge, see page 380](#).

The ScanLater Western Blot (WB) detection cartridge can be used for top reads only.

Analyzing Western Blot TRF Data

After you scan a membrane for Western Blot data, the data displays in the 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 [Viewing Western Blot Membrane Data on page 244](#).

Western Blot membrane data is saved as a TIFF image to allow you to use the image analysis tool of your choice for analysis. The SoftMax Pro Software includes a version of the ImageJ software from U.S. National Institute of Health (NIH). See [Analyzing Western Blot Images on page 249](#).

For best results, use the Molecular Devices ScanLater™ Western Blot Assay Kit that matches your application.

Blocking 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 cross react 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.

Handling Membranes

You must place the membrane in a Molecular Devices ScanLater™ membrane holder to scan a membrane. See [Loading the Membrane Holder on page 180](#).



Note: Use clean forceps to handle membranes by their edges only. Do not touch the membrane with gloved or bare hands.

The maximum size of a membrane that fits in the membrane holder is 109 mm x 77 mm.

Use standard blotting procedures for the membrane to prepare for the Western Blot. 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 for the scan.

SoftMax Pro Software controls Molecular Devices spectrophotometers and absorbance, luminescence, and fluorescence microplate readers and detection platforms.

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 n.n > Hardware User Guides**.

SoftMax Pro Software supports the following instruments:

- EMax Endpoint Microplate Reader, see page 316
- EMax Plus Microplate Reader, see page 317
- FilterMax F3 Multi-Mode Microplate Reader, see page 318 (DTX 800)
- FilterMax F5 Multi-Mode Microplate Reader, see page 321 (DTX 880)
- FlexStation 3 Multi-Mode Microplate Reader, see page 322
- Gemini EM Dual Scanning Microplate Spectrofluorometer, see page 323
- Gemini XPS Dual Scanning Microplate Spectrofluorometer, see page 323
- SpectraMax 190 Microplate Spectrophotometer, see page 324
- SpectraMax 340PC384 Microplate Spectrophotometer, see page 324
- SpectraMax ABS Microplate Spectrophotometer, see page 325
- SpectraMax ABS Plus Microplate Spectrophotometer, see page 325
- SpectraMax i3x Multi-Mode Microplate Reader, see page 328
- SpectraMax i3 Multi-Mode Microplate Reader, see page 326
- SpectraMax MiniMax 300 Imaging Cytometer, see page 329
- SpectraMax iD3 Multi-Mode Microplate Reader, see page 330
- SpectraMax iD5 Multi-Mode Microplate Reader, see page 333
- SpectraMax L Luminescence Microplate Reader, see page 335
- SpectraMax M2 Multi-Mode Microplate Reader, see page 338
- SpectraMax M2e Multi-Mode Microplate Reader, see page 338
- SpectraMax M3 Multi-Mode Microplate Reader, see page 339
- SpectraMax M4 Multi-Mode Microplate Reader, see page 339
- SpectraMax M5 Multi-Mode Microplate Reader, see page 340
- SpectraMax M5e Multi-Mode Microplate Reader, see page 340
- SpectraMax Mini Multi-Mode Microplate Reader, see page 341
- SpectraMax Paradigm Multi-Mode Microplate Reader, see page 342
- SpectraMax Plus 384 Microplate Spectrophotometer, see page 345
- VersaMax Microplate Spectrophotometer, see page 345
- VMax Kinetic Microplate Reader, see page 346
- StakMax Microplate Handling System, see page 347

The SpectraMax i3x supports user-installable detection cartridges for top reads and the SpectraMax Paradigm requires detection cartridges to do reads. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes for some applications.

See [Supported Detection Cartridges](#) on page 375.

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 plates.

EMax features include:

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

Read Mode	Read Type
Absorbance	Endpoint 

Adjusting the Lamp

To adjust the halogen lamp, select the **Operations** tab and click  **Configure** to display the Configuration dialog. You can adjust the intensity of the lamp for each of the installed filters in the instrument. You can view the positions and wavelengths of the installed filters. This dialog lists other instrument information including the serial number and firmware version.

You select the filters you install from the Settings dialog. See [Creating Absorbance Mode Protocols on page 160](#).

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. The Configuration dialog must remain open until the lamp adjustment completes.

EMax Plus Microplate Reader

The EMax Plus Microplate Reader is a filter-based visible absorbance microplate reader with eight standard filters for a range of applications. The instrument can read standard 96-well plates.

Read Mode	Read Type	
	Endpoint	Kinetic
Absorbance	✓	✓

Configuring Filters for the EMax, EMax Plus, and VMax Microplate Readers

EMax Endpoint Microplate Reader, EMax Plus Microplate Reader, and VMax Kinetic Microplate Reader have configurable filters. If you change the order of the filters, or add or remove filters, you need to use the Filter Configuration dialog to define the positions of the filters. If the filter settings in the Filter Configuration dialog are incorrect, the microplate reader generates incorrect data. For information about how to change the filters, see the *EMax Plus Microplate Reader User Guide*.

To define the filter positions:

1. Select the Operations tab and click  **Filters** to display the Filter Configuration dialog.
2. Enter the wavelength values in the correct positions for each filter you install. 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 in the Settings dialog.

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. The DTX 800 Multi-Mode Microplate Reader is an earlier model of the FilterMax F3.

FilterMax F3 features include:

- Visible absorbance at wavelengths between 340 nm and 650 nm.
- Fluorescence intensity for top reads at wavelengths between 340 nm and 650 nm.
- Glow-type luminescence at wavelengths between 400 nm and 650 nm.
- Measurements on samples in 96-well and 384-well plates.
- Linear and orbital plate shake.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

The filters are mounted on two types of interchangeable slides. The excitation slide filters are for absorbance and fluorescence measurements, the emission slide filters are for fluorescence and some luminescence measurements. Each slide can hold up to six filters. See [Configuring Filter Slides on page 318](#).

Read Mode	Read Type		
	Endpoint	Kinetic	Well Scan
Absorbance	✓	✓	✓
Dual Wavelength Luminescence	✓		
Fluorescence Intensity	✓	✓	✓
FRET	✓		
Luminescence	✓	✓	

Configuring Filter Slides

Use the Filter Settings dialog define a filter slide for the FilterMax F3 and FilterMax F5 in the software. When 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 you do not define the filter settings, the microplate reader generates incorrect data. See [FilterMax F3 Multi-Mode Microplate Reader on page 318](#) and [FilterMax F3 Multi-Mode Microplate Reader on page 318](#).



Note: You should not reconfigure standard filter slides.

The filters are mounted on two types of interchangeable slides. The excitation slide filters are for absorbance and fluorescence measurements, the emission slide filters are for fluorescence and some luminescence measurements. Each slide can hold up to six filters.



Note: Excitation and emission filter slides are different sizes to prevent installation in the incorrect position.

Each slide has a built in identification code that allows the software to recognize the slide and filter configuration. The software can store the definition for up to 31 excitation filter slides and 31 emission filter slides.

- For quantitation experiments, you must use a genomic filter slide that contains narrow bandwidth 260 nm and 280 nm filters.
- For experiments that use PathCheck technology, you must use a filter slide designed for PathCheck technology.

Access the Filter Settings dialog

Each of the following procedures is done in the Filter Settings dialog.

To access the Filter Slides dialog:

1. In the Ribbon, select the **Operations** tab and click **Info** to display the Instrument Information dialog.
2. Click **Edit Slide Configuration** to display the Filter Settings dialog.
3. Select one of the following:
 - Select **Excitation** to display the list of excitation slides.
 - Select **Emission** to display the list of emission slides.

Adding Filter Slides

To add and define filter slides:

1. Click **Add Slide** to add a new slide to the list.
2. In the Filter Slide Properties area on the right, in the **Slide ID** field, enter the identification number that is printed on the slide.
3. If needed, click **+** to display the list of filters installed on the slide and select a filter to display the filter properties on the right.
4. In the **Wavelength** field, enter the filter wavelength.
5. Click the **Techniques** drop-down and select the check box for each read mode for which the filter applies. The filter can be used only for experiments of the read modes you select. For the FilterMax F5, select **Polarization** for filter positions that have a polarization filter installed. If you do not select a read mode, the **Installed** field displays **No**, and you cannot use the filter position for experiments.
6. After you select the read modes, the **Installed** field displays **Yes** and the **Position** field displays the slide position. You cannot edit these fields.
7. In the **Bandwidth** field, enter the filter bandwidth.
8. In the **Order#** field, enter the manufacturer's part number for the filter for your reference.
9. Repeat to configure the rest of filters on the slide.

Exporting Filter Slides

You can export filter slide definitions to an .xml format and then import back into the software.

To export filter slide definitions:

1. Either:
 - To export the definition for a single slide, select the slide to export and click **Export Selected Slide**.
 - To export all slide definitions, click **Export Slides**.
2. In the Save As dialog, navigate to the folder where you want to save the .xml file and enter the file name in the **File Name** field. The default location for filter slide definitions is:
C:\ProgramData\Molecular Devices\SMP<n.n>\MultiMode\Detection Software\Filters
3. Click **Save**.

Removing Filter Slides

You cannot recover a filter slide definition after you remove it from the list. If you plan to use a filter slide definition in the future, export the definition before you remove it from the list.

To remove a filter slide definition:

1. In the slide list, select the slide to remove.
2. Click **Remove Slide**.

Importing Filter Slides

Follow the previous steps to import a filter slide definition from the .xml format file the software creates. Each filter slide definition must have a unique slide ID. If the filter slide ID 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:

1. Click **Import Slides**.
2. In the Open dialog, navigate to the folder where .xml file is saved and select the file.
The default location for filter slide definitions is:
C:\ProgramData\Molecular Devices\SMP<n.n>\MultiMode\Detection Software\Filters
3. Click **Open**.

Loading Slides (Replacing Slide Definitions)

When you use the Load Slides feature, the software deletes all current filter slide definitions and replaces the slide definitions with the definitions you import. If you plan to use one or more of the current filter slide definitions in the future, export the current slide definitions before you load the new definition and replace the current list.

You can load filter slide definitions from the .xml format file the software creates when you follow the steps above to export a slide definition. Slide definition files can contain definitions of one or more filter slides for excitation and emission filters.

To replace the current filter slide definitions:

1. Click **Load Slides**.
2. In the message that displays, click **Yes**.
3. In the Open dialog, navigate to the .xml file and select it.
The default location for filter slide definitions is:
C:\ProgramData\Molecular Devices\SMP<n.n>\MultiMode\Detection Software\Filters
4. Click **Open**.

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.

The DTX 880 Multi-Mode Microplate Reader is the earlier model of the FilterMax F5.

The filters are mounted on two types of interchangeable slides. The excitation slide filters are for absorbance and fluorescence measurements, the emission slide filters are for fluorescence and some luminescence measurements. Each slide can hold up to six filters. See [Configuring Filter Slides on page 318](#).

FilterMax F5 features include:

- UV and visible absorbance at wavelengths between 230 nm and 650 nm.
- Fluorescence intensity at wavelengths between 230 nm and 700 nm for top reads, or between 300 nm and 650 nm for bottom reads.
- Glow-type luminescence at wavelengths between 400 nm and 700 nm.
- Time-Resolved Fluorescence at wavelengths between 340 nm and 700 nm.
- Fluorescence polarization at wavelengths between 300 nm and 700 nm.
- Measurements on samples in 6, 12, 24, 48, 96, 384, and 1536-well plates.
- A deuterium light source that extends sensitivity into the deep UV band.
- PathCheck technology.
- Automatic read height (Z-height) optimization for top read.
- Temperature control in the plate chamber during measurements.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type		
	Endpoint	Kinetic	Well Scan
Absorbance	✓	✓	✓
Dual Wavelength Luminescence	✓		
Fluorescence Intensity	✓	✓	✓
Fluorescence Polarization	✓	✓	
FRET	✓		
Luminescence	✓	✓	
Time Resolved Fluorescence	✓		

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. The instrument combines fluid transfer with multi-detection optics to provide a microplate reader capable of doing a broad span of applications for the drug discovery and research environments.

The instrument's dual monochromators allow you to target the optimal assay excitation and emission wavelengths and eliminate the need to change expensive band pass filters between experiments.

FlexStation 3 features include:

- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Flex
Absorbance	✓	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓	✓
Fluorescence Polarization	✓	✓			
Luminescence	✓	✓	✓	✓	✓
Time Resolved Fluorescence	✓	✓	✓	✓	

Gemini EM Dual Scanning Microplate Spectrofluorometer

The Gemini EM Dual Scanning Microplate Spectrofluorometer can do a variety of fluorescent applications. The extreme flexibility and high sensitivity make the Gemini EM 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.

Gemini EM 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.
- Compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Fluorescence Intensity	✓	✓	✓	✓
Luminescence (secondary)	✓	✓	✓	✓
Time-Resolved Fluorescence (secondary)	✓	✓	✓	✓

Gemini XPS Dual Scanning Microplate Spectrofluorometer

The Gemini XPS Dual Scanning Microplate Spectrofluorometers can do a variety of fluorescent applications. The extreme flexibility and high sensitivity make the Gemini XPS useful for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology.

Gemini XPS 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.
- Compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Fluorescence Intensity	✓	✓	✓	✓
Luminescence (secondary)	✓	✓	✓	✓
Time-Resolved Fluorescence (secondary)	✓	✓	✓	✓

SpectraMax 190 Microplate Spectrophotometer

The SpectraMax® 190 Microplate Spectrophotometer 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 plates.

SpectraMax 190 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 technology reports the plate data as standard 1 cm cuvette values.
- Newer models compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type		
	Endpoint	Kinetic	Spectrum
Absorbance (UV and Visible)	✓	✓	✓

SpectraMax 340PC384 Microplate Spectrophotometer

The SpectraMax® 340PC384 Microplate Spectrophotometer 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 plates.

SpectraMax 340PC384 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 technology reports the plate data as standard 1 cm cuvette values.
- Newer models compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type		
	Endpoint	Kinetic	Spectrum
Absorbance	✓	✓	✓

SpectraMax ABS Microplate Spectrophotometer

The SpectraMax® ABS Microplate Spectrophotometer allows you to run both standard spectrophotometer and microplate reader applications on the same instrument. Read one sample or up to 384 at a time. The SpectraMax ABS supports 96-well or 384-well plates.

SpectraMax ABS 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 plates in just 5 seconds and 384-well plates in 16 seconds for ultra-fast sample throughput.
- Compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type		
	Endpoint	Kinetic	Spectrum
Absorbance (UV and Visible)	✓	✓	✓

SpectraMax ABS Plus Microplate Spectrophotometer

The SpectraMax® ABS Plus Microplate Spectrophotometer has a built-in cuvette port and plate drawer that allow you to run both standard spectrophotometer and microplate reader applications on the same instrument. Read one sample or up to 384 at a time. The SpectraMax ABS Plus supports standard cuvettes or 96-well or 384-well plates.

SpectraMax ABS Plus 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 plates in just 5 seconds and 384-well plates in 16 seconds for ultra-fast sample throughput.
- PathCheck technology reports the plate data as standard 1 cm cuvette values.
- Compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type		
	Endpoint	Kinetic	Spectrum
Absorbance (UV and Visible)	✓	✓	✓

SpectraMax i3 Multi-Mode Microplate Reader

The SpectraMax i3 Multi-Mode Microplate Reader is a monochromator-based, multi-mode detection platform that supports user-installable detection cartridges to expand its read capabilities. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes. Up to four detection cartridges can be installed in the detection cartridge drawer.

Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well plates. For micro-volume measurements, the instrument supports the SpectraDrop 24-well Low Volume Microplate and the SpectraDrop 64-well Low Volume Microplate. With some detection cartridges, the instrument can read 1536-well microplates.

