Agilent G3835AA MassHunter Mass Profiler Professional Software

Overview and Data Import Quick Start Guide

What is Agilent Mass Profiler Professional?

Agilent Mass Profiler Professional (MPP) is a powerful visualization and statistical analysis solution designed for the chemometric analysis of mass spectral data. MPP is applicable to all Agilent mass spectrometric instruments and the markets served by those instruments. MPP is ideally suited for applications characterized by complex sample matrices such as metabolomics, proteomics, natural products, food, beverages, flavors, fragrances, and environmental analyses.

MPP's powerful analytical capabilities fully exploit the high information content of chromatographic/mass spectrometry (MS) data to quickly and easily discover differences between sample groups, plot changing patterns of compound abundances over time, and develop multivariate models for class prediction. An integrated ID Browser that mirrors MassHunter's Qualitative Analysis functionality allows identification using LC/MS Personal Compound Databases such as METLIN or Forensics / Tox, and GC/MS libraries (NIST and Fiehn library). MPP allows you to display and analyze large GC/MS or LC/MS data sets on your personal computer, regardless of whether the peaks are identified or are unknown.

MPP addresses the needs of discovery analysis validation, the combination of statistical analysis with identification, and then provides features that allow you to put the compounds identified into a biological context using integrated pathway analyses.

Agilent Technologies

Mass Profiler Professional Quick Start Guides

This **Overview and Data Import Quick Start Guide** is one of three quick start guides developed to help you use Mass Profiler Professional with your data. The Overview and Data Import guides you through the steps to import your data and is common to all other workflows. It should be used when the other workflows are not appropriate or when ad hoc analysis is to be performed by an experienced chemometrician. Because this workflow is common to all workflows, the critical steps of filtering, alignment, normalization, and baselining are described in this quick start guide.

This quick start guide covers:

1. Overview to Mass Profiler Professional
   a. User interface
   b. License management
   c. Opening a recent or existing project
   d. Setting up your project
2. Importing your data
3. Next steps

The next two quick start guides are:

Analysis: Significance Testing and Fold Change. This guide guides you through a pre-defined set of filters and models to help you properly assign your data conditions and obtain an initial expression of differential analysis.

Class Prediction: Build and Test Model. This guide guides you through the process to create a class prediction model based on your data. Class prediction is the process where you use samples with a known class membership to establish rules that are used to classify new samples. The class prediction model can be exported to a model file that is used to automatically process new sample data using Agilent's Automated Class Prediction workflow.

Where to find new information

Online help

Press F1 To get more information about a pane, window, or dialog box place the cursor on the part of the pane, window, or dialog box of interest and press the **F1** key.

Help menu Click **Help > Documentation Index** to access the release notes, quick start guides, and HTML and PDF versions of the Mass Profiler Professional manual.

Documents

MPP Manual Agilent Mass Profiler Professional (Agilent publication n/a, March 2009). You can find a PDF copy of the MPP manual on the installation disk, in the **Manuals** folder.

Unbiased Differential Analysis Workflow Agilent Mass Profiler Professional - Metabolomics Discovery Workflow (Agilent publication 5990-7067EN, June 2011)

Qualitative Analysis Familiarization Guide Agilent MassHunter Workstation Software Qualitative Analysis (Agilent publication G3336-90018, Revision A, September 2011)

Quantitative Analysis Familiarization Guide Agilent MassHunter Workstation Software Quantitative Analysis (Agilent publication G3335-90061, Fourth Edition, April 2010)

Training

Quick Start Guides Use the quick start guides for MPP to get to know the program.

Training Courses Visit www.chem.agilent.com to view a listing of training courses for MPP.

1. Overview to Mass Profiler Professional

How do I get started with Agilent Mass Profiler Professional?

After installation of MPP, you can get started immediately using the preloaded demonstration experiment. The demonstrated experiment will familiarize you with the software functionality and workflow. The project called “Malaria” contains an experiment called “Malaria LCMS ESI+ pH 7” that consists of eight samples — four replicate healthy (control) samples and four replicate infected blood samples. You are encouraged to explore this demonstration project along with your own data to get to know MPP.

Quick Start Guide for Data Import

This quick start guide is the first of several quick start guides designed to help you quickly develop a working knowledge of MPP.

The data import workflow guides you through the steps to import your data. The data import workflow is common to all other workflows. It should be used when the other workflows are not appropriate or when ad hoc analysis is to be performed by an experienced chemometrician. Because this workflow is common to all workflows, the critical steps of filtering, alignment, normalization, and baselining are described in this quick start guide.

Since the advanced analysis operations available in the Workflow Browser do not guide you through the initial steps of condition assignment and differential analysis, it is not recommended to skip the “Analysis: Significance Testing and Fold Change” workflow.

A. User Interface

Note: Help and detailed information regarding the various fields and statistical treatments are available at anytime by pressing the **F1** key on the keyboard or by referring to the MPP User Manual.

Main Functional Areas

The main functional areas of Mass Profiler Professional are shown in Figure 3 on page 6.

The main MPP window consists of four parts: (1) the Menu Bar, (2) the Toolbar, (3) the Display Pane, and (4) the Status Bar.

1. Menu Bar The menu bar shown in Figure 1 provides actions that are used for managing your projects, experiments, pathways, and display pane views.

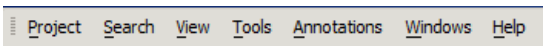


Figure 1 Menu bar

2. Toolbar The toolbar shown in Figure 2 is located below the menu bar and contains four sections of buttons for the following commonly used tasks:

Project section: New project, Open project, and Close project

Experiment section: New experiment and Add experiment

Entity List section: Create entity list from selection, Inspect selected entity, and Import entity list from file

Plots, Statistics, and Pathways section: Scatter plot, Profile plot, Histogram plot, Matrix plot, Venn diagram plot, Box-and-whisker plot, Heat map plot, Data spreadsheet, Summary statistics, Create new pathway, and Select data source for plots.

Figure 2 Toolbar

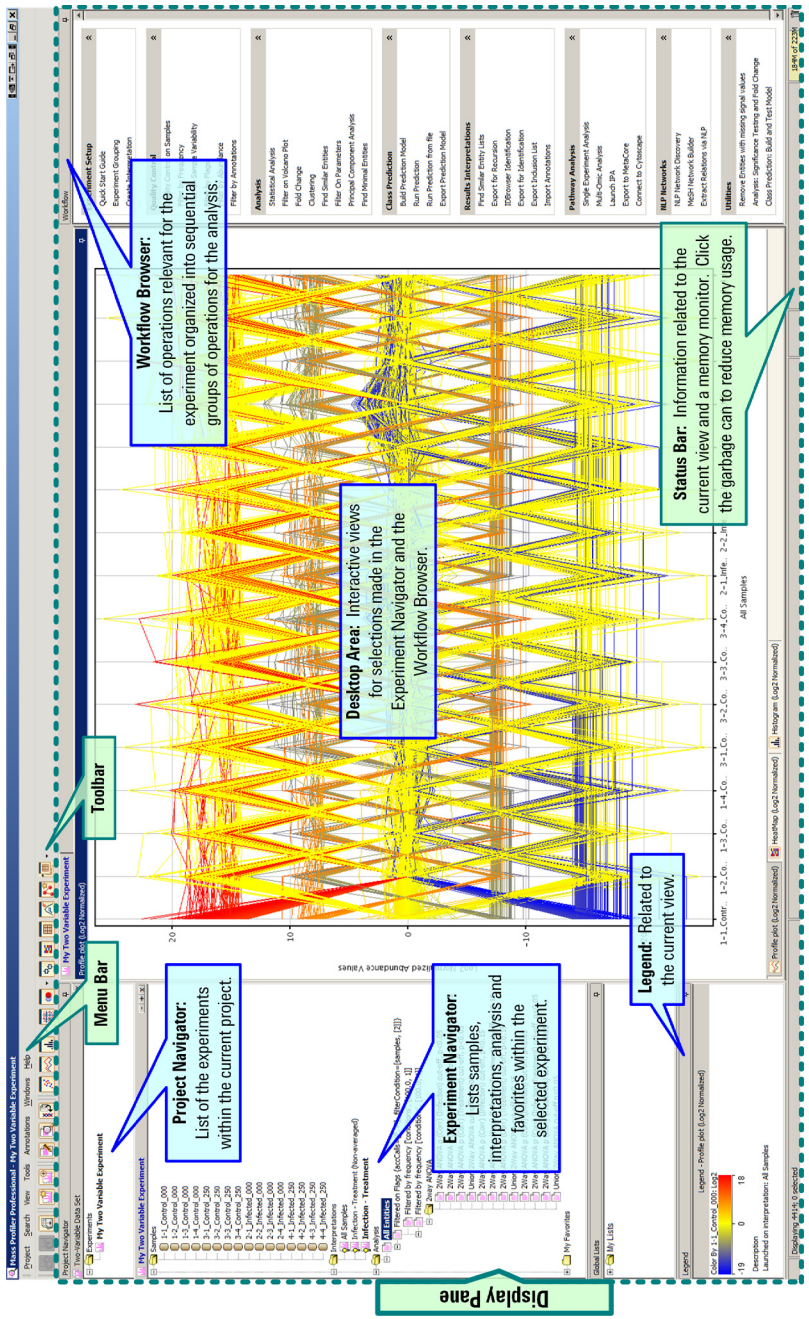


Figure 3 The main functional areas of the MPP software

3. Display Pane The display pane, see Figure 3 on page 6, is further divided into five areas — the Project Navigator, the Experiment Navigator, the Desktop Area, the Workflow Browser, and the Legend.

