Agilent RapidFire Analyzer

Quick Start Guide

What is RapidFire Analyzer?

RapidFire Analyzer is a data analysis program that allows you to rapidly process, review, and report semi-quantitative RF/MS/MS data acquired by an Agilent RapidFire 200/300/360/365 High-throughput Mass Spectrometry System in Multiple Reaction Monitoring (MRM) mode.

RapidFire Analyzer is primarily designed to quickly evaluate large panels of results and identify compromised samples. RapidFire Analyzer allows you to simultaneously view a large array of chromatographic peaks, perform calculations and normalizations, and highlight flagged errors.

Where to Find More Information

Help

The online Help provides in-depth information about how to configure and use RapidFire Analyzer.

To access online Help, click **Help** on the tool bar.

Technical Support

Technical support in United States and Canada:
1-800-227-9770, and select option **3**

Worldwide technical support: visit **www.agilent.com**

Installation

Before you begin

Make sure that your computer meets these minimum requirements:

- Windows 7 64-bit or Windows 10 64-bit operating systems
- 2 GHz or faster 64-bit processor, multicore preferred
- 4 GB RAM
- 40 GB hard drive capacity with 10 GB free space
- Graphics hardware acceleration with 1280 x 1024 or higher screen resolution
- .Net Version: 4.0 or higher
- RapidFire version 4.3.0.17235 or RapidFire version 5.0.0.18130 for synchronized (or sequence-mode) 6400 Series LC/MS only.

To install RapidFire Analyzer from a disc

1 Insert the installation disc into the disc drive.

   If the installation program does not automatically start, from the installation disc, double-click **setup.exe**,

2 In the **Welcome to the RapidFire Analyzer Setup Wizard** dialog box, click **Next**.

3 In the **End-User License Agreement** dialog box:

   a Carefully read the license agreement.

   b Print the agreement and then click **Back**.

   c Select **I accept the terms in the License Agreement** to accept the license agreement, and then click **Next**.

4 In the **Installation Scope** dialog box, choose whether to install for just yourself or for all users on this computer, and then click **Next**.

   • **Install just for you (***username***)** installs RapidFire Analyzer in your personal folder. The program is available to only your user account.You do not need administrator privileges for your computer.

   • **Install for all users of this machine** installs RapidFire Analyzer all users on this computer. You must have local administrator privileges for this computer.

NOTE If you are not able to install RapidFire Analyzer, contact your network administrator for assistance. Make sure that you have the appropriate local administrator privileges required to install the application on this computer.

5 In the **Destination Folder** dialog displays, choose whether to use the default folder or to specify a different location.

   • To accept the default location, click **Next** to continue.

   • To select a different location, click **Change**. Select the new location, and then click **OK**. Click **Next** to continue.

6 In the **Ready to install RapidFire Analyzer** dialog box, click **Install** to start the software installation process.

7 When the **Completed the RapidFire Analyzer Setup Wizard** dialog appears, click **Finish**.

8 Activate RapidFire Analyzer. See **"To activate RapidFire Analyzer"** on page 5.

To download and install RapidFire Analyzer

1 Use the link that Agilent provided you to visit the software download location.

   Agilent provides the link location when the software is purchased.

2 Select **RapidFire Analyzer** and click **Download**.

   If the computer that is to run RapidFire Analyzer cannot access the Internet, then from a computer that can, save the installation files to an accessible medium such as a USB flash drive or a server on your company Intranet.

3 Extract the RapidFire Analyzer installation files to your computer.

4 Double-click **setup.exe**.

5 Continue the installation at **step 2** of **"To install RapidFire Analyzer from a disc"** on page 4.

6 Activate RapidFire Analyzer. See **"To activate RapidFire Analyzer"** on page 5.

To activate RapidFire Analyzer

The first time you start RapidFire Analyzer after installation, you will need to enter a valid activation key. The activation key is supplied at the time of purchase.

Start the RapidFire Analyzer Activation

1 Start **RapidFire Analyzer**. See **"To start RapidFire Analyzer"** on page 14.

2 In the **RapidFire Analyzer Activation** dialog box, click **Yes** to continue.

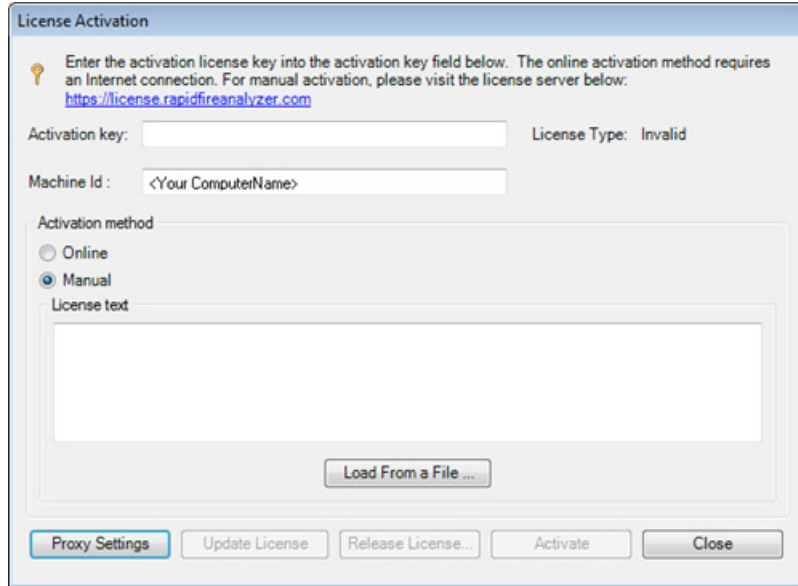   The **License Activation** dialog box appears (**Figure 1**).

