



Agilent G3835AA MassHunter Mass Profiler Professional Software

Application Guide

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What is Agilent Mass Profiler Professional?

Agilent Mass Profiler Professional (MPP) software is a powerful chemometrics platform designed to exploit the high information content of mass spectra (MS) data and can be used in any MS-based differential analysis to determine relationships among two or more sample groups and variables. MPP provides advanced statistical analysis and visualization tools for GC/MS, LC/MS, CE/MS, ICP-MS, and NMR data analysis. MPP also integrates smoothly with Agilent MassHunter Workstation, Spectrum Mill and ChemStation software and is the only platform that provides integrated identification/ annotation of compounds and integrated pathway analysis for metabolomic and proteomic studies. The system also enables Automated Sample Class Prediction that revolutionizes mass spectrometer-based qualitative analysis of unknown samples in many applications. MPP is ideally suited for applications characterized by complex sample matrices such as metabolomics, proteomics, natural products, food, beverages, flavors, fragrances, and environmental analyses.



Where is MPP used in your experiment?

MPP is used to import, organize, and analyze the data you acquired. Your unbiased differential analysis experiment may include the following steps with MPP beginning at step four: (1) prepare for your experiment, (2) acquire your data, (3) find the spectral features, (4) import and organize your data, (5) create your initial analysis, (6) identify the features, (7) save your project, and (8) perform advanced analysis operations. Figure 1 on page 2 shows the Agilent tools in your experiment.

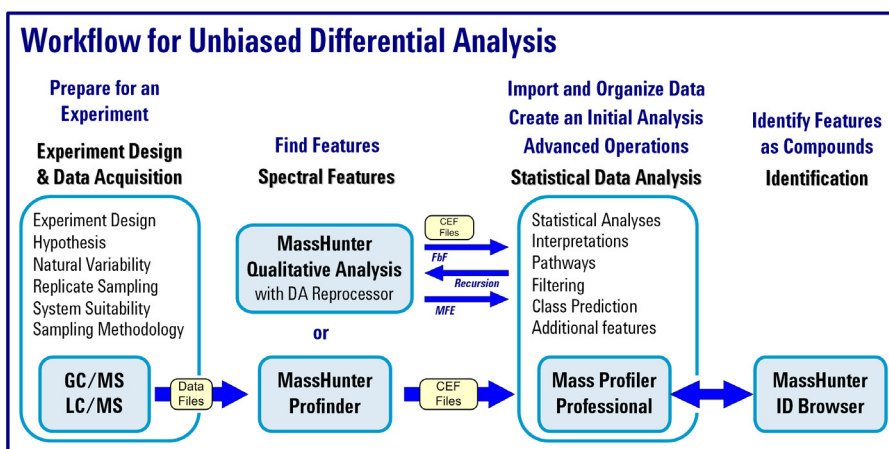


Figure 1 The steps involved in an unbiased differential analysis.

How do I use MPP to analyze my data?

MPP helps you analyze your data through the use of sequential dialog boxes and wizards as shown in Figure 2.

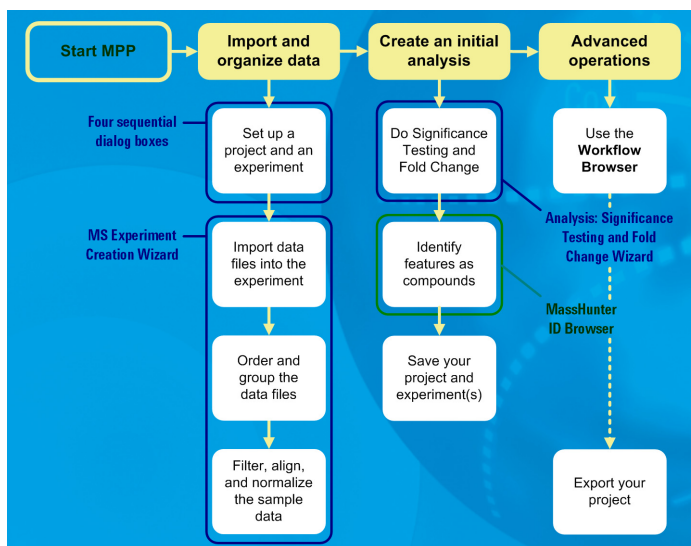


Figure 2 Overview of the wizards that help you use MPP.

Where do I get more information?

The Agilent workflow guides and overviews, online Help and user guides provide you with additional detail, techniques, and explanations to perform advanced analysis operations.