Project Navigator

Displays the current project and lists all the experiments with the project.

Experiment Navigator

Displays information related to Samples, Interpretation, Analysis, and My Favorites in folder related to the selected experiment in the Project Navigator. Each experiment within a project has a separate experiment navigator window.

Desktop

Display of one or more interactive views associated with the experiments. You can configure each view in the desktop area separately. Window views can be arranged using tile or cascade from the **Window** menu. The active view, noted using bold font in the Project Navigator and the Experiment Navigator, is represented by one of the views in the desktop area.
Right-clicking anywhere in the active view shows you a menu of options (Figure 4) to customize the view, copy the view to the system clipboard, and export the view as an image in one of the standard formats (jpg, png, jpeg, bmp, and tiff).

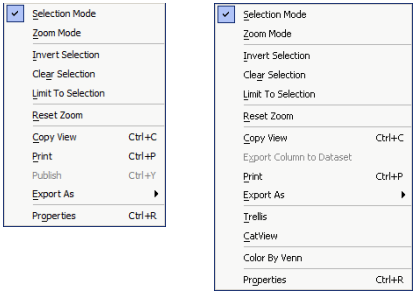


Figure 4 The menu of options available by right-clicking within the desktop view automatically adjusts to the type of view.

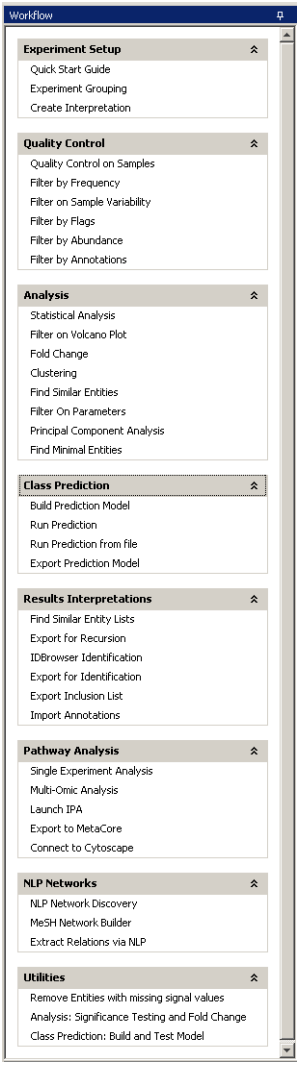


Figure 5 Workflow browser operations

Workflow Browser

The Workflow Browser (Figure 5) is organized into sequential groups of operations for the analysis of your data: Experiment Setup, Quality Control, Analysis, Class Prediction, Results Interpretations, Pathway Analysis (*optional*), NLP Networks (*optional*), and Utilities.

Legend

The Legend (Figure 6) shows the key (scale) to the use of color in the active desktop view. Right-clicking on the window title allows you to copy and export the legend as described in the Desktop area description.

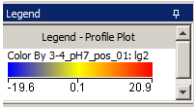


Figure 6 Legend

4. Status Bar The status bar (Figure 7) has three informative areas: the Status Area, the Ticker Area, and the Memory Monitor.

Status Area: Displays high-level information of the current view such as the number of rows and columns in table views and the number of entities or conditions selected in plot views.

Ticker Area: Displays the coordinates of the cursor in active plot views or the entity identification and value in table views.

Memory Monitor: Displays the total memory being used and the total memory allocated by MPP. You can click the garbage can icon at any time to reduce the memory usage.

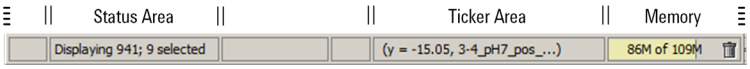


Figure 7 Status Bar

B. License Management

Activating a License

The first time you start MPP you need to select your license type and enter your **MassProfiler** and **GeneSpring** Order IDs. You can also select and enter any applicable proxy settings at this time.

License activation information is entered in the **Mass Profiler Professional License Activation** dialog box shown in Figure 8.

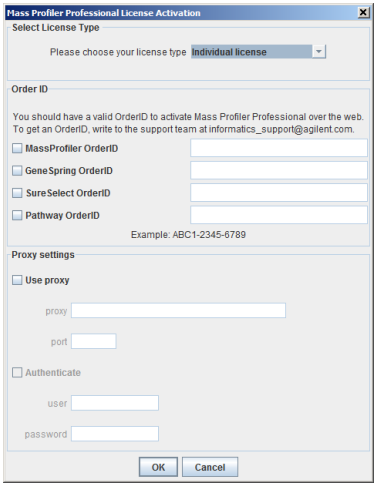


Figure 8 Mass Profiler Professional License Activation dialog box

Moving a License

MPP licensing is automatically enforced. You may move a license to another computer by surrendering it on the current computer and then activating it on the new computer. If you need to un-install MPP, it is always best to surrender the existing license before un-installing MPP.

To surrender your license:

1 Click **Help > License Manager** in the menu bar. See Figure 9 on page 10.

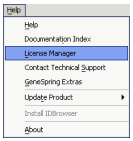


Figure 9 License Manager is available within the Help menu from the menu bar.

2 Click **Surrender** in the **License Manager** dialog box. You will then be presented with a dialog box where you can select MassProfiler as the product to surrender.

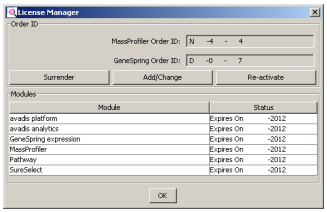


Figure 10 License Manager dialog box

3 Mark **MassProfiler** and **GeneSpring** when prompted to chose the products(orderIDs) to surrender.

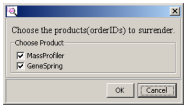


Figure 11 Product selection

4 Click **OK**. You will then have the opportunity in the next dialog box to confirm or decline surrendering your MPP license.

5 Click **Yes** in the **Confirm Surrender** dialog box.

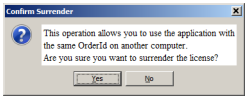


Figure 12 Confirm Surrender dialog box

Uninstalling ID Browser

If Mass Profiler Professional was previously installed on your computer, you must manually uninstall ID Browser before installing or re-installing Mass Profiler Professional.

If you are not sure whether ID Browser was previously installed, you can also determine whether ID Browser is currently installed by following the steps below.

1. From your Windows desktop click **Start > Control Panel**
2. Click **Programs and Features** (Programs and Features).
3. Find **Agilent MassHunter ID Browser** under the program **Name** column. If Agilent MassHunter ID Browser is not displayed in the list of programs you may stop, close the Programs and Features window, and install Mass Profiler Professional.
4. Click **Agilent MassHunter ID Browser** from the list of programs in the Programs and Features window as shown in Figure 13.

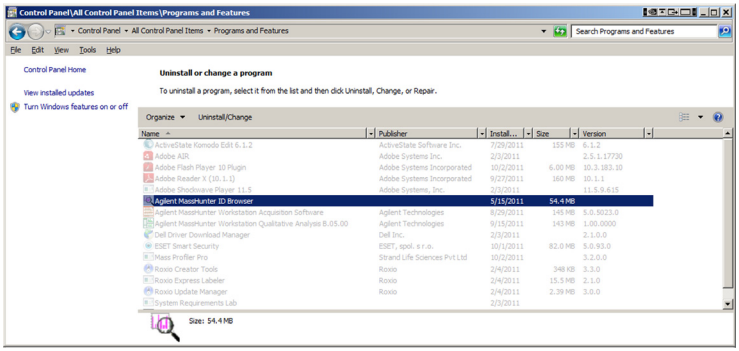


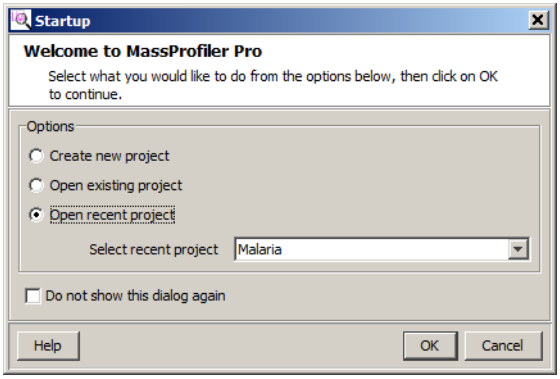
Figure 13 Select and delete Agilent MassHunter ID Browser from within Programs and Features on a PC operating with Windows 7.

5. Right-click the **Agilent MassHunter ID Browser** program and immediately select **Uninstall/Change**. ID Browser will be uninstalled from your computer.
6. Close the Programs and Features window.