Figure 1.   License Activation dialog box

3 Choose the activation method:

- Choose to activate online if the computer has access to the Internet.
- Choose to activate manually if the computer does not have acces to the Internet, or if you are not able to access the license server.

Online Activation

1 Type the valid activation key into the **Activation key** box.

The **License Type** displays **Invalid** because RapidFire Analyzer is currently not activated. The **Machine Id** identifies the name of **your machine**.

2 Click **Online Activation**.

3 Click **Activate**.

If the computer cannot access the license server, click **Proxy Settings** and check the settings. If you do not know the appropriate proxy settings, contact your network administrator for assistance. Enter the correct settings.

If the computer still cannot access the license server, manually activate the license key.

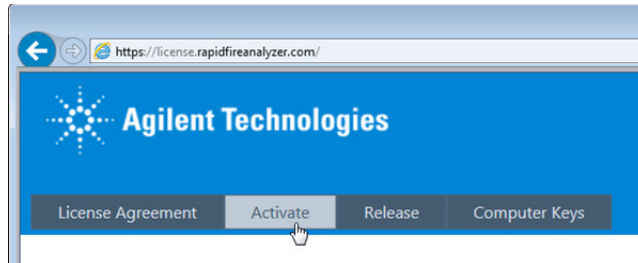**4** Click **OK** to close the **Key Activated** message.

The application closes and then restarts. RapidFire Analyzer is activated.

Manual Activation

1 Make a note of the computer name and the activation key.
2 On a computer with Internet access:

    a Go to **license.rapidfireanalyzer.com**.

    b Click **Activate**.

    c In the **License Activation** page, select the appropriate **RapidFire Analyzer** version.

    d Type in the valid **Activation Key**.

    e For **Machine Id**, type the computer name for the computer that is to run RapidFire Analyzer.

       If this license key is valid for multiple computers, type all computer names, one computer name per line. You cannot enter more computer names than the Activation Key is assigned.

    f Click **Activate**.

       The Activation Results page displays a list of computers that are activated, as well as those not activated.

    g Click **License Text**.
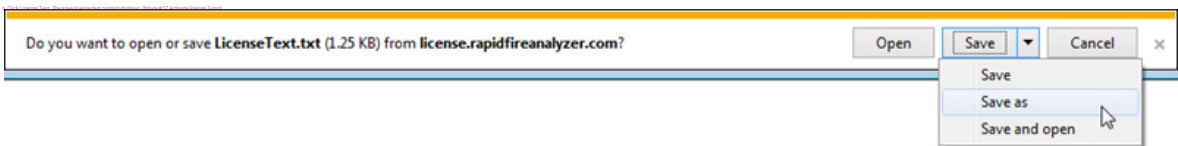
    h Save the **LicenseText.txt** file to a portable storage device, such as a USB flash drive.

3 On the computer that is to run RapidFire Analyzer:

    a In the **License Activation** dialog box, type the valid activation key into the **Activation key** box.

The **License Type** displays **Invalid** because RapidFire Analyzer is currently not activated. The **Machine Id** identifies the name of **your machine**.

b Type the **Machine Id** (computer name).

c For **Activation method**, click **Manual**.

d If needed, attach or insert the medium that contains **License Text.txt**.

e Click **Load From a File** and locate **LicenseText.txt** file on the portable storage device.

f Click **Open**.

The **License Text** displays in the **License text** box.

g Click **Activate**. The **Key Activated** message displays.

In the **License Activation** dialog box, the **License Type** changes from **Invalid** to **Permanent** or **Evaluation**. If you installed an evaluation copy, **Expiration** date displays.

h Click **OK** to close the **Key Activated** message.

The application closes and then restarts. RapidFire Analyzer is activated.

User Interface

RapidFire Analyzer Window

After data files are opened and injections are selected, various views are shown in the RapidFire Analyzer window (**Figure 2**).