- *Agilent MassHunter Mass Profiler Professional User Manual*. You can find a PDF copy in the MPP installation folder **C:\Program Files\Agilent\MassHunter\Workstation\Mass Profiler Professional\docs>manual**.
- *Agilent Metabolomics Workflow - Discovery Workflow Guide* (p/n 5990-7067EN, Revision B)
- *Agilent Metabolomics Workflow - Discovery Workflow Overview* (p/n 5990-7068EN, Revision B)
- *Class Prediction with Agilent Mass Profiler Professional Workflow Guide* (p/n 5991-1911EN)
- *Class Prediction with Agilent Mass Profiler Professional Workflow Overview* (p/n 5991-1912EN)
- MassHunter Profinder *Quick Start Guide* and online Help

1. Prepare for your Experiment

An experiment consists of the analysis of a set of replicate samples collected over a range of well defined parameters, treatments, and/or exposures known as independent variables, including parameter controls representing minimal or normal perturbations (control samples). The results of changes observed in the samples is designed to provide an answer to your hypothesis. The hypothesis may be proved or disproved by analyzing the correlation of the independent variables on the resulting expression of a large number of dependent variables - the features (compounds) that are measured in your samples. The results must be significant beyond natural variability.

After you obtain your samples, acquire your data, and find the features in your sample data, MPP takes you through data extraction, processing, and statistical analysis so that you can prove or disprove your hypothesis.

Elements to consider in planning your experiment

The hypothesis

The hypothesis is the question that is answered by your analysis. For example, the question may be a statement that proposes a possible correlation, or cause and effect, between a set of independent variables and the resulting features in your data.

Natural variability

It is important to understand how any one sample in your data represents the population as a whole. Because of natural variability and the uncertainties associated with both the measurement and the population, no assurance exists that any single sample from a population represents the mean of the population. Thus, increasing the sample size greatly improves the accuracy of the sample set in describing the characteristics of the population.

Replicate sampling

Sampling the entire population is not typically feasible because of constraints imposed by time, resources, and finances. On the other hand, fewer samples increase the probability of making a false positive or false negative correlation.

System suitability

System suitability involves collecting data to provide you with a means to evaluate and compensate for drift and instrumental variations to assure quality results. Techniques employed by your Agilent MassHunter software include (1) retention time alignment, (2) intensity normalization, (3) chromatographic deconvolution, and (4) baselining to produce the highest quality results. The best results are achieved by maintaining your instrument and using good chromatography.

Sampling methodology

Improved data quality comes from matching the sampling methodology to the experimental design so that replicate data is collected to span the parameter values for each parameter. A larger number of samples appropriate to the population under study results in a better answer to your hypothesis. An understanding of the methodologies used in sampling and using more than one method of sample collection have a positive impact on the significance of your results.

Where to find more information to help you prepare for your experiment

Step-by-step detail of the process for preparing for your experiment and performing an unbiased differential analysis is presented in the *Metabolomics Discovery Workflow* (5990-7067EN).

2. Find the Features in your Data

Before you analyze your data with MPP, the features (compounds) in your data must be extracted. For Agilent data, you can use either MassHunter Profinder or the MassHunter Qualitative Analysis program to find features.

MassHunter Profinder

To analyze Agilent LC- and CE-TOF/Q-TOF data in Mass Profiler Professional, MassHunter Profinder is the preferred program to extract features from your sample data. MassHunter Profinder is optimized for batch feature extraction and offers three different feature extraction workflows.

Refer to the MassHunter Profinder *Quick Start Guide* and online Help for details. on the Mass Profiler Professional Supplemental disc.

MassHunter Qualitative Analysis

The features in your sample data can be found and extracted by processing your data files with Agilent MassHunter Qualitative Analysis. MPP imports and analyzes the features that are saved in your .CEF files.

MassHunter Qualitative Analysis is used in conjunction with MassHunter DA Reprocessor to perform untargeted feature extraction, and additionally with MPP to perform recursive targeted feature extraction.

Feature finding with MassHunter Qualitative Analysis involves performing the following steps:

- 1 Create an untargeted Find by Molecular Feature (MFE) method in MassHunter Qualitative Analysis.
- 2 Run the MFE method using DA Reprocessor to extract and save the untargeted features from the sample data files.
- 3 Import, align, and filter the untargeted features using MPP.
- 4 Export the features from MPP for targeted, recursive finding in MassHunter Qualitative Analysis.

