Multi-Mode Analysis Software

SpectraMax® Paradigm® Multi-Mode Detection Platform
FilterMax™ Multi-Mode Microplate Readers

User Guide

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September 2010

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Safety

Introduction

This section provides safety information and instructions for the hardware and accessories of the system. It includes the following topics:

- Safety Terminology on page 3
- Chemical and Biological Safety on page 5
- Electrical Safety on page 5
- Moving Parts on page 6
- Cleaning on page 6
- Disposal and Recycling on page 6
- Maintenance on page 7
- Warnings and Cautions Found in this User Guide on page 7

Safety Terminology

The symbols displayed below and on the instrument should remind you to read and understand all safety instructions before attempting installation, operation, maintenance, or repair to this instrument.

WARNING! Paragraphs marked by "WARNING" alert you of a potential hazard to your personal safety if you do not adhere to the information stated within the paragraph.

CAUTION! Paragraphs marked by "CAUTION" indicate that there is a potential danger of equipment damage.

CAUTION! Paragraphs marked by "CAUTION" contain information about a possible software program failure, draw attention to a specific software setting or point out that a loss of data may occur if information stated within the paragraph is not adhered to or if procedures are executed incorrectly.

Note: Paragraphs marked by "Note" contain supplemental or explanatory information concerning the current topic or procedural step.

The symbols displayed below and on the instrument are reminders that all safety instructions should be read and understood before installation, operation, maintenance, or repair to this instrument is attempted.

WARNING! When present, this symbol indicates that a potential hazard to your personal safety exists if information stated within the "WARNING" paragraph is not adhered to or procedures are executed incorrectly.

WARNING! This icon accompanies text and/or other symbols dealing with potential damage to equipment. When present, it indicates that there is a potential danger of equipment damage, software program failure, or that a loss of data may occur if information stated within the "CAUTION" paragraph is not adhered to or procedures are executed incorrectly.

WARNING! HIGH VOLTAGE Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a high voltage source.

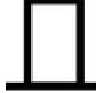
WARNING! BIOHAZARD Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a biological source.

WARNING! LASER LIGHT Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from a laser source.

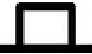
WARNING! SHARP OBJECTS Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from unblunted corners or other appendages on the outside or inside of the equipment.

WARNING! HOT SURFACE Paragraphs marked by this symbol indicate that a potential hazard to your personal safety exists from heated surfaces or other appendages on the outside or inside of the equipment.

WARNING! PROTECTIVE EARTH OR GROUND TERMINAL This symbol identifies the location of the protective earth or ground terminal lug on the equipment.



OFF POSITION OF PRINCIPAL POWER SWITCH This symbol graphically represents the equipment main power push-button switch when it is in the off position.



ON POSITION OF PRINCIPAL POWER SWITCH This symbol graphically represents the equipment main power push-button switch when it is in the on position.

Chemical and Biological Safety

Normal operation of the FilterMax 3 and FilterMax 5 Multi-Mode Microplate Readers and the SpectraMax Paradigm Multi-Mode Detection Platform may involve the use of materials that are toxic, flammable, or otherwise biologically harmful. When using such materials, observe the following precautions:

- Handle infectious samples according to good laboratory procedures and methods to prevent the spread of disease.
- Observe all cautionary information printed on the original solutions containers prior to their use.
- Dispose of all waste solutions according to your facility's waste disposal procedures.
- Operate the FilterMax 3 and FilterMax 5 Multi-Mode Microplate Reader, and the SpectraMax Paradigm Multi-Mode Detection Platform in accordance with the instructions outlined in this user guide, and take all the necessary precautions when using pathological, toxic, or radioactive materials.
- Splashing of liquids may occur; therefore, take appropriate safety precautions, such as using safety glasses and wearing protective clothing, when working with potentially hazardous liquids.
- Use an appropriately contained environment when using hazardous materials.
- Observe the appropriate cautionary procedures as defined by your safety officer when using flammable solvents in or near a powered-up instrument.
- Observe the appropriate cautionary procedures as defined by your safety officer when using toxic, pathological, or radioactive materials.

Note: Observe all warnings and cautions listed for any external devices attached or used during operation of the FilterMax 3 and FilterMax 5 Multi-Mode Microplate Readers and the SpectraMax Paradigm Multi-Mode Detection Platform. Refer to applicable external device user guides for operating procedures of that device.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact a Molecular Devices Service Engineer for any servicing of equipment requiring the removal of covers or panels.

To reduce risk of electrical shock, all devices employ a three-wire electrical cable and plug to connect the equipment to earth ground.

- Ensure that the wall outlet receptacle is properly wired and earth grounded.
- DO NOT use a three-to-two wire plug adapter.
- DO NOT use a two-wire extension cord or a two-wire multiple-outlet power strip.
- Disconnect power to the system before performing maintenance.
- DO NOT remove any panels; panels should be removed only by qualified service personnel.

WARNING! This symbol indicates the potential of an electrical shock hazard existing from a high voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) wall outlets. Use only multiplug power strips provided by the manufacturer.

Moving Parts

To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the Multi-Mode Analysis Software.
- Keep the Multi-Mode Analysis Software work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user guide for the Multi-Mode Analysis Software. Prior to cleaning equipment that has been exposed to hazardous material:

- Appropriate Chemical and Biological Safety personnel should be contacted.
- The Chemical and Biological Safety information contained in this user guide should be reviewed.

Disposal and Recycling

WARNING! It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that the device:

- Was put on the European Market after August 13, 2005.
- Is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Molecular Devices office for the proper decontamination information and take-back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.

Maintenance

Perform only the maintenance described in this user guide. Maintenance other than that specified in this user guide should be performed only by service engineers.

WARNING! It is your responsibility to decontaminate components of the Multi-Mode Analysis Software before requesting service by a Molecular Devices Service Engineer or returning parts to Molecular Devices for repair. Molecular Devices will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are retuned, they must be enclosed in a sealed bag stating that the contents are safe to handle and are not contaminated.

Warnings and Cautions Found in this User Guide

Please read and observe all cautions and instructions. Remember, the most important key to safety is to operate the FilterMax 3 and FilterMax 5 Multi-Mode Microplate Reader and the SpectraMax Paradigm Multi-Mode Detection Platform with care.

The WARNINGs and CAUTIONs found within this document are listed below.

WARNING! If the equipment is used in a manner not specified by Molecular Devices, the protection provided by the equipment may be impaired.

CAUTION! Settings that vary from the recommended Power Options Properties may introduce a risk of data transfer interruption and a loss of data.

CAUTION! In any situation (such as when operating the instrument with integrated systems) where automatic loading and ejection of the cartridge carrier may cause a potential equipment collision, we recommend disabling the Automatically load/eject cartridge carrier when running the Validation Plate feature, and to load and eject the cartridge carrier manually.

CAUTION! Shake low density plates, such as 6-well or 48-well plates, at low speed only. Shaking low density plates at higher speeds may cause liquid in wells to spill.

CAUTION! The plate height configured must not be less than that of the actual plate. Doing so may cause the FilterMax Multi-Mode Microplate Readers to collide with the plate during a Read Height Optimization. The SpectraMax Paradigm Multi-Mode Detection Platform has an auto-detection to prevent collision, if an incorrect plate height is entered for the SpectraMax Paradigm Multi-Mode Detection Platform an error message appears while running protocols using the defined labware.

CAUTION! Luminescence light levels visible to the human eye may cause damage to the detection system.

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Overview

1

Introduction

Multi-Mode Analysis Software configures and controls all measurement protocols and actions performed by the FilterMax Multi-Mode Microplate Readers and the SpectraMax Paradigm Multi-Mode Detection Platform. The software supports detection for absorbance, glow luminescence, fluorescence intensity, fluorescence polarization, and time-resolved fluorescence (TRF) measurements including HTRF. The measurement methods available to users depend on the capabilities of the instrument being controlled. Measurement results can be viewed in the Multi-Mode Analysis Software or easily exported to compatible applications such as Microsoft Excel.

Optional modules such as GxP Permissions lend support for electronic signature regulations such as 21 CFR Part 11.

This user guide covers the functionality supplied by the Multi-Mode Analysis Software and the Multi-Mode Analysis Software with GxP Permissions modules, including:

- Configuring and Controlling Instruments on page 51
- Setting Up and Using GxP Permissions on page 73
- Creating and Editing Detection Methods on page 87
- Creating and Editing Labware on page 127
- Creating and Running Protocols on page 145
- Viewing Measurement Results on page 215

Where to Begin

To correctly use the software it is important that initial configuration is done in a specific order.

1. Installing Multi-Mode Analysis Software on page 17.
2. Configuring Multi-Mode Analysis Software on page 32.
3. Configuring and Controlling Instruments on page 51.
4. Setting Up and Using GxP Permissions on page 73 (optional module).
5. Creating Detection Methods (FilterMax Multi-Mode Microplate Readers) on page 88 or Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform) on page 99. The measurement configuration parameters are stored in detection methods.
6. Creating and Editing Labware on page 127. Labware types must be configured and ready for use in protocols.
7. Creating Protocols on page 146 using the detection methods created in Step 5. A protocol stores all parameters required to perform a measurement, including technique types, detection methods, labware types, and preparation methods, such as shaking.
8. Running Protocols on page 197.
9. Viewing Measurement Results on page 215.
10. When required, create additional detection methods, labware, and protocols.

Installing, Using, and Configuring the Software

2

Overview

This section introduces users to the software and gives the instructions for:

- Installing Multi-Mode Analysis Software on page 17
- Using Multi-Mode Analysis Software on page 24
- Configuring Multi-Mode Analysis Software on page 32
- Deleting and Restoring Items on page 48

Installing Multi-Mode Analysis Software

The Multi-Mode Analysis Software installer provides the ability to install the software onto a new system or update from a previous version of the software.

Installing the software requires:

- Preparing to Install Multi-Mode Analysis Software on page 17
- Meeting System Requirements on page 18
- Installing Multi-Mode Analysis Software on Windows XP on page 19

Preparing to Install Multi-Mode Analysis Software

Before installing Multi-Mode Analysis Software, confirm that the host computer meets the minimum system requirements listed in Meeting System Requirements on page 18.

If upgrading from previous versions of Multi-Mode Analysis Software, see Upgrading From Previous Versions of the Software on page 19 before installing. The information in this section helps to ensure the update is successful.

Meeting System Requirements

To install and use the software successfully, the host computer must meet the minimum system requirements listed in Table 2-1. Where relevant, Table 2-1 also lists recommended specifications.

Table 2-1 Host Computer System Requirements

Component	Minimum System Requirements
CPU	Pentium III 600 Mhz
RAM	256 MB minimum 512 MB or more recommended
Hard Drive	600 MB free space
CD-ROM Drive	4X
Monitor	800x600 resolution
Keyboard	101 key
Mouse	IBM compatible
Serial Port USB Port	1 free serial port or 1 free USB port
Operating System	Microsoft Windows XP (Service Pack 2)
Operating System Language	English (U.S.)
Database	Microsoft SQL Server 2000 (Desktop Edition included on the installation CD) **Note:** SQL Server 2000 Desktop Edition has a 2GB storage limit. Contact Molecular Devices Technical Support if this limit is reached.
Web Browser	Microsoft Internet Explorer 6.0 or later (included on the installation CD)
Programs	Microsoft Excel 2003 recommended
Power Options Properties	Turn off hard disks: Never System standby: Never System hibernates: Never **Note:** System power options properties are set in **Control Panel > Power Options**. **CAUTION!** Settings that vary from the recommended Power Options Properties can introduce a risk of data transfer interruption and a loss of data.

Upgrading From Previous Versions of the Software

If upgrading from a previous Multi-Mode Analysis Software version, follow the steps in this section to make sure that the update is successful.

To update the software from a previous version:

1. Launch Multi-Mode Analysis Software and open Instrument Settings.
2. For FilterMax instruments: If the filter slides have been customized by installing new filters or moving existing filters to other slots, these settings need to be saved and later imported after software installation. In Instrument Settings, select the Filter Slides tab and select Export Slides before proceeding with installation. See Exporting and Importing All Filter Slide Configurations on page 61.
3. Install the software by following the steps in Installing Multi-Mode Analysis Software on page 17.

Note: Installing a newer version of Multi-Mode Analysis Software replaces the previous version.

4. When the installation is complete, launch Multi-Mode Analysis Software. The software automatically checks if any default detection methods, labware types, and protocols provided with the install have the same name as those imported to the database from the previous versions.

Note: If a prompt appears asking whether a detection method, labware type, or protocol should be overwritten, click **Yes** to overwrite the existing item or click **No** to keep the existing item in the database.

Note: SpectraMax Paradigm Multi-Mode Detection Platform: For each new detected cartridge, example Protocols and Methods are imported automatically. The automatic importing function may be disabled. To do this, go to the Multi-Mode Analysis Software main window. Then from the **File** menu click **Settings > Properties > Allow Protocol Auto-Import**.

Installing Multi-Mode Analysis Software on Windows XP

On a Windows XP system, the Multi-Mode Analysis Software installer uses a simple interface to guide the installation using the two CDs provided with your purchase. Installation proceeds in two phases:

- Installing Required Components and Multi-Mode Analysis Software on page 20
- Installing the Required Multi-Mode System Updater on page 22

Installing Required Components and Multi-Mode Analysis Software

To install the components and software:

1. Exit all Windows programs before starting installation.
2. Ensure the current user account has Administrator privileges. Accounts with Standard or Restricted access are not allowed to run the setup program. Contact the site system administrator for more information about account privileges.
3. Insert the installation CD 1 into the CD drive and browse to the contents of the CD.
4. Double-click on **Installer.exe**. The Multi-Mode Analysis Software Installer appears Figure 2-1. All components required to successfully install the software are listed along with the current status of each component:
   - A check icon indicates the correct version of the component is already installed on the system.
   - A caution icon indicates that an older version of the component is installed and must be updated before the software can be installed.
   - An X icon indicates that the component must be installed before the Multi-Mode Analysis Software can be installed.

Figure 2-1 Starting the Installation

5. Select **Update** or **Install** for the first component indicated. The components are installed one at a time and must be installed in the order listed.
6. Follow the steps in the component installer until the component installation is complete. Restart the system as required. For example, a restart might required after the Microsoft SQL Server installation.

Note: If a component installation requires restarting the system, restart before installing the next component listed. After the system restarts, browse to the contents of the installer CD and launch the installer again to continue installing components.

Note: Some components can give the option to Repair or Remove the component. First click Remove to remove the component and then click Install to reinstall the component.

7. Repeat Step 5 and Step 6 for each component required.

   When all components are installed correctly, the **Install Multi-Mode Software** button launches the Multi-Mode Analysis Software System Updater.
8. Click **Install Multi-Mode Software** to launch the Multi-Mode System Updater.

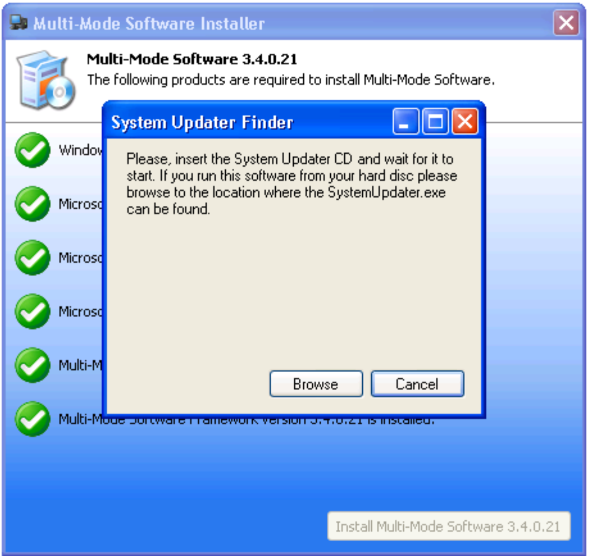


Figure 2-2 System Updater Window

9. Follow the steps as described in Installing the Required Multi-Mode System Updater on page 22.
10. In the Multi-Mode Analysis Software Installer, click **Finish**.

CAUTION! Settings that vary from the recommended Power Options Properties may introduce a risk of data transfer interruption and a loss of data.

11. Open the Power Options Properties by selecting **Start > Control Panel > Power Options**.
12. Set **Turn off Hard Disks** to **Never**.
13. Set **System standby** to **Never**.
14. Set **System hibernates** to **Never**.

The software is ready for use.

Installing the Required Multi-Mode System Updater

Upon installing, reinstalling, or updating Multi-Mode Analysis Software from a previous version, running the System Updater will provide vital updates to firmware, detection cartridge files and other components specific to your instrument's needs.

Note: If new detection platform products are purchased (such as SpectraMax Paradigm detection cartridges), or you intend to update your Multi-Mode Analysis Software installation, it is recommended that you use the Multi-Mode System Updater CD Package to consistently update your system.

Note: SpectraMax Paradigm Multi-Mode Detection Platform: Ensure the instrument is turned on, not in the standby mode, and all your detection cartridges are installed.

To run the System Updater:

1. If installing the Multi-Mode System Updater as a part of the Multi-Mode Analysis Software installation, remove installation CD 1 from the CD drive and insert CD 2 into the CD drive.

   If installing the System Updater separately, exit all Windows programs before starting system update as shown in Installing the Required Multi-Mode System Updater on page 22. Insert CD 2 into the CD drive and browse to the contents of the CD. Double click **installer.exe**

   The System Updater window appears Figure2.3.

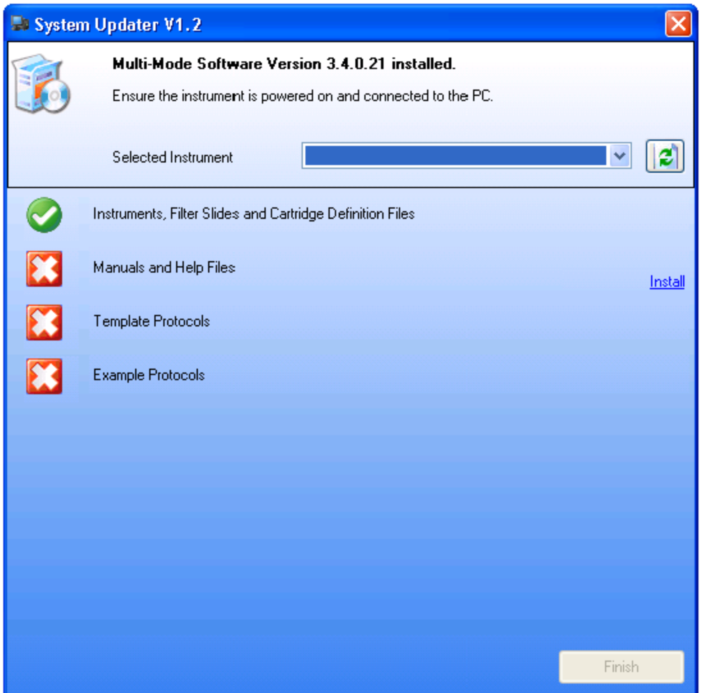


Figure 2-3 System Updater Window

Note: Depending on the host system's firewall configuration, a Windows firewall message might request that you unblock Multi-Mode System Updater's executable file, **SystemUpdater.exe**. If this message does show, allow unblocking.

2. Click the **Install** or **Update** links to add components as required by the System Updater.

Note: During the install/update process, the instrument might initialize and produce sounds while the System Updater runs.

3. When finished, the System updater window appears Figure 2-4.

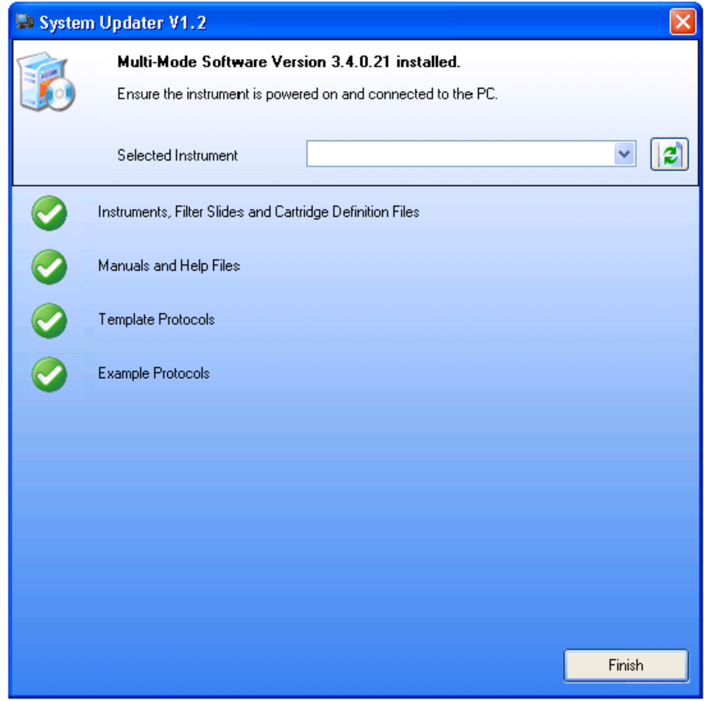


Figure 2-4 System Updater Window Showing Fully Updated Controller PC System

4. Upon completion of the entire update process, click **Finish**. The Multi-Mode Software Installer window appears.
5. Click **Finish**.

Note: If the System Updater ran as a part of the Multi-Mode Analysis Software installation, click **Finish** to close that installation also.

The software is ready for use.

Repairing or Removing the Multi-Mode Analysis Software Installation

In the event required components are missing or damaged, or if the software does not open or does not run correctly, repair should be made by uninstalling the Multi-Mode Analysis Software from the controlling PC system.

To repair or remove the software:

1. Exit all open Windows programs.
2. Make sure the current user account has Administrator privileges. Accounts with Standard or Restricted access are not permitted to modify or remove software. Contact the site system administrator for more information about account privileges.
3. If repairing the installation, insert the Multi-Mode Analysis Software installation CD into the CD-ROM drive.
4. From the Start menu, click **Settings > Control Panel**. The **Control Panel** appears.
5. In **Control Panel,** double-click **Add or Remove Programs**. The Windows **Add or Remove Programs** dialog appears.
6. In the **Add or Remove Programs** dialog, select **Multi-Mode Analysis Software**. Repair and removal options for Multi-Mode Analysis Software appear.
7. Click **Remove** and follow the instructions until the removal process is completed. Proceed to step 8.

Note: When removing the software, only files installed during the initial installation are removed. The software database and files created after the installation, such as exported measurement results, remain.

8. When finished, reinstall Multi-Mode Analysis Software from the original installation CD.

Using Multi-Mode Analysis Software

The Multi-Mode Analysis Software uses a simple interface that divides the main window into four basic sections: navigation pane, tool bar, selection and configuration pane, and preview pane (Figure2.5). The interface provides access to the selection lists that enables system functionality and comprehensive, context-sensitive online help.

This section covers:

- Launching the Software on page 25
- Using the Software Interface on page 26
- Accessing Online Help on page 31

Note: To correctly use the software it is important that the initial configuration is done in a particular order. Please see Where to Begin regarding important setup information.

Launching the Software

To launch Multi-Mode Analysis Software:

- From the Windows Start menu, click **Programs > Molecular Devices > Multi-Mode Analysis Software> Multi-Mode Analysis Software**. The Multi-Mode Analysis Software window appears (Figure 2-5).

Note: If the Multi-Mode Analysis Software is not found in the Start menu, the software may have been installed for a single user account on the system instead of all accounts. Check with the site system administrator or login to the user account with permission to access the software. See Installing Multi-Mode Analysis Software on Windows XP on page 19 for more information about installing the software for a single or multiple user accounts.

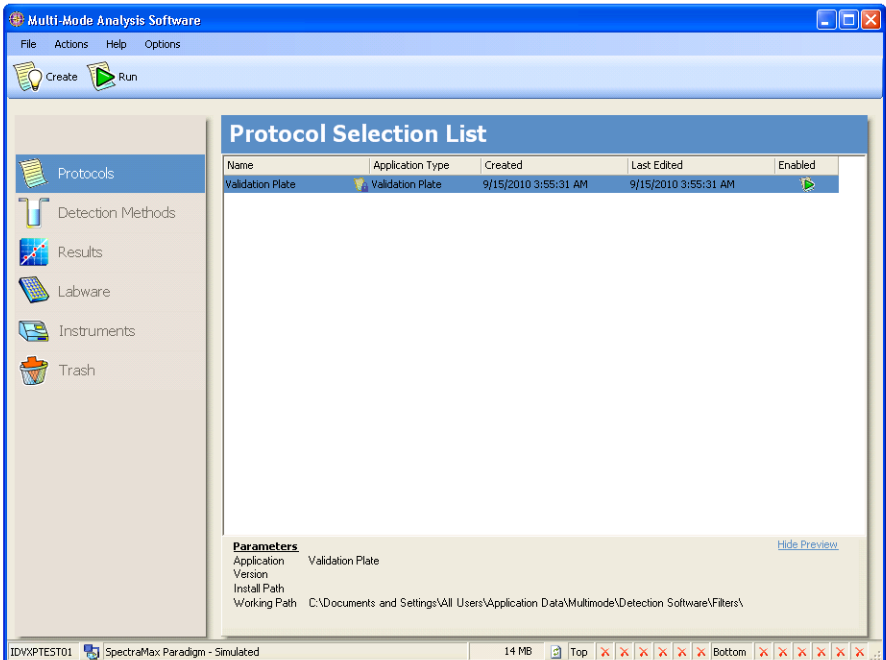


Figure 2-5 Multi-Mode Analysis Software Main Window

Note: The first time the software is launched, depending on the host system's firewall configuration, a Windows firewall message might request that you unblock Multi-Mode Analysis Software's executable file, Apex.exe. If this message does show, allow unblocking.

Using the Software Interface

Multi-Mode Analysis Software uses a simple interface that is divided into four basic sections:

- **Navigation Pane** (See About the Navigation Pane on page 26)
- **Tool Bar** (See About the Tool Bar on page 27)
- **Selection and Configuration Pane** (See About the Selection and Configuration Pane on page 31)
- **Preview Pane** (See About the Preview Pane on page 31)

The navigation pane provides access to the selection lists that provide the majority of the functionality built into the software. Items selected in the selection list determine the options available in the tool bar and configuration pane.

About the Navigation Pane

The navigation pane is the narrow pane on the left of the Multi-Mode Analysis Software window (Figure 2-5). Use the navigation pane to switch between selection lists.

Table 2-2 The Navigation Pane

Name	Button	Description
Protocols	Protocols	Contains the Protocols Selection List and provides the ability to define, run, edit, copy, delete, and print measurement protocols. See Creating and Running Protocols. A protocol stores all parameters required to perform a measurement, including technique types, detection methods, labware types, and preparation methods such as shaking.
Detection Methods	Detection Methods	Contains the Detection Method Selection List and provides the ability to create, edit, copy, and delete detection methods. See Creating and Editing Detection Methods. Measurement configuration parameters are stored in detection methods.
Results	Results	Contains the Results Selection List and provides the ability to view saved measurement results and modify data reduction parameters in the Result Viewer. Measurement results may be reevaluated using parameters different from those configured in the original protocol. See Viewing Measurement Results. Measurement results from each protocol run are stored in the Multi-Mode Analysis Software database and are accessed only from the Results Selection List.
Labware	Labware	Contains the Labware Selection List and provides the ability to create, edit, optimize, copy, and delete labware types. See Creating and Editing Labware.

Table 2-2 The Navigation Pane (cont'd)

Name	Button	Description
Instruments	Instruments	Contains the Instrument Selection List and provides the ability to manually control instrument actions (such as shaking, or loading and ejecting the plate carrier) and configure instrument settings and filter slides or detection cartridges. See Configuring and Controlling Instruments. The active instrument is controlled using the Instrument Selection List.
Users	Users	Appears in the navigation pane only when the Multi-Mode Analysis Software with GxP Permissions module is installed and enabled on the system. GxP Permissions is an integrated set of features that help Multi-Mode Analysis Software users comply with electronic signature regulations, such as 21 CFR Part 11. See Performing GxP Permissions User Actions in Multi-Mode Analysis Software on page 80.
Trash	Trash	Contains the Trash List containing labware, detection methods, and protocols pending deletion. This section provides the ability to restore or permanently delete items from the database. See Deleting and Restoring Items on page 48.

About the Tool Bar

The tool bar provides easy access to common software actions. The module chosen in the navigation pane determines which actions are available on the tool bar; for example, Optimize Labware is only available when the Labware module is active. A description of each tool bar is included for each view:

- Protocols Selection List Tool Bar. See Table 2-3.
- Detection Method Selection List Tool Bar. See Table 2-4.
- Results Selection List Tool Bar. See Table 2-5.
- Labware Selection List Tool Bar. See Table 2-6.
- Instrument Selection List Tool Bar. See Table 2-7.
- Users Tool Bar. See Table 2-8.
- Trash Tool Bar. See Table 2-9.

The Protocols Selection List tool bar provides access for creating, editing, and running protocols. A protocol stores all parameters required to perform a measurement, including technique types, detection methods, labware types, and preparation methods, such as shaking.

Table 2-3 Protocols Selection List Tool Bar

Button	Description
Create	Creates a new protocol, see Creating Protocols on page 146.
Run	Runs the currently selected protocol, see Running Protocols on page 197.
Edit	Edits the currently selected protocol, see Editing Protocols on page 211.
Copy	Copies the currently selected protocol, see Copying Protocols on page 212.
Print	Prints the configuration information for the currently selected protocol, see Printing Protocol Configuration Information on page 213.
Delete	Deletes the currently selected protocol, see Deleting Protocols on page 212.

The Detection Method Selection List tool bar provides access for creating and editing detection methods. Measurement configuration parameters are stored in detection methods.

Table 2-4 Detection Method Selection List Tool Bar

Button	Description
Create	Creates a new detection method, see Creating Detection Methods (FilterMax Multi-Mode Microplate Readers) on page 88 or Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform) on page 99.
Edit	Edits the currently selected detection method, see Editing Detection Methods on page 123.
Copy	Copies the currently selected detection method, see Copying Detection Methods on page 124.
Delete	Deletes the currently selected detection method, see Deleting Detection Methods on page 124.

The Results Selection List tool bar provides access to viewing, printing, and deleting results. Measurement results from each protocol run are stored in the Multi-Mode Analysis Software database and are accessed only from the Results Selection List.

Table 2-5 Results Selection List Tool Bar

Button	Description
Delete All	Deletes all displayed results, see Deleting Measurement Results on page 239.
Delete	Deletes currently selected result, see Deleting Measurement Results on page 239.
View	Views the currently selected result, see Viewing Measurement Results in the Result Viewer on page 217.
Print	Prints the currently selected result, see Printing Measurement Results on page 239

The Labware Selection List tool bar provides access to creating, copying, optimizing, and deleting labware.

Table 2-6 Labware Selection List Tool Bar

Button	Description
Create	Creates a new labware type, see Creating Labware on page 128.
Edit	Edits the currently selected labware type, see Editing Labware on page 133.
Copy	Copies the currently selected labware type, see Copying Labware on page 136.
Optimize	Optimizes the currently selected labware type, see Optimizing Labware on page 137.
Delete	Deletes the currently selected labware type, see Deleting Labware on page 136.

The Instrument Selection List tool bar provides access to adding, deleting, configuring, managing, and connecting to an instrument.

Table 2-7 Instrument Selection List Tool Bar

Button	Description
Add	Adds a new instrument to the instrument selection list, see Adding a New Instrument on page 52.
Delete	Deletes the currently selected instrument, see Deleting an Instrument on page 53.
Set Current	Sets the currently selected instrument to the current instrument, see Configuring the Current Instrument on page 54.
Settings	Configures instrument settings, see Configuring the FilterMax Multi-Mode Microplate Readers Instrument Settings on page 57 or Configuring SpectraMax Paradigm Multi-Mode Detection Platform System Information Settings on page 65.
Eject	Ejects the currently selected instrument's plate carrier, see Connecting to the Instrument on page 55.
Load	Loads the currently selected instrument's plate carrier, see Loading the Plate Carrier on page 55.
Init	Initialize the currently selected instrument, see Initializing the Instrument on page 56.
Connect	Connects to the currently selected instrument, see Enabling Simulation Mode on page 56.

The Users tool bar provides access to logging out the current user, changing the password, viewing the audit log, and reactivating disabled message boxes.

Table 2-8 Users Tool Bar

Button	Description
Logout	Logs out the current user, see Logging On and Off the System on page 81.
Change password	Changes the password of the user currently logged in, see Changing the Current User Password on page 82.
View audit entries	Views the audit log for the Multi-Mode Analysis Software, see Viewing and Searching the Multi-Mode Analysis Software Audit Log on page 83.
Reactivate disabled message boxes	Reactivates disabled message boxes for the current user, see Reactivating Disabled Message Boxes on page 83.

The Trash tool bar provides access for permanently removing and restoring items pending deletion from the database.

Table 2-9 Trash Tool Bar

Button	Description
Restore Item	Restores currently selected item, see Deleting and Restoring Items on page 48.
Restore All Items	Restores all items in trash, see Deleting and Restoring Items on page 48.
Delete	Permanently removes the selected item from the database, see Deleting and Restoring Items on page 48.
Delete All	Permanently removes all items in trash from the database, see Deleting and Restoring Items on page 48.

About the Selection and Configuration Pane

The selection and configuration pane is the large pane to the right of the navigation pane. Options available in this pane change depending on which module is currently selected in the navigation pane. For example, when Protocols is selected, the Protocol Selection List is displayed, which provides access to configured protocols and functionality specific to the Protocols module.

About the Preview Pane

The preview pane appears below the selection and configuration pane. It contains additional information about the selected object in the Protocols, Detection Methods, Labware, or Instruments selection lists. For example, when an instrument is selected in the Instrument Selection List, the parameters of the instrument appear in the preview pane. To hide the preview pane click **Hide Preview**. To display the preview pane click **Show Preview**.

Accessing Online Help

The Multi-Mode Analysis Software contains detailed online help that covers defining and editing labware, detection methods, and protocols, performing measurements, and exporting measurement results. The online help is context sensitive, which provides instant access to help for the active screen.

To access online help:

- Press **F1** at any time to display online help for the active screen.
- From the Help menu, select **Help > Contents** to display the table of contents.

Configuring Multi-Mode Analysis Software

After installing the software, physically connecting the instrument to a serial port on the host computer and turning the instrument on, instrument and software settings must be configured.

Configuration activities include:

- Configuring Instrument Settings on page 33
- Configuring Software Settings on page 35

To set up Multi-Mode Analysis Software:

1. From the Windows Start menu, select **Programs > Molecular Devices > Multi-Mode Analysis Software > Multi-Mode Analysis Software**. The Multi-Mode Analysis Software appears (Figure 2-6).

Note: If Multi-Mode Analysis Software is not found from the Windows **Start** menu, the software may have been installed for a single user account on the system instead of for all accounts. Check with the site system administrator or log in to the user account with permission to access the software. See Using Multi-Mode Analysis Software on page 24 for more information about installing the software for a single or multiple user accounts.

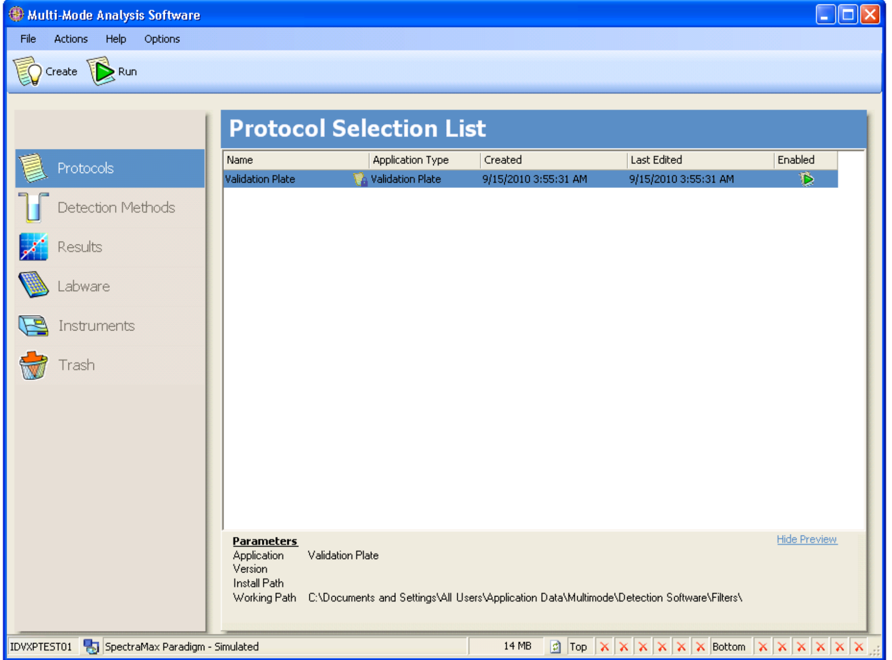


Figure 2-6 Multi-Mode Analysis Software Main Screen

2. If the Protocol Selection List (Figure 2-6) appears immediately, the instrument was automatically detected by the software. Proceed to Configuring Software Settings on page 35, to configure system settings.

   OR

   If a warning dialog appears (Figure 2-7), the instrument was not detected by the software. Click **OK** to work in simulation mode.

Note: Upon establishing a physical connection from the controlling PC, in many cases Multi-Mode Analysis Software may automatically detect and initialize the instrument.

Note: If the instrument is not detected check to see that the instrument is turned on, the instrument is connected to the controlling PC on which the software is installed, and that the instrument LED is not flashing. After turning on or plugging in the instrument click **Connect** on the Instrument Selection List toolbar. If the instrument does not connect automatically contact your local Molecular Devices Field Service Representative.

Figure 2-7 Warning Dialog Instrument Not Connected

Note: Upon software start up, the software will check if the instrument is locked, or is detected as a new instrument. If the software detects the instrument in either state, the unlock wizard will start up. Proceed to unlock the instrument before continuing.

Configuring Instrument Settings

Before an instrument is connected, a simulated instrument appears in the Instrument Selection List (Figure 2-8). When an actual instrument is detected by the software and has successfully connected, the simulated instrument is replaced in the list by the connected instrument.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Instrument Settings permission may configure instrument settings. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To configure instrument settings:

1. From the navigation pane, select **Instruments**. The Instrument Selection List appears (Figure 2-8).

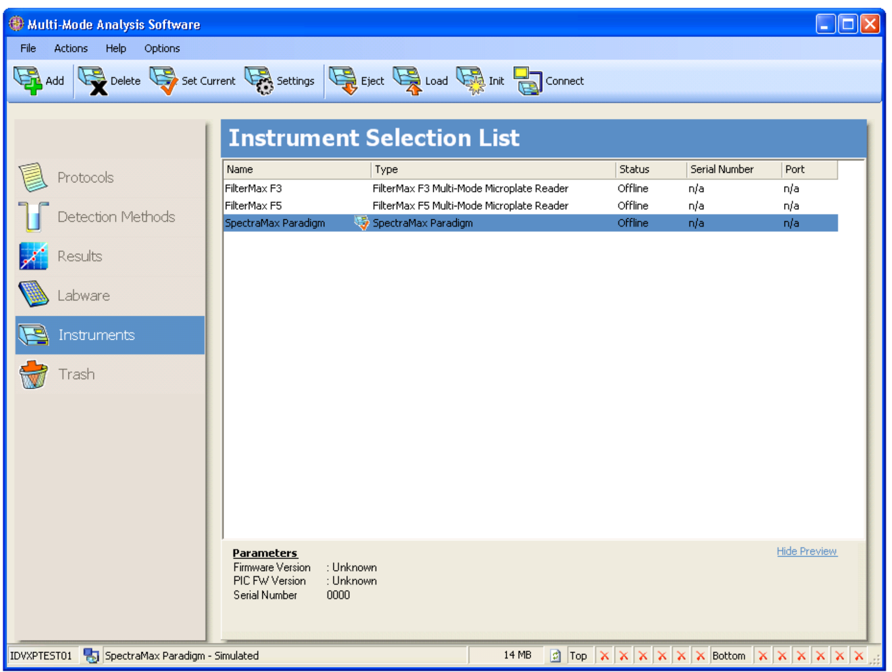


Figure 2-8 Instrument Selection List with Simulated Instrument Selected

2. From the tool bar, select **Settings**.

   OR

   From the menu bar select **Actions > Instrument Settings**.

3. The Instrument Settings dialog appears (Figure 2-9).

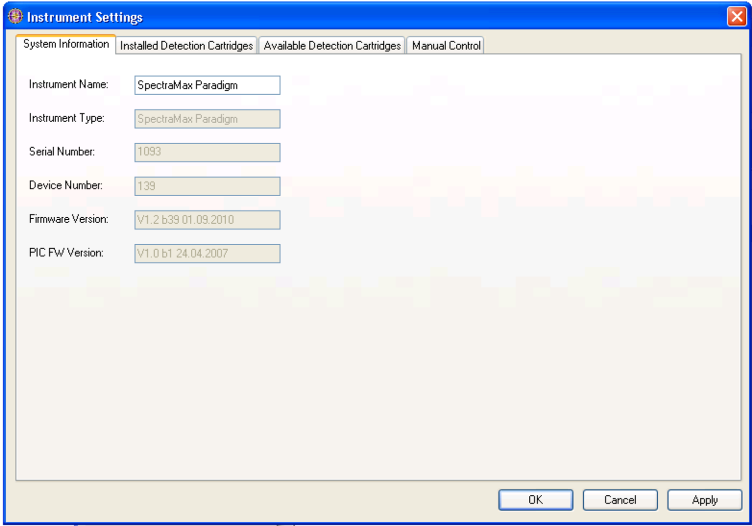


Figure 2-9 SpectraMax Paradigm Multi-Mode Detection Platform Instrument Settings

4. Select the **System Information** tab, if necessary.
5. In **Instrument Name**, modify the instrument's name if desired.
6. Click **OK** to close the Instrument Settings dialog.

Configuring Software Settings

The Multi-Mode Analysis Software can be customized using the options available in Software Settings (Figure 2-10). Use the menu in Software Settings to configure print options, default simulated data files, and the directory where measurement results are stored.

To configure Software Settings:

1. Select **File > Settings**. The Software Settings window appears (Figure 2-10).

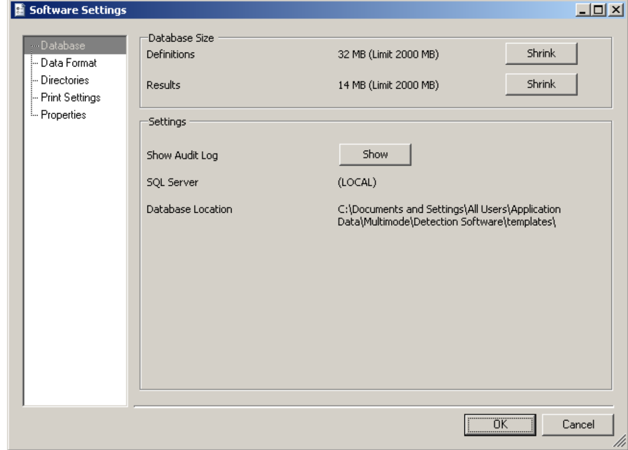


Figure 2-10 Software Settings Window

2. Configure the settings using the menus:
   - Selecting Simulated Data Files
   - Selecting a Directory for Saving Exported Measurement Results
   - Configuring Print Settings
   - Configuring Properties
   - Configuring the Data Format
   - Configuring Database Settings

Selecting Simulated Data Files

Protocols may be run in simulation mode, which allows the protocol configuration to be tested using simulated data before performing the protocol on actual samples. In simulation mode, all features for the instrument type currently selected in the Instrument Selection List are available, but measurement results are either randomly generated by the software or read from a data file.

Use the Directories menu option to select the default data files for simulated absorbance, luminescence, and fluorescence measurements (Figure 2-11).

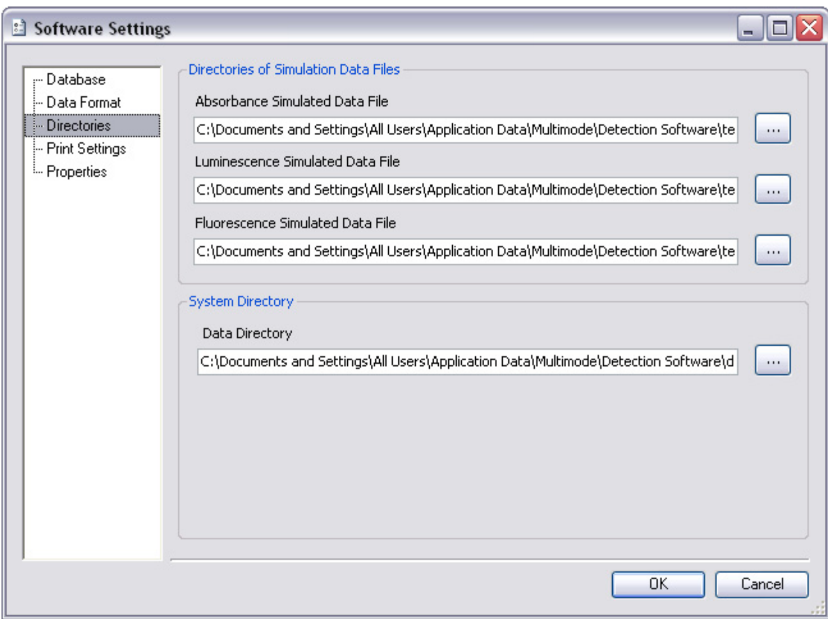


Figure 2-11 Selecting the Simulated Data Files

To select different simulated data files:

1. Select the **Directories** menu. The Simulated Data window appears (Figure 2-11).
2. In the desired field, enter the full path to the new simulated data file.

   For example: **c:\detection software templates\DefaultSimulatedData.dat**

   Any data file with a .dat extension may be selected, including prior measurement results. Proceed to step 3.

   OR

   Click the browse (...) button next to the desired measurement type. The Open dialog appears.

Note: Simulated data files are used when the number of measurement points in the simulated protocol run is the same as those present in the data file. When the number of measurement points is different, the software generates random data.

3. In the Open dialog, browse to and select the desired data file. Any data file with a .dat extension may be selected, including prior measurement results.
4. Click the **Open** button to select the data file and return to the Software Settings tab.
5. Repeat steps 2 through 4 for each simulated data file, as desired.
6. Click **OK** to set the new default data files.

Selecting a Directory for Saving Exported Measurement Results

Exported measurement results files, regardless of format, are saved into a single directory. The default storage directory is:

C:\Documents and Settings\All Users\Application Data\Multi-Mode\Detection Software\data

Use the **Directories** menu (Figure 2-12) to change the storage directory, as desired.

Note: All measurement results are also stored in the Multi-Mode Analysis Software database and may be accessed using the Result Viewer. See Viewing Measurement Results in the Result Viewer on page 217.

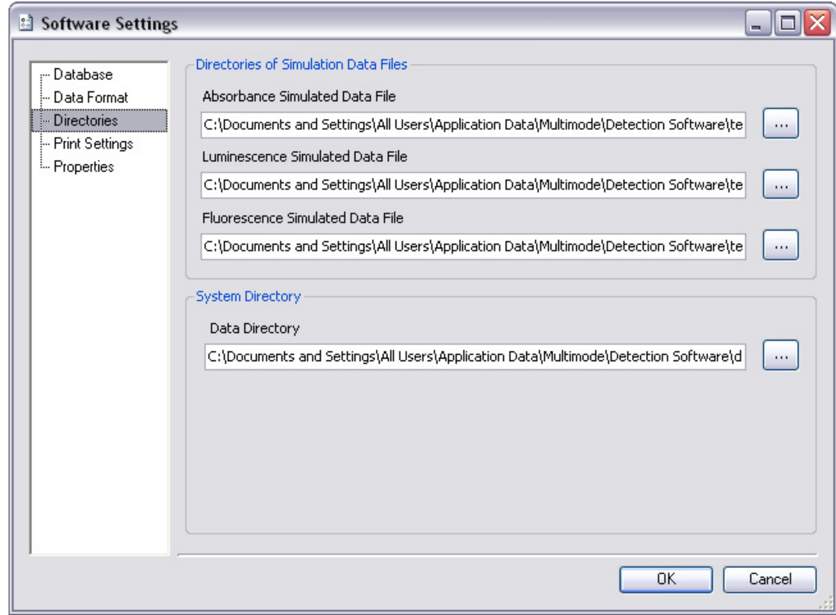


Figure 2-12 Directories Window for Data Storage Locations

To select a different storage directory:

1. Select the **Directories** menu. The directories window appears (Figure 2-12).
2. In the **Data Directory** path field, enter the complete path of the desired storage directory;
3. for example: **C:\documents\Multi-Mode measurement results\MyResults**

   OR

   Click the browse (...) button for a directory and use the Open window to browse to and select the desired directory.
4. Click **OK** to set the new storage directory.

Configuring Print Settings

Measurement results and protocol configurations may be printed. Printing parameters, such as headers and footers are configured in the Print Settings window (Figure 2-13).

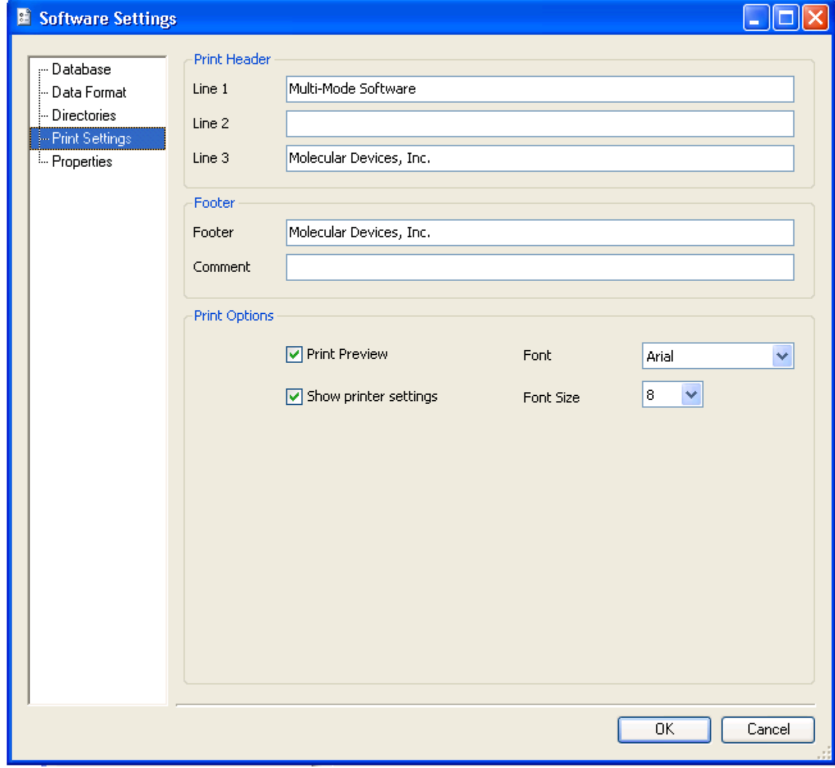


Figure 2-13 Print Settings Window

To configure print settings:

1. Select the **Print Settings** menu. The Print Settings window appears (Figure 2-13).
2. In the **Print Header** section, enter text for each header line, as desired. Header lines may be left blank.
3. In the **Footer** section, enter text for the Footer and Comment, as desired. The comment will display on printed pages below the footer. The footer and comment may be left blank.
4. In the **Print Options** section, select Print Preview to preview the page layout each time a protocol configuration or measurement results are printed.
5. In the **Print Options** section, select **Show printer settings** to display printing options each time a protocol or measurement results are printed.
6. In the **Print Options** section, select the desired Font and Font Size for printed text.
7. Click **OK** to save the new print settings.

Configuring Properties

Certain settings (described below) may be configured from the **Properties** window.

1. In the **Software Settings** window, select the **Properties** menu. The List View Settings and some other general settings appear (Figure 2-14).

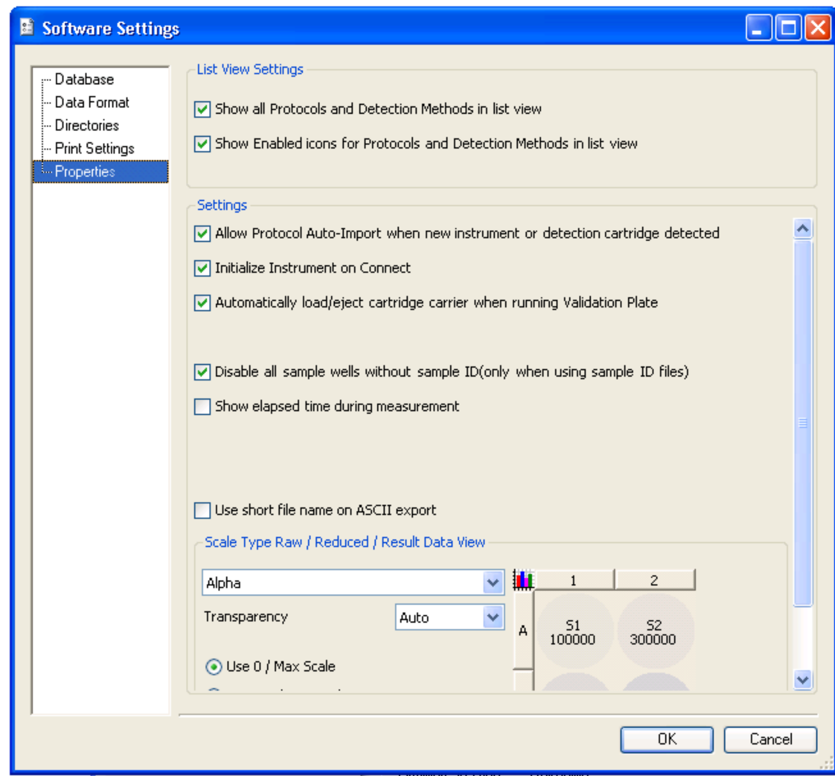


Figure 2-14 Software Settings Window

2. Select **Show all Methods and Protocols in list view** to display all methods and protocols on the Protocol Selection List window. Those protocols not available will be grayed out (Figure 2-15). When deselected unavailable methods and protocols are not displayed at all (Figure 2-16).
3. Select **Show enabled state icon in list view for methods and protocols** to display an enabled column in the Detection Methods List view and the Protocols Selection list view (Figure 2-17 and Figure 2-18). This allows the user to easily view if the detection method or protocol is currently enabled.
4. Select **Allow Protocol Auto-Import when new instrument or detection cartridge detected** to allow import of new example protocols when a new instrument or detection cartridge is detected.

Note: New cartridge detection is available for the SpectraMax Paradigm Multi-Mode Detection Platform only.

5. Select **Initialize Instrument on Connect** to allow initialization of the instrument when the software automatically connects to the instrument, or after the user clicks the **Connect** button.

6. Select **Automatically load/eject cartridge carrier when running a Validation Plate** to allow automatic loading and ejection of the cartridge carrier at appropriate times within the Validation Plate process.

   CAUTION! In any situation (such as when operating the instrument with integrated systems) where automatic loading and ejection of the cartridge carrier may cause a potential equipment collision, we recommend disabling the Automatically load/eject cartridge carrier when running the Validation Plate feature, and to load and eject the cartridge carrier manually.

   ---

7. Select **Disable all sample wells without sample ID** to disable processing of sample wells not bearing a sample ID.
8. Select **Show elapsed time during measurement** to allow indication of the elapsed time while running protocols. By deselecting this feature, the remaining time indicator will show in its place.
9. Click **OK** to save the new settings.

The **Show all Methods and Protocols in list view** option displays all methods and protocols in black, and those not available are grayed out (Figure 2-15).When deselected, unavailable methods and protocols are not shown in the Protocol Selection List or Detection Method List (Figure 2-16).

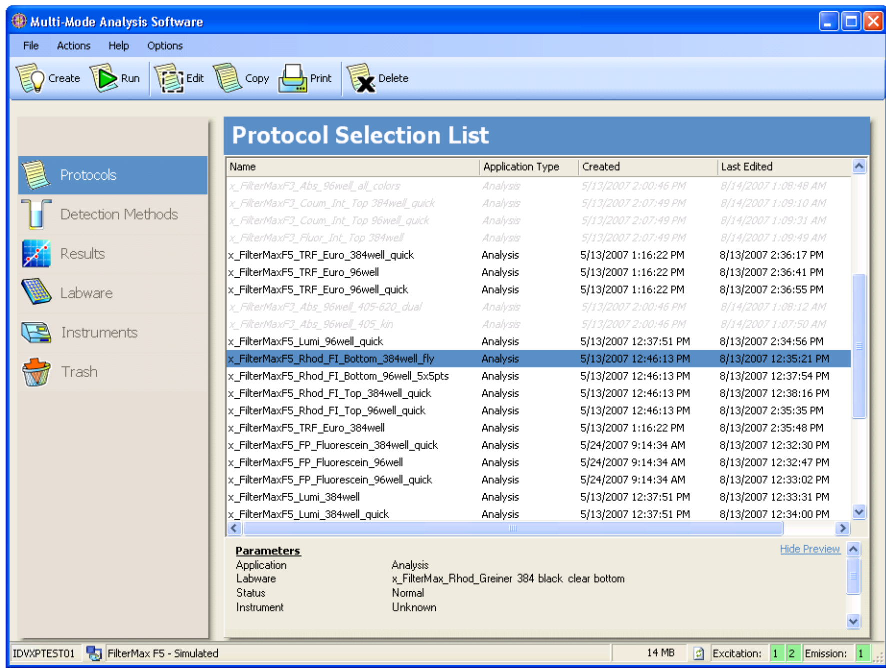


Figure 2-15 Protocol Selection List with Show all Methods and Protocols in list view selected

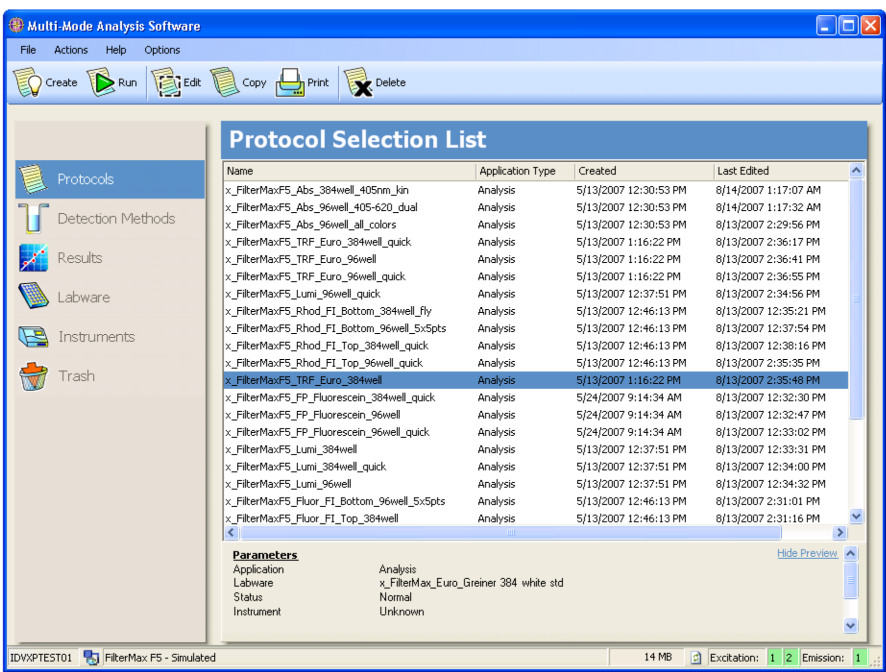


Figure 2-16 Protocol Selection List View with Show all Methods and Protocols in list view deselected

Note: Select multiple items by clicking them and holding down the SHIFT or CTRL keys.

The **Show enabled state icon in list view for methods and protocols** option when selected displays an enabled column in the Detection Methods list view and the Protocols list view (Figure 2-17 and Figure 2-18). This allows the user to easily view if the detection method or protocol is currently enabled.