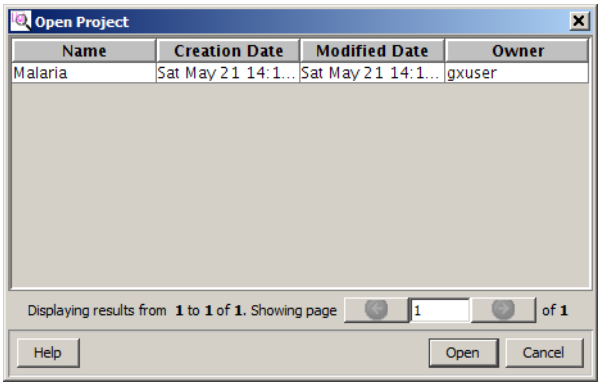
C. Opening a recent or existing project - Malaria Demo project

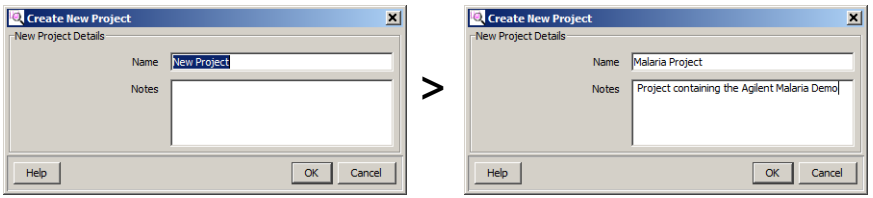
The feature files you need are located in the **samples** directory of the main installation folder **C:\Program Files\Agilent\Mass ProfilerPro\samples\Malaria Demo**.

Follow the steps below to **(1)** open the Malaria Demo project or to **(2)** import the Malaria Demo experiment into a new project.

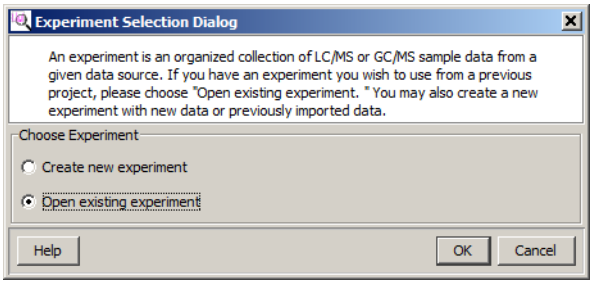
Steps	Detailed Instructions	Comments
1 Start Mass Profiler Professional.	Click the **MassProfilerPro icon** on your desktop, or click **Start > Programs > Agilent > MassProfilerPro > MassProfilerPro**.	• This will open the **Startup** dialog box.
Open a recent project: **2** Open the recent project named Malaria in the **Startup** dialog box. 	**a** Click the **Open recent project** button. **b** Select **Malaria** from the Select recent project list. **c** Click the **OK** button.	• **Open recent project** opens the project and the experiment that was stored in the project. • A project is a container for a collection of experiments. A project can have multiple experiments on different sample types and organisms.

Steps	Detailed Instructions	Comments
Open an existing project: **3** Open an existing project named Malaria in the **Startup** dialog box.	**a** Click the **Open existing project** button in the **Startup** dialog box in step 2 on page 12. **b** Click the **OK** button. **c** Select the **Malaria** project row from the **Open Project** dialog box. **d** Click the **Open** button.	• **Open existing project** allows you to open a project that is no longer displayed in the recent project list. • Opens the project and the experiment that was stored in the project. • When you have a large number of prior projects you may have to use the page navigation buttons in the **Open Project** dialog box. • A selected project will be highlighted by a color based on your PC's system settings.
Import into a new project: **4** **Alternate way to Open:** Create a new project in the **Startup** dialog box and import the Malaria project experiment. See "D. Setting up your project" on page 15 for creating a new project and a new experiment.	**a** Click the **Create new project** button in the **Startup** dialog box in step 2 on page 12. **b** Click the **OK** button. **c** Enter the new project details, **Name** and **Notes**, in the **Create New Project** dialog box. **d** Click the **OK** button.	• **Create new project** allows you to import an existing project and experiments into the new project. • Creating a new project allows you to enter new project detail information that will reflect your new data analyses.

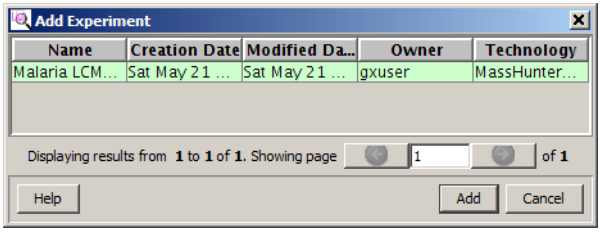




Steps	Detailed Instructions	Comments
	e Click the **Open existing experiment** button in the **Experiment Selection Dialog** dialog box.	



	f Select the **Malaria** experiment in the **Add Experiment** dialog box	• A selected experiment will be shown highlighted using a background color.



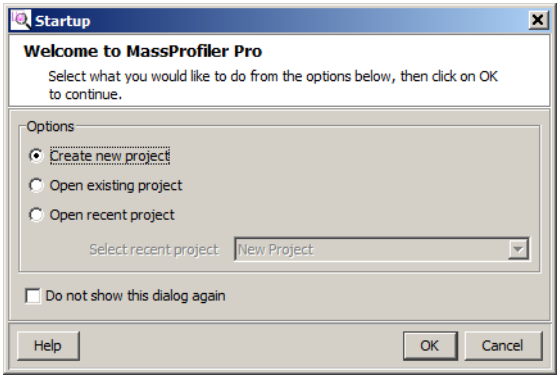
	g Click the **Add** button.	• The Malaria project is now imported and appears as if it were opened using Open recent project.

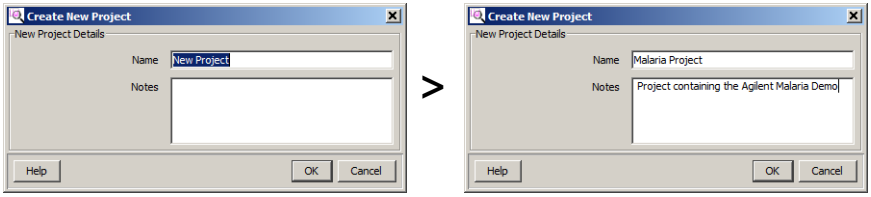
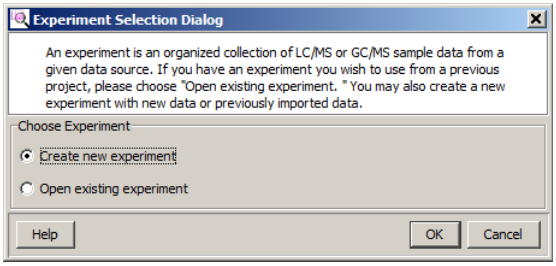
D. Setting up your project

You will be guided through four steps to create a new project and experiment to receive imported data:

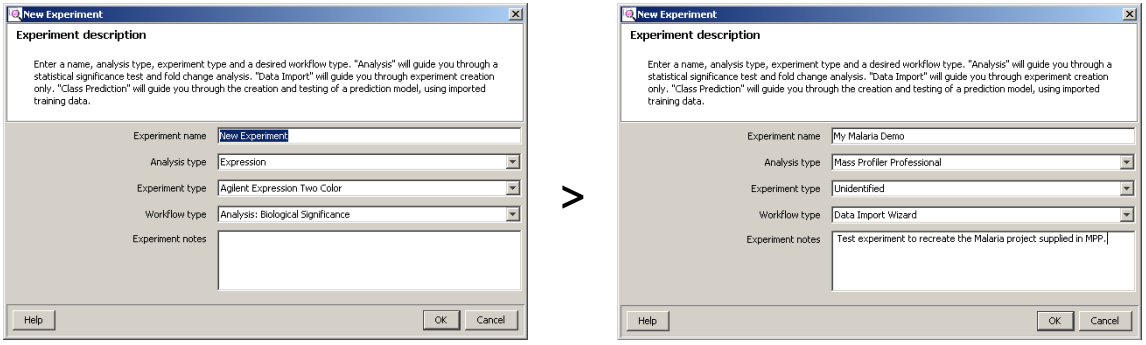
1. **Startup:** Select creation of a new project.
2. **Create New Project:** Type descriptive information about the project.
3. **Experiment Selection:** Select create a new experiment as part of the project.
4. **New Experiment:** Type and select custom information to store with the experiment.

Follow the steps below to setup your new project. The Agilent Malaria demo data set is used as an example in each step. You may substitute the demo information with information for your data.

Steps	Detailed Instructions	Comments
1 Start Mass Profiler Professional.	Click the **MassProfilerPro icon** on your desktop, or click **Start > Programs > Agilent > MassProfilerPro > MassProfilerPro**.	• This will open the Startup dialog box.
2 Create a new project in the **Startup** dialog box. 	**a** Click the **Create new project** button. **b** Click the **OK** button.	• **Create new project** allows you to import an existing project and experiments into the new project. • After closing an open project, you may create a new project from the (1) Menu bar by clicking **Project > New Project,** or (2) Toolbar by clicking the **New project** button. You will then be prompted to enter your project information in the next step. • A project is a container for a collection of experiments. A project can have multiple experiments on different sample types and organisms.

Steps	Detailed Instructions	Comments
3 Type descriptive information in the **New Project Details** in the **Create New Project** dialog box.	**a** Type `Malaria Project` in **Name**. **b** Type `Project containing the Agilent Malaria demo` in **Notes**. **c** Click the **OK** button.	• The project name and notes may be viewed and edited at any time using the **Project Inspector** dialog box by clicking **Project>Inspect Project** from the menu bar.
		