SpectraMax i3 features include:

- Built-in, 2x2 monochromators for absorbance, fluorescence, and luminescence reads
- Top and bottom read capability
- More capabilities with detection cartridges
- Adjustable read height (Z-height) for top reads
- Up to 1536-well format with some detection cartridges
- Temperature control
- PathCheck technology
- Compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Instrument Monochromator					
Absorbance	✓	✓	✓	✓	
Fluorescence Intensity	✓	✓	✓	✓	
Luminescence	✓	✓	✓	✓	
AlphaScreen 384 HTS, AlphaScreen 384 Std, and AlphaScreen 1536 HTS Detection Cartridges					
Screen	✓	✓	✓		
Cisbio HTRF Detection Cartridge					
TR-FRET	✓	✓	✓		
Dual Color Luminescence (LUM) (BRET2) and Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge					
Dual Wavelength Luminescence	✓	✓	✓		
Luminescence	✓	✓	✓		
Fluorescence Intensity (FL) (coum-fluor), Fluorescence Intensity (FL) (fluor-rhod), Fluorescence Intensity (FL) (Cy3-Cy5), Fluorescence Intensity (FL) (Cfp-Yfp), and Fluorescence Intensity GeneBLAzer Detection Cartridges					
Fluorescence Intensity	✓	✓	✓		
FRET	✓	✓	✓		

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Fluorescence Polarization (FP) (Fluorescein) and Fluorescence Polarization (FP) (Rhodamine) Detection Cartridges					
Fluorescence Polarization	✓	✓			
Glow Luminescence (LUM), Glow Luminescence (LUM) (96), and Glow Luminescence (LUM) (384) Detection Cartridges					
Luminescence	✓	✓	✓		
ScanLater Western Blot (WB) Detection Cartridge					
Time-Resolved Fluorescence					✓
Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge					
Dual Wavelength Time-Resolved Fluorescence	✓	✓			
Time-Resolved Fluorescence	✓	✓	✓		

SpectraMax i3x Multi-Mode Microplate Reader

The SpectraMax i3x Multi-Mode Microplate Reader is a monochromator-based, multi-mode detection platform. The monochromator supports top reads for absorbance and luminescence reads in addition to top or bottom reads for fluorescence intensity reads. You can install detection cartridges in a detection cartridge drawer to expand the SpectraMax i3x read capabilities. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes. You can install up to four detection cartridges for top reads.

The SpectraMax MiniMax 300 Imaging Cytometer adds imaging capability to the SpectraMax i3x to visually inspect samples and to run cell-based assays at cellular or whole-cell resolution. Depending on the application, the instrument can read 6, 12, 24, 48, 96, and 384-well plates. For micro-volume measurements, the instrument supports the SpectraDrop 24-well Low Volume Microplate and the SpectraDrop 64-well Low Volume Microplate. With some detection cartridges, the instrument can read 1536-well microplates.

SpectraMax i3x features include:

- Built-in, 2x2 monochromators for absorbance, fluorescence, and luminescence reads
- Top and bottom read capability
- Adjustable read height (Z-height) for top reads
- Up to 1536-well format with some detection cartridges
- Temperature control
- PathCheck technology
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

See [Read Modes and Read Types on page 277](#) and [Supported Detection Cartridges on page 375](#).

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Instrument Monochromator					
Absorbance	✓	✓	✓	✓	
Fluorescence Intensity	✓	✓	✓	✓	
Luminescence	✓	✓	✓	✓	
AlphaScreen 384 HTS, AlphaScreen 384 Std, and AlphaScreen 1536 HTS Detection Cartridges					
Screen	✓	✓	✓		
Cisbio HTRF Detection Cartridge					
TR-FRET	✓	✓	✓		
Dual Color Luminescence (LUM) (BRET2) and Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge					
Dual Wavelength Luminescence	✓	✓	✓		
Luminescence	✓	✓	✓		

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Fluorescence Intensity (FL) (coum-fluor), Fluorescence Intensity (FL) (fluor-rhod), Fluorescence Intensity (FL) (Cy3-Cy5), Fluorescence Intensity (FL) (Cfp-Yfp), and Fluorescence Intensity GeneBLAzer Detection Cartridges					
Fluorescence Intensity	✓	✓	✓		
FRET	✓	✓	✓		
Fluorescence Polarization (FP) (Fluorescein) and Fluorescence Polarization (FP) (Rhodamine) Detection Cartridges					
Fluorescence Polarization	✓	✓			
Glow Luminescence (LUM), Glow Luminescence (LUM) (96), and Glow Luminescence (LUM) (384) Detection Cartridges					
Luminescence	✓	✓	✓		
ScanLater Western Blot (WB) Detection Cartridge					
Time-Resolved Fluorescence					✓
SpectraMax Injector Cartridge					
Luminescence	✓	✓			
Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge					
Dual Wavelength Time-Resolved Fluorescence	✓	✓			
Time-Resolved Fluorescence	✓	✓	✓		
Transmitted Light (TL) Detection Cartridge with the SpectraMax MiniMax 300 Imaging Cytometer					
Imaging					

SpectraMax MiniMax 300 Imaging Cytometer

You can attach the SpectraMax MiniMax 300 Imaging Cytometer to the bottom of the SpectraMax i3x to add imaging capability 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 plate wells. The cytometer supports 96-well and 384-well, flat-bottom, clear-bottom plates.

The measurement is primarily fluorescent with quantification of cell size, shape, area, and intensity. Brightfield, transmitted light imaging supports label-free quantification. 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 cytometer can use transmitted light and two fluorescent channels during the same plate read to acquire image data. You can select the channels for the acquisition. The StainFree Cell Detection Algorithm uses proprietary transmitted light analysis technology to eliminate cell staining for cell counting and confluency measurements.

The SpectraMax Transmitted Light (TL) detection cartridge enables you to do brightfield transmitted-light imaging. See [Imaging - Transmitted Light Detection Cartridge on page 379](#).

SpectraMax iD3 Multi-Mode Microplate Reader

The SpectraMax iD3 Multi-Mode Microplate Reader is a monochromator-based detection platform. Use the instrument touchscreen interface to run protocols without a connected computer. The SpectraMax iD3 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.

SpectraMax iD3 features include:

- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓

You can operate the instrument via the SoftMax Pro Software and the QuickSync Tool. See [Installing the QuickSync Tool on page 331](#).



Note: When you operate the instrument via a computer running the SoftMax Pro Software, the instrument touchscreen is disabled.

SpectraMax iD3 Considerations

When you use the SoftMax Pro Software to operate the SpectraMax iD3, your company network configuration could prevent the connection 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 **System Information** 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 display the SoftMax Pro Options dialog.
4. In the list on the left, click **SpectraMax iDx Control**.
5. Select the **Enables Control of SpectraMax iDx Instruments** check box.
6. Enter the IP address of the instrument in one of the fields. There are five fields to enter up to five IP addresses.
7. Click **OK**.
8. Select the **Home** tab and click **Instrument** to display 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.

For users that use the SoftMax Pro Software - GxP edition the user must have the Lock/Unlock Instrument permission to lock and unlock the instrument touchscreen. In the Ribbon on the GxP tab, the following icons lock and unlock the instrument touchscreen:

- 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 you must operate the instrument from a computer running the SoftMax Pro Software - GxP edition.
- Click  **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to use the instrument touchscreen to run experiments.



Note: The instrument remains locked until the user with the appropriate permission

clicks  **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an instrument that is locked in GxP mode.

Installing the QuickSync Tool

When you use the SpectraMax® iD3 Multi-Mode Microplate Reader and SpectraMax® iD5 Multi-Mode Microplate Reader, use the QuickSync tool to use the raw data the instrument collects in the SoftMax Pro Software for further analysis.

The QuickSync Tool is a separate software application that you install and run on the computer running the SoftMax Pro Software. Contact Molecular Devices to obtain the installation file.

To install the QuickSync Tool:

1. Locate and double-click **QuickSyncInstaller.exe** file to start the install.
2. When the *Do you want to allow this app to make changes to your computer?* message displays, click **Yes**. The installation starts.
3. On the Completing the Setup page, click **Finish**.

The QuickSync Tool icon  appears on the computer desktop.

Using the QuickSync Tool

Use the QuickSync tool to use the raw data the SpectraMax iD3 and SpectraMax iD5 export for further analysis. The QuickSync Tool software is a separate installation. See [Installing the QuickSync Tool on page 331](#).

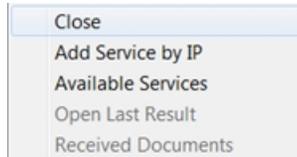
The instrument acquires an IP address when you connect the instrument to your intranet. After you synchronize the computer with the instrument, the instrument exports result data to the computer for further analysis.



Note: The microplate reader cannot have a USB flash drive in the USB slot below the touchscreen.

To use the QuickSync tool:

1. Use the instrument to run the read and leave the results displayed on the touchscreen.
2. On the computer running the SoftMax Pro Software, double-click the  on the desktop or in the task bar. A message appears that states QuickSync Ready!
3. Click ^ by the computer clock (Show Hidden Icons) to display a smaller version of the  in the computer tray.
4. Right-click  in the tray to display a menu and select **Available Services** to display the list of SpectraMax iD3 and SpectraMax iD5 instruments on your intranet and/or the instrument to which you connect the computer through an Ethernet cable.



5. 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.
6. On the upper left of the instrument touchscreen, touch **Export**. The computer running the SoftMax Pro Software displays a confirmation message.
7. On the computer running the SoftMax Pro Software, right-click  and select **Open Last Result**.

SpectraMax iD5 Multi-Mode Microplate Reader

The SpectraMax iD5 Multi-Mode Microplate Reader has a touchscreen interface that provides integrated instrument control, data display, and the ability to export results over your network for statistical data analysis.

There are several instrument configurations.

- Base includes filters for tunable fluorescence polarization read mode using the monochromator and filters to measure europium and terbium using the time-resolved fluorescence read mode.
- SpectraMax Injector System with SmartInject (factory installed or by a Molecular Devices field representative)
- Enhanced TRF Extension Module (you can install)
- Bottom Luminescence (factory installed)



Note: The SpectraMax iD5 is certified for use with Cisbio Bioassays' HTRF (Homogeneous Time-Resolved Fluorescence) technology. HTRF is a proprietary TR-FRET technology.

SpectraMax iD5 features include:

- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

You can operate the instrument via the SoftMax Pro Software and the QuickSync Tool. See [Installing the QuickSync Tool on page 331](#).

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Absorbance	✓	✓	✓	✓	
Dual Wavelength Luminescence	✓	✓	✓		
Fluorescence Intensity	✓	✓	✓	✓	
Fluorescence Polarization	✓	✓			
FRET	✓	✓	✓		
Luminescence	✓	✓	✓		
Time Resolved Fluorescence	✓	✓	✓	✓	✓
TR-FRET (Requires Enhanced TRF Module)	✓	✓	✓		

You must use a computer running the SoftMax Pro Software to operate the instrument for injector protocols and for the TR-FRET and Western Blot read modes.



Note: When you operate the instrument via a computer running the SoftMax Pro Software, the instrument touchscreen is disabled.

SpectraMax iD5 Considerations

When you use SoftMax Pro Software to operate the SpectraMax iD5, 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 iD5 to your company intranet. You may have to perform the following steps to select the instrument.

1. On the SpectraMax iD5 instrument touchscreen, navigate to the **Maintenance** page **System Information** 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 display the SoftMax Pro Options dialog.
4. In the list on the left, click **SpectraMax iDx Control**.
5. Select the **Enables Control of SpectraMax iDx Instruments** check box.
6. Enter the IP address of the instrument in one of the fields. There are five fields to enter up to five IP addresses.
7. Click **OK**.
8. Select the **Home** tab and click **Instrument** to display the Instrument Connection dialog.
9. Click **Refresh** if needed and the SpectraMax iD5 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.

For users that use the SoftMax Pro Software - GxP edition the user must have the Lock/Unlock Instrument permission to lock and unlock the instrument touchscreen. In the Ribbon on the GxP tab, the following icons lock and unlock the instrument touchscreen:

- 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 you must operate the instrument from a computer running the SoftMax Pro Software - GxP edition.
- Click  **GxP Mode Off** to release the lock from the instrument touchscreen and allow users to use the instrument touchscreen to run experiments.



Note: The instrument remains locked until the user with the appropriate permission

clicks  **GxP Mode Off** to stop the GxP mode. You cannot use the Instrument Connection dialog to disconnect from an instrument that is locked in GxP mode.

SpectraMax L Luminescence Microplate Reader

The SpectraMax L Luminescence Microplate Reader is a high-performance microplate reader that measures luminescence in 96 and 384-well plates. When the instrument is equipped with the variable-volume injectors option, it can measure both flash and glow luminescence.

- 96-well Standard plates - 10 μ L to 200 μ L
- 384-well and 96-well half area plates - 10 μ L to 80 μ L

High-precision peristaltic pumps ensure precise injection volumes and rapid sample mixing. Continuous monitoring between injection and measurement provides for extremely fast reactions and reduced delay time.

Optional multiple photomultiplier tubes (PMTs) and multiple injector configurations allow increased throughput and wavelength-dependent assays such as BRET1 and BRET2.

The extreme flexibility and high sensitivity of the instrument makes it ideal for applications within the fields of biochemistry, cell biology, immunology, molecular biology, and microbiology. Typical applications include luciferase reporter gene, ATP, Ca²⁺, aequorin, and ELISAs.

The instrument can accommodate SBS-standard 96 and 384-well plates with dimensions of 128.2 mm long, 86.0 mm wide, and heights from 14 mm to 15 mm.

In the SoftMax Pro Software, the SpectraMax L has a unique set of icons on the Ribbon and a unique set of settings in the Settings dialog.

SpectraMax L features include:

- Greater than nine orders of dynamic range
- Reads both 96 and 384-well plates
- Dual injection into both 96 and 384-well plates
- Programmable injection speeds
- AutoWash flush of tubing and tips when the instrument is turned off
- Landscape and portrait plate orientation
- Optional dual PMT configuration with filter positions for instrument wavelength dependent assays such as BRET
- Optional dual PMT configuration for higher throughput
- Optional injectors up to four injectors
- Isothermal temperature control in the plate chamber
- Optional barcode reader to read labels on plates
- Compatible with the StakMax Microplate Handling System.
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Dual Read	Endpoint	Kinetic	Fast Kinetic
Dual Wavelength Luminescence	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓

See [SpectraMax L Protocols on page 336](#)

SpectraMax L Ribbon

The SpectraMax L has a unique set of icons on the Ribbon. For a description of each setting, see the SoftMax Pro Software application help.

Home Tab

-  **Simulator Configuration** - When you operate a SpectraMax L in Offline or Simulator mode, use the Simulator Configuration dialog to define the instrument configuration for simulated protocols. You can use offline and simulator mode to define protocols that you save for use later. The settings for the protocols you save must be compatible with the instrument configuration of the instrument on which you intend to run the protocol.
- **Instrument Configuration** - When you connect the computer to the instrument, use the Instrument Configuration dialog to view the instrument components that are available for protocol settings.

Operations Tab

- Click  **Calibration** to display the Calibration dialog. Use the Calibration dialog to calibrate the internal light emitting diodes and do custom multi-PMT normalization plate calibration.
- Click  **Wash Injectors** to display the Wash Injectors dialog. Use the Wash Injectors dialog to wash the injectors.
- Click  **Prime Injectors** to display the Prime Injectors dialog. Use the Prime Injectors dialog to prime the injectors.
- Click  **Reverse Injectors** to display the Reverse Injectors dialog. Use the Reverse Injectors dialog to recover the reagent from the injector tubing.
- Click  **Injector Tubing** to display the Injectors Pump Tubing dialog where you to determine when to replace injector tubing. Use the Injectors Pump Tubing dialog to view when it is time to replace the injector tubing.
- Click  **Change Aperture** to change the plate drawer aperture from 96-well plates to 384-well plates or vice-versa. The instrument configuration has either a manual aperture or an automatic aperture. For the manual configuration you must use the Change Aperture command when you switch plate formats. The command instructs the reader to check whether a plate is in the drawer. If no plate is present, the plate carriage moves away from the read head to allow you to manually change the aperture. See the *SpectraMax L User Guide* for instructions to manually change the aperture.
- Click  **Shake Settings** to display the Shake Plate Settings dialog. Use the Shake Plate Settings dialog to custom define how to shake a plate. This shake process is independent of a protocol. When you create a protocol, use the Settings dialog to define how to shake the plate as part of the protocol.

SpectraMax L Protocols

The SpectraMax L instrument has multiple hardware configurations and the instrument configuration determines which read modes and read types are available.

When you operate the instrument Offline or Simulator mode, you define the instrument configuration from the Simulator Configuration icon on the Home tab.

When you connect the computer to the instrument, you can view the instrument hardware components from the Instrument Configuration icon on the Home tab.

Read Modes

The instrument hardware configuration determines which read modes are available.

- **Lumi** (Luminescence): For all instrument configurations you can select the Lumi read mode. When the instrument has two PMTs and no filters, the instrument runs Lumi protocols that use both PMTs to read 2 wells simultaneously and it assumes the reads of the PMTs are comparable.
- **Lumi2W** (Luminescence Dual Wavelength): When the instrument has two PMTs and the PMTs are equipped with filters you can select the Lumi2W read mode to use the PMTs one at a time to detect the two different wavelengths.