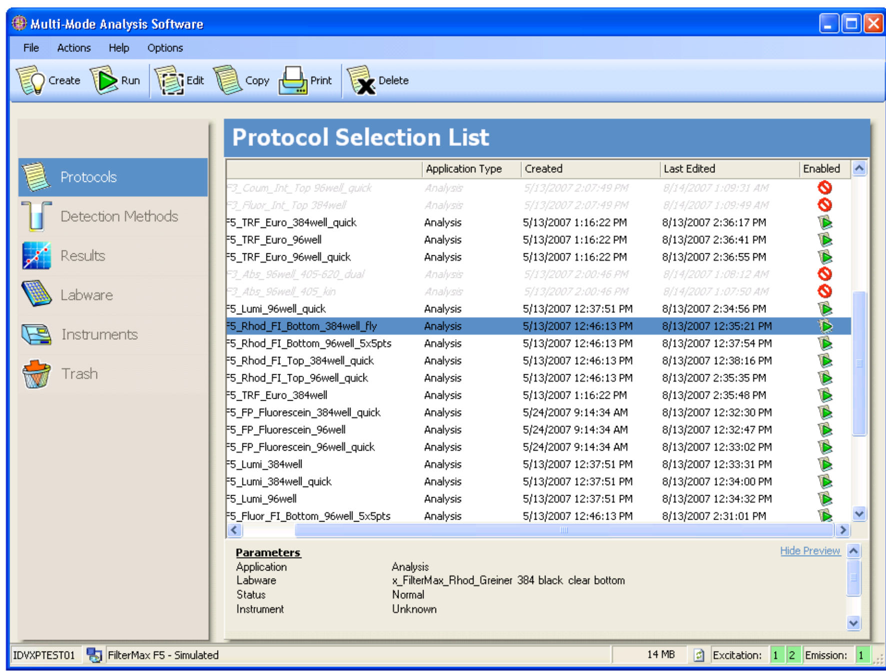


Figure 2-17 Protocol Selection List View with Show enabled state icon in list view for methods and protocols selected

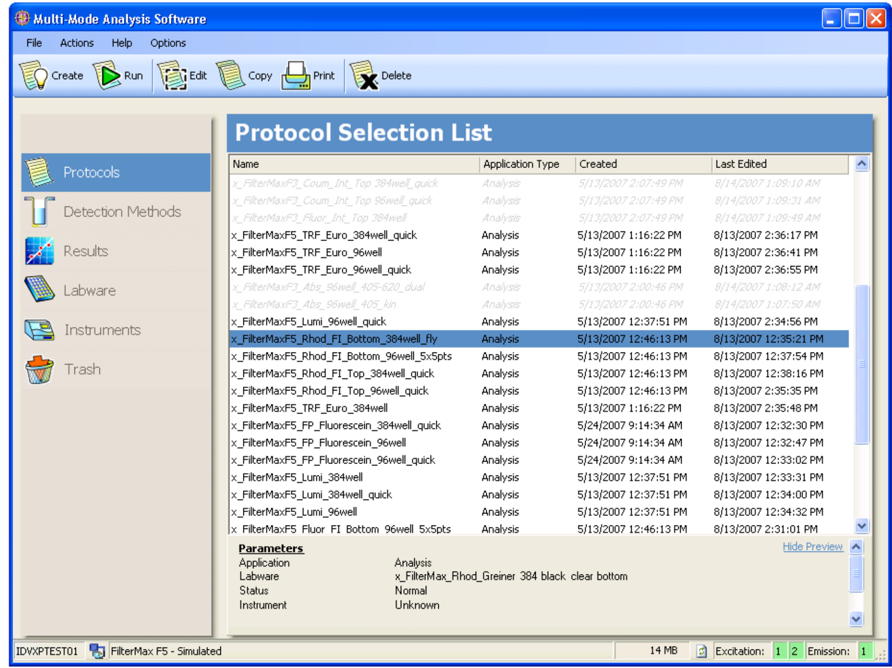


Figure 2-18 Protocol Selection List View with Show enabled state icon in list view for methods and protocols deselected

Configuring the Data Format

Measurement results may be saved in Excel or .dat (data) file formats. Data Format (Figure 2-19) provides options to specify how data files are formatted, such as the delimiter character in .dat files and how to display cycle data for kinetic measurements in Excel files.

Note: When using Microsoft Excel 2002 or higher, the Multi-Mode Analysis Software will format the Excel worksheets with the appropriate column width and apply formatting to display the status of a measurement value (such as bold and colors).

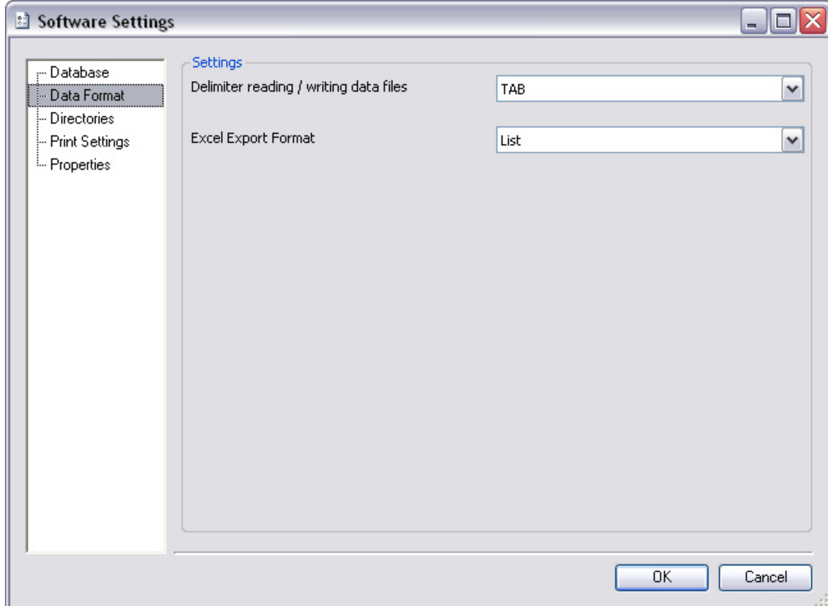


Figure 2-19 Configuring Data Format

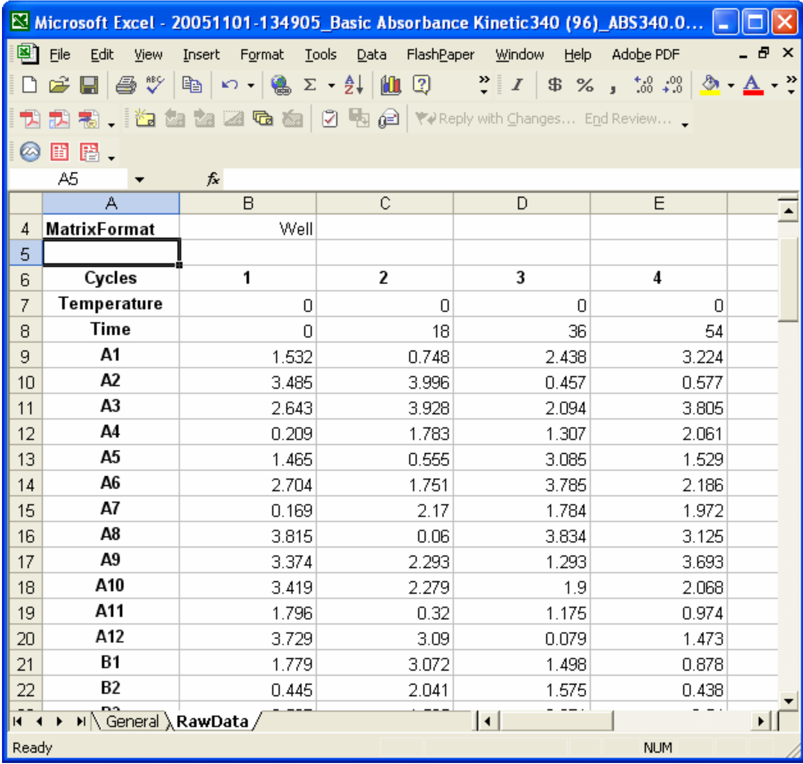
To configure data file formats:

1. In the **Delimiter reading / writing data files** field, select the character to use to separate each column when reading and writing .dat files. Available delimiters are:
   - **Tab**
   - **Comma** (,)
   - **Semicolon** (;)
   - **Pipe** (|)

2. In the **Export Excel format** field, select the desired option:

- **List**: Results from each cycle are displayed side-by-side adjacent to the well in a column layout (Well, Cycle1, Cycle2, Cycle3, etc.) on one Excel spreadsheet.

Note: Area Scan and Wavelength Scan (when the Wavelength Scan points exceeds 250) cannot be exported in **List** format due to column limitations in Excel.



	A	B	C	D	E
4	MatrixFormat	Well			
5					
6	Cycles	1	2	3	4
7	Temperature	0	0	0	0
8	Time	0	18	36	54
9	A1	1.532	0.748	2.438	3.224
10	A2	3.485	3.996	0.457	0.577
11	A3	2.643	3.928	2.094	3.805
12	A4	0.209	1.783	1.307	2.061
13	A5	1.465	0.555	3.085	1.529
14	A6	2.704	1.751	3.785	2.186
15	A7	0.169	2.17	1.784	1.972
16	A8	3.815	0.06	3.834	3.125
17	A9	3.374	2.293	1.293	3.693
18	A10	3.419	2.279	1.9	2.068
19	A11	1.796	0.32	1.175	0.974
20	A12	3.729	3.09	0.079	1.473
21	B1	1.779	3.072	1.498	0.878
22	B2	0.445	2.041	1.575	0.438

Figure 2-20 Excel Format - List

- **All Cycles on one Sheet**: Cycles are displayed in a plate layout format, with each subsequent cycle displayed below the data for the previous cycle.

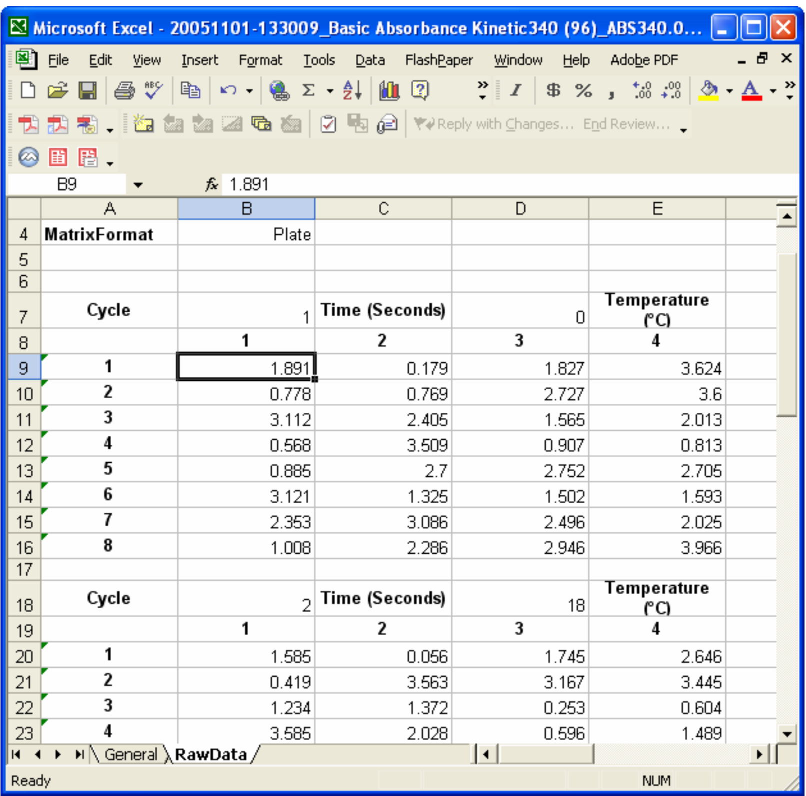
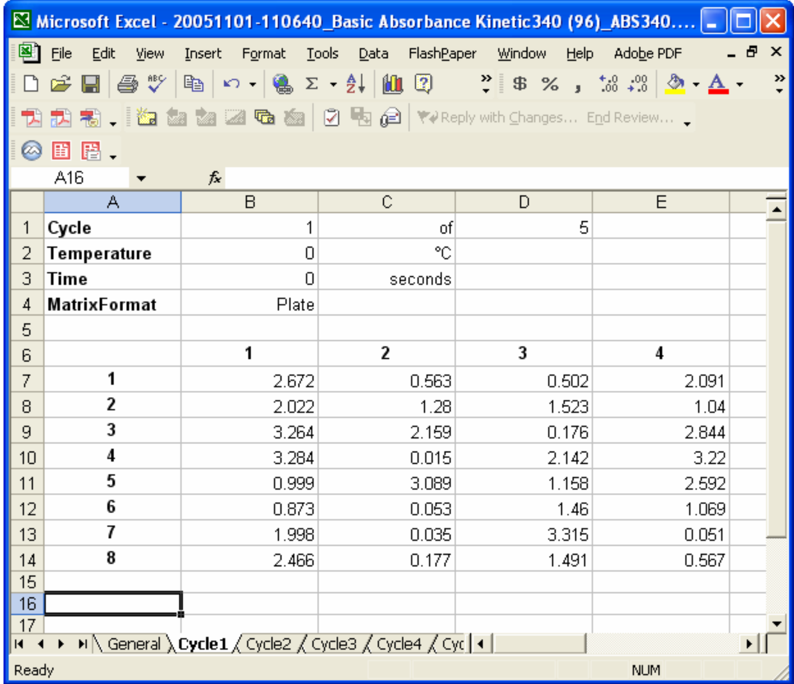


Figure 2-21 Excel Format - All Cycles On One Sheet

- **One Sheet per Cycle**: cycles are displayed in a plate layout format, with each cycle displayed separately on a new worksheet labeled with the cycle number.



	A	B	C	D	E
1	Cycle	1	of	5	
2	Temperature	0	°C		
3	Time	0	seconds		
4	MatrixFormat	Plate			
5					
6		1	2	3	4
7	1	2.672	0.563	0.502	2.091
8	2	2.022	1.28	1.523	1.04
9	3	3.264	2.159	0.176	2.844
10	4	3.284	0.015	2.142	3.22
11	5	0.999	3.089	1.158	2.592
12	6	0.873	0.053	1.46	1.069
13	7	1.998	0.035	3.315	0.051
14	8	2.466	0.177	1.491	0.567
15					
16					
17					

Figure 2-22 Excel Format - One Sheet Per Cycle

Configuring Database Settings

The current database size and audit log for the database can be viewed using the Database settings (Figure 2-23). Database settings also provides options for shrinking the database.

Note: Compressing a database does not affect items in the Trash Selection list.

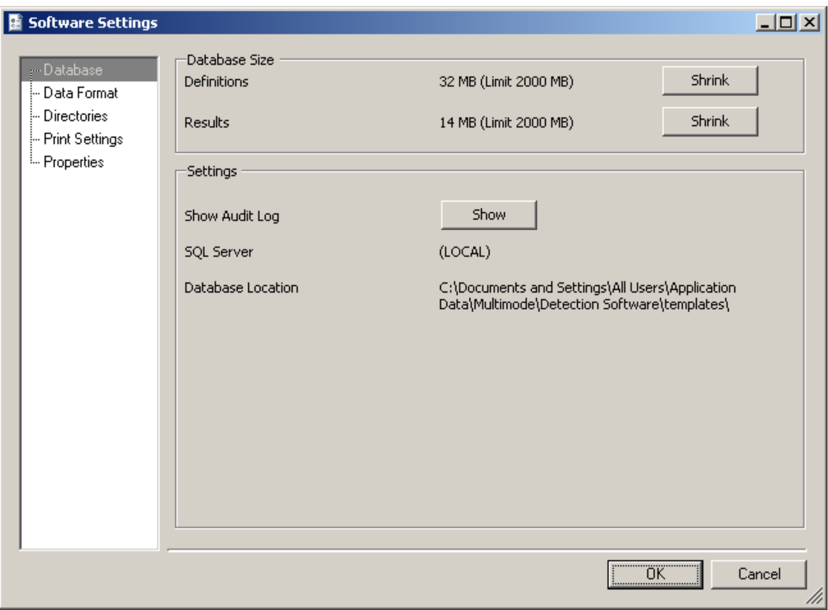


Figure 2-23 Configuring Database Settings

To configure database settings:

1. Select the **Database** menu. Database Settings appear (Figure 2-23).
2. To compress the size of the database containing labware types, detection methods, and protocols click **Shrink** for **Size Database Definition**. Once the database is compressed, click **OK**.
3. To compress the size of the database containing results click **Shrink** for **Size Database Result**. Once the database is compressed, click **OK**.
4. To view the audit log for the database click **Show**. The Audit Viewer appears the audit log for the database (Figure 2-24).

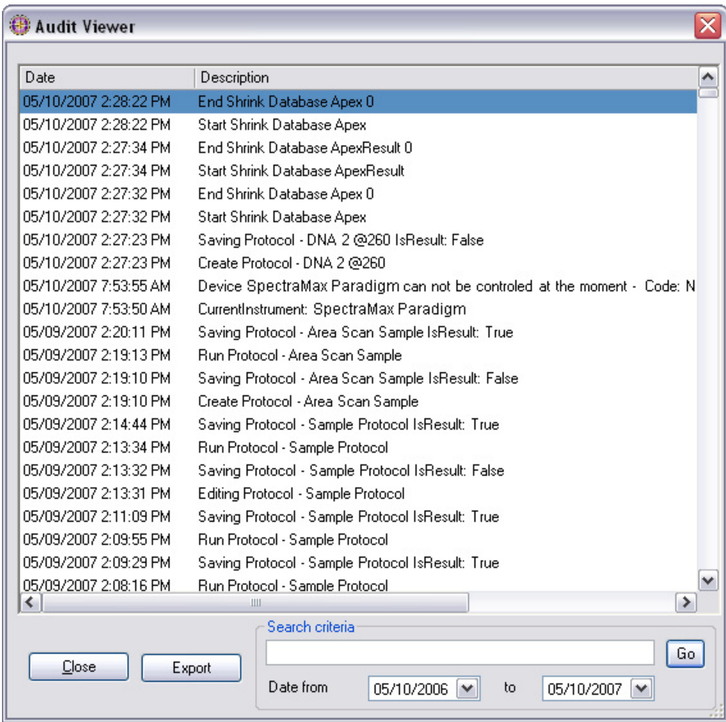


Figure 2-24 Database Audit Log

Deleting and Restoring Items

The Trash Selection List contains labware, detection methods and protocols that are pending permanently deletion from the database. Items may be restored for use or permanently removed from the database using this window.

To restore an item pending deletion:

1. From the navigation pane, select **Trash**. The Trash List appears.
2. Select the item to restore.

   OR

   Click **Restore All Items** to restore all items in the Trash List for use in the software. All items in the Trash List are restored.
3. Click **Restore Item**. The selected item in the Trash List is restored.

Note: Select multiple items by clicking them and holding down the SHIFT or CTRL keys.

To permanently remove an item from the database:

1. From the navigation pane, select **Trash**. The Trash List appears.
2. Select the item to permanently remove from the database.

   OR

   Click **Delete All** to permanently delete all items in the Trash List from the database. All items in the Trash List are permanently removed from the database.
3. Click **Delete**. The selected item in the Trash List is permanently removed from the database.
4. To compress the database after items are removed from the database see Configuring Database Settings on page 47.

Configuring and Controlling Instruments

3

Overview

Before defining measurement protocols, detection methods, and labware, or running protocols on an instrument using Multi-Mode Analysis Software, the instrument must be configured.

The Instrument Selection List provides access for configuring instrument settings and performing common instrument actions, such as loading and ejecting the plate carrier, and for configuring instrument settings.

Use the Instrument Selection List for:

- Managing Instruments on page 52
- Controlling Instrument Actions on page 54
- Configuring the FilterMax Multi-Mode Microplate Readers Instrument Settings on page 57
- Configuring SpectraMax Paradigm Multi-Mode Detection Platform Instrument Settings on page 64
- Configuring SpectraMax Paradigm Multi-Mode Detection Platform System Information Settings on page 65

To configure and manually control instruments:

- From the navigation pane, select **Instruments**. The Instrument Selection List appears (Figure 3-1).

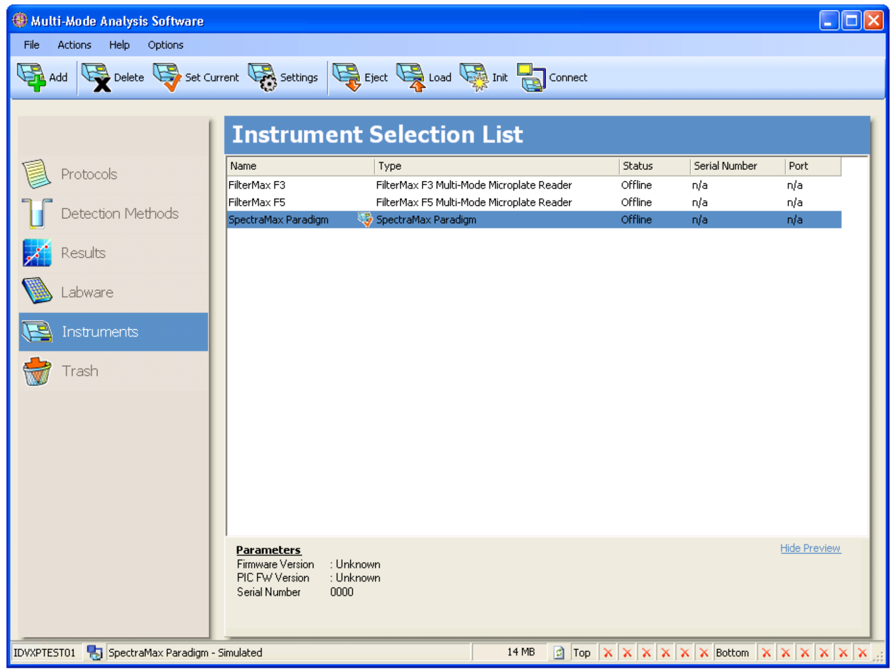


Figure 3-1 Instrument Selection List

All instruments that have been connected to the host computer and configured in the software are displayed in the Instrument Selection List. When an instrument is not currently connected to the computer that is selected, the software automatically enters simulation mode. See Enabling Simulation Mode on page 56. This allows protocols, detection methods, and labware to be defined, edited, and tested for the selected instrument even though it is not physically connected to the host computer.

Note: Select the current instrument for the software. The labware, detection methods, protocols, and results are specific to each instrument. To configuring the current instrument see Configuring the Current Instrument on page 54.

Managing Instruments

Adding a New Instrument

When an instrument is connected to the PC and turned on it is automatically installed and added to the Instrument Selection list for use.

Note: If a simulated instrument already exists in the Instrument Selection List and the actual instrument of the same type is connected to the PC and turned on, all detection methods and protocols for the simulated instrument will be associated with the connected instrument.

Adding an instrument adds a new simulated instrument to the Instrument Selection list for use in building and creating methods, labware, and protocols. Two instruments of the same type may be added and are identified by their serial number and instrument name. Two simulated instruments of the same type may not be added.

To manually add an instrument:

1. From the tool bar, click **Add a new Instrument to the list**.

   OR

   From the menu bar select **Actions > Add a new Instrument to the list**.

   OR

   Right-click on the Instrument Selection list and select **Add a new Instrument to the list** from the menu that appears.
2. The Add Instrument Wizard dialog appears (Figure 3-2).

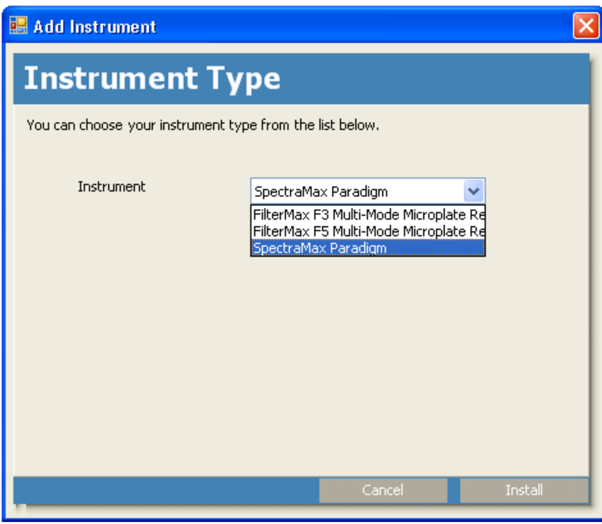


Figure 3-2 Add Instrument Wizard Dialog

3. Using the **Instrument Type** field, select the instrument to be added.
4. Click the **Install** button.
5. The instrument is added to the instrument selection list. To configure the Instrument Settings, see Configuring the FilterMax Multi-Mode Microplate Readers Instrument Settings on page 57 or Configuring SpectraMax Paradigm Multi-Mode Detection Platform Instrument Settings on page 64.

Deleting an Instrument

Deleting an instrument removes the instrument from the Instrument Selection list and all associated methods and protocols are disabled. Protocols and methods from a deleted instrument are automatically associated with an instrument of the same type and configuration (detection cartridges or filter slides).

Note: If an instrument is connected and on, the instrument cannot be deleted. It will be temporarily removed from the Instrument Selection List, and when the list is refreshed the software will retrieve all defined and connected instruments. Only simulated instruments can be permanently removed from the Instrument Selection List.

To delete an instrument:

1. Select the instrument to be deleted.
2. From the tool bar, click **Delete the currently selected instrument**.

   OR

   From the menu bar select **Actions > Delete the currently selected instrument**.

   OR

   Right-click on the instrument and select **Delete the currently selected instrument** from the menu that appears.
3. The Instrument Delete dialog appears, click **Yes** to delete the instrument.
4. The instrument is deleted from the Instrument Selection list, along with disabling all associated detection methods and protocols.

Configuring the Current Instrument

Configuring the current instrument readies the instrument for use. It displays the available detection methods and protocols for the instrument, and disables other instruments' detection methods and protocols. Detection methods and protocols can only be created for the current instrument.

To configure the current instrument:

1. On the Multi-Mode Analysis Software main window, select the instrument for configuration.
2. From the tool bar, click the **Set Current** button.

   OR

   From the menu bar select **Actions > Sets the selected Instrument to Current Instrument**.

   OR

   Right-click on the instrument and from the menu that appears select **Sets the selected Instrument to Current Instrument**.
3. The instrument is now configured as the current instrument.

Controlling Instrument Actions

Instrument actions, such as ejecting or loading the plate carrier and initializing the instrument, can be performed directly from the Instrument Selection List using the buttons on the tool bar.

Actions that may be controlled include:

- Connecting to the Instrument on page 55
- Connecting to the Instrument on page 55
- Loading the Plate Carrier on page 55
- Initializing the Instrument on page 56
- Enabling Simulation Mode on page 56

Note: Before connecting to any instrument, ensure all connections are secure and that the instrument is unlocked and powered up.

Note: Only the current instrument can be controlled. Set the desired instrument to the current instrument. See Configuring the Current Instrument on page 54.

Connecting to the Instrument

When started, the Multi-Mode Analysis Software automatically establishes communication with the instrument or enters simulation mode when no instrument is detected. A connection to an instrument may be established manually after physically connecting a different instrument to the computer, or when switching from simulation mode. See Enabling Simulation Mode on page 56.

To connect to the current instrument:

- From the tool bar, click the **Connect** button. The button remains depressed while the selected instrument is connected and not in simulation mode.

  OR

  From the menu bar select **Actions > Connect to the instrument**.

  OR

  Right-click on the instrument and from the menu that appears select **Connect to the instrument**.

Ejecting the Plate Carrier

Ejecting the plate carrier moves the plate carrier outside the instrument to allow access for placement or removal of a microplate.

To eject the plate carrier from the current instrument:

- From the tool bar, click the **Eject** button.

  OR

  From the menu bar select **Actions >** Eject the plate carrier.

  OR

  Right-click on the instrument and from the menu that appears select **Eject the plate carrier**.

Loading the Plate Carrier

Loading the plate carrier retracts the plate carrier and microplate back into the instrument in preparation of performing a measurement.

To load the plate carrier on the current instrument:

- From the tool bar, click the **Load** button.

  OR

  From the menu bar select **Actions > Load the plate carrier**.

  OR

  Right-click on the instrument and from the menu that appears select **Load the plate carrier**.

Initializing the Instrument

Initializing the instrument moves the optics and microplate transports to home positions. The instrument is initialized automatically each time it turned on. If necessary, the instrument may be initialized manually; for example, after an emergency stop has been performed.

Note: When a hardware error occurs, turning the instrument off and on is the recommended initialization method.

To manually initialize the current instrument:

- From the tool bar, click the **Init** button.

  OR

  From the menu bar select **Actions > Initialize the instrument**.

  OR

  Right-click on the instrument and from the menu that appears select **Initialize the instrument**.

Enabling Simulation Mode

The Multi-Mode Analysis Software can operate in simulation mode whether or not an instrument is connected. Simulation mode enables all features supported by the instrument currently selected in the Instrument Selection List, but measurement results are generated randomly or read from a file. See Selecting Simulated Data Files on page 35 for more information about selecting simulated data files.

Note: Only the current instrument can be controlled, set the desired instrument to the current instrument. See Configuring the Current Instrument on page 54.

To enable simulation mode:

- From the tool bar, click **Simulate the current instrument**. The button remains depressed while the instrument is in simulation mode.

  OR

  From the menu bar select **Actions > Simulate the current instrument**.

  OR

  Right-click on the current instrument and from the menu that appears select **Simulate the current instrument**.

To exit simulation mode, reconnect to the instrument. See Connecting to the Instrument on page 55.

Note: If no instrument is connected to the controlling PC, then Multi-Mode Analysis Software may only run in simulation mode.

Configuring the FilterMax Multi-Mode Microplate Readers Instrument Settings

Filter slides used by the FilterMax Multi-Mode Microplate Readers are configured in Instrument Settings. Configuring Instrument Settings informs Multi-Mode Analysis Software about the instrument and the configuration of filter slides and individual filters. Instrument activities – such as microplate shaking and the ejection or loading of filter slides – may also be controlled manually.

Note: To configure instrument settings for the SpectraMax Paradigm Multi-Mode Detection Platform see Configuring SpectraMax Paradigm Multi-Mode Detection Platform Instrument Settings on page 64.

Note: Only the current instrument can be controlled. Set the desired instrument to the current instrument. See Configuring the Current Instrument on page 54.

Note: When GxP Permissions is enabled on the system, only users assigned a role supported by the Instrument Settings permission may configure instrument settings. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To configure current instrument settings:

1. From the tool bar, click the **Settings** button.

   OR

   From the menu bar select **Actions > Instrument Settings**.

   OR

   Right-click on the current instrument and select **Instrument Settings** from the menu that appears.
2. The **Instrument Settings** window appears.
3. Configure instrument settings on the three tabs as described in the following sections:

   - Configuring the FilterMax Multi-Mode Microplate Readers Instrument Settings on page 57
   - Defining and Editing Filter Slides on page 58
   - Manually Controlling the FilterMax Multi-Mode Microplate Readers on page 62

Modifying and Viewing System Information

The **System Information** tab (Figure 3-3) contains information about the instrument.

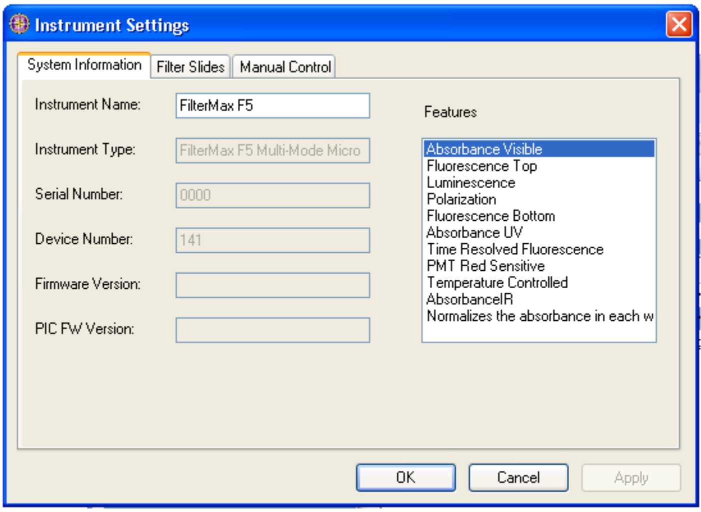


Figure 3-3 FilterMax Multi-Mode Microplate Readers Instrument Settings

To configure basic instrument settings:

1. In the Instrument Settings window, select the **System Information** tab.
2. In the **Instrument Name** field, modify the alias for the instrument's name as desired.

Other fields are read-only (grayed out) and thus cannot be modified.

Table 3-1 Instrument Settings fields

Field	Description
Instrument Name	The alias for the instrument name.
Instrument Type	The model of the instrument
Serial Number	The serial number of the instrument.
Device Number	The device number of the instrument.
Firmware Version	The firmware version loaded for the instrument.
PIC FW Version	The instrument PIC processor firmware version.
Features	The types of measurements the instrument is capable of performing.

Defining and Editing Filter Slides

The **Filter Slides** tab (Figure 3-4) is used to add, remove, and configure filter slides and the filters installed on a filter slide. Slide definitions may also be imported and exported.

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

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

When exchanging slides, an identification code built into the slide allows Multi-Mode Analysis Software to recognize the new slide and filter configuration. When a slide with a new configuration is inserted, or the filters on a slide change, the slide must be configured in the **Filter Slides** tab. Up to 31 excitation filter slides and 31 emission filter slides may be stored in Multi-Mode Analysis Software at one time.

Note: For FilterMax Multi-Mode Microplate Readers to create or run quantitation protocols, a genomic filter slide, which contains narrow bandwidth 260 nm and 280 nm filters) must be installed and configured.

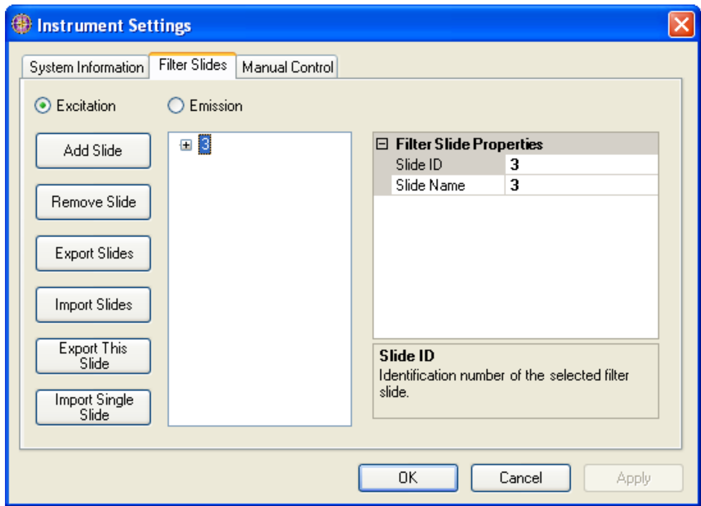


Figure 3-4 Instrument Settings - Filter Slides

This section covers:

- Adding Filter Slides on page 59
- Configuring Filter Slides on page 60
- Removing Filter Slides on page 61
- Exporting and Importing All Filter Slide Configurations on page 61
- Exporting and Importing Single Filter Slide Configurations on page 61

Adding Filter Slides

When a new filter slide is used with the instrument for the first time, it must be added so that the Multi-Mode Analysis Software may identify the slide and filter configuration.

To add filter slides:

1. Select the type of filter slide to add: **Excitation** or **Emission**. The list of filter slides displays all slides of the selected type currently stored in memory.
2. Click **Add Slide**. A new filter slide appears on the list of slides in the central pane.
3. Configure the slide following the steps in Configuring Filter Slides on page 60.

Configuring Filter Slides

The **Filter Slide Properties** pane shows filter name and ID for the currently selected slide, and displays information about the filters installed on the slide. When a new slide is added, or the filter configuration on a slide changes, the slide must be configured.

CAUTION! It is recommended not to reconfigure standard filter slides.

To configure a filter slide:

1. Select the type of filter slide to configure: **Excitation** or **Emission**. The list of filter slides displays all configured slides of the selected type.
2. Select the desired filter slide to configure from the list. The he **Filter Slide Properties** pane displays information about the selected slide (Figure 3-4).
3. In the **Slide ID** field, enter the identification number printed on the slide.
4. In the **Slide Name** field, enter a name by which to identify the filter slide.
5. In the list of filter slides, click the **+** to the left of the filter slide name to display the list of filters installed on the slide.
6. Select a filter to configure. Filter properties for the selected filter is displayed on the **Filter Properties** pane (Figure 3-5).

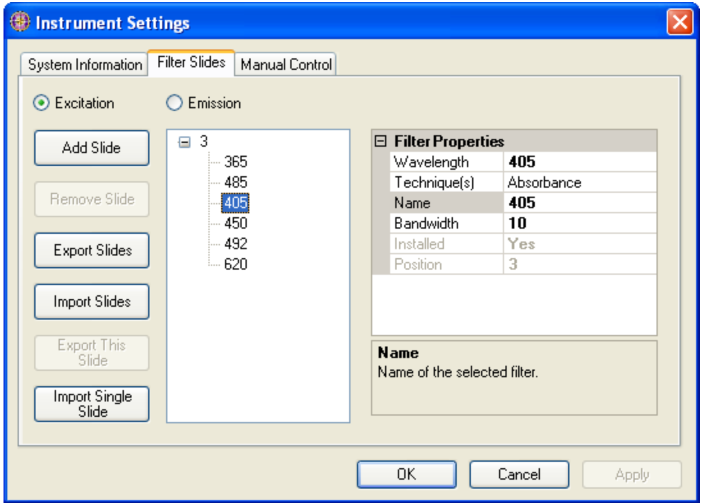


Figure 3-5 Configuring Filter Properties

7. In the **Wavelength** field, enter the wavelength of the filter.
8. Click in the **Techniques** field and then click the down arrow to display a list of the available detection techniques.
9. Select all techniques for which the filter applies. The filter can be used only for measurements of the selected technique types. When techniques are selected, the read-only **Installed** field appears **Yes**. When **No** is appears, no techniques are selected and the filter may not execute any techniques.

Note: FilterMax 5 Multi-Mode Microplate Reader only: Select **Polarization** only for filter positions where a polarization filter is installed.

10. In the **Name** field, enter a name for the selected filter. Filter names default to the wavelength entered, but may be renamed as desired.
11. In the **Bandwidth** field, enter the bandwidth (in nanometers) of the selected filter.
12. Repeat steps 6 through 11 to configure additional filters on the slide.

Removing Filter Slides

If a filter slide is no longer used with an instrument, it can be removed from the Multi-Mode Analysis Software.

To remove a filter slide:

1. Select the type of filter slide to remove: **Excitation** or **Emission**. The list of filter slides displays all slides of the selected type.
2. Select the desired filter slide to remove from the list. The **Filter Slide Properties** window displays information about the selected slide.
3. Click **Remove Slide**. The selected filter slide is removed from the list.

Exporting and Importing All Filter Slide Configurations

Information for all excitation and emission filter slides configured for the instrument may be exported to an XML file and imported at a later time to restore that configuration or share the filter slide configuration with another instrument. Importing the filter slide configuration from an XML file replaces the current configuration for all filter slides with the configuration from the file.

Note: If necessary, the default filter slide configuration may be restored by importing the file for the type of instrument in use located in **Documents and Settings\All Users\Application Data\Multi-Mode\Detection Software\Filters**.

To export all filter slides:

1. Select **Export Slides**. The Save As dialog appears.
2. In the **Save As** window, select the desired directory and enter a file name.
3. Click **Save**. Slide information is saved as an XML file with the specified path and file name.

To import all filter slides from a previously exported file:

1. Select **Import Slides**. The Open dialog appears.
2. In the Open dialog, browse to and select the desired XML file to import.
3. Click **Open**. The filter slides defined in the XML file are imported into the filter slide list and replace all existing filter slides.

Exporting and Importing Single Filter Slide Configurations

Configuration information for a single filter slide may be exported to an XML file and imported to restore that configuration or share the configuration with another instrument.

To export a single filter slide configuration:

1. In the list of filter slides, select the slide desired to export.
2. Select **Export This Slide**. The Save As dialog appears.
3. In the Save As dialog, select the desired directory and enter a file name.
4. Click **Save**. Slide information is saved as an XML file with the specified path and file name.

To import a single filter slide configuration:

1. If the list of filter slides currently loaded contains a slide with the same ID as a slide configuration for import, delete that currently loaded slide. Every filter slide ID stored in the software must be unique.
2. Select **Import Single Slide**. The Open dialog appears.
3. In the Open dialog, browse to and select the desired XML file to import.
4. Click **Open**. The selected filter slide is imported and added to the list of filter slides for the current instrument.

Manually Controlling the FilterMax Multi-Mode Microplate Readers

The Manual Control tab (Figure 3-6) provides options to control the actions of the connected instrument. These actions include microplate shaking and ejecting or loading filter slides.

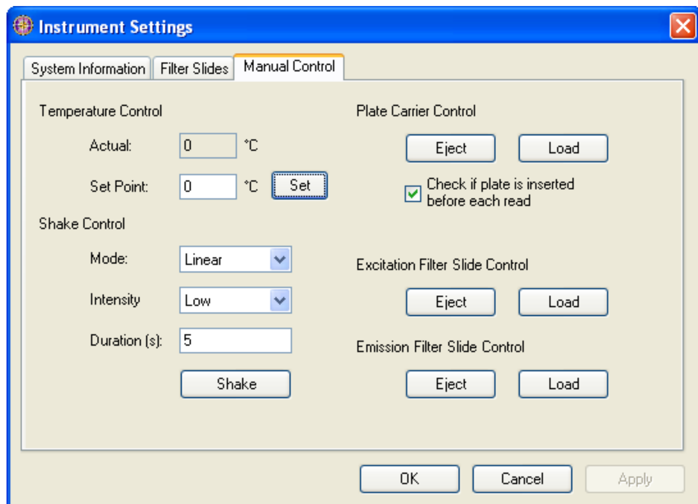


Figure 3-6 FilterMax Multi-Mode Microplate Readers Instrument Settings - Manual Control Tab

Manual Control is divided into five subsections:

- Temperature Control (FilterMax 5 Multi-Mode Microplate Reader only) on page 63
- Shake Control on page 63
- Plate Carrier Control on page 64
- Excitation Filter and Emission Filter Slide Control on page 64

Temperature Control (FilterMax 5 Multi-Mode Microplate Reader only)

Temperature Control is used to set the microplate chamber temperature. The temperature is set by heating the microplate chamber; cooling the chamber is not supported. Depending on the light source used to perform measurements configured in the protocol, the temperature may range from 3°C (5.4°F) or 4°C (7.2°F) above ambient to 45°C (113°F). The **Actual** field displays the current temperature of the instrument.

To set the temperature:

1. In the **Set Point** field, enter the desired temperature in Celsius.
2. Click **Set**. Temperature control is activated for the instrument and begins to heat to the desired temperature. The set temperature is maintained until it is changed or the instrument is powered off.

Note: A minimum of 15 minutes is required for the instrument to reach the desired temperature from ambient. The actual time required depends on the relative change in temperature.

The FilterMax 3 Multi-Mode Microplate Reader does not support heating to a set temperature.

To turn off temperature control:

- In the **Set Point** field, enter 0, then click **Set**.

  OR

  Turn the power to the instrument off and on.

Shake Control

Shake Control is used to manually shake the microplate loaded in the instrument.

To manually perform a shaking operation:

1. In the **Mode** field, select the desired shaking mode:
   - **Linear**: shakes from side to side.
   - **Orbital**: shakes in a circular pattern.
   - **Squared**: shakes in a square pattern, moving clockwise or counter-clockwise at right angles.

   CAUTION! Shake low-density plates, such as 6- or 48-well plates, at Low speed only. Shaking low density plates at higher speeds may cause liquid in wells to spill.

2. In the **Intensity** field, select the desired shaking intensity: Low, Medium, or High.
3. In the **Duration** field, enter the length of time (in seconds) for shaking.
4. Click **Shake**. The instrument immediately shakes the microplate according to these settings.

Plate Carrier Control

Plate Carrier Control provides options to eject or load the plate carrier. It also features an option to sense that a microplate is in the plate carrier before starting a measurement.

To manually control the plate carrier:

- Click **Eject** to extend the plate carrier outside the instrument.
- Click **Load** to retract the plate carrier into the instrument.
- Select **Check if plate is inserted before each read** to sense if a microplate is in the plate carrier before starting measurement.

Excitation Filter and Emission Filter Slide Control

Excitation Filter Slide Control and Emission Filter Slide Control are used to manually eject or load the excitation or emission filter slides.

To manually eject or load the excitation or emission filter slide:

- Click **Eject** from the desired filter slide control section to unload the filter slide from the filter compartment and partially open the compartment door.

Note: To remove the filter slide, it is still necessary to grasp it by the tab and pull it until it is free of the geared track. Store the removed filter slide in a protected, dust-free area, preferably in the original packaging.

- Click **Load** from the desired filter slide control section to retract the filter slide into position.

Configuring SpectraMax Paradigm Multi-Mode Detection Platform Instrument Settings

Instrument settings and detection cartridges used by the SpectraMax Paradigm Multi-Mode Detection Platform are configured in **Instrument Settings**. Configuring Instrument Settings informs Multi-Mode Analysis Software about the instrument, such as the configuration of detection cartridges. Instrument activities – such as microplate shaking and the ejection or loading of filter slides – may also be controlled manually.

Note: To configure the FilterMax Multi-Mode Microplate Readers, see Configuring the FilterMax Multi-Mode Microplate Readers Instrument Settings on page 57.

Note: Only the current instrument can be controlled, set the desired instrument to the current instrument. See Configuring the Current Instrument on page 54.

Note: When GxP Permissions is enabled on the system, only users assigned a role supported by the Instrument Settings permission may configure instrument settings. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To configure current instrument settings:

1. From the tool bar, click **Settings**.

   OR

   From the menu bar select **Actions > Instrument Settings**.

   OR

   Right-click on the current instrument and select **Instrument Settings** from the menu that appears.
2. The **Instrument Settings** window appears.
3. Configure instrument settings on the four tabs as described in the following sections:
   - Configuring SpectraMax Paradigm Multi-Mode Detection Platform System Information Settings on page 65
   - Viewing Installed Detection Cartridges on page 66
   - Defining and Editing the Available Detection Cartridges on page 66
   - Manually Controlling the SpectraMax Paradigm Multi-Mode Detection Platform Instrument on page 69

Configuring SpectraMax Paradigm Multi-Mode Detection Platform System Information Settings

The System Information tab (Figure 3-7) contains information about the instrument.

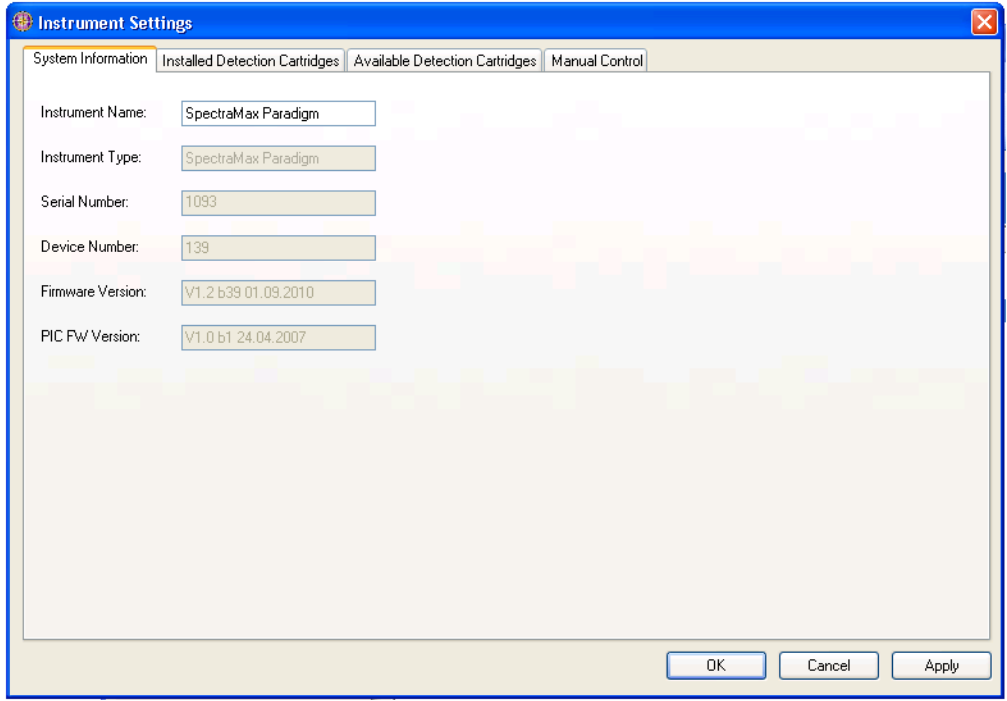


Figure 3-7 Instrument Settings - System Information Settings

To configure System Information settings:

1. In the Instrument Settings dialog, select the **System Information** tab.
2. In the **Instrument Name** field, modify the alias for the instrument’s name as desired.
3. Click **Apply**. The instrument information fields are automatically populated with information about the connected instrument. Refer to Table 3-2 for more information about each field.

Table 3-2 Instrument Settings fields

Field	Description
Instrument Name	The alias for the instrument name.
Instrument Type	The model of the instrument
Serial Number	The serial number of the instrument.
Device Number	The device number of the instrument.
Firmware Version	The firmware version loaded for the instrument.
PIC FW Version	The instrument PIC processor firmware version.

Viewing Installed Detection Cartridges

The Installed Detection Cartridges tab appears detection cartridges currently installed in the instrument. Detection Cartridges are automatically detected and added to the Installed Detection Cartridge tab and Available Detection Cartridges tab when they are inserted into the instrument.

Defining and Editing the Available Detection Cartridges

The Available Detection Cartridges tab (Figure 3-8) is used to add detection cartridges to **My Detection Cartridges** for convenient configuration of methods and protocols. The detection cartridges do not need to be installed in the instrument, however to configure a detection cartridge it is necessary to add it to **My Detection Cartridges**.

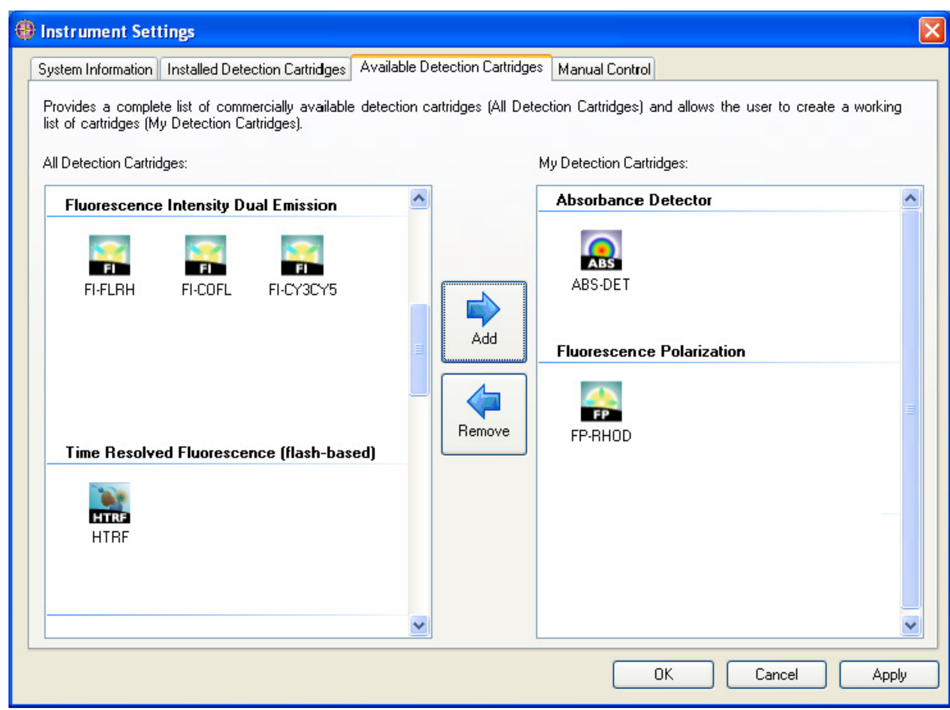


Figure 3-8 Instrument Settings - Available Detection Cartridges

This section covers:

- Adding Detection Cartridges to the list of Available Detection Cartridges on page 67
- Removing Detection Cartridges from the list of Available Detection Cartridges on page 68

Adding Detection Cartridges to the list of Available Detection Cartridges

To use a detection cartridge with the instrument, the detection cartridge must be added to **My Detection Cartridges** so that it can be used in protocols. Only the detection cartridges contained within the **My Detection Cartridges** will be available for creating Detection Methods and Protocols.

Note: When a detection cartridge is installed in the instrument the software automatically adds the detection cartridge to **My Detection Cartridges.**

Note: Any available detection cartridge can be added to **My Detection Cartridges** for use in creating detection methods and protocols. The detection cartridge can be placed in the instrument at a later point in time. Fluorescence Intensity detection methods are based upon a Top Read or Bottom Read, it is important to install the cartridge in the proper Top Read or Bottom Read detection cartridge transport according to the created protocol.

To manually add detection cartridges to **My Detection Cartridges**:

1. Click on the Available Detection Cartridges tab.
2. A list of all detection cartridges available is displayed in **All Detection Cartridges**. The list of installed detection cartridges is displayed within **My Detection Cartridges**. Select the detection cartridges to add from **All Detection Cartridges**.
3. Click **Add**. The selected detection cartridges are added to **My Detection Cartridges**. They are now available for use in a detection method, see Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform) on page 99.

Removing Detection Cartridges from the list of Available Detection Cartridges

In simulation mode, removing a detection cartridge from **My Detection Cartridges** disables its use. All Detection Methods and Protocols associated with the detection cartridge will become unavailable for use in simulation mode. Associated Detection Methods and Protocols are not deleted, they are simply removed from the view. If the detection cartridge is added to **My Detection Cartridges** again, all Detection Methods and Protocols associated with it will become available again.

Note: When an instrument is connected and not in simulation mode, removing a detection cartridge from **My Detection Cartridges** is only temporary. As soon as the connection is refreshed the detection cartridge will be added to **My Detection Cartridges** and it will become available again.

To remove detection cartridges from **My Detection Cartridges**:

1. Click on the **Available Detection Cartridges** tab.
2. A list of all detection cartridges available is displayed in **All Detection Cartridges**. The list of installed detection cartridges is displayed within **My Detection Cartridges**. Select the detection cartridges to remove from **My Detection Cartridges**.
3. Click **Remove**. The detection cartridges are removed from **My Detection Cartridges**. All detection methods and protocols that use this detection cartridge are now unavailable.

Manually Controlling the SpectraMax Paradigm Multi-Mode Detection Platform Instrument

The Manual Control tab (Figure 3-9) provides options to control the actions of the connected instrument, such as shaking microplates and ejecting or loading detection cartridges.

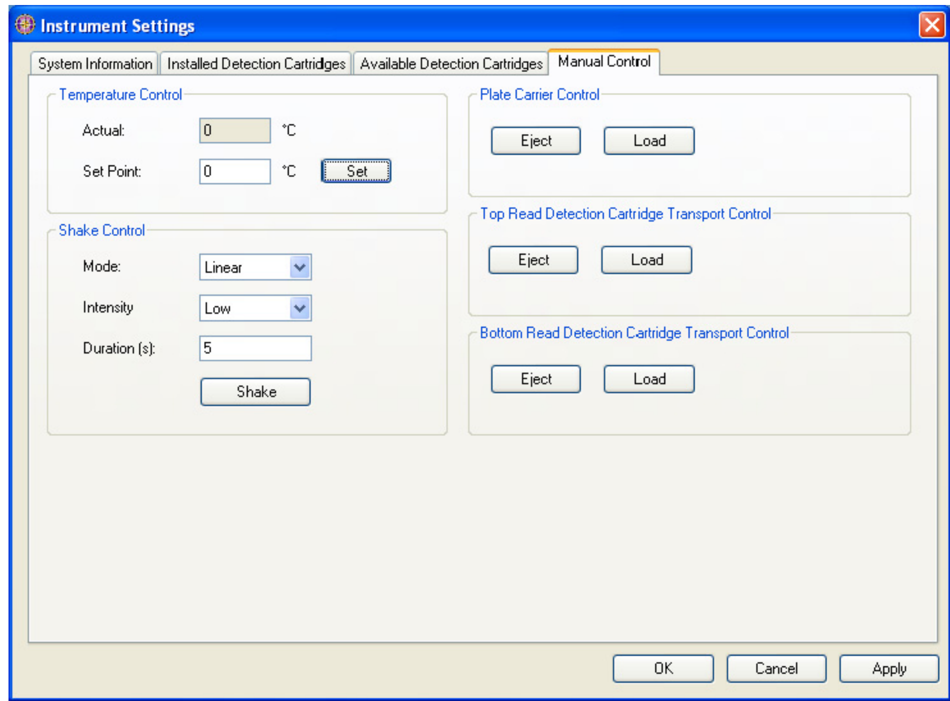


Figure 3-9 SpectraMax Paradigm Multi-Mode Detection Platform Instrument Settings - Manual Control

Manual Control is divided into five subsections:

- Temperature Control on page 70
- Shake Control on page 70
- Plate Control on page 71
- Top Read Detection Cartridge Transport Control on page 71
- Bottom Read Detection Cartridge Transport Control on page 71

Temperature Control

Temperature Control is used to adjust microplate chamber temperature. The temperature is set by heating the microplate chamber; cooling the chamber is not supported. Depending on the light source used to perform measurements configured in the protocol, the temperature may range from 3°C (5.4°F) or 4°C (7.2°F) above ambient to 45°C (113°F). The **Actual** field displays the current temperature of the instrument.

To set the temperature:

1. In the **Set Point** field, enter the desired temperature in Celsius.
2. Click **Set**. Temperature control is activated for the instrument and begins to heat to the desired temperature. The set temperature is maintained until it is changed or the instrument is powered off.

Note: A minimum of 15 minutes is required for the instrument to reach the desired temperature from ambient. The actual time required depends on the relative change in temperature.

To turn off temperature control:

- In the **Set Point** field, enter 0, then click **Set**.

  OR

  Turn the power to the instrument off and on.

Shake Control

Shake Control is used to manually shake the microplate loaded in the instrument.

To manually perform a shaking operation:

1. In the **Mode** field, select the desired shaking mode:
   - **Linear**: shakes from side to side.
   - **Orbital**: shakes in a circular pattern.

Note: Squared shaking (available in FilterMax Multi-Mode Microplate Readers) is not supported on the SpectraMax Paradigm Multi-Mode Detection Platform.

CAUTION! Shake low-density plates, such as 6-well or 48-well plates, at low speed only. Shaking low density plates at higher speeds may cause liquid in wells to spill.

2. In the **Intensity** field, select the desired shaking intensity: **Low**, **Medium**, or **High**.
3. In the **Duration** field, enter the length of time (in seconds) for shaking.
4. Click **Shake**. The instrument immediately shakes the microplate according to these settings.

Plate Control

Plate Control provides options to eject or load the plate carrier. It also features an option to sense that a microplate is in the plate carrier before starting a measurement.

To manually control the plate carrier:

- Click **Eject** to extend the plate carrier outside the instrument.
- Click **Load** to retract the plate carrier inside the instrument.
- Select **Check if plate is inserted before each read** to sense if a microplate is in the plate carrier before starting each measurement.

Top Read Detection Cartridge Transport Control

The Top Read Detection Cartridge Transport Control is used to manually eject or load detection cartridges into the Top Read detection cartridge transport.

To manually eject or load the Top Read detection cartridge transport:

- Click **Eject** from the Top Read Detection Cartridge Transport Control section to unload a detection cartridge from the Top Read compartment.
- Click **Load** from the Top Read Detection Cartridge Transport Control section to retract the detection cartridge into position. If a detection cartridge is inserted into the instrument it will automatically be detected.

Bottom Read Detection Cartridge Transport Control

The Bottom Read Detection Cartridge Transport Control is used to manually eject or load detection cartridges into the Bottom Read detection cartridge transport.

To manually eject or load the Bottom Read detection cartridge transport:

- Click **Eject** from the Bottom Read Detection Cartridge Transport Control section to unload a detection cartridge from the Bottom Read transport compartment.
- Click **Load** from the Bottom Read Detection Cartridge Transport Control section to retract the detection cartridge into position. If a detection cartridge is inserted into the instrument it will automatically be detected.

Setting Up and Using GxP Permissions 4

Overview

To assist users in complying with electronic signature regulations, such as 21 CFR Part 11, the optional GxP Permissions module for Multi-Mode Analysis Software may be purchased from Molecular Devices. When GxP Permissions is enabled for Multi-Mode Analysis Software, users must have a valid user account and password on the system to access the software. Each user is assigned roles that contain specific permissions which determine the software actions the user may perform.

GxP Permissions provides support for closed systems only; access over a network is not supported. In a location where several systems are present, GxP Permissions must be installed and enabled separately for each system where compliance is desired. Users require separate accounts on each system they need to access.

Note: Compliance with regulations, such as 21 CFR Part 11, requires implementing site processes beyond the control of the software.

A single system administrator sets up the level of support provided by GxP Permissions, creates and manages roles assigned to user accounts, and configures GxP Permissions system parameters.

This section covers:

- Enabling GxP Permissions on page 73
- Performing System Administration Tasks on page 75

Enabling GxP Permissions

A single system administrator account may be configured for GxP Permissions. The system administrator enables the system by setting the desired support level. Three support levels are available:

- **No Support**: GxP Permissions is disabled; user accounts are not required.
- **GxP Permissions**: User accounts are required to log into the software, but passwords are not required to sign items.

Note: GxP Permissions without password checks may not provide adequate support to comply with 21 CFR Part 11 or other regulations. Each site must evaluate the level of support required for a given system.

- **GxP Permissions, with password checks for signing and check-in**: User accounts are required to log into the software, and passwords must be entered for confirmation when prompted.

Note: Compliance with regulations, such as 21 CFR Part 11, requires implementing site processes beyond the control of the software.

To enable GxP Permissions by setting a support level:

1. Log off and close all Molecular Devices applications.
2. Place the GxP Permissions CD in the drive, and browse to the contents of the CD.
3. Double-click GxP Permissions -- Support Options.exe. The Administrator Login appears.
4. If GxP Permissions is being enabled for the first time, in Administrator Login, enter Password. A message explaining that the password must be changed appears.

   OR

   If GxP Permissions has been enabled previously, in Administrator Login, enter the administrator password and click **OK**. Support Options appear. Proceed to step 5.

Note: If the Administration Password is lost, forgotten, or not known, follow the steps in Restoring the Administrator Password on page 79.

5. In the message box, click **OK**. The Change Password dialog appears.
6. In the upper field, enter a new password.
7. In the lower field, re-enter the new password and click **OK** to confirm. The Support Options dialog appears (Figure 4-1).