4 Select the option to create a new experiment in the **Choose Experiment** in the **Experiment Section Dialog** dialog box.	**a** Click the **Create new experiment** button. **b** Click the **OK** button.	• You may create or add a new experiment from the Menu bar by clicking **Project > New Experiment,** or Toolbar by clicking the **New experiment** button, or the **Add experiment** button. You will then be prompted to enter your experiment information in the next step. • If you click the **Open existing experiment** button, you are prompted for the experiment to add to the project as described in "Import into a new project," step 4 under "C. Opening a recent or existing project - Malaria Demo project" on page 12.
		

Steps	Detailed Instructions	Comments
5 Type and select information that guides the experiment creation in the **New Experiment** dialog box.	**a** Type the descriptive name `Malaria Demo` for the experiment in **Experiment name**. This name may be different from the project name previously entered. **b** Select **Mass Profiler Professional** for **Analysis type**. **c** Select **Unidentified** for the **Experiment type**. **d** Select **Data Import Wizard** for **Workflow type**. *Analysis: Significance Testing and Fold Change* is covered in another Quick Start Guide. **e** Type `Agilent demonstration data` in **Experiment notes**. **f** Click the **OK** button.	• The selection of experiment type determines how Mass Profiler Professional manages the data. • **Unidentified** is the proper experiment type selection when the compounds have only been identified by their molecular features of neutral mass and retention time. • **Combined (Identified + Unidentified)** is the proper experiment type when you are unsure if the data has been **Identified** in full or in part, or when MassHunter Qualitative Analysis has been previously used to identify some of the compound features.



- Your new project is now set up.
- You will be ***immediately*** guided through importing your data as described in "2. Importing your data" on page 18.

2. Importing your data

Import data into a new project and experiment

The Data Import Wizard will guide you through the necessary parameters and values that will prepare the data for analysis by ad hoc interactive processing or subsequent processing by the other workflow types.

Up to eleven steps are involved in the experiment creation. The steps you will use with your experiment depends on your experiment description and data source. Importing data involves only the steps presented below:

Step 1. Select Data Source: This allows you to select the data source that will be used for the experiment.

Step 2. Select Data to Import: Select the feature sample files.

Step 5. Sample Reordering: You may deselect individual samples and reorder the selection to group the samples, such as with respect to the independent variables.

Step 6. Experiment Grouping: This allows you to define the sample grouping with respect to the independent variables including the replicate structure of your experiment.

Step 7. Filtering: Filters the molecular features by abundance, mass range, number of ions per feature, and by charge state.

Step 8. Alignment: This allows you to align the features across the samples based on retention time and mass based on tolerances. This step is omitted when the experiment type is “identified” because identified compounds are treated as aligned by identification

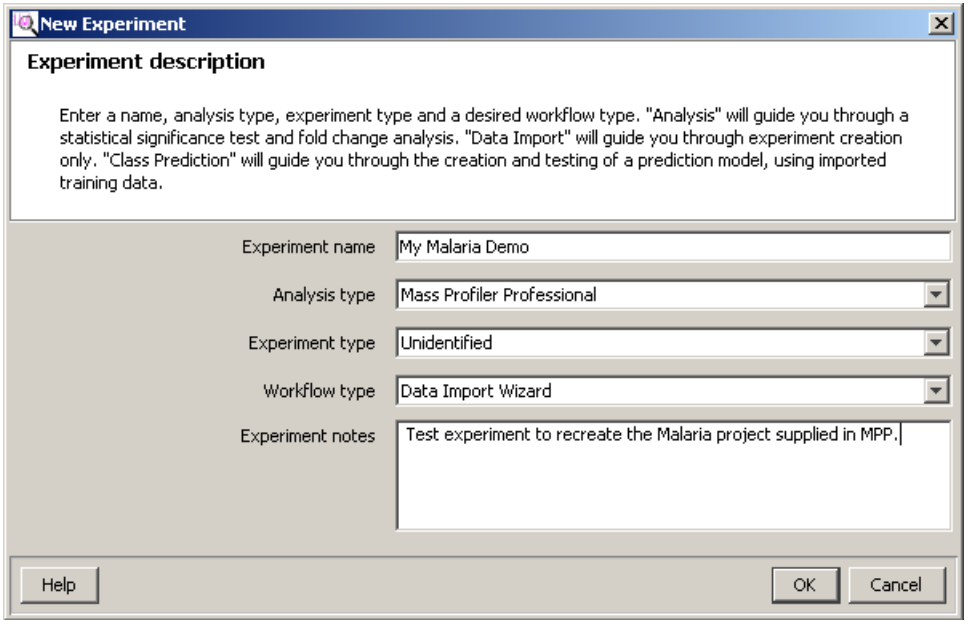
Step 9. Sample Summary: Displays mass versus retention time plot, spreadsheet, and compound frequency for the distribution of aligned and unaligned entities in the samples. Compound Frequency charts provide a quick view into the effectiveness of the alignment of unidentified experiment types. The use of the back and next buttons in the wizard allow different alignment and filtering options to be selected and the results to be reviewed.

Step 10. Normalization Criteria: Scales the sample features to a value calculated by the specified algorithm or an external scalar.

Step 11. Baselining Options: Compares each sample to a representative value calculated across all of the samples or the control samples.

Follow the steps below to import your data. The Agilent Malaria demo data set is used as an example in each step.

Steps	Detailed Instructions	Comments
1 Create a new project.	• If you need to create a new project for your data refer to section "1. Overview to Mass Profiler Professional - D. Setting up your project."	• A project is a container for a collection of experiments. A project can have multiple experiments on different sample types and organisms.
2 Create a new experiment.	**a** Click the **Create new experiment** button or **Project > New Experiment**. **b** Click the **OK** button.	
3 Type and select information that guides the experiment creation in the **New Experiment** dialog.	**a** Type the descriptive name of your data, such as `My Malaria Demo` for the demo data in **Experiment name**. **b** Select **Mass Profiler Professional** for **Analysis Type**.	• Table 1 and Table 2 on page 20 show selection and entry options available for the **New Experiment** dialog box



Steps	Detailed Instructions	Comments

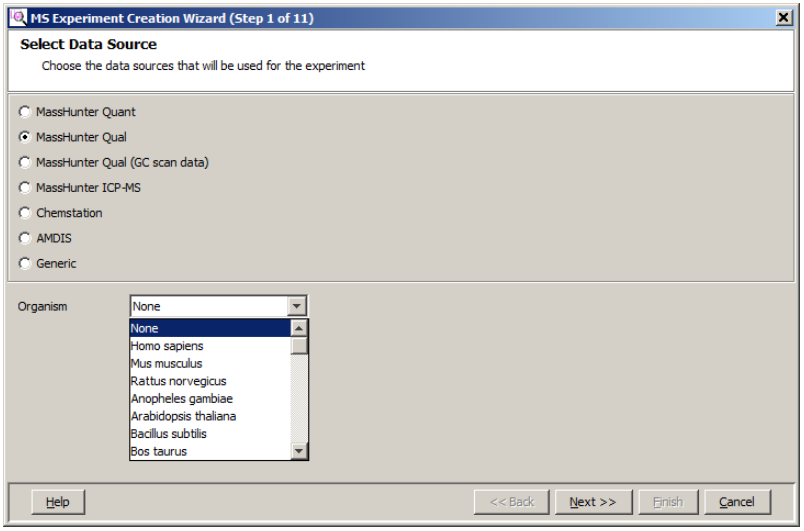
Table 1 Table of selections and entries for the New Experiment dialog box

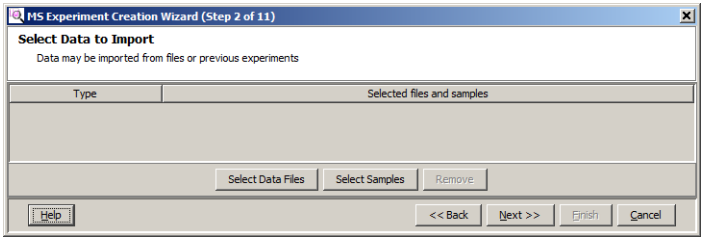
Dialog Box Field	Your Choices	Comments
Experiment name	<none>	Edit field to describe this experiment
Analysis type	Mass Profiler Professional <other choices depending on Order IDs>	"Mass Profiler Professional" must be selected
Experiment type	Combined (Identified and Unidentified) Identified Unidentified	<see next table>
Workflow type	Analysis: Significance Testing and Fold Change Class Prediction: Build and Test Model Data Import Wizard	
Experiment notes		Edit field to enter other experimental notes

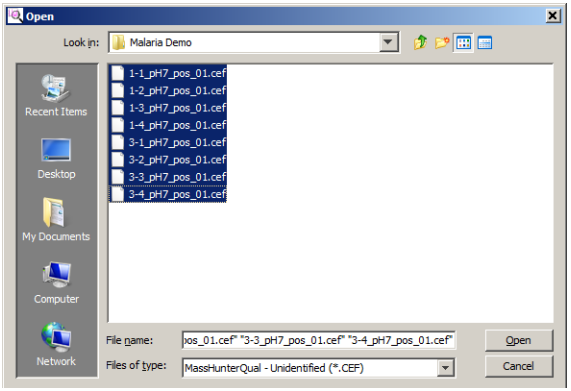
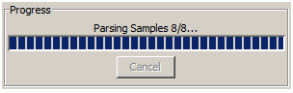
Table 2 Table of data sources and file extensions based on Experiment Type