Read Types

For all instrument configurations, the following read types are available:

- **Endpoint:** A read of each well is taken in the center of each well, either at all wavelengths if filters are not used, or at specific wavelengths if filters are used. Raw data values are reported as relative light units (RLU). Each well can receive up to two injections, and then is read once. You can set a delay time after each injection, from 0 to 3,600 seconds for the M-injection, and from 0.1 to 3,600 seconds for the P-injection.
- **Dual Read:** Each well receives two injections, each followed by a read. The first injection is the P-injection with a subsequent read. The second injection is the M-injection with a subsequent read. You can set a delay time between injection and read, from 0.1 to 3,600 seconds for the P-injection, and from 0 to 3,600 seconds for the M-injection.
- **Kinetic:** The instrument collects data over time with multiple reads taken in the center of each well at regular intervals. Each well can receive multiple injections and reads. You can specify single injections, followed by single reads, for multiple time points. The minimum integration interval is dependent on the number of wells you select, the integration time you select, and the number of injections you select.
- **Fast Kinetic:** The instrument performs repeated reads of one or more wells up to a 200 point maximum integration. All reads of a single well are made before the next well is read. You cannot pre-read a plate with the Fast Kinetic read type. You can specify multiple injections and multiple reads, for multiple times. Integration time can be set from 0.01 to 3600 seconds.

For a description of each setting, see the SoftMax Pro Software application help.

SpectraMax M2 Multi-Mode Microplate Reader

The SpectraMax M2 Multi-Mode Microplate Reader contains two monochromators and dual-mode cuvette ports. Area-well scanning read types and the PathCheck technology let homogeneous and heterogeneous plate assays be done in one flexible system.

SpectraMax M2 features include:

- Dual-mode cuvette port
- Two monochromators
- PathCheck technology and well volume sensors
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓
Time-Resolved Fluorescence	✓	✓	✓	✓

SpectraMax M2e Multi-Mode Microplate Reader

The SpectraMax M2e Multi-Mode Microplate Reader contains two monochromators, dual-mode cuvette ports, and top-plate and bottom-plate read capability. Area-well scanning read types and the PathCheck technology let homogeneous and heterogeneous plate assays be done in one flexible system.

SpectraMax M2e features include:

- Top-read and bottom-read
- Dual-mode cuvette port
- Two monochromators
- PathCheck technology and well volume sensors
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓
Time-Resolved Fluorescence	✓	✓	✓	✓

SpectraMax M3 Multi-Mode Microplate Reader

The SpectraMax M3 Multi-Mode Microplate Reader is a modular, dual-monochromator microplate reader platform that offers a wide range of high performance multi-mode capabilities ideal for life science research and drug discovery screening.

SpectraMax M3 features include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- Temperature control
- PathCheck technology
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓

SpectraMax M4 Multi-Mode Microplate Reader

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

SpectraMax M4 features include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- Temperature control
- Plate shake
- PathCheck technology
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Luminescence	✓	✓	✓	✓
Time-Resolved Fluorescence	✓	✓	✓	✓

SpectraMax M5 Multi-Mode Microplate Reader

The SpectraMax M5 Multi-Mode Microplate Reader is a dual-monochromator microplate reader platform that offer a wide range of high performance multi-mode capabilities ideal for life science research and drug discovery screens.

SpectraMax M5 features include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- PathCheck technology
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Fluorescence Polarization	✓	✓		
Luminescence	✓	✓	✓	✓
Time-Resolved Fluorescence	✓	✓	✓	✓

SpectraMax M5e Multi-Mode Microplate Reader

The SpectraMax M5e Multi-Mode Microplate Reader is a dual-monochromator microplate reader platform that offer a wide range of high performance multi-mode capabilities ideal for life science research and drug discovery screens.

SpectraMax M5e features include:

- Three-mode cuvette port for assay development
- Dual monochromator tunability
- PathCheck technology
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity	✓	✓	✓	✓
Fluorescence Polarization	✓	✓		
Luminescence	✓	✓	✓	✓
Time-Resolved Fluorescence	✓	✓	✓	✓

SpectraMax Mini Multi-Mode Microplate Reader

The SpectraMax Mini Multi-Mode Microplate Reader contains a monochromator for absorbance reads and accommodates a filter cube for fluorescence or luminescence reads. Kinetic and well scan read types, along with PathCheck technology, enable a wide range of life science applications. The instrument allows reads that include shaking and temperature control.

SpectraMax Mini features include:

- Monochromator
- Filter cube
- Temperature control
- PathCheck technology

Read Mode	Read Type			
	Endpoint	Kinetic	Well Scan	Spectrum
Absorbance (UV and Visible)	✓	✓	✓	✓
Fluorescence Intensity (requires a Fluorescence filter cube)	✓	✓	✓	
Luminescence (requires the Luminescence filter cube)	✓	✓	✓	

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 you configure the microplate reader for specific applications, and the cartridges expand the capabilities of the microplate reader. The detection cartridges you install define the measurement capabilities of the microplate reader.

You can install up to six detection cartridges in each of the two detection cartridge drawers of the SpectraMax Paradigm.

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.

SpectraMax Paradigm features 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 technology
- AlphaScreen
- Compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Absorbance Detection Cartridge					
Absorbance	✓	✓	✓	✓	
AlphaScreen 384 HTS, AlphaScreen 384 Std, and AlphaScreen 1536 HTS Detection Cartridges					
Screen	✓	✓	✓		
Cisbio HTRF Detection Cartridge					
TR-FRET	✓	✓	✓		
Dual Color Luminescence (LUM) (BRET2) and Dual Color Luminescence (LUM) (Chroma-Glo) Detection Cartridge					
Dual Wavelength Luminescence	✓	✓	✓		
Luminescence	✓	✓	✓		
Fluorescence Intensity (FL) (coum-fluor), Fluorescence Intensity (FL) (fluor-rhod), Fluorescence Intensity (FL) (Cy3-Cy5), Fluorescence Intensity (FL) (Cfp-Yfp), and Fluorescence Intensity GeneBLAzer Detection Cartridges					
Fluorescence Intensity	✓	✓	✓		
FRET	✓	✓	✓		

Read Mode	Read Type				
	Endpoint	Kinetic	Well Scan	Spectrum	Membrane
Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge					
Dual Wavelength Fluorescence Intensity	✓	✓	✓		
Fluorescence Intensity	✓	✓	✓		
FRET	✓	✓	✓		
Fluorescence Polarization (FP) (Fluorescein) and Fluorescence Polarization (FP) (Rhodamine) Detection Cartridges					
Fluorescence Polarization	✓	✓			
Glow Luminescence (LUM), Glow Luminescence (LUM) (96), and Glow Luminescence (LUM) (384) Detection Cartridges					
Luminescence	✓	✓	✓		
Multi-Mode (MULTI) Detection Cartridge					
Fluorescence Intensity	✓	✓	✓		
Luminescence	✓	✓	✓		
Time-Resolved Fluorescence	✓	✓	✓		
ScanLater Western Blot (WB) Detection Cartridge					
Time-Resolved Fluorescence					✓
Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge					
Dual Wavelength Time-Resolved Fluorescence	✓	✓			
Time-Resolved Fluorescence	✓	✓	✓		
Tunable Wavelength (TUNE) Detection Cartridge					
Fluorescence Intensity	✓	✓	✓	✓	
Luminescence	✓	✓	✓	✓	
Time-Resolved Fluorescence	✓	✓	✓	✓	

See [Read Modes and Read Types](#) on page 277 and [Supported Detection Cartridges](#) on page 375.

Removing Software Locks

At this point you have removed the physical transport locks, installed the SoftMax Pro Software, connected the cables, and powered on the instrument. 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.



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.



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 remove the software locks and initialize the instrument:

1. 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.
2. Start the SoftMax Pro Software. See [Starting the Software on page 9](#).
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.
3. After the computer and the instrument successfully connect, the Instrument Unlocking Procedure wizard displays.
4. If the Instrument Unlocking Procedure wizard does not display, use the Instrument Connection dialog to select and connect to the instrument. See [Selecting an Instrument on page 64](#).



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

5. 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 plate 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 Multi-Mode Microplate Reader User Guide*.

To lock the drawers for shipment, use the Instrument Information dialog. See [Instrument Information on page 373](#).

SpectraMax Plus 384 Microplate Spectrophotometer

The SpectraMax Plus 384 Microplate Spectrophotometer has a built-in cuvette port and plate 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 plates.

SpectraMax Plus 384 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 plates in just 5 seconds and 384-well plates in 16 seconds for ultra-fast sample throughput.
- PathCheck technology
- Newer models compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type		
	Endpoint	Kinetic	Spectrum
Absorbance (UV and Visible)	✓	✓	✓

VersaMax Microplate Spectrophotometer

The VersaMax Microplate Spectrophotometer 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 plates.

VersaMax 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.
- Newer models compatible with the StakMax Microplate Handling System
- Compliance validation package see [SpectraTest Validation Plates on page 370](#)

Read Mode	Read Type	
	Endpoint	Kinetic
Absorbance	✓	✓

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 plates.

VMax features include:

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

Read Mode	Read Type	
	Endpoint	Kinetic
Absorbance	✓	✓

StakMax Microplate Handling System

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

Access the StakMax Software from the Operations tab in the SoftMax Pro Software. See [StakMax Software](#) on page 348.



Compatible Instruments

The StakMax Microplate Handling System is compatible with the following instruments:

- Gemini™ EM Dual Scanning Microplate Spectrofluorometer
- Gemini™ XPS Dual Scanning Microplate Spectrofluorometer
- SpectraMax® ABS Microplate Spectrophotometer
- SpectraMax® ABS Plus Microplate Spectrophotometer
- SpectraMax® i3x Multi-Mode Microplate Reader
- SpectraMax® iD3 Multi-Mode Microplate Reader
- SpectraMax® iD5 Multi-Mode Microplate Reader
- SpectraMax® L Luminescence Microplate Reader
- SpectraMax® M2 Multi-Mode Microplate Reader
- SpectraMax® M2e Multi-Mode Microplate Reader
- SpectraMax® M3 Multi-Mode Microplate Reader
- SpectraMax® M4 Multi-Mode Microplate Reader
- SpectraMax® M5 Multi-Mode Microplate Reader
- SpectraMax® M5e Multi-Mode Microplate Reader
- SpectraMax® Paradigm® Multi-Mode Microplate Reader

Newer models of the following instruments are compatible with the StakMax Microplate Handling System:

- SpectraMax® 190 Microplate Spectrophotometer (Serial number begins with NNR)
- SpectraMax® 340PC384 Microplate Spectrophotometer (Serial number begins with LNR)
- SpectraMax® Plus 384 Microplate Spectrophotometer (Serial number begins with MNR)
- VersaMax™ Microplate Spectrophotometer (Serial number begins with BNR)

For more information, see the *StakMax Microplate Handling System User Guide*.

StakMax Software



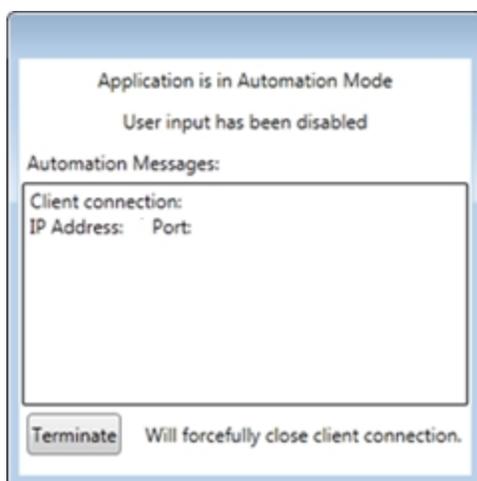
Note: When you connect the microplate handler to the AquaMax Microplate Washers, the AquaMax Software controls the StakMax Microplate Handler. See the *AquaMax 2000 Microplate Washer/AquaMax 4000 Microplate Washer User Guide*.

The StakMax Software is included with the SoftMax Pro Software to integrate the StakMax Microplate Handling System with the Molecular Devices microplate reader.

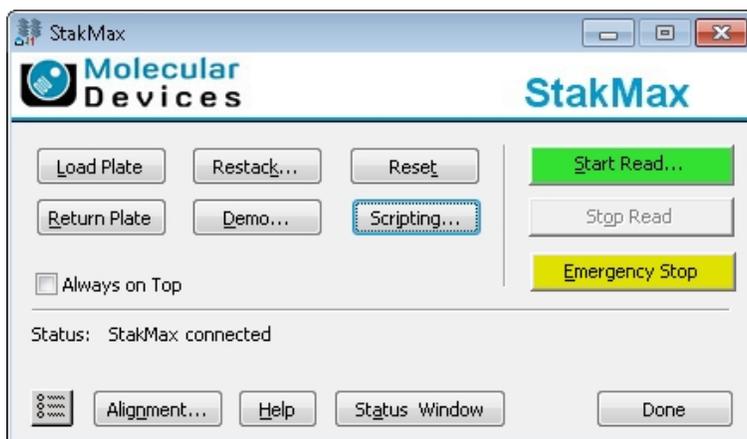


Note: When you use the SoftMax Pro Software - GxP edition, StakMax specific commands and scripts are not tracked in the audit trail and do not have user permission settings. Please implement standard work as required by your organization.

To start the StakMax Software from the SoftMax Pro Software, select the **Operations** tab and click **Plate Stacker**. The SoftMax Pro Software goes into Automation mode and prevents user interaction with the SoftMax Pro Software.



The StakMax dialog displays on top of the SoftMax Pro Software along with the microplate handler connection status.



Load Plate

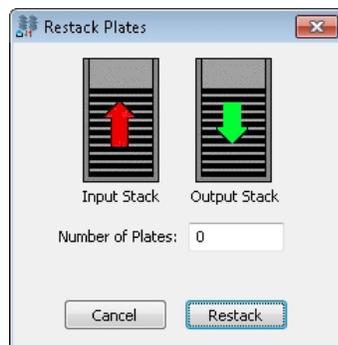
Click **Load Plate** to move one plate from the input stack to the microplate reader. Click this button to confirm that the plate alignment between the input stack and the microplate reader is still accurate.

Return Plate

Click **Return Plate** to move one plate from the microplate reader to the output stack. Click this button to clear a plate that was left in the microplate reader, or to determine if a component is out of sync.

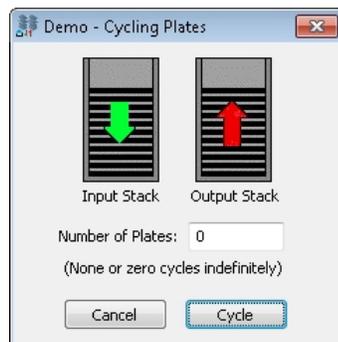
Restack Plates

Click **Restack** to move plates from the output stack to the input stack. Use the Restack Plates dialog to specify the number of plates to restack. To move all plates, leave the **Number of Plates** field blank or enter **0** (zero).



Demo

Click **Demo** to continuously move plates from the microplate handler to the microplate reader and back. This switches the plate source between the input stack and the output stack as they fill and empty. Use Demo mode to test the plate movement. Do not use Demo mode for normal operation. Use the Demo - Cycling Plates dialog to enter the number to move in the **Number of Plates** field. To move all plates, leave the **Number of Plates** field blank or enter **0** (zero).



Reset

Click **Reset** to reset the microplate handler to the initial start-up state. In most cases, the software automatically resets the system if an error occurs. Use the **Reset** button only in rare cases when the microplate handler does not reset itself.

Scripting

Click **Scripting** to create and edit scripts to run the microplate handler. The Scripting dialog displays to enable you write scripts that are a series of commands to control and automate more complex runs. See [Scripting on page 363](#).

Start Read

Click **Start Read** to starts a plate read. This button is green when the software and instrument are ready to start a read. The Read Plate dialog displays. See [Reading Plates on page 362](#).

Stop Read

Click **Stop Read** to stop the read in progress and reset the microplate handler and the microplate reader. This button turns red after a read starts. The Status line displays **Read Plate Session Stopped** after the read stops.

- If you stop the read before a read starts and while the microplate handler is in motion, no data saves.
- If you stop the read after a read starts, interrupting the read, the data does not automatically save. You can manually save the collected data.
- If you stop the read after a read completes, the data automatically saves if you define the protocol to use the Auto Export or Auto Save feature. You can manually save the collected data.

Emergency Stop

Click **Emergency Stop** to immediately shut down the entire system, including the microplate handler and the microplate reader. After you click **Emergency Stop**, clear objects that are in the way, remove plates that are in the drawer, and then click **Reset** in the StakMax dialog.

Settings

Click  to display the StakMax Settings dialog where you define how to name documents when you install a barcode reader, whether to append the date and time to script commands, and whether to display a prompt during script editing. See [Settings on page 351](#).