- 5 Create a targeted Find by Formula (FbF) method in MassHunter Qualitative Analysis.
- 6 Run the FbF method using DA Reprocessor to re-extract and save the targeted features from the sample data files.

Non-Agilent Data

You can use Mass Profiler Professional to process non-Agilent data. Once imported into Mass Profiler Professional, you can do statistical analysis and visualizations on non-Agilent data in the same way that you analyze Agilent data, except *you are not able to do*:

- Spectral visualization
 - Compound identification (using ID Browser)
 - Create a recursion list (for further mining of the data once interesting features are identified)
 - Create an MS/MS inclusion list
- 1 Use your non-Agilent data acquisition and analysis program to extract the features in your sample data.
 - 2 Export your non-Agilent data to a spreadsheet file in comma-separated, tab-separated, or Excel (.xls and .xlsx) format. Make sure that these required columns exist in the spreadsheet in this order:
 - RT
 - Mass
 - Compound Name
 - Formula
 - CAS ID
 - One or more Signal columns with non-specific column headers and only numeric values in each column

Please note that:

- Mass Profiler Professional recognizes only the named required columns (**RT**, **Mass**, **Compound Name**, **Formula**, and **CAS ID**), plus these optional columns: **KEGG ID**, **ChEBI ID**, **HMP ID**, **Lipid ID**, **NCBI gi ID**, and **Swiss-Prot ID**.

2. Find the Features in your Data

- Mass Profiler Professional identifies all other columns that contain numeric values as signal columns. However, you can clear any column that Mass Profiler Professional wrongly identifies as a signal column.
- Only the protein entities with a valid **UniprotKB Accession** values specified in the **Swiss-Prot ID** column are considered for mapping in pathway analysis. **UniprotKB Entry name** is not considered for mapping.
- A generic file can contain one or more samples and many such files can be used to create an experiment. Given a set of generic files, if the same sample name occurs multiple times in the same file or across files, Mass Profiler Professional uses only the first instance (in alphanumeric file name order) of the sample.
- Mass Profiler Professional supports Identified, Unidentified, and Combined (Identified + Unidentified) types of experiment creation for Generic data. Select one of these three experiment types during Experiment Description. The first entry that appears in any one of the **Compound Name**, **CASID**, **Swiss Prot ID**, and **Formula** columns (in that order) is used to name the entity. If no entry appears in any of these four columns, the compound is considered to be unidentified.

Mass	RT	Compound Name	Formula	CAS ID	KEGG ID	ChEBI ID	HMP ID	Lipid ID	Swiss Prot ID	1-1_pH7_pos_01	1-2_pH7_pos_01
410.0033	0.298625									43892	34
352.0715	0.3075	Griseofulvin		126-07-8						41515	37
693.9807	0.32									237861	282
695.9493	0.32025									112335	9C
433.9571	0.318625									126290	125
611.9776	0.321375									391212	43E
529.9748	0.321375									815284	1053
791.9571	0.321875									184395	171
541.0607	0.419	cyclic adenosine diphosphate ribose		119340-53-3							
427.0293	0.45175	Zidovudine diphosphate		106060-89-3							
265.9593	0.74725	2,3-Diphospho-D-Glyceric Acid		138-81-8							
559.0709	0.929	N1-(5-Phospho-D-ribosyl)-AMP		1109-75-7							
427.0292	0.936	Zidovudine diphosphate + 0.936		106060-89-3							
122.048	1.066625	Niacinamide		98-92-0						573554	523
346.1166	5.071375	Nifedipine		21829-25-4						95548	73
332.0699	6.865624	Fluorescein								22123	27
418.2698	10.325	Simvastatin		79902-63-9							
174.1119	0.358	Arginine		74-79-3						143150	197
348.0486	0.575	Inosine 5'-monophosphate (IMP)		131-99-7						624724	571
344.1836	8.48075	Granisetron metabolite 1								81795	83

Figure 3 Example spreadsheet to import non-Agilent data. Click this image to open the spreadsheet file for use as a template.