Experiment Type	Data Source	File Types	Comments
Identified	MH Quant		Compounds identified by MassHunter Quantitative Analysis
	Chemstation	*.FIN	Compounds identified by Chemstation Quantification or Screener processes
	MH Qual	*.CEF	Find by Formula
	MH Qual (GC Scan)	*.CEF	Identify by Unit Mass Library
	ICP-MS	*.CSV	Identified by ICP-MS software
	AMDIS	*.FIN	Compound identified by an AMDIS target library
	Generic	*.XLS *.XLSX *.CSV *.TXT	Entries identified by Compound (column C), Formula (column D), CASID (column E)
Unidentified	MH Qual	*.CEF	Find By Molecular Feature Extractor (MFE)
	MH Qual (GC Scan)	*.CEF	Find by Chromatographic Deconvolution
	ICP-MS	*.CSV	Identified by ICP-MS software
	AMDIS	*.ELU	Components identified by AMDIS that are not identified by an AMDIS target library
	Generic	*.XLS *.XLSX *.CSV *.TXT	Entries NOT identified by Compound (column C), Formula (column D), CASID (column E)
Combined	MH Qual	*.CEF	Find By Molecular Feature Extractor (MFE) and Find By Formula
	MH Qual (GC Scan)	*.CEF	Find by Chromatographic Deconvolution and Library Search
	ICP-MS	*.CSV	Identified by ICP-MS software
	AMDIS	*.FIN *.ELU	Targets and components discovered by AMDIS
	Generic	*.XLS *.XLSX *.CSV *.TXT	Combination of entries identified by and not identified by Compound (column C), Formula (column D), CASID (column E)

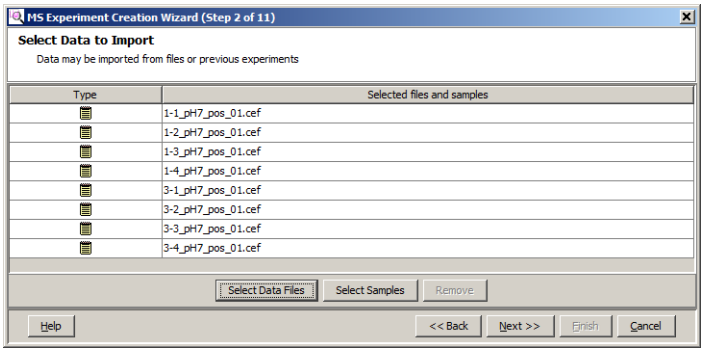
Steps	Detailed Instructions	Comments
	c Select your **Experiment type**, such as **Unidentified** for the demo data. **d** Select **Data Import Wizard** for **Workflow type**. • **NOTE:** The same import steps apply if you select **Analysis: Significance Testing and Fold Change.** **e** Type information relevant to your experiment in **Experiment notes**, such as `Test experiment to recreate the Malaria project supplied in MPP data.` **f** Click the **OK** button.	• **Unidentified** is the proper selection when the compounds have only been identified by their molecular features of neutral mass and retention time. • **Combined (Identified + Unidentified)** is the proper selection when you are unsure if the data is identified in full or in part or when MassHunter Qualitative Analysis has been used previously to identify some of the compound features.
4 Select the data source in the **MS Experiment Creation Wizard (Step 1 of 11)**.	**a** Click the description that matches the source of your data. • If you are using the sample Malaria data set click **MassHunter Qual**. • If you are using your own data set, click the source of the sample files. **b** Select the organism represented by your data. **c** Click the **Next >>** button.	• The available data sources will depend on your selection of **Experiment type** in the previous step shown in Table 2. • Selection of an **Organism** is important if you plan to use pathways. • To control your progress through the wizard dialog boxes: • Click the **Next>>** Next >> button to go to the next step. • Click the **<<Back** << Back button to return to prior steps and make modifications to your settings and previous entries. • Click the **Cancel** Cancel button to end the MS Experiment Creation without saving. You may then restart creating a new project and experiment.

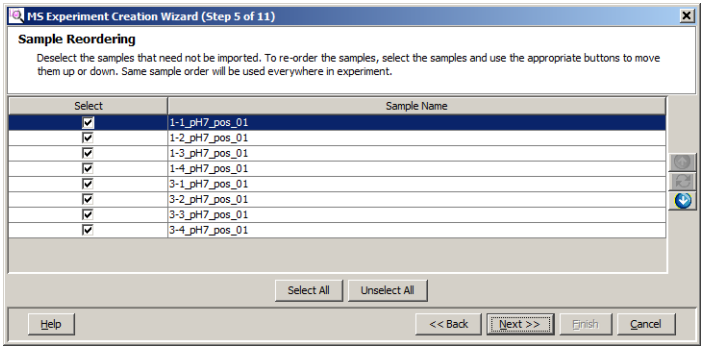


Steps	Detailed Instructions	Comments
5 Select the sample data to import in the **MS Experiment Creation Wizard (Step 2 of 11)**.	**a** Click the **Select Data Files** button.	• The file type you need to select will depend on the data source you selected in the **MS Experiment Creation Wizard (Step 1 of 11).** • See Table 2 on page 20 for a comprehensive list of data sources you may select from based on your experiment type.
	**b** The **Open** dialog box will most likely already point to the **samples** directory in the main Mass Profiler Professional installation directory, but if not, browse to **C:\Program Files\Agilent\MassProfilerPro\samples** and then select the **Malaria Demo** folder. **c** Browse for the proper data file types based on your data source selection.	

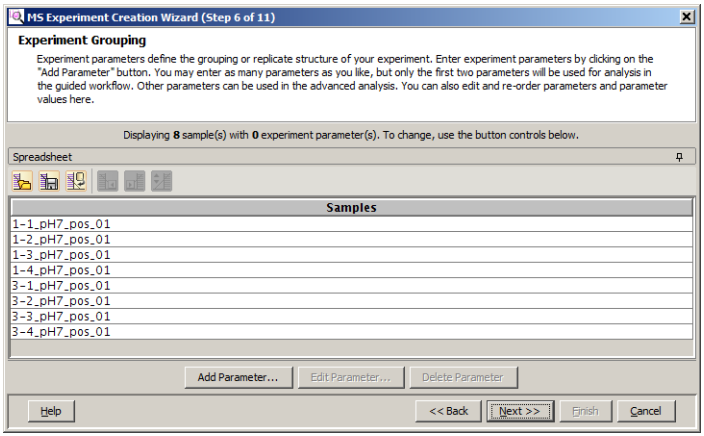
Steps	Detailed Instructions	Comments
	d Click the sample molecular feature **data files** to import into the experiment. The example Malaria data files are: • 1-1_pH7_pos_01.cef • 1-2_pH7_pos_01.cef • 1-3_pH7_pos_01.cef • 1-4_pH7_pos_01.cef • 3-1_pH7_pos_01.cef • 3-2_pH7_pos_01.cef • 3-3_pH7_pos_01.cef • 3-4_pH7_pos_01.cef	• The Open dialog box uses conventional buttons and icons that allow you to navigate and view your file system. • You may select a continuous range of files with a click on a first file and a Shift-click on a last file that includes the range of files you want to select. • You may select discontinuous, individual files with a Ctrl-click on any file.
	**e** Click the **Open** button to load the selected files for further preparation.	• A progress indicator will be displayed while the files are being imported into Mass Profiler Professional.
		

Steps	Detailed Instructions	Comments
	f Click the **Next >>** button.	• You can review and make changes to your selection during the next step before finalizing the experiment creation. • Replicate samples are from the collection of multiple identical samples from a population. When replicate samples are evaluated a result is obtained that more closely approximates the true value of the population.
6 Review and order the selected files that will be imported in the **MS Experiment Creation Wizard (Step 5 of 11)**.	**a** Click one or more samples that you want to reorder. **b** Click the **Up** or **Down** buttons to reorder the selected sample or samples. **c** Repeat the reordering actions as often as necessary to obtain your order. **d** Mark the sample names that will be imported into your experiment. **e** Click the **Next >>** button.	• **NOTE:** This step presents the only opportunity you have to reorder your samples. After completing the Data Import Wizard you will need to create a new project and repeat this process to reorder your samples. • You may select a continuous range of files with a click on a first file and a Shift-click on a last file that includes the range of files you want to select. • You may Ctrl-click any sample name to select multiple samples. • Click the **Restore** button at any time after any sample reorder to return the sample order to your starting point when this step was begun.