Alignment

Click **Alignment** before you use the microplate handler for the first time to align the instrument with the microplate reader. The Alignment wizard provides instructions to complete the alignment process. Use the Alignment wizard one time after instrument installation. This process takes approximately 15 minutes. See [Aligning the Gripper on page 352](#).

Status Window

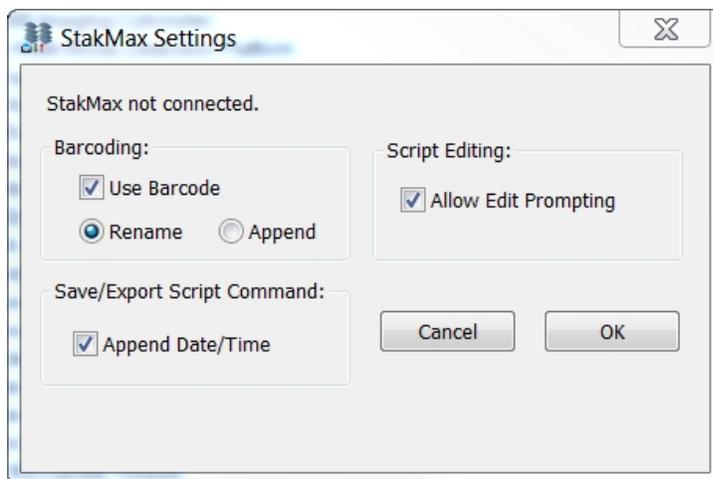
Click **Status Window** to display the Status dialog. The Status dialog displays information about the current run including low-level commands and error messages. Use the Status dialog to print the status, clear the status, and view the status history. To save status information, select the text, copy (Ctrl+C) the text, and then paste (Ctrl+V) the text into a text editor such as Microsoft Word or Notepad. See [Status Dialog Error Codes on page 360](#).

Done

Click **Done** to exit the StakMax Software, and then click **Terminate** in the SoftMax Pro Software Automation dialog.

Settings

Click  to display the StakMax Settings dialog where you define how to name Plate sections in the data files when you install a barcode reader in the microplate handler, whether to append the date and time to script commands, and whether to display a prompt when you edit scripts.



Barcoding

Select the **Use Barcode** check box to use the microplate handler barcode reader to identify plates.

- Select **Rename** to overwrite the Plate section name with the barcode information.
- Select **Append** to add the barcode information to the Plate section name.

Script Editing

The Allow Edit Prompting option is applicable when you use the Scripting feature. See [Scripting on page 363](#).

- Select the **Allow Edit Prompting** check box to have the software display a message each time you click **Remove** or **Remove All** in the Script Editor dialog.
- Clear the **Allow Edit Prompting** check box to not display messages when you delete a script command in the Script Editor dialog.

Save/Export Script Command

The Save/Export Script Command feature helps with record keeping, for backup purposes, and to reduce the risk of overwriting existing script files.

- Select the **Append Date/Time** check box to have the software add the date and time to the script file name when the file saves. Example: The script file: **user.pda** saves as **user_19.01.22-07-36-41 PM.pda**.
- Clear the **Append Date/Time** check box to have the software add a 1, 2, 3, and so on, to the script file name to prevent overwriting script files.

Aligning the Gripper

The StakMax Software Alignment wizard provides instructions to complete the alignment process. Use the Alignment wizard one time after instrument installation. This process takes approximately 15 minutes.

You do not need to align the SpectraMax iD3 and SpectraMax iD5.



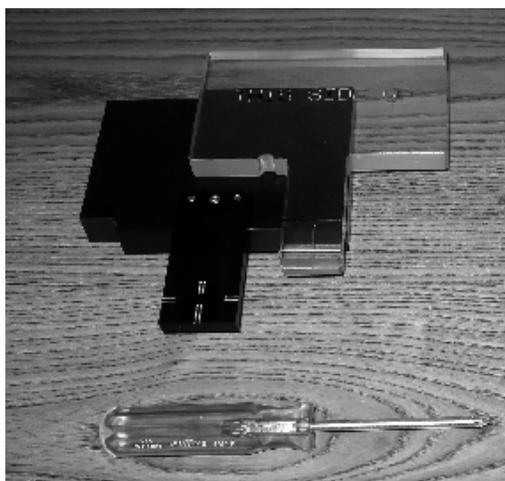
Note: Do not use this alignment process for the SpectraMax i3x and SpectraMax Paradigm. See the application online help for instructions.

The following are in the accessory kit:

- Clear alignment tool
 - Black alignment tool
 - Phillips screwdriver
 - Plate
-



Note: Do not use the purple plate adapter when you perform the alignment. Use the purple plate adapter only when you read a plate in the top read mode for specific instruments.



Make sure you have the two alignment tools and other parts available before you start the alignment process.

To align the gripper:

1. Start the SoftMax Pro Software.
2. In the SoftMax Pro Software, select the **Operations** tab and click **Plate Stacker**. After a few seconds, the StakMax dialog displays. See [StakMax Software on page 348](#).

3. Loosen the silver thumbscrew on the cover that is between the microplate handler arm and the input stack.



4. Lift off the cover from the microplate handler arm.



5. In the StakMax dialog, click **Alignment** to start the Alignment wizard. See [StakMax Alignment Wizard on page 354](#).
6. After you finish the alignment, replace the cover on the microplate handler arm and tighten the thumbscrew.



After you complete the gripper alignment, run a functional check of the gripper. See [Checking Gripper Function on page 359](#).

StakMax Alignment Wizard



Note: Do not use this alignment process for the SpectraMax i3x and SpectraMax Paradigm. See the application online help for instructions.

To use the StakMax Alignment Wizard:

1. In the SoftMax Pro Software, select the **Operations** tab and click **Plate Stacker**. After a few seconds, the StakMax dialog displays.



2. In the StakMax dialog, click **Alignment** to start the Alignment wizard.



On the Align Tool Placement page:

- a. Open the microplate reader plate drawer.
- b. Place the black alignment tool onto the plate tray. Leave the plate drawer open.
- c. Click **Next**.

3. On the Gripper Position page:



- a. Click **Move Gripper Over to Carriage**.
- b. Click **Next**.

4. On the Clear Alignment Tool Insertion page:



- a. Click **Open Gripper Jaws**.



- b. Place the clear alignment tool in the gripper so that the notch is aligned with the gripper foot.
- c. Click **Next**.

5. On the Alignment page:



- a. Look straight down at the alignment tools and move the stacker so that the single cross hairs on the alignment tool are between the double cross hairs on the black alignment tool.
- b. Tighten the screws on the StakMax baseplate.
- c. Click **Next**.

6. On the Remove Clear Alignment Tool page:

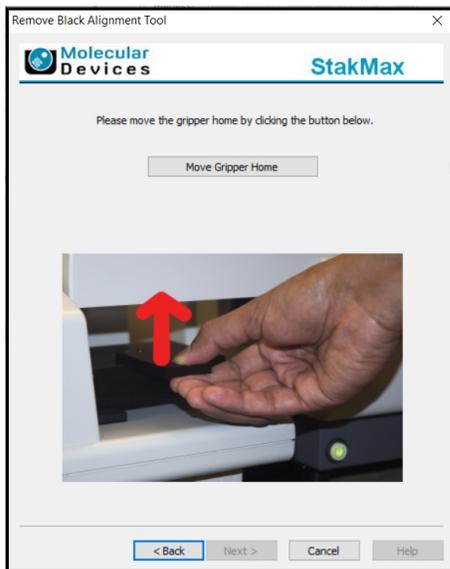


- a. Click **Remove Clear Alignment Tool**.
- b. Remove the clear alignment tool from the instrument.

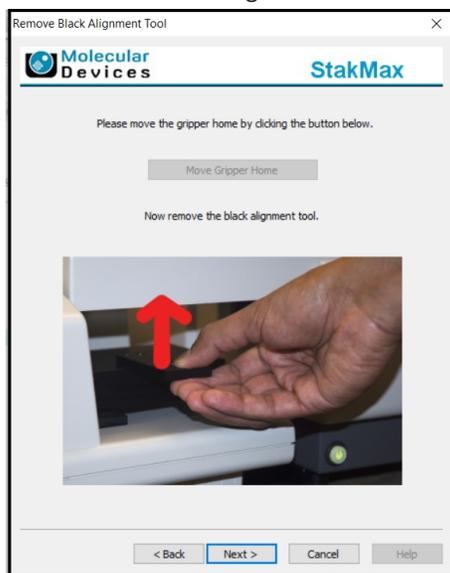


- c. Click **Next**.

7. On the Remove Black Alignment Tool page:

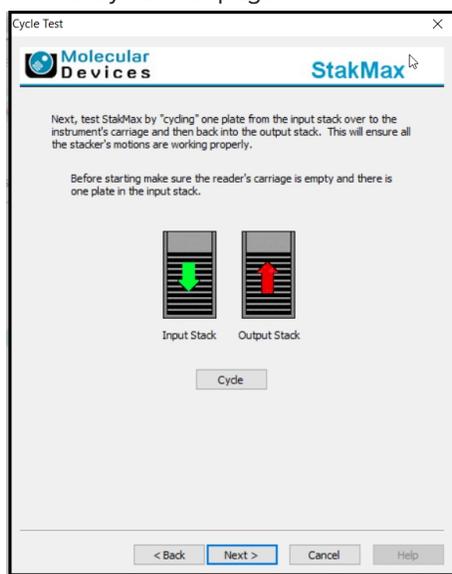


- a. Click **Move Gripper Home**.
- b. Remove the black alignment tool from the instrument.

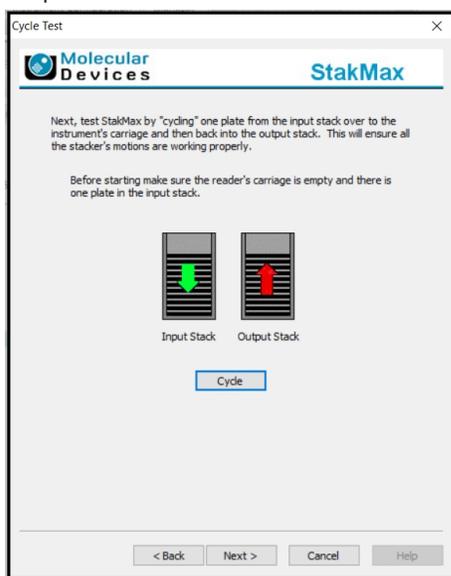


- c. Click **Next**.

8. On the Cycle Test page:



- a. Click **Cycle**.
- b. Wait for the plate to move from the input stack, into the instrument, and then to the output stack.



- c. Click **Next**.

9. On the Calibration Finished page, Click **Finish**.



After you complete the gripper alignment, run a functional check of the gripper.

Checking Gripper Function

After you align the gripper, do the following functional check to make sure that the microplate handler is operational.

You need five empty plates.

To do a gripper functional check:

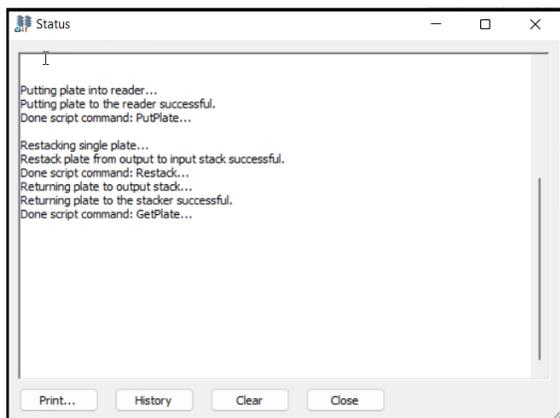
1. Turn on the microplate reader and the StakMax Microplate Handler.
2. Start the SoftMax Pro Software.
3. Select the **Operations** tab and click **Plate Stacker**. After a few seconds, the StakMax dialog displays.
4. Place five plates in the input stack or the magazine.
5. Click **Scripting** to display the Scripting dialog.
6. Click **Open**.
7. Navigate to and select the **Functional_Check.txt** script in the following location:
C:\ProgramData\Molecular Devices\SMP<n.n>\StakMax Scripts and then click **Open**.
8. Click **Run**.
 - The microplate handler loads the first of the 5 plates into the plate drawer.
 - The drawer closes then re-opens.
 - The microplate handler picks up the plate and places it in the output stack.
 - This process continues for all 5 plates.
 - After the plates are processed, they are moved from the output stack and are re-stacked in the input stack.
 - This process repeats 10 times for a total of 50 plate load and unload cycles.

If the plates transfer and stack properly, the microplate handler passes the functional check.

9. Click **Done**.

StakMax Status

Use the Status dialog to view the status of the StakMax Microplate Handler operations.



The following options are available:

- Click **Print** to print the contents of the Status dialog.
- Click **History** to view historical status information.
- Click **Clear** to clear the information from the Status dialog.

Status Dialog Error Codes

Use the Status dialog to diagnose StakMax Microplate Handler errors. To access the Status dialog, click **Status Window** in the StakMax dialog.

200 Error: A problem was encountered during Reset

The homing routine could not find a state change on the home flag. A sensor or flag problem is the likely source.

Solution:

Press the power interrupt button.

201 Error: Call Molecular Devices Technical Support at +1-800-635-5577

A timer resource was not available or not apparently used in the code.

Solution:

Contact Molecular Devices Technical Support.

202 Error: A problem occurred during a motion

A stall occurred during motion. The encoder and step counts exceeded the maximum threshold.

Solution:

Check for an object blocking movement and then realign the microplate handler.

203 Error: A problem occurred with the X axis

The X axis motion did not cause a state change on the edge-detect switch. Indicates a poorly gripped plate or that a plate is not present.

Solution:

Check to make sure plate is properly gripped and then realign the microplate handler. If the error persists, contact Molecular Devices Technical Support.

204 Error: A problem was encountered during Reset

Cannot move axis if it has not been homed.

Solution:

Press the power interrupt button.

205 Error: A problem occurred during a motion

Cannot move axis if it is currently in motion.

Solution:

Press the power interrupt button.

206 Error: A Problem occurred with the EPROM

Generic EPROM error.

Solution:

Contact Molecular Devices Technical Support.

207 Error: A problem occurred during a motion

Carriage drop off exceeds the maximum travel of the X axis.

Solution:

Realign the microplate handler.

208 Error: A problem occurred during a motion

The jaws snapped shut after opening.

Solution:

Check for an object blocking movement and then realign the microplate handler.

209 Error: A Problem occurred with the EPROM

EPROM space is larger than portion of FLASH memory allocated.

Solution:

Contact Molecular Devices Technical Support.

210 Error: A problem occurred during a motion

Moving gripper to the upward position did not find home.

Solution:

Check for an object blocking movement and then realign the microplate handler.

211 Error: A Problem occurred with the EPROM

EPROM did not have the proper checksum and cannot be trusted. This should prevent operation of the unit.

Solution:

Contact Molecular Devices Technical Support.

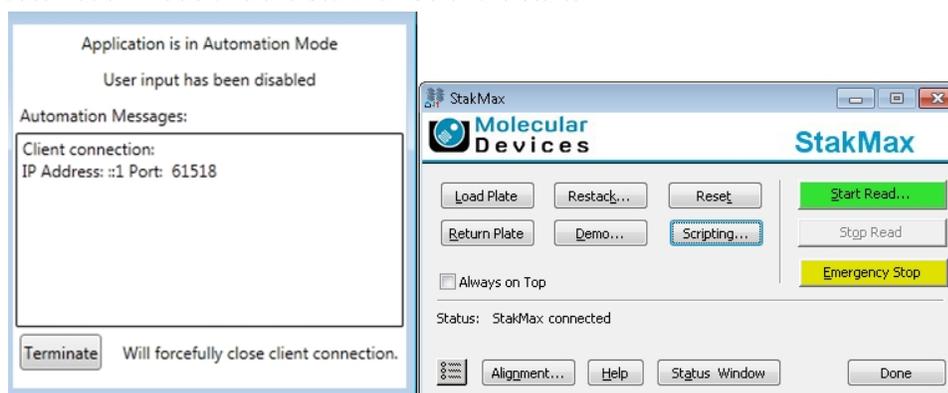
Reading Plates



CAUTION! You should define the protocol to use the Auto Export or Auto Save feature to prevent data loss before you start the StakMax Software. See the *SoftMax Pro Data Acquisition and Analysis Software User Guide* or the application help.