3. Import and Organize your Data

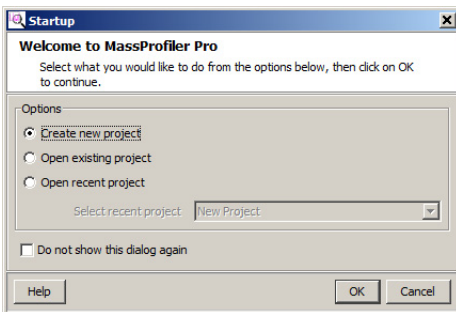
Create a new project and experiment for your data

You are guided through four sequential dialog boxes to create a new project and experiment to receive your data:

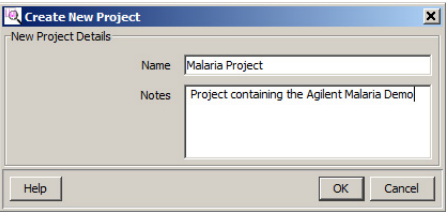


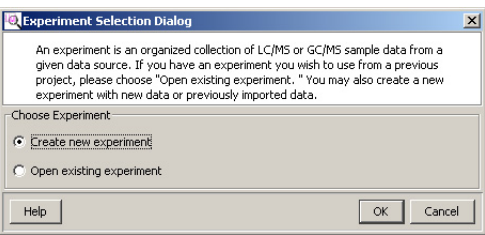
- 1 **Startup:** Select the option to create a new project.
- 2 **Create New Project:** Type descriptive information about your project.
- 3 **Experiment Selection:** Select the option to create a new experiment as part of your project.
- 4 **New Experiment:** Set up the information to store with your experiment and to guide the analysis process.

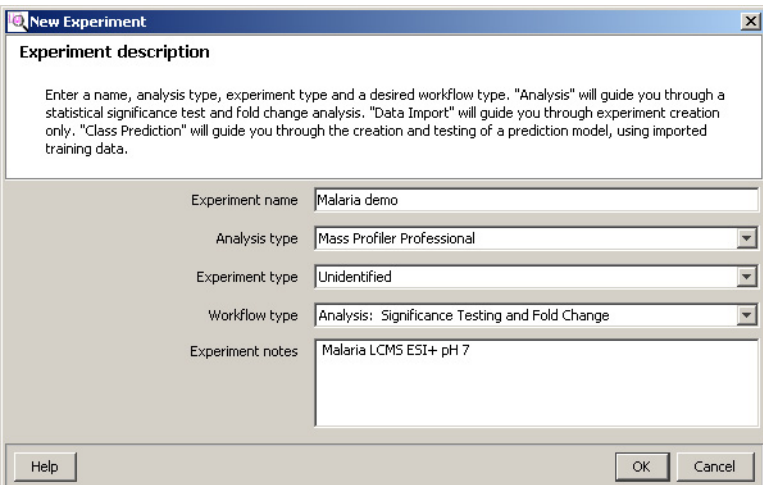
Follow the steps below to setup your new project. The Agilent *Malaria Demo* data set is used as an example in each step. You are encouraged to substitute the demo information and data files with your own data.

Steps	Detailed Instructions	Comments
1 Start Mass Profiler Professional.	a Click the Mass Profiler Professional icon  on your desktop.	<ul style="list-style-type: none"> When MPP starts, if you choose, you are immediately guided through four sequential dialog boxes to create a new project and experiment.
2 Create a new project from the Startup dialog box.	a Click Create new project . b Click OK .	<ul style="list-style-type: none"> Create new project provides you with the option to create a new experiment or import an experiment from an existing project into the new project. After closing an open project, you may create a new project from the Menu bar: click Project > New Project, or from the Toolbar: click the New project button .



3. Import and Organize your Data

Steps	Detailed Instructions	Comments
<p>3 In the Create New Project dialog box, enter your project information.</p>	<p>a Type Malaria Project or your project information in Name. b Type descriptive information in Notes. c Click OK.</p>	<ul style="list-style-type: none"> The project name and notes may be viewed and edited at any time using the Project Inspector by clicking Project > Inspect Project from the menu bar.
		
<p>4 In the Experiment Selection Dialog dialog box, create a new experiment.</p>	<p>a Click Create new experiment. b Click OK.</p>	<ul style="list-style-type: none"> You can also create a new experiment in your project from the: <ul style="list-style-type: none"> Menu bar: Click Project > New Experiment. Toolbar: Click the New experiment button . Open existing experiment opens a project and the experiment(s) that are stored in the project. You may also click the Add experiment button  to add an existing experiment to your project.
		