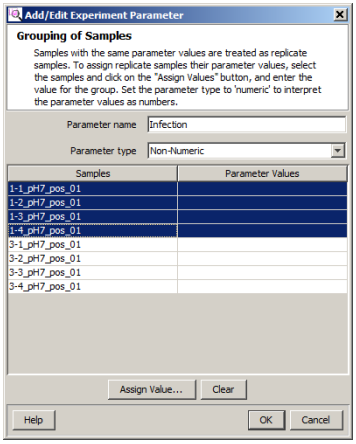




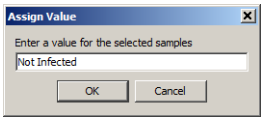
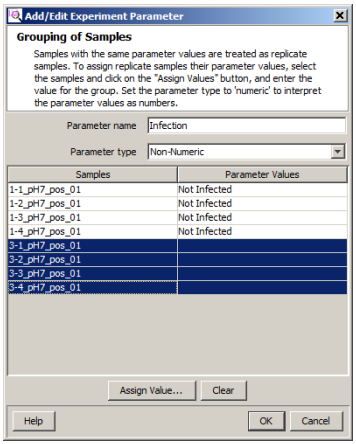
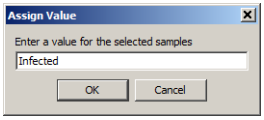
Steps	Detailed Instructions	Comments
7 Enter the sample grouping with respect to the independent variables and the replicate structure of your experiment in the **MS Experiment Creation Wizard (Step 6 of 11)**.	**a** Click the **Add Parameter...** button. **b** Type a name for your **Parameter name** in the **Add/Edit Experiment Parameter** dialog box. `Infection` is typed in the example using the Malaria data set.	• **NOTE:** Grouping at this time is optional. You may add grouping or change your grouping at any time. • An independent variable is an essential element, constituent, attribute, or quality in a data set that is deliberately controlled in an experiment. An independent variable is referred to as a parameter and is assigned a parameter name. • The attribute values within an independent variable are referred to as parameter values. Samples with the same parameter values within a parameter name are treated as replicates.

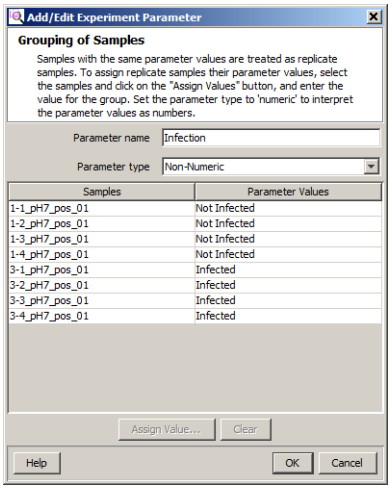
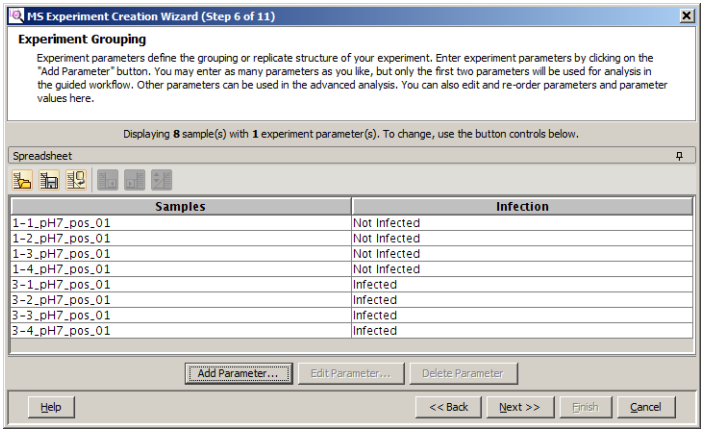


	c Click your replicate **Samples** that share the same first parameter value in your data. For example: • 1-1_pH7_pos_01 • 1-2_pH7_pos_01 • 1-3_pH7_pos_01 • 1-4_pH7_pos_01 **d** Select the **Parameter type** for your grouping. **Non-Numeric** is selected for the example Malaria data set. **e** Click the **Assign Value...** button.	**Parameter Type** options: • Select **Non-Numeric** if the grouping is not a quantitative value. • Select **Numeric** if the grouping value will be quantitative or a value that reflects a degree of proportionality among the samples with respect to an independent variable. • Entry of numerical parameter values for a numeric parameter type will allow some data plots to be scaled by the parameter value.

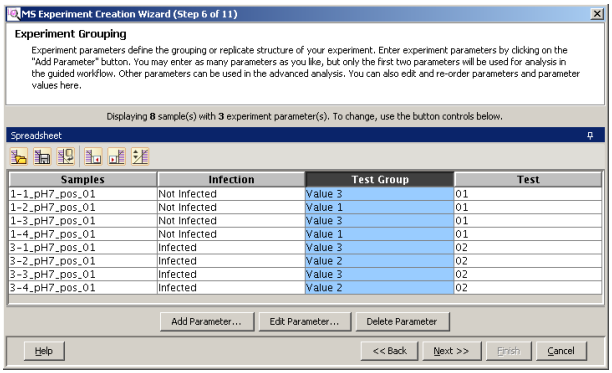


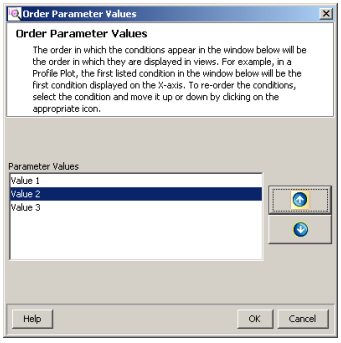
	• You may select a continuous range of files with a click on a first file and a Shift-click on a last file that includes the range of files you want to select. • You may select discontinuous, individual files with a Ctrl-click on any file.

Steps	Detailed Instructions	Comments
	**f** Type the value for your first grouping in the **Assign Value** dialog box. For the Malaria data set the first value typed is `Not Infected`. **g** Click the **OK** button. **h** Click your replicate **Samples** that share the same second parameter value in your data. For example: • 3-1_pH7_pos_01 • 3-2_pH7_pos_01 • 3-3_pH7_pos_01 • 3-4_pH7_pos_01 **i** Select the **Parameter type** for your grouping. **Non-Numeric** is selected for the example Malaria data set. **j** Click the **Assign Value...** button. **k** Type the value for your second grouping in the **Assign Value** dialog box. For the Malaria data set the second value typed is `Infected`. **l** Click the **OK** button. **m** Repeat the value assignment steps until you have assigned a parameter name, type, and value to all of your samples. **n** Review your entries and grouping assignment accuracy in the **Add/Edit Experiment Parameter** dialog box. **o** Repeat the value assignments for individual or multiple samples as necessary to make corrections or changes. **p** Click the **OK** button when the grouping for this parameter is complete.	• In this example the samples will be assigned a value representing the Infection Parameter name. • The highlighted samples will be assigned the value typed in the **Assign Value** dialog box. • In this example the samples will be assigned a value representing the Infection Parameter name.
		
		

Steps	Detailed Instructions	Comments
	**q** Repeat the **Add Parameter...** steps if your data has more than one independent variable. • Click the **Add Parameter...** button. • Repeat the steps above until you have assigned a parameter name, type, and value to all of your data. Before continuing, review the following OPTIONAL and HIGHLY RECOMMENDED step 8 "Re-ordering parameter values in the **Spreadsheet**" on page 28 and step 9 "Saving and importing experiment grouping information in the **Spreadsheet**" on page 29**.** These steps provide advanced instructions to manage your parameters and parameter name assignments. **r** Click the **Next >>** button when you have completed the experiment grouping.	• You may review and edit the parameters and parameter value assignments. • You may change the value of any sample, or group of samples, by highlighting the sample and clicking on the **Assign Value...** or **Clear** button. • **NOTE:** You may add grouping or change your grouping at any time after you complete the Data Import Wizard.
		

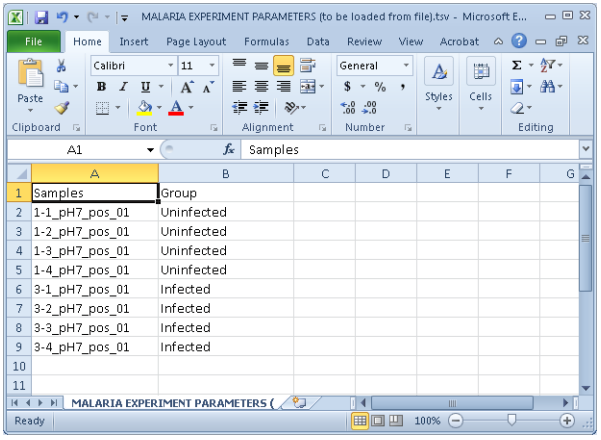
Steps	Detailed Instructions	Comments
8 OPTIONAL: Re-ordering parameter values in the **Spreadsheet**.	**a** Click any one value in the parameter column to select the whole parameter column. **b** Re-order the parameter columns by selecting a parameter column and then clicking on the **Left** or **Right** button.	• When you have more than one parameter associated with your samples, each parameter and its values will be displayed in a separate column in the completed **MS Experiment Creation Wizard (Step 6 of 11)** dialog box. • When the parameter column is selected the column will be highlighted.