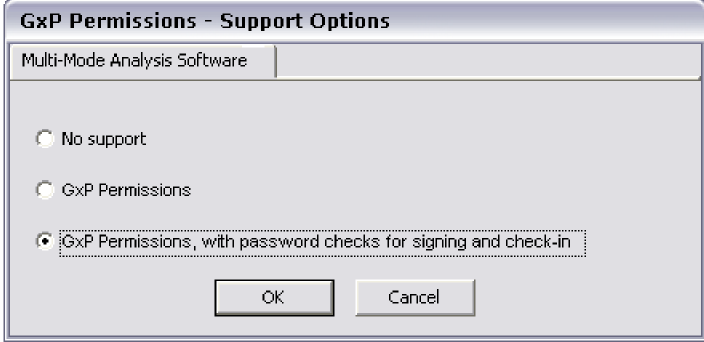


Figure 4-1 Support Options Dialog

8. Select the **Multi-Mode Analysis Software** tab, as necessary. Multiple tabs appear only when several software applications with GxP Permissions support are installed on the system.
9. Select the level of support:
   - **No support**: User accounts are not required to access Multi-Mode Analysis Software. Users have access to all software operations and functionality. System activity is logged in the audit trail and may be viewed in the Audit Log. See Viewing the System Activity Audit Log on page 79.
   - **GxP Permissions**: Enables the use of user accounts and permissions for Multi-Mode Analysis Software. Users must log in to use the software and may access only features and actions for which they have permission. Actions performed in the software, such as signing a labware type or protocol, do not require password confirmation.

Note: GxP Permissions without password checks may not provide adequate support to comply with 21 CFR Part 11 or other regulations. Each site must evaluate the level of support required for a given system.

- **GxP Permissions, with password checks for signing and check-in**: Enables the use of user accounts and permissions and electronic signatures for Multi-Mode Analysis Software. Users must log in to use the software and may access only features and operations for which they have permission. Support for 21 CFR Part 11 or other regulations is provided by requiring password checks for operations such as signing a detection method.

Note: When other applications that support GxP Permissions are installed on the system, each must be configured with the same support level as Multi-Mode Analysis Software.

Note: Regulations, such as 21 CFR Part 11, contain additional requirements for account management beyond the control of this software.

10. Click **OK** to activate the support level chosen and close Support Options.

Note: After GxP Permissions is enabled for the first time, the administrator must create at least one user account to access Multi-Mode Analysis Software. See Administering User Accounts and Roles on page 75 for detailed instructions.

Performing System Administration Tasks

On a system with GxP Permissions enabled, a single administrator account provides the ability to perform GxP Permissions system administration tasks, including:

- Administering User Accounts and Roles on page 75
- Restoring the Administrator Password on page 79
- Viewing the System Activity Audit Log on page 79

Administering User Accounts and Roles

GxP Permissions system administration tasks are performed in the Account Management application included as part of the GxP Permissions installation. The system administrator sets up and configures user accounts, passwords, and roles, and configures system settings, such as automatic password expiration and system logout time.

A single system administrator password is used on a controlling system. System administration tasks may be performed only on the computer where Account Management is installed; access to Account Management over a network is not supported.

Note: If the administrator requires access to Multi-Mode Analysis Software, a separate user account must be created.

Note: Regulations, such as 21 CFR Part 11, contain additional requirements for account management beyond the control of this software.

This section covers accessing Account Management and configuring roles that are assigned to user accounts. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76. Refer to the Account Management online help for detailed information about GxP Permissions system administration tasks.

To open Account Management:

1. Close all Molecular Devices applications.
2. In the Windows Start menu, select **Settings > Control Panel**. the **Control Panel** appears.
3. In **Control Panel**, double-click on **Administrative Tools**. The **Administrative Tools** window appears.
4. In the **Administrative Tools** window, double-click on **Account Management**. The **Administrator Login** window appears.
5. Enter the administrator password and click **OK**. The **Account Management** window appears (Figure 4-2).

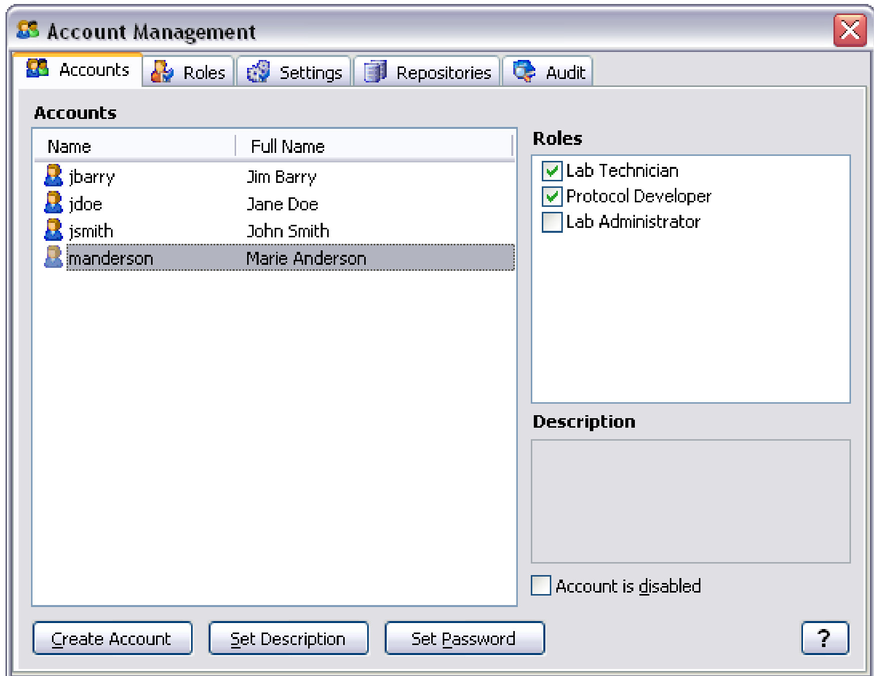


Figure 4-2 Account Management

Configuring Roles for Multi-Mode Analysis Software User Accounts

Multi-Mode Analysis Software permissions, which control access to software actions, are installed as a part of Multi-Mode Analysis Software as the GxP Permissions module. Permissions are not assigned directly to user accounts. Instead the system administrator assigns permissions to roles, which are then assigned to accounts as desired.

Roles may be assigned multiple permissions. When several software applications that support GxP Permissions are installed on the same system,

permissions from different applications may be assigned to the same role. A permission may be assigned to as many roles as desired.

Three preconfigured roles are installed with the GxP Permissions module (refer to Table 4-3). These roles may be assigned to user accounts as is, or edited, renamed, or deleted as desired.

Note: Refer to the Account Management online help for more detailed information. To access online help, press F1 or click the **Help** (question mark) button in the lower right corner of the Account Management window.

To configure roles:

1. In the Account Management window, select the **Roles** tab (Figure4.3).

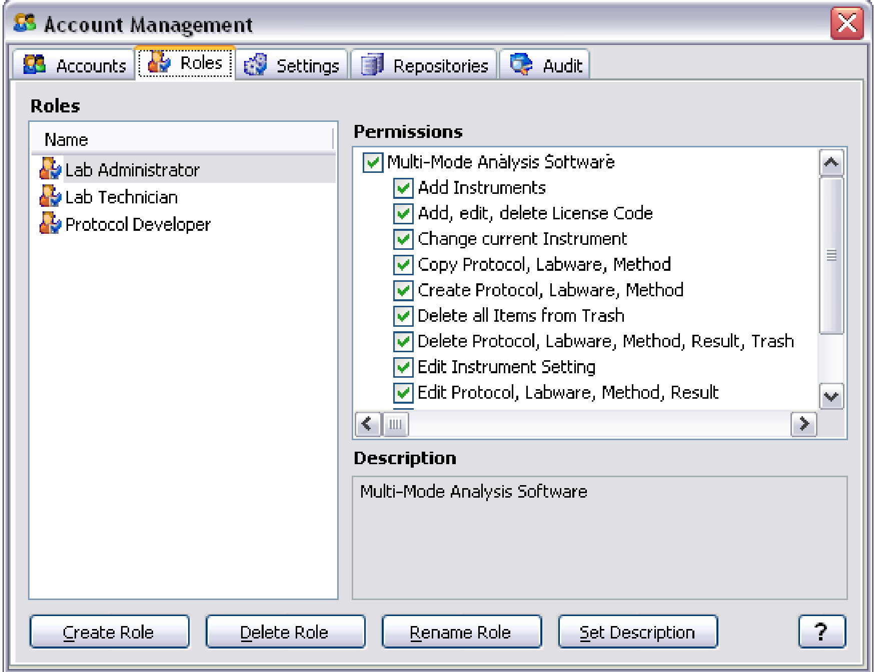


Figure 4-3 Configuring Roles

2. In the Roles section, click the **Create Role** button or select an existing role to edit. Table 4-1 describes the default roles provided with the software.
3. While ensuring the appropriate role is highlighted in the **Roles** pane, in the **Permissions** pane select the permissions to match the selected role. Table 4-2 describes the permissions available for Multi-Mode Analysis Software.
4. Repeat steps 2 and 3 for each role being configured.
5. When all roles are configured as desired, select the **Accounts** tab.
6. In the **Accounts** tab, click the **Create Account** button or select an existing user account.
7. While ensuring the appropriate role is highlighted in the **Accounts** pane, in the **Roles** pane, select the desired roles to assign to the account (Figure 4-3).

Table 4-1 Multi-Mode Analysis Software Default Roles

Role	Description
Lab Administrator	Contains all Multi-Mode Analysis Software permissions. Users assigned this role may perform all software actions.
Protocol Developer	Contains the Multi-Mode Analysis Software Copy, Create, and Edit permissions. Users assigned this role may create and edit protocols, labware, and detection methods, but may not sign or delete items.
Lab Technician	No Multi-Mode Analysis Software permissions are assigned. Users assigned this role may run protocols and view measurement results, but not create, edit, delete, or sign items or change instrument settings.

Table 4-2 Multi-Mode Analysis Software Permissions

Permission	Description
Add Instruments	Allows users to manually add new simulated instruments to the Software. **Note:** Auto-detected instruments are added to the list automatically when they are connected.
Add, edit, delete License Code	Allows users to add, edit, or delete license codes.
Change current Instrument	Allows user to set the current instrument. See Configuring the Current Instrument on page 54.
Copy Protocol, Labware, Method	Allows users to make copies of protocols, labware types, and detection methods.
Create Protocol, Labware, Method	Allows users to create protocols, labware types, and detection methods.
Delete all Items from Trash	Allows users to permanently remove all items in the Trash Selection List from the database.
Delete Protocol, Labware, Method, Result, Trash	Allows users to delete protocols, labware types, detection methods, results, and permanently remove individual items in the Trash Selection List from the database.
Edit Instrument Setting	Allows users to edit Instrument Settings. See Configuring and Controlling Instruments.
Edit Protocol, Labware, Method, Result	Allows users to edit protocols, labware types, detection methods, and results.
Optimize Labware	Allows users to optimize labware. See Optimizing Labware on page 137.
Sign Protocol, Labware, Method, Result	Allows users to electronically sign protocols labware types, detection methods, and results. See Adding Electronic Signatures and Comments to Items on page 84.
View Audit Entry	Allows users to view the Multi-Mode Analysis Software audit log using the Audit Viewer. See Viewing and Searching the Multi-Mode Analysis Software Audit Log on page 83.

Restoring the Administrator Password

Only one GxP Permissions administrator account exists on a system with GxP Permissions installed. If the administrator password is lost or forgotten, technical support must be contacted to restore access to the Account Management application.

To restore the administrator password:

1. Place the GxP Permissions CD in the drive, and browse to the contents of the CD.
2. Double-click on **Administrator Password Restore.exe**. The Administrator Password Restore dialog appears (Figure 4-4).

Figure 4-4 Administrator Password Restore Dialog

3. Contact technical support and provide the code displayed in the upper field of the Administrator Password Restore dialog.

CAUTION! Leave the Administrator Password Restore dialog open until technical support supplies a new code. The new code is based on the code displayed in the upper field, which changes each time the Administrator Password Restore dialog is opened.

4. In the lower field of the Administrator Password Restore dialog, enter the new code provided by technical support.
5. Click **OK** to close the Administrator Password Restore dialog and accept the new code.
6. Follow any additional instructions provided by technical support.

Viewing the System Activity Audit Log

The system administrator may view the Audit Log, which displays the audit trail for all user activity in software applications that support GxP Permissions. System activity is logged, even when GxP Permissions is set to **No Support**. See Enabling GxP Permissions on page 73.

Note: Other than changing the level of support for GxP Permissions, Administrator activity is not saved in the Audit Log. Administrator activity is viewed in the Audit tab of the Account Management application.

Note: User activity within Multi-Mode Analysis Software may be viewed in the Audit Viewer. See Viewing and Searching the Multi-Mode Analysis Software Audit Log on page 83.

To view the Audit Log:

1. In the Windows Start menu, select **Settings > Control Panel**. the Control Panel window appears.
2. In the Control Panel, double-click on **Administrative Tools**. The Administrative Tools window appears.
3. In the Administrative Tools window, double-click on **Audit Log**. The Audit Log appears (Figure 4-5).

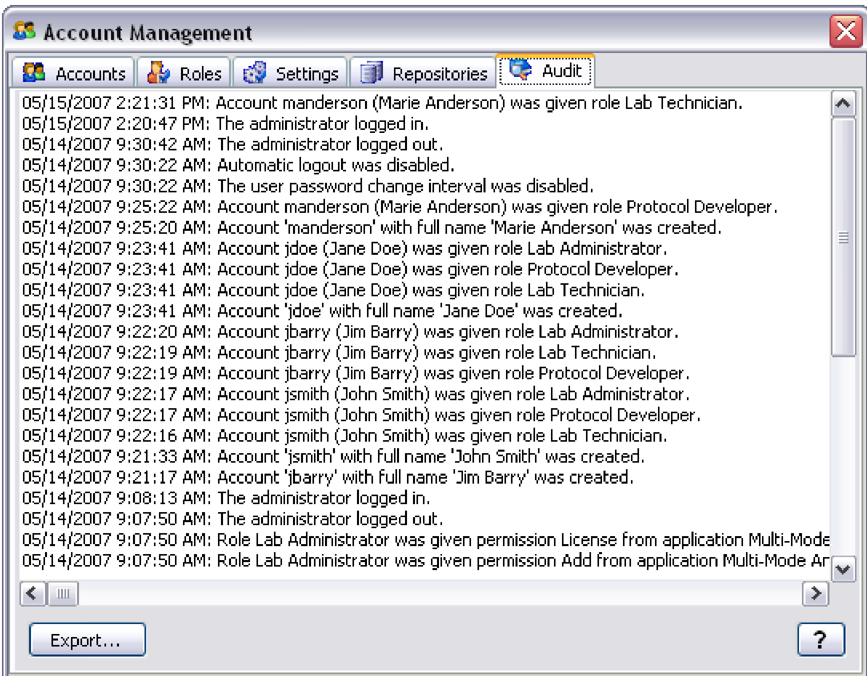


Figure 4-5 GxP Permissions Audit Log

4. As desired, click **Export** to export the entire audit log to a text file. The exported file may be opened, read, and printed in any application that supports text files.

Note: When the Audit Log is open while applications that support GxP Permissions are open, system activity is logged, but not refreshed on the screen automatically.

Performing GxP Permissions User Actions in Multi-Mode Analysis Software

When GxP Permissions is enabled, users are required to log in to Multi-Mode Analysis Software. Permissions configured in the roles assigned to accounts determine which actions are available to users. The User Selection List provides access to GxP Permissions actions (Figure 4-6).

GxP Permissions user actions include:

- Logging On and Off the System on page 81
- Changing the Current User Password on page 82
- Viewing and Searching the Multi-Mode Analysis Software Audit Log on page 83
- Reactivating Disabled Message Boxes on page 83
- Adding Electronic Signatures and Comments to Items on page 84

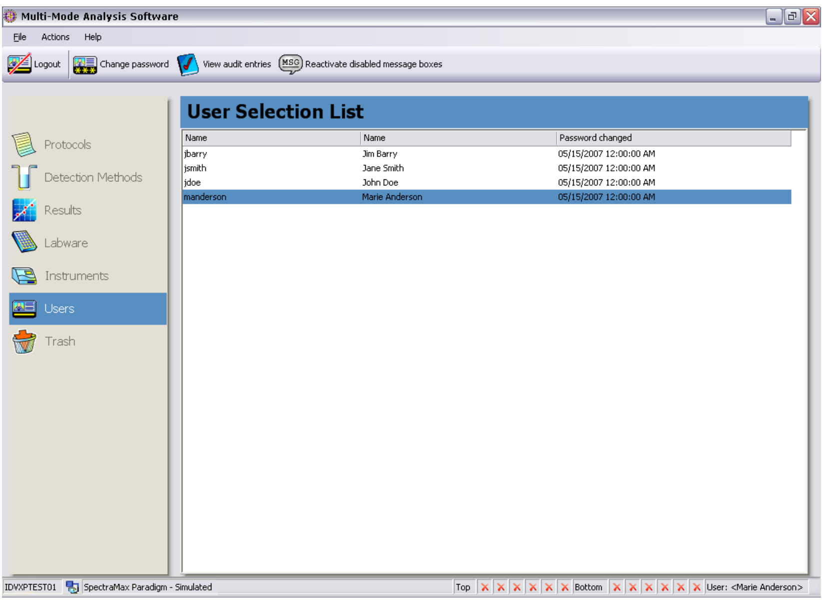


Figure 4-6 Accessing User Actions

Logging On and Off the System

When GxP Permissions is enabled, users must log on before Multi-Mode Analysis Software or other installed applications that support GxP Permissions may be accessed. Only one user may be logged onto the system at time. Once logged in, the current user may access all applications installed on the system supported by GxP Permissions.

This section covers:

- Logging On the System on page 81
- Logging Off the System on page 82
- Handling Disabled Accounts on page 82

Logging On the System

When the software launches or is idle with no user logged on, the Logon dialog appears.

To log on the system:

1. Enter the desired account User Name and Password.

Note: The first time a user logs on using a new account, or after having the password changed by the administrator, the Multi-Mode Analysis Software prompts for the password to be changed.

The new password must be different from the original and may include alphanumeric characters and spaces up to 250 characters in length. Passwords are not case sensitive.

2. Click **OK**. The user is logged on to the Multi-Mode Analysis Software and any other supported software applications installed on the system. The current user's full name appears in the title bar next to instrument status (Figure 4-6).

Logging Off the System

The current user must log off the system before another user may log on. Logging off automatically logs the user off all installed applications that support GxP Permissions.

To log off the system and close the software:

- Select **File > Exit**.

To log off the system and leave the software open for the next user:

1. In the navigation pane, click **Users** to access the User Selection List.
2. From the tool bar, click **Log out current user**. The user is logged out of the Multi-Mode Analysis Software and any other supported software applications installed on the system.

   OR

   From the menu bar select **Actions > Log out current user**.

   OR

   Right-click in the User Selection List and select **Log out current user**.

Handling Disabled Accounts

The administrator may manually disable user accounts in Account Management, or configure accounts to be automatically disabled after a number of logon attempts for the account fail.

When an account is automatically disabled, an Administrator Notification appears. The administrator password must be entered before Multi-Mode Analysis Software may be accessed by the disabled account.

Changing the Current User Password

The user currently logged into the system may change their password.

To change the password:

1. In the navigation pane, click **Users** to access the User Selection List.
2. From the tool bar, click **Change password of user currently logged in**. The Change Password dialog appears.

   OR

   From the menu bar select **Actions > Change password of user currently logged in**.

   OR

   Right-click in the User Selection List and select **Change password of user currently logged in**.
3. In the upper field, enter the new password desired.
4. In the lower field, re-enter the new password to confirm.
5. Click **OK** to change the password.

Viewing and Searching the Multi-Mode Analysis Software Audit Log

Users assigned a role with the View Audit Entry permission may view or search the audit log of user activity within Multi-Mode Analysis Software. Refer to Table 4-2 for more information about the permissions available to users.

The Multi-Mode Analysis Software audit log lists actions performed within the software only. To view a log of system-wide GxP Permissions activity, use the system Audit Log. See Viewing the System Activity Audit Log on page 79.

To view records in the log or search for specific records:

1. In the navigation pane, click **Users** to access the User Selection List.
2. From the tool bar, click **View the audit log for this software**. The Audit Viewer appears.

   OR

   From the menu bar select **Actions > View the audit log for this software**.

   OR

   Right-click in the User Selection List and select **View the audit log for this software**.
3. In Audit Viewer, perform the action desired. Table 4-3 describes the actions available.

Table 4-3 Audit Log Actions

Action	Description
Close	Close the Audit Viewer.
Export	Export records currently displayed in the Audit Viewer to an XML file.
Search Criteria	Enter the desired search terms and click Go. All records containing matching terms are listed. Searching for terms within records may also be limited to specific dates configured in Date from...to.
Date from...to	Limit the records displayed to those falling between the specified dates. Searching for records by date may also be combined with terms entered in Search Criteria.

Reactivating Disabled Message Boxes

Message boxes that have been disabled by users may be reactivated.

Note: Message boxes are disabled by selecting **Don't show this message again** when the message box is displayed.

To reactivate disabled dialog boxes:

1. In the navigation pane, click **Users** to access the User Selection List.
2. From the tool bar, click **Reactivate Disabled Message Boxes**.

   OR

   From the menu bar select **Actions > Reactivate Disabled Message Boxes**.

   OR

   Right-click in the User Selection List and select **Reactivate Disabled Message Boxes**.
3. The Message dialog appears, confirming that the message boxes are reactivated.

Adding Electronic Signatures and Comments to Items

Protocols, measurement results, detection methods, and labware types may signed be by users assigned a role with the Sign permission. Signing an item adds a user's comments and electronic signature to the audit trail and prevents the item from being edited or deleted. An item may be signed multiple times by multiple users.

Users assigned a role with the Sign permission may also view existing signatures for signed items and unlock any signature attached to an item. An unlocked signature is deactivated and moved from Signatures to History; signatures are never permanently removed from the system. When all signatures for an item are unlocked, the item is no longer signed and may be edited again.

To sign an item or view or unlock an existing electronic signature:

1. From the desired Selection List, select the item to sign.
2. From the tool bar, click **Sign the selected <item>**.
   OR
   From the menu bar select **Actions > Sign the selected <item>**.
   OR
   Right-click on the selected item and select **Sign the selected <item>**.
3. The Sign the Selected Item dialog appears (Figure 4-7).

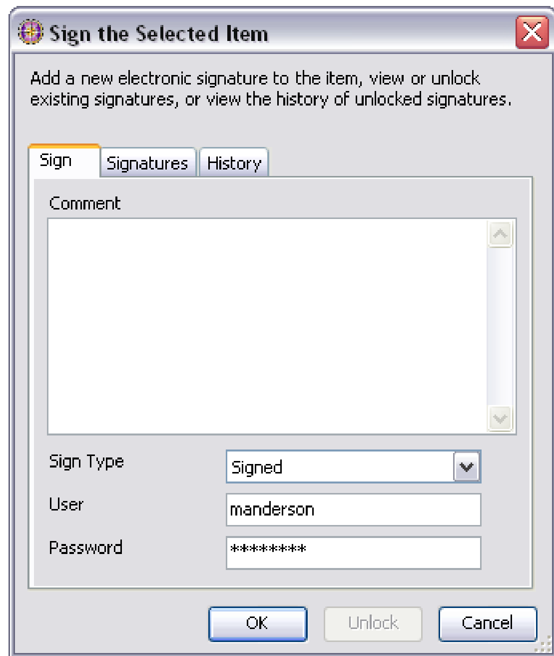


Figure 4-7 Signing an Item

4. Select the desired action:
   - Sign the item. See Signing Items on page 85.
   - View or unlock an existing signature. See Viewing or Unlocking Signatures for an Item on page 85.
   - View unlocked signatures. See Viewing Unlocked Signatures on page 86.

Signing Items

To sign an item:

1. In the Sign tab, enter a Comment. A comment is required to complete an electronic signature.
2. Select the type of signature: **Sign** or **Approved**. The selected type is saved in the audit trail.
3. If the **Password** field is visible, enter the password for the user account.

Note: Passwords are required only when GxP Permissions, with password checks for signing and check-in is the selected support level. See Enabling GxP Permissions on page 73.

4. Click **OK** to sign the item.

Viewing or Unlocking Signatures for an Item

Users assigned a role with the Sign permission may view active signatures for an item in the Signatures tab. Users with the Sign permission may also unlock any signature attached to an item. An unlocked signature is deactivated and moved from Signatures to History. Signatures are never permanently removed from the system. Unlocking all signatures attached to an item allows the item to be edited again.

To view existing signatures:

1. Select the desired item and open Sign the Selected Item following the steps in Adding Electronic Signatures and Comments to Items on page 84.
2. Select the **Signatures** tab (Figure 4-8).

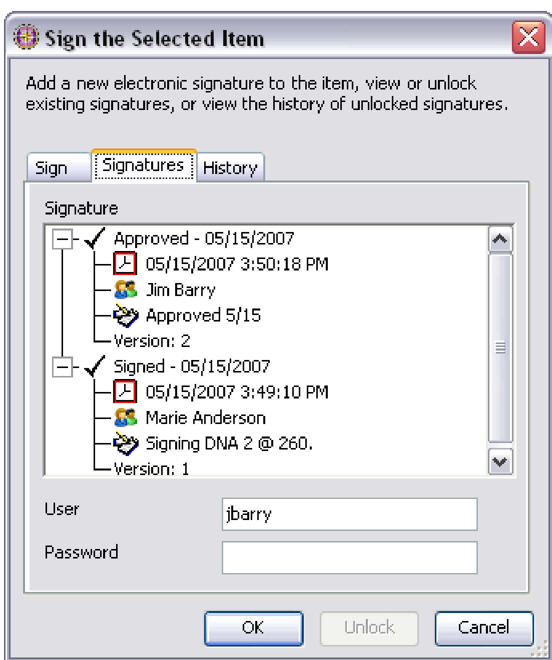


Figure 4-8 Viewing Active Signatures

3. Click on the **+** or - to the left of a signature to expand or collapse details about the signature. Details for each signature are listed on four lines:
   - date and time signature was entered
   - the full name of the user signing the item
   - user comments
   - version of the item
4. If the **Password** field is visible, enter the password for the user account.

Note: Passwords are required only when GxP Permissions, with password checks for signing and check-in is the selected support level. See Enabling GxP Permissions on page 73.

5. To unlock the selected signature, click **Unlock**. Unlocked signatures can be viewed in the History tab.

Viewing Unlocked Signatures

Unlocked signatures are never permanently removed from the system. Users assigned a role with Sign permission may view unlocked signatures for an item in the History tab.

1. Select the desired item and open the Sign the Selected Item dialog following the steps in Adding Electronic Signatures and Comments to Items on page 84.
2. Select the **History** tab.
3. Click on the **+** or - to the left of a signature to expand or collapse details about the signature. Details for each signature are listed on four lines:
   - date and time the signature was created
   - date and time the signature was unlocked
   - the full name of the user signing the method
   - comments entered by the use
   - version of the item

Creating and Editing Detection Methods

Overview

Multi-Mode Analysis Software stores measurement configuration parameters in detection methods. Stored parameters include the method technique (for example, absorbance), FilterMax Filters or SpectraMax Paradigm Detection Cartridges used, and parameters specific to the selected method, such as integration time. The software supports absorbance, luminescence, and fluorescence method techniques. The method techniques available for configuration depend on the capabilities of the instrument being controlled.

Detection methods are created and edited using the Method Editor. Configured detection methods are listed in the Detection Method Selection List and are available for use in measurement protocols. See Creating and Running Protocols on page 145.

Note: When GxP Permissions is enabled on the system, only users assigned with Sign, Copy, Create, Delete, and Edit permissions may perform all of the actions covered in this section. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

Viewing Available Detection Methods

To view available detection methods and access the Method Editor:

From the navigation pane, select **Detection Methods**. The Detection Method Selection List appears (Figure 5-1) containing detection methods for the currently selected instrument. To change the current instrument see Configuring the Current Instrument on page 54.

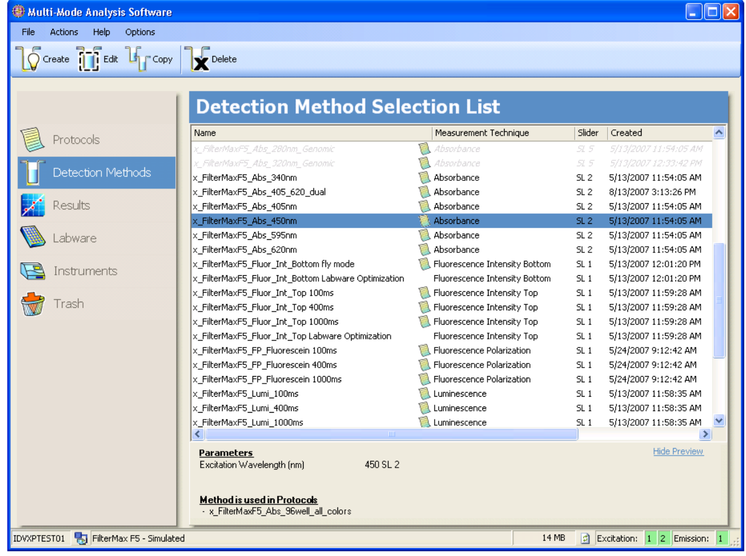


Figure 5-1 Accessing Detection Method Actions

Note: For the SpectraMax Paradigm Multi-Mode Detection Platform, detection methods will only display as enabled if the detection cartridge is listed within **My Detection Cartridges**. See Defining and Editing the Available Detection Cartridges on page 66.

All detection method functions are accessed from the Detection Method Selection List. Available detection method functions are:

- Creating Detection Methods (FilterMax Multi-Mode Microplate Readers) on page 88
- Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform) on page 99
- Editing Detection Methods on page 123
- Copying Detection Methods on page 124
- Deleting Detection Methods on page 124
- Exporting and Importing Detection Methods on page 125

Creating Detection Methods (FilterMax Multi-Mode Microplate Readers)

Detection methods are created in the Method Editor, which guides the creation process with a wizard-type interface. Creating a new detection method requires:

- Selecting a Method Technique (FilterMax Multi-Mode Microplate Readers) on page 89
- Selecting the Method Type (FilterMax Multi-Mode Microplate Readers) on page 90

  The Method Type is configured for absorbance detection methods only
- Defining Method Parameters (FilterMax Multi-Mode Microplate Readers) on page 91
- Signing a Detection Method (FilterMax Multi-Mode Microplate Readers) on page 98 (Optional)

  This is used to prevent methods from being edited or deleted. Methods can be signed only when GxP Permissions is enabled on the system.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Create permission may create new detection methods.

To sign detection methods, users must be assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To create and define a new detection method:

1. Click the **Detection Methods** selection list button.
2. From the tool bar, click **Create**.

   OR

   From the menu bar select **Actions > Create a new method**.

   OR

   Right-click in the Detection Method Selection List and select **Create a new method**.
3. The Method Editor appears (Figure 5-2).

Selecting a Method Technique (FilterMax Multi-Mode Microplate Readers)

In the Method Editor, the type of detection method to create is selected in Method Technique (Figure 5-2). Only techniques supported by the instrument currently selected in the Instrument Selection List are available for configuration. See Configuring and Controlling Instruments on page 51.

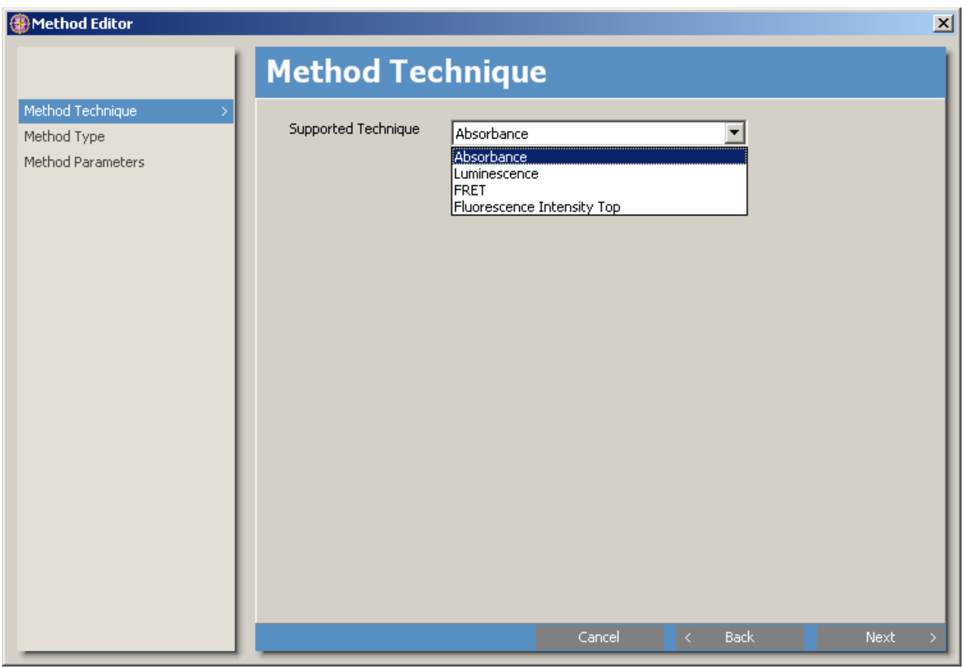


Figure 5-2 Selecting a Method Technique

To select a method technique:

1. In Supported techniques, select the desired detection method.
2. If defining an absorbance method, click **Next** to select the Method Type. See Selecting the Method Type (FilterMax Multi-Mode Microplate Readers) on page 90.

   OR

   If defining a luminescence or fluorescence method, click **Next** to define Method Parameters. See Defining Method Parameters (FilterMax Multi-Mode Microplate Readers) on page 91.

Selecting the Method Type (FilterMax Multi-Mode Microplate Readers)

When defining an absorbance detection method, use Method Type to select whether a monochromatic or bichromatic method will be defined (Figure 5-3).

Note: Method Type appears only when defining absorbance methods.

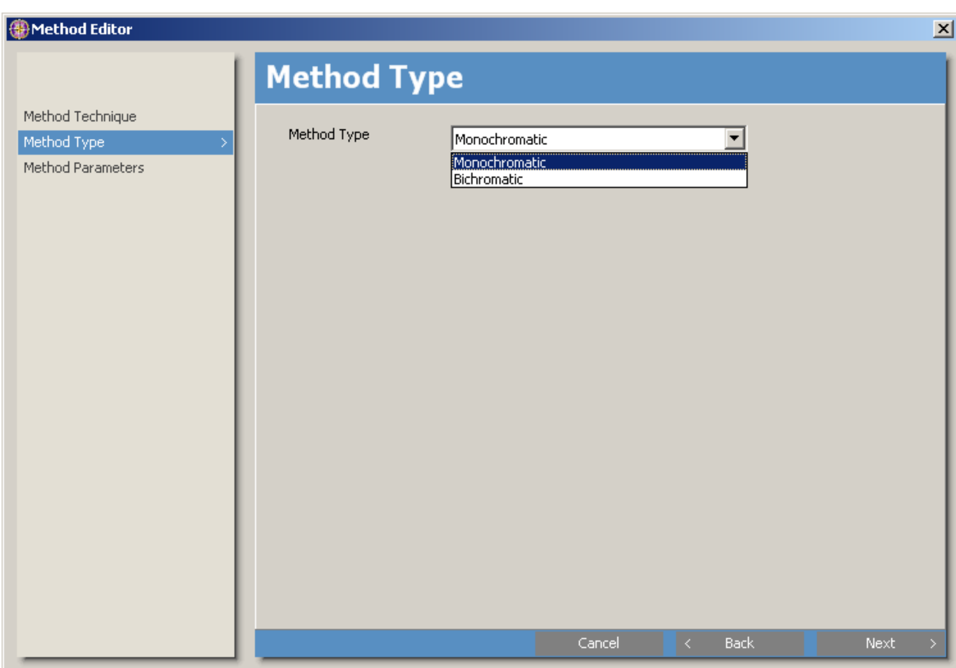


Figure 5-3 Selecting an Absorbance Method Type

To select a method type:

1. In the Method Editor, click on **Method Type**.
2. Select the desired method: **Monochromatic** or **Bichromatic**.

   If you want to use PathCheck® Pathlength Measurement Technology, select **Monochromatic**. See PathCheck® Pathlength Measurement Technology on page 245.

Note: Monochromatic methods perform a single-wavelength measurement.

Bichromatic methods perform a second measurement at a reference wavelength, which is subtracted from the first to calculate the final result.

3. Click **Next** to define Method Parameters. See Defining Method Parameters (FilterMax Multi-Mode Microplate Readers) on page 91.

Defining Method Parameters (FilterMax Multi-Mode Microplate Readers)

Detection method parameters, such as filters and integration time, are defined in Method Parameters. The parameters available for configuration depend on the technique selected in Method Technique. See Selecting a Method Technique (FilterMax Multi-Mode Microplate Readers) on page 89.

Use Method Parameters for:

- Defining Absorbance Method Parameters on page 91
- Defining Luminescence Method Parameters on page 92
- Defining Fluorescence Intensity Top Method Parameters on page 93
- Defining Fluorescence Intensity Bottom Method Parameters (FilterMax 5 Multi-Mode Microplate Reader only) on page 95
- Defining Fluorescence Polarization Method Parameters (FilterMax 5 Multi-Mode Microplate Reader only) on page 96
- Defining Time-Resolved Fluorescence Method Parameters (FilterMax 5 Multi-Mode Microplate Reader only) on page 97

Defining Absorbance Method Parameters

A monochromatic absorbance method performs an absorbance measurement at a single wavelength. A bichromatic method performs a second measurement at a reference wavelength. This measurement is subtracted from the first to calculate the final result.

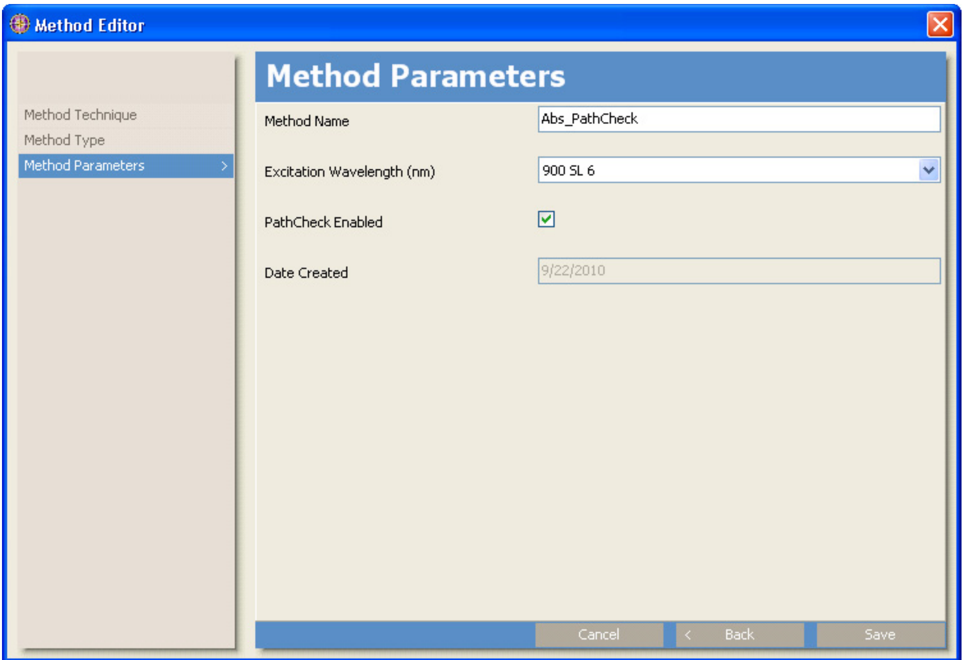


Figure 5-4 Defining Absorbance Method Parameters

To define absorbance method parameters:

1. Enter a **Method Name** (Figure 5-4).

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example an absorbance measurement @ 260 ms might read ABS_260.

2. In the **Excitation Wavelength (nm)** field, select the measurement filter.

   The PathCheck Pathlength Measurement Technology requires a filter of 900 nm and 998 nm. See Defining and Editing Filter Slides on page 58.

3. If a bichromatic measurement is being defined, in the **Reference Excitation Filter (nm)** field, select the reference filter.

Note: The filters available are those installed on the excitation filter slide loaded in the instrument and configured for absorbance techniques in Instrument Settings. See Defining and Editing Filter Slides on page 58.

4. To enable PathCheck Pathlength Measurement Technology, select the **PathCheck Enabled** check box. See PathCheck® Pathlength Measurement Technology on page 245.
5. Click **Save** to save the new absorbance detection method. The new method appears in the Detection Method Selection List.

Defining Luminescence Method Parameters

A luminescence method performs glow luminescence measurements on samples. Generally, luminescence measurements do not require a filter; however, cutoff filtration using an emission filter may be specified to eliminate photoluminescence generated by the microplate itself, if desired.

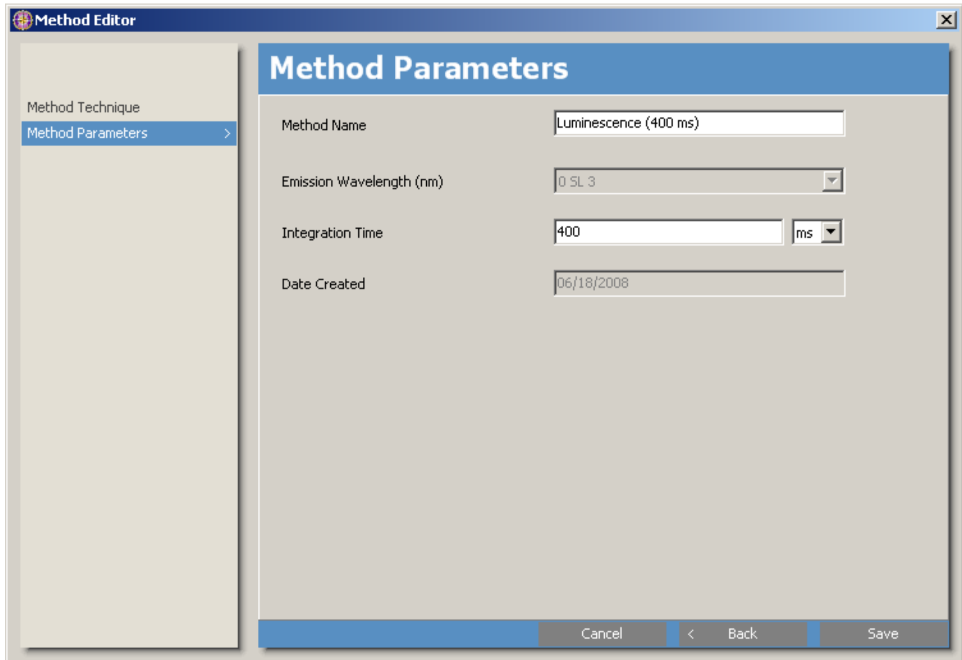


Figure 5-5 Defining Luminescence Method Parameters

To define luminescence method parameters:

1. Enter a **Method Name** (Figure 5-5).

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example a luminescence measurement @ 400 ms might read LUM_400.

2. In the **Emission Filter (nm)** field, select 0 when no cutoff filtration is desired. Most luminescence measurements do not require cutoff filtration.

   OR

   In the **Emission Filter (nm)** field, select the cutoff filter.

Note: The filters available are those installed on the emission filter slide loaded in the instrument and configured for luminescence techniques in Instrument Settings. See Defining and Editing Filter Slides on page 58.

3. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) the signal is collected from samples. Set the integration time within the range of 50 μseconds to 3600 seconds.
4. Click **Save** to save the new luminescence detection method. The new method appears in the Detection Method Selection List.

Defining Fluorescence Intensity Top Method Parameters

In a fluorescence intensity top method, the source light is focused by an objective lens and directed through an excitation filter above the plate. The filter passes only the wavelength necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by the photo multiplier tube. When performing a fluorescence intensity top method on a FilterMax 5 Multi-Mode Microplate Reader, excitation of samples from below the plate is stopped.

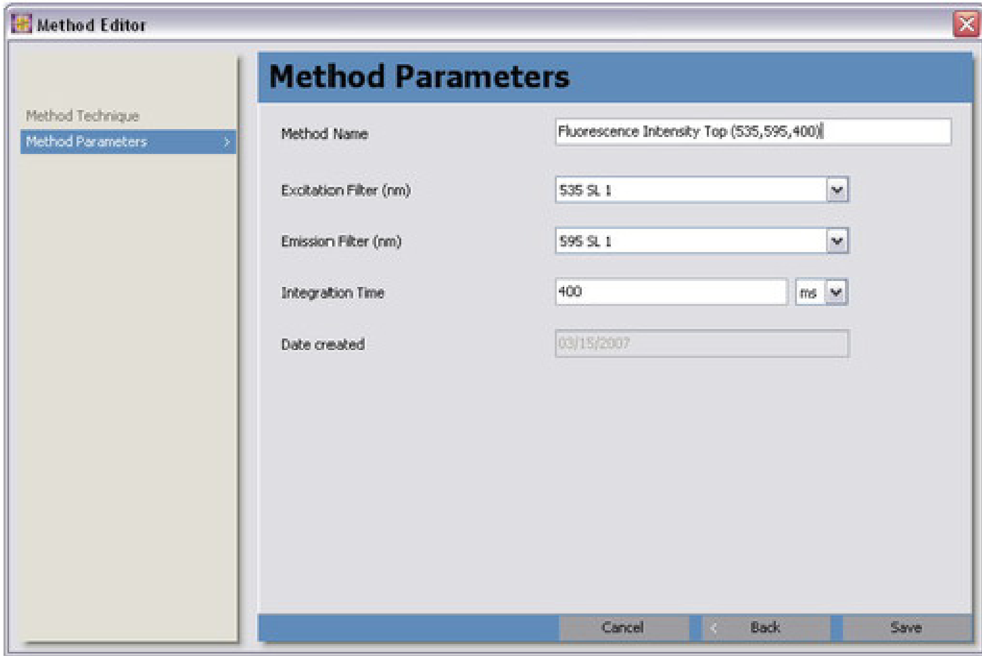


Figure 5-6 Defining Fluorescence Intensity Top Method Parameters

To define fluorescence intensity top method parameters:

1. Enter a **Method Name** (Figure 5-5).

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example a fluorescence intensity top measurement @ 400 ms might read FI_T_400.

2. Select the **Excitation Filter (nm).**
3. Select the **Emission Filter (nm).**

Note: The filters available are those installed on the slides loaded in the instrument and configured for fluorescence techniques in Instrument Settings. See Defining and Editing Filter Slides on page 58.

4. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) the signal is collected from samples. Set the integration time within the range of 50 µseconds to 3600 seconds.
5. Click **Save** to save the new fluorescence intensity top detection method. The new method appears in the Detection Method Selection List.

Defining Fluorescence Intensity Bottom Method Parameters (FilterMax 5 Multi-Mode Microplate Reader only)

In a fluorescence intensity bottom method, the source light is directed through an excitation filter, which passes only the wavelengths necessary to excite samples, and focused by an objective lens below the plate. The objective lens collects the resulting fluorescence from below the plate and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by the photo multiplier tube. When performing a fluorescence intensity bottom method on a FilterMax 5 Multi-Mode Microplate Reader, excitation of samples from above the plate is stopped.

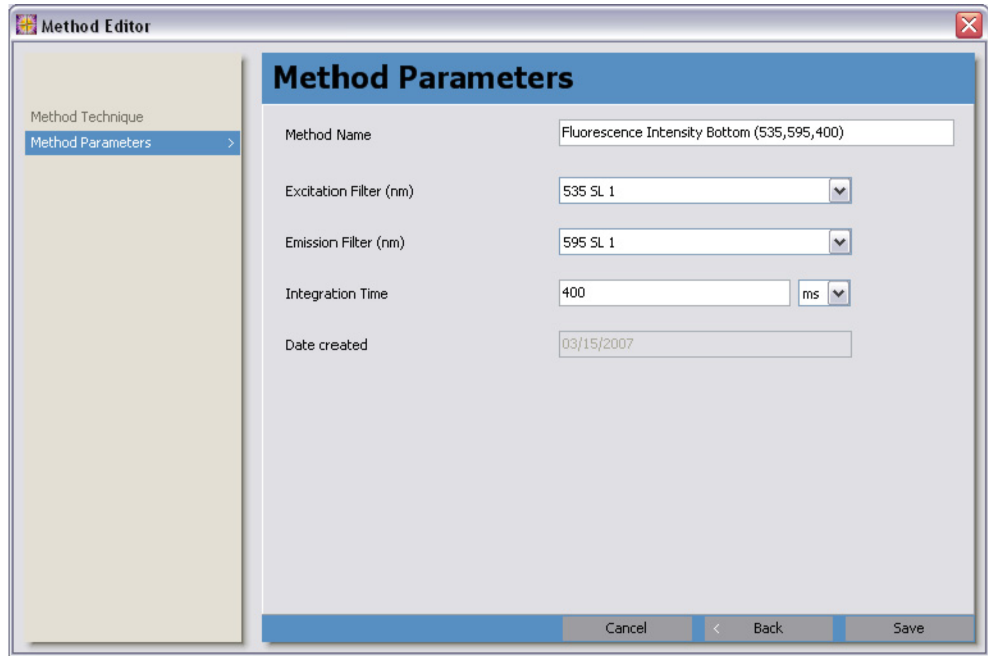


Figure 5-7 Defining Fluorescence Intensity Bottom Method Parameters

To define fluorescence intensity bottom method parameters:

1. Enter a **Method Name** (Figure 5-7).

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example a fluorescence intensity bottom measurement @ 400 ms might read FI_B_400.

2. Select the **Excitation Filter (nm).**
3. Select the **Emission Filter (nm).**

Note: The filters available are those installed on the slides loaded in the instrument and configured for fluorescence techniques in Instrument Settings. See Defining and Editing Filter Slides on page 58.

4. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) the signal is collected from samples. Set the integration time within the range of 50 µseconds to 3600 seconds.

5. Click **Save** to save the new fluorescence intensity bottom detection method. The new method appears in the Detection Method Selection List.

Defining Fluorescence Polarization Method Parameters (FilterMax 5 Multi-Mode Microplate Reader only)

A fluorescence polarization method measures two orthogonal (perpendicular) polarization states by performing two sequential fluorescence intensity measurements from above the plate.

Light is directed through an excitation filter (which passes only the wavelength necessary for excitation) and a polarizing filter. The fluorescence resulting from the excitation of the sample is passed through two emission filters equipped with polarizing filters to distinguish the parallel and perpendicular polarization states.

The polarized signals are then detected sequentially by the photo multiplier tube.

Note: The read direction settings configured in a protocol determine when polarization states are measured during a run. See Configuring Labware Layout Settings on page 151.

When **Read by well** is selected, both states are measured for each sample before proceeding to the next sample. When **Read by row** or **Read by column** is selected, the parallel states are measured for all samples in a row or column before measuring the perpendicular states of the same group of samples.

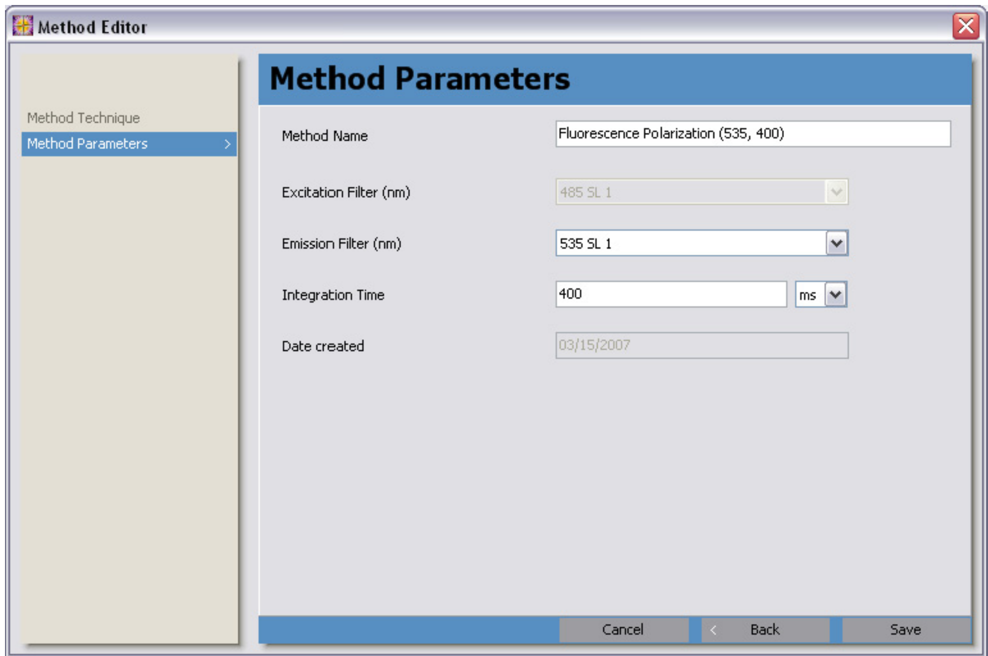


Figure 5-8 Defining Fluorescence Polarization Method Parameters

To define fluorescence polarization parameters:

1. Enter a **Method Name** (Figure 5-8).

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example an fluorescence polarization measurement @ 400 ms might read FP_400.

2. Select the **Excitation Wavelength (nm).**
3. Select the **Emission Wavelength (nm).**

Note: The wavelength filters available are those installed on the excitation and emission wavelength filter slides loaded in the instrument and configured for fluorescence polarization techniques in Instrument Settings. See Defining and Editing Filter Slides on page 58.

4. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) the signal is collected from samples. Set the integration time within the range of 50 µseconds to 3600 seconds.
5. Click **Save** to save the new fluorescence polarization detection method. The new method appears in the Detection Method Selection List.

Defining Time-Resolved Fluorescence Method Parameters (FilterMax 5 Multi-Mode Microplate Reader only)

In a time-resolved fluorescence measurement, the excitation light source is turned off and the measurement is performed from above the plate after a specified delay. Several of these excitation/measurement cycles may be performed on each sample. When multiple excitation/measurement cycles are performed, the results from all cycles are used to calculate a single measurement result for each sample.

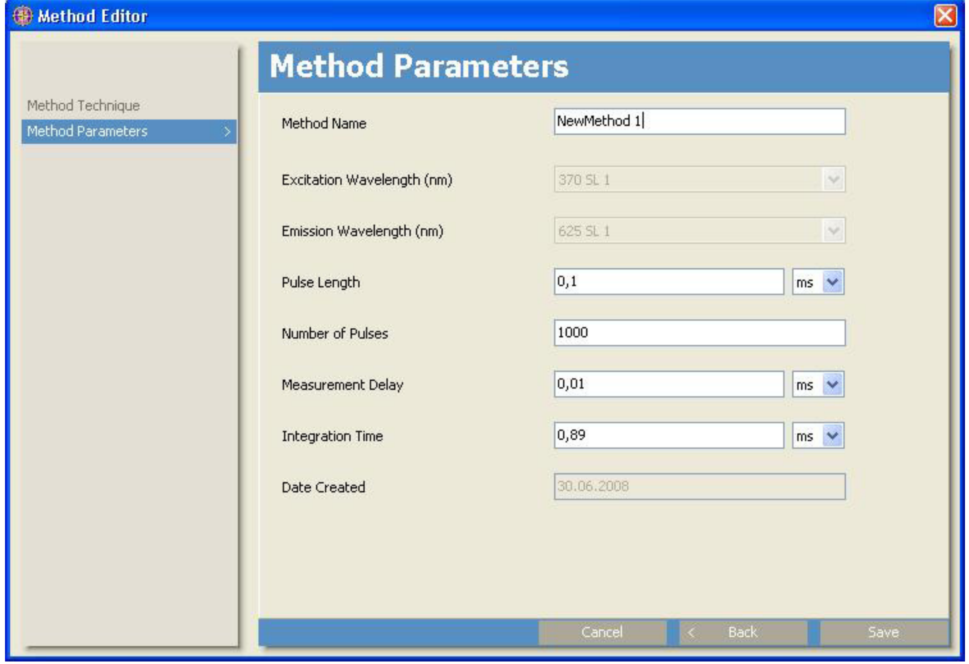


Figure 5-9 Defining Time-Resolved Fluorescence Method Parameters

To define time-resolved fluorescence parameters:

1. Enter a **Method Name** (Figure 5-9).

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example a time-resolved fluorescence measurement @ 0.89 ms might read TRF_0.89.

2. Select the **Excitation Wavelength (nm).**
3. Select the **Emission Wavelength (nm).**

Note: The wavelength filters available are those installed on the excitation and emission wavelength filter slides loaded in the instrument and configured for time-resolved fluorescence techniques in Instrument Settings. See Defining and Editing Filter Slides on page 58.

4. In the **Pulse Length** field, enter the length of time (in seconds, milliseconds, or microseconds) that the LED light source remains turned on.
5. In the **Number of Pulses** field, enter the number of excitation/measurement cycles performed for each well in the measurement. The number of pulses sets the exposure received by samples during measurements.
6. In the **Measurement Delay** field, enter the interval (in seconds, milliseconds, or microseconds) between switching off the light source and performing the measurement. Set the delay within the range of 1 µs to 7.5 ms.
7. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) each sample is measured. Set the integration time within the range of 50 µs to 7.5 ms.
8. Click **Save** to save the new time-resolved fluorescence detection method. The new method appears in the Detection Method Selection List.

Signing a Detection Method (FilterMax Multi-Mode Microplate Readers)

When GxP Permissions is enabled on the controlling computer system, detection methods may be signed to prevent methods from being edited or deleted. Detection methods may be signed at any time after the configuration is complete.

Detection methods may be signed by users who are assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To sign labware:

1. In the Detection Method Selection List, select the detection method to sign.
2. From the tool bar, click **Sign the selected method**.

   OR

   From the menu bar select **Actions > Sign the selected method**.

   OR

   Right click on the selected labware and select **Sign the selected method**.
3. The **Sign the Selected Item** dialog appears.
4. In the **Sign the Selected Item** dialog, add comments and an electronic signature by following the instructions in Adding Electronic Signatures and Comments to Items on page 84.

Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform)

Detection methods are created in the Method Editor, which guides the creation process with a wizard-type interface. Creating a new detection method requires:

- Selecting a Detection Cartridge (SpectraMax Paradigm Multi-Mode Detection Platform) on page 100
- Selecting the method technique, configuring the method type (absorbance detection methods only), and defining method parameters based upon the selected cartridge:
  - Absorbance (ABS) Detection Cartridge on page 101
  - Fluorescence Polarization (FP) Detection Cartridge on page 102
  - Multi-Mode (MULTI) Detection Cartridge on page 104
  - Fluorescence Intensity (FI) Detection Cartridge on page 109
  - Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge on page 110
  - Time Resolved Fluorescence (TRF) Detection Cartridge on page 112
  - Cisbio HTRF® Detection Cartridge on page 116
  - Luminescence (LUM) Detection Cartridge on page 118
  - AlphaScreen Detection Cartridge on page 121
- Signing a Detection Method (SpectraMax Paradigm Multi-Mode Detection Platform) on page 122 (Optional)

  This is used to prevent methods from being edited or deleted. Methods can only be signed when GxP Permissions is enabled on the system

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Create permission may create new detection methods.

To sign detection methods, users must be assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To create and define a new detection method:

1. From the tool bar, click **Create**.

   OR

   From the menu bar select **Actions > Create a new method**.

   OR

   Right-click in the Detection Method Selection List and select **Create a new method**.
2. The Method Editor appears (Selecting a Detection Cartridge (SpectraMax Paradigm Multi-Mode Detection Platform) on page 100).

Selecting a Detection Cartridge (SpectraMax Paradigm Multi-Mode Detection Platform)

In the Method Editor, the detection cartridges contained within **My Detection Cartridges** display in the detection cartridge list (Figure 5-10). Detection cartridges installed on the machine, but not within **My Detection Cartridges** are not displayed in the detection cartridge list. See Defining and Editing the Available Detection Cartridges on page 66.

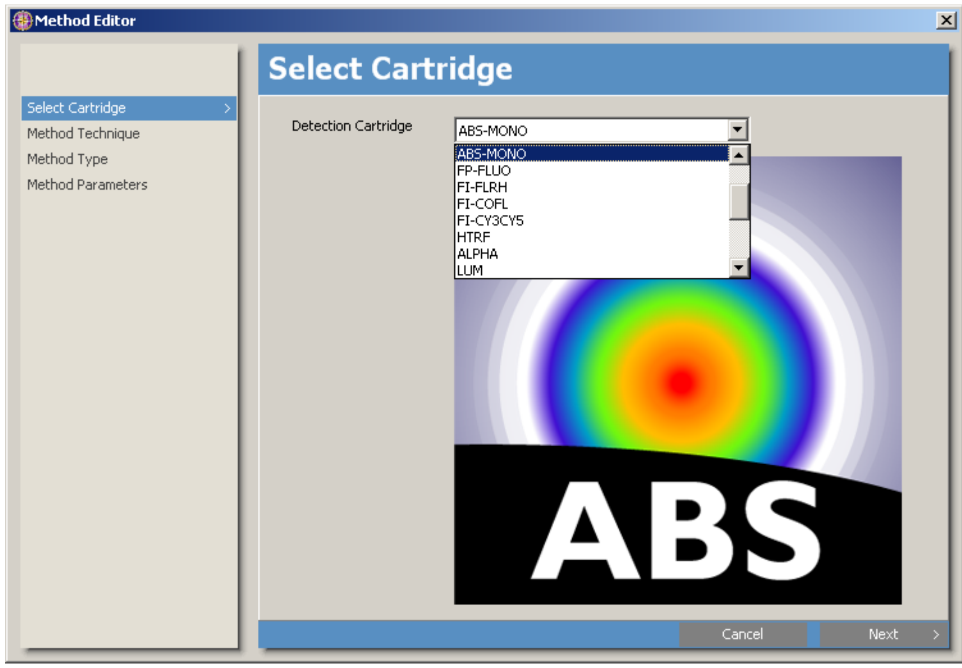


Figure 5-10 Selecting a Detection Cartridge

To select a detection cartridge:

1. In the **Detection Cartridge** field, select the desired detection cartridge.
2. Define the Method Technique, Method Type (for absorbance detection cartridges), and Method Parameters based upon the selected cartridge:
   - Absorbance (ABS) Detection Cartridge on page 101
   - Fluorescence Polarization (FP) Detection Cartridge on page 102
   - Multi-Mode (MULTI) Detection Cartridge on page 104
   - Fluorescence Intensity (FI) Detection Cartridge on page 109
   - Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge on page 110
   - Time Resolved Fluorescence (TRF) Detection Cartridge on page 112
   - Cisbio HTRF® Detection Cartridge on page 116
   - Luminescence (LUM) Detection Cartridge on page 118
   - AlphaScreen Detection Cartridge on page 121

Absorbance (ABS) Detection Cartridge

Note: For additional information regarding absorbance detection cartridges please see the detection cartridge’s user guide.