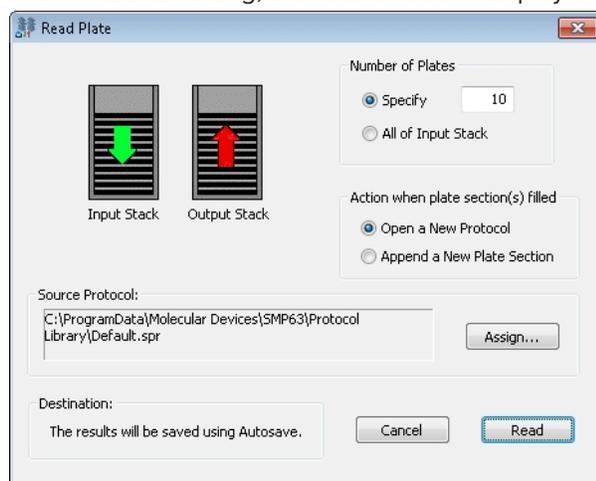
You should not do data analysis or other operations in the SoftMax Pro Software until the process completes. As with other robotic plate handling systems, it is best to do data analysis on a different computer from the computer that runs the microplate handler.

1. Power on the microplate handler and then power on the microplate reader.
2. Load plates into the input magazine.
3. In the SoftMax Pro Software, open the protocol to run and then click  to define the **Auto Export** or **Auto Save** settings to prevent data loss. See the *SoftMax Pro Data Acquisition and Analytics Software User Guide* or the application help.
4. Select the **Operations** tab and click **Plate Stacker**. The SoftMax Pro Software enters automation mode and the StakMax Software starts.



The StakMax dialog displays the status as the software connects to the StakMax Microplate Handler.

5. In the StakMax dialog, click **Start Read** to display the Read Plate dialog.



6. Select a **Number of Plates** option.
 - Select **Specify** and enter the number of plates to read.
 - Select **All of Input Stack** to read all of the plates in the input stack.

7. Select an **Action When Plate Sections Filled** option.
 - Select **Open a New Protocol** to save the data from each plate or set of plates to a different data file.
Example: You have 50 sample plates and the protocol has one Plate section, the software opens 49 copies of the protocol and saves the data to 50 documents. Alternatively, you have 4 sets of 5 plates (1 control plate and 4 sample plates) and the protocol has 5 Plate sections, the software opens 4 instances of the protocol for each set of plates and saves the data to 4 documents. This allows you to automate the processing of plate sets without a script.
 - Select **Append a Plate Section** to save the data from all plates to the same document.
Example: You have 50 sample plates and the protocol has one Plate section, the software adds 49 Plate sections to the original protocol, and the data from all plates save in a single document.
8. In the Source Protocol area, click **Assign** to display the Choose a Protocol for Reading dialog or the Open dialog. Select the protocol to read and click **Open**. The file path to the protocol displays in the Source Protocol field.



Note: The Destination area should display **The Results Will Be Saved Using Auto Save**. If not, click Cancel and enable either Auto Export or Auto Save.

9. Click **Read**. The Status line displays the read status.

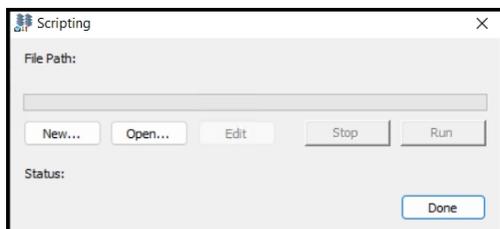


Note: If you leave a plate in the microplate reader and try to read a new plate, the system detects that a plate is present and moves the new plate back to the input stack where it came from. Click **Return Plate** to move the plate from the microplate reader to the output stack and then restart the read.

Scripting

Use the Scripting dialog to manage scripts and to run scripts. Scripting allows you to automate a series of commands that control reads.

Click **Scripting** in the StakMax dialog to display the Scripting dialog.



The file path to the script displays.

To manage scripts:

- Click **New** or **Edit** to display the Script Editor dialog. See [Script Editor on page 364](#).
- Click **Open** to display the Open a Script dialog. Navigate to and select the script file to run and then click **Open**.
- Click **Run** to run the script.

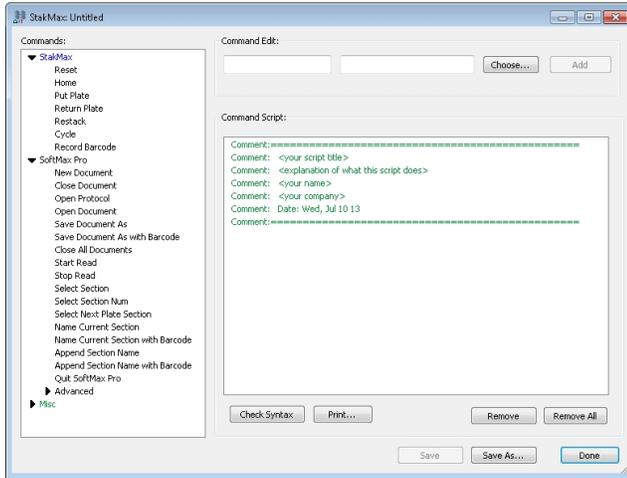
As the script is runs, the **Stop** button allows you to stop the run before it completes.



Note: If the script has unsaved changes on the Script Editor, the unsaved commands do not run.

Script Editor

Use the Scrip Editor dialog to manage scripts.



In the Scripting dialog click **New** or **Edit** to display the Script Editor dialog. The Script Editor has three areas:

- The **Commands** area lists the commands you can use in a script.
- The **Command Edit** fields allow you to add commands to the script.
- The **Command Script** field displays the script and allows you to modify the commands.

StakMax Commands

Command	Description
Reset	Resets the microplate handler to its initial start-up state and position.
Home	Returns all axes of the microplate handler to their home positions.
Put Plate	Opens the microplate reader plate drawer and moves one plate from the microplate handler input stack to the microplate reader plate drawer.
Return Plate	Opens the microplate reader plate drawer and moves one plate from the microplate reader plate drawer to the microplate handler output stack.
Restack	Moves the entered number of the plates from the microplate handler output stack to the input stack. If you do not enter a value, all plates in the output stack move to the input stack.
Cycle	Moves the entered number of plates from the microplate handler input stack to the output stack. If you do not enter a value, all plates in the input stack move to the output stack.
Record Barcode	Sets the barcode variable in the script to that of the plate that was most recently scanned by the barcode reader.

SoftMax Pro Commands

Command	Description
New Document	Creates a new protocol with the default protocol settings.
Close Document	Closes the current protocol.
Open Protocol	Opens the protocol from the path you enter.
Open Document	Opens the document from the path you enter.
Save Document As	Saves the current document with the name and file path you enter.
Save Document As with Barcode	Saves the current document with the name and file path you enter plus adds the current barcode to the end of the file name.
Close All Documents	Closes all documents.
Start Read	Starts the read.
Stop Read	Stops the read that is in progress.
Append Read	Appends data to the current read.
Select Section	Selects a specific section in the document.
Select Section Num	Selects a section by number.
Select Next Plate Section	Selects the next available Plate section.
Name Current Section	Gives the name you enter to the current section.
Name Current Section with Barcode	Gives the name you enter to the current section and adds the current barcode to the end of the name.
Append Section Name	Adds a text string to the end of the section name.
Append Section Name with Barcode	Adds the current barcode to the end of the section name.
Quit SoftMax Pro	Exits the SoftMax Pro Software.
Start Workflow	Starts a workflow.

Expand the list of SoftMax Pro commands to display the Advanced SoftMax Pro commands.

Advanced SoftMax Pro Commands

Command	Description
Open Drawer	Opens the microplate reader plate drawer.
Close Drawer	Closes the microplate reader plate drawer.
Set Temperature	Sets the temperature for a microplate reader plate drawer. To turn the incubator off, enter zero (0).
Shake Plate	Shakes the plate inside the microplate reader. Enter "on" to start shaking and "off" to stop shaking. Use the Wait command to enter the number of seconds to shake the plate.
New Plate Section	Creates a new Plate section in the protocol.
New Notes Section	Creates a new Note section in the protocol.
New Experiment	Creates a new experiment in the protocol.
Import Plate Template	Imports a plate template into the current Plate section.
Export Section	Exports the data from the current section to the file path you enter.
Export Section With Barcode	Exports the data from the current section to the file path you enter and adds the current barcode to the end of the name
Log On	When you use the SoftMax Pro Software - GxP edition, this command logs on the user you enter. Syntax: username/password/project
Log Off	When you use the SoftMax Pro Software - GxP edition, this command logs the current user off.

Miscellaneous Commands

Command	Description
Comment	Adds a comment or note to the current script.
Begin Repeat	Indicates the start of a set of commands that are to be repeated the number of times you enter.
End Repeat	Indicates the end of a set of commands that are to be repeated.
Wait	Pauses the script for the number of seconds you enter before proceeding to the next action.

Managing Scripts

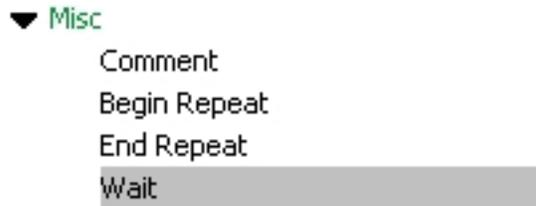
To create or edit a script:

1. In the **Command Script** area, click the line above where you want to insert the new command. The new command displays in the line below the line you select. To place the command at the top of the script, do not select a line.

```
Shake Plate:on
Shake Plate:off
```

Example: Select "Shake Plate: On" to add the command to the next line

2. In the **Commands** list, select the command to add.



Example: Select "Wait"

The command displays in the left side **Command Edit** field and its description displays below the field.

A screenshot of the 'Command Edit' field in the software. The field contains the text 'Wait' followed by a numerical input field containing the value '5'. To the right of the input field are two buttons: 'Choose...' and 'Add'. Below the input field, the text 'Wait: (# seconds required).' is displayed.

Example: Command Edit "Wait" with a 5 second duration

3. If the command requires a value, enter the value in the right side **Command Edit** field.
4. For the following commands: Open Protocol, Open Document, Save Document As, Save Document As with Barcode, and Set Protocol Folder, click **Choose** to display the Open dialog. Navigate to and select the path to the file or folder.



Note: The StakMax Software uses the file extensions for SoftMax Pro Software version 5.x and earlier (.pda or .eda). To use a script with version 6.x or 7.0.x, you must edit the file name in the path statement to use the appropriate version 6.x or 7.0.x file extension (.sda or .sdax) before you add the command to the script.

5. For the Comment command: Click **Comment** in the Commands list to add the Comment command to the left side **Command Edit** field. In the left side **Command Edit** field enter a colon (:) after the Comment command and then enter the comment text after the colon. Comments do not initiate actions in the script.

A screenshot of the 'Command Edit' field in the software. The field contains the text 'Comment: Repeat 3 times' followed by an empty input field. To the right of the input field are two buttons: 'Choose...' and 'Add'. Below the input field, the text 'Script comment, remark or note (not an actual command).' is displayed.

Example: Comment command "Repeat 3 times" (for reference only)

- Click **Add** to add the command to the script.

```
Shake Plate:on
Wait:5
Shake Plate:off
```

Example: "Wait" command displays in the script

- Click **Check Syntax** to check the syntax of the individual commands in the script. If the syntax is invalid see [Managing Commands on page 369](#).
- Click **Save As** to display the Save Script As dialog. Name the script file, navigate to, and select the folder where you want to save the script file. Scripts files save as text (.txt) files.



Note: If you use an existing script as a template for a new script, save the script file with a different file name or in a different folder to preserve the original script.

After you name and save the script file, click **Save**.

- Click **Print** to display the Print dialog where you select the printer and enter the number of copies to print. Click **OK** to print the script.

Repeat Loops

To add a repeat loop, define the beginning of the loop, end of the loop, and the number of times to repeat the loop.

- In the **Command Script** field, click the line directly above where you want the repeat loop to start.
- From the Commands list, select the **Begin Repeat** command to display the Begin Repeat command in the left side Command Edit field.
- In the right side **Command Edit** field, enter the number of times to repeat the loop.
- Click **Add** to add the Begin Repeat command to the script.
- In the **Command Script** field, click the last line of the repeat loop.
- From the Commands list, select the **End Repeat** command to display the End Repeat command in the left side Command Edit field.
- Click **Add** to add the End Repeat command to the script below the last line of the repeat loop.

New Document

Select Next Plate Section

```
Begin Repeat:3
```

```
Put Plate
```

```
Shake Plate:on
```

```
Wait:5
```

```
Shake Plate:off
```

```
Record Barcode
```

```
Start Read
```

```
Return Plate
```

```
Save Document As with Barcode:C:\Data\ReadResults.sda
```

```
End Repeat
```

```
Close Document
```

Repeat a read three times



Note: Nesting of repeat loops is not supported. The syntax check does not detect an unpaired Begin Repeat or End Repeat as an error because the syntax check validates each individual command.

Managing Commands

Use the Command Script area on the Script Editor dialog to edit, move, or remove commands from the script. You can edit a command value or change the command execution sequence.

In the Command Script area, double click a command to make it editable. Enter the value for the command. Make sure that a colon (:) follows the command name and precedes the value.

Wait:5

Edit the "Wait" command

Move a Command

Drag and drop commands to change the command sequence. A horizontal bar indicates where the command is placed.

Start Read
Return Plate
End Repeat
Save Document As with Barcode: C:\Data\ReadResults.sda
Close Document

Move a command in the script

Remove a Command

Select the command to remove and then click **Remove**. When you select the Allow Edit Prompting check box in the Settings dialog, you must click **OK** to confirm the removal. See [Settings on page 351](#).

To clear the Command Script area, click **Remove All**.

Check Command Syntax

Click **Check Syntax** to check the syntax of each individual command in the script. When a syntax error exists, the erroneous command line appears highlighted in the Command Script area. An invalid command can be a typo or an unknown or missing parameter. If the command requires a value, make sure that a colon (:) follows the command name and precedes the value.

Shake Plate

The Shake Plate command is missing the "on" or "off" value



Note: The syntax check validates each individual command. It does not detect the validity or logic of the sequence of the commands.

SpectraTest Validation Plates

Molecular Devices microplate readers are designed to provide consistent performance for many years. Use the SpectraTest Validation Plates from Molecular Devices to validate the optical performance your instrument.

Each SpectraTest Validation Plate is a comprehensive optical validation package. The American Association for Laboratory Accreditation (A2LA) has granted accreditation to the Laboratory Quality System under ISO/IEC 17025 for validation plate calibration and re-calibration processes.

The SoftMax Pro Software Protocol Library includes instrument specific protocols that automatically read the validation plate, perform the required test measurements, and make the required calculations. The software also enables you to customize the test report format.

Validation Packages Part Numbers

Part Number	Item Name	Compatible Instruments
n/a*	SpectraTest ABS1 Absorbance Validation Plate	FlexStation 3, SpectraMax 190, SpectraMax 340PC384, SpectraMax ABS, SpectraMax ABS Plus, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384, VersaMax
0200-6191	SpectraTest ABS2 Absorbance Validation Plate	FlexStation 3, SpectraMax ABS, SpectraMax ABS Plus, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384
0200-5060	SpectraTest FL1 Fluorescence Validation Plate	FlexStation 3, Gemini EM, Gemini XPS, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e
0200-6186	SpectraTest LM1 Luminescence Validation Plate	FlexStation 3, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax L, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e
0200-2420	Cuvette Absorbance Validation Set	SpectraMax ABS Plus, SpectraMax M2, SpectraMax M2e, SpectraMax M3, SpectraMax M4, SpectraMax M5, SpectraMax M5e, SpectraMax Plus 384
0200-7200	Multi-Mode Validation Plate	FilterMax F3, FilterMax F5, SpectraMax i3*, SpectraMax i3x*, SpectraMax iD5*, SpectraMax Paradigm * Specific read modes or cartridges.

* Discontinued. Use the SpectraTest ABS2 Absorbance Validation Plate.



The user guide for each instrument describes instrument maintenance.

Instrument Calibration

Use the Calibration dialog to have the instrument step 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 Operations tab contains a Calibration icon when the instrument supports the air calibration process.



Note: Air-calibration applies to absorbance reads. You do not need a plate or cuvette for instrument calibration. The cuvette chamber must be empty for a cuvette calibration.

The software calculates air-calibration values and stores these values in the firmware. The instrument uses the stored air-calibration values when you use Speed Read and when the Calibrate icon is disabled in the Settings dialog. See [Speed Read Settings on page 127](#) and [Calibrate on page 144](#).

1. Select the Operations tab and click **Calibration** to display the Calibration dialog.
2. Select **Plate** to calibrate for plates 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**.