<p>5 In the New Experiment dialog box, enter and select information that guides your experiment creation.</p>	<p>a Type a descriptive name for the experiment in Experiment name. b Select Mass Profiler Professional for Analysis type. c Select Unidentified or Combined (Identified + Unidentified) for the Experiment type. d Select Analysis: Significance Testing and Fold Change for Workflow type. e Type descriptive information in Experiment notes. f Click OK.</p>	<ul style="list-style-type: none"> Regardless of your personal expertise, it is recommended to select the Analysis: Significance Testing and Fold Change for the Workflow type to provide you with quality control to your analysis that improves your results. At the conclusion of the Analysis: Significance Testing and Fold Change workflow, you may save your project and customize your entire analysis using the operations available in the Workflow Browser.

Steps	Detailed Instructions	Comments
		<ul style="list-style-type: none"> • Table 1 on page 12 and Table 2 on page 12 show the selection and entry options available to you for the New Experiment dialog box • Experiment type (see also Table 2) determines how Mass Profiler Professional manages the data: <ul style="list-style-type: none"> • Select Unidentified when the compounds have only been identified by their molecular features of neutral mass and retention time. • Select Identified when the compounds have been identified by compound, formula, and/or CAS number. • Select Combined (Identified + Unidentified) 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. • If you selected Analysis: Significance Testing and Fold Change or Data Import Wizard for the Workflow type in the New Experiment dialog box, you immediately begin the data import process.

3. Import and Organize your Data

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

Dialog Box Option	Your Choices	Comments
Experiment name	<none>	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 Table 2 on page 12>
Workflow type	Analysis: Significance Testing and Fold Change Class Prediction: Build and Test Model Data Import Wizard	
Experiment notes		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
	Profinder/MH Qual	.CEF	Find by Formula
	MH Qual (GC Scan)	.CEF	Identify by Unit Mass Library
	ICP-MS	.CSV	Identified by IP-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)

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

Experiment Type	Data Source	File Types	Comments
Unidentified	Profinder/MH Qual	.CEF	Find by Molecular Feature Extractor (MFE)
	MH Qual (GC Scan)	.CEF	Find by Chromatographic Deconvolution
	ICP-MS	.CEF	Identified by IP-MS software
	AMDIS	.ELU	Components identified by AMDIS that are not identified by an AMDIS target library
	Generic	.XLS .XLSX .CSV .TXT	Entries <i>not</i> identified by Compound (column C), Formula (column D), CASID (column E)
Combined	Profinder/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	.CEF	Identified by IP-MS software
	AMDIS	.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)

3. Import and Organize your Data

Import and organize your data

After you set up your project and create an experiment, the **MS Experiment Creation Wizard** (Figure 4) immediately guides you through the necessary steps to organize your experiment, import your data, define your experiment variables, and prepare your data for analysis; data preparation includes grouping, filtering, alignment, normalization, and baselining.

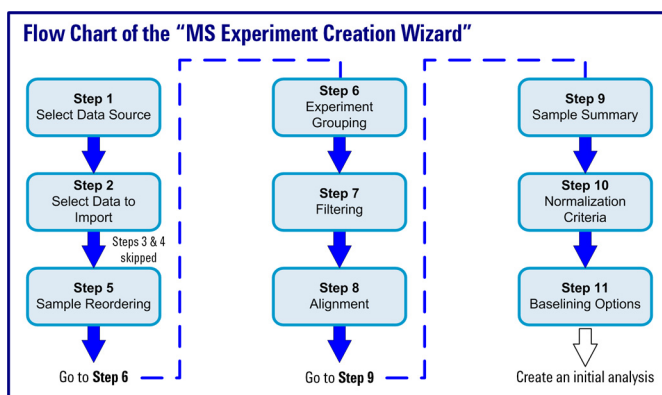


Figure 4 MS Experiment Creation Wizard

Steps	Detailed Instructions	Comments
1 Select the data source that generated the molecular features for your experiment in the MS Experiment Creation Wizard (Step 1 of 11) .	<p>a Click MassHunter Qual and select Homo sapiens for the Organism if you are using the <i>Malaria Demo</i> data set.</p> <p>b Click Next.</p>	<ul style="list-style-type: none">• If you are using your own data set, click the source of your sample files, and select the Organism of the sample files or select None.• Note that selecting an Organism is most important when you use the Pathway Analysis features of MPP.• If you are importing a non-Agilent data file, click Generic.