Click areas in the RapidFire Analyzer window for overview information. See **"RapidFire Analyzer Menu"** on page 10 for a list of tasks that you can do in this window.
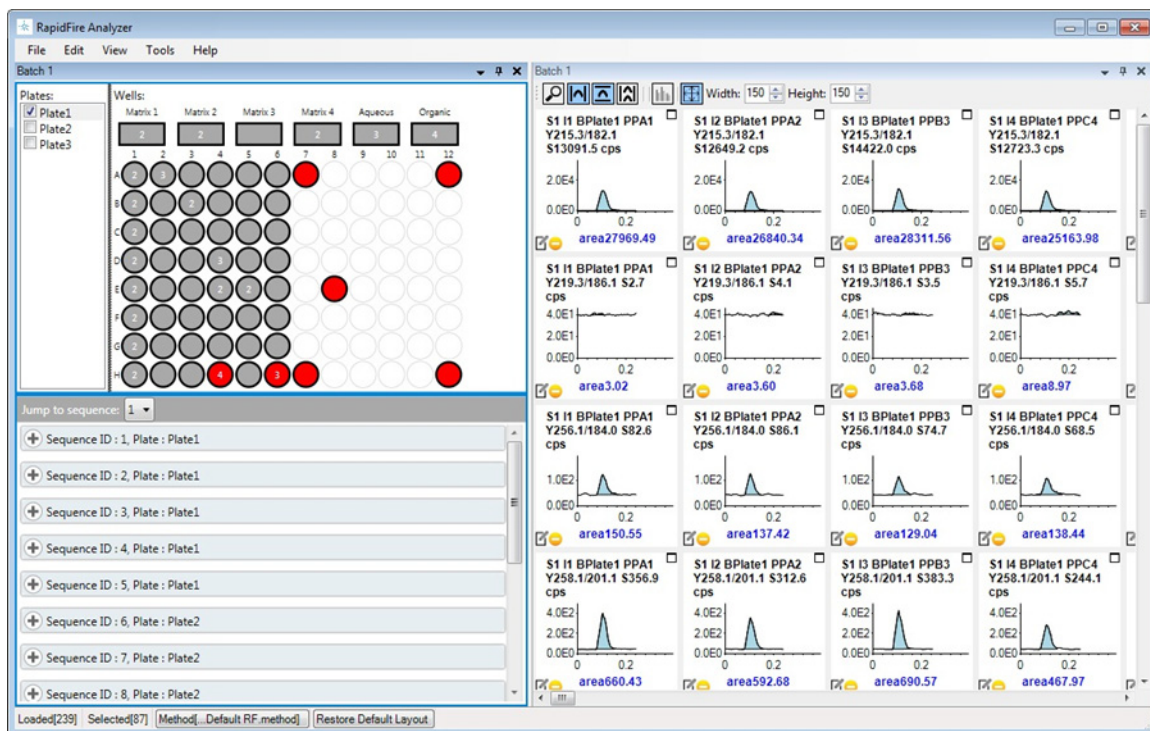

Figure 2.   RapidFire Analyzer with opened data files and selected injections

RapidFire Analyzer Menu

Table 1 describes the RapidFire Analyzer menus.

Table 1   RapidFire Analyzer menus

Menu	Submenu		Description
File	Open		Displays the **Open RapidFire Files** dialog box. Locate the **RFDatabase.xml** file you want to open and select it. Click **Open** to open the raw data file.
	Close	All Batches	Closes all batches currently open.
		Active Batch	Closes only the active open batch. If you have more than one batch open, the program closes only the active batch.
	Recent Data Files		Displays previously-opened **RFDatabase.xml** raw data files.
	Exit		Closes all open batches of samples and exits the program.
Edit	Calculations		Displays the **Calculations Editor**. Allows you to create or change calculations displayed in the injections list.
	Select All Injections		Selects all injections for all plates in the active batch.
	Deselect All Injections		Clears the selection of all injections in the active batch.
View	Logging	Console	Displays the **Logging Console**. The console displays RapidFire Analyzer event information and errors.
		File	Opens a copy of the error log file (**ASPROLog.txt**) in a text-readable file format using Windows Notepad.
		Settings	Displays the **Log System Settings** dialog box where you can configure the error settings.
	Containers		Displays open batches. If multiple batches are opened, a specific Batch can be hidden by clearing the check box. The batch can be viewed by re-selecting it.
	Group Views		Displays the **Chromatogram View** for the Active Batch. Clear the check box to suppress the Chromatogram views.
Tools	Preferences		Displays the **Workflow Preferences** dialog box. It allows you to customize how chromatograms are displayed and labeled in the Chromatogram view.
	Settings		Displays the **Application Settings** dialog. It allows you to set the default Method for the Application.

Table 1   RapidFire Analyzer menus (Continued)

Menu	Submenu	Description
Help	RapidFire Analyzer Help	Displays the **RapidFire Analyzer Online Help**.
	License Activation	Displays the **License Activation** dialog box.
	About RapidFire Analyzer	Displays the **RapidFire Analyzer** copyright information.