To create an absorbance detection method using an absorbance detection cartridge:

1. The only supported method technique using an absorbance detection cartridge is absorbance. Click **Next** in Method Technique to define the Method Type.
2. When defining an absorbance detection method, use **Method Type** to select whether a **Monochromatic** or **WavelengthScan** method will be defined (Figure 5-11).
   - A **Monochromatic** absorbance method performs an absorbance measurement at a single wavelength.
   - A **WavelengthScan** absorbance method performs a series of absorbance measurement scans within a specified range configured in the protocol.

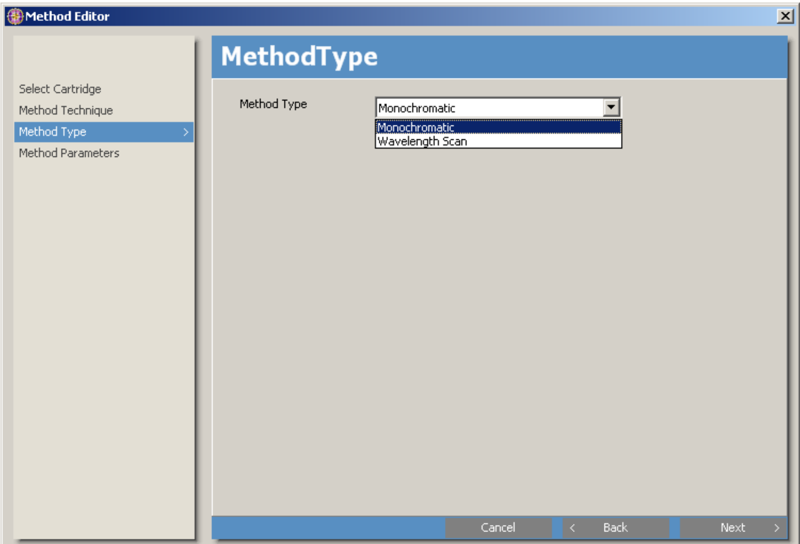


Figure 5-11 Absorbance Detection Cartridge Method Type

3. Click **Next** to define the Method Parameters.
4. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example a monochromatic measurement @ 260 might read ABS_260, while an Absorbance scan measurement between 260 -360 might read ABS_260-350 Scan.

5. For a **Monochromatic** absorbance method, in the **Excitation Wavelength (nm)** field, specify the wavelength.

   OR

   For a **Wavelength Scan** absorbance method, in the **Minimum Wavelength (nm)** and **Maximum Wavelength (nm)** fields, specify the wavelength.
6. To enable PathCheck Pathlength Measurement Technology, select the **PathCheck Enabled** check box. See PathCheck® Pathlength Measurement Technology on page 245.
7. Click **Save** to save the new absorbance detection method. The new method appears in the Detection Method Selection List.

Fluorescence Polarization (FP) Detection Cartridge

A fluorescence polarization method measures two orthogonal (perpendicular) polarization states by performing two simultaneous fluorescence intensity measurements from above the plate.

Light is directed through an excitation filter, which passes only the wavelength necessary for excitation, and a polarizing filter. The fluorescence resulting from the excitation of the sample is passed through two emission filters equipped with polarizing filters to distinguish the parallel and perpendicular polarization states.

The polarized signals are then detected simultaneously by the photo multiplier tubes.

Note: For additional information regarding the fluorescence polarization detection cartridges please see the detection cartridge's user guide.

To create a fluorescence polarization detection method using a fluorescence polarization detection cartridge:

1. The only supported method technique using a fluorescence polarization detection cartridge is fluorescence polarization. Click **Next** to define the Method Parameters (Figure 5-12).

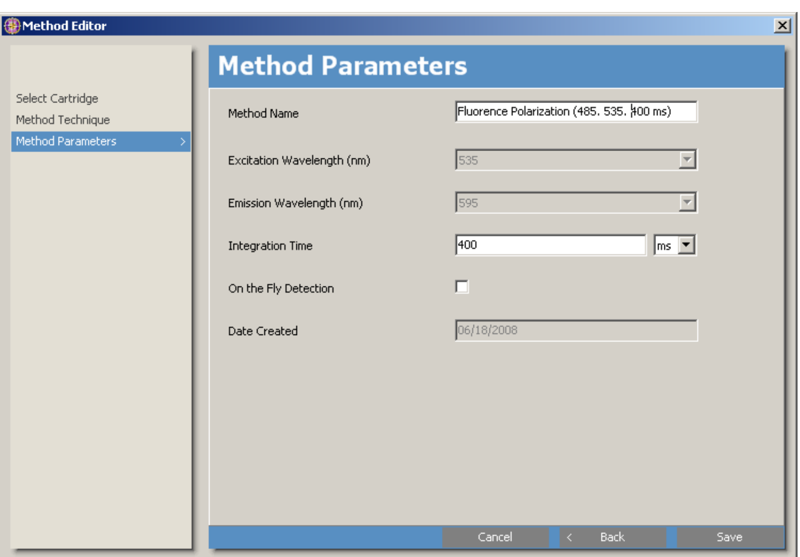


Figure 5-12 Fluorescence Polarization Detection Cartridge Method Parameters

2. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example an fluorescence polarization measurement @ 400 ms might read FP_400.

3. Optionally, to use on-the-fly detection select **On the Fly Detection**.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

4. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However there is a trade-off between the data quality (for example, CVs vs. dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

OR

Specify the measurement time per well (in seconds, milliseconds, or microseconds) using the **Integration Time** field.

5. Click **Save** to save the new fluorescence polarization detection method. The new method appears in the Detection Method Selection List.

Multi-Mode (MULTI) Detection Cartridge

The Multi-Mode detection cartridge allows for three detection techniques:

- **Luminescence:** A luminescence method performs glow luminescence measurements on samples.
- **Fluorescence Intensity:** In a fluorescence intensity method, the source light is focused by an objective lens and directed through an excitation filter above or below the plate. The filter passes only the wavelength necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by the photo multiplier tubes. When performing a fluorescence intensity method on a SpectraMax Paradigm Multi-Mode Detection Platform, excitation of samples from below the plate may be stopped depending on the mounting position of the Multi-Mode detection cartridge.
- **Time Resolved Fluorescence:** In a time-resolved fluorescence measurement, the excitation light source is turned off and the measurement is performed from above the plate after a specified delay. Several of these excitation/measurement cycles may be performed on each sample. When multiple excitation/measurement cycles are performed, the results from all cycles are used to calculate a single measurement result for each sample.

Note: It is necessary for the detection cartridge to be installed in the Upper Read Detection Cartridge Transport for Fluorescence Intensity Top Detection Methods and in the Lower Read Detection Cartridge Transport for Fluorescence Intensity Bottom Detection Methods.

Note: For additional information regarding the Multi-Mode detection cartridge please see the detection cartridge’s user guide.

To create a detection method using the Multi-Mode detection cartridge:

- In the supported technique select from **Luminescence**, **Fluorescence Intensity Top**, **Fluorescence Intensity Bottom**, or **Time Resolved Fluorescence** (Figure 5-13).

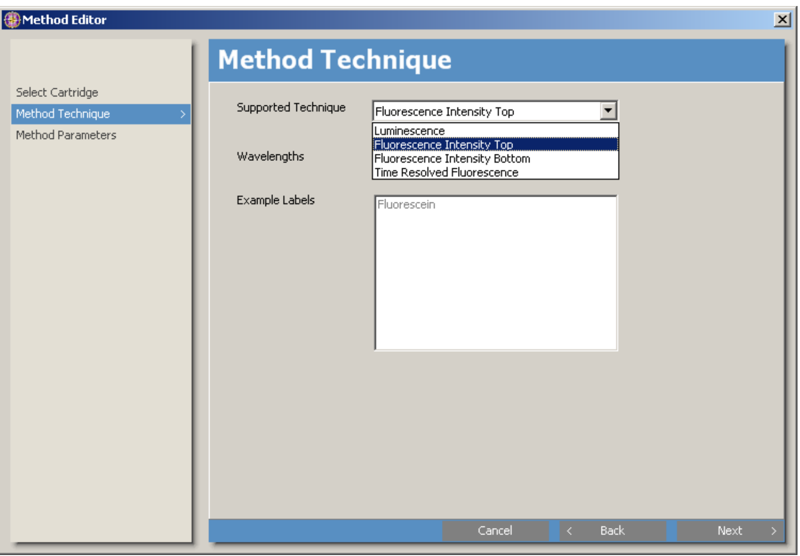


Figure 5-13 Multi-Mode Detection Cartridge Method Technique

For Luminescence (Figure 5-14):

1. Before using the Luminescence technique, determine whether to take measurements using the **On the Fly Detection** feature.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

2. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However there is trade-off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

3. If not using on-the-fly detection, specify the measurement time per well (in seconds, milliseconds, or microseconds) using the **Integration Time** field.
4. Click **Save** to save the new luminescence detection method. The new method appears in the Detection Method Selection List.

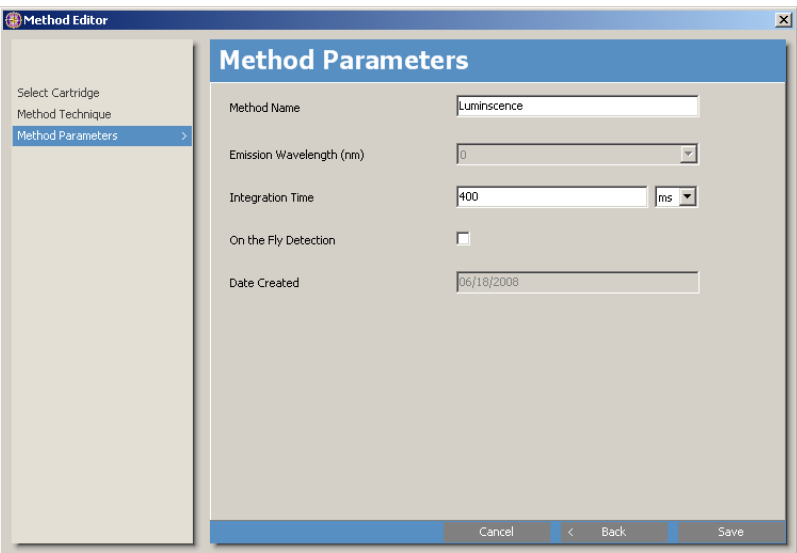


Figure 5-14 Luminescence Method Parameters

For Fluorescence Intensity Top or Fluorescence Intensity Bottom (Figure 5-15):

1. Before using the Fluorescence Intensity Top or Fluorescence Intensity Bottom techniques, determine whether to take measurements using the **On the Fly Detection** feature.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

2. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However there is trade-off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

3. If not using on-the-fly detection, specify the measurement time per well (in seconds, milliseconds, or microseconds) using the **Integration Time** field.
4. Click **Save** to save the new fluorescence intensity detection method. The new method appears in the Detection Method Selection List.

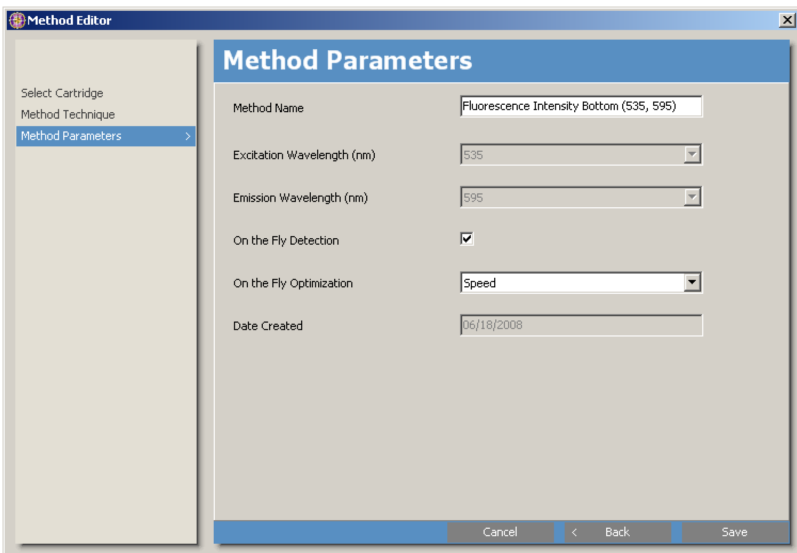


Figure 5-15 Fluorescence Intensity Bottom Method Parameters

For Time Resolved Fluorescence (Figure 5-17):

1. Before using the Time Resolved Fluorescence technique, determine whether to take measurements using the **On the Fly Detection** feature.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

2. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However there is trade-off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

3. In the **Method Name** field, enter a name for the method.
4. In the **Pulse Length** field, enter the length of time (in seconds, milliseconds, or microseconds) that the LED light source remains turned on (t_1 on Figure 5-16).
5. If **On the Fly Detection** is not selected, in the **Number of Pulses** field, enter the number of excitation/measurement cycles performed in the measurement. The number of pulses sets the exposure received by samples during measurements.
6. In the **Measurement Delay** field, enter the interval between (in seconds, milliseconds, or microseconds) switching off the light source and performing the measurement (t_2 on Figure 5-16).

7. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) each sample is measured. Set the integration time within the range of 50 µs to 7.5 ms (t_3 on Figure 5-16).

Note: Measurement Delay + Integration Time + Pulse Delay => Minimum Pulse Period ($t_2 + t_3 + t_4 => t_5$)

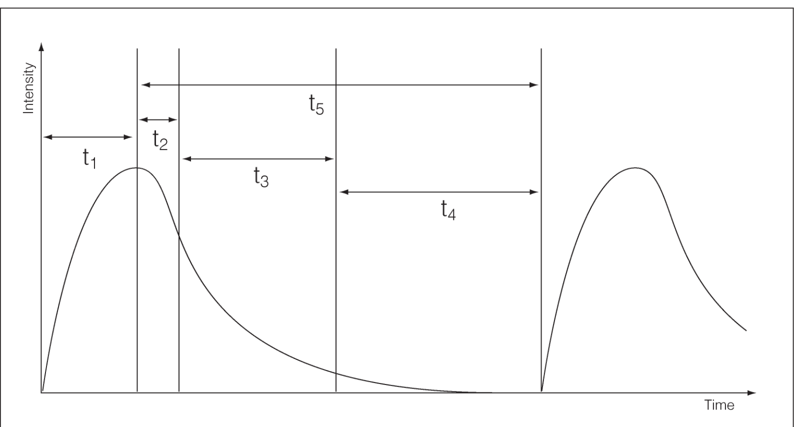


Figure 5-16 Time Resolved Fluorescence

8. Click **Save** to save the new time resolved fluorescence detection method. The new method appears in the Detection Method Selection List.

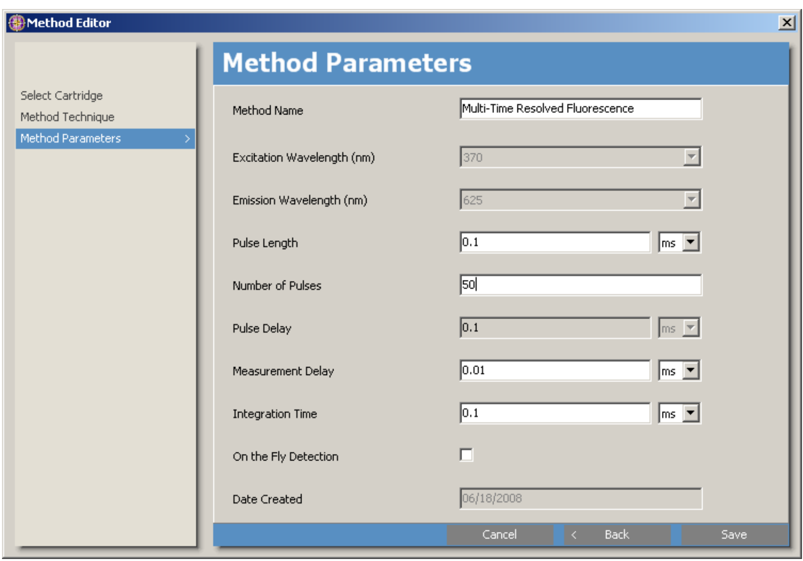


Figure 5-17 Time Resolved Fluorescence Method Parameters

Fluorescence Intensity (FI) Detection Cartridge

In a fluorescence intensity detection method, the source light is focused by an objective lens and directed through an excitation filter above or below the plate, depending upon the mounting position of the cartridge. Reading of the plate is determined by the method type, fluorescence intensity top methods read above the plate and fluorescence intensity top methods read below the plate. The filter passes only the wavelength necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by the photo multiplier tube.

Note: It is necessary for the Fluorescence Intensity detection cartridge to be installed in the Upper Read Detection Cartridge Transport for the Fluorescence Intensity Top Detection Method. It is necessary for the Fluorescence Intensity detection cartridge to be installed in the Bottom Read Detection Cartridge Transport for the Fluorescence Intensity Bottom detection method.

Note: For additional information regarding the fluorescence intensity detection cartridge please see the detection cartridge's user guide.

To create a fluorescence intensity detection method using a fluorescence intensity detection cartridge:

1. In the **Supported technique** field select **Fluorescence Intensity Dual-Label**, **FRET**, **Fluorescence Intensity Top**, or **Fluorescence Intensity Bottom**.
2. Click **Next** to define the Method Parameters (Figure 5-18).

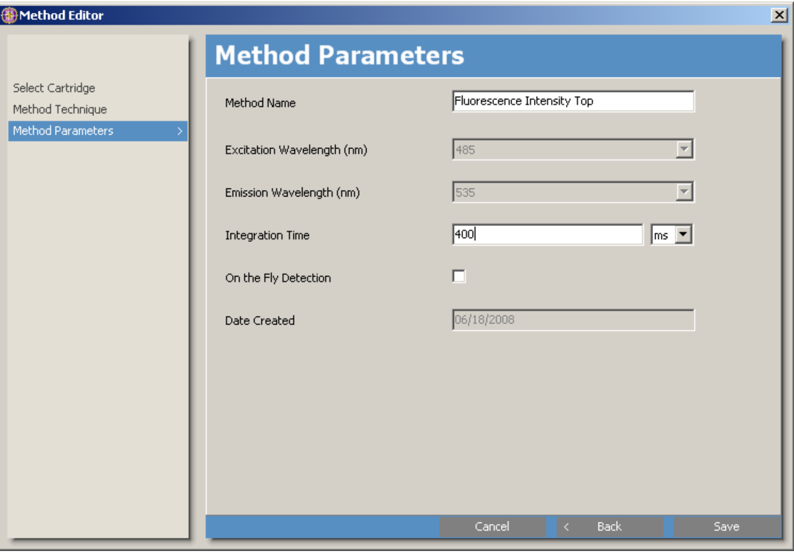


Figure 5-18 Fluorescence Intensity Detection Cartridge Method Parameters

3. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example, an absorbance measurement @ 260 might read ABS_260.

4. To use on-the-fly detection select **On the Fly Detection**.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

5. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However, there is trade off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

6. Specify the measurement time per well (in seconds, milliseconds, or microseconds) using the **Integration Time** field.
7. Click **Save** to save the new fluorescence intensity detection method. The new method appears in the Detection Method Selection List.

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

In a fluorescence intensity dual-label detection method, the source light is focused by an objective lens and directed through an excitation filter above or below the plate, depending upon the mounting position of the cartridge. The filter passes only the wavelength necessary to excite samples. The objective lens collects the resulting fluorescence and directs it through an emission filter to separate background light from the specific wavelengths generated by samples. This signal is detected by the photo multiplier tubes.

Note: It is necessary for the Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge to be installed in the Upper Read Detection Cartridge Transport for reading from above the plate. It is necessary for the Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge to be installed in the Bottom Read Detection Cartridge Transport for reading from the below plate.

Note: For additional information regarding the Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge, please see the detection cartridge user guide.

To create a fluorescence intensity dual-label detection method using a Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge:

1. In the Supported technique field, select **Fluorescence Dual-Label**. Other detection methods might be available also, such as **FRET**, **Fluorescence Intensity Top**, or **Fluorescence Intensity Bottom**.
2. Click **Next** to define the Method Parameters (Figure 5-19).

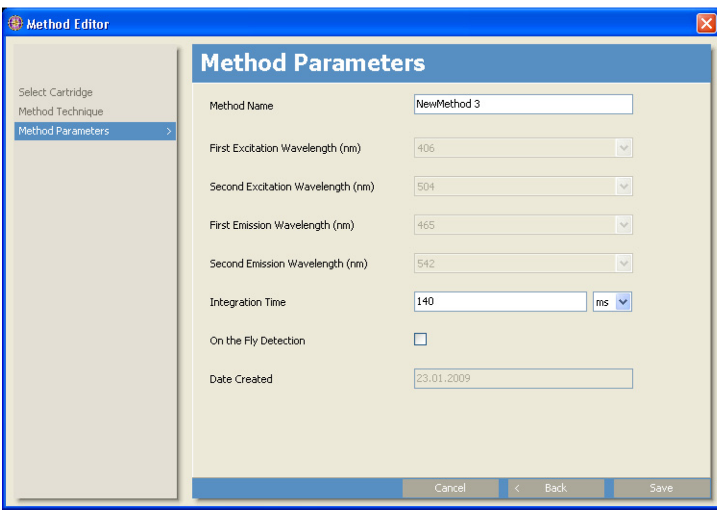


Figure 5-19 Fluorescence Intensity Dual Label (FI-DL) (MultiTox-Fluor) Detection Cartridge Method Parameters

3. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods, it is important to use a consistent and informative naming convention. For example, an absorbance measurement @ 260 might read ABS_260.

4. To use on-the-fly detection select **On the Fly Detection**.

Note: On the Fly Detection yields considerably faster read times since the plate moves continuously as each well is measured, as opposed to the stop and go mode where the plate stops moving for each read.

5. If using on-the-fly detection, specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate; however, there is trade off between the data quality (that is, CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

6. Specify the measurement time per well (in seconds, milliseconds, or microseconds) using the Integration Time field.

7. Click Save to save the new fluorescence intensity dual-label detection method. The new method appears in the Detection Method Selection List.

Time Resolved Fluorescence (TRF) Detection Cartridge

In a time resolved fluorescence measurement, the excitation light source is turned off and the measurement is performed from above the plate after a specified delay. Several of these excitation/measurement cycles may be performed on each sample. When multiple excitation/measurement cycles are performed, the results from all cycles are used to calculate a single measurement result for each sample.

The time resolved fluorescence measurement cycle consists of five parts:

- **Pulse Length**: the time that the excitation light source is turned on (t_1 on Figure 5-19).
- **Measurement Delay**: the time between the excitation light source being turned off and when the measurement begins (t_2 on Figure 5-19).
- **Integration Time**: the measurement time (t_3 on Figure 5-19).
- **Pulse Delay**: the time between the measurement stopping (integration time) and when the excitation light source is turned on for the next measurement (t_4 on Figure 5-19). This value is read only and is adjusted based upon the Minimum Pulse Period, Measurement Delay, and Integration time.
- **Minimum Pulse Period**: The minimum time required between the excitation light source being turned off and when the excitation light source is turned on again (t_5 on Figure 5-19). This value is based upon each detection cartridge and is not editable.

Note: Measurement Delay + Integration Time + Pulse Delay ≥ Minimum Pulse Period ($t_2 + t_3 + t_4 \geq t_5$)

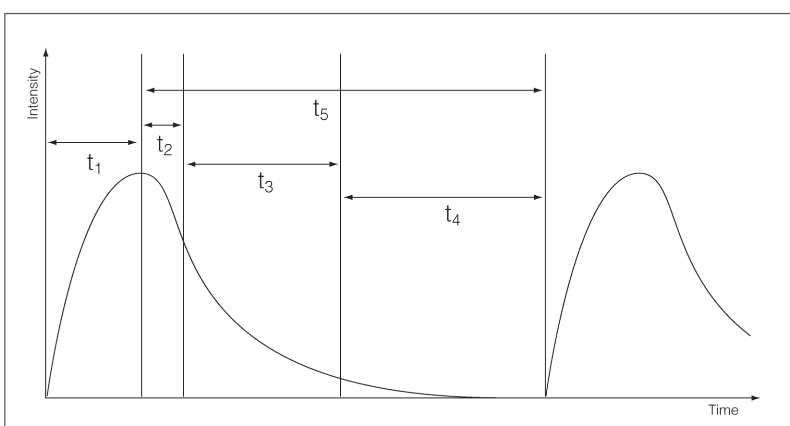


Figure 5-20 Time Resolved Fluorescence

The dual-time resolved fluorescence measurement cycle consists of seven parts:

- **Pulse Length**: the time that the excitation light source is turned on (t_1 on Figure 5-20).
- **Measurement Delay (First Window)**: the time between the excitation light source being turned off and when the first measurement begins (t_2 on Figure 5-20).
- **Integration Time (First Window)**: the measurement time (t_3 on Figure 5-20) that each sample is measured for the first time.
- **Measurement Delay (Second Window)**: the time between the excitation light source being turned off and when the second measurement begins ($t_2+t_3+t_6$ on Figure 5-20).
- **Integration Time (Second Window)**: the measurement time (t_7 on Figure 5-20) each sample is measured the second time.
- **Pulse Delay**: the time between the measurement stopping (integration time) and when the excitation light source is turned on for the next measurement (t_4 on Figure 5-20). This value is read only and is adjusted based upon the Minimum Pulse Period, Measurement Delay, and Integration time.
- **Minimum Pulse Period**: The minimum time required between the excitation light source being turned off and when the excitation light source is turned on again (t_5 on Figure 5-20). This value is based upon each detection cartridge and is not editable.

Note: First Measurement Delay + First Integration Time + Second Measurement Delay + Second Integration Time + Pulse Delay ≥ Minimum Pulse Period ($t_2 + t_3 + t_6 + t_7 + t_4 \geq t5$)

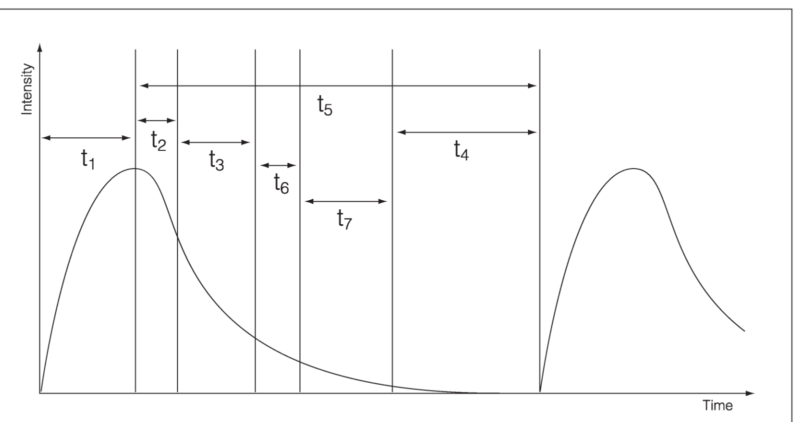


Figure 5-21 Dual-Time Resolved Fluorescence

Note: For additional information regarding the time resolved fluorescence detection cartridge please see the detection cartridge’s user guide.

To create a time resolved fluorescence detection method using a time resolved fluorescence enabled detection cartridge:

1. If applicable, in the **Supported Technique** field select **Time Resolved Fluorescence** or **Time Resolved Fluorescence Dual**.
2. If applicable, in the **Wavelengths** field specify the Excitation and Emission Wavelength.
3. Click **Next** to define the Method Parameters (Figure 5-21).

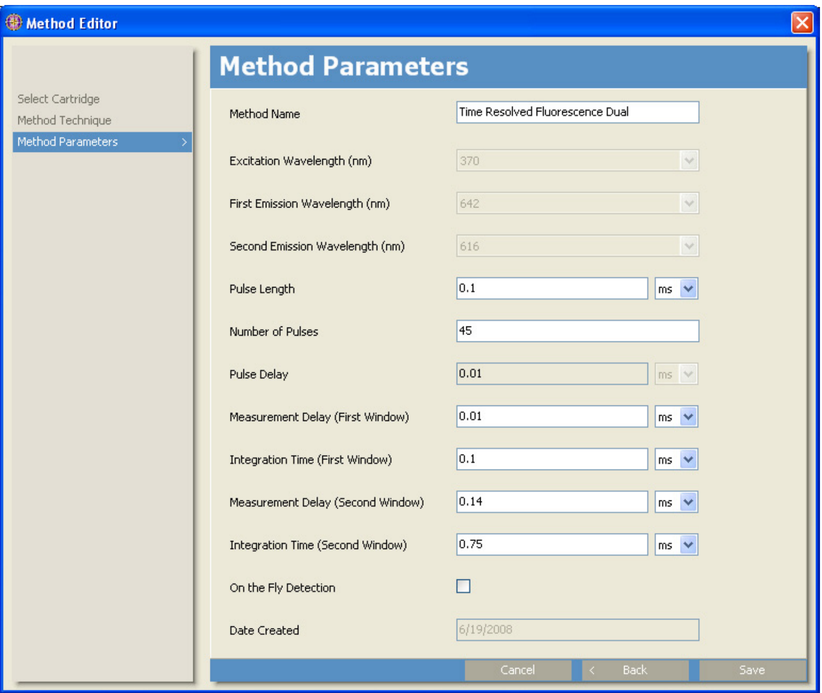


Figure 5-22 Time Resolved Fluorescence Method Parameters - Time Resolved Fluorescence

4. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example an absorbance measurement @ 260 might read ABS_260, while an Absorbance scan measurement between 260 -360 might read ABS_260-350 Scan.

5. To use on-the-fly detection select **On the Fly Detection**.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

6. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However, there is trade off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

7. In the **Pulse Length** field, enter the length of time (in seconds, milliseconds, or microseconds) that the light source remains turned on (t_1 in Figure 5-19 and Figure 5-20).
8. If **On the Fly Detection** is not selected, in the **Number of Pulses** field, enter the number of excitation/measurement cycles performed in the measurement. The number of pulses sets the exposure received by samples during measurements.
9. In the **Measurement Delay** field, enter the time interval (in seconds, milliseconds, or microseconds) between switching off the light source and performing the measurement (t_2 in Figure 5-19 and Figure 5-20).
10. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) each sample is measured. Set the integration time within the range of 10 µs to 10 ms (t_3 in Figure 5-19 and Figure 5-20).
11. For dual time resolved fluorescence, in the second **Measurement Delay** field, enter the time interval (in seconds, milliseconds, or microseconds) between switching off the light source and performing the second measurement ($t_2+t_3+t_6$ in Figure 5-20).
12. For dual time resolved fluorescence, in the second **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) each sample is measured the second time. Set the integration time within the range of 10 µs to 10 ms (t_7 in Figure 5-20).
13. Click **Save** to save the new time resolved fluorescence detection method. The new method appears in the Detection Method Selection List.

Cisbio HTRF® Detection Cartridge

HTRF (homogeneous time resolved fluorescence) is a measurement technique based upon fluorescence resonance energy transfer (FRET) using the advantage of time resolved floresecence.

The homogeneous time resolved fluorescence measurement cycle consists of five parts:

1. **Pulse Length**: the time that the excitation light source is turned on (t_1 on Figure 5-22).
2. **Measurement Delay**: the time between the excitation light source being turned off and when the measurement begins (t_2 on Figure 5-22).
3. **Integration Time**: the measurement time (t_3 on Figure 5-22).

- **Pulse Delay**: the time between the measurement stopping (integration time) and when the excitation light source is turned on for the next measurement (t_4 on Figure 5-22). This value is read only and is adjusted based upon the Minimum Pulse Period, Measurement Delay, and Integration time.
- **Minimum Pulse Period**: The minimum time required between the excitation light source being turned off and when the excitation light source is turned on again (t_5 on Figure 5-22). This value is based upon each detection cartridge and is not editable.

Note: Measurement Delay + Integration Time + Pulse Delay => Minimum Pulse Period ($t_2 + t_3 + t_4 => t_5$)

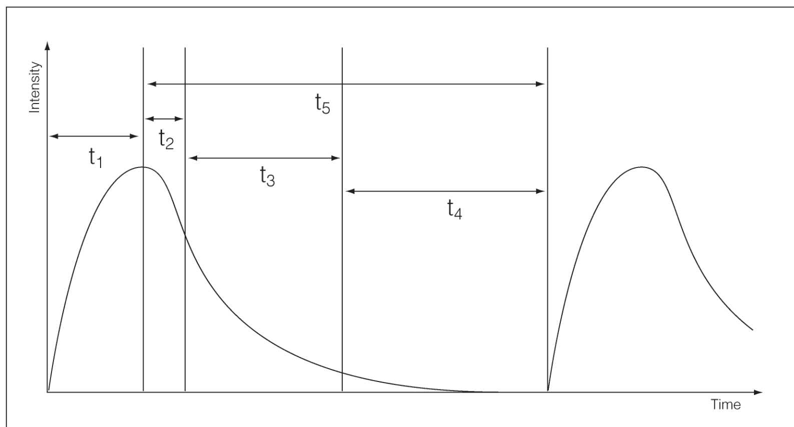


Figure 5-23 Homogeneous Time Resolved Fluorescence

Note: For additional information regarding the homogeneous time resolved fluorescence detection cartridge please see the detection cartridge's user guide.

To create a homogeneous time resolved fluorescence detection method using a homogeneous time resolved fluorescence enabled detection cartridge:

1. The only supported method technique using a homogeneous time resolved fluorescence detection cartridge is homogeneous time resolved fluorescence. Click **Next** to define the Method Parameters (Figure 5-23).

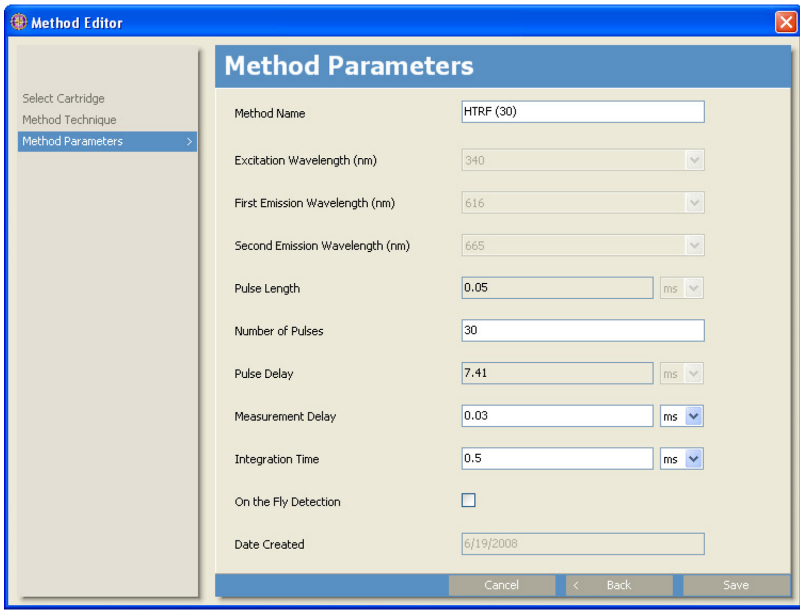


Figure 5-24 Homogeneous Time Resolved Fluorescence Method Parameters

2. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example an absorbance measurement @ 260 might read ABS_260, while an Absorbance scan measurement between 260 -360 might read ABS_260-350 Scan.

3. To use on-the-fly detection select **On the Fly Detection**.

Note: On the Fly Detection yields considerably faster read times while the plate moves continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

4. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However, there is trade off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

5. If **On the Fly Detection** is not selected, in the **Number of Pulses** field, enter the number of excitation/measurement cycles performed in the measurement. The number of pulses sets the exposure received by samples during measurements.
6. In the **Measurement Delay** field, enter the interval (in seconds, milliseconds, or microseconds) between switching off the light source and performing the measurement (t_2 in Figure 5-22).
7. In the **Integration Time** field, enter the length of time (in seconds, milliseconds, or microseconds) each sample is measured. Set the integration time within the range of 10 µs to 10 ms (t_3 in Figure 5-22).
8. Click **Save** to save the new homogeneous time resolved fluorescence detection method. The new method appears in the Detection Method Selection List.

Luminescence (LUM) Detection Cartridge

In a luminescence method, the intensity at which light is emitted from a chemiluminescent or bioluminescent reaction is measured. The light output is measured as the rate of photons per time and is expressed as counts per second. In glow luminescence reactions, the light output decays slowly with time. Since the light is emitted as a result of a chemical reaction, no excitation light and no excitation filters are required to measure luminescence. In a dual glow luminescence method, different wavelength bands at the same time are measured.

Note: There are different types of Luminescence detection cartridges available for the SpectraMax Paradigm Multi-Mode Detection Platform. All are designed and optimized for different applications and microplate formats (for more details, refer to the respective detection cartridge manuals).

To create a detection method using the Luminescence detection cartridge:

1. In the supported technique select **Luminescence** or **Dual Luminescence** (Figure 5-25).

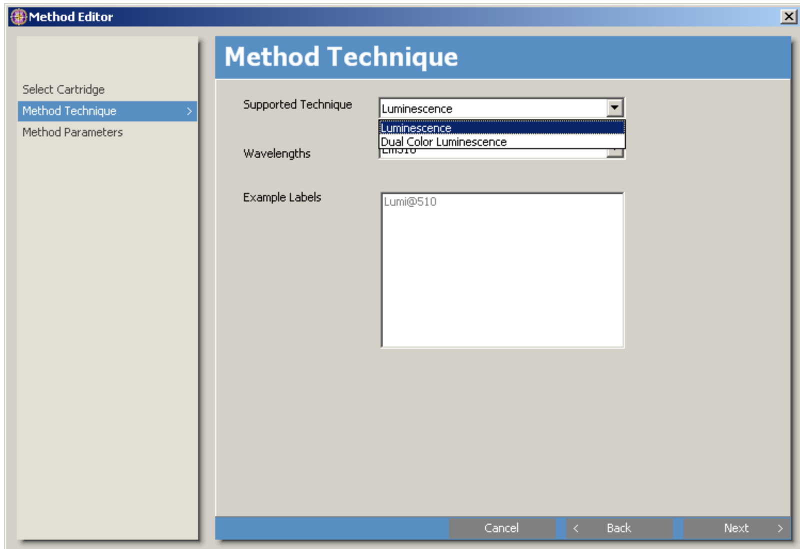


Figure 5-25 Luminescence Detection Cartridge Method Technique

2. As required, select the detection wavelengths using the **Wavelengths** field.
3. Click **Next** to define the Method Parameters (Figure 5-26).
4. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods it is important to use a consistent and informative naming convention. For example Absorbance measurement @ 260 might read ABS_260, while an Absorbance scan measurement between 260 -360 might read ABS_260-350 Scan.

5. To use on-the-fly detection select **On the Fly Detection**.

Note: On the Fly Detection yields considerably faster read times due to the plate moving continuously as each well is measured, as opposed to stop and go mode where the plate stops moving for each read.

6. If using on-the-fly detection specify whether the detection method should be optimized for **Speed** or **Performance** using the **On the Fly Optimization** field.

Note: Selecting **Speed** results in the fastest possible read time per plate. However, there is trade off between the data quality (i.e. CVs or dynamic range) and read speed because each well is sampled for shorter integration times. Selecting **Performance** results in a faster read time than not using **On the Fly Detection**, but not as fast as the **Speed** mode. **Performance** provides considerably better results than **Speed** for demanding assays.

OR

If not using on-the-fly detection, specify the measurement time per well (in seconds, milliseconds, or microseconds) using the **Integration Time** field.

7. Select **Attenuation** to apply a neutral density optical filter to the reading, which will reduce the intensity of all samples.

Note: Using attenuation will shift the linear dynamic range to higher sample concentrations. A similar effect can be achieved by using black plates instead of white plates. Samples appearing even stronger in signal although attenuation is applied are outside the measurement range.

Note: The attenuation option is available only for luminescence top reading.

CAUTION! Luminescence light levels visible to the human eye may cause damage to the detection system.

8. Click **Save** to save the new luminescence detection method. The new method appears in the Detection Method Selection List.

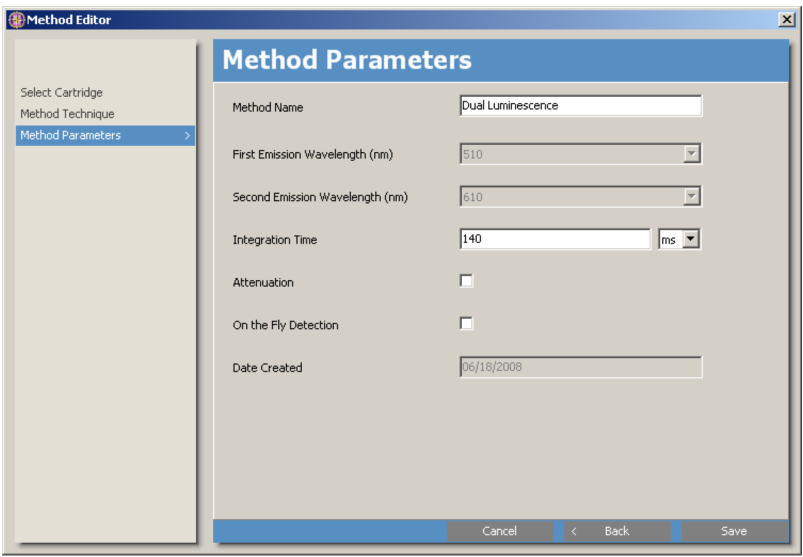


Figure 5-26 Dual Luminescence Method Parameters

AlphaScreen Detection Cartridge

In an AlphaScreen method, the intensity at which light is emitted from the bead-based AlphaScreen assay is measured. The light output is measured as the rate of photons per time and can be normalized to counts per second. In AlphaScreen reactions, the light output decays slowly with time. Since the light is emitted as a result of a photochemical reaction, excitation light is exposed to the sample once, before signal is integrated for a specified time.

Note: There are different types of AlphaScreen detection cartridges available for the SpectraMax Paradigm Multi-Mode Detection Platform. All are designed and optimized for different microplate formats (for more details, refer to the AlphaScreen Detection Cartridge user guide).

To create a detection method using the AlphaScreen detection cartridge:

1. In the **Supported technique**, select **AlphaScreen** (Figure 5-27).

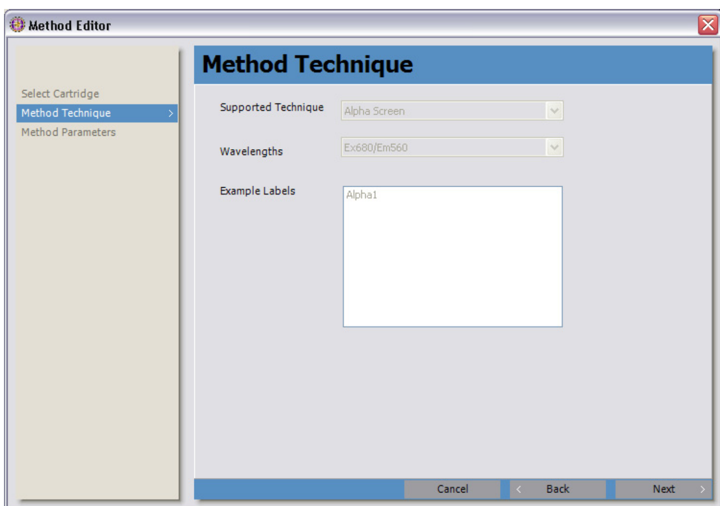


Figure 5-27 AlphaScreen Detection Cartridge Method Technique

2. As required, select the detection wavelengths using the **Wavelengths** field.
3. Click **Next** to define the Method Parameters (Figure 5-28).
4. In the **Method Name** field, enter a name for the method.

Note: When naming detection methods, it is important to use a consistent and informative naming convention. For example, Absorbance measurement @ 260 might read ABS_260, while an Absorbance scan measurement between 260 and 350 might read ABS_260-350 Scan.

5. In the **Excitation Time** field, type the length of time in milliseconds that you want the laser beam to be on.
6. In the **Integration Time** field, type the elapsed time in milliseconds from when the laser beam is turned off to when you want the signal to be detected.
7. To normalize raw data from counts into counts per second, select **Normalization**.

8. Select **Interlaced Reading** when you expect a high assay dynamic range in white plates.

Note: Interlaced Reading processes every other well in a checkerboard fashion and adds another plate run to cover the wells left from the first run. Using this process minimizes crosstalk from the possible afterglow of an adjacent well that may have a strong emission when the critical well is read. **Interlaced Reading** is the suggested read mode when expecting high assay dynamic range in white plates.

9. Click **Save** to save the new AlphaScreen detection method. The new method appears in the Detection Method Selection List.

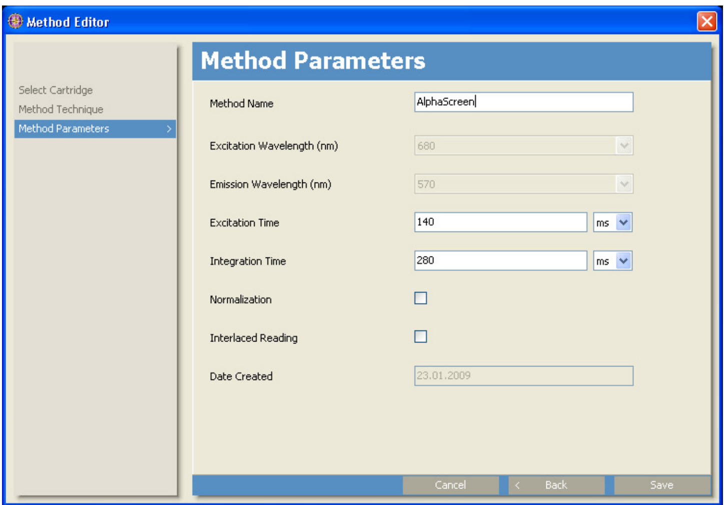


Figure 5-28 AlphaScreen Method Parameters

Signing a Detection Method (SpectraMax Paradigm Multi-Mode Detection Platform)

When GxP Permissions is enabled on the system, detection methods may be signed to prevent method properties from being edited or methods from being deleted. Detection methods may be signed at any time after the configuration is complete.

Detection methods may be signed by users who are assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To sign a detection method:

1. In the Detection Method Selection List, select the detection method to sign.
2. From the tool bar, click **Sign the selected method**.

   OR

   From the menu bar select **Actions > Sign the selected method**.

   OR

   Right click on the selected labware and select **Sign the selected method**.
3. The Sign the Selected Item dialog appears.
4. In the Sign the Selected Item dialog, add comments and an electronic signature by following the instructions in Adding Electronic Signatures and Comments to Items on page 84.

Editing Detection Methods

Parameters configured in user-defined detection methods may be edited; however, the method technique may not be changed. Default methods installed with the software and methods used in protocols may be edited, but not renamed.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Delete permission may delete user-defined detection methods. Signed methods may not be deleted. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To edit a detection method:

1. In the Detection Method Selection List, select the detection method to edit.
2. From the tool bar, click **Edit**.

   OR

   From the menu bar select **Actions > Edit the selected method**.

   OR

   Right-click on the selected detection method and select **Edit the selected method**.
3. The Method Editor appears.
4. Edit the method parameters as desired. For more information about the parameters for a specific detection method, refer to the section that covers defining the desired detection method:
   - Creating Detection Methods (FilterMax Multi-Mode Microplate Readers) on page 88
   - Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform) on page 99
5. Click **Save** to close the Method Editor and save the changes.

Copying Detection Methods

Copies may be made of existing detection methods. After a copy has been created, it may be used as a template for a new detection method using the same method technique.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Delete permission may delete user-defined detection methods. Signed methods may not be deleted. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To make a copy of a detection method:

1. In the Detection Method Selection List, select the detection method to copy.
2. From the tool bar, click **Copy**.

   OR

   From the menu bar select **Actions > Make a copy of the selected method**.

   OR

   Right-click on the selected detection method and select **Make a copy of the selected method**.

Note: The default name format for copied detection methods is Copy of OriginalName. To change the name, edit the detection method. See Editing Detection Methods on page 123.

Deleting Detection Methods

User-defined detection methods may be deleted from the Detection Method Selection List. Some detection methods may not be deleted, including:

- methods used in protocols.
- default methods installed with Multi-Mode Analysis Software.
- methods signed on systems with GxP Permissions enabled.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Delete permission may delete user-defined detection methods. Signed methods may not be deleted. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

When a detection method is deleted it is moved to the trash selection list. To permanently remove or restore items for deletion see Deleting and Restoring Items on page 48.

To delete a detection method:

1. In the Detection Method Selection List, select the detection method to delete.
2. From the tool bar, click **Delete**.

   OR

   From the menu bar select **Actions > Delete the selected method**.

   OR

   Right-click on the selected detection method and select **Delete the selected method**.

Note: Multiple items may be selected for deletion by holding down the CTRL or SHIFT key while selecting each item desired.

3. A dialog box appears. Click **Yes** to delete the selected detection method.
4. To permanently remove the detection method see Deleting and Restoring Items on page 48

Exporting and Importing Detection Methods

A user-defined detection method can be exported to an XML file and imported later to restore that configuration or share it with a copy of Multi-Mode Analysis Software installed on another system.

Default detection methods installed with Multi-Mode Analysis Software are present on all systems and may not be deleted or overwritten. For this reason, importing default detection methods from an XML export file is not permitted.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Delete permission may delete user-defined detection methods. Signed methods may not be deleted. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To export a detection method:

1. In the Detection Method Selection List, select the detection method to export.
2. From the **File** menu, click **Export > Detection Method**. The Browse for Folder dialog appears.
3. In the Browse for Folder dialog, browse to the folder where the exported detection method will be saved.

   OR

   Click **Make New Folder** to create a new folder where the exported detection method will be saved.
4. Click **OK** to export the detection method. The exported detection method is saved using the default file name format, Method_MethodName.xml.

Note: To import the file at a later date, the filename must not be changed.

To import a detection method from an exported XML file:

1. From the File menu, click **Import > Detection Method**. The Open dialog appears.

Note: For SpectraMax Paradigm Multi-Mode Detection Platform, the detection cartridge used in the imported detection method must be contained within the list of available detection cartridges in **My Detection Cartridges** configured in instrument settings. To add a detection cartridge to the list of available detection cartridges see Adding Detection Cartridges to the list of Available Detection Cartridges on page 67.

2. In the Open dialog, browse to and select the desired XML file to import.
3. Click **Open**. The detection method is imported to the Detection Method Selection List.

Creating and Editing Labware

6

Overview

Multi-Mode Analysis Software supports a wide range of labware, with many common microplate formats already preconfigured and ready for use in protocols. Configured labware types are listed in the Labware Selection List and are available for use in protocols.

New labware types may be created at any time using the Labware Editor. The Labware Editor also provides the ability to edit and delete existing labware types not used in protocols, make copies of labware types, and optimize labware dimensions to compensate for slight dimensional variations that may exist between production lots.

The types of labware supported depend on the capabilities of the instrument:

- FilterMax 3 Multi-Mode Microplate Reader: Supports 96-well and 384-well microplates.
- FilterMax 5 Multi-Mode Microplate Reader: Supports 6-well to 384-well microplates.
- The SpectraMax Paradigm Multi-Mode Detection Platform supports 6-well to 1536-well microplates, depending on the installed detection cartridges.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Create permission may define labware. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

Labware actions are accessed from the Labware Selection List (Figure6.1). Labware actions include:

- Creating Labware on page 128
- Editing Labware on page 133
- Copying Labware on page 136
- Deleting Labware on page 136
- Optimizing Labware on page 137
- Exporting and Importing Labware on page 143

To define and edit labware:

- From the navigation pane, click **Labware**. The Labware Selection List appears (Figure 6-1).

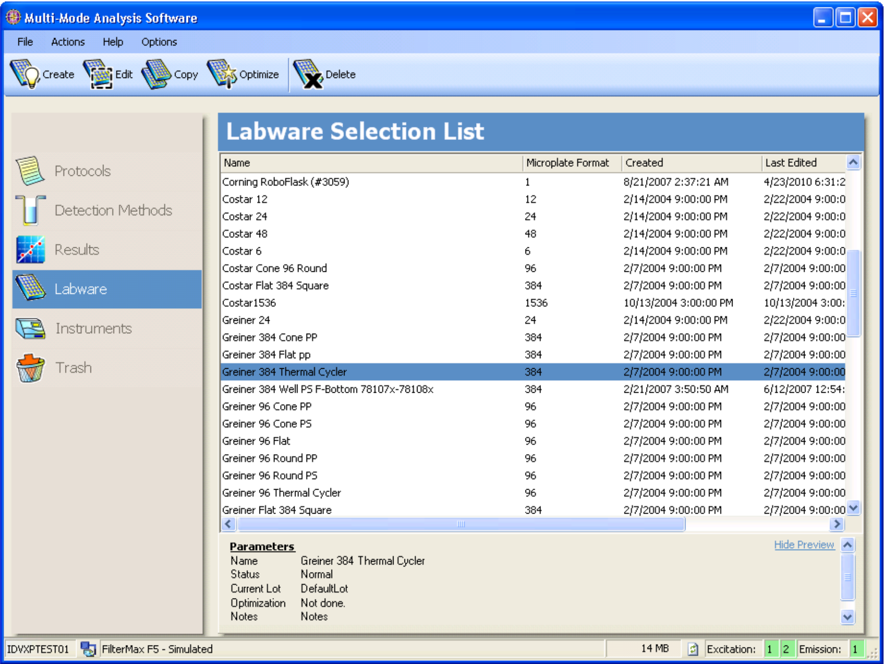


Figure 6-1 Accessing Labware Actions

Creating Labware

New types of labware are created in the Labware Editor, which guides the creation process with a wizard-type interface. Creating labware includes:

- Defining Labware Information on page 129
- Configuring Offsets and Well Dimensions for the Default Labware Lot on page 131
- Signing Labware on page 133 (Optional)

  This is used to prevent labware from being edited or deleted. Labware may be signed only when GxP Permissions is enabled on the system.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Create permission may create new labware definitions. To sign labware, users must be assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To create and define new labware:

1. From the tool bar, click **Create**.
   OR
   From the menu bar select **Actions > Create a new labware type**.
   OR
   Right-click in the Labware Selection List and select **Create a new labware type**.
2. The Labware Editor display (Figure 6-2).

Note: After installing Multi-Mode Analysis Software, profiles for commonly used labware load to the system upon start up. Further labware profiles may be loaded by importing them. For details see Exporting and Importing Labware on page 143.

Defining Labware Information

Use Labware Information to define labware names, dimensions, well parameters, and supported measurement techniques (Figure 6-2).

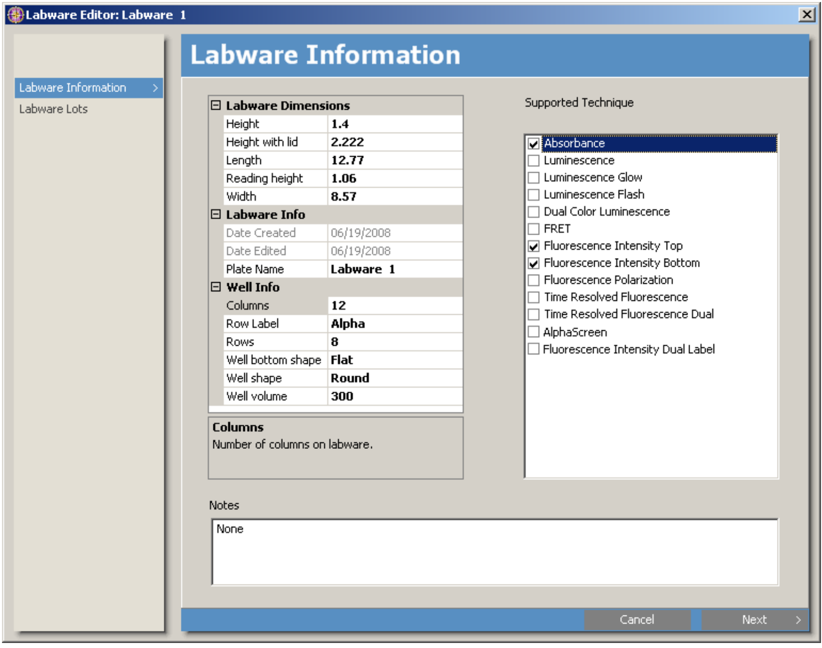


Figure 6-2 Defining Plate Dimensions and Information

To define Labware Information:

1. If necessary, click the **+** next to Labware Info to display the fields in the category.
2. Enter the **Plate Name**. A name must be entered to proceed to the second configuration screen, Labware Lots.

3. If necessary, click the **+** next to Labware Measurements to display the fields in the category.

Note: More information about the field being defined is displayed below the property grid (Figure 6-2).

If information text is too long to be displayed entirely, the text box may be enlarged by dragging the upper border of the Notes box.

CAUTION! The plate height configured must not be less than that of the actual plate. Doing so may cause the FilterMax Multi-Mode Microplate Readers to collide with the plate during a Read Height Optimization. The SpectraMax Paradigm Multi-Mode Detection Platform has an auto-detection to prevent collision, if an incorrect plate height is entered for the SpectraMax Paradigm Multi-Mode Detection Platform an error message appears while running protocols using the defined labware.

4. In the **Height** field enter the height of the plate without the lid. All labware dimensions are entered in centimeters.
5. In the **Height with lid** field enter the height of the plate with lid in centimeters.
6. In the **Length** field enter the length of the plate in centimeters.
7. In the **Reading height** field enter the height from the top of the plate at which the plate is read.
8. In the **Width** field enter the width of the plate.
9. If necessary, click the **+** next to **Well Info** to display the fields in the category.
10. In the **Columns** field enter the number of columns on the plate.
11. In the **Row Label** field select **Alpha** or **Numeric** for the row label.
12. In the **Rows** field enter the number of rows on the plate.
13. Click in either column of **Well bottom shape**, then click on the down arrow and select the shape of well bottoms: **Flat**, **Cone**, or **Round**.
14. Click in either column of **Well shape**, then click on the down arrow and select the shape of the wells: **Round**, **Square**, or **Cone**.
15. Enter the maximum **Well volume** in microliters.
16. In **Supported Techniques**, select all measurement techniques compatible with the plate being defined. The labware being configured will only be available for use in protocols configured with compatible measurement techniques.

Note: See General Labware Selection Guidelines on page 131 for more information about selecting the appropriate labware for the desired techniques.

17. As desired, in **Notes**, enter information about the labware or configuration.
18. Click **Next** to define the default row and column offsets and well dimensions for the labware type in Labware Lots. See Configuring Offsets and Well Dimensions for the Default Labware Lot on page 131.

General Labware Selection Guidelines

When creating labware, select only measurement techniques compatible with the microplate being defined. Each measurement technique requires labware of a specific color and/or material be used.

Table 6-1 provides general labware color and material guidelines for each measurement technique. Along with these basic guidelines, always select microplates with a surface treatment suitable for the desired application, and follow any additional guidelines provided by the plate manufacturer.

Table 6-1 General Microplate Selection Guidelines

Measurement Technique	Supported Plate Color	Additional Considerations
Absorbance	clear, white with clear bottom, or black with clear bottom	Clear polystyrene or film plates with transparent bottoms are suitable. Polypropylene or PVC plates do not provide sufficient optical quality.
Luminescence Glow Type	solid black or solid white	Black plates are recommended unless the signal is weak enough to require the higher sensitivity of white plates. However, with strong signals, white plates may produce crosstalk.
Fluorescence Intensity Top	solid black	N/A
Fluorescence Intensity Bottom	black with clear bottom	N/A
Fluorescence Polarization	solid black	Microplates must not be covered with a lid or plastic film during fluorescence polarization measurements.
Time-Resolved Fluorescence	solid white	N/A
Time-Resolved Fluorescence Dual	solid white	N/A

Configuring Offsets and Well Dimensions for the Default Labware Lot

Use Labware Lots to define row and column offsets and well dimensions (Figure 6-3). The offsets and dimensions entered when new labware is created define the default labware lot (DefaultLot). After the new labware has been saved, additional lots may be created by optimizing the labware to compensate for dimensional variations between different production lots. See Optimizing Labware on page 137.

In Labware Lots, x and y offsets are defined for all four corners of the labware. An x offset is the distance from the edge of the microplate to the center of wells on the first row; a y offset is the distance from the edge of plate to the center of wells in the first column. Well dimensions defined include well depth, length, and width, as well as distances between rows and columns.