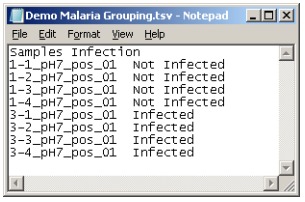




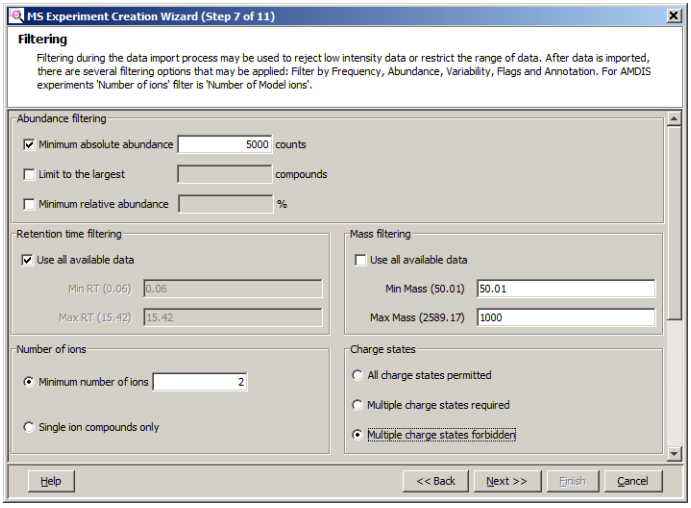
c Re-order the parameter values by selecting a parameter column and then clicking on the **Re-order parameter values** button.
d Click one or more values that you want to reorder.
e Click the **Up** or **Down** buttons to reorder the selected value or values.
f Click the **OK** button when the order for this parameter is complete.

Steps	Detailed Instructions	Comments
9 OPTIONAL: Saving and importing experiment grouping information in the **Spreadsheet**.	**a** Once you have setup the experiment parameters and parameter values, you may save them to a .tsv file by clicking the **Save experiment parameters** button. **b** When you create your experiment parameter grouping, you may load the values from a .tsv file instead of using the MPP user interface by clicking the **Load experiment parameters** button. **c** Experiment parameter grouping may also be found in some sample files by clicking the **Import parameters from samples** button.	• An example experiment grouping file that may be found in the Malaria demo directory under the named "MALARIA EXPERIMENT PARAMETERS (to be loaded from file).tsv" • The .tsv file is organized using tab separated values (tsv) that may be created, edited, and viewed using Microsoft Excel or Notepad. • Creating and editing experiment parameter groupings may be more convenient for you using Excel.

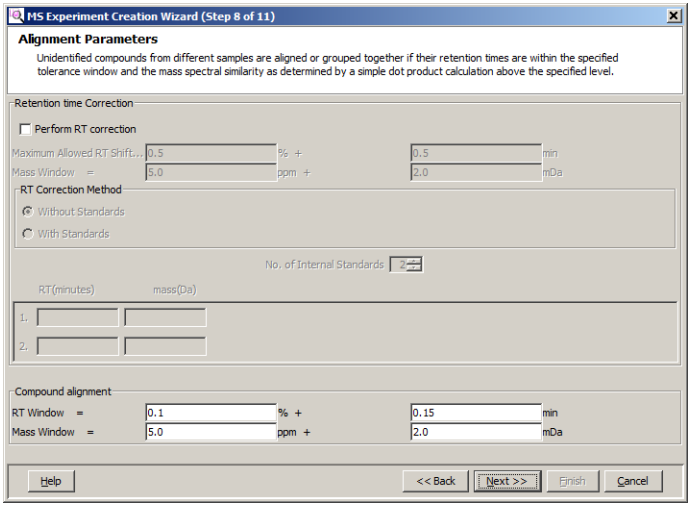




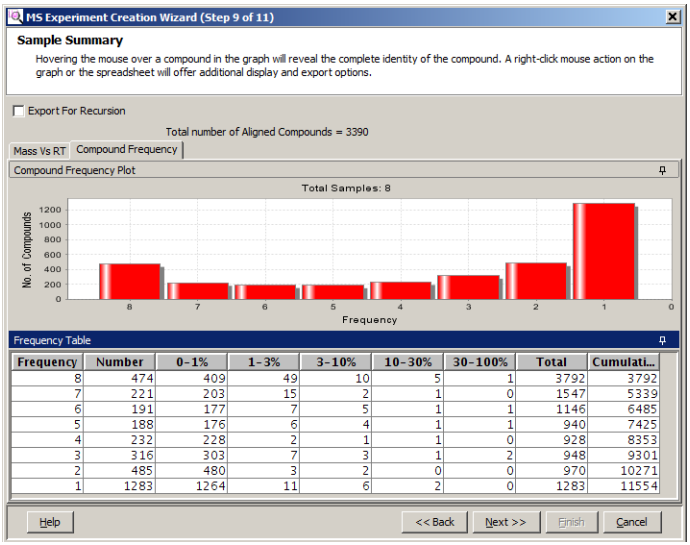
Steps	Detailed Instructions	Comments
10 Select and enter the data filter parameters in the **MS Experiment Creation Wizard (Step 7 of 11)**.	**a** Mark the **Minimum absolute abundance** check box under the Abundance filtering heading. • **NOTE:** Example values are for the Malaria data set. **b** Type a value of `5000` **counts**. **c** Clear the **Limit to the largest** and **Minimum relative abundance** check boxes. **d** Mark the **Use all available data** check box under the Retention time filtering heading. **e** Clear the **Use all available data** check box and type `50.01` for the **Min Mass** and `1000` for the **Max Mass** under the Mass filtering heading. **f** Click the **Minimum number of ions** button and type `2` under the Number of ions heading. The mass filter need not be set to include reference ions. **g** Click the **Multiple charge states forbidden** button under the Charge states heading. **h** Click the **Next >>** button.	• The filtering parameters dialog box will be unique for each experiment type. More information may be found in the online help. • MassHunter Qualitative Analysis data used in this example presents the most active fields. • Filtering during the data import process may be used to reject low-intensity data or restrict the range of data. • In a find by molecular feature generated data file the term abundance actually refers to the feature volume. • In a find by formula generated data file the term abundance actually refers to the feature chromatographic area. • Filtering by maximum mass may improve the statistical analysis by rejecting masses that are not significant to the experiment. This is especially relevant to metabolomic samples. • The filter parameters may be cleared to preserve the prior filtering that was used to generate the feature data file. • Filtering works with both GC/MS and LC/MS data. • After data is imported, several additional filtering options may be applied: Abundance, Retention Time, Mass, Flags, Number of ions, Mass and Minimum Quality Score.



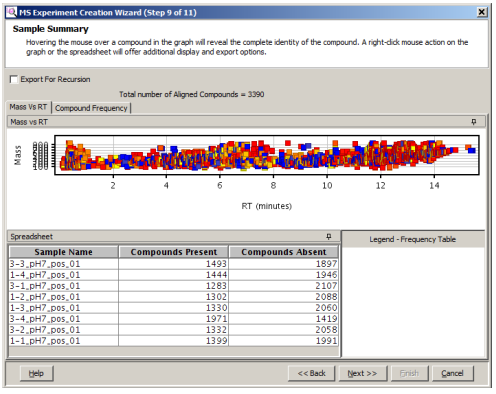
Steps	Detailed Instructions	Comments
11 Select and enter the retention time and mass alignment parameters in the **MS Experiment Creation Wizard (Step 8 of 11)**.	**a** Clear the **Perform RT correction** check box under the Retention Time correction heading. • **NOTE:** Example values are for the Malaria data set. **b** Type `0.1` **%** and `0.15` **min** for RT Window under the Compound alignment heading. • Smaller RT Window values result in reduced compound grouping among the samples leading to a larger list of unique compounds in the experiment **c** Type `5.0` **ppm** and `2.0` **mDa** for Mass Window. • It is not recommended to set the mass window less than 2.0 mDa for higher masses. **d** Click the **Next >>** button.	• The alignment parameters dialog box will be unique for each experiment type. More information may be found in the online help. • MassHunter Qualitative Analysis data used in this example presents more active fields. GC/MS data alignment will involve retention time difference and mass spectral match factor. • A larger retention time shift may be used to compensate for less than ideal chromatography. • If retention time correction is used, it is recommended to perform retention time correction with standards provided that at least two widely spaced standards exist, and those standards must be present in every sample. With standards the correction is based on a piecewise linear fit • Unidentified compounds from different samples are aligned or grouped together if (1) their retention times are within the specified tolerance window and (2) the mass spectral similarity, as determined by a simple dot product calculation, are above the specified level. • The alignment methodologies for the data types are explained in Section 2.1.5 in the Mass Profiler Professional User Manual. • Retention alignment rewrites the retention times in the data file so that the user input or algorithmically selected features are used to correct the retention times.



Steps	Detailed Instructions	Comments
12 View and review the compounds present and absent in each sample in the **MS Experiment Creation Wizard (Step 9 of 11)**.	**a** Clear the **Export for Recursion** check box. • It is not recommended to export the compounds for recursion at this step in the MS Experiment Creation. Better results are obtained after the data has been filtered for significance in the following steps. **b** Click the **Next >>** button.	• This step allows you to see a summary of the compounds present and absent in each of the samples based on the experiment parameters including the application of the filter and alignment parameters. • It is useful to click the **<< Back** button to make changes in the Filtering (Step 7 of 11) page and the Alignment (Step 8 of 11) page parameters and then return to this Sample Summary (Step 9 of 11) page several times to develop a feel for how each of the parameters affects the compound summary. You can even independently assess the effects of retention time alignment versus compound alignment. • Replicates should have similar numbers of compounds present and absent. You can see this easily if the files have a systematic naming system that allows replicates to be sorted together. • Any part of the sample summary may be exported by **Right-Clicking** on that part of the summary and clicking **Export As** and clicking on the image type. The option **Image** allows you to enter the image file location, file name, image size and resolution to meet you organization and publication requirements.