RapidFire Analyzer Views

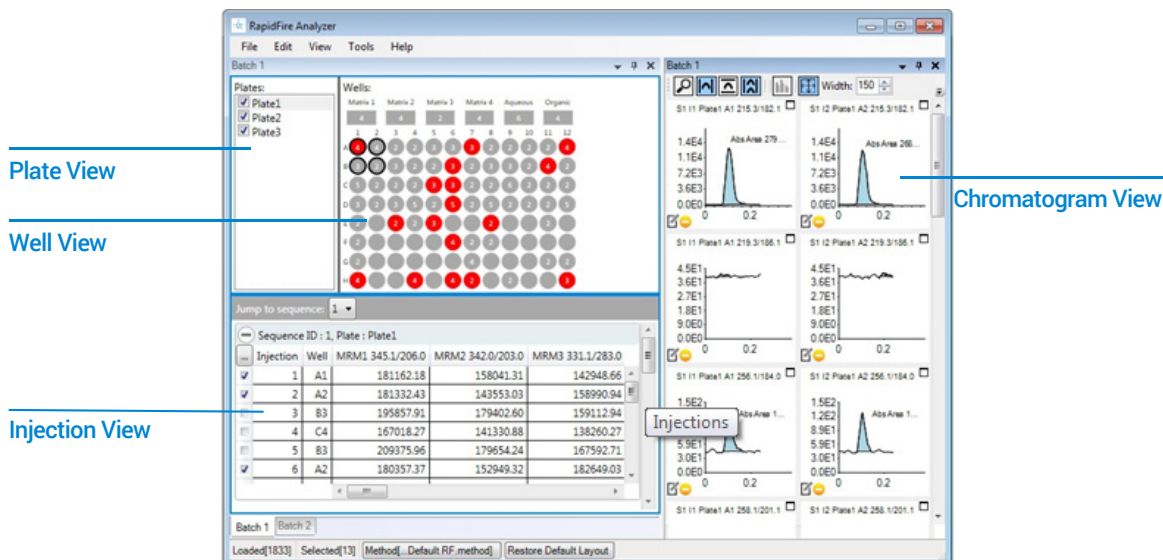RapidFire Analyzer provides several Views to work with your data.

Plate View	Displays the available plates for the open data file, also known as the **Active Batch**. The wells for the selected plates are displayed in the Well View.
Well View	Displays a summary of the selected plates. See **Table 2** for a description of the information that is displayed.
Injection View	Displays all injections, regardless of plate selection. The chromatograms for the selected injections are displayed in the Chromatogram View.
Chromatogram View	Displays the chromatograms for the injections that are selected in the Injection View.

Table 2   Description of well indicators

Image	Image Description	Indication
	White	No injections are present
	Grey background with no number	A single injection is present.
	Number on any background	The number of injections that are present.
	Grey background	No special condition.
	Red background	At least one injection contains an error or warning, for example, a sip-sensor error.
	Yellow background	At least one injection has been manipulated. Possible manipulations include deletion or manual integration.
	Black outline	All injections are selected and displayed in the Chromatogram View.
	Blue outline	At least one injection is selected and displayed in the Chromatogram View.

Basic Tasks

Before you do these tasks, make sure:

- The software is properly installed
- The software is activated with a valid activation key.

To start RapidFire Analyzer

- Double-click the **RapidFire Analyzer** icon on the desktop.
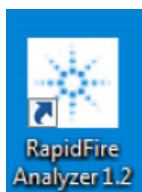
To open data files in RapidFire Analyzer

1 In RapidFire Analyzer, click **File** > **Open > RapidFire RD DB(.xml)**.
2 In the **Open File** dialog box, find the folder that contains the raw data.
3 Select **RFDatabase.xml**, and then click **Open**.

When you open an RFDatabase.xml data file, RapidFire Analyzer applies all settings for sample processing from the default method. The default method file included with RapidFire Analyzer is **RapidFire Default_Agilent.method**. For the majority of samples, these settings produce satisfactory results.

To open data files using a saved method different than the default

1 In RapidFire Analyzer, click **Tools > Settings**.

2 In the Application Settings dialog box, browse to and select the desired .method file and then click **Open**.

3 Click **OK**.

4 Open a data file as described above.

Please note that this process requires the alternative method file to have already been created and saved. See **"To view, change, save, and apply methods"** on page 20.

To open data files using a specific calculations method

Use the **Calculations** tab to define the calculation template for each sample series imported and processed.

1 Click **Tools** > **Preferences**.

2 In the **Workflow Preferences** dialog box, click the **Calculations** tab.
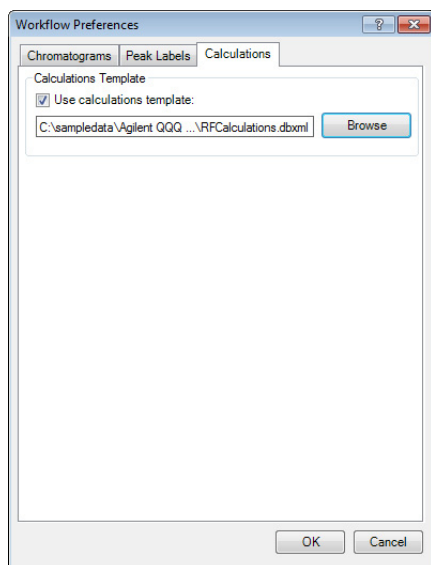
Figure 3.   Calculations tab

3 Select **Use calculations template**.

4 Click **Browse** and select the calculations template to use.

5 Click **OK** to save the changes.

6 Open a data file.

See **"To open data files in RapidFire Analyzer"** on page 14 or **"To open data files using a saved method different than the default"** on page 15.