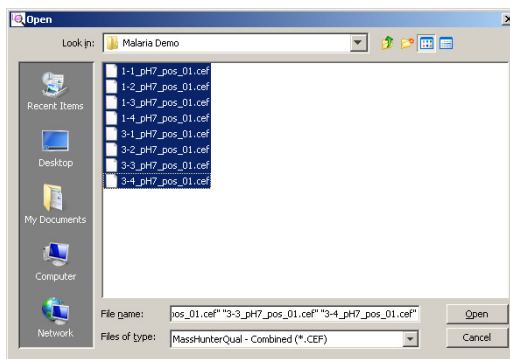
The screenshot shows the 'MS Experiment Creation Wizard (Step 1 of 11)' dialog box. The title bar reads 'MS Experiment Creation Wizard (Step 1 of 11)'. The main area is titled 'Select Data Source' and contains the instruction 'Choose the data sources that will be used for the experiment'. There are four radio button options: 'MassHunter Qual' (selected), 'MassHunter ICP-MS', 'AMDIS', and 'Generic'. Below these is a dropdown menu for 'Organism' with 'Homo sapiens' selected. At the bottom, there are buttons for 'Help', '<< Back', 'Next >>', 'Finish', and 'Cancel'.

3. Import and Organize your Data

Steps

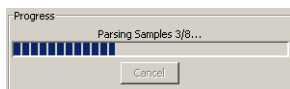
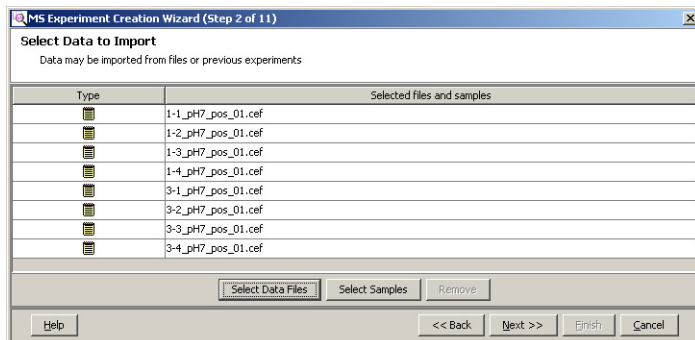
Detailed Instructions

Comments



d Click **Open** to load the selected files.




e Click **Next**.

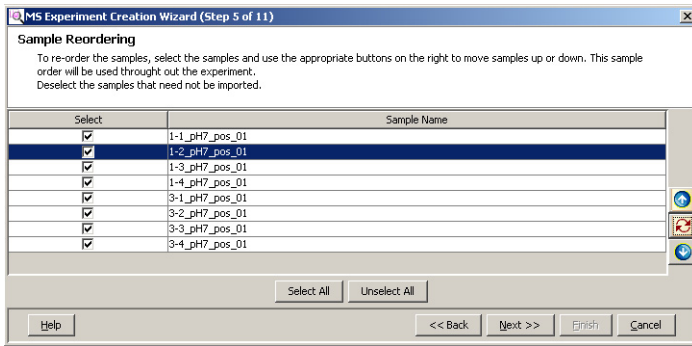


- Replicate samples are from the collection of multiple identical samples from a population. When replicate samples are evaluated a result is obtained that more closely approximates the true value of the population.

- You can review and make changes to your selection during the next step before finalizing the experiment creation.

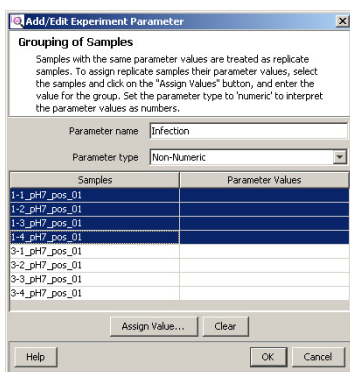
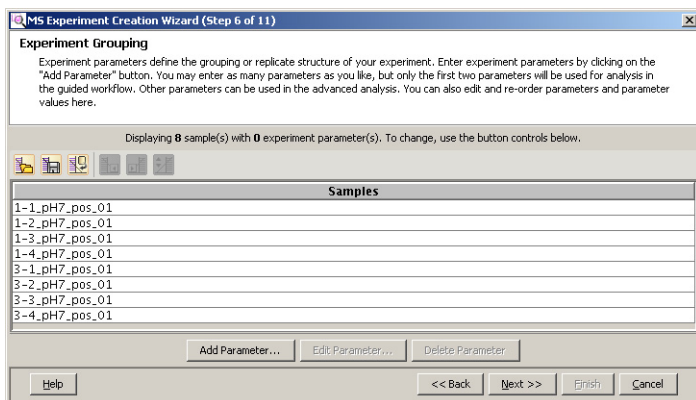
- A progress indicator is shown while your files are imported into MPP.