Calibrating SpectraMax L Instruments

For the SpectraMax L instrument, do the following for calibration.

Calibrate the instrument once a year for maximum performance. Select the Operations tab and click Calibration to display the Calibration dialog. There are two calibration methods:

- Select **Internal Light Emitting Diodes** to calibrate using the instrument's onboard LEDs. This method does not require sample preparation.
- Select **Custom Multi-PMT Normalization Microplate** when the instrument has multiple PMTs and you want to use the light spectrum from your own assays to calibrate the instrument, rather than the light spectrum from the factory configured LEDs.

Calibrate with Internal Light Emitting Diodes

To calibrate the internal light emitting diodes:

1. Remove any plate from the plate drawer.
2. Select the Operations tab and click **Calibration** to display the Calibration dialog.
3. Select the **Internal Light Emitting Diodes** Calibration Method option.
4. Click **OK**. Calibration is done automatically.

Custom Multi-PMT Normalization Microplate

Create your own custom calibrations if you prefer to calibrate using the light spectrum from your assays rather than the light spectrum from the factory configured LEDs. Custom calibrations are used from the Settings dialog in the Sensitivity category. See [SpectraMax L Protocols on page 336](#).

There are two different PMT Calibration Factor options:

- Select **Auto Calculation** to use your own assay materials for calibration, but you want the software to automatically calculate a PMT calibration factor.
- Select **Manual Set** to use your own assay materials for calibration, and you want to manually calculate the PMT calibration factor based on the signal obtained from each PMT.

Auto Calculation

Use this option to use your own assay materials for calibration and have the software automatically calculate a PMT calibration factor.

1. Remove any plate from the plate drawer.
2. Select the Operations tab and click **Calibration** to display the Calibration dialog.
3. Select the **Custom Multi-PMT Normalization Microplate** Calibration Method option.
4. Click the **Custom Calibration #** drop-down and select the custom calibration to define. You can define two different custom calibrations.
5. Select the **Auto-Calculation** PMT Calibration Factor option.
6. In the **Dark Adapt Delay** field, enter the number of seconds to enable the sample to adapt to the dark interior of the plate drawer before the calibration starts. This allows the plate autophosphorescence to subside.
7. Click **OK**.
8. A message displays to tell you to insert a 96-well plate into the instrument with material of greater than 100k RLU in wells B2, G2, B11, and G11. After you insert the plate, click **OK**.

Manual Set

Use this option to use your own assay materials for calibration and manually calculate the PMT calibration factor based on the signal obtained from each PMT. You can read all or part of a plate that contains your assay material (should be greater than 100k RLU) to determine these values and then determine a factor, or multiplier, for each PMT that equalizes the RLU values for each PMT.

1. Select the Operations tab and click **Calibration** to display the Calibration dialog.
2. Select the **Custom Multi-PMT Normalization Microplate** Calibration Method option.
3. Click the **Custom Calibration #** drop-down and select the custom calibration to define. You can define two different custom calibrations.
4. Select the **Manual Set** PMT Calibration Factor option.
5. Enter the values for each available PMT from your calculations. Available fields are dependent the instrument configuration.
6. Click **OK**.

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 FilterMax F3, FilterMax F5, SpectraMax i3, SpectraMax i3x, SpectraMax iD3, SpectraMax iD5, SpectraMax Mini, and SpectraMax Paradigm. To update the PIC or instrument firmware, click the **Update** button. For more information about how to update firmware, contact Molecular Devices support.

FilterMax F3, FilterMax F5, and SpectraMax iD5

The software discovers the installed excitation and emission filter slides and displays their configurations in 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.

For the FilterMax F3 and FilterMax F5, click  to the left of the slide definition to eject or retract a filter slide. To edit the filters in a slide, or to define more slides, click **Edit Slide Configuration**. See [Configuring Filter Slides on page 318](#).

SpectraMax i3 and SpectraMax i3x

The software discovers the installed detection cartridges and displays them in 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.

When you use the StakMax Microplate Handling System, you can lock or unlock the detection cartridge drawer:

- When the microplate handler is installed, the detection cartridge drawer must be locked. To lock the cartridge drawer, click **Lock Drawers for StakMax**.
- When you need to open the detection cartridge drawer, remove the microplate handler from the detection platform and then unlock the drawer. To unlock the cartridge drawer, click **Unlock Drawers for StakMax**.
- Click **StakMax Alignment Wizard** to align the StakMax Microplate Handler.

For more information, see the *StakMax Microplate Handling System User Guide*.

SpectraMax Mini

The SpectraMax Mini ships with at least one filter cube and you can purchase additional filter cubes. You install a fluorescence filter cube to do fluorescence intensity reads or install the luminescence filter cube to do luminescence reads.

In the Instrument Information dialog, **Filter Cube** displays the filter cube that you install in the instrument.

SpectraMax Paradigm

The software discovers the installed detection cartridges and displays them in 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.

When you use the StakMax Microplate Handling System, you can lock or unlock the detection cartridge drawers:

- 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**.
- Click **StakMax Alignment Wizard** to align the StakMax Microplate Handler.

For more information, see the *StakMax Microplate Handling System User Guide*.

To lock all drawers closed, click **Lock Instrument for Shipment** and follow the on-screen instructions. To prepare an instrument for shipment, contact Molecular Devices support.

For information about how to unlock the drawers, see [Removing Software Locks on page 343](#).

To align the transfer position of the StakMax Microplate Handling System with the plate drawer of the SpectraMax Paradigm, click **StakMax Alignment Wizard**. For how to use the StakMax Alignment wizard, see the *StakMax Microplate Handling System User Guide*.



Appendix B: Supported Detection Cartridges

The SpectraMax i3x supports user-installable detection cartridges for top reads and the SpectraMax Paradigm requires detection cartridges to do reads. Each detection cartridge contains the light source, optics, and electrical components needed to do specific read modes for some applications.

The SoftMax Pro Software supports the following detection cartridges:

- [Absorbance Detection Cartridge](#), see page 375
- [AlphaScreen Detection Cartridges](#), see page 376
- [Fluorescence Intensity Detection Cartridges](#), see page 376
- [Fluorescence Polarization Detection Cartridges](#), see page 378
- [FRET Detection Cartridges](#), see page 378
- [Imaging - Transmitted Light Detection Cartridge](#), see page 379
- [Luminescence Detection Cartridges](#), see page 379
- [ScanLater Western Blot Detection Cartridge](#), see page 380
- [SpectraMax Injector Cartridge](#), see page 381
- [Time Resolved Fluorescence Detection Cartridges](#), see page 382
- [TR-FRET Detection Cartridge](#) on page 383

For detailed cartridge specifications, see the *SpectraMax i3x Multi-Mode Detection Platform User Guide* or the *SpectraMax Paradigm Multi-Mode Microplate Reader User Guide*.

Contact Molecular Devices to have custom detection cartridges designed to meet the specific needs of your application.

Absorbance Detection Cartridge

The Absorbance detection cartridge is for the SpectraMax Paradigm only and combines wavelength scanning and a broad spectrum wavelength range necessary to address nucleic acid, protein quantitation, ELISA, immunoassay, and proliferation/viability applications.

The cartridge uses a monochromator and has two components. You must install both components:

- The detection component (ABS-DET) measures the absorbance. Install this component in one slot in the top cartridge drawer.
- The excitation component (ABS-MONO) sets the absorbance wavelengths. Install this component in two slots in the bottom cartridge drawer.

The cartridge supports the following read types:

- [Endpoint Read Type](#), see page 277
- [Kinetic Read Type](#), see page 278
- [Well Scan Read Type](#), see page 279
- [Spectrum Read Type](#), see page 279

AlphaScreen Detection Cartridges

There are three AlphaScreen® Detection Cartridges that use a 680 nm laser diode to provide a sensitive read system for AlphaScreen assays. The design isolates each well and provides the optimal performance for AlphaScreen assays. These 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 cartridge occupies one slot in the cartridge drawer. For the SpectraMax Paradigm you can install it in either cartridge drawer (top for top reads or bottom for bottom reads) but it cannot occupy the rear-most slot. For best results, you should install the cartridge in the top drawer.

These cartridges support the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)
- [Well Scan Read Type, see page 279](#)

ALPHASCREEN and ALPHALISA are registered trademarks of PerkinElmer, Inc.

Fluorescence Intensity Detection Cartridges

The following Fluorescence Intensity (FI) detection cartridges use an ultra-high power LED for the excitation of various fluorescent labels for the fluorescence intensity read mode. The standard dual-emission design provides simple, straightforward fluorescence resonance energy transfer (FRET) measurement. These cartridges occupy one slot in the cartridge drawer.

- Fluorescence Intensity (FI) (coum-fluor) Detection Cartridge
- Fluorescence Intensity (FI) (fluor-rhod) Detection Cartridge (no longer available for purchase)
- Fluorescence Intensity (FI) (Cy3-Cy5) Detection Cartridge (no longer available for purchase)
- Fluorescence Intensity (FI) (Cfp-Yfp) Detection Cartridge

Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge

The Fluorescence Intensity (FI) GeneBLAzer detection cartridge is designed for use with GeneBLAzer assays from Invitrogen. The standard dual-emission design provides Fluorescence Resonance Energy Transfer (FRET) measurement. This cartridge occupies one slot in the cartridge drawer.

Multi-Mode (MULTI) Detection Cartridge

The Multi-Mode (MULTI) detection cartridge is for the SpectraMax Paradigm only and supports several read modes including fluorescence intensity. This cartridge is optimized for 96-well and 384-well plates. It occupies three slots in either cartridge drawer (top drawer for top reads and bottom drawer for bottom reads).

This cartridge also supports the following read modes:

- [Luminescence Read Mode, see page 301](#)
- [Time-Resolved Fluorescence Read Mode, see page 304](#)

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 cartridge is for the SpectraMax Paradigm only and occupies one slot in either cartridge drawer (top drawer for top reads and bottom drawer for bottom reads).

This cartridge is optimized for detection of the label set used in the MultiTox-Fluor Multiplex Cytotoxicity Assay from Promega:

- AFC (amino-fluorocoumarin)
- R110 (rhodamine 110)

The cartridges listed above support the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)
- [Well Scan Read Type, see page 279](#)

Tunable Wavelength (TUNE) Detection Cartridge

The Tunable Wavelength (TUNE) detection cartridge is for the SpectraMax Paradigm only and supports several read modes including fluorescence intensity. Spectral optimization helps you get the maximum signal-to-background ratio for most fluorophores or luminescence labels that are compatible with the wavelength ranges. This cartridge occupies three slots in either the top or bottom cartridge drawer (top drawer for top reads and bottom drawer for bottom reads). In addition to the endpoint, kinetic, and well scan read types, this cartridge also supports the [Spectrum Read Type on page 279](#).

This cartridge also supports the following read modes:

- [Luminescence Read Mode, see page 301](#)
- [Time-Resolved Fluorescence Read Mode, see page 304](#)

Fluorescence Polarization Detection Cartridges

There are two Fluorescence Polarization (FP) detection cartridges that support the fluorescence polarization read mode for specific labels, depending on the cartridge. For best results you should use solid black plates.

- Fluorescence Polarization (FP) (Fluorescein) Detection Cartridge
- Fluorescence Polarization (FP) (Rhodamine) Detection Cartridge

Each cartridge occupies one slot in the cartridge drawer. For the SpectraMax Paradigm you can install it in either cartridge drawer (top for top reads or bottom for bottom reads) but it cannot occupy the rear-most slot. For best results, you should install the cartridge in the top drawer.

These cartridges support the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)

FRET Detection Cartridges

The following detection cartridges use an ultra-high power LED for the excitation of various fluorescent labels. The standard dual-emission design provides simple, straightforward fluorescence resonance energy transfer (FRET) measurement. These cartridges occupy one slot in the cartridge drawer.

- 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

Fluorescence Intensity (FI) GeneBLAzer Detection Cartridge

The Fluorescence Intensity (FI) GeneBLAzer detection cartridge is designed for use with GeneBLAzer assays from Invitrogen. The standard dual-emission design provides Fluorescence Resonance Energy Transfer (FRET) measurement. This cartridge occupies one slot in the cartridge drawer.

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 cartridge is optimized for detection of the label set used in the MultiTox-Fluor Multiplex Cytotoxicity Assay from Promega:

- AFC (amino-fluorocoumarin)
- R110 (rhodamine 110)

This cartridge is for the SpectraMax Paradigm only and occupies one slot in either cartridge drawer (top for top reads and bottom for bottom reads).

These cartridges support the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)
- [Well Scan Read Type, see page 279](#)

Imaging - Transmitted Light Detection Cartridge

The Transmitted Light (TL) detection cartridge provides white LED illumination to do brightfield, transmitted-light imaging when you use the SpectraMax MiniMax 300 Imaging Cytometer. The StainFree Cell Detection Algorithm uses proprietary transmitted light analysis technology to eliminate cell staining for cell counting and confluency measurements.

This cartridge is for the SpectraMax i3x when instrument is installed with the SpectraMax MiniMax 300 Imaging Cytometer and cannot be used in the SpectraMax Paradigm.

The cartridge occupies one slot in the cartridge drawer and supports the Imaging read mode.

Luminescence Detection Cartridges

There are three Glow Luminescence (LUM) detection cartridges that support the luminescence read mode. For best results you should use solid white plates. The Glow Luminescence detection cartridges are individually optimized for a 1536-well, 384-well, or 96-well plate format:

- Glow Luminescence (LUM) detection cartridge
- Glow Luminescence (LUM) detection cartridge (384)
- Glow Luminescence (LUM) detection cartridge (96)

Each cartridge occupies one slot in the cartridge drawer. For the SpectraMax Paradigm you can install it in either cartridge drawer (top for top reads or bottom for bottom reads) but it cannot occupy the rear-most slot. For best results, you should install the cartridge in the top drawer.

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

The Dual Color Luminescence (LUM) (Chroma-Glo™) Detection Cartridge supports the luminescence read mode and is designed to provide the optimal performance when you use Promega Chroma-Glo™ reagents. This cartridge supports both luminescence and dual wavelength luminescence reads.

This cartridge occupies one slot in the cartridge drawer. For the SpectraMax Paradigm you can install it in either cartridge drawer (top for top reads or bottom for bottom reads) but it cannot occupy the rear-most slot. For best results, you should install the cartridge in the top drawer for LUM reads.

Chroma-Glo is a trademark of Promega Corporation.

Dual Color Luminescence (LUM) (BRET2) Detection Cartridge

The Dual Color Luminescence (LUM) (BRET2™) Detection Cartridge is designed to provide the optimal performance when you use Perkin Elmer BRET² reagents. This cartridge supports both luminescence and dual wavelength luminescence reads. For best results, you should use solid white plates.

This cartridge occupies one slot in the cartridge drawer. For the SpectraMax Paradigm you can install it in either cartridge drawer (top for top reads or bottom for bottom reads) but it cannot occupy the rear-most slot. For best results, you should install the cartridge in the top drawer for LUM reads.

BRET² is a trademark of PerkinElmer, Inc.

Multi-Mode (MULTI) Detection Cartridge

The Multi-Mode (MULTI) detection cartridge is for the SpectraMax Paradigm only and supports several read modes including luminescence. This cartridge optimized for 96-well and 384-well plates. It occupies three slots in the cartridge drawer and is .

This cartridge also supports the following read modes:

- [Fluorescence Intensity Read Mode, see page 287](#)
- [Time-Resolved Fluorescence Read Mode, see page 304](#)

These cartridges support the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)
- [Well Scan Read Type, see page 279](#)

Tunable Wavelength (TUNE) Detection Cartridge

The Tunable Wavelength (TUNE) detection cartridge is for the SpectraMax Paradigm only and supports several read modes including luminescence. Spectral optimization helps you get the maximum signal-to-background ratio for most fluorophores or luminescence labels that are compatible with the wavelength ranges. In addition to the endpoint, kinetic, and well scan read types, this cartridge also supports the [Spectrum Read Type on page 279](#).

This cartridge occupies three slots in the cartridge drawer and you should install it in the top cartridge drawer for glow luminescence reads.

This cartridge also supports the following read modes:

- [Fluorescence Intensity Read Mode, see page 287](#)
- [Time-Resolved Fluorescence Read Mode, see page 304](#)

ScanLater Western Blot Detection Cartridge

The ScanLater™ Western Blot (WB) Detection Cartridge supports the Time-Resolved Fluorescence read mode for Western Blot membranes.

The Molecular Devices ScanLater™ Western Blot Assay Kit is a novel system for protein analysis. 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 support.moleculardevices.com.

The cartridge occupies two slots in the cartridge drawer.