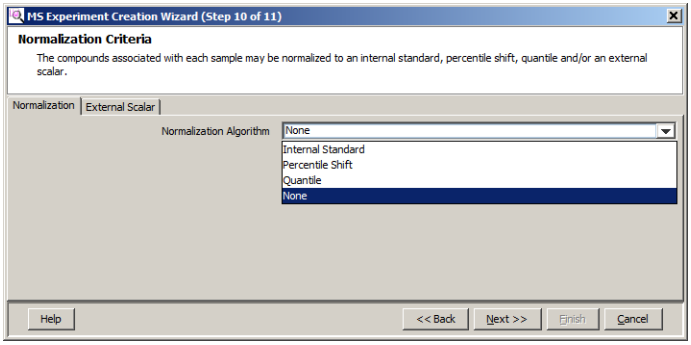


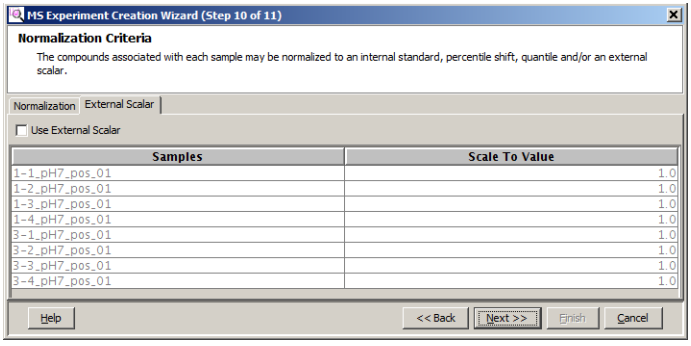
Frequency	Number	0-1%	1-3%	3-10%	10-30%	30-100%	Total	Cumulati...
8	474	409	49	10	5	1	3792	3792
7	221	203	15	2	1	0	1547	5339
6	191	177	7	5	1	1	1146	6485
5	188	176	6	4	1	1	940	7425
4	232	228	2	1	1	0	928	8353
3	316	303	7	3	1	2	948	9301
2	485	480	3	2	0	0	970	10271
1	1283	1264	11	6	2	0	1283	11554



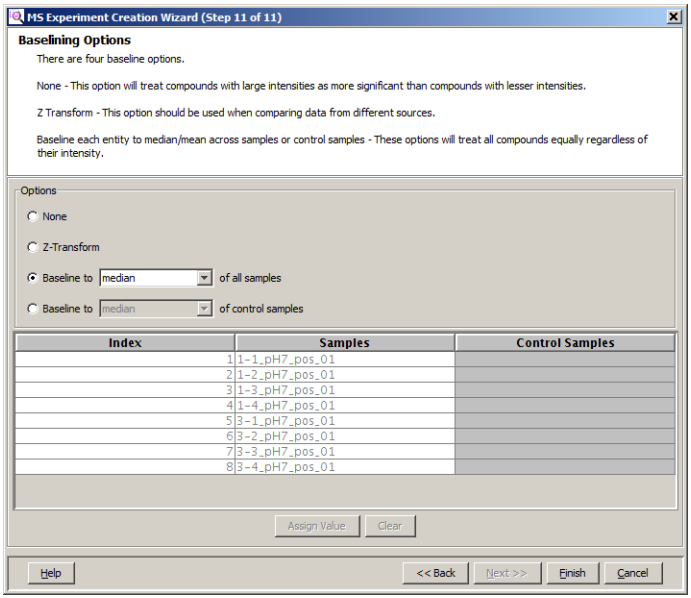
Sample Name	Compounds Present	Compounds Absent
3-3_pH7_pos_01	1493	1897
1-4_pH7_pos_01	1444	1946
3-1_pH7_pos_01	1283	2107
1-2_pH7_pos_01	1302	2088
1-3_pH7_pos_01	1330	2060
3-4_pH7_pos_01	1971	1419
3-2_pH7_pos_01	1332	2058
1-1_pH7_pos_01	1399	1991

Steps	Detailed Instructions	Comments
13 View and review the compounds present and absent in each sample in the **MS Experiment Creation Wizard (Step 10 of 11)**.	**a** Select **None** for the Normalization Algorithm in the Normalization tab. **b** Clear the **Use External Scalar** check box in the External Scalar tab. **c** Click the **Next >>** button.	• You may use normalization and external scalar techniques to reduce the variability caused by sample preparation and instrument response.

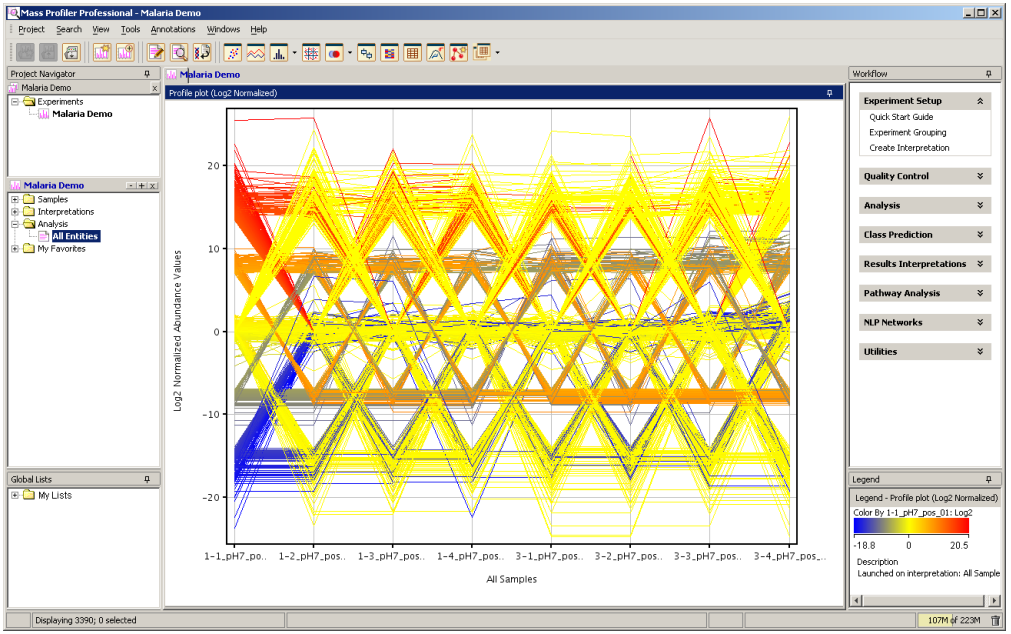




Steps	Detailed Instructions	Comments
14 Select whether to compare features in each sample to the response of the features across multiple samples in the **MS Experiment Creation Wizard (Step 11 of 11)**.	**a** Click the **Baseline to ____ of all samples** button. **b** Select **median** for the Baseline to ____of all samples. **c** Click the **Finish** button Finish .	• There are four baselining options: • **None:** Recommended if only a few features in the samples exist. • **Z-Transform:** Recommended if the data sets are very dense, data where very few instances of compounds are absent from any sample, such as a quantitation data set from recursion. • **Baseline to ____ of all samples:** The abundance for each compound is normalized to its selected statistical abundance across all of the samples. This has the effect of reducing the weight of very large and very small compound features on later statistical analyses. • **Baseline to ____ of control samples:** The abundance for each compound is normalized to its selected statistical abundance across just the samples selected as the control samples. This has the effect of weighting the compound features to a known value that is considered to be normal in the population while reducing the effect of large and small compound features.



Steps	Detailed Instructions	Comments
15 Export your project. This is the end of the Data Import Wizard.	**a** Your MPP screen view will appear with a profile plot of your data. **b** It is recommended that you export your project by clicking **Project > Export Project.** • The project may be loaded by clicking **Project > Import Project** after you close an open project or when you start a new session of MPP.	• Your project is now ready to begin your analysis. • Exporting your project allows you to quickly return to the "saved" point in your analysis. You may export frequently. • **NOTE:** If you selected **Analysis: Significance Testing and Fold Change** for the **Workflow type** in the **New Experiment** dialog box in Step 3, you will immediately start the **Significance Testing and Fold Change** workflow.



3. Next steps

Generate your initial differential analysis

After you create your project and import your data into an experiment, you are ready to generate your initial differential expression. The “Analysis: Significance Testing and Fold Change Quick Start Guide”(G3835-90003) guides you through a pre-defined set of filters and models to help you properly assign your data conditions and obtain an initial expression of differential analysis.

Steps	Detailed Instructions	Comments
1 Continue to your first analysis by following the **Analysis: Significance Testing and Fold Change** wizard.	**a** Obtain a copy of the “Analysis: Significance Testing and Fold Change Quick Start Guide” (G3835-90003). **b** Click **Analysis: Significance Testing and Fold Change** under the Utilities section of the Workflow Browser. **c** Follow the steps presented in the quick start guide.	• Regardless of you personal expertise, the **Analysis: Significance Testing and Fold Change** wizard provides you with a quality control to your analysis that improves your results.
2 Customize your analysis using operations available within the **Workflow Browser**.		• Operations available within the **Workflow Browser** allow you to customize your experiment.

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In this book

The *Overview and Data Import Quick Start Guide* presents first steps to use the MassHunter Mass Profiler Professional Software

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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