To configure offsets and well dimensions:

1. If necessary, click the **+** next to **Labware Lot Measurements** to display the fields in the category. More information about the field being defined is displayed below the property grid (Figure 6-3).

Note: The fields in Labware Lot Info may not be configured when creating new labware.

2. Enter column and row x and y offsets for each of the four corner wells. All offsets and well dimensions are entered in millimeters.

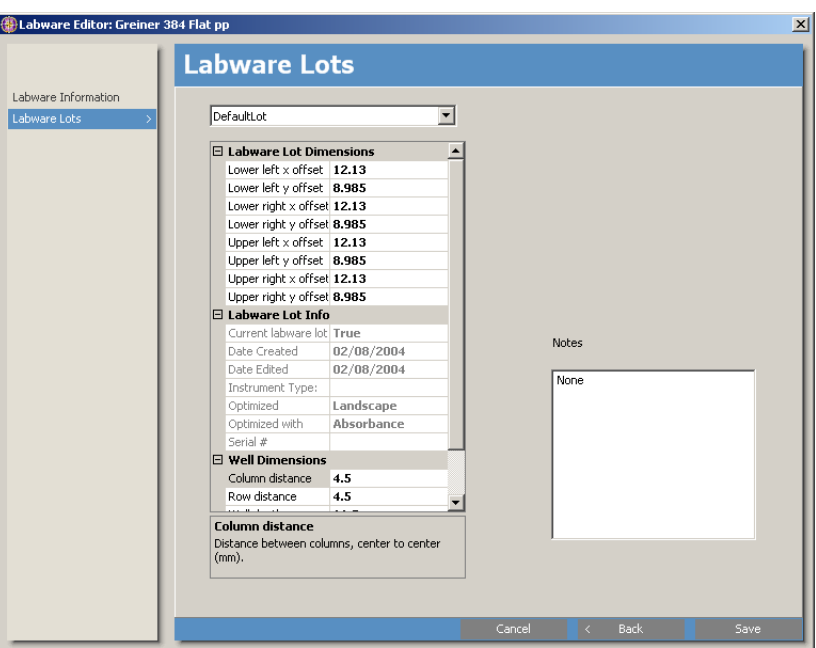


Figure 6-3 Defining Offsets and Well Dimensions

3. If necessary, click the **+** next to **Well Measurements** to display the fields in the category.
4. In **Column Distance**, enter the distance between columns (well center to well center).
5. In **Row Distance**, enter the distance between rows (well center to well center).
6. Enter the **Well depth**.
7. In **Well Width**, enter the diameter of the well in the direction of the rows on the plate.
8. In **Notes**, enter information about the labware lot or configuration, if desired.
9. Click **Save** to save the new labware and close the Labware Editor.

Signing Labware

When GxP Permissions is enabled on the system, labware may be signed to prevent labware properties from being edited. Signed labware may not be optimized to create new labware lots unless all signatures attached to the labware are unlocked, which changes the labware status to unsigned.

Labware may be signed by users who are assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To sign labware:

1. In the Labware Selection List, select the labware type to sign.
2. From the tool bar, select **Sign the selected labware type**.

   OR

   From the menu bar select **Actions > Sign the selected labware type**.

   OR

   Right-click on the selected labware and select **Sign the selected labware type**.
3. The Sign the Selected Item dialog appears.
4. In the Sign the Selected Item dialog, add an electronic signature by following the instructions in Adding Electronic Signatures and Comments to Items on page 84.

Editing Labware

Dimensions and information for user-defined labware not used in measurement protocols may be edited. Dimensions and information may be viewed, but not edited, for:

- default labware installed with Multi-Mode Analysis Software.
- labware used in protocols.
- labware that has been signed on a system with GxP Permissions enabled.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Edit permission may edit labware definitions. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

All labware in the Labware Selection List may be optimized to create new labware lots. See Optimizing Labware on page 137. Labware lots compensate for dimensional variations between production lots. When multiple lots exist for a labware type, the active lot may be changed. Labware lot properties may be edited for all labware types except those that have been signed.

Labware is edited in the Labware Editor (Figure 6-5). Editing includes:

- Viewing and Editing Labware Information on page 134
- Selecting and Editing Labware Lots on page 135

To view and edit labware dimensions and information:

1. In the Labware Selection List, select the labware to edit.
2. From the tool bar, click **Edit**.

   OR

   From the menu bar select **Actions > Edit the selected labware type**.

   OR

   Right-click on the selected labware and select **Edit the selected labware type**.

Viewing and Editing Labware Information

Use Labware Information to view and edit labware dimensions, information, and supported techniques. Plate information for default labware included in the software installation and labware used in protocols may be viewed, but not edited.

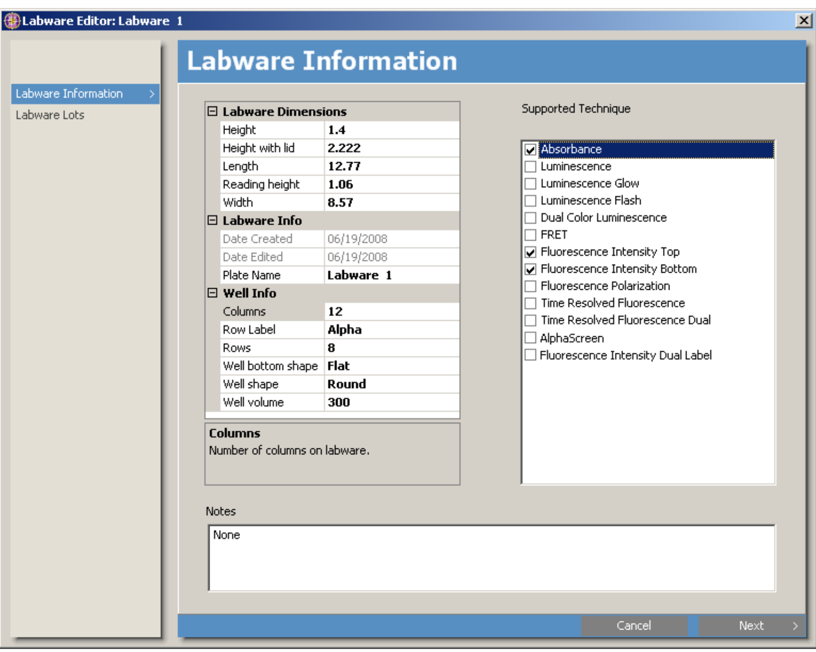


Figure 6-4 Editing Labware Dimensions and Well Information

To edit labware information:

1. In the property grid, edit labware dimensions and information as desired. See Defining Labware Information on page 129 for more information about the fields available in the property grid.
2. In Supported Techniques, change the measurement techniques supported by the labware, if desired.

Note: See General Labware Selection Guidelines on page 131 for more information about labware/technique compatibility.

3. Edit the labware **Notes**, if desired.
4. Click **Next** to select and edit labware lots and save changes made to the labware. See Selecting and Editing Labware Lots on page 135.

Selecting and Editing Labware Lots

Use Labware Lots to select the active lot and/or edit and save changes made in the Labware Editor (Figure 6-5). Lots can be selected and edited for all labware, including labware used in measurement protocols.

Note: Labware Lots are created by optimizing labware. See Optimizing Labware on page 137.

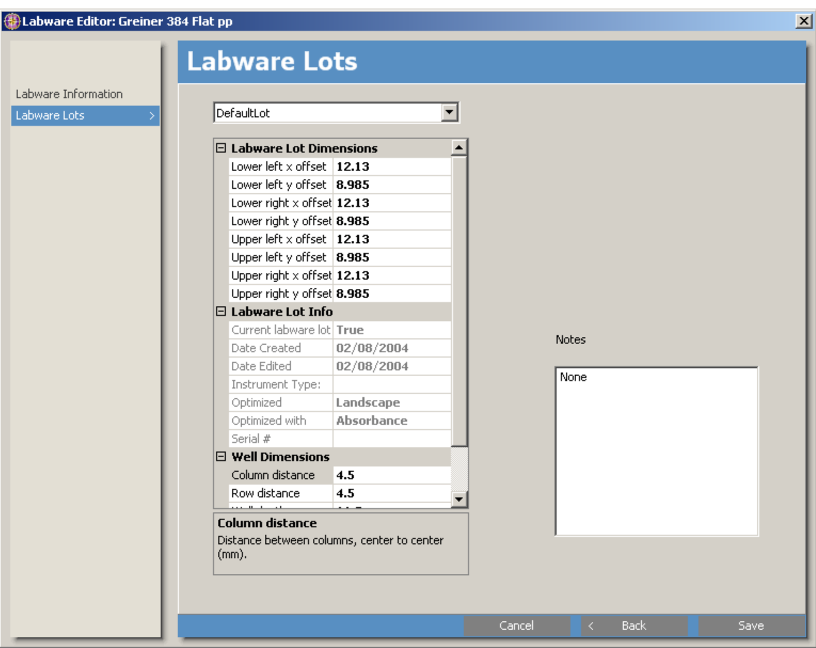


Figure 6-5 Configuring Offsets and Well Dimensions in Labware Lots

To select and edit lots:

1. From the pull-down menu, select the lot to use or edit. The default lot created when the labware was defined and all lots configured using Optimizing Labware are available. See Optimizing Labware on page 137.
2. Click in Current labware lot and select **True**, if necessary. True must be selected to save changes made to the labware lot.
3. In the property grid, edit lot dimensions and information as desired. See Configuring Offsets and Well Dimensions for the Default Labware Lot on page 131, for more information about the fields available in the property grid.
4. Edit the lot **Notes**, if desired.
5. Click **Save** to save changes made in the Labware Editor.

Copying Labware

Labware can be copied and then used as a template for a new labware type by editing the dimensions and parameters in the Labware Editor. See Editing Labware on page 133.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Copy permission may create copies of labware definitions. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To make a copy of a labware type:

1. In the Labware Selection List, select the labware type to copy.
2. From the tool bar, click **Copy**.

   OR

   From the menu bar select **Actions > Make a copy of the selected labware type**.

   OR

   Right-click on the selected labware type and select **Make a copy of the selected labware type**.

Note: The default name format for copied labware types is Copy of OriginalName. To change the name, edit the labware type. See Editing Labware on page 133.

Deleting Labware

User-defined labware may be deleted from the Labware Selection List. Some labware may not be deleted:

- labware used in protocols.
- default labware installed with Multi-Mode Analysis Software.
- labware that has been signed on a system with GxP Permissions enabled.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Delete permission may delete user-defined labware. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

When labware is deleted it is moved to the trash selection list. To permanently remove or restore items for deletion see Deleting and Restoring Items on page 48.

To delete labware:

1. In the Labware Selection List, select the labware to delete.
2. From the tool bar, click **Delete**.
   OR
   From the menu bar select **Actions > Delete the selected labware type**.
   OR
   Right-click on the selected labware and select **Delete the selected labware type**.

Note: Multiple items may be selected for deletion by holding down the CTRL or SHIFT key while selecting each item desired.

3. The Message dialog appears.
4. Click **Yes** to delete the selected labware.
5. To permanently remove the labware see Deleting and Restoring Items on page 48.

Optimizing Labware

Microplate dimensions may vary slightly between production lots, which potentially affects measurement accuracy. Multi-Mode Analysis Software allows labware dimensions to be optimized by determining the centers of the four corner wells on the plate. Each time a labware type is optimized, a new labware lot is created with dimensions specific to that lot.

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

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Optimize permission may optimize labware to create new labware lots. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for details about permissions.

New labware lots may be created by optimizing labware for labware types that have been signed. See Signing Labware on page 133.

Labware is optimized in Optimizing Labware, which guides the process with a wizard-type interface. Optimizing labware includes:

- Creating a Copy of the Labware to be Optimized on page 138
- Start the Optimization Wizard on page 138
- Selecting the Detection Method on page 138
- Preparing and Loading the Labware on page 139
- Performing the Optimization Read on page 140
- Selecting the Centers of the Four Corner Wells on page 141
- Verifying Well Centers on page 142

Creating a Copy of the Labware to be Optimized

To create a copy of the labware to be optimized:

1. In the Labware Selection List, select the labware to optimize.
2. From the tool bar, click **Make a copy of the selected labware type**.

   OR

   From the menu bar select **Actions > Make a copy of the selected labware type**.

   OR

   Right-click on the selected labware and select **Make a copy of the selected labware type**.
3. As desired, rename the copied labware. See Editing Labware on page 133.

Start the Optimization Wizard

To start the optimization wizard:

1. In the Labware Selection List, select the copied version of the labware to optimize.
2. From the tool bar, click **Optimize the selected labware type**.

   OR

   From the menu bar select **Actions > Optimize the selected labware type**.

   OR

   Right-click on the selected labware and select **Optimize the selected labware type**.

Selecting the Detection Method

Labware is optimized by performing area scan measurements of the four corner wells of the microplate and then defining the well centers using images of the wells generated by the measurements. To ensure the most accurate optimization is performed, use Select Detection Method to select the most appropriate detection method for the optimization (Figure 6-6).

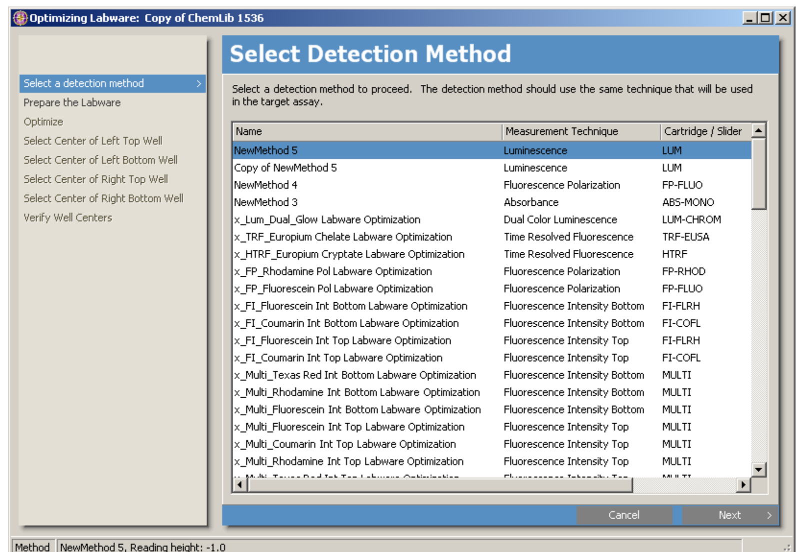


Figure 6-6 Selecting the Detection Method for Labware Optimization

To select the detection method:

1. To optimize labware for use in absorbance, fluorescence, or luminescence protocols, select a detection method configured in the protocol.
2. Click **Next** to Prepare the Labware. See Preparing and Loading the Labware on page 139.

Preparing and Loading the Labware

Labware dimensions are optimized by reading the four corner wells of the plate. Prepare Labware provides controls to load and eject labware from the instrument and to select the orientation of the plate on the microplate carrier (Figure 6-7).

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

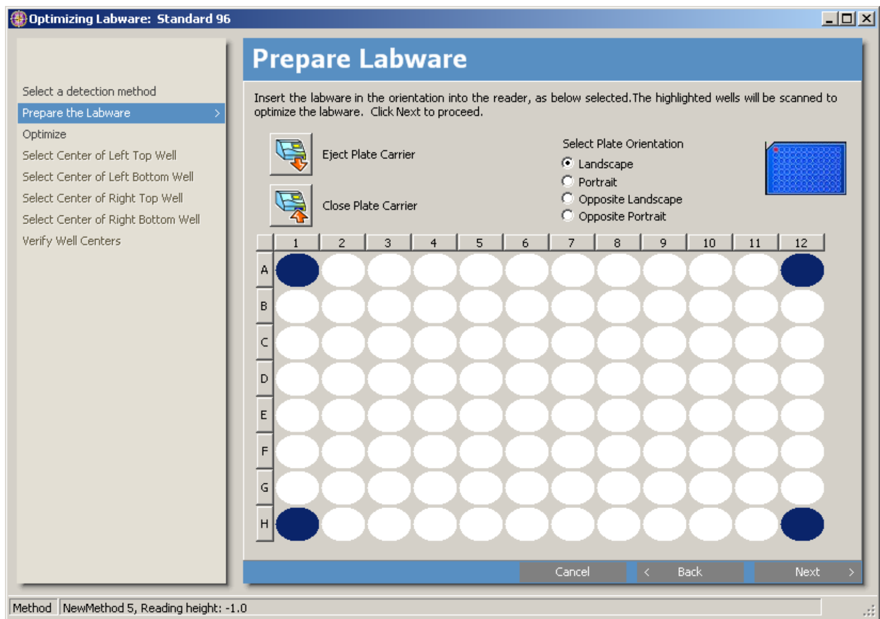


Figure 6-7 Preparing the Labware for Optimization

To prepare labware for optimization:

1. Click **Eject Plate Carrier** to move the microplate carrier outside the instrument.
2. Fill the corner wells of the plate with identical samples. To ensure accuracy, samples must be appropriate for the selected detection method. Sample concentration and volume must be identical in each well.
3. Place the microplate to be optimized on the plate carrier.
4. Click **Close Plate Carrier** to load the microplate into the instrument.
5. In Select Plate Orientation, select the orientation of the plate on the microplate carrier. The selected orientation is displayed graphically to the right of the screen, with well A1 highlighted in red.
6. Click **Next** to start the optimization. See Performing the Optimization Read on page 140. The optimization read begins automatically.

Performing the Optimization Read

Optimization in Progress displays the status of the optimization read and provides the ability to cancel the optimization in progress (Figure 6-8). The optimization read requires several minutes to complete.

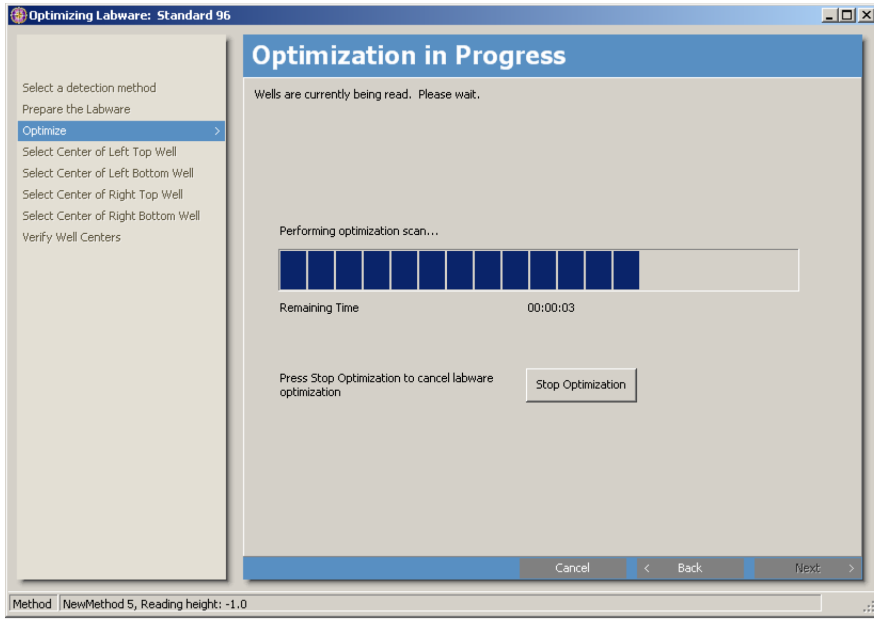


Figure 6-8 Labware Optimization In Progress

To cancel the optimization process and close the Optimizing Labware dialog without saving the optimization data click the **Stop Optimization** button.

When the optimization read is complete:

- Click **Next** to select the centers of the four corner wells. See Selecting the Centers of the Four Corner Wells on page 141.

Selecting the Centers of the Four Corner Wells

Use Select Center to precisely define the centers of the corner wells read in the optimization Figure 6-9. Select Center displays an image of the well generated by the optimization read. Well centers are defined graphically by dragging cross hairs to the position visually identified as the center. Select Center is performed for each corner well individually.

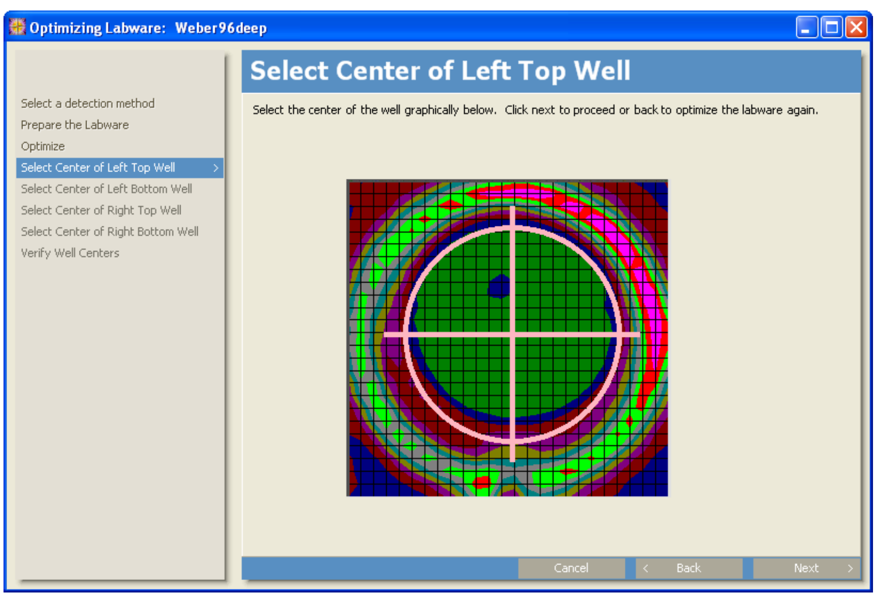


Figure 6-9 Selecting the Well Center

To define the centers of the wells:

1. Place the cursor in the well image.
2. Click-and-drag the cross hairs to the desired center of the well.

Note: The cross hairs will not display if the cursor is not in the well image.

3. Click **Next** to define the centers of the remaining well reads.
4. When all four well centers are defined, **Verify Well Centers** appears. See Verifying Well Centers on page 142.

Verifying Well Centers

Use Verify Well Centers to verify that the x and y offsets and distances between rows and columns are correct (Figure 6-10). The offsets, distances, and lot name may be edited in Verify Well Centers.

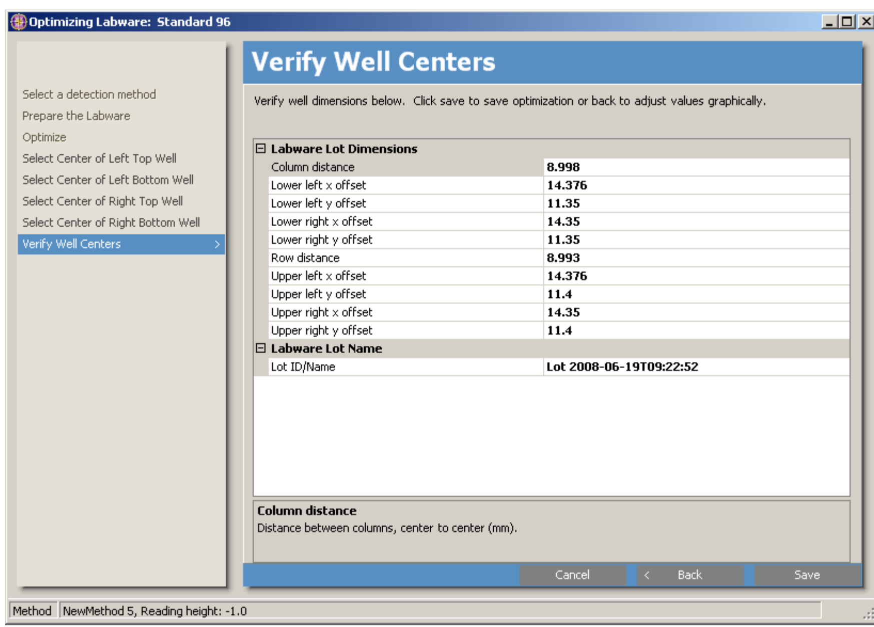


Figure 6-10 Verifying Well Centers

1. If necessary, click the **+** next to **Labware Lot Measurements** to display the fields in the category.

Note: More information about the field being defined is displayed below the property grid (Figure 6-10).

2. In **Column distance**, verify the distance between columns and edit the dimension. All offsets and well dimensions are entered in centimeters, if desired.
3. Verify the x and y offsets for the lower two wells and edit the dimensions, if desired.
4. In **Row distance**, verify the distance between rows and edit the dimension, if desired.
5. Verify the x and y offsets for the upper two wells and edit the dimensions, if desired.
6. If necessary, click the **+** next to **Labware Lot Name** to display the default name assigned to the new labware lot.
7. Enter a new **Lot ID/Name**, if desired.
8. Click **Save** to save the optimization data and create the new labware lot.

Note: To use the optimized lot in a measurement protocol, open the labware for editing and select the new Labware Lot. See Selecting and Editing Labware Lots on page 135.

Exporting and Importing Labware

User-defined labware can be exported to an XML file and imported later to restore that configuration or share it with a copy of Multi-Mode Analysis Software installed on another system.

Default labware installed with Multi-Mode Analysis Software is present on all systems and may not be edited, deleted, or overwritten. For this reason, importing default labware from an XML export file is not permitted.

Note: When GxP Permissions is enabled, signed labware may be exported for use on another system; however, electronic signatures are not retained, which allows labware to be edited when imported to another system. Because signed labware may not be deleted or overwritten, importing signed labware into the system from which it was originally exported is not permitted.

To export labware:

1. In the Labware Selection List, select the labware to export.
2. From the **File** menu, select **Export > Labware**. The Browse for Folder dialog appears.
3. In the **Browse for Folder** dialog, browse to the folder where the exported labware will be saved.

   OR

   Click **Make New Folder** to create a new folder where the exported labware will be saved.
4. Click **OK** to export the labware. The exported labware is saved using the default file name format, Labware__LabwareName.xml. To import the file at a later date, the filename must not be changed.

To import labware from an exported XML file:

1. From the **File** menu, select **Import > Labware**. The Open dialog appears.
2. In the Open dialog, browse to and select the desired XML file to import.
3. Click **Open**. The labware is imported to the Labware Selection List.

Creating and Running Protocols

7

Overview

A protocol stores all parameters required to perform a measurement, including technique type, detection methods, labware type, and preparation methods, such as shaking. Multiple measurements, including kinetic and scan measurements, may be configured in a single protocol. Protocols also specify how measurement results are viewed, exported, or printed when a protocol run completes. This section includes instructions for configuring the analysis features. Detection methods and labware must be configured before the protocol is created. See Creating and Editing Detection Methods on page 87 and Creating and Editing Labware on page 127 for additional information.

Note: Always verify the parameters configured in a protocol. Failing to verify all configured parameters may result in incorrect measurement results.

Protocols are listed in the Protocol Selection List (Figure 7-1), which provides access to all protocol actions:

- Creating Protocols on page 146
- Creating a Protocol from a Template on page 196
- Running Protocols on page 197
- Editing Protocols on page 211
- Copying Protocols on page 212
- Deleting Protocols on page 212
- Printing Protocol Configuration Information on page 213
- Exporting and Importing Protocols on page 214

To select protocols and access protocol functions:

- From the navigation pane, click **Protocols**. The Protocol Selection List appears (Figure 7-1).

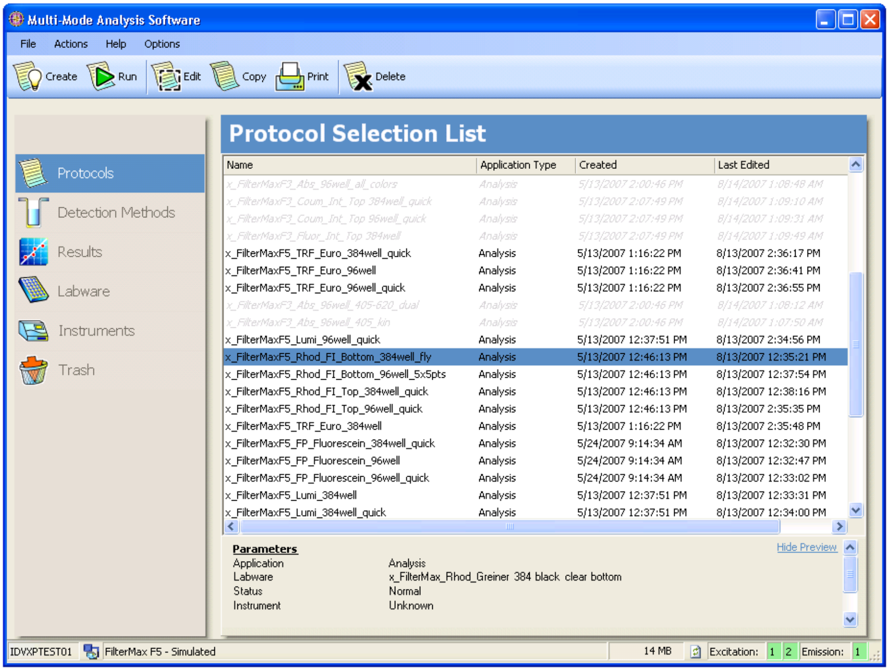


Figure 7-1 Protocol Selection List

Creating Protocols

New protocols are defined in Create Protocol, which guides the creation process with a wizard-type interface. Creating a new protocol requires:

- Configuring General Settings on page 148
- Selecting the Technique Type on page 149 (for Analysis protocols)
- Selecting the Labware Type Used in the Protocol on page 150
- Configuring Labware Layout Settings on page 151
- Adding Detection and Preparation Methods for Analysis Protocols on page 155

  OR

  Configuring Methods for Quantitation Protocols on page 165
- Configuring the Data Reduction on page 172
- Configuring Output Settings on page 187

Several analysis options may be selected and configured to transform measurement data as desired when configuring Analysis and Quantitation protocols:

- Configuring Variables on page 170
- Configuring a Transformation Formula on page 177
- Configuring Concentration on page 179
- Configuring Cutoff Values on page 183
- Configuring Validation Rules on page 185

To create and configure a new protocol:

1. From the tool bar, click **Create**.
   OR
   From the menu bar select **Actions > Create a new protocol**.
   OR
   Right-click in the Protocol Selection List and select **Create a new protocol**.
2. The Select Protocol Type dialog appears (Figure 7-2).

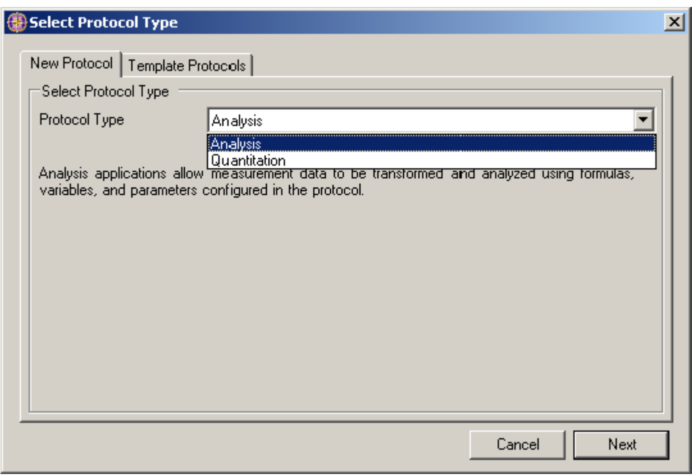


Figure 7-2 Selecting the Protocol Type

3. There are two options for creating protocols, from the **New Protocol** tab and **Template Protocols** tab. To create a new (non-template) protocol, continue to step 4. To create a protocol using a template protocol see Creating a Protocol from a Template on page 196.

Note: Template protocols are used to create a protocol based upon a selected template. Pre-defined settings appear as the protocol is configured.

Note: By disabling the **Menu Options > Show Example Protocols**, all example protocols (starting with **x_**) are hidden from view in the **Protocol Selection** List.

4. Select the **Protocol Type**:
   - **Analysis**: Analysis applications allow measurement data to be transformed and analyzed using formulas, variables, and parameters configured in the protocol.
   - **Quantitation**: Quantitation applications measure the purity and/or concentration of proteins or nucleic acid samples, such as DNA or RNA.

Note: For the FilterMax Multi-Mode Microplate Readers, to create or run quantitation protocols a genomic filter slide, which contains narrow bandwidth 260nm and 280nm filters must be installed and configured.

5. Click **Next** to configure the protocol. The Create Protocol dialog appears, displaying General Settings. See Configuring General Settings on page 148.

Configuring General Settings

Use General Settings to define the protocol name and enter any related notes about the protocol (Figure 7-3). Analysis Options to include in the protocol are available for selection.

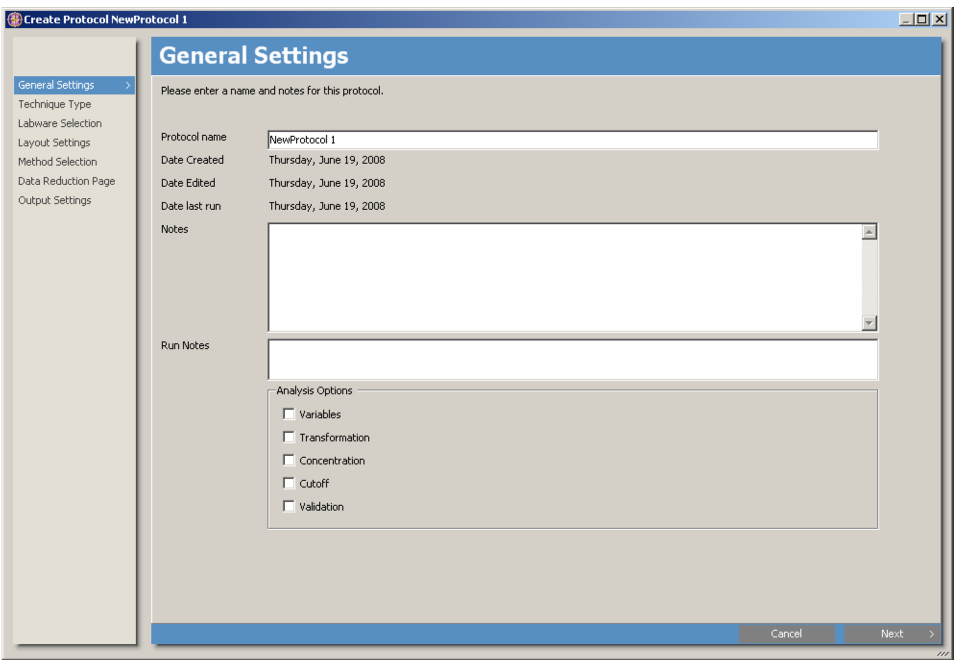


Figure 7-3 Defining a Protocol Name and Entering Notes About the Protocol

To configure the general settings for a new protocol:

1. In **Protocol name**, enter a unique name for the protocol. Duplicate protocol names are not permitted.
2. In **Notes**, enter a description for the protocol, if desired.
3. In **Run Notes**, enter further notes specific to this run, if desired. These notes will appear when the protocol starts up.
4. Select the desired Analysis Options for configuration in the protocol:
   - **Variables**: define up to ten numeric values that may be used in any formula configured in the protocol. See Configuring Variables on page 170.

Note: The Variables option is only available for Analysis applications.

   - **Transformation**: configure an algebraic formula to transform measurement data. See Configuring a Transformation Formula on page 177.

Note: The Transformation option is only available for Analysis applications.

- **Concentration**: values or formulas for quantitation of sample concentration with a standard curve. See Configuring Concentration on page 179.
- **Cutoff**: configure cutoff formulas to classify measured samples according to defined cutoff values. See Configuring Cutoff Values on page 183.
- **Validation**: configure up to ten validation formulas to evaluate if a protocol run meets the specified conditions required to be valid. See Configuring Validation Rules on page 185.

Note: Only Analysis Options that have been selected appear in the Create Protocol navigation pane and are available for configuration in the protocol.

5. Click **Next** to select the Technique Type. See Selecting the Technique Type on page 149.

Selecting the Technique Type

Use Technique Type to select the measurement technique to be performed by the protocol (Figure 7-4). Only techniques supported by the instrument and for which detection methods have already been defined are available. For more information about technique types, see Creating and Editing Detection Methods on page 87.

Note: Technique Type is only configured for Analysis protocols. For Quantitation protocols see Selecting the Labware Type Used in the Protocol on page 150.

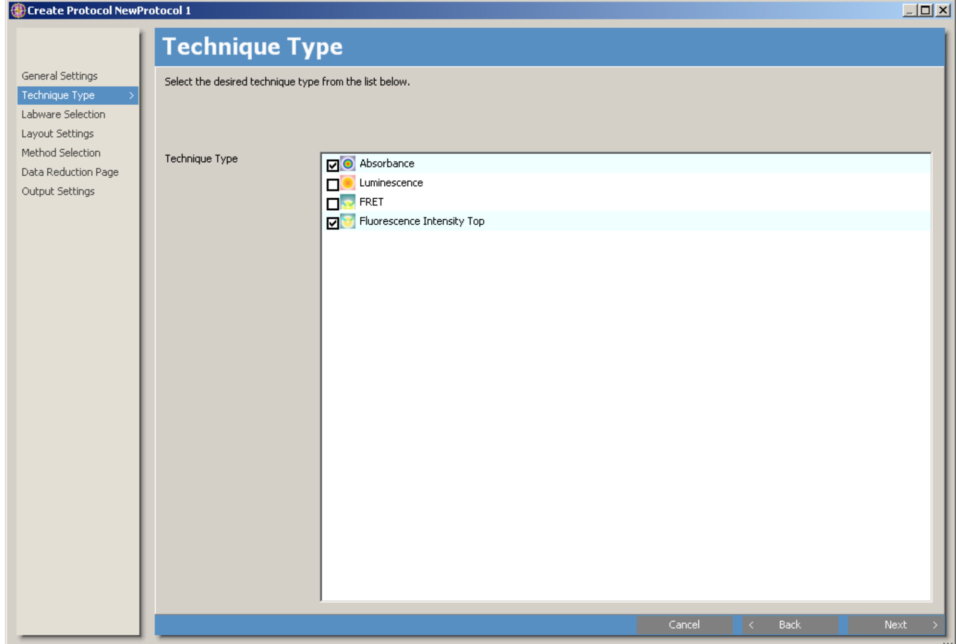


Figure 7-4 Selecting the Measurement Technique

To select a technique type:

1. In **Technique Type**, select the desired measurement techniques. Multiple techniques may be selected to create protocols using different detection techniques.

Note: By selecting different technique types following options while creating a protocol are filtered (such as available labware, detection methods, etc.).

Note: If editing an existing protocol, selecting a different technique type removes any detection methods previously configured in Method Selection. New detection methods must be configured before the protocol may be used to perform measurements. See Adding Detection and Preparation Methods for Analysis Protocols on page 155.

2. Click **Next** to select the Labware Type. See Selecting the Labware Type Used in the Protocol on page 150.

Selecting the Labware Type Used in the Protocol

Use Labware Selection to select the type of labware used in the protocol (Figure 7-5). Labware must be configured prior to configuring the protocol. Only labware configured for the selected protocol and technique type is available. Labware cannot be edited once it is used in a protocol. See Creating and Editing Labware on page 127 for detailed information about creating and configuring labware.

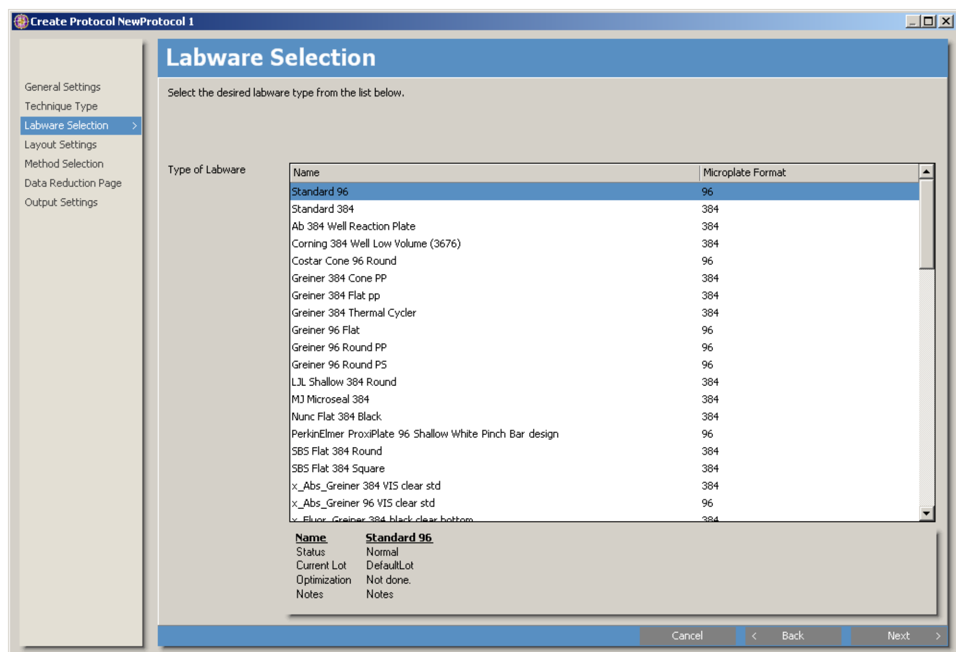


Figure 7-5 Selecting the Type of Labware Used in the Protocol

To select labware:

1. Select the desired **Type of Labware** from the list.

Note: If the labware is not optimized a Warning dialog appears. To continue without optimizing the labware click Yes. To optimize the labware, click No and then Cancel to exit the Create Protocol Wizard and optimize the labware first. See Optimizing Labware on page 137. Labware optimization can be done after the protocol is created. The protocol always uses the active labware lot.

2. Click **Next** to configure the Labware Layout Settings. See Configuring Labware Layout Settings on page 151.

Configuring Labware Layout Settings

Use Layout Settings to configure how wells on the plate are read (Figure 7-6). Settings include configuring well types and locations, replicates, and the layout of well identifiers on the plate.

To configure labware layout settings:

1. To import a plate layout from an existing protocol, click **Import Layout** and select the desired protocol from the list that appears. Only protocols with compatible plate layouts are listed.

   OR

   To create a new or edit an existing plate layout, select the desired wells to label. To select:

   - **all wells on the plate**: click the small button in the upper left corner of the plate layout display (Figure 7-6).
   - **all wells in a single column or row**: click the desired column or row header. Multiple columns or rows may be selected by holding down the **CTRL** key while selecting each header desired.
   - **individual wells**: click on the desired well. Multiple wells may be selected by holding down the **CTRL** key while clicking on each well desired.
   - **groups of wells**: click and drag over the desired group of wells. Multiple groups may be selected by holding down the **CTRL** key while dragging over each desired group.

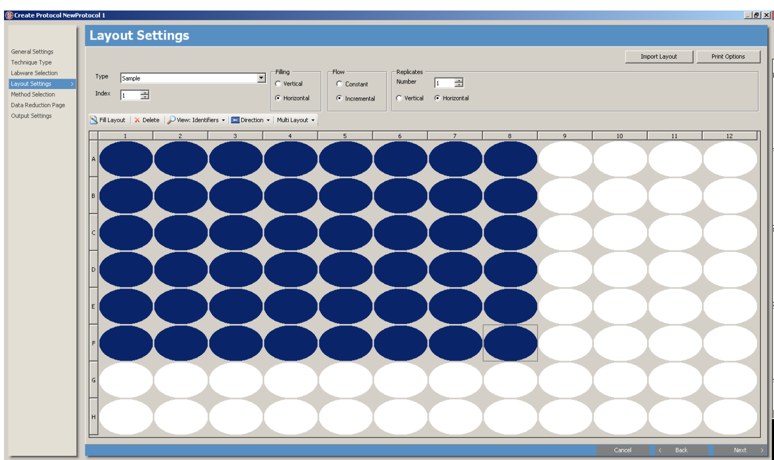


Figure 7-6 Configuring Layout Settings

2. In **Index**, select the initial label number for the sequence of selected wells.
3. In **Filling**, select the desired direction for labeling the selected wells:
   - **Vertical**: labels wells column by column.
   - **Horizontal**: labels wells row by row.
4. In **Flow**, select how the index is applied to wells in the selection.
   - **Constant**: all well identifiers in the selection are assigned the current index number.
   - **Incremental**: increases each well identifier in the selection by one.
5. In **Replicates**, select the number of replicates for each sample and the layout orientation on the plate:
   - **Vertical**: replicates are arranged vertically in columns.
   - **Horizontal**: replicates are arranged horizontally in rows.

Note: Replicate parameters are available for configuration only when **Incremental Flow** is selected.

6. Select the type of wells to add to the plate layout from the **Type** field and clicking **Fill** or by right-clicking on a selected well:
   - **Standard**: a well with a known concentration used to develop or correct a standard curve. Up to twelve standards may be configured on a plate.
   - **Control**: a well with a known, expected signal used to verify the results of the plate.
   - **Positive Control**: a control well in which a known amount of target reagent generates a signal to verify positive results measured in sample wells.

- **Negative Control**: a control well lacking the target reagent that generates little to no signal; verifies negative results measured in sample wells.
   - **Sample**: a well containing a sample to measure.
   - **Blank**: a well filled with reagents but no reacting sample. Blank wells are used to measure background noise. When blanks are configured, background correction is automatically applied to measurement results.
   - **Empty**: a well that is left empty.
   - **Advanced Controls** (Blue/Green/Red): For use with wells of a known, expected color signal to verify the results of the plate.
7. If configuring Blank wells, select the type of **Blank Validity** desired: **Plate**, **Row**, or **Column.** The mean value of all blank wells in the selected validity option is subtracted from sample wells to provide background correction.

Note: By choosing **Blank**, **Blank Validity** replaces **Filling**, **Flow**, and **Replicate** options in **Layout Settings** when configuring Blank wells.

8. If necessary, click **Delete** to delete existing labels from the selected wells.
9. Repeat steps 1 through 8 to define additional well selections, as desired.
10. Configure **Dilution Factors**, if desired. See Configuring Dilution Factors on page 154.

11. Select the desired reading direction using the Direction menu:
    - **Read by row** reads plates row-by-row.
    - **Read by column** reads plates column-by-column.
    - **Read by well** reads each well individually before reading the next well, which is useful for short-interval kinetic, area scan, and wavelength scan measurements.

Note: If adding a wavelength scan to the protocol, the reading direction must be set to well mode.

12. To use a multi-plate layout, using the **Multi Layout** menu select **Yes**. To view the multiplate layout click **View**. A multi-plate layout places all the controls on the first plate and the following plates only have samples. The number of plates in a multi-plate layout is specified when the protocol is run, see Running a Protocol on an Instrument on page 197.

Note: In a multi-plate layout the multiple plates are treated like one large plate. All controls are on the first plate and the following plates only have samples. The limit for a multi-plate layout is 100 plates.

13. Click **Print Options** and select which information about plate layouts will display in printed reports.
    - **Print all Wells in List Format**: Well label identifiers will display in a list.
    - **Print all Wells in Layout Format**: Well label identifiers appear in a layout.

Configuring Dilution Factors

If wells are diluted, the dilution factor can be set for each well. As desired, the dilution factor can be used in the Data Reduction step.

To configure dilution factors for the wells on a plate:

1. In the **View:** menu, select **Dilutions**. The plate map display changes to show the well identifier and the dilution ratio. Initially, the dilution ratio for all wells is 1/1, or undiluted.

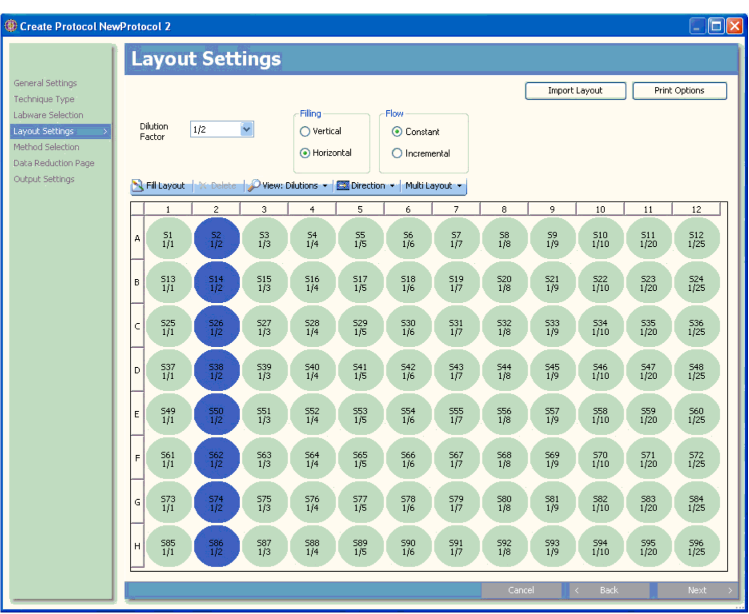


Figure 7-7 Configuring Dilution Factors for a Plate

2. Select the well or wells for which to set the dilution ratio.
3. In the **Dilution Factor** field, select the desired dilution ratio.
4. In **Filling**, select whether to fill across rows first or down columns first. Options are:
   - **Vertical**: fill the dilution ratio for wells vertically down columns before filling the dilution ratio in the next column.
   - **Horizontal**: fill the dilution ratio for wells horizontally across rows before filling the dilution ratio in the next row.
5. In **Flow**, specify whether to use a constant dilution factor or to increment each well by the Dilution Factor. Options are:
   - **Constant**: set the dilution ratio of all selected wells to the Dilution Factor.
   - **Incremental**: increment the dilution ratio for each well by the Dilution Factor, starting with an undiluted well. For example, if the Dilution Factor is 1/2, four wells are selected, and the Flow is incremental, the dilution ratio for those four wells will be 1/1, 1/2, 1/4, and 1/8.
6. Click **Fill** to set the dilution ratio for the selected wells using the current **Dilution Factor**, **Filling**, and **Flow** settings.
7. Repeat Step 1 through Step 5 to configure the dilution ratio for all wells on the plate.
8. Click **Next** to configure the Detection and Preparation Methods. See Adding Detection and Preparation Methods for Analysis Protocols on page 155 or Configuring Methods for Quantitation Protocols on page 165.

Adding Detection and Preparation Methods for Analysis Protocols

Use Method Selection to select and add detection and preparation methods. The estimated duration time for the protocol is displayed in the Estimated Time Field of the protocol. Selecting **Per Cartridge** sorts the detection methods by cartridge type versus detection method type. Selecting **Show only installed Methods** displays only enabled detection methods in the selection list when connected to an instrument.

Note: If configuring a protocol in Quantitation mode, see Configuring Methods for Quantitation Protocols on page 165.

To add a method:

1. Select the measurement type using the tabs:
   - **Single**: One read per well (endpoint measurement).
   - **Kinetic**: Kinetic measurements perform a specified series of measurements on each sample at specified intervals. Final measurement results are calculated from raw data using a data reduction method. For more information about data reductions see Configuring the Data Reduction on page 172.
   - **Area Scan**: Area scans read a number of measurement points arranged in a grid pattern across each well. Linear scans read a number of points in a linear axis crossing the center of each well.
   - **Wavelength Scan**: Wavelength scan enables scan measurements between 230-1000nm in 1nm increments on each well.

Note: If adding a wavelength scan the reading direction must be set to well mode.

2. Available detection and preparation methods display within the Select Method section on the left-side of the window. To add a detection or preparation method, click-and-drag the method to the protocol list in the center of the window.

   OR

   Select the detection or preparation method and click the **Add a method to this protocol** button (Figure 7-8).

   OR

   Select the detection or preparation method and press **Enter** or **Space** to add the method.

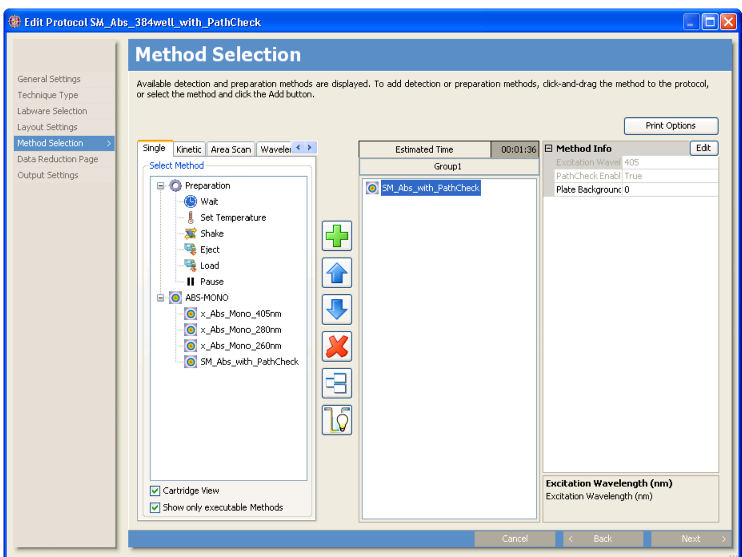


Figure 7-8 Adding a Single Detection Method

3. Select the method in the protocol and as required configure it using the Method Info section in the right pane.

   OR

   If adding a preparation method, in the protocol pane select the preparation method. The preparation info replaces the Method Info in the right pane. Configure the preparation method properties, as applicable:

   - **Shake**: See Configuring Shake Properties on page 163 to configure the Method Properties for the Shake preparation method.
   - **Set Temperature**: See Configuring Set Temperature Properties (FilterMax 5 Multi-Mode Microplate Reader and SpectraMax Paradigm Multi-Mode Detection Platform only) on page 163 to configure the Method Properties for the Set Temperature preparation method.
   - **Wait**: halts operations on the instrument for a specified length of time before continuing to the next action in the method sequence. See Configuring Wait Properties on page 164 to configure the Method Properties for the Wait preparation method.
   - **Eject**: moves the plate carrier outside the instrument to allow access for placement or removal of a microplate. Eject has no Method Properties to configure.

Note: The Eject preparation method should be followed by a Pause preparation method. If there is no Pause, the next command in the sequence is executed immediately after completion of the Eject command.

   - **Load**: retracts the plate carrier and microplate inside the instrument in preparation for performing a measurement. Load has no Method Properties to configure.
   - **Pause**: halts operations on the instrument for an indefinite length of time and displays a message, requiring user interaction to continue to the next action in the method sequence. See Configuring Pause Properties on page 164 to configure the Method Properties for the preparation method.

Note: A new detection method can be created at this time by clicking the **Create** button. The Method Editor appears allowing the creation of a new detection method for the current instrument. See Creating Detection Methods (FilterMax Multi-Mode Microplate Readers) on page 88 or Creating Detection Methods (SpectraMax Paradigm Multi-Mode Detection Platform) on page 99 for more information regarding creating a detection method.

Note: To change method parameters, in the Method Information window select the method and then click the **Edit** button.

4. If adding a kinetic detection method, in the protocol select **Kinetic** for the detection method. Kinetic Info appears in the right pane. Configure Kinetic Info by following the instructions in Configuring Kinetic Method Properties on page 159.

   OR

   If adding an area or linear scan detection method, in the protocol select **Area Scan** for the detection method. Area Scan Info appears in the right pane. Configure Area Scan Info by following the instructions in Configuring Area Scan Method Properties on page 160 or Configuring Linear Scan Method Properties on page 161.

   OR

   If adding a wavelength scan detection method, in the protocol select **Wavelength Scan** for the detection method. Wavelength Scan Info appears in the right pane. Configure Wavelength Scan Info by following the instructions in Configuring Wavelength Scan Method Properties on page 162.

Note: If adding a wavelength scan the reading direction must be set to well mode.

5. Add and configure additional detection or preparation methods by repeating steps 1 through 5, as desired.

Note: Edit selected methods by clicking the **Edit** button near the top right corner of the **Method Selection** window. See Figure 7-9.

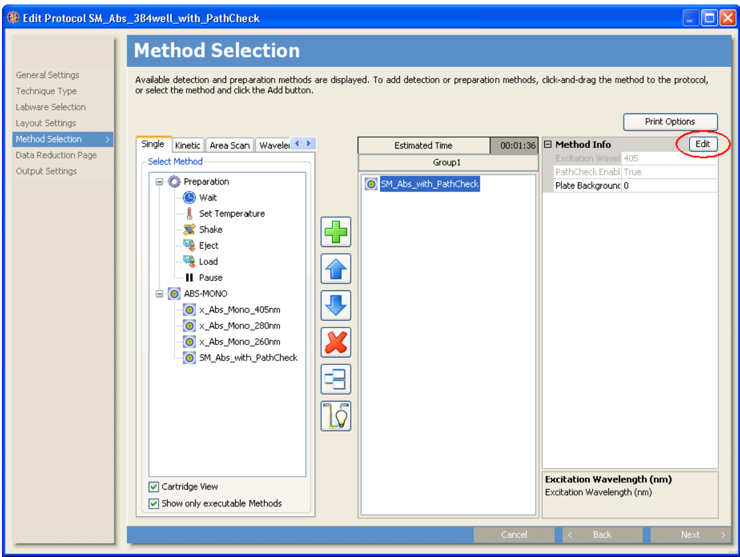


Figure 7-9 Method Selection window Edit button

6. To change the order in which detection and preparation methods are executed in the sequence, in the protocol, select the method to be moved and click the blue up or down arrows.

   OR

   In in the protocol, right-click on the desired method and select **Move Up** or **Move Down**.

   OR

   In the protocol, click on the desired method and drag the method up or down into the desired position.

Note: To move a kinetic, area scan, or wavelength scan method, select **Kinetic**, **Area Scan**, or **Wavelength Scan** above the detection method desired to move, not the method itself.

7. To remove a detection or preparation method from the protocol, in the protocol select the method to be removed and click the red delete button.

   OR

   In the protocol, right-click on the desired detection or preparation method and select **Remove**.

Note: To delete a kinetic, area scan, or wavelength scan method, select **Kinetic**, **Area Scan**, or **Wavelength Scan** above the detection method desired to delete, not the method itself.

8. To add a group, select the desired detection or preparation method for creating a new group. Click on Group, and a new group will be created. Two or more groups can be created in a protocol, there is no limit to the number of groups that can be created.

Note: The group option is used for integration or multi-step analysis purposes, providing a break in the protocol without ending the protocol. Data obtained from each group can be used together in the Analysis.

9. If you are using a method with PathCheck Pathlength Measurement Technology enabled and you have determined a plate background constant for your microplate, you can enter the value in the **Plate Background** field. See Use Plate Background Constant on page 247.
10. Click on the **Print Options** button to select the method information and measurement results data included in printouts:
    - **Print Method Parameters**: details about the configured method, including technique type and filters used.
    - **Print Graphical View**: kinetic or scan graphs of results for all measured samples. Available for kinetic and scan measurements only.
    - **Raw Data**: results from each kinetic cycle or scan point. Available when kinetic measurements, scan measurements, or multiple detection methods are configured in the sequence.
    - **Print Well Status**: status indicating whether the well was read successfully.
11. Click **Next** to configure the Variables (if selected in the General Settings, see Configuring Variables on page 170) or to configure the Data Reduction, see Configuring the Data Reduction on page 172.

Configuring Method Properties

Detection methods and preparation methods that are added to a protocol may have method properties that need to be configured.

- Configuring Kinetic Method Properties on page 159
- Configuring Area Scan Method Properties on page 160
- Configuring Linear Scan Method Properties on page 161
- Configuring Wavelength Scan Method Properties on page 162
- Configuring Shake Properties on page 163
- Configuring Set Temperature Properties (FilterMax 5 Multi-Mode Microplate Reader and SpectraMax Paradigm Multi-Mode Detection Platform only) on page 163
- Configuring Wait Properties on page 164
- Configuring Pause Properties on page 164

Configuring Kinetic Method Properties

Kinetic measurements perform a specified series of measurements on each sample at specified intervals. Final measurement results are calculated from raw data using a data reduction method.

- **FilterMax Multi-Mode Microplate Readers**: Kinetic measurements may be configured for all FilterMax Multi-Mode Microplate Readers method types except time-resolved fluorescence, FRET and area scans.
- **SpectraMax Paradigm Multi-Mode Detection Platform**: Kinetic measurements may be configured for all SpectraMax Paradigm Multi-Mode Detection Platform method types except area scans and absorbance wavelength scans.

To configure properties for a kinetic measurement:

1. Select Kinetic for the detection method being configured. Kinetic Info appears in the right pane.
2. In Kinetic Info enter the number of **Kinetic Cycles** to be performed. Kinetic measurements may be set to perform 2 to 100 cycles.
3. In Kinetic Info enter the desired **Kinetic Interval** in seconds. The interval is the length of time between each measurement of the same well.

Note: The minimum kinetic interval is populated automatically in **Kinetic Interval**, and is determined by the labware type and layout settings configured in the protocol. The maximum interval between measurement cycles is 65,535 seconds.

Configuring Area Scan Method Properties

Area scan measurements may be configured for absorbance and fluorescence intensity detection methods. Area scans read a number of measurement points arranged in a grid pattern across each well.

Note: Properties (Resolution and Point Selection) are fixed when using on-the-fly detection methods.

To configure properties for an area scan:

1. Select **Area Scan** for the detection method being configured. Area Scan Info appears in the right pane.
2. To configure the type of scan measurement and the number of points measured, in Method Properties, click in **Scan Points** and click the configuration button. Scan Selection Editor appears (Figure 7-10).