Western Blot reads use the time-resolved fluorescence read mode with a membrane read type. See [Membrane Read Type on page 278](#).

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

- **Read Empty Plates/Cuvettes** to start a read.
- **Create/Save Data Documents** to save the data document before the read.
- **Overwrite Plate/Cuvette Data** to do a high-resolution ROI read.

See [SoftMax Pro Software - GxP Edition on page 251](#).

SpectraMax Injector Cartridge

The SpectraMax® Injector Cartridge adds injector capability to the SpectraMax i3x. You can use the injectors and optics in this cartridge for luminescence reads from the top of the sample or use the injectors in the cartridge along with the instrument's built-in monochromator for fluorescence intensity reads from the bottom of the sample.

This cartridge is for the SpectraMax i3x only and cannot be used in the SpectraMax Paradigm. Install the SpectraMax Injector cartridge in the two rear-most slots (3 and 4) in the cartridge drawer.

When you install the SpectraMax Injector cartridge, you can create protocols that inject and read well by well to reduce signal loss. Injectors deliver a specified volume of a reagent to the wells of a plate. Use the injectors to deliver the reagent to initiate a reaction that occurs rapidly and results in a luminescent or fluorescent signal that the instrument must quickly detect.

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

The SpectraMax 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.

To define the settings for a read with injection, you must use the Acquisition View in the Settings dialog. See [Acquisition View and Reads With Injectors on page 156](#).

Time Resolved Fluorescence Detection Cartridges

Time Resolved Fluorescence (TRF-EUSA) Detection Cartridge

The Time Resolved Fluorescence (TRF-EUSA) detection cartridge supports the time-resolved fluorescence read mode for Europium and Samarium. It is ideal for protein interaction, GPCR, and enzyme activity applications. For best results you should use solid black plates.

This cartridge occupies one slot in the cartridge drawer. For the SpectraMax Paradigm you can install it in either cartridge drawer (top for top reads or bottom for bottom reads). For best results, you should install the cartridge in the top drawer.

These cartridge support the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)
- [Well Scan Read Type, see page 279](#) (Not available for dual-label assays.)

Multi-Mode (MULTI) Detection Cartridge

The Multi-Mode (MULTI) detection cartridge is for the SpectraMax Paradigm only and supports several read modes including Time Resolved Fluorescence. This cartridge is optimized for 96-well and 384-well plates. It occupies three slots in either cartridge drawer (top drawer for top reads and bottom drawer for bottom reads).

This cartridge also supports the following read modes:

- [Fluorescence Intensity Read Mode, see page 287](#)
- [Luminescence Read Mode, see page 301](#)

Tunable Wavelength (TUNE) Detection Cartridge

The Tunable Wavelength (TUNE) detection cartridge is for the SpectraMax Paradigm only and supports several read modes including Time-Resolved Fluorescence. This cartridge occupies three slots in either the top or bottom cartridge drawer (top drawer for top reads and bottom drawer for bottom reads). In addition to the endpoint, kinetic, and well scan read types, this cartridge also supports the [Spectrum Read Type on page 279](#).

This cartridge also supports the following read modes:

- [Fluorescence Intensity Read Mode, see page 287](#)
- [Luminescence Read Mode, see page 301](#)

TR-FRET Detection Cartridge

Cisbio HTRF Detection Cartridge

The Cisbio HTRF® Detection Cartridge uses a high-energy Xenon flash lamp for sensitive reads of HTRF reagents. The standard dual-emission design gives the most accurate results in short overall read time. This cartridge provides Time-Resolved Fluorescence read mode 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.

This is a dual-emission cartridge that occupies two slots in the cartridge drawer.

HTRF is a registered trademark of Cisbio Bioassays.

The cartridge supports the following read types:

- [Endpoint Read Type, see page 277](#)
- [Kinetic Read Type, see page 278](#)
- [Well Scan Read Type, see page 279](#)



Appendix C: Exporting File Details

Whether you save the export as a .txt or .xls, the information presents as tab-separated values. This topic describes the basic information that is included in a .txt or .xls file export format.

For a copy of the SoftMax Pro .xml schema, contact Molecular Devices.

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

- `##BLOCKS= n`
where *n* is the number of sections that are in the export file.

The software exports the sections you select to export in the sequence that the sections display in the document.

Each section displays the following:

- Header line
The header line displays the section type: Note, Plate, or Group, and the title of the section you define in the document. Plate sections header lines contain additional information.
- Data lines
The data lines follow the header line and contain the text or data from the section. The layout and format of the data lines depend on the section type, export type, read mode, and read type.
- End line
The end line of each section displays the text: `~End`

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

Plate Export Formats

The following describes the format of Plate sections you export to a .txt or .xls file. For a copy of the SoftMax Pro .xml schema, contact Molecular Devices.

- 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 [Plate Header Fields on page 386](#).
- 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 393](#).

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.

Absorbance 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 document
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains Columns output format or 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 (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
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
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

Absorbance Read Mode (continued)

Position	Name	Description
19.	Number of wells	Contains the total number of wells in the plate, 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

Fluorescence Intensity and AlphaScreen 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 document
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains Columns output format or 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 read from the bottom of the plate and FALSE if read 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
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 plate, such as 96 or 384

Fluorescence Intensity and AlphaScreen Read Mode (continued)

Position	Name	Description
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 read
27.	PMT gain	Contains Automatic, High, Medium, Low, or Manual 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

Luminescence, Time-Resolved Fluorescence, and Fluorescence Polarization 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 document
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains Columns output format or 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

Luminescence, Time-Resolved Fluorescence, and Fluorescence Polarization Read Mode (continued)

Position	Name	Description
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
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 plate, 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 read

**Luminescence, Time-Resolved Fluorescence, and Fluorescence Polarization Read Mode
(continued)**

Position	Name	Description
26.	PMT gain	Contains Automatic, High, Medium, Low, or Manual 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
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

Imaging 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 document
3.	Export version	Contains the version of the export application used for this export
4.	Export format	Contains Time Format for Columns output format or Plate Format 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
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 plate, 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	...
0:01:58	##	1.564	3.344	2.378	...	2.744	0.113	3.872	...
...	##
Raw Data									
0:00:00	##	3.928	3.645	1.956	...	3.622	2.195	0.519	...
0:01:58	##	2.891	3.824	2.782	...	3.203	1.105	0.281	...
...	##
Reduced Data									
		A1	A2	A3	...	B1	B2	B3	...
		203.45455	-18.91525	-28.13251	...		106.53005	82.585516	...

Kinetic Data Exported in Column Format

For plate formats, the data displays in a table to indicate the columns and rows of the plate. 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				wavelength 2			
		1	2	3	...	1	2	3	...
0:00:00	Temperature	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	...
	
		1	2	3	...	1	2	3	...
		203.45455	-18.91525	-28.13251	...	106.53905	82.585516	121.96918	...
		74.597843	107.62096	-88.65948	...	376	-77.66718	-83.94453	...
		-134.6718	68.810478	-52.5208	...	246.51772	60.674884	48.51772	...
	

Kinetic Data Exported in Plate Format

Group Section Export Formats

The following is the format of Group sections that you export to a .txt or .xls file. For a copy of the SoftMax Pro .xml schema, contact Molecular Devices.

The header line contains the type of section, such as Group, and the title of the section as defined in the document.

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

The following describes the format of Note sections that you export to a .txt or .xls file. For a copy of the SoftMax Pro .xml schema, contact Molecular Devices.

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.



Appendix D: 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. Select a category from the Category list to filter the list of curve fits.

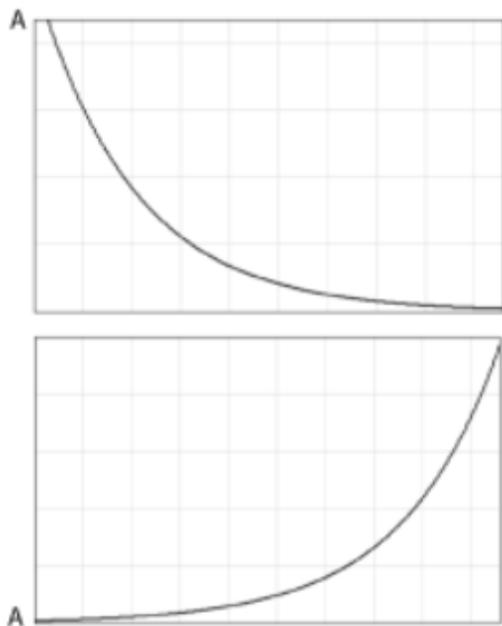
No Fit

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 397](#)
- [Exponential Decay on page 398](#)



$$y = A \times e^{Bx}$$

y = Concentration

x = Time

A = Y_0

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.

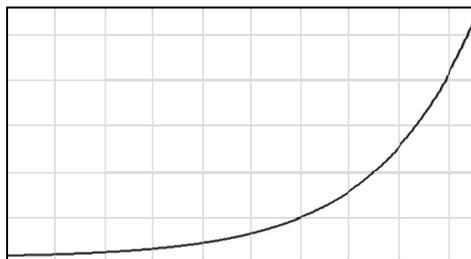
Mathematically, a 2-parameter exponential curve fit is a special case of a 3-parameter exponential curve fit. See [3-Parameter Exponential on page 399](#).

For the best results, you should 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:

- [2-Parameter Exponential on page 396](#)



$$y = A \times e^{Bx}$$

y = Concentration

x = Time

A = Y_0

B = k

For exponential growth, $B > 0$

You can calculate doubling time with the following equation:

$$\text{Doubling time} = B \times \ln(2) = B \times 0.693$$

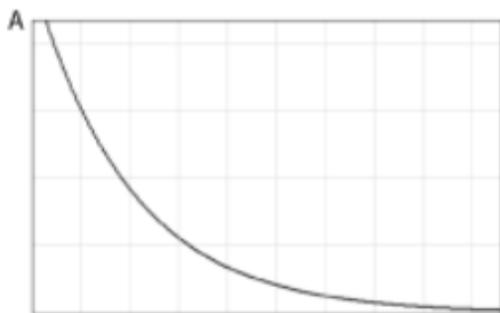
Mathematically, this is a special case of a 3-parameter exponential curve fit. See [3-Parameter Exponential on page 399](#).

For the best results, you should 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 396](#)



$$y = A \times e^{Bx}$$

y = Concentration

x = Time

A = Y_0

B = k

For exponential decay, $B < 0$

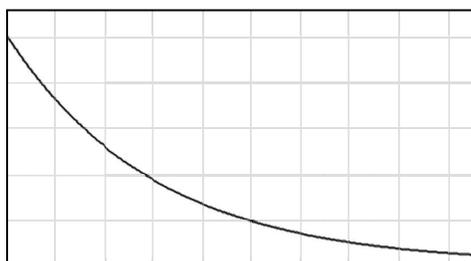
Mathematically, this is a special case of a 3-parameter exponential curve fit. See [3-Parameter Exponential on page 399](#).

For the best results, you should 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 software)
- 3P Exponential
- [One-Phase Exponential Decay, see page 400](#)
- [One-Phase Exponential Association, see page 401](#)



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

x = Time (usually)

y = Specific + Nonspecific Binding or Response



Note: In previous versions of the software, it was possible to select a linearized version of this fit. This option is not available in SoftMax Pro Software version 6.

One-Phase Exponential Decay

The **One-Phase Exponential Decay** curve fit is also known as:

- [3-Parameter Exponential](#), see page 399
- 3P Exponential



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

x = Time (usually)

y = Specific + Nonspecific Binding or Response

A = $Y_0 = Y_{\max} = \text{Plateau} + \text{Span}$

B = $-\text{Span}$

C = $1/k = 1/\text{decay constant}$

The software expresses C as the reciprocal of k, so it has same units as X.

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 401](#).

For the best results, you should 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 399
- 3P Exponential



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

x = Time (usually)

y = Specific + Nonspecific Binding or Response

$A = Y_0 = Y_{\min}$

$B = \text{Span} = Y_{\max} - Y_0$

$C = 1/k = 1/\text{association constant}$

A one-phase exponential association curve fit is commonly used for pseudo-first order kinetics of ligand/receptor interaction or substrate/enzyme interaction.

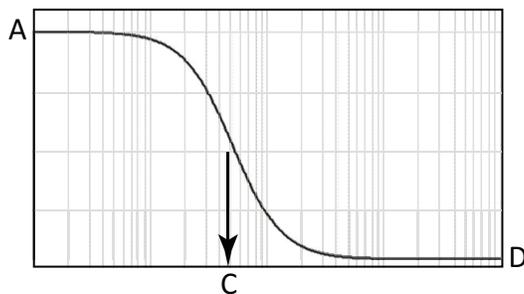
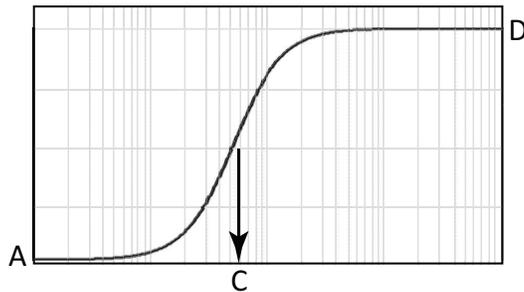
The curve is the same as one-phase exponential decay, except B is positive instead of negative. See [One-Phase Exponential Decay on page 400](#).

For the best results, you should 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 Logistic

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 = EC₅₀/IC₅₀

D = Second Asymptote



Note: In the 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. 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 EC₅₀/IC₅₀ 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 EC₅₀ 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 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 404.

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

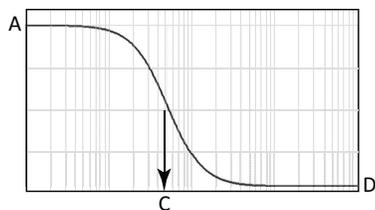
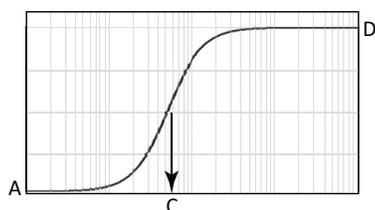
3-Parameter Dose-Response Curves are special cases of a 4-Parameter Logistic equation with one parameter fixed. See [4-Parameter Logistic on page 402](#).

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)



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

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.

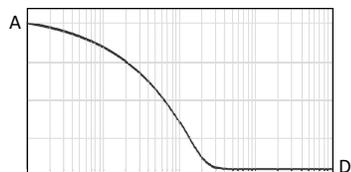
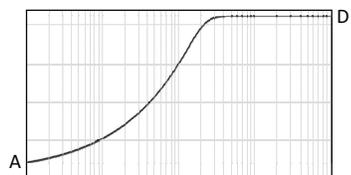
For the best results, you should exceed the minimum number of standards required for a curve fit. The minimum number of standards required mathematically for a 4-parameter curve fit algorithm is 4.

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 Logistic** 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 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. 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 EC₅₀/IC₅₀ 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. The EC₅₀ cannot be estimated.

Unlike the 4-parameter logistic equation, C is not the IC₅₀ value. The IC₅₀ value needs to be calculated separately. See the *SoftMax Pro Data Acquisition and Analysis Software Formula Reference Guide*.

For the best results, you should 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.

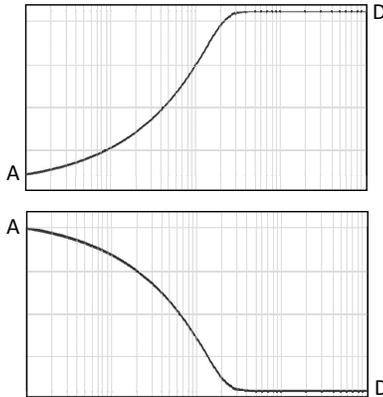
The minimization method for the 5-parameter equation is based on the Levenberg-Marquardt Method. See *The Art of Scientific Computing* by William H. Press, Brian P. Flannery, Saul A Teukolski, and William T. Vetterling, published by Cambridge University Press, New York, 1988.

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 EC₅₀ value is given by C.

A **5-Parameter Logistic Alternate** equation is also known as:

- Five-Parameter Alternate Logistic Equation
- 5P Alternate



$$y = D + (A - D) \left\{ 1 + \left(2^{\frac{1}{G}} - 1 \right) \left(\frac{X}{C} \right)^{\frac{1}{2G} \left(1 - 2^{\frac{1}{G}} \right)} \right\}^{-G}$$

A = First Asymptote

B = Slope Factor

C = EC₅₀/IC₅₀

D = Second Asymptote

G = Asymmetry Factor

In the 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. A and D can swap between top and bottom according to the shape of the curve, whether rising or falling with increasing concentration. If either A or D is 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 EC₅₀/IC₅₀ 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 EC₅₀/IC₅₀ value.