Steps	Detailed Instructions	Comments
<p>3 Review and order the sample files based on the independent variables in your experiment in the MS Experiment Creation Wizard (Step 5 of 11).</p>	<p>a Click one or more samples that you want to reorder.</p> <p>b Click the Up  or Down  button to reorder the selected sample(s).</p> <p>c Repeat the reordering actions as often as necessary to obtain your order.</p> <p>d Mark the sample names that you want to import into your experiment.</p> <p>e Click Next.</p>	<ul style="list-style-type: none"> • Note: This step is the only opportunity to reorder your samples. After completing the data import, create a new project or experiment 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. • Click the Restore  button at any time to return the sample order to your starting point when this step was begun.



3. Import and Organize your Data

Steps	Detailed Instructions	Comments
4 Define 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) .	<p>a Click Add Parameter.</p>	<ul style="list-style-type: none"> • Note: Grouping at this time is optional. You may add grouping or change your grouping during the Analysis: Significance Testing and Fold Change Wizard or at any time thereafter. • An independent variable is an essential element, constituent, attribute, or quality in a data set that is deliberately controlled in your 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 value and the same parameter name are treated as replicates. • Parameter Type options: <ul style="list-style-type: none"> • Select Non-Numeric if the grouping is not a quantitative value. • Select Numeric if the grouping value is quantitative or a value that reflects a degree of proportionality among the samples with respect to an independent variable. A numeric parameter type allows some data plots to be scaled by the parameter values.

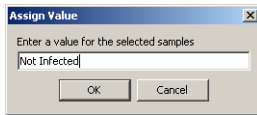


- b Type a name for your **Parameter name** in the **Add/Edit Experiment Parameter** dialog box. Type **Infection** for the *Malaria Demo*.
- c Click your replicate **Samples** that share the same first parameter value in your data. For example:
- 1-1_pH7_pos_01
 - 1-2_pH7_pos_01
 - 1-3_pH7_pos_01
 - 1-4_pH7_pos_01
- d Select the **Parameter type** for your grouping. **Non-Numeric** is selected for the *Malaria Demo*.
- e Click **Assign Value**.

Steps

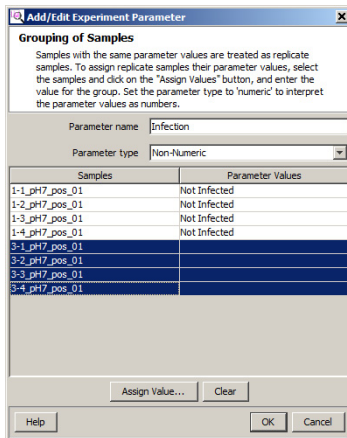
Detailed Instructions

Comments

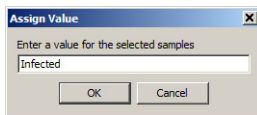


- f** Type the value for your first grouping in the **Assign Value** dialog box. For the *Malaria Demo* type *Not Infected*.
- g** Click **OK**.

- In this example the samples are assigned parameter values representing the Infection parameter.
- The highlighted samples are assigned the value typed in the **Assign Value** dialog box.



- 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** Click **Assign Value**.



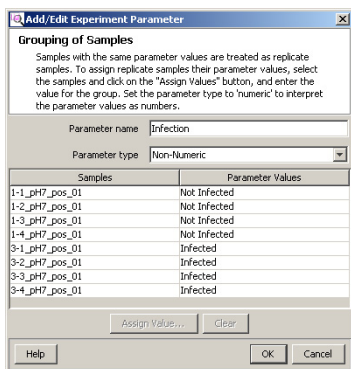
- j** Type the value for your second grouping in the **Assign Value** dialog box. For the *Malaria* data type *Infected*.
- k** Click **OK**.
- l** Repeat the value assignment steps with your own data until you have assigned a parameter name, type, and value to all of your samples.
- m** Review your entries and grouping assignment accuracy in the **Add/Edit Experiment Parameter** dialog box.
- n** Repeat the value assignments for individual or multiple samples as necessary to make corrections or changes.
- o** Click **OK** when the grouping for this parameter name is complete.