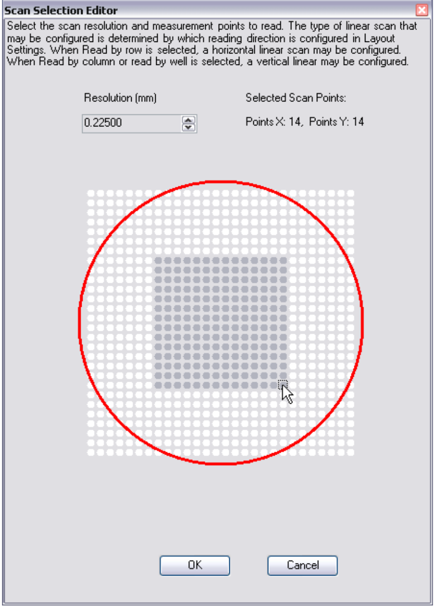


Figure 7-10 Configuring an Area Scan

3. In **Resolution (mm)**, use the up and down arrows to select the proximity of measurement points. selecting a smaller value increases the number of measurement points available; selecting a larger value decreases the number of points available. Available resolutions are determined by the

type of labware selected for use in the protocol. Detection methods with “On the fly” detection selected have fixed resolution and scan points. See Selecting the Labware Type Used in the Protocol on page 150 and Configuring Labware Layout Settings on page 151 for more information.

4. Click anywhere inside the well boundary, except the center row, and drag until the desired number of measurement points is selected (Figure 7-10).
5. Click **OK** to save the scan configuration and close the Scan Selection Editor.

Configuring Linear Scan Method Properties

Linear scan measurements may be configured for absorbance and fluorescence intensity detection methods. Linear scans read a number of points in a linear axis crossing the center of each well.

To configure properties for a linear scan:

1. Select Area Scan for the detection method being configured. Area Scan Info appears in the right pane.
2. To configure the type of scan measurement and the number of points measured, in Method Properties, click in **Scan Points** and click the configuration button. Scan Selection Editor appears (Figure 7-11).

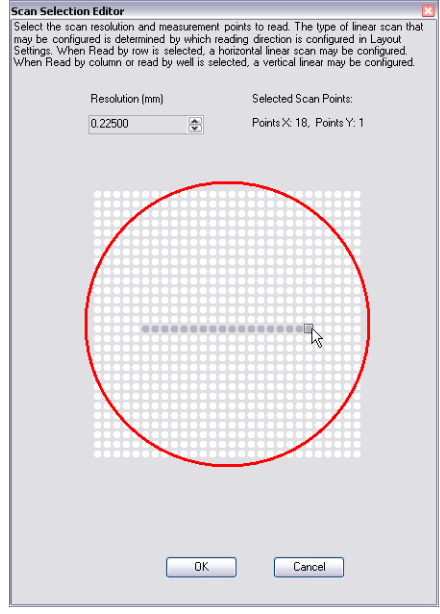


Figure 7-11 Configuring a Linear Scan

3. In **Resolution (mm)**, use the up and down arrows to select the proximity of measurement points. selecting a smaller value increases the number of measurement points available; selecting a larger value decreases the number of points available. Available resolutions are determined by the type of labware selected for use in the protocol. Detection methods with “On the fly” detection selected have fixed resolution and scan points. See Selecting the Labware Type Used in the Protocol on page 150 and Configuring Labware Layout Settings on page 151 for more information.
4. To configure a linear scan, click anywhere on the center row or column inside the well boundary and drag towards the boundary until the desired number of measurement points is selected (Figure 7-11).

Note: The type of scan that may be configured is determined by the reading direction settings in Layout Settings. See Configuring Labware Layout Settings on page 151. Horizontal linear scans along the center row may be configured only when Read by row is selected, while vertical linear scans along the center column may be configured only when Read by column or Read by well is selected.

5. Click **OK** to save the scan configuration and close the Scan Selection Editor.

Configuring Wavelength Scan Method Properties

Wavelength scan measurements enables scans between the defined start wavelength and end wavelength in user configurable increments on each well.

Note: If adding a wavelength scan the reading direction must be set to well mode.

To configure properties for a wavelength scan:

1. Select the wavelength scan detection method to configure. Wavelength Scan Info appears in the right pane (Figure 7-12).

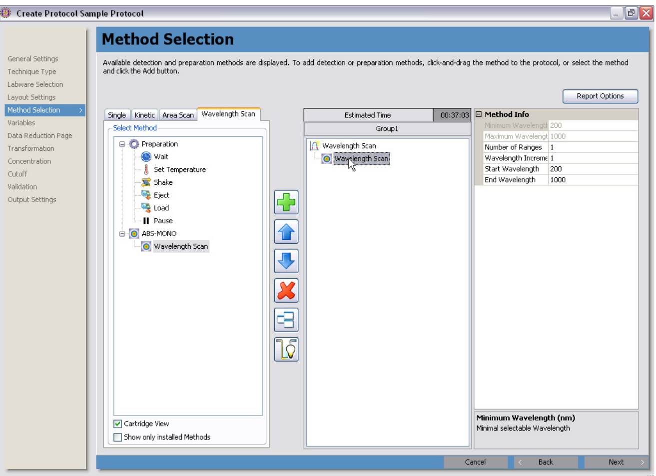


Figure 7-12 Wavelength Scan Detection Method Properties

2. In Method Info enter the number of wavelength ranges in **Number of Ranges**, Up to three wavelength ranges can be defined so that the spectrum can be divided into separate parts (such as 230-300 and 900-1000).
3. In Method Info enter the **Wavelength Increment** in nm. The wavelength step interval is the increment between each wavelength measurement of the same well.
4. In Method Info enter the **Start Wavelength** in nm. The starting wavelength must me smaller than the end wavelength.
5. In Method Info enter the **End Wavelength** in nm.
6. As required for multiple wavelength ranges, repeat Step 4 through Step 5.

Configuring Shake Properties

A Shake preparation method may be placed in the desired location in the sequence, or attached to a kinetic measurement when interval shaking between each measurement cycle is desired.

To configure properties for a Shake preparation method:

1. Select the desired Shake preparation method in the protocol. Shake Info appears in the right pane.

   CAUTION! Shake low density plates, such as 6- or 48-well plates, at low speed only. Shaking low density plates at higher speeds may cause liquid in wells to spill.

2. In **Shake Intensity**, select the desired intensity of shaking: **Low**, **Medium**, or **High**.
3. In **Shake Interval**, enter the length of time in seconds (0-18000) to shake the microplate.

   Note: Shaking efficiency for 24- to 384-well plates has been improved in Multi-Mode Analysis Software version 2.0 and above. The Shake Interval for protocols originally created for use with version 1.0 may need to be reduced.

4. In **Shake Mode**, select the desired shaking pattern.
   - **Linear**: shakes from side to side.
   - **Orbital**: shakes labware in a circular pattern.
   - **Squared**: shakes labware in a square pattern, moving at right angles (FilterMax Multi-Mode Microplate Readers only).

Configuring Set Temperature Properties (FilterMax 5 Multi-Mode Microplate Reader and SpectraMax Paradigm Multi-Mode Detection Platform only)

A Set Temperature preparation method sets the temperature inside the microplate chamber by heating the chamber; cooling the chamber is not supported. Depending on the light source used in the protocol, the set temperature may range from 3°C (5.4°F) or 4°C (7.2°F) above ambient to 45°C (113°F). A minimum of 15 minutes is required for the instrument to reach the desired temperature. The actual time required depends on the relative change in temperature. The instrument may be configured to wait for it to reach the desired temperature or continue immediately to the next command in the method sequence.

Note: Set Temperature preparation methods are useful for kinetic measurements intended to measure the effects of temperature changes on samples.

The FilterMax 5 Multi-Mode Microplate Reader does not support heating to a set temperature.

To configure properties for a Set Temperature preparation method:

1. Select the Set Temperature preparation method in the protocol. Temperature Info appears in the right pane.
2. In **Set Temperature**, enter the desired microplate chamber temperature in degrees Celsius. In protocols that perform measurements only at visible wavelengths (>359 nm), the minimum temperature that may be set is 3°C (5.4°F) above ambient. When the protocol performs measurements in the UV range the minimum temperature is 4°C (7.2°F) above ambient. The maximum temperature that may be set is 45°C (113°F).

Note: The temperature remains at the current setting until overridden by another Set Temperature preparation method, by changing the temperature in Manual Control, or by turning the instrument off and on.

3. In **Wait for Temperature**, select the desired option:
   - **True**: wait until the FilterMax 5Multi-Mode Microplate Reader or SpectraMax Paradigm Multi-Mode Detection Platform reaches the Set Temperature before executing the next command.
   - **False**: immediately execute the next command.

Configuring Wait Properties

A Wait preparation method halts actions on the instrument for a specified length of time prior to executing the next command. Properties for a Wait preparation method include the length of time to wait before continuing with the protocol.

To configure properties for a Wait preparation method:

1. In Method Selection, select the desired **Wait** preparation method. Method Properties for the selected method appear.
2. In **Wait Time**, enter the length of time in seconds the instrument should wait before executing the next command.

Note: Wait Time must be between 1 and 3600 seconds.

Configuring Pause Properties

A Pause preparation method halts actions on the instrument for an indefinite length of time and display a message, requiring user interaction to continue to the next action in the method sequence. Properties for a Pause preparation method include the message to display during the pause.

To configure properties for a Pause preparation method:

1. Select the desired Pause preparation method in the protocol. Pause Info appears in the right pane.
2. In **Comment**, enter the text for the message to display when the Pause is executed during the protocol run.

Configuring Methods for Quantitation Protocols

In a Quantitation protocol, Method Selection (Figure 7-13) is used to select the quantitation methods to perform as part of the measurement. Configuring a quantitation method includes selecting the desired applications to run and configuring the parameters for the selected applications. Parameters may include a Normalization Factor, or effective sample height, which transforms the microplate OD values to a comparable value for the same sample in a 1cm cuvette. This Normalization Factor is applied as a divisor of the sample reading.

Note: To create or run quantitation protocols for a FilterMax Multi-Mode Microplate Reader, a genomic filter slide, which contains narrow bandwidth 260nm and 280nm filters must be installed and configured.

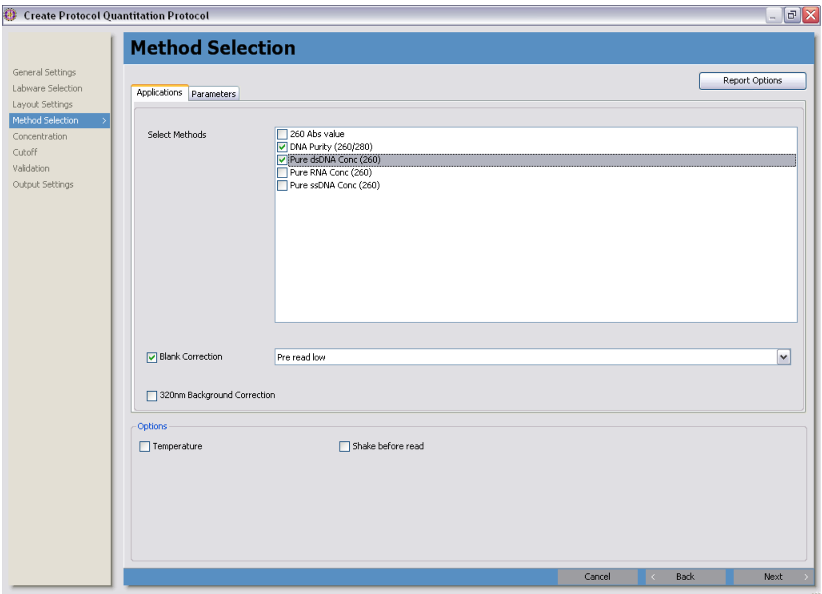


Figure 7-13 Configuring a Quantitation Method in Method Selection

To select and configure a quantitation method:

1. In Method Selection, select the **Applications** tab (Figure 7-13).
2. In Select Methods, select the desired quantitation applications to run:
   - **260 Abs Value**: net absorbance value at 260 nm. This selection is required when the Analysis option Concentration is selected in General Settings and samples are quantified against standards supplied in the plate layout. See Configuring Labware Layout Settings on page 151.
   - **DNA Purity**: measures absorbance at both 260 nm and 280 nm and determines the ratio as an indicator of nucleic acid purity. Pure DNA has a ratio around 1.8, while pure RNA has a ratio around 2.0.
   - **Pure dsDNA Concentration (260)**: measures absorbance at 260 nm and determines the concentration of pure dsDNA by normalizing the measurement values to equivalent 1cm cuvette measurements and transforming the equivalent values into concentrations. Requires input of a normalization factor and reciprocal coefficient of extinction.

- **Pure RNA Concentration (260)**: measures absorbance at 260 nm and determines the concentration of pure RNA by normalizing the measurement values to equivalent 1 cm cuvette measurements and transforming the equivalent values into concentrations. Requires the input of a normalization factor and reciprocal coefficient of extinction.
- **Pure ssDNA Concentration (260)**: measures absorbance at 260 nm and determines the concentration of pure ssDNA by normalizing the measurement values to equivalent 1cm cuvette measurements and transforming the equivalent values into concentrations. Requires the input of a normalization factor and reciprocal coefficient of extinction.

3. If no blank wells were added in Layout Settings, alternative Blank Correction options are available. Select **Blank Correction** to enable it for the protocol and select the desired type of correction to use:

Note: Reliable results require blank subtraction prior to performing further calculations. If the plate layout does not include blank wells, it is recommended that Blank Correction is selected.

- **Pre read high**: requires a pre read plate filled with blanks that is read prior to the measurement plate; subtracts the buffer measurement value of a well from the analyte measurement value of the same well for each well read. Use this option when blank variability from well to well is high.

Note: The blank plate should come from the same batch as the measurement plate with blank replicates dispensed into all wells. A blank replicate should contain all components common to all samples except for the analyte. Leaving blank wells empty may be an approximation and should be validated for each assay.

Note: When running a quantitation protocol with a pre read, the blank plate is measured first. After reading the blank plate, the plate carrier opens and the protocol pauses to allow the blank plate to be removed and the measurement plate to be loaded.

- **Pre read low**: requires a pre read plate filled with blanks that is read prior to the measurement plate; averages the buffer measurement values from all wells read and subtract the average from the analyte measurement value for each well. Use this option when blank variability from well to well is low, or relatively constant.

Note: The blank plate should come from the same batch as the measurement plate with blank replicates dispensed into all wells. A blank replicate should contain all components common to all samples except for the analyte. Leaving blank wells empty may be an approximation and should be validated for each assay.

Note: When running a quantitation protocol with a pre read, the blank plate is measured first. After reading the blank plate, the plate carrier opens and the protocol pauses to allow the blank plate to be removed and the measurement plate to be loaded.

- **Fixed Constant**: subtracts a fixed constant for 260 nm, 280 nm, and 320 nm (if 320 nm Background Correction is selected) measurements from each well. Use this option when the average blank value is stable for the given set of assay conditions. Enter the desired constants for 260 and 280, and 320. Default values are displayed in Table 7-1.

Table 7-1 Default blank values for Fixed Constant[a]

Wavelength	Blank Value
260	0.065
280	0.047
320	0.035

a Values are based on using TE buffer in a Costar 96-well microplate.

4. Select **320 nm Background Correction** to subtract the blanked measurement value at 320 nm from the value of each well. This background correction partially compensates for the turbidity of samples and is in addition to blank subtraction. Selection of this option results in an additional measurement being made at 320 nm.

Note: Reading background at 320nm does not substitute for blank correction, as the 320nm sample read is typically different from the blank reads at 260 nm and 280nm.

5. Select the **Parameters** tab to display the parameter information. Only the parameters for the methods selected on the Applications tab are displayed.
6. Configure the **Parameters** as appropriate. The application each parameter is associated with is identified in the front of each parameter name. See Table 7-2.

Table 7-2 Quantitation Parameters by Application Type

Application	Parameter	Default Value
260 Abs Value $abs = (A[260] - B[260]) - (A[320] - B[320])$ A[xxx] = sample measurement at xxx nm B[xxx] = blank measurement at xxx nm	Has no parameters.	N/A
DNA Purity $R = \frac{(A[260] - B[260]) - (A[320] - B[320])}{(A[280] - B[280]) - (A[320] - B[320])}$ A[xxx] = sample measurement at xxx nm B[xxx] = blank measurement at xxx nm	Normalization Factor [cm] (also called effective sample height)	Default: 0.29[a]Valid range: 0.01 to 1
Pure dsDNA Conc (260) $conc = \frac{abs}{norm}\frac{1}{E}dilution$ abs = absorbance value at 260 nm, as defined in 260 Abs Value above norm = normalization factor 1/E = reciprocal coefficient of extinction dilution = dilution factor	Normalization Factor [cm] (also called effective sample height)	Default: 0.29[a] Valid range: 0.01 to 1
	Reciprocal Coefficient of Extinction [(µg/mL) / (OD/cm)]	Default: 50 Valid range: 1 to 100

Table 7-2 Quantitation Parameters by Application Type (cont'd)

Application	Parameter	Default Value
Pure RNA Conc (260) $conc = \frac{abs}{norm}\frac{1}{E}dilution$	Normalization Factor [cm] (also called effective sample height)	Default: 0.29aValid range: 0.01 to 1
abs = absorbance value at 260 nm, as defined in 260 Abs Value above norm = normalization factor 1/E = reciprocal coefficient of extinction dilution = dilution factor	Reciprocal Coefficient of Extinction [(µg/mL) / (OD/cm)]	Default: 40 Valid range: 1 to 100
Pure ssDNA Conc (260) $conc = \frac{abs}{norm}\frac{1}{E}dilution$	Normalization Factor [cm] (also called effective sample height)	Default: 0.29[a]Valid range: 0.01 to 1
abs = absorbance value at 260 nm, as defined in 260 Abs Value above norm = normalization factor 1/E = reciprocal coefficient of extinction dilution = dilution factor	Reciprocal Coefficient of Extinction [(µg/mL) / (OD/cm)]	Default: 37 Valid range: 1 to 100

a Default value for normalization factor is based on 100 µL of DNA in a 96-well plate. This value is dependent on a number of factors, including volume of DNA, buffer viscosity, plate type, and well shape, and should be determined empirically and modified for the specific application. See Determining the Normalization Factor. See Table 7-3 for typical values for DNA in a 96-well plate.

Table 7-3 Typical Values for Normalization Factor[a]

Volume of DNA	Normalization Factor (Effective Sample Height)
100 µL	0.29 cm
150 µL	0.44 cm
200 µL	0.59 cm

a Values are based on volume of DNA in TE buffer in a Costar 96-well microplate. See Determining the Normalization Factor for instructions on determining normalization factors for different labware types.

7. As desired, in Options select **Temperature** to set the temperature of the instrument before reading. Properties for Temperature appears.
8. As necessary, in Set Temperature, enter the desired microplate chamber temperature in degrees Celsius. In protocols that perform measurements only at visible wavelengths (>359 nm), the minimum temperature that may be set is 3°C (5.4°F) above ambient. When the protocol performs measurements in the UV range the minimum temperature is 4°C (7.2°F) above ambient. The maximum temperature that may be set is 45°C (113°F).

Note: The temperature remains at the current setting until overridden by another Set Temperature preparation method, by changing the temperature in Manual Control, or by turning the instrument off and on.

9. As necessary, in Wait for Temperature, select the desired option:
   - **True**: wait until instrument reaches the Set Temperature before reading the plate.
   - **False**: immediately read the plate.
10. As desired, in Options select **Shake Before Read** to shake the microplate before reading, if desired.

CAUTION! Shake low density plates, such as 6-well or 48-well plates, at low speed only. Shaking low density plates at higher speeds may cause liquid in wells to spill.

11. As necessary, in **Shake Intensity**, select the desired intensity of shaking: **Low**, **Medium**, or **High**.
12. As necessary, in **Shake Interval**, enter the length of time in seconds (**0-18000**) to shake the microplate.

Note: Shaking efficiency for 24-well to 384-well plates has been improved in versions 2.0 and above. The Shake Interval for protocols originally created for use with version 1.0 of the software may need to be reduced.

13. As necessary, in **Shake Mode**, select the desired shaking pattern.
   - **Linear**: shakes from side to side.
   - **Orbital**: shakes labware in a circular pattern.
   - **Squared** (FilterMax Multi-Mode Microplate Readers only): shakes labware in a square pattern, moving at right angles.
14. Click **Report Options** and select the method information and measurement results data included in printouts:
   - **Method Information**: details about the configured method, including technique type and filters used.
   - **Raw Data**: results from each kinetic cycle or scan point. Available for kinetic and scan measurements only.
   - **Print Status**: status indicating whether or not the well was read successfully.
15. Click **Next** to configure the Variables (if selected in the General Settings, see Configuring Variables on page 170) or to configure the Data Reduction, see Configuring the Data Reduction on page 172.

Determining the Normalization Factor

The normalization factor is a value that relates the measurement value of a microplate well to its equivalent value in a 1cm cuvette. This normalization factor depends on the microplate type, well shape and dimensions, sample volume and viscosity, and other factors, and can be determined experimentally by comparing the value of a sample in a cuvette to the same sample measured in a microplate.

Note: It is recommended for best results that the assay conditions for the microplate are replicated as closely as possible when determining the normalization factor. Therefore, use a DNA standard diluted in assay buffer with UV-transparent microplates and quartz glass cuvettes. The microplate used with the instrument should be optimized (see Performing the Optimization Read on page 140) and measurements made using the 260 nm filter on the genomic filter slide.

To experimentally determine the normalization factor:

1. Aliquot a sample and a sample blank into separate 1cm pathlength cuvettes.

Note: Select a sample concentration within the dynamic range of the spectrophotometer at 1cm pathlength and in the instrument.

2. Measure the sample and the sample blank in a spectrophotometer. Record the blanked value (absorbance of the sample minus absorbance of the blank) as the **Absorbance value per 1cm**.
3. Aliquot a volume of the same sample and sample blank equal to the volume that will be read with unknown samples into several wells of the microplate which will be used for measurements. This enables replicates to be performed and yields a more accurate result.
4. Measure the microplate on the instrument. Record the blanked value (absorbance of the sample minus absorbance of the blank) as the **Absorbance value of the plate**.
5. Calculate the normalization factor by dividing the **Absorbance value of the plate** (step 4) by the **Absorbance value per 1 cm** (step 2). For example:

Table 7-4 Example Calculation of Normalization Factor

	Blank	Sample	Absorbance (Sample - Blank)	Normalization Factor
1 cm cuvette	0.066	0.816	0.750 (step 2)	
microplate	0.064	0.314	0.250 (step 4)	0.250/0.750 = 0.333 (step 5)

Configuring Variables

Up to ten variables may be defined for use in formulas configured in the protocol (Figure 7-14). Variables are typically used with test kits that have cutoff values or standard correction values based on lot number.

Note: Variables appear only when selected in General Settings for an Analysis protocol. See Configuring General Settings on page 148.

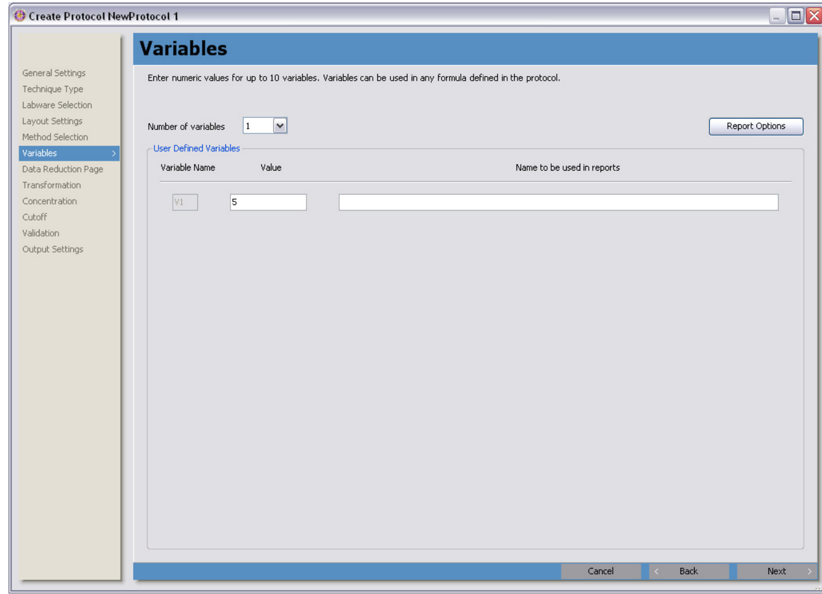


Figure 7-14 Configuring Variables in an Analysis Protocol

To configure variables:

1. Select the **Number of variables** to be configured. Up to ten variables may be configured. Entry fields for each variable appear.
2. For each variable, enter the desired numeric **Value**.
3. For each variable, enter the desired **Name to be used in reports**.
4. Click **Report Options** and select **Definition** to include all variable names, values, and names used in reports in printouts, if desired.
5. Click **Next** to configure the Data Reduction. See Configuring the Data Reduction on page 172.

Configuring the Data Reduction

Data reduction allows for multiple data calculations of measurement results from the detection methods in the protocol. Raw data results can be summarized to a single value. Results from the data reduction are used in analysis. Passes allow for additional data reduction using a previous data reduction. Results from the data reduction are displayed in the results and can be used for transformation, concentration, cutoff values, or for validation.

The raw data from each detection method is assigned a character that is listed besides the detection method in the Select Methods pane (For example, the first detection method is labeled “A”, the second “B”, and so on). The formula A+B+C would add the raw data from the three detection methods represented by A, B, and C. Detection methods that include multi-point data results can be expanded to view the additional labels (Figure 7-15).

Multipoint methods should have a data reduction (kinetic, area scan, wavelength scan). If the user does not enter a data reduction, the software creates a standard data reduction once the protocol is saved.

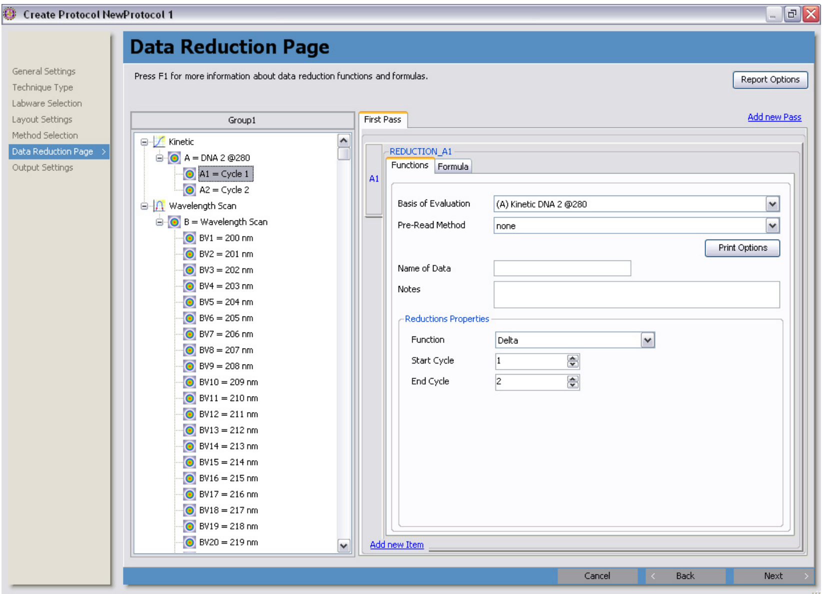


Figure 7-15 Data Reduction Page with Labels

To configure the data reductions:

1. In Data Reduction Method click **Add new** to use a data reduction method.
2. Select either the **Functions** tab or the **Formula** tab. The **Functions** tab provides access to predefined data reduction methods. The **Formula** tab allows for a manually creating a data reduction formula.

Note: The **Functions** tab is accessible for certain types of detection methods and only on the first pass of the data reduction.

3. If using the **Functions** tab (Figure 7-16):
   - Select the desired **Basis of Evaluation** using the selection box.

Note: Only detection methods that can be used as a basis of evaluation are available for selection.

- As necessary, select a **Pre-Read Method**. The Pre-Read method will subtract the measurement values of the Pre-Read method from the measurement values of the method selected in Basis of Evaluation.
- When necessary, using the Pre-Read Method menu select whether the Pre-Read method will subtract the average for all wells in the Pre-Read method (**Mean**), or subtract on a well-by well basis. (**Well**).
- Enter a name for the Data using the **Name of Data** field. This information is used later during data output in the reduced data results.
- As necessary, enter any notes about the data reduction using the **Notes** field.
- In the Reduction Properties section, using the **Function** field select the predefined data reduction method to use.

Note: Each type of read mode has its own set of predefined data reduction functions. See Table A-1 on page 241 for details about the data reduction methods available for sequence measurements.

- As necessary, configure the function's parameters using the fields displaying below the **Function** field.

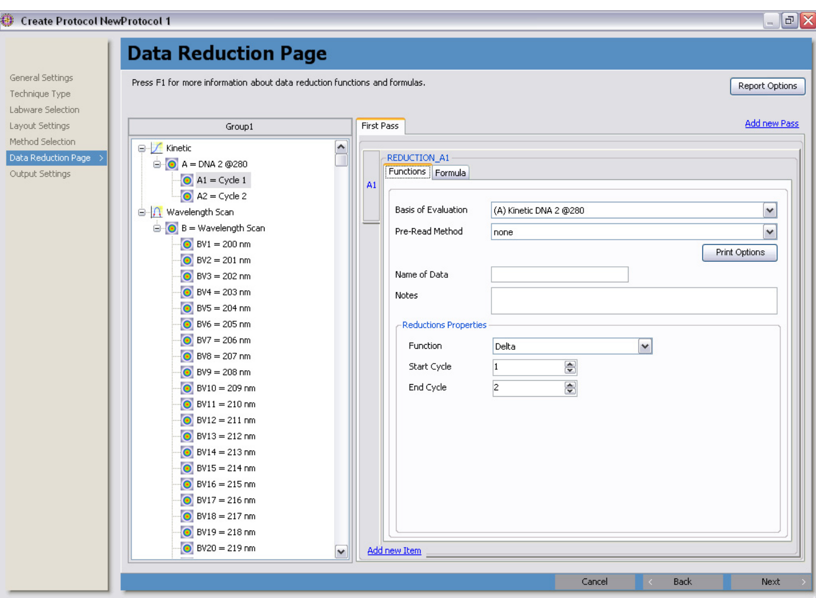


Figure 7-16 Functions Tab

OR

4. If using the Formula tab (Figure 7-17):

- Enter a formula for the desired data reduction using any of the mathematical operators (except for <, >, or =) or mathematical functions listed in Mathematical Operators and Functions on page 249.

Note: The **Formula** field can be used to create ratios, such as A/B, or corrected ratios, such as (A–C)/(B–C).

- Enter a name for the Data using the **Name of Data** field. This information is used later during data output in the reduced data results.
- Enter the units for the data using the **Name of Units** field. This information is used for information purposes only during data output.
- As necessary, enter any notes about the formula using the **Notes** field.
- Select the wells to apply the data reduction formula to using the **Calculate Formula for Controls** section. Only the selected well types will be used in the current data reduction formula.

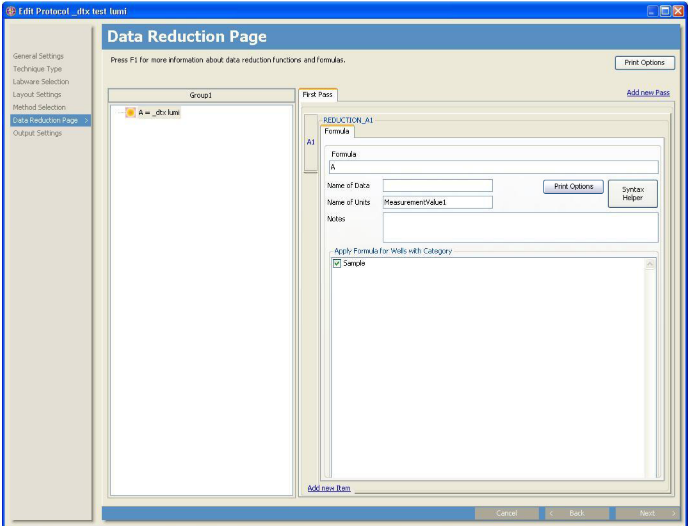


Figure 7-17 Formula Tab

5. To add an additional data reduction click **Add new**.

OR

To add an additional pass of data reduction formulas click **Add new Pass**.

OR

To add an additional pass of data reduction formulas click **Add new Pass**.

Note: Up to twenty-six data reductions may be added for each pass. Up to twenty-six passes can be added.

6. Optionally, click the **Syntax Helper** button, and the **Syntax Helper** window (Figure 7-18) appears. Syntax Helper helps in the creation of the correct syntax for accessing advanced data values. Access raw measurement data, blanked data (if blanks are used) or data resulting from any previous data reduction.

Note: Select the insertion point inside of the formula before starting Syntax Helper.

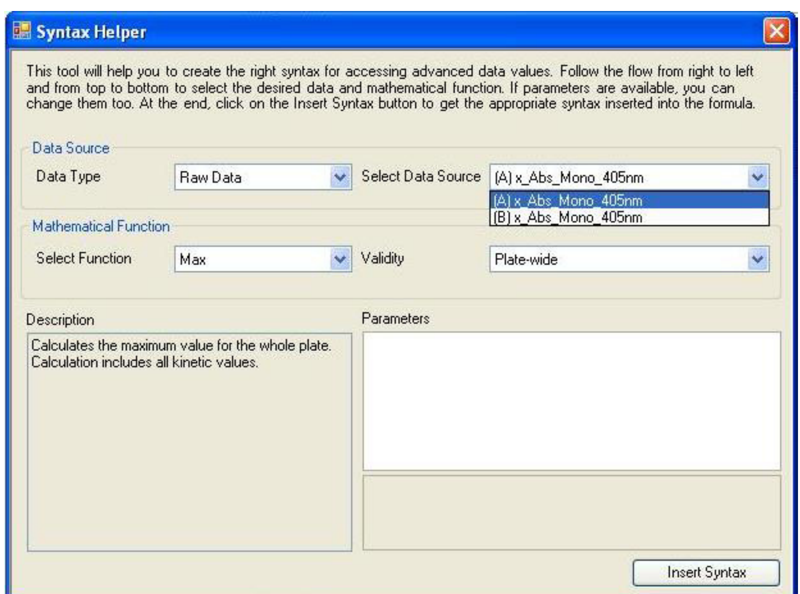


Figure 7-18 Syntax Helper window

Available mathematical functions (Figure 7-19) included in Syntax Helper are minimum, maximum, mean, median, standard deviation or coefficient of variation.

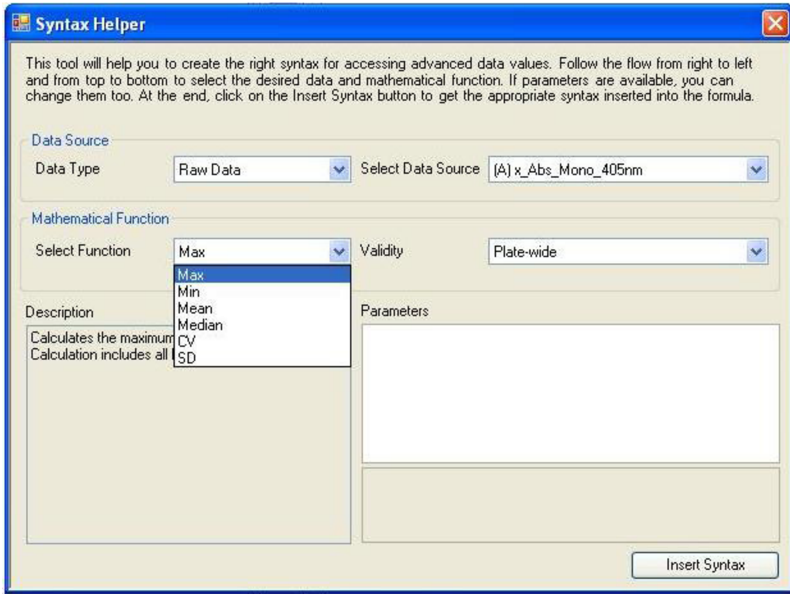


Figure 7-19 Syntax Helper mathematical functions selection

Select attributes from the drop-down menus (Figure 7-20) to combine data with mathematical functions and validity constraints to render a desired configuration.

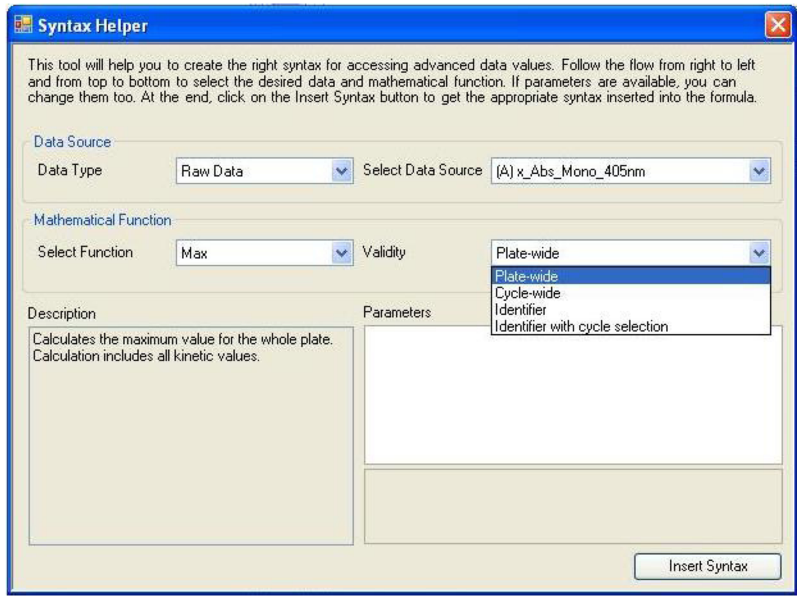


Figure 7-20 Syntax Helper Validity

Note: Depending on the type of the data and mathematical function, validity can be plate-wide (valid for the whole plate), cycle wide (valid for the selected cycle), identifier (for example, maximum value of the specified control). Depending on function type and selected validity there are additional parameters available to specify a cycle for which the function should be applied.

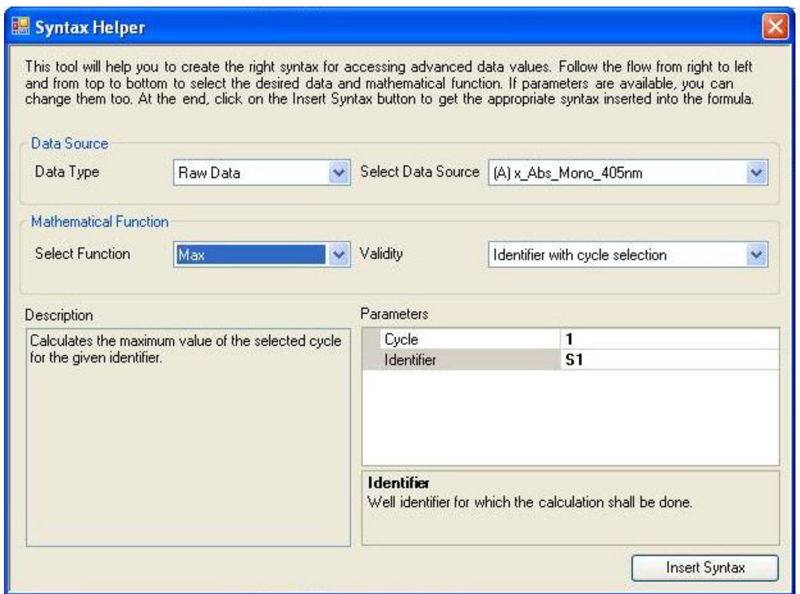


Figure 7-21 Syntax Helper parameters

Conclude by clicking on the **Insert Syntax** button to apply the syntax to the formula at the point of insertion.

7. Add and configure additional passes by repeating steps 2 through 6.
8. Click **Next**.

Configuring a Transformation Formula

Use Transformation to configure an algebraic formula (X' =) that is applied to every well in a set of reduced data (X) (Figure 7-22). The transformation formula must include X, and may include mathematical operators, but not relational or logical operators. See Mathematical Operators and Functions on page 249 for more information about supported mathematical operators.

Note: X represents the result value of a data reduction selected in the **Basis of Evaluation** field.

Note: Transformation is available for configuration only when selected in General Settings for an Analysis protocol. See Configuring General Settings on page 148.

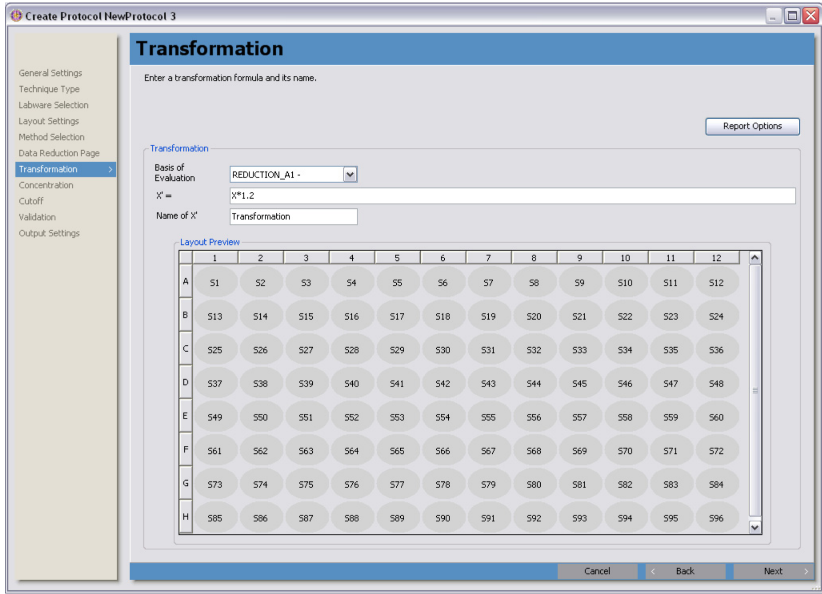


Figure 7-22 Configuring a Transformation Formula

To configure a transformation formula:

1. In the **Basis of Evaluation** field, select the desired data reduction to use for the transformation.
2. In **X' =**, enter the desired transformation formula. See Mathematical Operators and Functions on page 249 for detailed information about mathematical operators supported by the software.
3. In **Name of X'**, enter a new name for the transformation formula. The name appears in printed reports and in protocol configuration screens where a basis for evaluation must be selected.
4. Click **Report Options** and select the transformation information included in printouts:
   - **Definition**: the transformation formula and name are listed.
   - **Print in Matrix**: the transformed result calculated for each sample will display in a measurement results matrix corresponding to the plate layout.
   - **Print in List**: the transformed result calculated for each sample will display in a list of measurement results.
   - **Print Status**: status indicating whether or not the well was read successfully.

Configuring Concentration

Use Concentration to select a curve fitting method and configure concentration and response formulas for standards (Figure 7-23).

Note: Concentration is available for configuration only when selected in General Settings for an Analysis or Quantitation protocol. See Configuring General Settings on page 148.

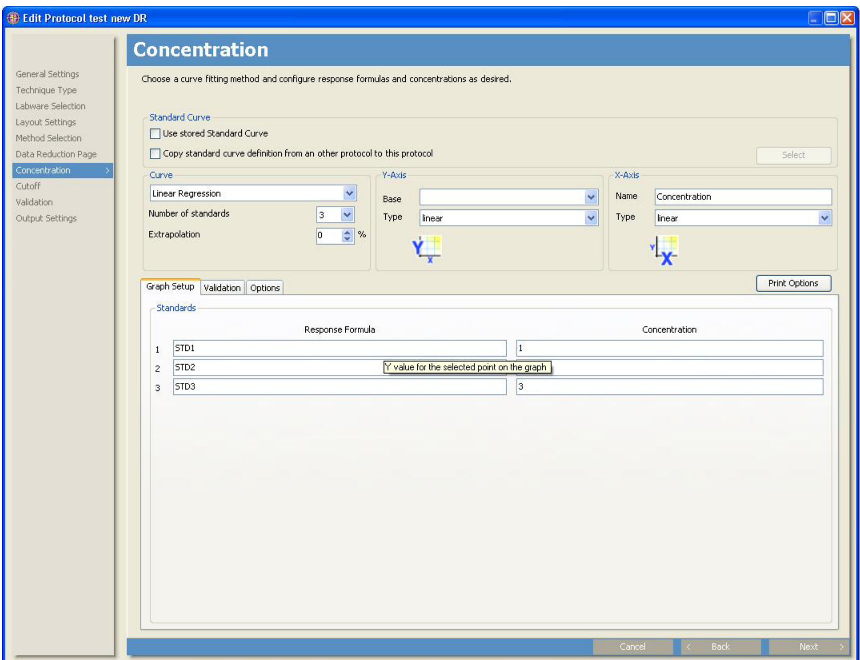


Figure 7-23 Configuring Concentration Parameters

Note: Standards may be deleted or inserted with a special key stroke combination. Use CTRL+Y to delete a standard and CTRL+N to insert a standard.

To configure a standard curve and standards:

1. To use a stored Standard Curve select Use stored Standard Curve. Click **Select** to select the standard curve. Using the Standard Curve Record Selection Form dialog select the specific record to use as the standard curve or **Always use the most recent record from the Database**. Click **Next** to continue.

   OR

   To use the standard curve definition from another protocol select **Copy Standard curve definition from another protocol to this protocol**. Click **Select** to select the protocol to copy the standard curve from. Click **Next** to continue.

   OR

   Select a **Curve Type**: **Point to Point**, **Linear Regression**, **Cubic Spline**, **Four Parameter Fit**, or **Polynomial**. See Table 7-5 more information about curve fitting methods.

Table 7-5 Curve Fitting Methods

Method	Description	Example
Linear regression	Construction of a straight line using the least squares method with the highest possible approximation to all standard points. Requires a minimum of 3 standard points.	
Point to Point	Direct connection of all standard points. Requires a minimum of 3 standard points.	
Cubic Spline	All standard points are connected by the best fitting curve. Can only be used for nonlinear and nonsigmoid functions. Requires a minimum of 3 standard points.	
4 Parameter Fit	This procedure can be used only to characterize sigmoid curves. The curve is calculated according to the formula: $y_i = \frac{(a-d)}{\left[1+\left(\frac{x_i}{c}\right)^b\right]} + d$ a = zero dose response (upper asymptote) d = infinite dose response (lower asymptote) c = dose level which results in a response midway between a and d b = slope factor Requires a minimum of 3 standard points. The X and Y axes are fixed: X = logarithm Y = linear	
Polynomial	Calculates the least squares fit through points using the formula: $y = b + c_1x + c_2x^2 + c_3x^3 + \ldots + c_6x^6$ Requires a minimum of n+1 standard points, where n is the order of the polynomial.	

2. Select the **Number of standards** to configure. Up to 12 standards may be configured in a protocol. Standards configured in the labware layout are automatically added to Concentration.
3. In **Extrapolation**, enter a percentage value to extrapolate the standard curve above and below the highest and lowest standard points in the curve, if desired. Extrapolation may be configured for all curve fitting methods.
4. In **Y-Axis**, select the **Base** for the axis.
   - **Reduction**: the reduced data from the protocol run.
   - **Transformation**: the value calculated using the transformation formula configured in Transformation. This option is available only when Transformation is configured in the protocol. See Configuring a Transformation Formula on page 177.

Note: Transformation is the default name for transformation formulas. If a different name is entered in Transformation, that name appears in Base. See Configuring a Transformation Formula on page 177.

5. In **Y-Axis**, select the **Type** of scale for the Y-Axis: **linear** or **logarithmic**.

Note: Type is not available when configuring a four parameter fit curve.

6. In **X-Axis**, enter a **Name** for the axis, if desired.
7. In **X-Axis**, select the **Type** of scale for the X-Axis: **linear** or **logarithmic**.

Note: Type is not available when configuring a four parameter fit curve.

8. Click **Report Options** and select the concentration information and data included in printouts:
   - **Definition**: curve type, parameters, and statistics, such as intercept and slope.
   - **Graph**: the standard curve.
   - **Print in Matrix**: the transformed result calculated for each sample will display in a measurement results matrix corresponding to the plate layout.
   - **Print in List**: the transformed result calculated for each sample will display in a list of measurement results.
   - **Print Status**: status indicating whether or not the well was read successfully.
9. In the Graph Setup tab, edit each **Response Formula** and the corresponding **Concentration**. Response formulas may contain any controls, standards, or variables defined in the test, as well as numerical constants and mathematical operators. A response formula is often simply the value of a measured standard, which is expressed as STD1, STD2, or STD3.

Note: See Mathematical Operators and Functions on page 249 for detailed information about mathematical operators supported by the software.

10. If configuring a linear regression curve fitting method, select the **Validation** tab to validate the protocol based on acceptable coefficient of correlation, if desired (Concentration Options Tab on page 183).

Note: The **Validation** tab appears only when configuring a linear regression curve fitting method.

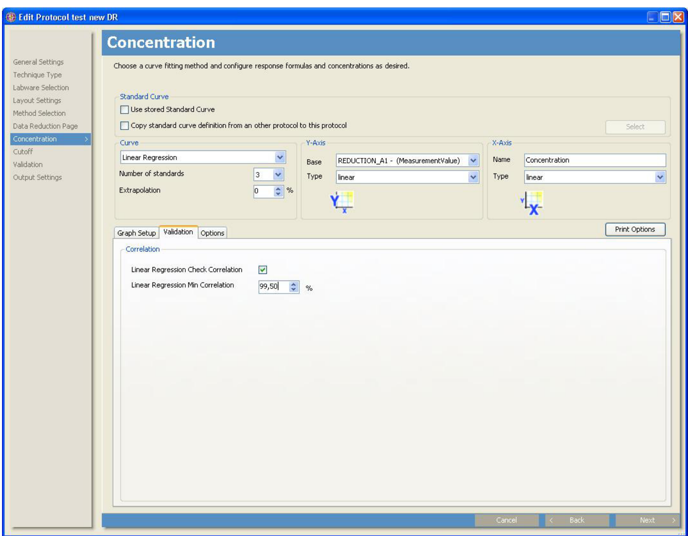


Figure 7-24 Configuring Standard Curve Validation Parameters

11. Select **Linear Regression Check Correlation** to enable validation.
12. In **Linear Regression Min Correlation**, enter the minimum correlation percentage value for the test to be valid.
13. In the **Options** tab, it is possible to configure an algebraic formula that is applied to the calculated concentration of every well (Figure 7-28). The formula shall include X, and may include mathematical operators, but not relational or logical operators. For detailed information about mathematical operators supported by the software, see Mathematical Operators and Functions on page 249.

Note: X represents the result value of the concentration calculation. For example: X/DF => divides the concentration values of every well by the dilution factor (DF), which is entered in the plate layout settings.

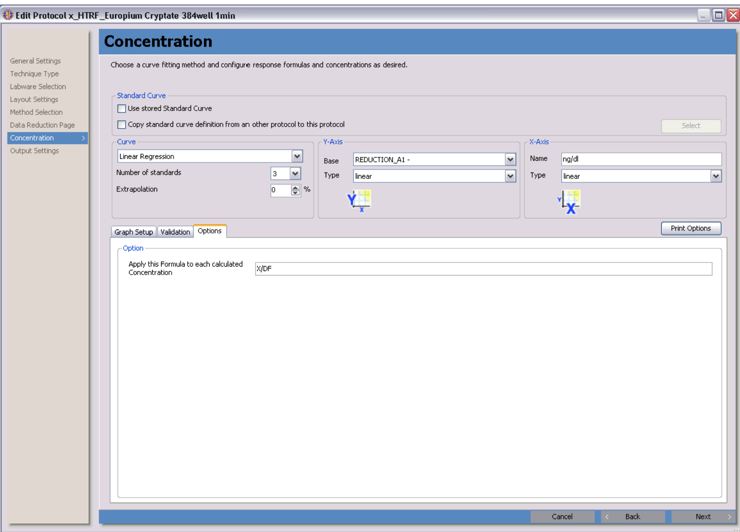


Figure 7-25 Concentration Options Tab

Configuring Cutoff Values

Use Cutoff to configure qualitative evaluations that classify measured samples into groups according to defined cutoff formulas or values (Figure 7-26). Up to ten cutoff groups may be configured.

Note: Cutoff is available for configuration only when selected in General Settings for an Analysis or Quantitation protocol. See Configuring General Settings on page 148.

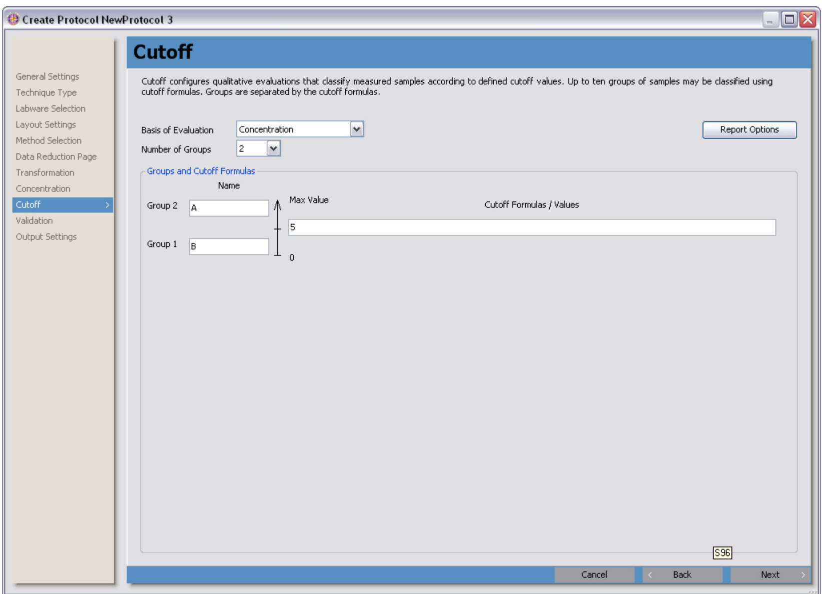


Figure 7-26 Configuring Cutoff Values

To configure cutoff groups and formulas:

1. Select the **Basis of Evaluation**:
    - **Concentration**: the value calculated using the standard curve configured in Concentration. This option is available only when Concentration is configured in the protocol. See Configuring Concentration on page 179.
    - **Transformation**: the value calculated using the transformation formula configured in Transformation. This option is available only when Transformation is configured in the protocol. See Configuring a Transformation Formula on page 177.
    - **Reduction**: reduced data from the protocol run. See Configuring the Data Reduction on page 172.

Note: Transformation is the default name for transformation formulas. If a different name is entered in Transformation, that name appears in Basis of Evaluation. See Configuring a Transformation Formula on page 177.

2. Select the **Number of groups** to configure.
3. Enter a **Name** for each group.
4. Enter the formulas and/or values used to classify samples into groups. The cutoff formula or value entered represents the maximum value included the group being configured.

Note: The results calculated from the formulas or the values entered must ascend. Results or values that do not ascend will generate an error during protocol runs.

5. Click **Report Options** and select the cutoff information and data included in printouts:
    - **Definition**: the basis for measurement, group names, and cutoff values and/or formulas configured.
    - **Print in Matrix**: the cutoff group classification for each sample appears in a measurement results matrix corresponding to the plate layout.
    - **Print in List**: the cutoff group classification for each sample appears in a list of measurement results.
    - **Print Status**: status indicating whether or not the well was read successfully.

Configuring Validation Rules

Use Validation to configure up to ten validation rules (Figure 7-27). Protocol runs that do not meet the conditions specified in the rules are marked as invalid.

Note: Validation is available for configuration only when selected in General Settings for an Analysis or Quantitation protocol. See Configuring General Settings on page 148.

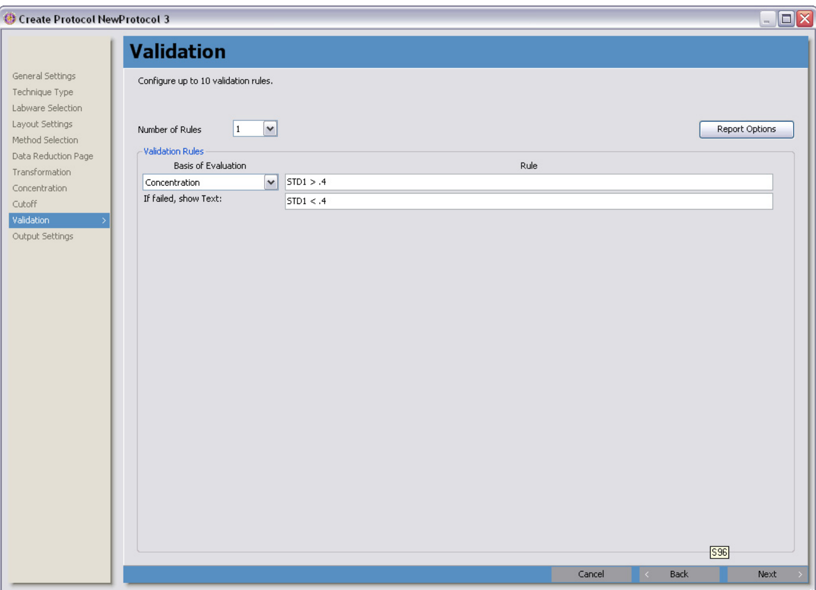


Figure 7-27 Configuring Validation Rules

To configure validation rules:

1. Select the **Number of formulas** to configure. Up to ten formulas may be configured.
2. Select the **Basis of Evaluation** for the first formula:
    - **Concentration**: the value calculated using the standard curve configured in Concentration. This option is available only when Concentration is configured in the protocol. See Configuring Concentration on page 179.
    - **Transformation**: the value calculated using the transformation formula configured in Transformation. This option is available only when Transformation is configured in the protocol. See Configuring a Transformation Formula on page 177.
    - **Data Reduction Results**: reduced data from the protocol run. See Configuring the Data Reduction on page 172.

Note: Transformation is the default name for transformation formulas. If a different name is entered in Transformation, that name appears in Basis of Evaluation. See Configuring a Transformation Formula on page 177.

3. Enter the first formula. Formulas may contain:

- any controls, standards, or variables defined in the protocol. For controls and standards, use the same labels displayed in **Layout Settings**; for example, **STD1** for standard 1, **C2** for control 2, **P5** for positive control 5, or **N2** for negative control 1.
- numeric constants.
- mathematical operators and functions and logical operators. See Mathematical Operators and Functions on page 249 for a complete list of supported operators and functions.

Table 7-6 Example Test Validation Formulas

Application	Validation Formula
The results from a protocol run are valid only if the mean absorption value of the positive control wells P2 is less than or equal to 0.8 OD.	P2<=0.8
The results from a protocol run are valid only if both controls are within the linear range of the instrument.	0.1<=C1 AND C1<=3.0 AND 0.1<=C2 AND C2<=3.0

4. Repeat steps 2 through 3 for any additional formulas.

5. Click **Report Options** and select **Definition** to include the validation configuration and state (pass/fail) for all rules in printouts.

Configuring Output Settings

Use Output Settings to specify which measurement result export, view, and print actions are performed automatically when a protocol run completes (Figure 7-28). An external software application, such as a notification utility, may also be configured to execute when a run completes.

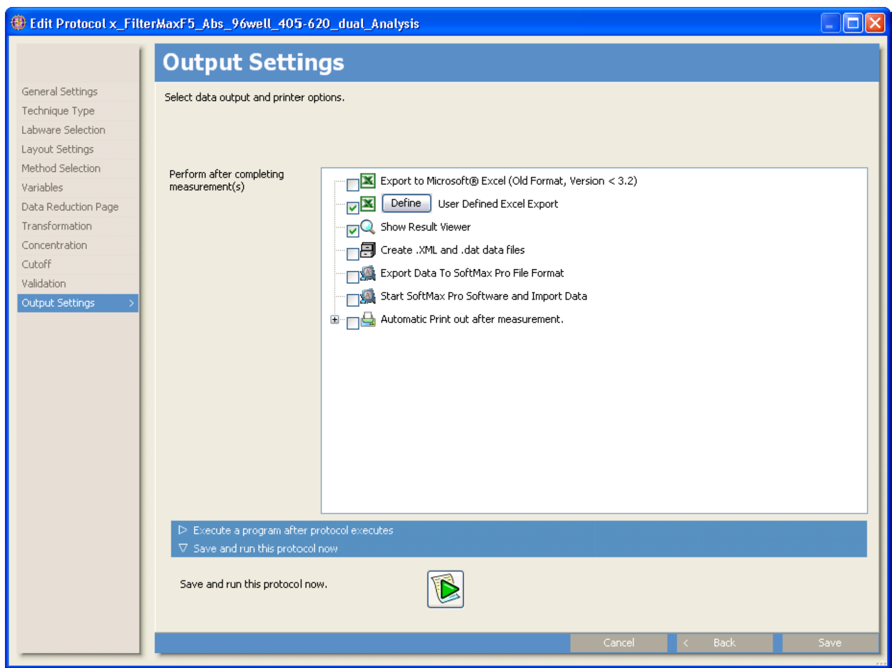


Figure 7-28 Configuring Output Settings

To configure Output Settings:

1. Select export, view, and print output options as desired. Table 7-7 describes the options available. Further output options are described in Table 7-8, Table 7-9, and Table 7-10.
2. Click **Execute a program after protocol executes** to display options for configuring an external software application to run after the protocol run is completed. See Configuring a Program to Execute after a Protocol Run Completes on page 195 for more information.
3. Click **Run protocol now** to run the protocol. See Running Protocols on page 197 for more information about running protocols.

Note: When creating or editing a protocol, the protocol must be saved before it can be run. After saving the protocol, Run Protocol appears for protocols that were edited; new protocols must be selected in the Protocol Selection List before they may be run.