Please note that this process requires the calculations template to already exist. See **"To view, change, and save calculations methods"** on page 25.

To work with chromatograms

- To expand a single chromatogram, click the **Expand** button in the top right of each chromatogram pane.

  You can also double-click anywhere on the chromatogram.
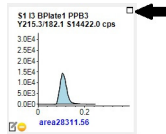
- To close an expanded chromatogram, click the **Close** button in the top right of the pane.

  You can also double-click anywhere on the expanded chromatogram.
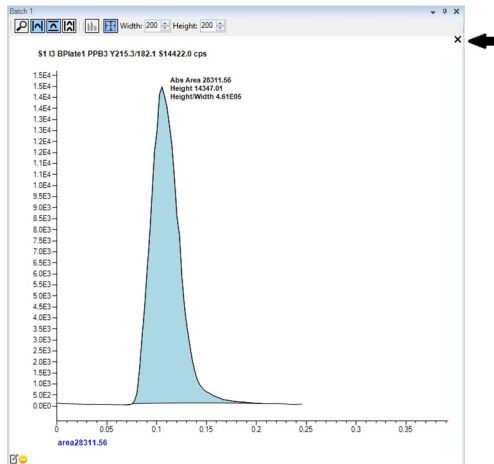
- To act on chromatograms, refer to **Table 3**.

See **Figure 4** on page 19 to see multiple chromatograms in Chromatogram View.

🔍	If you have selected zoom in the Y or X axis, or both, click this button to unzoom the chromatogram view.
∿	Click this button to zoom the chromatogram in the X direction. You can also click the ⌃ button to zoom in both X and Y direction.
⌃	Click this button to zoom the chromatogram in the Y direction. You can also click the ∿ button to zoom in both X and Y direction
∿⌃	Click this button to synchronize all views across all chromatograms in the Chromatogram view.
📊	Compares two or more rows. By default, the Compare button is not available. Press and hold the CTRL key and click at least two rows using the mouse. The Compare button is enabled after two rows are selected which is indicated by a blue line around the outside of each row of selected chromatograms. Click the Compare button. RapidFire Analyzer creates an updated chromatogram display containing only the selected rows. The Y axis scaling is made the same in all cases allowing you to see the relative differences in chromatographic peak intensities.
⊞	Maintains the height/width aspect ratio for the chromatograms in the Chromatogram view. If this button is selected, changing either the Height or Width will change both values at the same time and reflect the size changes. If this button is not selected, you can independently change the Height and Width.
Width: 150	Sets the Width for the chromatograms in the Chromatogram view. If the Maintain aspect ratio button is selected, changing either the Width or Height values will change both.
Height: 150	Sets the Height for the chromatograms in the Chromatogram view. If the Maintain aspect ratio button is selected, changing either the Width or Height values will change both.