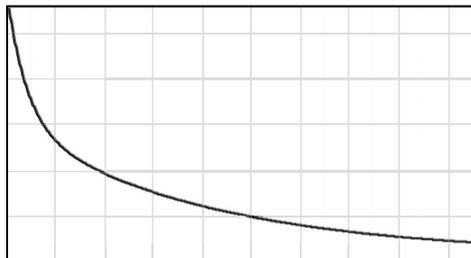
A 5-parameter logistic alternate equation assumes an asymmetric sigmoidal data set with clear lower and upper asymptotes. If either asymptote is not clearly defined or is missing (as in a marginal data set), it is nearly impossible to estimate where the asymptote lies, or if exists at all. Likewise the EC₅₀ cannot be estimated.

For the best results, you should 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:

- [Two-Phase Exponential Decay](#), see page 408
- 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 of the sum of a fast and a slow exponential decay.

For the best results, you should 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:

- [Bi-Exponential, see page 407](#)
- 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.

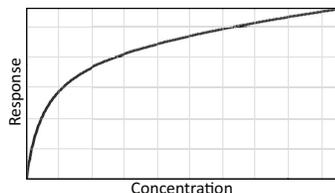
The two-phase exponential decay curve fit is commonly used when the decay is the result of the sum of a fast and a slow exponential decay.

For the best results, you should 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:

- [Two-Site Equilibrium Binding \(specific binding\)](#), see page 409
- [Michaelis-Menten with Two Isozymes](#), see page 410



$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$

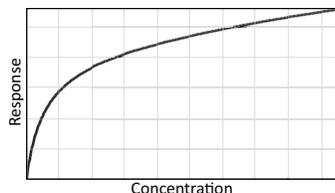
A bi-rectangular curve fit is commonly used for estimation of maximum (specific) binding and K_d values in the presence of two binding sites: high-affinity and low-affinity. It is also used for evaluation of enzyme activity, as function of $[S]$ when two isozymes are present.

For the best results, you should 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 409](#)



$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$

y = Specific binding

A = Maximum binding (high affinity)

B = K_d (high)

C = Maximum binding (low affinity)

D = K_d (low)

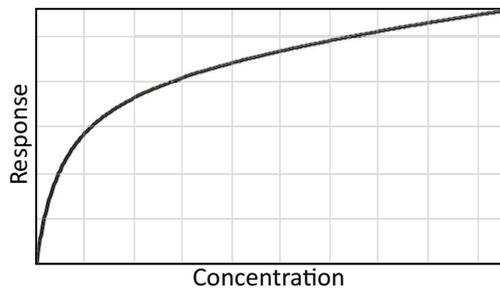
A two-site equilibrium binding (specific binding) curve fit is commonly used for estimation of maximum (specific) binding and K_d values in the presence of two binding sites: high-affinity and low-affinity.

For the best results, you should 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 409](#)



$$y = \frac{Ax}{x + B} + \frac{Cx}{x + D}$$

$$Y = V_i$$

$$A = V_{i\max} (1)$$

$$B = K_m (1)$$

$$C = V_{i\max} (2)$$

$$D = K_m (2)$$

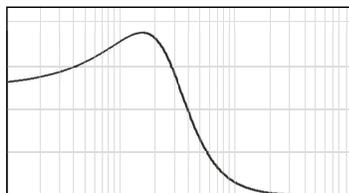
A Michaelis-Menten with two isozymes curve fit is commonly used for evaluation of enzyme activity, as function of [S] when two isozymes are present.

For the best results, you should 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 = EC₅₀/IC₅₀

D = Second Asymptote

G = Hormesis Factor

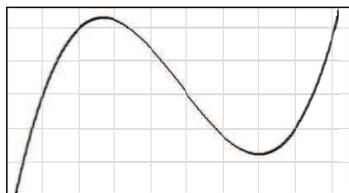
A brain cousens curve fit is used for studies of test systems that show hormesis (opposite responses at low and high doses). An example is when an inhibitor shows an enhanced response at low doses and an inhibitory response at high doses.

For the best results, you should 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



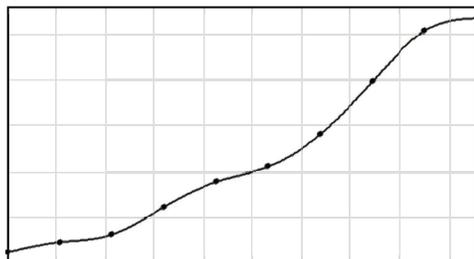
$$y = A + Bx + Cx^2 + Dx^3$$

A cubic curve fit can be used for standard curves, but there is risk of multiple inflection points, and ambiguity in InterpX results.

For the best results, you should 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

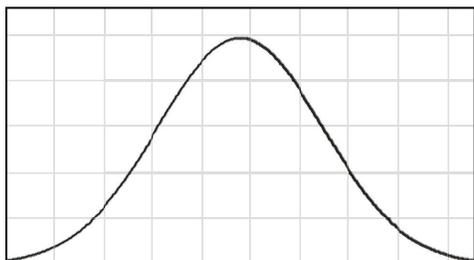
A **Cubic Spline** curve fit is a series of cubic segments joining adjacent pairs in a set of points such that the first and second derivatives match at the joints. There is no simple formula for the entire function.



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

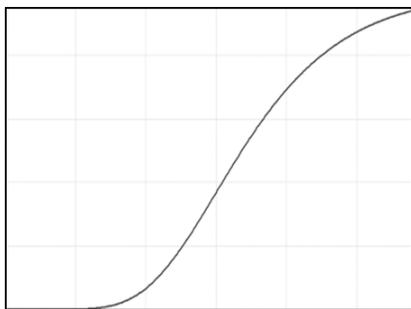
D = Baseline

Peak width at half-height = $\text{ParmC}(\text{Plot@Graph}) * 2 * \text{Sqrt}(\text{Ln}(2))$

For the best results, you should 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.

Gompertz

A **Gompertz** function is typically used to model cellular growth kinetics.



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

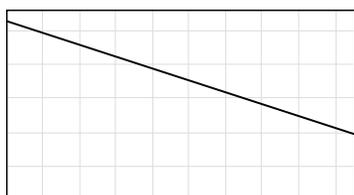
A = Upper Asymptote

B = Maximum Specific Growth Rate * e

C = Lag Time (intercept on x-axis of tangent through the inflection point)

Linear

A **Linear** curve fit is also known as: Polynomial First Order



$$y = A + Bx$$

A = Intercept

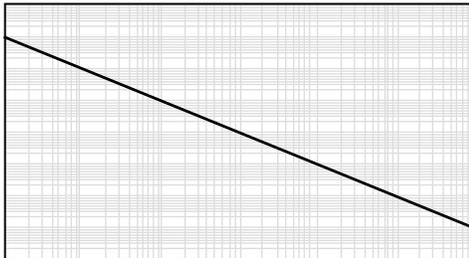
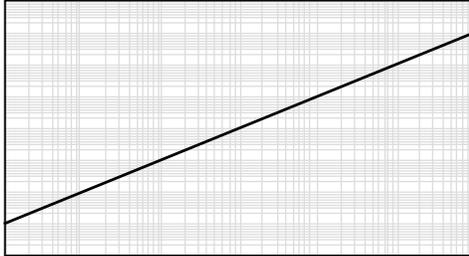
B = Slope

Use a linear fit when the values seem to lie on or are scattered around a straight line.

For the best results, you should 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 reads on the Y-axis (the response) and the logarithm of the dose on the X-axis.



$$\text{Log}(y) = A + B \times \text{Log}(x)$$

A = Intercept

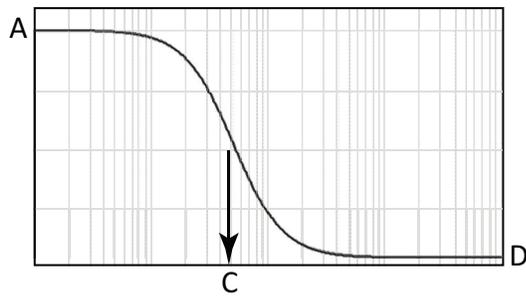
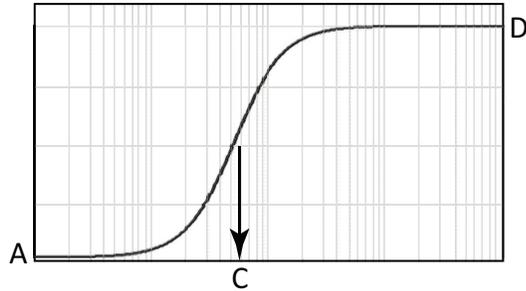
B = Slope

Log in this equation is the common or base-10 logarithm.

For the best results, you should 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 Logistic](#) on page 402.

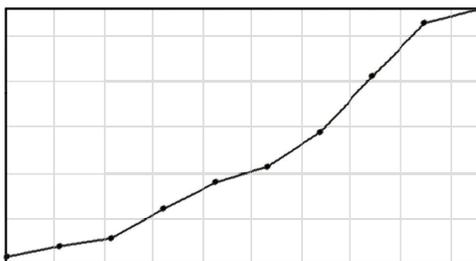


$$y = D + \frac{A - D}{1 + \left(\frac{x}{C}\right)^B}$$

- A = First Asymptote
- B = Slope Factor/Hill Coefficient
- C = EC₅₀/IC₅₀
- D = Second Asymptote

Point-to-Point

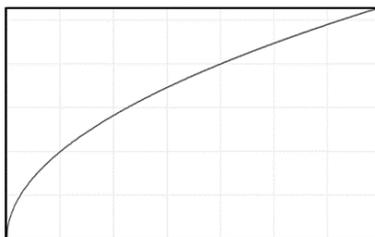
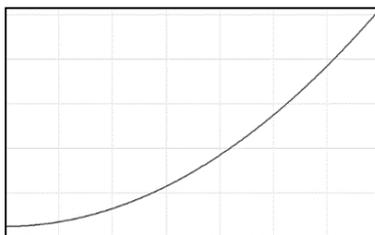
The **Point-to-Point** curve fit uses a linear equation to fit each pair of data points.



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.

Power

The **Power** curve fit is a three-parameter power function.



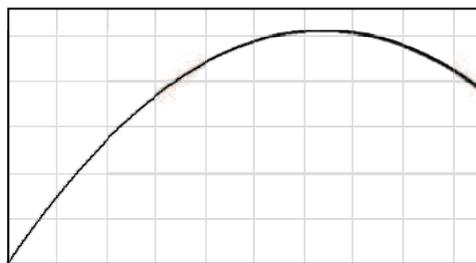
$$y = A + Bx^C$$

A = Intercept (C > 0)

The upper and lower curves are for C > 1 and 0 < C < 1 respectively.

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

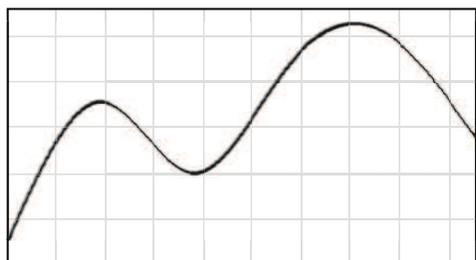
C = a measure of the curvature of the parabola

The quadratic fit is useful for standard curves.

For the best results, you should 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



$$y = A + Bx + Cx^2 + Dx^3 + Gx^4$$

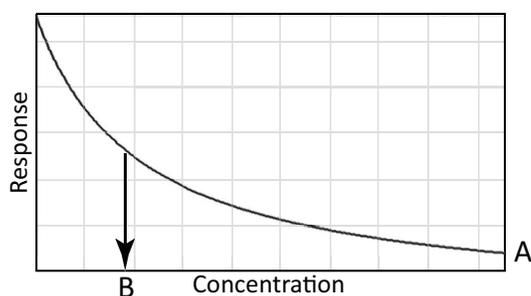
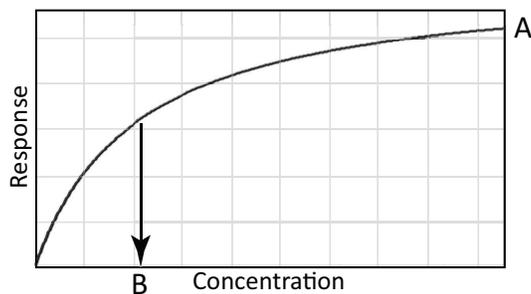
A quartic curve fit can be used for standard curves, but there is risk of multiple inflection points, and so ambiguity in InterpX results.

For the best results, you should 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:

- [Michaelis-Menten](#), see page 419
- [One-Site Equilibrium Binding \(specific binding\)](#), see page 419



$$Y = \frac{Ax}{x + B}$$

A rectangular hyperbola curve fit is commonly used estimation of V_i max, K_m , maximum binding, and K_d .

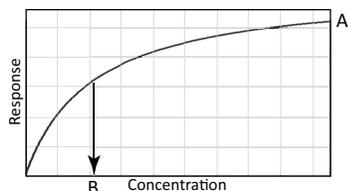
A rectangular hyperbola curve fit is a special case of the 4-parameter curve fit where $B = 1$, $D = 0$, and x is $1/\text{concentration}$.

For the best results, you should 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 418](#)



$$y = \frac{Ax}{x + B}$$

$$y = V_i$$

$$A = V_{i\max}$$

$$B = K_m$$

A Michaelis-Menten curve fit is commonly used estimation of $V_{i\max}$ and K_m .

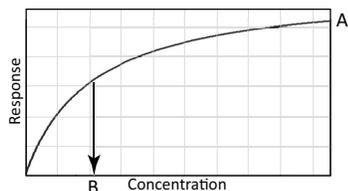
A Michaelis-Menten curve fit is a special case of the 4-parameter curve fit where $B = 1$, $D = 0$, and x is $1/\text{concentration}$.

For the best results, you should 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 418](#)



$$y = \frac{Ax}{x + B}$$

$$y = \text{Specific Binding}$$

$$A = \text{Maximum Binding}$$

$$B = K_d$$

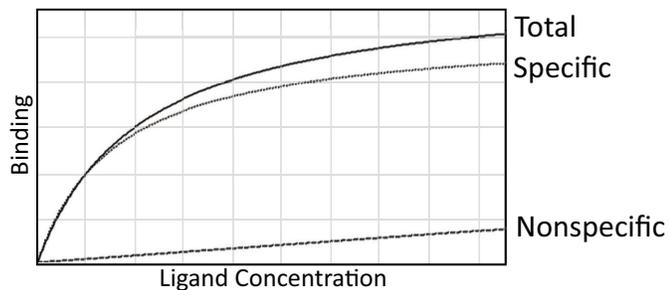
A one-site equilibrium binding (specific binding) curve fit is commonly used estimation of maximum binding and K_d .

For the best results, you should 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 421



$$y = \frac{Ax}{x + B} + Cx$$

y = Total binding

A = Specific Binding B_{max}

B = Kd (specific binding)

C = nonspecific binding coefficient

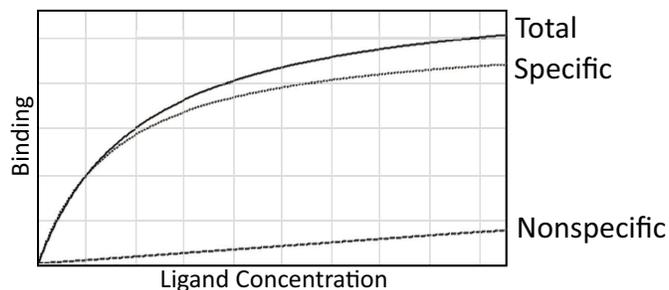
A rectangular hyperbola plus linear curve fit is commonly used for estimation of maximum binding and Kd (of specific binding) in the presence of nonspecific binding.

For the best results, you should 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 420](#)



$$y = \frac{Ax}{x + B} + Cx$$

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 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 .



$$y = A + B \times \text{Log}(x)$$

A = y-intercept at $x=1$

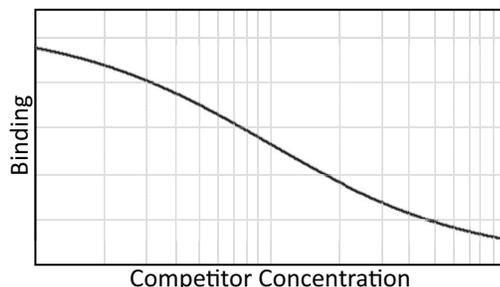
B = Slope

Log in this equation is the common or base-10 logarithm.

For the best results, you should 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.



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

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 404](#).

For the best results, you should 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.

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