Table 7-7 Output Options

Output Option	Description
Export to Microsoft Excel	Saves results in a format compatible with Microsoft Excel according to the Data Format configuration in Software Settings. See Configuring the Data Format on page 43, and automatically opens Excel after completing a protocol run. **Note:** Versions of Excel prior to Office 2000 are not supported by the Export to Microsoft Excel function, but can open measurement results stored in tab-delimited data .dat files. **Note:** When using Microsoft Excel 2002 or higher, the Multi-Mode Analysis Software will format the Excel worksheets with the appropriate column width and apply formatting to display the status of a measurement value (such as bold and colors).
User Defined Excel Export	Saves results in a format compatible with Microsoft Excel with customizable output attributes. Upon clicking the User Defined Excel Export checkbox, click the Define button to invoke the Excel Export Definition Dialog. Within this dialog designate more specific output attributes as needed. For more details, see User Defined Excel Export on page 189.
Show Result Viewer	Automatically opens the measurement results in the Result Viewer after completing a protocol run. See Viewing Measurement Results in the Result Viewer on page 217.
Create XML and DAT data files	Automatically exports measurement results to a tab-delimited data (*.dat) file and an XML file. These files may be opened by software applications compatible with tab-delimited data or XML files. **Note:** The directory where the data files are saved is configured in System Settings. See Selecting a Directory for Saving Exported Measurement Results on page 37.
Export Data to SoftMax Pro File Format	Automatically exports measurement results to a text file in a format that can be used to import the data into SoftMax Pro Software. See Exporting Data to SoftMax Pro File Format on page 193. **Note:** The directory where the data file is saved is configured in System Settings. See Selecting a Directory for Saving Exported Measurement Results on page 37.
Start SoftMax Pro Software and Import Data	Automatically exports measurement results to a formatted text file, and then opens SoftMax Pro Software and imports the data from the text file after completing a protocol run. See Starting SoftMax Pro Software and Importing Data on page 194.
Print options	Automatically prints the results after completing a protocol run.

User Defined Excel Export

Upon completion of a protocol run, results may be exported into a user-defined format for viewing in Microsoft Excel. A preview of the exported results may be seen in the Preview window at the lower left portion of the Export Option Dialog window (Figure 7-29).

To configure User Defined Excel Export:

1. In **Output Settings**, click the **Define** button. The Excel Export Definitions Dialog window appears (Figure 7-29).

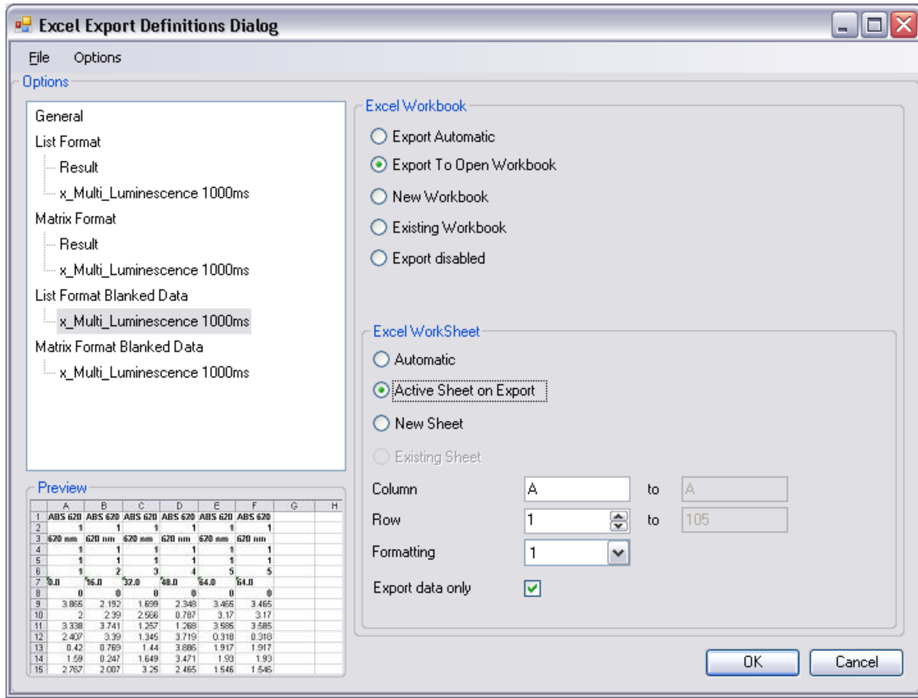


Figure 7-29 Export Option Dialog window

The **Options** list to the left of the Excel Export Definitions Dialog window allows for selection of data layout options. These options may be selected on the left portion of the window:

- General
- List Format
- Matrix Format
- List Format Blanked Data
- Matrix Format Blanked Data

Further options specific to workbook and worksheet export properties may be combined to meet specific needs. These further export options are summarized in:

- Workbook Export Options on page 190
- Worksheet Export Options on page 191

Workbook Export Options

Options (Figure 7-30) specific to workbook export properties are available as follows:

Table 7-8 Workbook Export Options

Properties	Description
Export Automatic	Multi-Mode Analysis Software automatically determines the file name and exports data to that file name.
Export to Open Workbook	Data results are exported to the currently open workbook on the controlling PC.
New Workbook	Data results are exported to a new workbook on the controlling PC.
Existing Workbook	Data results are exported to an existing workbook on the controlling PC.
Export disabled	Data results will not be exported to an Excel file.

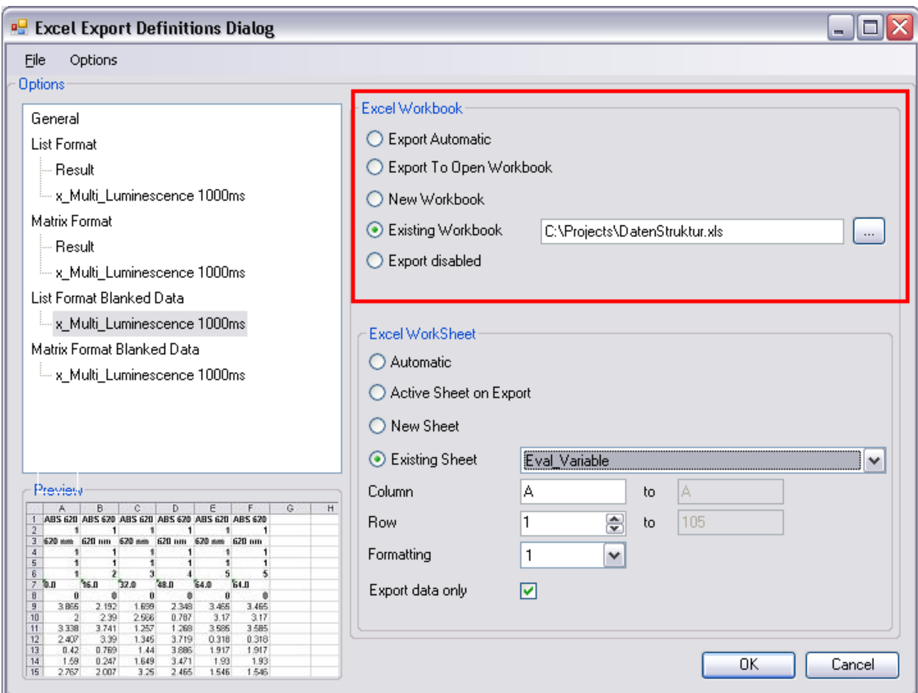


Figure 7-30 Workbook options

Worksheet Export Options

Options (Figure 7-31) specific to worksheet export properties are available as follows:

Table 7-9 Worksheet Export Options

Properties	Description
Automatic	Multi-Mode Analysis Software automatically determines the worksheet name and exports data to a sheet with that name.
Active Sheet on Export	Data results are exported to the currently open worksheet on the controlling PC.
New Sheet	Data results are exported to a new worksheet on the controlling PC.
Existing Sheet	Data results are exported to an existing worksheet selected by the user on the controlling PC.

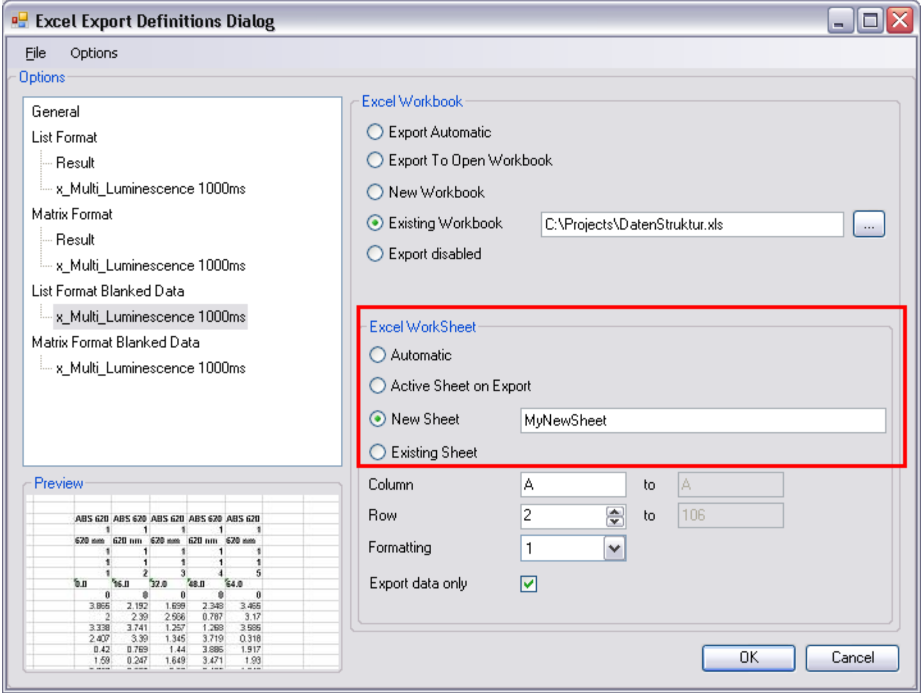


Figure 7-31 Worksheet options

New Sheet and Existing Sheet Export Options

Further worksheet options (Figure 7-32) allow for selection of data layout options for both new and existing sheets.

Table 7-10 Further Worksheet Export Options

Properties	Description
Column	Determines the start column for export.
Row	Determines the start row for export.
Formatting	Allows export of data using user-defined formatting assigned to a numbered template.
Export data only	Allows export of data without well, horizontal and vertical labels.
Export Mean Data	Allows export of all mean values of each data reduction step.
Export CV Data	Allows export of CV values of each data reduction step.
Export SD Data	Allows export of Standard Deviation values of each data reduction step.

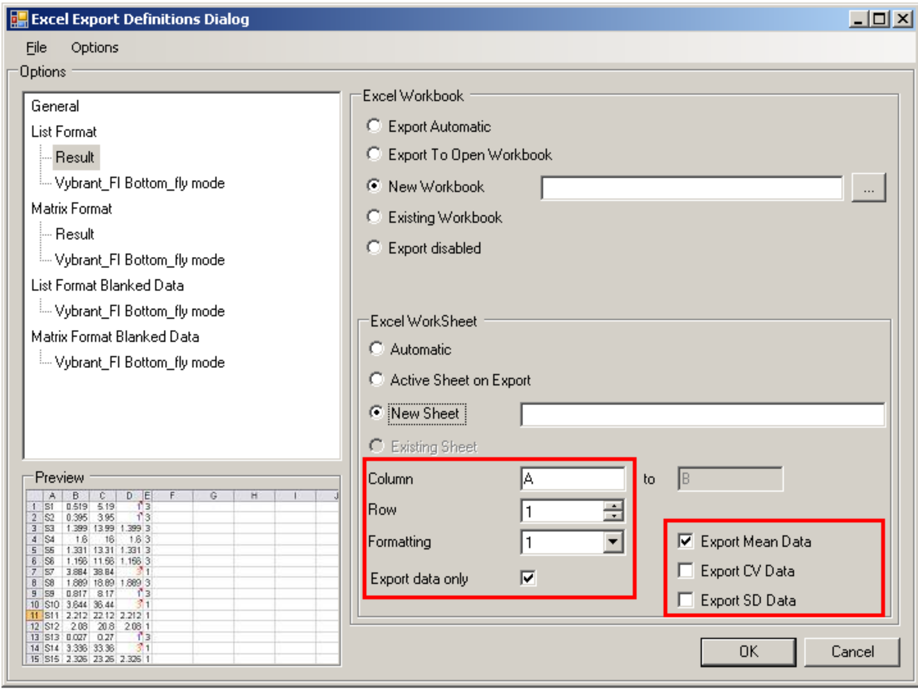


Figure 7-32 Further Worksheet Options

Note: New Sheet and Existing Sheet only: A preview of the selected format is shown in the Preview window.

Exporting Measurement Data to SoftMax Pro Software

At the conclusion of a read, Multi-Mode Analysis Software can export the raw data, plate settings, wavelength settings, kinetic parameters, and scan parameters to a text file that can then be imported into SoftMax Pro Software. Results, reductions, and template settings are not exported. Certain data might be altered to match the parameters for the data differ in each program. For example, in Multi-Mode Analysis Software, you can specify a shorter interval time for a kinetic read than can be specified in SoftMax Pro Software.

Note: Before importing Multi-Mode Analysis Software data into SoftMax Pro Software, you must select an instrument in SoftMax Pro Software that supports the read types, methods, and modes that were used in the Multi-Mode Analysis Software protocol.

There are two methods of exporting Multi-Mode Analysis Software data to SoftMax Pro Software:

- **Export Data to SoftMax Pro File Format** exports measurement results to a text file in a format that can be used to import the data into SoftMax Pro Software.
- **Start SoftMax Pro Software and Import Data** exports measurement results to a formatted text file, and then opens SoftMax Pro Software and imports the data from the text file.

Exporting Data to SoftMax Pro File Format

At the conclusion of a read, Multi-Mode Analysis Software can export the data to a text file that can then be imported into SoftMax Pro Software. To do this, select the **Export Data to SoftMax Pro File Format** check box in the **Output Settings** window. See Configuring Output Settings on page 187.

The text file is save in the directory where Multi-Mode Analysis Software saves its measurement results export files. See Selecting a Directory for Saving Exported Measurement Results on page 37.

The exported text file is given a unique name based on the **Result Name** and **Protocol Name** for the read.

For example, if the **Result Name** is **20100922-021736** and the **Protocol Name** is **x_FilterMaxF5_Abs_384well_405_kin**, then the text file will have the following name: **20100922-021736_x_FilterMaxF5_Abs_384well_405nm_kin SMP.txt**

When you save the data from the Results Viewer, the raw data is exported again to a formatted text file. For information about saving data from the Results Viewer, see Saving Measurement Results on page 228.

For information about importing a text file into SoftMax Pro Software, see the user guide provided with the program.

Starting SoftMax Pro Software and Importing Data

If you have SoftMax Pro installed on the same computer as Multi-Mode Analysis Software, then you can export the data at the conclusion of the read and then start SoftMax Pro Software to automatically import the data. To do this, select the **Start SoftMax Pro Software and Import Data** check box in the **Output Settings** window. See Configuring Output Settings on page 187.

Before starting SoftMax Pro Software, an export file is generated and saved. For information about how to locate this export file, see Exporting Data to SoftMax Pro File Format on page 193.

After Multi-Mode Analysis Software finishes the read and exports the data, SoftMax Pro Software starts and then automatically imports the data from the text file.

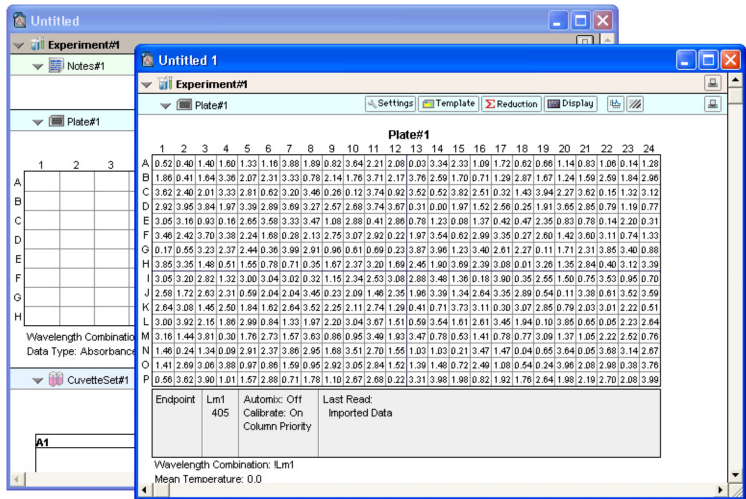


Figure 7-33 Data imported into SoftMax Pro Software

You can immediately work with the imported data in SoftMax Pro Software, or save the data to work with it later. For information about working with data in SoftMax Pro Software, see the user guide provided with the program.

Configuring a Program to Execute after a Protocol Run Completes

Output Settings can be configured to open an external software application after completing a protocol run. If the selected application supports entering commands using a command line interface, a specific command for the application may also be configured.

To configure a program to run after a protocol is executed:

1. In **Output Settings**, click **Execute a program after protocol executes**. Two configuration fields appear (Figure 7-34).

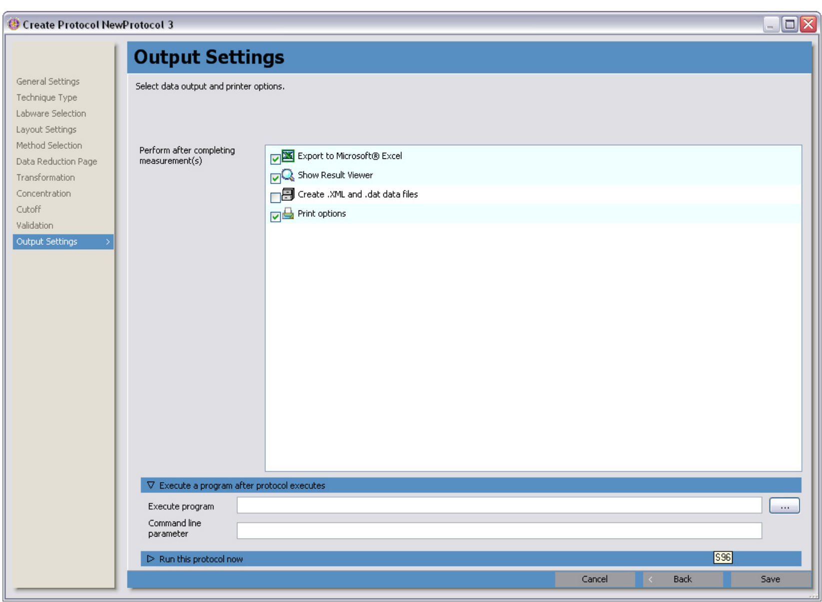


Figure 7-34 Configuring an External Software Application in Output Settings

2. In **Execute Program**, enter the complete path of the program to run; for example, C:\Windows\System32\Net.exe. Net.exe is a small utility supplied with Windows operating systems that sends messages to computers on the same network.

   OR

   Select **Choose an external program to run at the end of measurement**. The Open dialog appears.
3. In the Open dialog, browse to the location of the desired application and select it.
4. Click **Open** to return to Output Settings. The selected path appears in **Execute Program**.
5. If the selected application supports command line parameters, in Command line parameter, enter the desired parameter. For example, if using Net.exe, entering the parameters "**SEND**" "**Workstation1**" **"Finished"** instructs the application to send the message, **"**Finished,**"** to a computer named Workstation1 when the protocol run completes.

Signing a Protocol

When GxP Permissions is enabled on the system, protocols may be signed to prevent the configured parameters from being edited. Protocols may be signed at any time after the configuration is complete.

Protocols may be signed by users who are assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To sign a protocol:

1. In the Protocol Selection List, select the protocol to sign.
2. From the tool bar, click **Sign the selected protocol**.

   OR

   From the menu bar select **Actions > Sign the selected protocol**.

   OR

   Right-click on the selected labware and select **Sign the selected protocol**.
3. The Sign the Selected Item dialog appears.
4. In the Sign the Selected Item dialog, add comments and an electronic signature by following the instructions in Adding Electronic Signatures and Comments to Items on page 84.

Creating a Protocol from a Template

Template protocols are used to create a protocol based upon the selected template. Pre-defined settings appear as the protocol is configured.

To create a protocol from a template:

1. From the tool bar, click **Create**.

   OR

   From the menu bar select **Actions > Create a new protocol**.

   OR

   Right-click in the Protocol Selection List and select **Create a new protocol**.
2. The Select Application Type dialog appears.
3. Select the **Templates** tab.

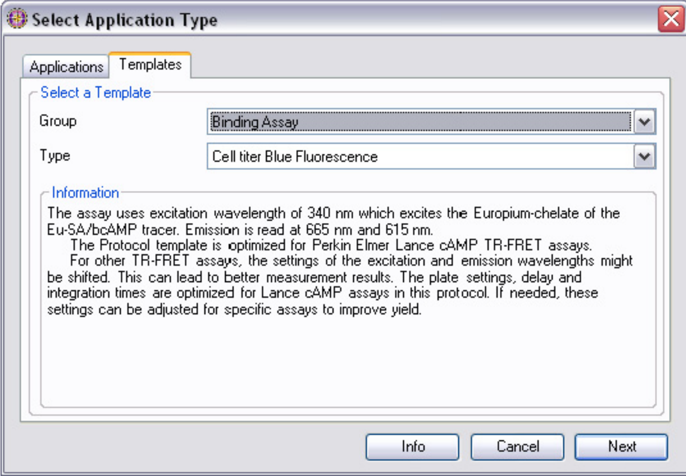


Figure 7-35 Selecting the Application Type - Templates Tab

4. Select the **Group** and **Type** for the template. To obtain more information about the template protocol click on **Info**.

5. Click **Next** to configure the protocol. The Create Protocol dialog appears, displaying General Settings. See Configuring General Settings on page 148.

Note: Pre-defined settings appear as the protocol is configured. See Creating Protocols on page 146 for detailed information about configuring protocol parameters.

Running Protocols

Saved protocols may be accessed and run at any time, either on an instrument or in simulation mode. The run options available are different for each mode. For more information, refer to:

- Running a Protocol on an Instrument on page 197
- Running a Protocol When Simulation Mode is Enabled on page 209

Running a Protocol on an Instrument

Running a protocol on an instrument performs measurements on samples and outputs results data following the parameters configured in the protocol.

Note: To create or run quantitation protocols for a FilterMax Multi-Mode Microplate Readers instrument, a genomic filter slide, which contains narrow bandwidth 260 nm and 280 nm filters, must be installed and configured.

To run a protocol on an instrument:

1. In the Protocol Selection List, select the protocol to run.
2. From the tool bar, click **Run**.

Note: When running an Analysis protocol with variables configured, Variables appear as the first screen in Run Protocol. Change the values of the variables, if desired, and click **Next** to continue. Prepare to Run appears.

OR

From the menu bar select **Actions > Run the selected protocol**.

OR

Right-click on the selected protocol and select **Run the selected protocol** from the menu.

3. The Prepare to Run dialog appears (Figure 7-36).

Figure 7-36 Preparing to Run a Protocol on an Instrument

4. Enter a new unique **Result Name**, if desired. The name must not match any previous result names, including those pending deletion.
5. Specify the **Number of Samples** to measure per plate.
6. Specify the **Number of Plates** to read. Changing the number of plates to read will increase or decrease the number of plates to read as specified in the plate layout of the protocol.
7. Use the **Read Sample ID's** button to import .DAT file format samples.

Note: If a multi-plate layout is specified in the plate layout only the first plate will contain controls and all following plates will contain only samples. If a multi-plate layout is not selected in the plate layout for every plate will contain the controls specified in the plate layout. For more information see Configuring Labware Layout Settings on page 151.

Note: Clicking on the plate layout icon provides an expanded view of the microplate in its current configuration. Limited editing of the plate is allowed from this expanded view.

8. As required, select **Plate is lidded**.

CAUTION! It is important to select Plate is lidded when the plate has a lid due to the low reading height.

9. Use **Eject Plate Carrier** and **Close Plate Carrier** to load the microplate into the instrument, if necessary.

Note: If running a quantitation protocol with either **Pre read** low or **Pre read** high selected for blank correction, insert the blank plate first. During the protocol run, there will be a pause which requests the measurement plate be inserted.

10. Select the orientation that matches how the plate is positioned on the plate carrier:
    - **Landscape**: the long edges of the plate run parallel to the front of the instrument, with well A1 located in the upper left corner.
    - **Portrait**: the short edges of the plate run parallel to the front of the instrument, with well A1 located in the upper right corner.
    - **Opposite Landscape**: the edges of the plate run parallel to the front of the instrument, with well A1 located in the lower right corner.
    - **Opposite Portrait**: the short edges of the plate run parallel to the front of the instrument, with well A1 located in the lower left corner.

Note: Labware optimization should be performed for the selected plate orientation.

Note: Well A1 is identified in the plate graphic by a red highlight. Clicking on the layout preview will zoom the view of the plate layout.

11. When preparing to run a luminescence, fluorescence top, fluorescence bottom (SpectraMax Paradigm Multi-Mode Detection Platform only), fluorescence polarization, or time-resolved (TRF) protocol, select **Optimize Read Height** to automatically determine and set the optimal read height used in the protocol run. See Optimizing Read Height (FilterMax 5 Multi-Mode Microplate Reader) on page 200 or Optimizing Read Height (SpectraMax Paradigm Multi-Mode Detection Platform) on page 203.

Note: Optimize Read Height is available for the FilterMax 5 Multi-Mode Microplate Reader and SpectraMax Paradigm Multi-Mode Detection Platform only.

Note: SpectraMax Paradigm Multi-Mode Detection Platform only — Optimize Read Height is not available for Luminescence detection cartridges. For Luminescence detection cartridges the optimum read height is the closest distance between the read head and the microplate, as derived from the labware definition, and confirmed by the plate height detection.

12. Click **Run** to start the protocol run. The Run Protocol runtime window appears (Figure 7-37).

Figure 7-37 Run Protocol Runtime Display

Optimizing Read Height (FilterMax 5 Multi-Mode Microplate Reader)

The FilterMax 5 Multi-Mode Microplate Reader features an objective lens that may be moved up and down to optimize the read height used in luminescence, fluorescence intensity top, fluorescence polarization, and time-resolved fluorescence protocols. Read height is the distance between the top or bottom (using bottom reading) surface of the microplate being read and the surface of the objective lens. Optimizing read height matches the focus of the optics with the sample volume. This maximizes the raw signal, which yields the highest precision and maximum sensitivity.

Read height is optimized using the Read Height Optimization Wizard (Figure 7-40). A single sample with a known maximum signal and volume is placed on the same type of microplate used in the protocol. The sample is measured using the same, or very similar, detection method used in the protocol.

The optimized read height is saved in the protocol and is used for all subsequent runs of the protocol until reset by performing a new optimization or manually selecting a read height option.

To optimize the read height:

1. In Run Protocol, select **Optimize Read Height**. When clicking on Run, the Read Height Optimization Wizard appears (Figure 7-40).
2. Select the well measured to perform the optimization. Prepare Labware appears (Figure 7-38).

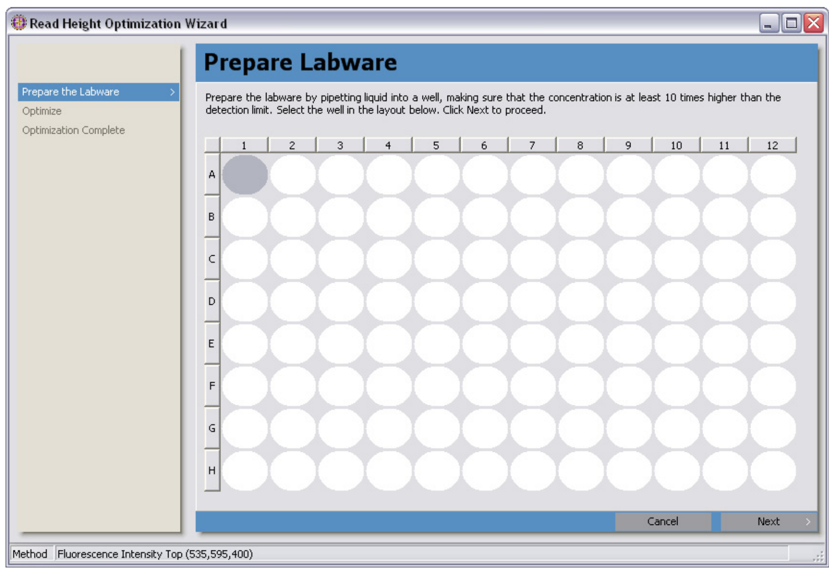


Figure 7-38 Selecting the Well Read in the Read Height Optimization

3. Use a standard or sample position with a good signal or pipette liquid with a known maximum signal to a single well on the microplate used in the optimization. The concentration of the optimization sample should be at least ten times greater than the detection limit. The sample volume should be the same as that of samples measured in the protocol. If using a layout with standards, the standard well closest to the center of the plate is pre-selected. If not using a layout with standards, the first sample is pre-selected.

Note: When optimizing reading height for a fluorescence protocol, make sure the optimization sample is the same fluorescent substance the detection method is configured to detect.

4. Load the plate into the instrument.
5. In Prepare Labware, select the well containing the optimization sample (Figure 7-38).

6. Click **Next** to start the optimization. Optimization in Progress appears (Figure 7-39). The optimization may take several seconds or up to several minutes depending on the detection methods used.

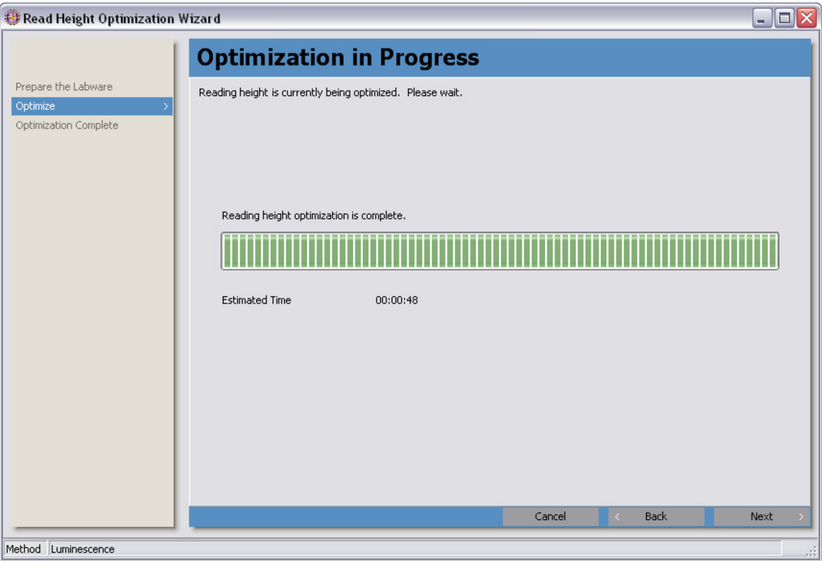


Figure 7-39 Read Height Optimization in Progress

7. When the read is finished, Optimization Complete appears, displaying the Optimized Read Height (Figure 7-40). While the optimization is in progress, click **Cancel** to stop the optimization read and close the wizard, if desired.

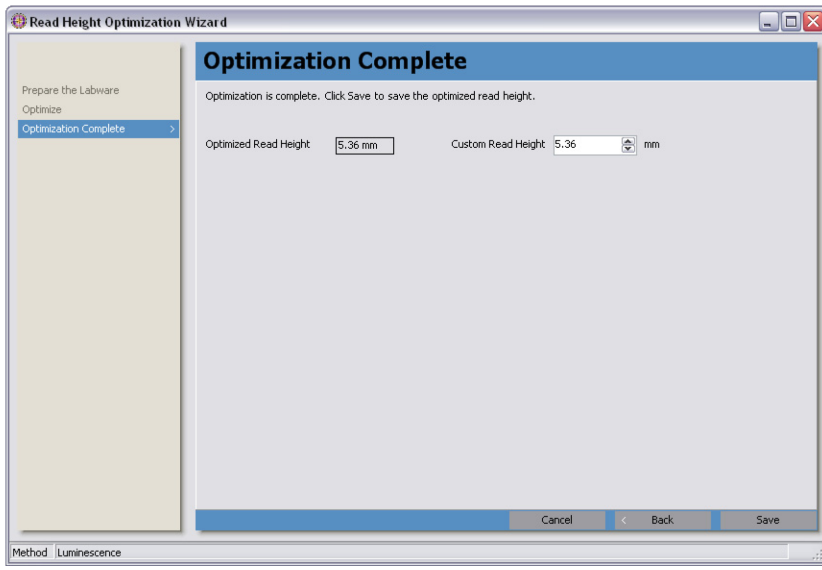


Figure 7-40 Read Height Optimization Completed

8. In the Optimization Complete window the **Optimized Read Height** is displayed. As desired, modify the **Custom Read Height**.
9. Click **Save** to save the optimized read height in the protocol. The optimized read height is used for all subsequent runs of the protocol until reset by performing a new optimization or manually selecting a read height option.

Optimizing Read Height (SpectraMax Paradigm Multi-Mode Detection Platform)

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

Read height is optimized using the Read Height Optimization Wizard (Figure 7-41). A single sample with a known maximum signal is placed on the same type of microplate used in the protocol. The sample is measured using the same, or very similar, detection method used in the protocol.

The optimized read height is saved in the protocol and is used for all subsequent runs of the protocol until reset by performing a new optimization or manually selecting a read height option.

To optimize read height:

1. In Run Protocol, select **Optimize Read Height**. When clicking on Run, the Read Height Optimization Wizard appears.

Note: SpectraMax Paradigm Multi-Mode Detection Platform only — Optimize Read Height is not available for Luminescence detection cartridges. For Luminescence detection cartridges the optimum read height is the closest distance between the read head and the microplate, as derived from the labware definition, and confirmed by the plate height detection.

2. Select the well measured to perform the optimization. Prepare Labware appears (Figure 7-41).

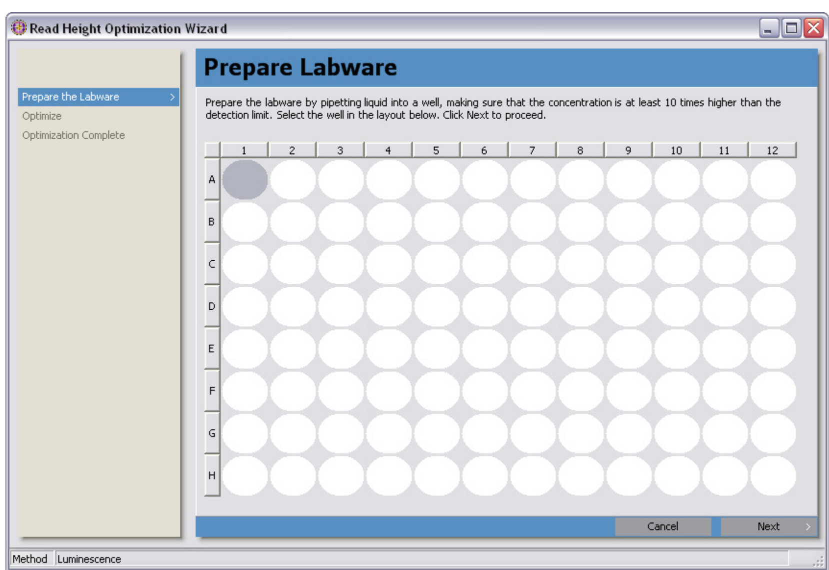


Figure 7-41 Selecting the Well Read in the Read Height Optimization

3. Use a standard or sample position with a good signal or pipette liquid with a known maximum signal to a single well on the microplate used in the optimization. The concentration of the optimization sample should be at least ten times greater than the detection limit. The sample volume should be the same as that of samples measured in the protocol. If using a layout with standards, the middle most standard well is pre-selected. If not using a layout with standards, the first sample is pre-selected.

Note: When optimizing reading height for a fluorescence protocol, make sure the optimization sample is the same fluorescent substance the detection method is configured to detect.

4. Load the plate into the instrument.
5. In Prepare Labware, select the well containing the optimization sample (Figure 7-41).
6. Click **Next** to start the optimization. Optimization in Progress appears (Figure 7-42). The optimization may take several seconds or up to several minutes depending on the detection methods used.

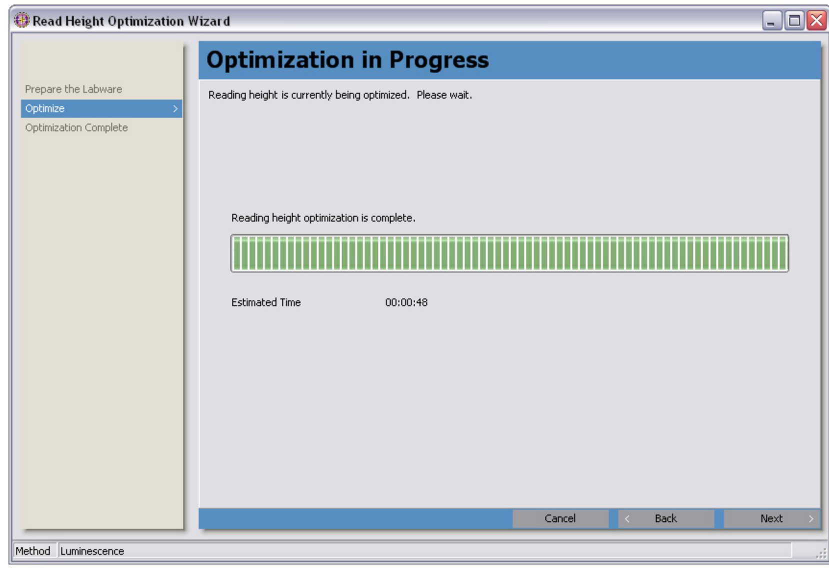


Figure 7-42 Read Height Optimization in Progress

7. When the read is finished, Optimization Complete appears, displaying the Optimized Read Height (Figure 7-43). While the optimization is in progress, click **Cancel** to stop the optimization read and close the wizard, if desired.

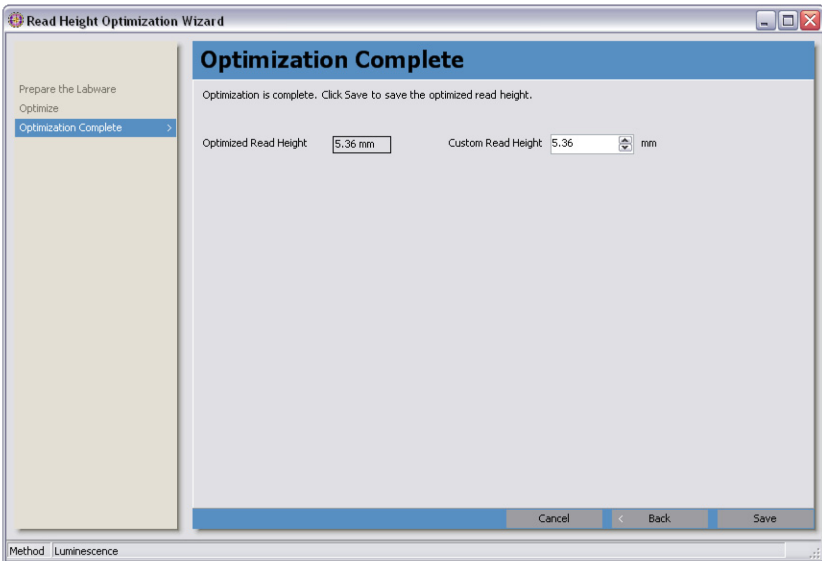


Figure 7-43 Read Height Optimization Completed

8. In Optimization Complete, click **Save** to save the optimized read height in the protocol. The optimized read height is used for all subsequent runs of the protocol until reset by performing a new optimization or manually selecting a read height option.

Viewing the Run Protocol Runtime Display

Once a protocol run begins, the Run Protocol runtime display shows information about the status and results of the current run.

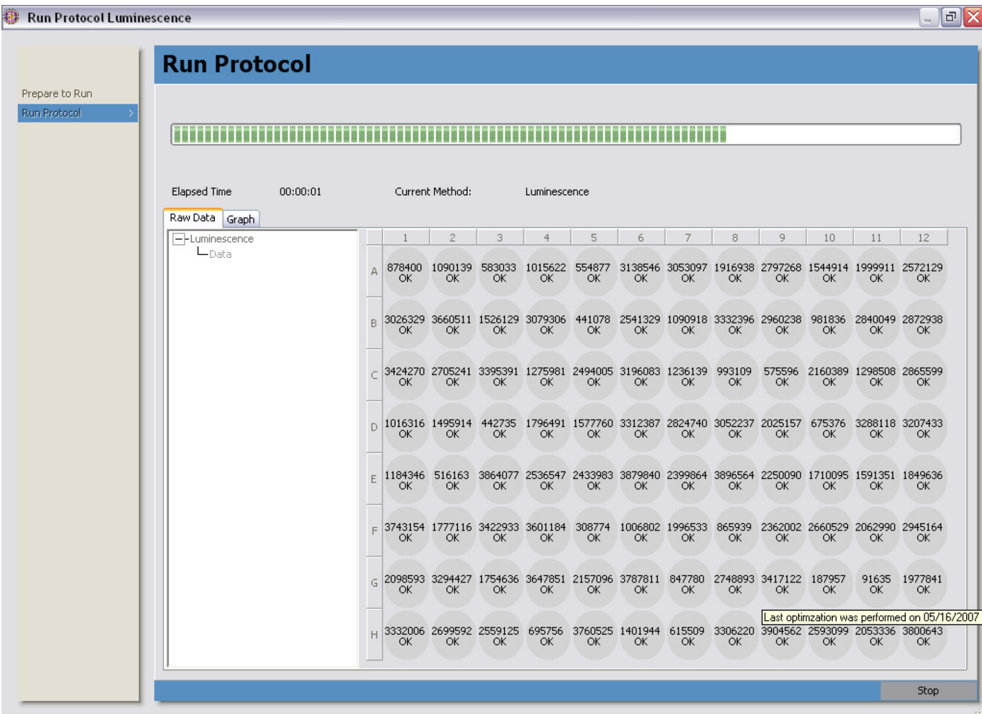


Figure 7-44 Run Protocol Runtime Display

Run Protocol displays the following information:

- **Elapsed Time**: how much time has elapsed since the protocol started, in HH:MM:SS format.
- **Temperature**: the current temperature inside the instrument, in ºC.
- **Current Method**: the current detection or preparation method that is being executed.
- **Raw Data** (tab): dynamically displays the raw data for the selected detection method as it is read.
- **Graph** (tab): dynamically graphs the raw data as it is read for Kinetic or Scan measurements.

The protocol run may be stopped at any time using the **Stop** button at the bottom right of the screen.

During certain preparation methods, such as **Wait** and **Pause**, a **Continue** or **Skip** button appears. The **Continue** button functions as described below depending on the Current Method:

- **Pause**: the protocol run pauses and displays a message. The protocol remains paused until the user selects **Continue** to resume the protocol (Figure 7-45).

Note: In Quantitation protocols using **Pre read low** or **Pre read high** for blank correction, **Pause** is used to exchange the blank plate with the measurement plate.

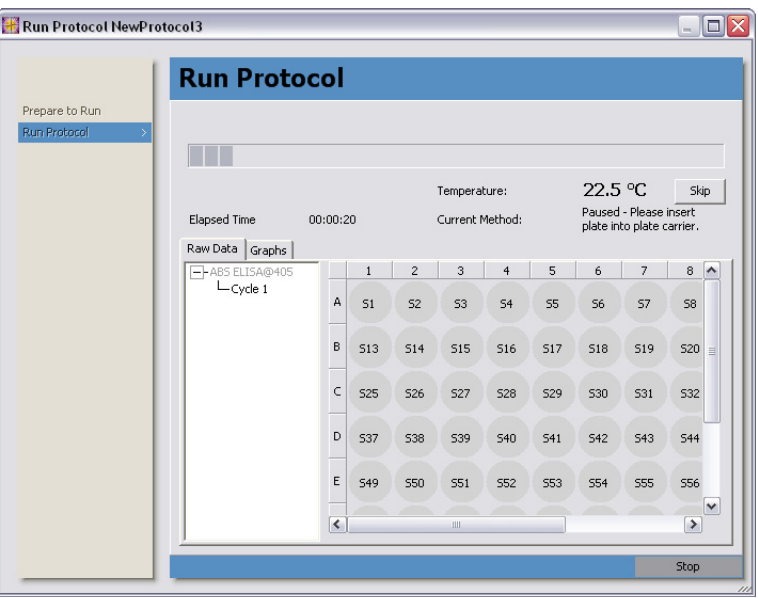


Figure 7-45 Pause During a Protocol Run

- **Wait**: the protocols waits a predefined length of time as specified in the protocol definition before continuing the protocol run. The user may choose to end the wait and continue before the full length of time passes by clicking **Skip** (Figure 7-46).

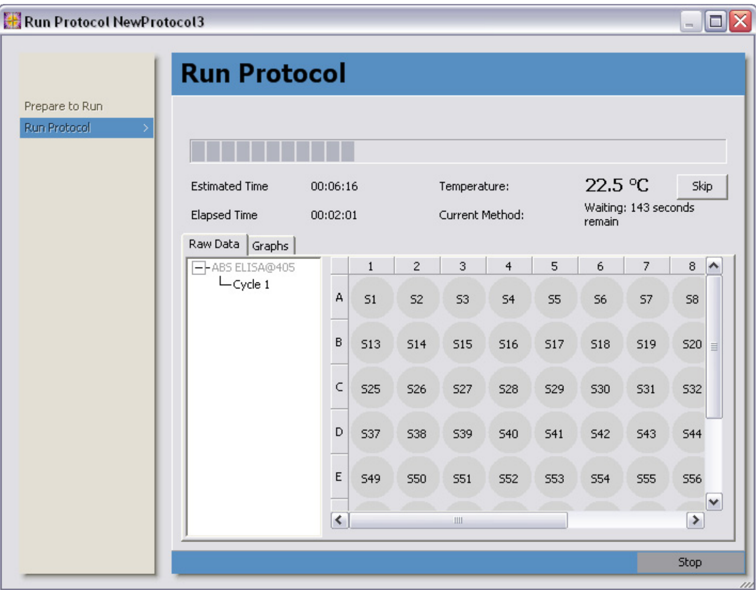


Figure 7-46 Wait During a Protocol Run

- **Set Temperature**: if the protocol is configured to set the temperature and **Wait** is **True**, the protocol pauses until the **Set Temperature** is reached. The user may resume the protocol at any time by clicking **Skip**, and the protocol continues at the current temperature; that is, it does not reach the desired **Set Temperature** (Figure 7-47).

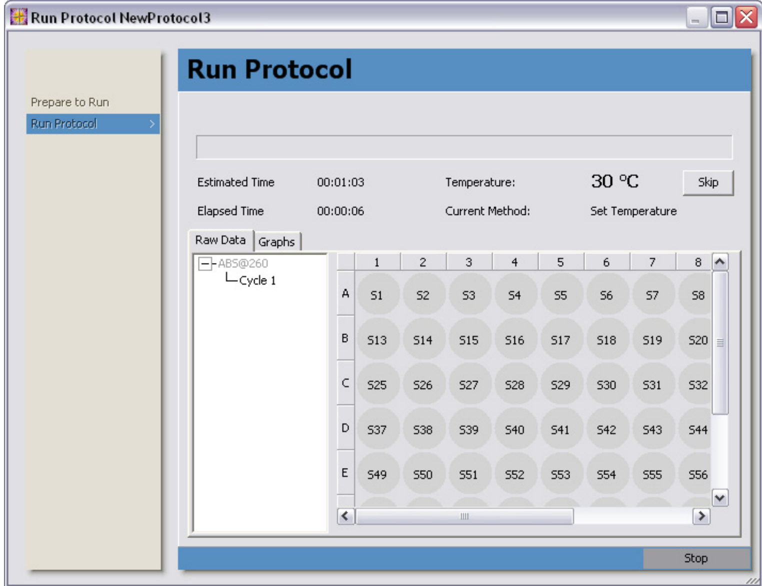


Figure 7-47 Set Temperature During a Protocol Run

Running a Protocol When Simulation Mode is Enabled

Running a protocol in simulation mode allows the protocol configuration to be tested using simulated data before performing the protocol on actual samples. Simulated data is either generated randomly or read from a data file.

Simulation mode is automatically enabled when the host computer is not connected to an instrument. When an instrument is connected, simulation mode may be enabled manually in Instruments. See Enabling Simulation Mode on page 56.

To run a protocol in simulation mode:

1. In the Protocol Selection List, select the protocol to run.
2. From the tool bar, click **Run the selected protocol**.

Note: When running an Analysis protocol with variables configured, Variables appears as the first screen in Run Protocol. Change the values of the variables, if desired, and click **Next** to continue. Prepare to Run appears.

   OR

   From the menu bar select **Actions > Run the selected protocol**.

   OR

   Right-click on the selected protocol and select **Run the selected protocol**.

3. Prepare to Run appears (Figure 7-48).

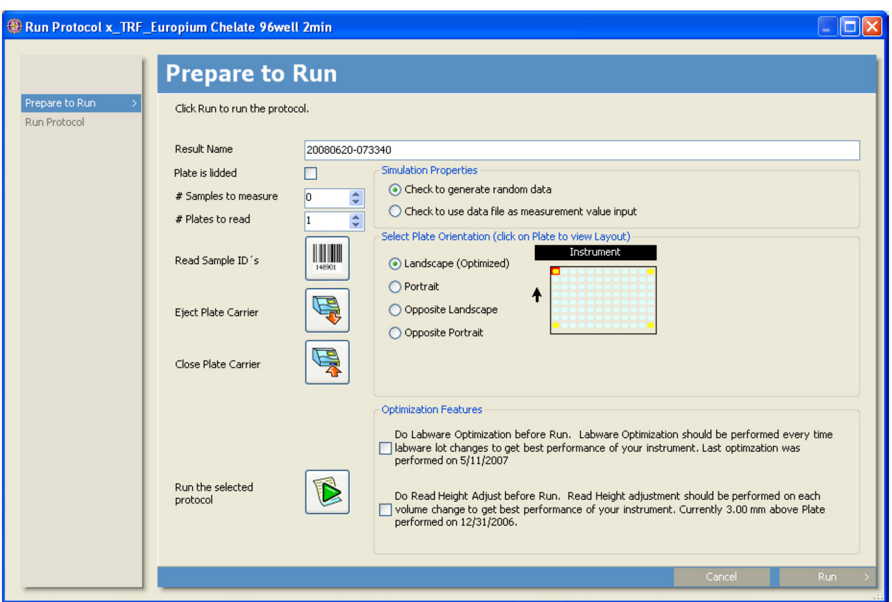


Figure 7-48 Preparing to Run a Protocol in Simulation Mode

4. Enter a new unique **Result Name**, if desired.
5. Select **Check to generate random data** to run the protocol using random data generated by the software, if desired.
6. In Use this data simulation file for this read:
    - Leave the directory path of the simulated data path as configured to use the data file selected in Software Settings. See Selecting Simulated Data Files on page 35.
    - Browse to the location where the desired data file is saved and select it.

Note: Results from prior measurements saved in .dat format may be used as simulated data files. See **Configuring Output Settings**. Simulated data files are used when the number of measurement points in the simulated protocol run is the same as those present in the data file. When the number of measurement points is different, the software generates random data.

When a different simulated data file is selected in Prepare to Run Protocol, the file is used for the current simulated run only. After the simulated run has finished, the data file defaults to the file selected in Software Settings. See **Selecting Simulated Data Files**.

7. Click **Run** or **Next** to run the selected protocol.

Editing Protocols

Parameters configured in a protocol may be edited. When the GxP Permissions module is enabled on the system, signed protocols may not be edited.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Edit permission may edit protocol definitions. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To edit a protocol:

1. In the Protocol Selection List, select the protocol to edit.
2. From the tool bar, click **Edit the selected protocol**.
   OR
   From the menu bar select **Actions > Edit the selected protocol**.
   OR
   Right-click on the selected protocol and select **Edit the selected protocol**.
   OR
   Double-click on the selected protocol.
3. The Edit Protocol window appears (Figure 7-49).

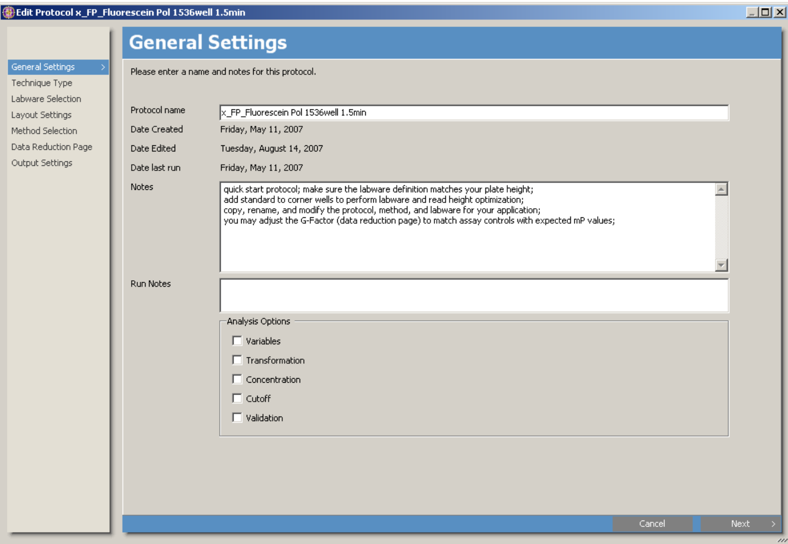


Figure 7-49 Editing an Fluorescence Polarization Protocol

4. Edit the parameters in each Edit Protocol screen as desired. See Creating Protocols on page 146 for detailed information about configuring protocol parameters.
5. Click **Save** to close Edit Protocol and save the changes.

Copying Protocols

Copies may be made of existing protocols. A copy of an existing protocol may be used as a template for a new protocol using the same technique.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Copy permission may create copies of protocols. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

Signed protocols may be copied. Copies of signed protocols are unsigned and may be edited.

To make a copy of a protocol:

1. In the Protocol Selection List, select the protocol to copy.
2. From the tool bar, click **Make a copy of the selected protocol**. A copy of the selected protocol appears in the Protocol Selection List.

   OR

   From the menu bar select **Actions > Make a copy of the selected protocol**.

   OR

   Right-click on the selected protocol and select **Make a copy of the selected protocol**.

Note: The default name format for copied protocols is Copy of OriginalName. To change the name, open the protocol for editing and enter the new protocol name. See Editing Protocols on page 211.

Deleting Protocols

Protocols that are no longer used to perform measurements may be deleted from the Protocol Selection List. When the optional GxP Permissions module is enabled on the system, signed protocols may not be deleted.

Note: When GxP Permissions is enabled on the system, only users assigned a role containing the Delete permission may delete protocols. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To delete a user-defined protocol:

1. In the Protocol Selection List, select the protocol to delete.
2. From the tool bar, click **Delete the selected protocol**.

   OR

   From the menu bar select **Actions > Delete the selected protocol**.

   OR

   Right-click on the selected protocol and select **Delete the selected protocol**.
3. The delete message dialog appears.
4. Click **Yes** to delete the selected protocol. The protocol is moved to the Trash list (to permanently remove the protocol from the database see Deleting and Restoring Items on page 48.

Note: Multiple protocols may be selected by holding down the CTRL key while selecting each protocol desired.

Printing Protocol Configuration Information

Information about the protocol configuration and sample layout may be printed separately from measurement results.

To print protocol configuration information:

1. In the Protocol Selection List, select the protocol to print.
2. From the tool bar, click **Print**.

   OR

   From the menu bar select **Actions > Print the selected protocol**.

   OR

   Right-click on the selected protocol and select **Print the selected protocol**.

Note: Depending on how Print Settings are configured, Print or Print Preview may display before the protocol configuration prints. See Configuring Print Settings on page 38 for more information about enabling and disabling Print and Print Preview.

3. If **Show Printer Settings** and **Print Preview** are not enabled in Print Settings, no additional configuration is required.

   OR

   If the Print dialog appears, configure printing options as desired and click **OK**.

Note: Print is the dialog box that appears when Show Printer Settings is selected.

   OR

   If the Print Preview dialog appears, use the tool bar controls to change the magnification, layout view, or pages displayed, if desired.

4. In the Print Preview dialog, click **Print** to print out the measurement results.
5. In the Print Preview dialog, click **Close** to close the window.

Note: Clicking **Close** without first clicking **Print** cancels printing.

Exporting and Importing Protocols

A protocol can be exported to an XML file, which may be imported at a later time to restore the configuration saved in the file or shared with a copy of Multi-Mode Analysis Software installed on another system. Detection methods and labware configurations used in the protocol are also saved in the export file.

Protocols that use default detection methods and labware installed with Multi-Mode Analysis Software may be imported and exported; however, default methods and labware are not imported with the protocol because these items are present on all systems and may not be edited, deleted, or overwritten.

Note: When GxP Permissions is enabled, signed protocols may be exported for use on another system; however, electronic signatures attached to the protocol are not retained, which allows the protocol to be edited. Importing a signed method to the system from which it was originally exported is not permitted because signed methods may not be deleted or overwritten.

To export a protocol:

1. In the Protocol Selection List, select the protocol to export.
2. From the File menu, click **Export > Protocol**. The Browse for Folder dialog appears.
3. In the Browse for Folder dialog, browse to the folder where the exported protocol will be saved.

   OR

   Click **Make New Folder** to create a new folder where the exported protocol will be saved.
4. Click **OK** to export the protocol. The exported protocol is saved using the default file name format, Protocol_ProtocolName.xml. To import the file at a later date, the filename must not be changed.

To import a protocol from an exported XML file:

1. From the File menu, select **Import > Protocol**. The Open dialog appears.
2. In the Open dialog, browse to and select the desired XML file to import.
3. Click **Open**. The protocol, as well as detection methods and labware used in the protocol, are imported. Any default detection methods or labware used in the protocol are not imported because default methods and labware may not be edited, deleted, or overwritten. Instead, the imported protocol uses the same default methods and labware stored in the software.

Viewing Measurement Results

8

Overview

Measurement results from each protocol run are stored in the Multi-Mode Analysis Software database and are accessed from the Results Selection List (Figure 8-1), which also provides access to result actions and the ability to search for specific results based on names and/or dates.

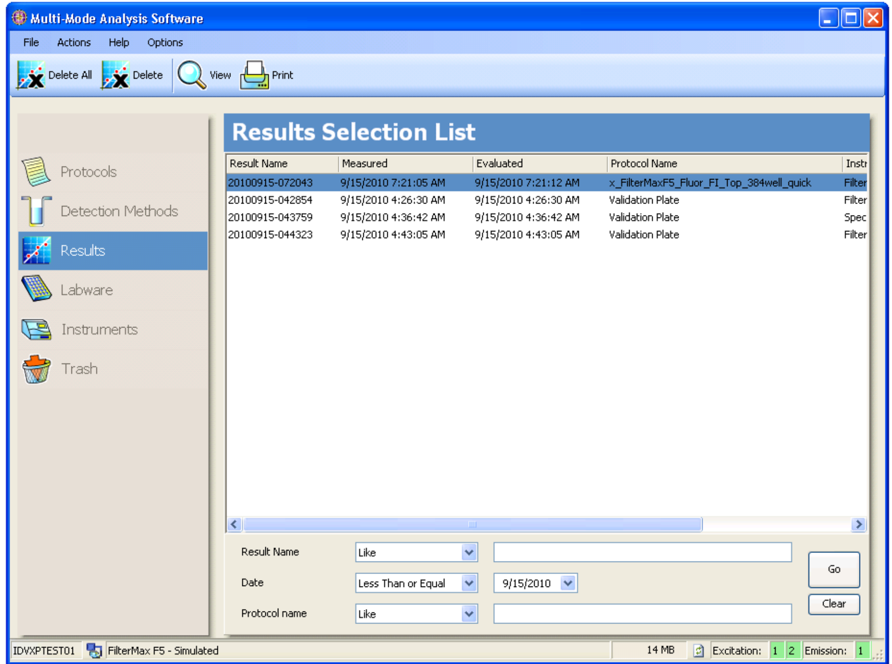


Figure 8-1 Accessing Measurement Result Actions

Measurement results selected from the list are viewed in the Result Viewer, which, depending on measurement type, displays raw data from each measurement technique, measurement cycle or point, reduced data, and graphs. Results calculated using a data reduction method may be reevaluated using different parameters.

Results may be reevaluated by editing analysis parameters configured in the protocol.

Result actions covered in this section include:

- Viewing Measurement Results in the Result Viewer on page 217
- Viewing and Reevaluating Results from an Analysis Application on page 229
- Viewing Exported Measurement Results on page 235

Note: This section includes viewing measurement results exported to Microsoft Excel from the Result Viewer and results exported to XML or data files at the completion of a protocol run.

- Signing Measurement Results on page 238
- Deleting Measurement Results on page 239
- Printing Measurement Results on page 239

To view measurement results:

1. From the navigation pane, click **Results**. The Results Selection List appears (Figure 8-1).
2. In the Results Selection List, select the desired results and from the tool bar, click **View**. The Result Viewer appears (Figure 8-2).

   OR

   Use the search options to locate specific results. Table 8-1 describes the search options available. Results matching the search parameters appear in the Results Selection List.

Table 8-1 Measurement Results Search Options

Search Option	Description
Result Name	Enter a partial or complete Result Name. Searching for result names may also be limited to specific dates configured in Date.
Date	Select the desired range option: Less Than or Equal, Equal, Greater Than or Equal, or Between, then select the desired dates.
Protocol Name	Enter a partial or complete Protocol Name. Searching for protocol names may also be limited to specific dates configured in Date.
Go	Perform the search. Matching results appear in the Results Selection List.
Clear	Clear search results and display all measurement results in the Results Selection List.