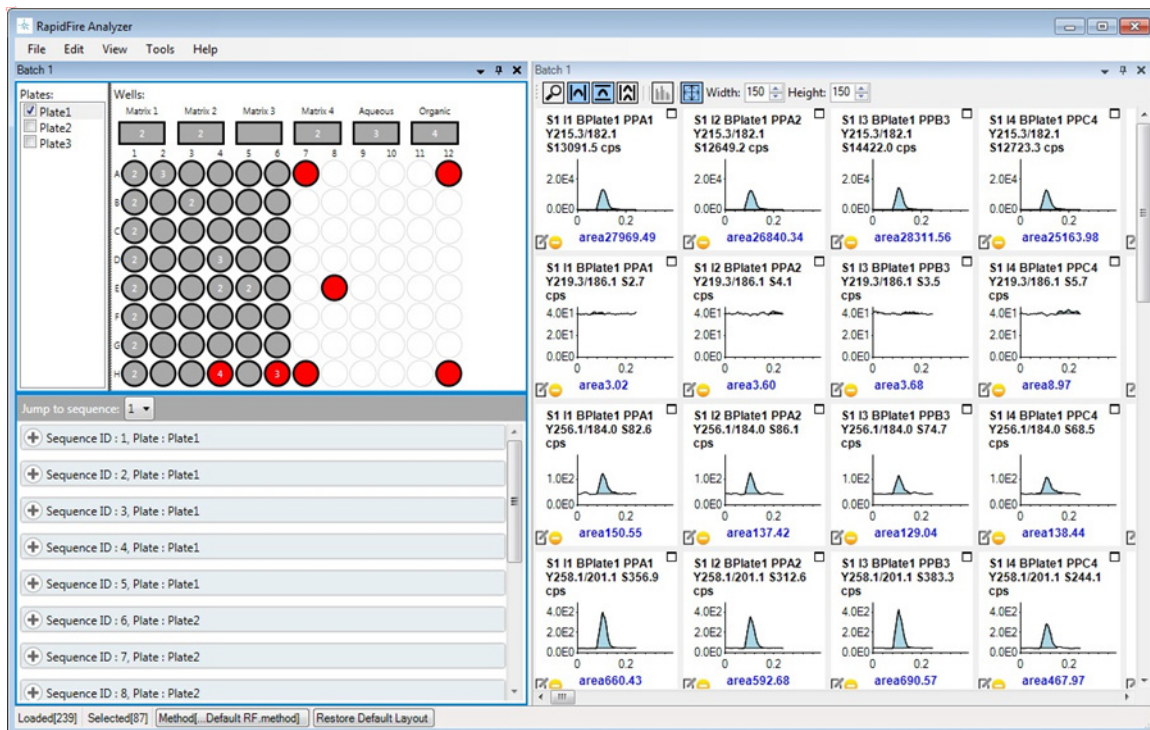**Table 3   Chromatogram Actions**

Figure 4.   Chromatogram View with multiple chromatograms

To view, change, save, and apply methods

To view and change methods

1 Open a RapidFire data file. See the "To open data file" topics near the start of **"Basic Tasks"** on page 14.

2 Open an RFDatabase.xml data file.

3 Select one or more injections.

4 Identify a chromatogram to edit.

5 In the chromatogram pane, click the **Method Editor** button.
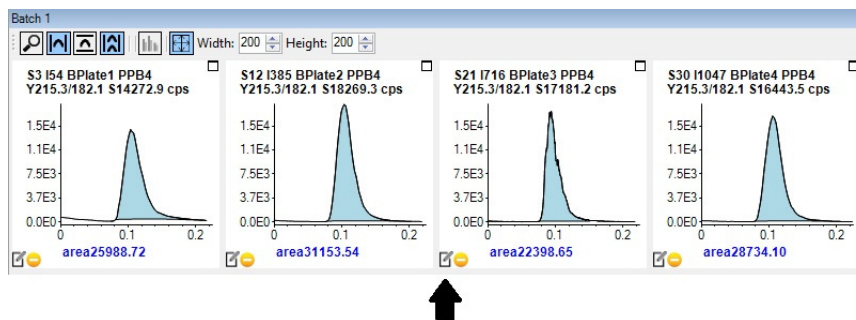
The Method Editor dialog displays. The **Select MRM to use for tuning** drop down panel displays the MRM of the pane in which you clicked the **Edit Method** button.

6 Adjust the settings as needed. The changes are reflected in the Chromatogram View as you make changes.

To save the method as a new method file for future use

1 Click **File > Save To File**.

2 In the File name box, type a name for the new method.

3 Click **Save**.

All method settings, including the adjusted settings, are saved in a new filename.method file. This method can be used to process future data. See **"To open data files using a saved method different than the default"** on page 15.

To apply the method settings to the open data file

1 Under the Chromatogram View, select either **Sequence**, **Plate**, or **Batch**.

2 Click **Process**. The changes are applied to the entire Sequence, Plate, or Batch.

To close the Method Editor without processing the data or saving any changes to the method, either click **Cancel** or click **File > Close**.

Note that the changes cannot be applied to a single injection. To change a single injection, you need to manually integrate it. See **"To remove peak integration and reintegrate"** on page 23 for more information.

Figure 5. Method Editor

To remove peak integration and reintegrate

If you need to reintegrate an individual chromatographic peak, you can remove the integration and then use Manual Integration to reintegrate it.
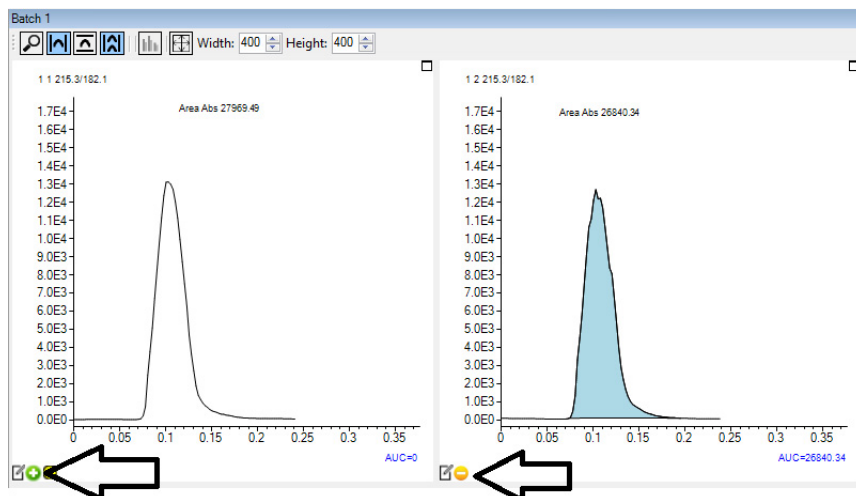

Figure 6.   Reprocessing (left) and removing (right) MRM integration

- To remove an integration, in the individual chromatogram pane, click the orange **Remove Peak Integration** button. See **Figure 6**, right-side pane.

- To reintegrate the injection without applying any changes to the default parameters, click the bright green **Reprocess the injection** button. See **Figure 6**, left-side pane.

- To manually integrate the injection, right-click the bottom of one side of the curve and drag to the other side of the curve.