Viewing Measurement Results in the Result Viewer

Measurement results accessed from the Results Selection List are viewed in the Result Viewer (Figure 8-2). Results are displayed in a series of tabs. The tabs displayed depend on the measurement types configured in the protocol; for example, reduced data is not displayed when data reduction is not configured.

Results may also be printed, saved, or exported to a Microsoft Excel spreadsheet using the Result Viewer actions. Results calculated using data reduction may be reevaluated by editing data reduction parameters or selecting a different method.

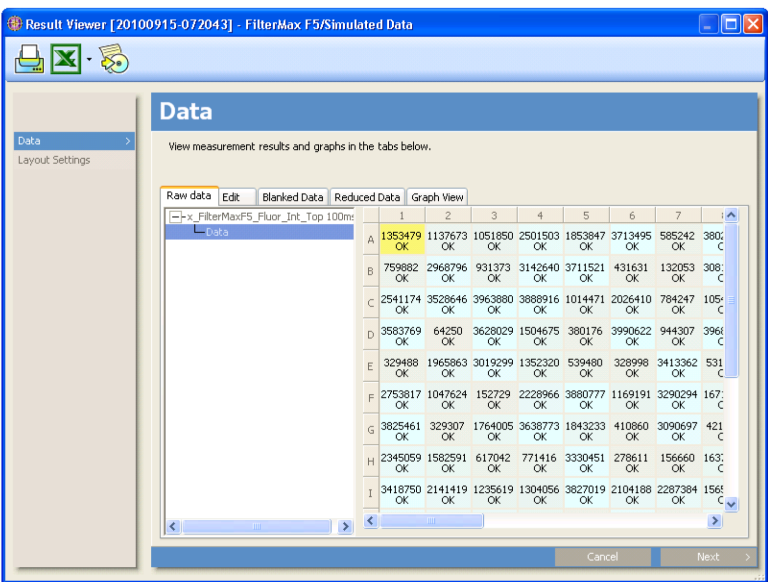


Figure 8-2 Viewing Raw Data

This section covers Result Viewer functionality provided in the Data screen, which is displayed for analysis applications, including:

- Viewing Raw Data on page 218
- Viewing Blanked Data on page 219
- Viewing Reduced Data on page 221
- Viewing Mean Data on page 223
- Viewing Graphs on page 224
- Recalculating Data Reduction on page 227
- Exporting Measurement Results to Microsoft Excel on page 228
- Saving Measurement Results on page 228

Note: The Result Viewer also appears transformed measurement results for analysis options configured in the protocol. See Viewing and Reevaluating Results from an Analysis Application on page 229.

Viewing Raw Data

The Raw Data tab appears all data measured during the protocol run (Figure 8-3). Depending on the measurement types configured in the protocol, the left pane lists all detection methods, measurement cycles, or wells in a tree view. Selecting a method, cycle, or well displays measured values and status in the right panel. A result may be rejected as an outlier when the value falls outside of the expected result.

Note: Results for single-point measurements are displayed in Raw Data.

To view raw data:

1. Select the **Raw Data** tab, if necessary.

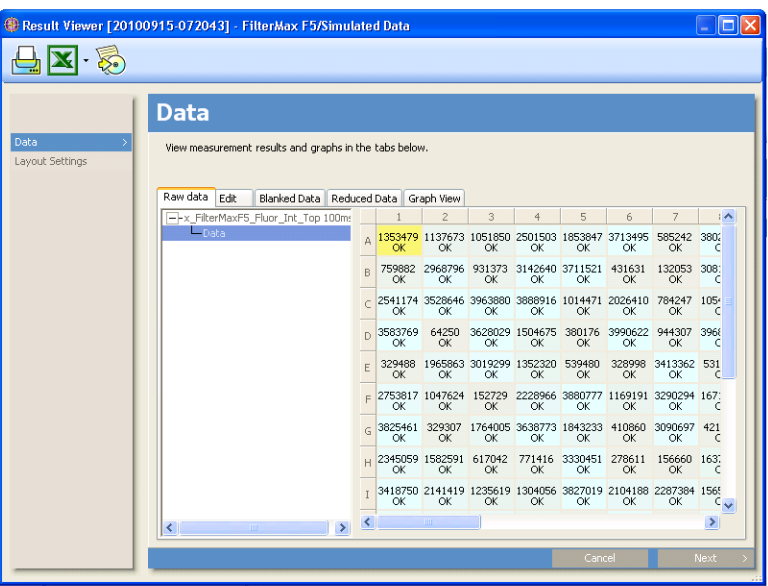


Figure 8-3 Viewing Raw Data

2. From the tree view in the left pane, select the detection method, measurement cycle, or well desired to view. Results and status appear in the right pane. Table 8-2 describes the status indicators that may be displayed.

Table 8-2 Well Status Indicators

Status	Description
OK	The sample was measured successfully.
Error	The sample was not measured because an instrument error occurred.
Overflow	No result is available because the value exceeds the indication limit.
Underflow	No result is available because reduced data could not be calculated.
Extrapolated	The result fell within the extrapolation percentage set for a standard curve.
Not Evaluated	The measurement result was not evaluated.
Rejected	The sample was rejected as an outlier by a user. Samples may be rejected in any tab displaying a matrix view of measurement results.
Unused	The sample was not selected for measurement in the protocol.

Viewing Blanked Data

The Blanked Data tab displays the well identifier, and the blanked data for each well measured during the protocol run (Raw Data – Blank Data = Blanked Data). The Blanked Data tab displays this information in a matrix corresponding to the plate layout (Figure 8-4). Depending on the measurement types configured in the protocol, the left pane lists all detection methods, measurement cycles, or wells in a tree view. Selecting a method, cycle, or well displays measured values and status in the right panel.

Note: The Blanked Data tab is only available if the plate layout has blank wells specified in the plate layout.

To view blanked data:

1. Select the **Blanked Data** tab, if necessary.

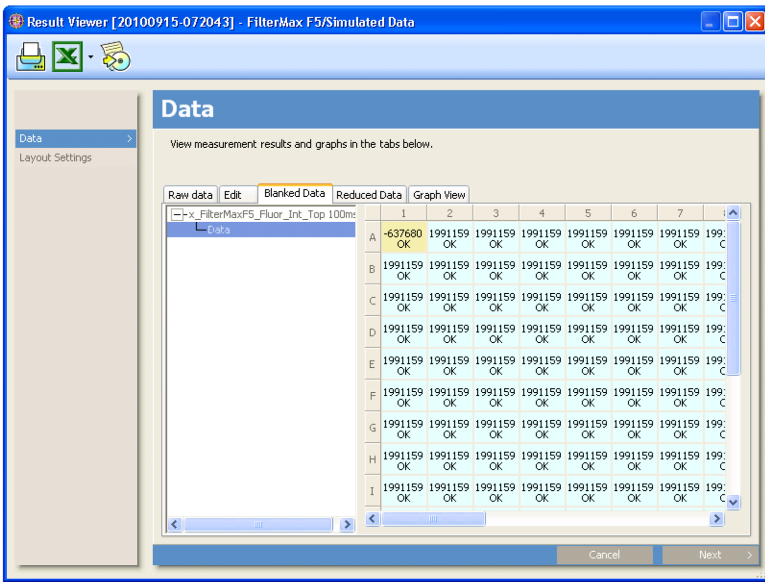


Figure 8-4 Viewing Blanked Data

2. From the tree view in the left pane, select the detection method, measurement cycle, or well desired to view. Blank results and the well status appear in the right pane. Table 8-3 describes the status indicators that may be displayed.

Table 8-3 Well Status Indicators

Status	Description
OK	The sample was measured successfully.
Error	The sample was not measured because an instrument error occurred.
Overflow	No result is available because the value exceeds the indication limit.
Underflow	No result is available because reduced data could not be calculated.
Extrapolated	The result fell within the extrapolation percentage set for a standard curve.
Not Evaluated	The measurement result was not evaluated.
Rejected	The sample was rejected as an outlier by a user. Samples may be rejected in any tab displaying a matrix view of measurement results.
Unused	The sample was not selected for measurement in the protocol.

Viewing Reduced Data

The Reduced Data tab is displayed when the detection methods configured in the protocol use data reductions (Figure 8-5). You can view PathCheck® Pathlength Measurement Technology adjusted results in the Reduced Data tab. Wells may also be rejected as outliers when the calculated value falls outside of the expected result. Reduced data for the plate may be recalculated with outliers removed, if desired.

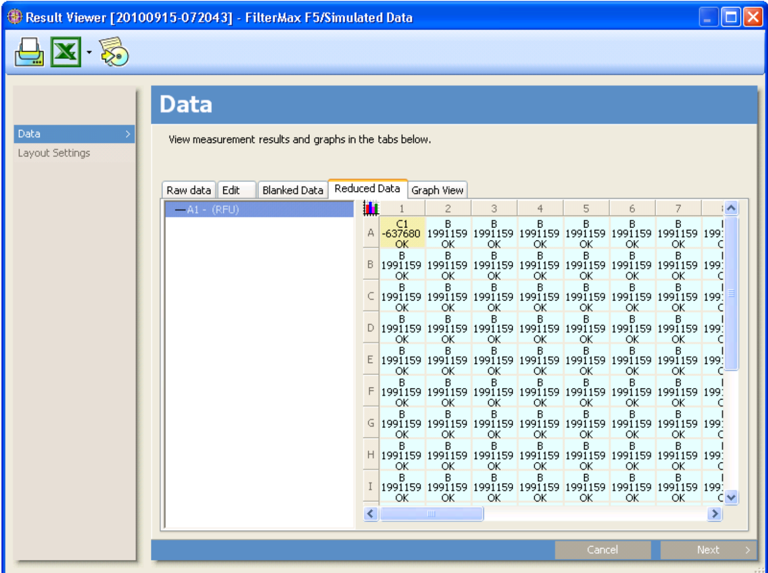


Figure 8-5 Viewing Reduced Data for a measurement Performed in a Multiwavelength Protocol

Note: Reduced data may be also shown as 2D or 3D graphic by clicking the graphic symbol at the top left corner of the plate grid.

For a Quantitation protocol, each Quantitation application that was performed as part of the protocol is listed in Reduced Data. Results for each application type can be viewed by selecting the desired application.

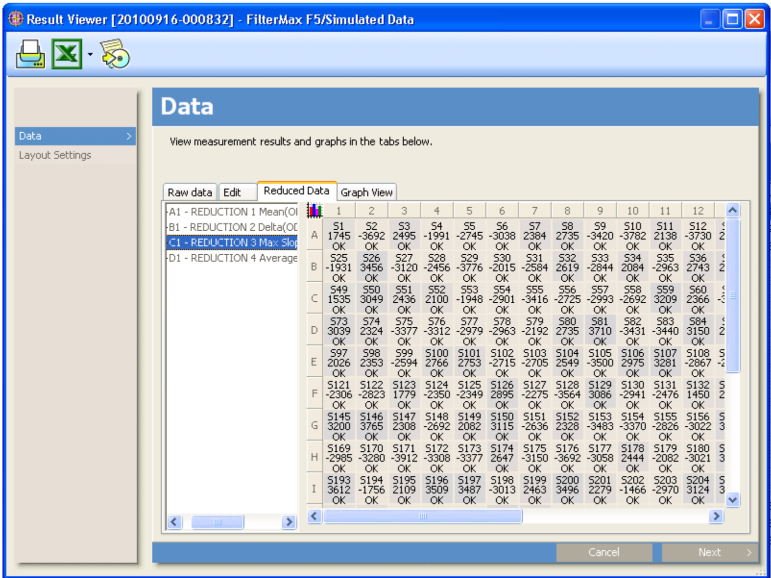


Figure 8-6 Viewing Reduced Data for a Measurement Performed in a Quantitation protocol

To view reduced data, reject outliers, and recalculate reduced data:

1. Select the **Reduced Data** tab, if necessary.
2. If multiple detection methods with data reduction techniques are configured in the protocol, select the desired data reduction technique from the left pane. Reduced data for selected method appear in the right pane.

   OR

   For a Quantitation protocol, if multiple Quantitation applications are selected for the protocol, select the desired application. Reduced data for the selected Quantitation application is displayed.
3. To reject a well as an outlier, right-click on the desired well and select **Reject Well**. A red X appears over the well and the Reevaluate current measurement results button appears on the tool bar.

Note: Multiple wells may be rejected as outliers. Rejected wells may be included in the measurement results again by right-clicking on each well desired and selecting **Accept Well**.

4. From the tool bar, click **Reevaluate current measurement results** to recalculate the results with outliers removed.

Note: The **Reevaluate** button appears on the tool bar only when a parameter in the results, such as data reduction technique, has been edited.

Viewing Mean Data

The Mean Data and Mean Data List tabs display the well identifier, mean (reduced) value, well status, coefficient of variation (CV%), and standard deviation for replicate groups. The Mean Data tab displays this information in a matrix corresponding to the plate layout (Figure 8-7); the Mean Data List tab displays the same information in a list. Mean data are calculated and displayed for each result of the data reduction.

Note: The Mean Data tab is only available if the protocol has replicates in the plate layout.

Wells may be rejected as outliers when the calculated value falls outside the expected result. The results may be recalculated with outliers removed, if desired.

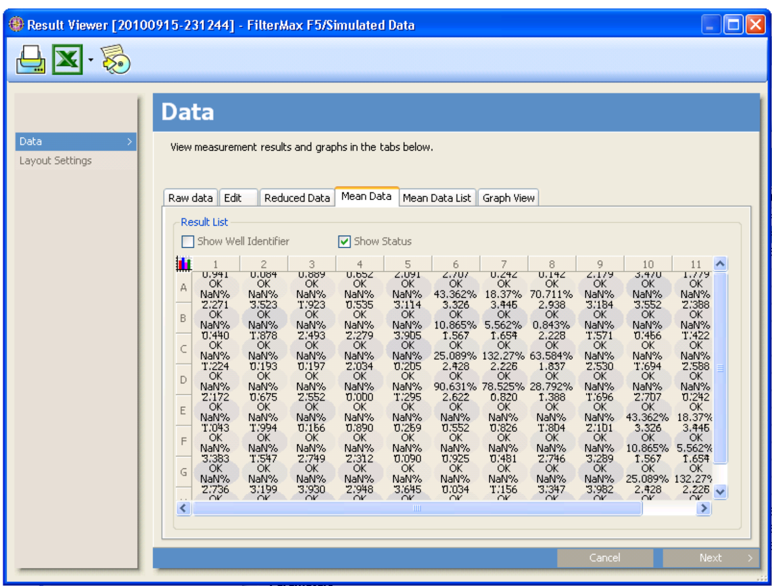


Figure 8-7 Viewing Mean Data

To view mean data:

1. Select the **Mean Data** tab, if necessary.
2. Select **Show Status** to show the measurement status for each well, if desired. Table 8-2 describes the status indicators that may be displayed.
3. Select **Show Well Identifier** to show identifiers for each well, if desired.
4. To reject a well as an outlier, right-click on the desired well and select **Reject Well**. A red **X** appears over the well and the **Reevaluate current measurement results** button activates on the tool bar.

Note: Multiple wells may be rejected as outliers. Rejected wells may be included in the measurement results again by right-clicking on each well desired and selecting **Accept Well**.

5. Click **Reevaluate current measurement results** to recalculate the results with outliers removed.

Note: The **Reevaluate** button appears on the tool bar only when a parameter in the results, such as an outlier, has been changed.

Note: If Sample-ID was used on the run protocol screen, Sample-ID is also displayed here.

Viewing Graphs

The Graphs tab displays graphs for all measured wells on a plate (Figure 8-8). The detection methods configured in the protocol determine the types of graphs displayed.

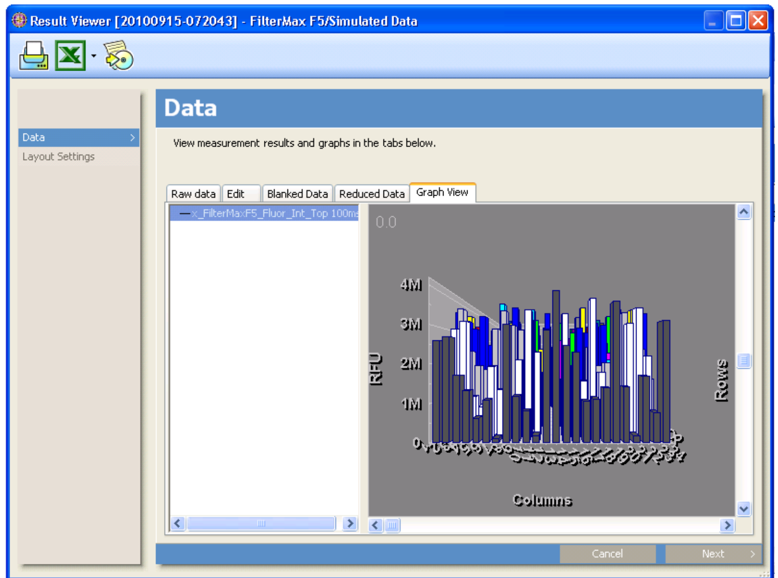


Figure 8-8 Viewing Kinetic Measurement Graphs

Detailed graphs for individual samples, all samples in a column or row, or all samples on the plate may be viewed and printed. To view detailed graphs for:

- **an individual sample**: click in the desired well. Depending on measurement type, a two- or three-dimensional graph of the selected result appears. See Viewing Two-Dimensional Graphs on page 225 or Viewing Three-Dimensional Graphs on page 226.

Note: Detailed graphs for individual samples may be viewed for all measurement types, even when the graphs displayed appear to be empty.

- **a row or column**: click in the desired row or column header. A three-dimensional graph of results for the row or column appears. See Viewing Three-Dimensional Graphs on page 226.

Note: Rows and columns may not be selected for multiwavelength and area scan measurement results.

- **all samples on the plate**: click in the upper left corner of the plate layout (Figure 8-10). A three-dimensional graph of all results on the plate appears. See Viewing Three-Dimensional Graphs on page 226.

Note: All samples may not be selected for multiwavelength and area scan measurement results.

Viewing Two-Dimensional Graphs

Results for individual single-point, kinetic, and linear scan measurements are displayed in two-dimensional graphs (Figure 8-9). Two-dimensional graphs may be printed.

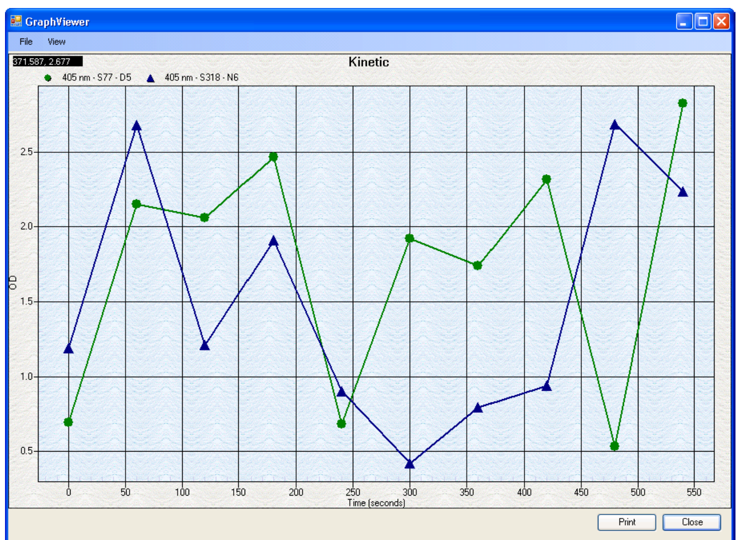


Figure 8-9 Viewing a Two-Dimensional Absorbance Graph

To print the graph:

1. From the **File** menu, select **Print**.

Note: Depending on how Print Settings are configured, Print and/or Print Preview may display before the protocol configuration prints. See Configuring Print Settings on page 38 for more information about enabling and disabling Print and Print Preview.

2. If **Show Printer Settings** and **Print Preview** are not enabled in Print Settings, the graph prints automatically.

   OR

   If the Print dialog appears, configure printing options as desired and click **OK**.

Note: Print is the dialog box that appears when **Show Printer Settings** is selected.

   OR

   If Print Preview appears, use the tool bar controls to change the magnification, layout view, or pages displayed, if desired. See Printing Measurement Results on page 239 for more information about Print Preview.

Viewing Three-Dimensional Graphs

Selecting an individual area scan graph, a row, column, or all samples in the Graph tab displays a three-dimensional graph (Figure 8-10). Three-dimensional graphs may be rotated horizontally or vertically and printed.

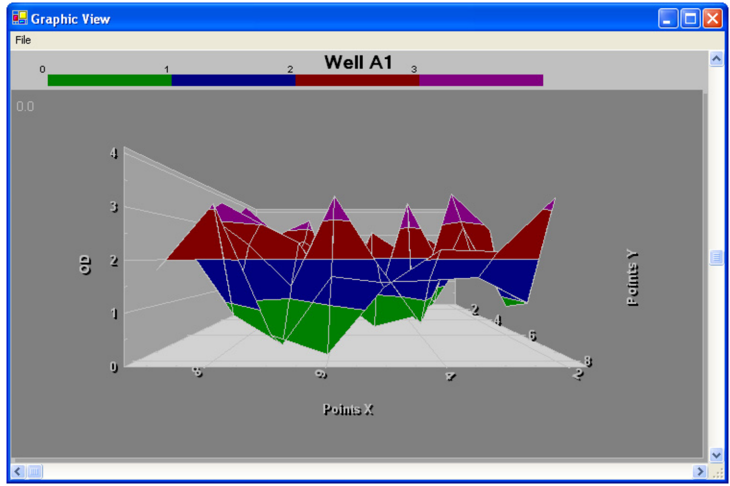


Figure 8-10 Viewing a Three-Dimensional Area Scan Graph

To rotate the graph:

1. Use the vertical scroll bar to rotate the graph vertically.
2. Use the horizontal scroll bar to rotate the graph horizontally.
3. Double-click on the graph to start and stop a continuous animated horizontal rotation of the graph.

To print the graph:

1. From the File menu, click **Print**.

   OR

   Right-click on the graph and select **Export Dialog** and then select **Print**.

Note: Depending on how Print Settings are configured, Print and/or Print Preview may display before the protocol configuration prints. See Configuring Print Settings on page 38 for more information about enabling and disabling Print and Print Preview.

2. If **Show Printer Settings** and **Print Preview** are not enabled in Print Settings, the graph prints automatically.

   OR

   If the Print dialog appears, configure printing options as desired and click **OK**.

Note: Print is the dialog box that appears when **Show Printer Settings** is selected.

   OR

   If the Print Preview dialog appears, use the tool bar controls to change the magnification, layout view, or pages displayed, if desired. See Printing Measurement Results on page 239 for more information about Print Preview.

Recalculating Data Reduction

The Edit tab allows reduced data to be recalculated by editing the parameters of the current data reduction method or by selecting a different data reduction method (Figure 8-11). Data reduction may be recalculated for any detection method configured with data reduction in the protocol.

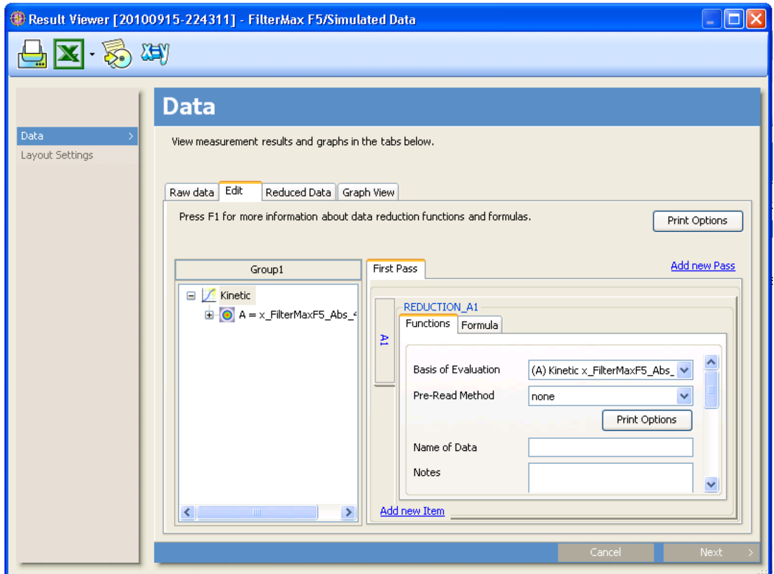


Figure 8-11 Changing Data Reduction Parameters in the Results Viewer

To change parameters:

1. Click the **Edit** tab, if necessary.

Note: Refer to Data Reduction Techniques on page 241 for details about the data reduction methods available for different measurement types.

2. In the Edit tab, click **Report Options** and select the method information and measurement results data included in printouts:
   - **Method Information**: details about the configured method, including technique type and filters used.
   - **Graph**: graphs of results for all measured samples.
   - **Raw Data**: results from each detection method, kinetic cycle, or well scanned.
3. Click **Reevaluate current measurement results** to recalculate the results using the new data reduction configuration.

Note: The **Reevaluate** button appears on the tool bar only when a parameter in the results, such as a data reduction technique, has been edited.

Exporting Measurement Results to Microsoft Excel

Results displayed in the Result Viewer may be exported to Microsoft Excel. Exported results are saved in the directory selected in Directory Settings. See Selecting a Directory for Saving Exported Measurement Results on page 37.

To export results to Excel:

1. From the tool bar, select **Export the current measurement results to Excel**. Excel opens automatically.
2. In Excel, note that exported results appear in multiple workbooks. A workbook may contain general information and raw data for a single detection method, or general information and reduced data.

Note: See Exporting Measurement Results to Microsoft Excel on page 228 for information about viewing exported results in Excel.

Saving Measurement Results

The measurement results currently being viewed may be saved to the database from within the Result Viewer, which allows results that have been reevaluated by editing data reduction or analysis parameters to be saved with a different name. Optionally, parameters edited in the Result Viewer may be saved in the original protocol definition.

To save measurement results:

1. From the tool bar, click **Save the current measurement results to the database**. The question dialog appears, asking if the existing results should be overwritten.
2. Click **Yes** to overwrite existing results. The results are overwritten and a message dialog appears, asking if parameters edited in the Result Viewer should also be saved to the protocol definition. Proceed to Step 3.

Note: When GxP Permissions is enabled on the system, measurement results that have been signed may not be overwritten. See Signing Measurement Results for more information about signing results.

OR

Click **No** to save the results with a different name. Result Name appears.

3. Enter a new name for the results and click **OK**. The results are saved to the database. The message dialog appears, asking if parameters edited in the Result Viewer should also be saved to the protocol definition.
4. Click **Yes** to save the new parameters to the protocol definition.

Note: When GxP Permissions is enabled on the system, parameters changed in the Result Viewer may not be saved to protocols that have been signed.

OR

Click **No** to ignore any changes made and retain the original parameters configured in the protocol.

Viewing and Reevaluating Results from an Analysis Application

The Result Viewer appears measurement results, transformed data from the analysis options configured in the protocol, and the parameters currently configured for each analysis option.

Multiple "what if?" analyses on transformed data may be performed by editing the parameters configured for an analysis option and then reevaluating the data with the new parameters.

This section covers:

- Viewing Results From an Analysis Protocol on page 230
- Reevaluating Results from an Analysis Protocol on page 233

Note: See Viewing Measurement Results in the Result Viewer on page 217 for information about viewing results reported in the Data screen, recalculating data reduction, and exporting and saving results.

Viewing Results From an Analysis Protocol

All analysis options configured in the protocol appear in the navigation pane of the Result Viewer (Figure 8-12). Transformed data and parameters for the selected analysis option appear in a series of tabs.

To view analysis results and parameters:

1. In the navigation pane, select the desired analysis option. Only analysis options configured in the protocol are listed. A series of tabs with results from the selected analysis option appears (Figure 8-12).

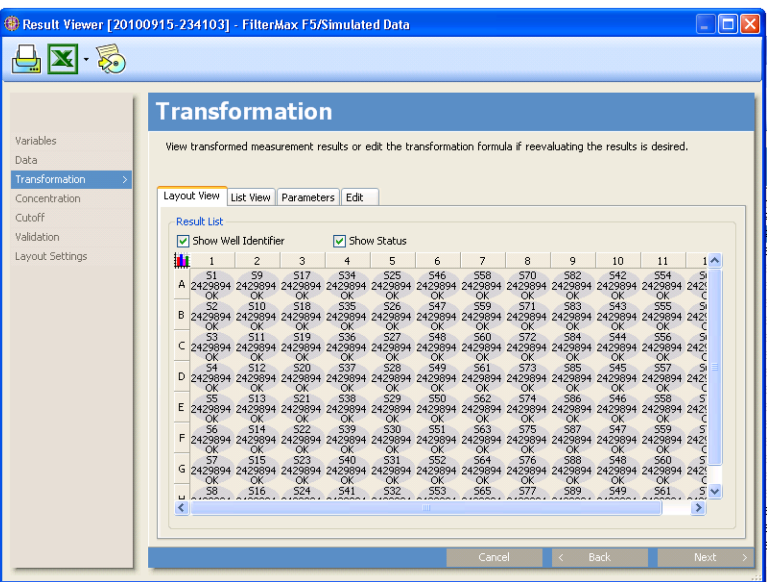


Figure 8-12 Viewing Existing Transformation Parameters in the Results Viewer

2. Select the tab desired to view. Table 8-4 describes each tab and lists which analysis options display the tab.

Table 8-4 Measurement Results Tabs Displayed for Analysis Options

Tab	Description	displays In
Layout View	displays transformed results and measurement status for the selected analysis option in a matrix corresponding to the plate layout. **Note:** Results may be rejected as outliers in any Layout View tab. To reject a result, right click on the desired sample and select Reject Well. Results may be reevaluated with outliers removed.	Transformation Concentration Cutoff
List View	displays transformed results and measurement status for the selected analysis option in a list.	Transformation Concentration Cutoff
Standard Curve	displays a graph of the standard curve. See Changing the Standard Curve Graph View on page 231 for information about customizing the graph display.	Concentration
Parameters	displays the parameters used to calculate the transformed data currently displayed in the Layout View and List View tabs.	Variables Transformation Concentration Cutoff Validation
Edit	Edit the parameters configured for the analysis option using the same configuration screen that appears in the Create Protocol wizard. See Reevaluating Results from an Analysis Protocol on page 233.	Variables Transformation Concentration Cutoff Validation

Changing the Standard Curve Graph View

The standard curve graph view may be changed by zooming in on a selected region or changing view options.

To zoom in on a region of the graph:

1. Position the cursor at the desired starting point for the region, then click and hold the mouse button down. The cursor icon changes to a magnifying glass.
2. Drag the mouse until the desired region is selected. The selected region is highlighted in black.
3. Release the mouse button. The selected region is displayed.

To zoom out and view the graph at the original size:

- Right-click on the graph and select **Undo Zoom** from the menu that appears.

To change view options:

- Right-click on the graph and select a view option from the menu that appears. Table 8-5 describes the view options available.

Table 8-5 Standard Curve Graph View Options

<table>
<tr><th>View Option</th><th>Description</th></tr>
<tr><td>Viewing Style</td><td>Select how the graph is displayed: in Color, Monochrome, or Monochrome with symbols.</td></tr>
<tr><td>Numeric Precision</td><td>Select the numeric precision of graph data displayed on the screen or exported to text files. Precision up to three decimal positions may be specified.</td></tr>
<tr><td>Plotting Method</td><td>Select how the graph is plotted. Line is the default method.</td></tr>
<tr><td>Data Shadows</td><td>Enable shadows that give the graph a 3-D appearance.</td></tr>
<tr><td>Grid Options</td><td>Customize the grid display. Grid lines may be displayed, hidden, or changed to a different style, such as thick, thin, or dashed.</td></tr>
<tr><td>Include Data Labels</td><td>Select to display labels for data points on the curve.</td></tr>
<tr><td>Mark Data Points</td><td>Select to mark each data point with a small circular symbol.</td></tr>
<tr><td>Undo Zoom</td><td>Select to display a zoomed graph at the original size. Available only when the graph is zoomed.</td></tr>
<tr><td>Maximize</td><td>Select to display a full-screen version of the graph. Close the maximized view by pressing the Esc key or clicking on the title bar of the maximized window.</td></tr>
<tr><td>Customization Dialog</td><td>Open the Customization dialog box. Customization options are grouped in a series of tabs:
• General: Enter a title for the graph, change the viewing style (color or monochrome), set the numeric precision up to 3 decimal positions, and change the grid appearance.
• Plot: Change the plotting method, and enable or disable 3-D shadows and data point markers.
• Subsets: This tab contains no configurable options.
• Axis: Change the properties of the X- and Y-axes.
• Font: Change the font used for titles and labels.
• Color: Change the color of any graph attribute except data points and lines.
• Style: Change the colors and styles of data points and lines displayed in the graph.

Note: Many of the options available in Customization are the same as those in the menu that appears when right-clicking on the graph.</td></tr>
<tr><td>Export Dialog</td><td>Open the Export dialog box.</td></tr>
</table>

Reevaluating Results from an Analysis Protocol

Multiple "what if?" analyses on transformed data may be performed by editing the parameters configured for an analysis option and then reevaluating the data with the new parameters. The parameters used to calculate the transformed data currently displayed in the Result Viewer are viewed in the Parameters tab. Parameters are edited in the Edit tab.

To reevaluate measurement results:

1. In the left pane, select an analysis option to edit. Only analysis options configured in the protocol are listed. The selected analysis option appears (Figure 8-13).

Note: Select Data to view measurement results and edit data reduction parameters configured in the protocol. See Viewing Measurement Results in the Result Viewer on page 217 for more information.

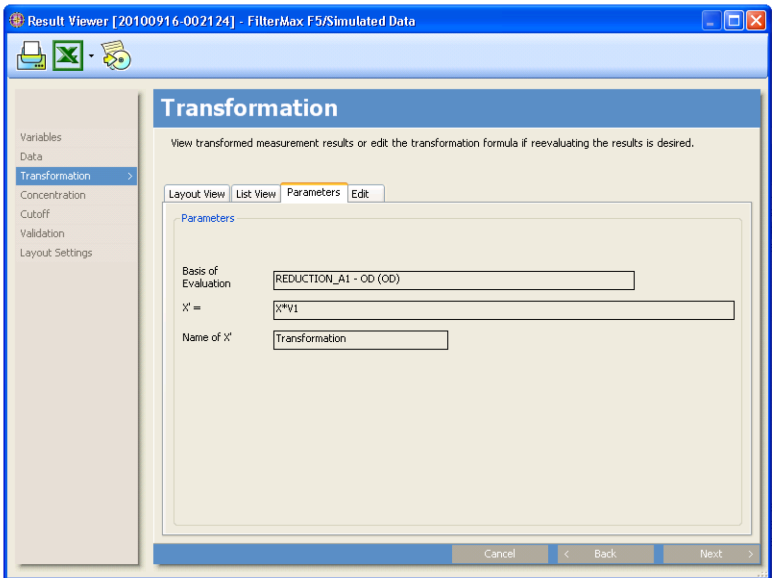


Figure 8-13 Viewing Existing Transformation Parameters in the Results Viewer

2. Select the **Layout View** or **List View** tab to view the current transformed data. Layout View displays data in a matrix corresponding to the plate layout; List View displays the same data in a list.
3. Select the **Parameters** tab to view the parameters used to calculate the transformed data currently displayed; for example, Figure 8-13 shows transformation parameters.

4. Select the **Edit** tab to edit parameters as desired (Figure 8-14). Parameters in the Edit tab are identical to those in the corresponding screen in the protocol configuration. Refer to the appropriate section for more information:
    - Configuring Variables on page 170
    - Configuring a Transformation Formula on page 177
    - Configuring Concentration on page 179
    - Configuring Cutoff Values on page 183
    - Configuring Validation Rules on page 185

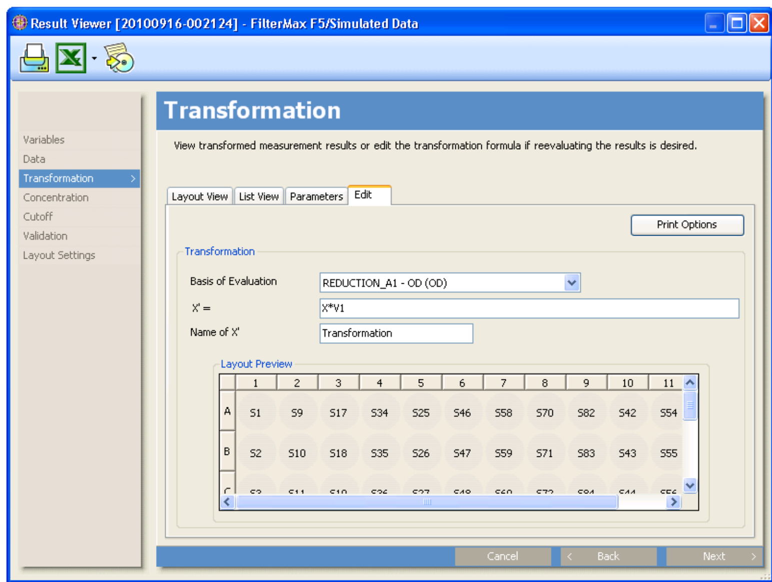


Figure 8-14 Figure8.14 Editing Transformation Parameters in the Results Viewer

5. Edit additional analysis options, if desired.
6. From the tool bar, click **Reevaluate current measurement results** to recalculate the results using the edited parameters.

Note: The **Reevaluate** button appears on the tool bar only when a parameter in the results, such as the transformation formula, has been changed.

7. Optionally, save the reevaluated results with a different name to preserve a record of changes made to the parameters. See Saving Measurement Results on page 228.

Viewing Exported Measurement Results

Measurement results may be exported manually from the Results Viewer or automatically at the end of a protocol run when export options are configured in Output Settings.

Two file export and save options are available:

- **Export to Microsoft Excel**: saves results in a format compatible with Microsoft Excel, and automatically opens Excel. See Viewing Measurement Results in Microsoft Excel on page 235 for more information about viewing exported results in Excel.

Note: Multi-Mode Analysis Software automatically determines the appropriate export method based on the version of Microsoft Office installed on the host computer. XML (.xml) files are exported when Office XP is installed. When Office 2000 installed, the measurement results are copied into a new spreadsheet which must be saved in Excel. Versions of Excel prior to Office 2000 are not supported by the Export to Microsoft Excel function, but can open measurement results stored in tab-delimited data (.dat) files.

- **Create XML and DAT data files**: saves results in XML and tab-delimited data (.dat) files, which may be opened by compatible software applications.

Note: See Configuring Output Settings on page 187 for more information about configuring export and file options.

To view saved measurement results:

1. Open the desired software application.
2. Browse to the directory where exported measurement results are stored, and open the desired file. Exported measurement results are stored in the data directory selected in Software Settings. See Selecting a Directory for Saving Exported Measurement Results on page 37.

Viewing Measurement Results in Microsoft Excel

When measurement results for a protocol run are exported to Excel, multiple workbooks (spreadsheets) are created. A workbook is created for the raw data read for each detection method configured in the protocol. Reduced and transformed data are included in a separate workbook.

This section covers:

- Viewing Protocol and Measurement Information on page 236
- Viewing Raw Data on page 237
- Viewing Reduced and Transformed Data on page 237

Viewing Protocol and Measurement Information

Information about the system and parameters configured in the protocol is saved in the General worksheet. General is included in all worksheets exported from a set of measurement results (Figure 8-15).

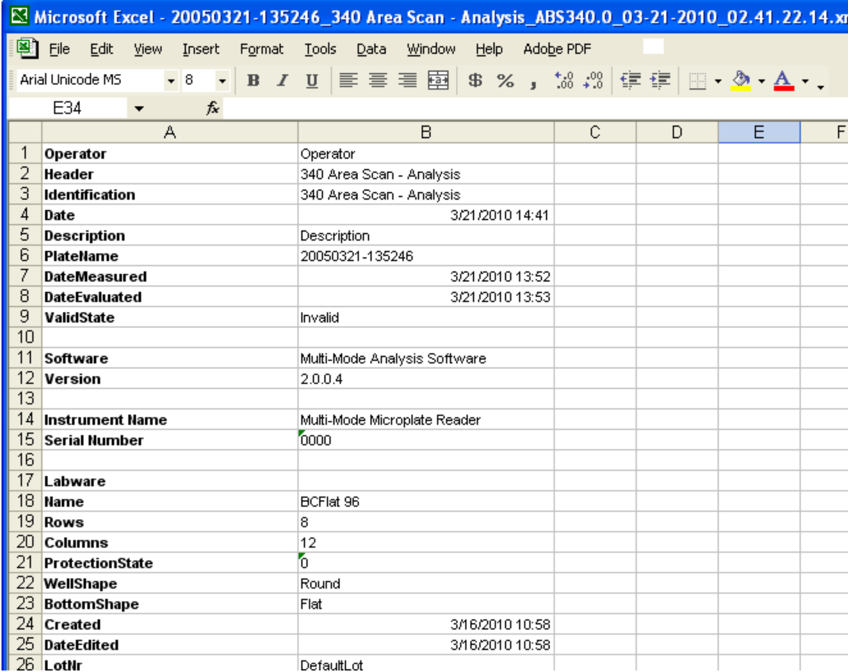


Figure 8-15 Viewing Protocol Information in the General Worksheet (excerpt)

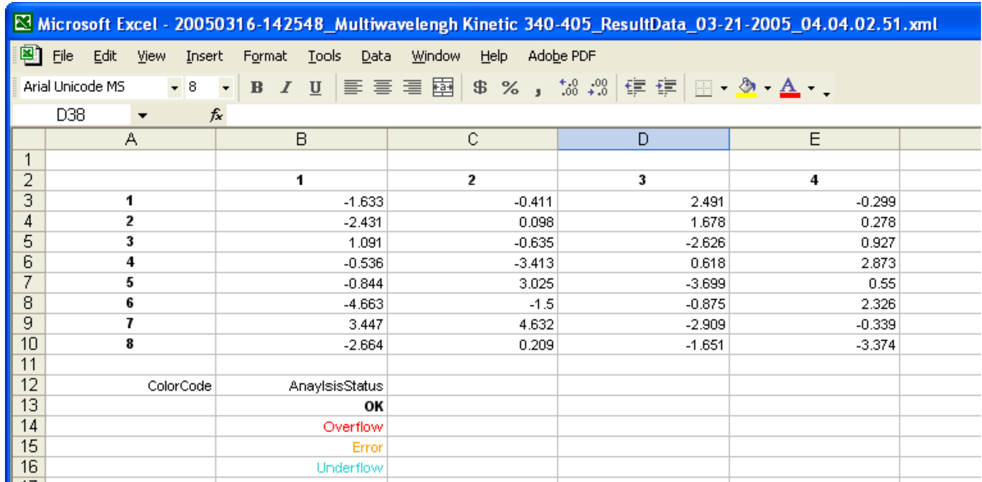
To view the General worksheet:

1. In Excel, access either workbook containing the desired results.
2. Select the **General** tab in the lower left corner of the window.

Viewing Raw Data

Measurement results exported to Excel display raw data in matrices corresponding to the plate layout (Figure 8-16). Data from each measurement cycle or point is presented in a separate sheet within the workbook.

Note: Results from protocols containing multiple detection methods export raw data from each detection method to a separate workbook. The title of workbooks containing raw data always includes the name of the detection method.



	A	B	C	D	E
1					
2		**1**	**2**	**3**	**4**
3	**1**	-1.633	-0.411	2.491	-0.299
4	**2**	-2.431	0.098	1.678	0.278
5	**3**	1.091	-0.635	-2.626	0.927
6	**4**	-0.536	-3.413	0.618	2.873
7	**5**	-0.844	3.025	-3.699	0.55
8	**6**	-4.663	-1.5	-0.875	2.326
9	**7**	3.447	4.632	-2.909	-0.339
10	**8**	-2.664	0.209	-1.651	-3.374
11					
12	ColorCode	AnaylsisStatus			
13		**OK**			
14		Overflow			
15		Error			
16		Underflow			

Figure 8-16 Viewing Reduced Data (excerpt)

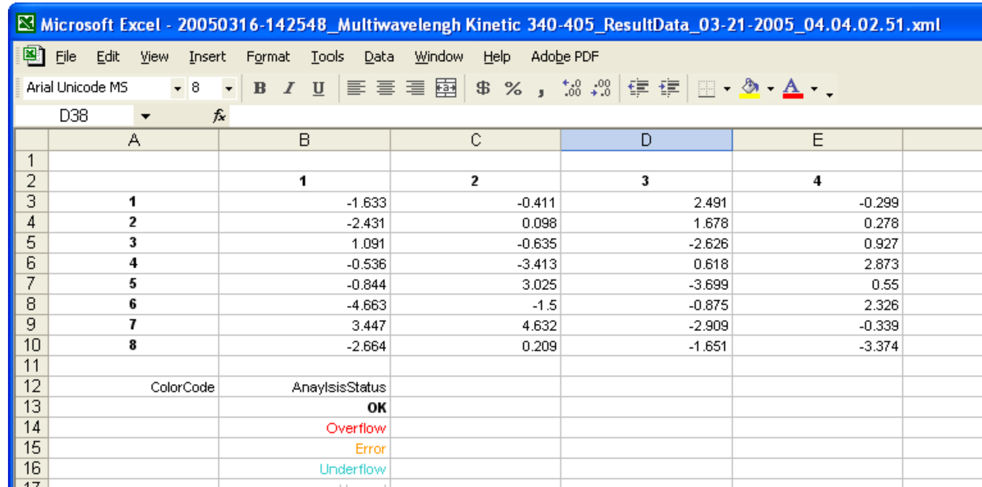
To view raw data:

1. In Excel, access the workbook containing raw data for the desired measurement results. Titles of workbooks containing raw data always contain the name of a specific detection method configured in the protocol; for example, ABS340 (Figure 8-16).
2. In the worksheet, select the **Cycle** tab for the desired measurement cycle.

Viewing Reduced and Transformed Data

Reduced and transformed data are exported to a different Excel workbook than raw data. Reduced data generally appears in a sheet named Measurement; transformed data appears in sheets with names corresponding to the analysis options configured in the protocol.

Note: Transformed data appears in results for protocols with analysis options configured.



	A	B	C	D	E
1					
2		1	2	3	4
3	1	-1.633	-0.411	2.491	-0.299
4	2	-2.431	0.098	1.678	0.278
5	3	1.091	-0.635	-2.626	0.927
6	4	-0.536	-3.413	0.618	2.873
7	5	-0.844	3.025	-3.699	0.55
8	6	-4.663	-1.5	-0.875	2.326
9	7	3.447	4.632	-2.909	-0.339
10	8	-2.664	0.209	-1.651	-3.374
11					
12	ColorCode	AnaylsisStatus			
13		OK			
14		Overflow			
15		Error			
16		Underflow			

Figure 8-17 Viewing Reduced Data (excerpt)

To view reduced or transformed data:

1. In Excel, access the workbook containing reduced or transformed data for the desired measurement results. Workbooks containing reduced or transformed data always contain ResultData in the title.
2. In the worksheet, click the desired tab to view.

Signing Measurement Results

On systems with the GxP Permissions module enabled, measurement results may be signed to prevent them from being deleted or overwritten. Signed results from protocols configured with data reduction and/or analysis options may be reevaluated; however, reevaluated results must be saved using a different name. Reevaluated results are not signed by default.

Results may be signed at any time by users who are assigned a role containing the Sign permission. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76 for more information about roles and permissions.

To sign measurement results:

1. In the Results Selection List, select the measurement results to sign.
2. From the tool bar, click **Sign the selected result**.

   OR

   From the menu bar select **Actions | Sign the selected result**.

   OR

   Right click on the desired results and select **Sign the selected result**.
3. The Sign the Selected Item dialog appears.
4. In Sign the Selected Item, add an electronic signature by following the instructions in Adding Electronic Signatures and Comments to Items on page 84.

Deleting Measurement Results

Measurement results may be deleted from the Results Selection List. When the GxP Permissions module is enabled on the system, results that have been signed may not be deleted. See Signing Measurement Results on page 238.

Note: When GxP Permissions is enabled, only users assigned a role containing the Delete permission may delete measurement results. See Configuring Roles for Multi-Mode Analysis Software User Accounts on page 76.

To delete measurement results:

1. In the Results Selection List, select the results to delete.
2. From the tool bar, click **Delete**.

   OR

   From the menu bar select **Actions > Delete the selected result**.

   OR

   Right-click on the desired results and select **Delete the selected result**.

Note: Multiple items may be selected for deletion by holding down the CTRL or SHIFT key while selecting each item desired.

3. The delete message dialog appears.
4. Click **Yes** to delete the selected results. The selected results are moved to the Trash list. To permanently remove the results from the database see Deleting and Restoring Items on page 48.

Printing Measurement Results

Measurement results may be printed from the Results Selection List or the Result Viewer. Printed reports include information about the protocol and all Report Options configured in the protocol.

Depending on how Print Settings are configured, Print and/or Print Preview may display before the measurement results print. See Configuring Print Settings on page 38 for information about enabling and disabling Print and Print Preview.

To print measurement results:

1. To print from the Results Selection List, select the results to print and click the print button on the tool bar.

   OR

   To print from the Result Viewer, click the print button on the tool bar.
2. If Print appears, configure printing options as desired and click **OK**.

   OR

   If the Print Preview dialog appears, use the tool bar controls to change the magnification, layout view, or pages displayed, if desired.
3. In the Print Preview dialog, click **Print** to print out the measurement results.
4. In the Print Preview dialog, click **Close** to close the window.

Note: Clicking **Close** without first clicking **Print** cancels the printout.

Data Reduction Techniques

Supported Data Reduction Techniques

The tables in this section describe the data reduction techniques supported by the software:

- Table A-1 describes the techniques available for scan measurements and measurement sequences configured in protocols.
- Table A-2 describes the techniques available for kinetic measurements.
- Table A-3 describes techniques available for fluorescence polarization measurements performed on a FilterMax 5 Multi-Mode Microplate Reader or a SpectraMax Paradigm Multi-Mode Detection Platform.

Table A-1 Scan Measurement Data Reduction Techniques

Data Reduction Technique	Description	Parameters
Delta	Difference between the first and last points measured in a well.	N/A
Mean	Determines the mean value per sample from the points measured.	N/A
Peak Value	Used to detect the highest measured value per sample.	Smoothing Points
Standard Deviation	Calculates the standard deviation for each well.	N/A
Coefficient of Variation	Calculates the coefficient of variation for each well.	N/A

Table A-2 Kinetic Data Reduction Techniques

Data Reduction Technique	Description	Parameters
Average Slope	Determines the average slope of the reaction curve by calculating the average of all linear regressions calculated over each group of Smoothing Points in the kinetic reading sequence. A decreasing slope shows a decline.	Smoothing Points
Delta	Difference between the first and last kinetic measurements in a protocol run.	N/A
Delta Max Slope	Difference between the first measurement and the center point of the maximum slope. **Note:** The center point of the maximum slope is calculated by determining the center point between the smoothing points of the regression line with the maximum slope.	Smoothing Points
Delta Time Absolute	Time elapsed from one preselected measurement value to another.	Lower Limit Upper Limit
Delta Time Max Slope	Time difference in seconds between the first measurement and the occurrence of the center point of the maximum slope. **Note:** The center point of the maximum slope is calculated by determining the center point between the smoothing points of the regression line with the maximum slope.	Smoothing Points
Delta Time Relative	Time elapsed in seconds from the first measurement to reaching a set increase/decrease amount from the first measurement value.	In-/Decrease
Max Declining Slope	Determines the maximum declining rate of the reaction curve by calculating a linear regression over each group of Smoothing Points in the kinetic reading sequence.	Smoothing Points
Max Inclining Slope	Determines the maximum inclining rate of the reaction curve by calculating a linear regression over each group of Smoothing Points in the kinetic reading sequence.	Smoothing Points
Max Slope	Maximum slope of the curve in measurement value/min. The line with the highest slope is calculated, along with maximum reaction speed. **Note:** The accuracy of this calculation depends on the number of measurement cycles selected.	Smoothing Points
Mean	Determines the mean value per sample within a sequence of measurements.	N/A
Time Peak	Used to detect the time elapsed until the peak value is reached.	Smoothing Points
Peak Value	Used to detect the highest value per sample within a sequence of measurements.	Smoothing Points

Table A-3 Fluorescence Polarization Data Reduction Techniques (FilterMax 5 Multi-Mode Microplate Reader and SpectraMax Paradigm Multi-Mode Detection Platform)

Data Reduction Technique	Description	Parameters
Polarization	Ratio of the difference between the parallel and the perpendicular polarization intensity[a] components divided by the sum of the two orthogonal fluorescence intensity components. Polarization is calculated according to the formula: $P(mP) = \frac{[I(parallel) - (G\text{-}factor) \times I(perpendicular)]}{I(parallel) + (G\text{-}factor) \times I(perpendicular)} \times 1000$	G-Factor[b] Threshold[c]
Total Intensity	Provides raw fluorescence intensity[a] measurements in the parallel and the perpendicular polarization planes with respect to the plane of linearly polarized excitation light. Total intensity is calculated according to the formula: $Total\ Intensity = I(parallel) + (G\text{-}factor) \times 2 \times I(perpendicular)$	G-Factor[b]
Anisotropy	The ratio of the difference between the parallel and perpendicular polarization intensity[a] components divided by the sum of the fluorescence intensity parallel to the excitation plane plus the fluorescence intensity perpendicular to the excitation plane multiplied by two. Anisotropy is calculated according to the formula: $A = \frac{I(parallel) - (G\text{-}factor) \times I(perpendicular)}{I(parallel) + (G\text{-}factor) \times 2 \times I(perpendicular)}$	G-Factor[b] Threshold[c]

a In polarization data reduction techniques, intensity defines sample raw data minus the average of blank replicate raw data.

b The G-factor factors out differences in detection efficiency between the polarization planes. The default G-factor is derived from fluorescein measurements performed on several instruments. If a more accurate G-factor has been determined for the connected instrument, it may be entered in the data reduction method configuration.

c Threshold defines the minimum number of counts; values measured below the threshold are noise.

PathCheck® Pathlength Measurement Technology

B

Overview

(SpectraMax® Paradigm® Multi-Mode Detection Platform and FilterMax™ F5 Multi-Mode Microplate Reader only)

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

$$A = \varepsilon bc$$

where A is the absorbance, ε is the molar absorbtivity of the sample, b is the pathlength and c is the concentration of the sample. In short, 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 perform extinction-based assays and also makes it confusing to compare results between microplate readers and spectrophotometers reading cuvettes.

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

In a microplate, pathlength is dependent on the liquid volume, so absorbance is proportional to both the concentration and the pathlength of the sample. Standard curves are often used to determine analyte concentrations in vertical-beam photometry of unknowns, yet errors can still arise from pipetting the samples and standards. The PathCheck® Pathlength Measurement Technology feature automatically determines the pathlength of aqueous samples in the microplate and normalizes the absorbance in each well to a pathlength of 1 cm. This patented approach to correcting the microwell absorbance values is accurate to within 3% of the values obtained directly in a 1 cm cuvette.

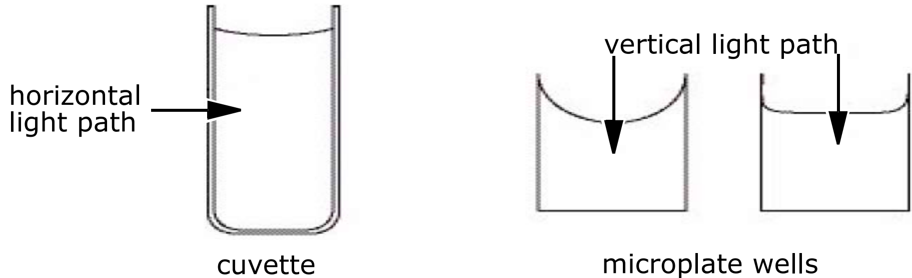


Figure B-1 Cuvette and Microwell light paths

Reference measurements made using factory-stored values derived from deionized water can be used to normalize the OD data for microplate wells. PathCheck Pathlength Measurement Technology is used to normalize the data acquired from absorbance endpoint microplate readings to a 1 cm pathlength, correcting the OD for each well to the value expected if the sample were read in a 1 cm cuvette.

The SpectraMax Paradigm Multi-Mode Detection Platform and FilterMax F5 Multi-Mode Microplate Reader offer a water-constant method of pathlength correction. For the FilterMax F5 Multi-Mode Microplate Reader, the water constant is stored in the instrument. For the SpectraMax Paradigm Multi-Mode Detection Platform, the water constant is stored in the Absorbance Detection Cartridge.

The actual pathlength, *d*, of a solvent is found from the following equation:

$$d(cm) = \frac{Sample(OD_{1000} - OD_{900})}{k}$$

When the water constant is used for pathlength correction, the value of *k* is obtained from the instrument or cartridge. This constant is saved in the instrument or cartridge in the factory and may differ slightly from instrument to instrument or cartridge to cartridge.

After the pathlength *d* is found, the following equation is used for the pathlength correction:

$$\frac{OD}{cm} = \frac{OD_{sample}}{d(cm)}$$

Using the PathCheck Pathlength Measurement Technology Water Constant

The PathCheck Pathlength Measurement Technology on the SpectraMax Paradigm Multi-Mode Detection Platform and FilterMax F5 Multi-Mode Microplate Reader uses a water constant reference. Be aware that if your sample matrix contains an organic solvent such as ethanol or methanol, the estimated pathlengths will be lower than the true values, and PathCheck Pathlength Measurement Technology normalized values will be higher than the corresponding 1 cm values.

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

To enable PathCheck for Absorbance readings, you must first set up a detection method with the PathCheck Pathlength Measurement Technology enabled. See Creating and Editing Detection Methods on page 87.

When you edit a protocol that uses PathCheck Pathlength Measurement Technology, and you have determined a plate background constant for your microplate, you can enter the value in the **Plate Background** field. See Use Plate Background Constant on page 247 and Creating Protocols on page 146.

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

Background Constant Subtraction and Blanking Considerations

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

Use Plate Background Constant

To determine Plate Background Constants:

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

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

PathCheck Pathlength Measurement Technology and Interfering Substances

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

Turbidity is the most common interference. If you can detect any turbidity in your sample, you should not use the PathCheck Pathlength Measurement Technology. Turbidity elevates the 900 nm measurement more than the 1000 nm measurement and causes an erroneously low estimate of pathlength.

Samples that are highly colored in the upper visible spectrum can have absorbance extending into the near infrared (NIR) and can interfere with the PathCheck Pathlength Measurement Technology. Examples include Lowry assays, molybdate-based assays, and samples containing hemoglobins or porphyrins. In general, if the sample is distinctly red or purple, you should check for interference before using the PathCheck Pathlength Measurement Technology. See Determining Color Interference on page 248.

Organic solvents could interfere with the PathCheck Pathlength Measurement Technology if they have absorbance in the region of the NIR water peak. Solvents such as ethanol and methanol do not absorb in the NIR region, so they do not interfere, except for causing a decrease in the water absorbance to the extent of their presence in the solution. However, if the solvent absorbs between 900 nm and 1000 nm, the interference would be similar to the interference of highly colored samples. If you are considering adding an organic solvent other than ethanol or methanol with the SpectraMax Paradigm Multi-Mode Detection Platform, you are advised to run a Spectrum scan between 900 nm and 1000 nm to determine if the solvent would interfere with the PathCheck Pathlength Measurement Technology. Spectrum scan is not available with the FilterMax Multi-Mode Microplate Readers.

Determining Color Interference

To determine possible color interference, do the following:

1. Measure the OD at 900 nm and 998 nm (both measured with air reference).
2. Subtract the 900 nm value from the 998 nm value.
3. Do the same for pure water.

If the delta OD for the sample differs significantly from the delta OD for water, then it is advisable not to use the PathCheck Pathlength Measurement Technology feature.

Mathematical Operators and Functions

Supported Mathematical Operators and Functions

The tables in this section describe the mathematical operators and functions that may be used in formulas:

- Table C-1 describes the mathematical and logical operators supported.
- Table C-2 describes the mathematical functions supported.

Note: In formulas where multiple parameters are configured, separate parameters with a semicolon; for example, max(S3;S5).

Table C-1 Mathematical and Logical Operators Table

Operator	Description
+	Sums two numbers.
=	Compares two expressions to determine if they are equal.
/	Divides two numbers and returns a numeric result.
<>	Compares two expressions to determine if they are not equal.
>	Compares two expressions to determine if one is greater than the other.
^{3}	Compares two expressions to determine if one is greater than or equal to the other.
<	Compares two expressions to determine if one is less than another.
£	Compares two expressions to determine if one is less than or equal to another.
*	Multiplies two numbers.
-	Performs subtraction of two expressions.
-	Unary negation operator indicating the negative value of a numeric expression
AND	Logical AND operator. Performs a logical conjunction on two expressions.
NOT	Logical NOT operator. Performs logical negation on an expression.
OR	Logical OR operator. Performs logical disjunction on two expressions.

Table C-2 Mathematical Functions

Function	Description
abs(number)	Returns the absolute value of a number.
acos(number)	Returns the arccosine of a number.
asin(number)	Returns the arcsine of a number.
atan(number)	Returns the arctangent of a number.
cos(number)	Returns the cosine of a number.
exp(number)	Returns e (the base of natural logarithms) raised to a power.
log(number)	Returns the natural logarithm of a number.
max(num1;num2)	Returns the greater of two supplied numeric expressions.
min(num1;num2)	Returns the lesser of two supplied numeric expressions.
pow(base, exponent)	Returns the value of a base expression taken to a specified power.
sin(number)	Returns the sine of a number.
sqrt(number)	Returns the square root of a number.
tan(number)	Returns the tangent of a number.