   If the injection is manually integrated, the Manual Modification flag displays (**Figure 7**). The Well View shows a yellow condition indicator for that injection. However, if the well also experiences a more serious error (such as a sip-sensor error or a calculations or boundary error), the Well View shows a red condition indicator instead.

   The Manual Modification flag displays whenever the injection integration differs from the original integration, regards of whether the injection is removed or is manually integrated.

   If you manually integrate, and then reprocess an injection, the manual peak reintegrations are lost.
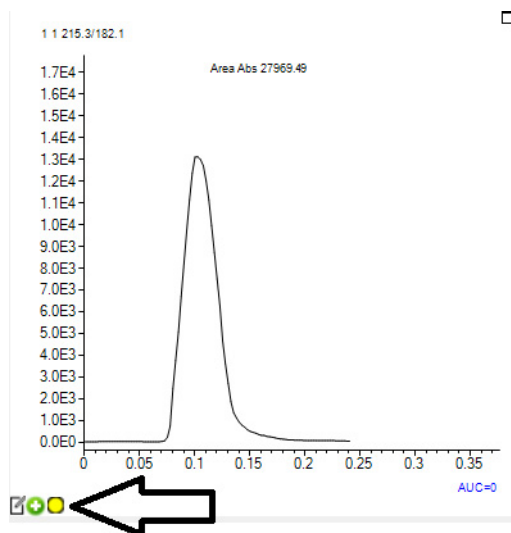
Basic Tasks
To remove peak integration and reintegrate

Figure 7. Manual Modification indicator

To view, change, and save calculations methods

The Calculations method can be used to calculate the ratio of the areas under the curve (AUC) of two different MRMs, for example, to normalize with an internal standard or to calculate percent conversion of a substrate to a product. Use the Calculations Editor to make changes to the calculations for the injections of an entire sequence.

Because the MRM transitions can vary from sequence to sequence, the calculation formula can be set only for one sequence at a time. These changes are stored in the **RFCalculations.dbxml** file saved in the same directory as the raw data, and are available the next time that an **RFDatabase.xml** data file is opened. Any customized calculations are written to this file and used the next time the raw data is processed. This file can be deleted if you want to omit all customized calculations.

1 Click **Edit** > **Calculations.**

2 In the **Calculations Editor** dialog, find the Sequence you want to change and click the **Expand** button (**Figure 8**).
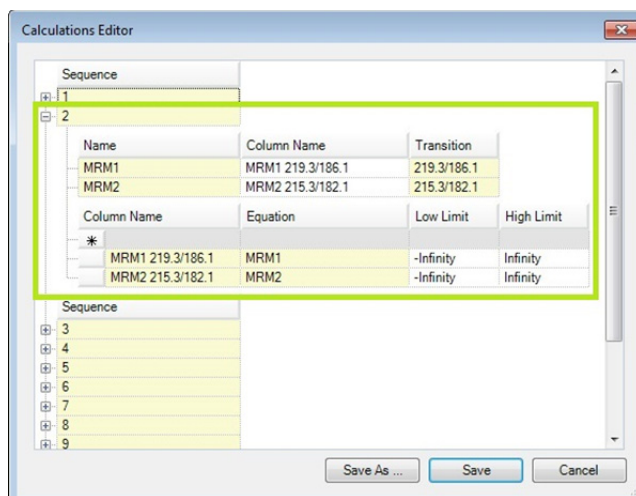

Figure 8.   Calculations Editor

Two tables are displayed for the sequence:

- The first table contains the experiment data. In **Figure 8**, the first table shows a row for each MRM. In Sequence 2, two MRMs were run on the instrument. The information in pale yellow cells cannot be changed. Only the **Column Name** can be changed in the first table.

- The second table allows you to change the **Low Limits** and the **High Limits**. If the corresponding MRM value falls below the low limit or above the high limit, the error is indicated by a color change on the displayed plate map, the sequence list, and the chromatogram entries.

You can add additional rows to the second table (**Figure 9**).

Sequence		
2		

Name	Column Name	Transition
MRM1	MRM1 219.3/186.1	219.3/186.1
MRM2	MRM2 215.3/182.1	215.3/182.1

Column Name	Equation	Low Limit	High Limit
*			
MRM1 219.3/186.1	MRM1	-Infinity	Infinity
MRM2 215.3/182.1	MRM2	-Infinity	Infinity

Figure 9.   Details of Sequence 2

3 To add a row, click the **cell** below **Column Name**. Type the **name** of the new row. Complete the **Equation**, **Low Limit**, and **High Limit** text boxes.

4 Save the changes:

- Click **Save** to save the changes to the default Calculations method.
- Click **Save As** to save the changes as a template in a specific folder.

This calculations method can be used to process future data. See **"To open data files using a specific calculations method"** on page 16.

Changes saved to the file are reflected in the sequence. New column headers appear at the beginning of the MRM data, just after the **Injection** and **Well** column (**Figure 10**).

Figure 10.   Sequence with new column from the Calculations method

To customize the viewable columns for a sequence

RapidFire Analyzer allows you to display all or some columns of information for an individual sequence.

1   In the **Jump to sequence** list, click a sequence **number**.

2   In the **Sequence View**, click the **Column Chooser** button to the left of the first column (**Figure 11**).

Figure 11.   Select sequence to view

3   In the Display Options dialog box, select or clear the check boxes for the sequence parameters to display. By default, all parameters are selected (**Figure 12**).

    The **Injection** and **Well** columns are not listed because they cannot be hidden.
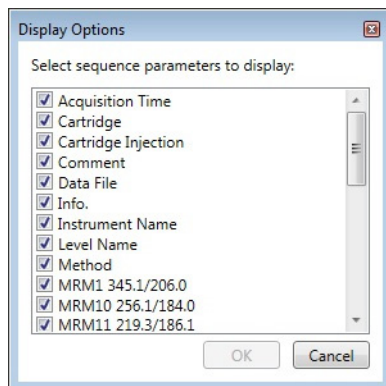
Figure 12. Display Options dialog box

4 Click **OK** to apply the changes and close the dialog box.

The Display Options changes are applied for as long as the Active Batch is open. If the Active Batch is closed, the Display Options changes are lost. The next time you open the Batch, all columns appear for all sequences in the Sequence View.

Customizing Workflow Preferences

The Workflow Preferences dialog box allows you to control the display of the chromatograms.

To customize chromatogram viewing preference

Use the **Chromatogram** tab to control the default display settings for all chromatograms.

1 Click **Tools > Preferences**.

2 In the **Workflow Preferences** dialog box, click the **Chromatograms** tab.
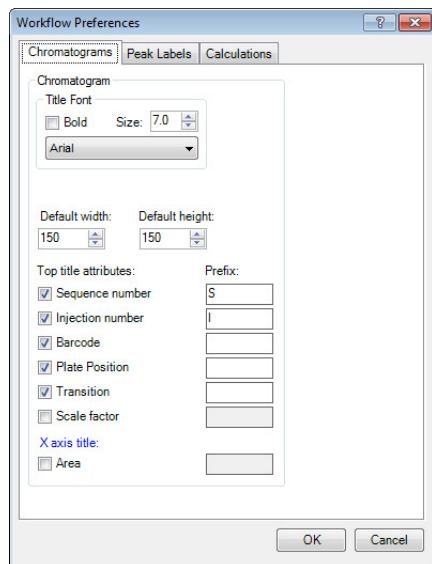

Figure 13. Chromatograms tab

3 Change the settings as needed.

4 Click **OK** to save the changes.

The changes are applied to the **Chromatogram View**.

To customize peak labeling preference

Use the **Peak Labels** tab to control the default display settings for all chromatograms when viewing multiple chromatograms at one time.

1 Click **Tools** > **Preferences**.

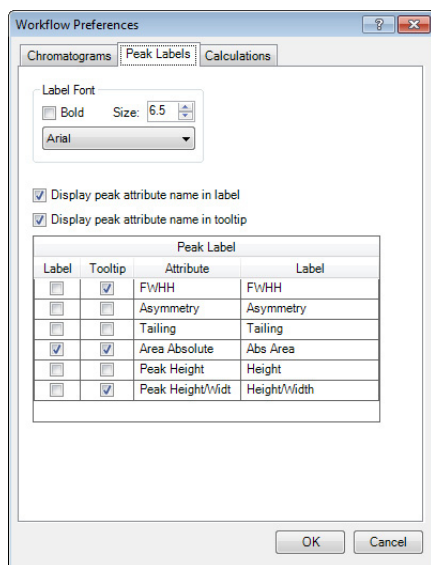**2** In the **Workflow Preferences** dialog box, click the **Peak Labels** tab.

Figure 14. Peak Labels tab

3 Change the settings as needed.

4 Click **OK** to save the changes.

The changes are applied to the **Chromatogram View**.

Generating Reports

You can generate two types of report:

- an .xls report that contains all injection information
- a set of .csv reports, one for each plate

To generate a report for all injections in a batch

1   In the **Injection View**, expand a sequence to display individual injections.
2   Right-click any column for any injection in the sequence.
3   Click **Export To Excel**.
4   In the **Save As** dialog, select a folder to save the report and type a new **File Name** for the report.
5   Click **Save**.

The saved .xls report contains all injection information, including all user-defined values added in the Calculations Editor. The file is saved as an Excel workbook file.

To generate a set reports for each plate in a batch

1   In the **Injection View**, expand a sequence to display individual injections.

2   Right-click any column for any injection in the sequence.

3   Click **Export Plate Peak Data**.

4   In the **Browse For Folder** dialog box, select an existing folder or create a new folder.

   To make a new folder, click **Make New Folder**, and type a **New Folder** name.

5   Click **Save** to save the report.

RapidFire Analyzer creates one .csv file in the selected folder for each plate. Each report contains the MRM results from the sips taken from the plate. "-1" indicates no aspiration was performed on the well.

The individual reports are saved in text format and are named **Plate1.csv**, **Plate2.csv** and so on. You can use any text editor or program that can read .csv files to view the files.

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In This Book

This guide contains information to run the RapidFire Analyzer 1.2 software.

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Agilent