3. Import and Organize your Data

Steps

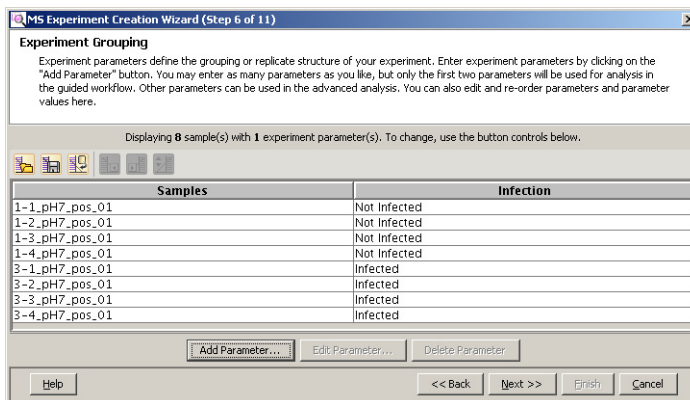
Detailed Instructions



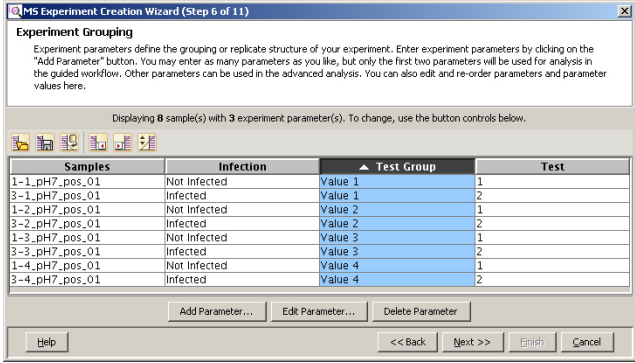



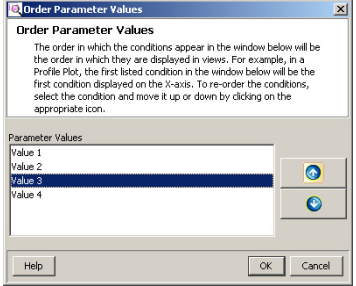


Comments



- p** Repeat **Add Parameter** if your data has more than one independent variable.
- Click **Add Parameter**.
 - Repeat the steps above until you have assigned a parameter name, type, and value to all of your data.
- Review step 5 *OPTIONAL: Re-order your parameter values* and step 6 *OPTIONAL: Saving and importing experiment grouping information in a spreadsheet*. These steps provide advanced instructions to manage your parameters and parameter name assignments using the wizard toolbar and a spreadsheet application.
- q** Click **Next** when you have completed your experiment grouping.

- You may change the value of any sample, or group of samples; highlight the sample and click **Assign Value** or **Clear**.
- Note:** You may add grouping or change your grouping during the **Analysis: Significance Testing and Fold Change Wizard** and at any time thereafter.



Steps	Detailed Instructions	Comments																																				
5 OPTIONAL: Re-order your parameter values.	<p>a Click any one value under the parameter column to select the whole parameter column.</p> <p>b Re-order the parameter column, click the Left  or Right  button.</p>	<ul style="list-style-type: none"> When you have more than one parameter associated with your samples, each parameter and its values is displayed in a separate column in the MS Experiment Creation Wizard (Step 6 of 11) dialog box. When the parameter column is selected the column is highlighted. 																																				
 <p>The screenshot shows the 'MS Experiment Creation Wizard (Step 6 of 11)' dialog box. The 'Experiment Grouping' section is active, displaying a table with 8 samples and 3 experiment parameters. The 'Test Group' column is highlighted. Below the table are buttons for 'Add Parameter...', 'Edit Parameter...', and 'Delete Parameter...'. At the bottom are 'Help', '<< Back', 'Next >>', 'Finish', and 'Cancel' buttons.</p> <table border="1" data-bbox="132 621 762 760"> <thead> <tr> <th>Samples</th> <th>Infection</th> <th>Test Group</th> <th>Test</th> </tr> </thead> <tbody> <tr><td>1-1_pH7_pos_01</td><td>Not Infected</td><td>Value 1</td><td>1</td></tr> <tr><td>3-1_pH7_pos_01</td><td>Infected</td><td>Value 1</td><td>2</td></tr> <tr><td>1-2_pH7_pos_01</td><td>Not Infected</td><td>Value 2</td><td>1</td></tr> <tr><td>3-2_pH7_pos_01</td><td>Infected</td><td>Value 2</td><td>2</td></tr> <tr><td>1-3_pH7_pos_01</td><td>Not Infected</td><td>Value 3</td><td>1</td></tr> <tr><td>3-3_pH7_pos_01</td><td>Infected</td><td>Value 3</td><td>2</td></tr> <tr><td>1-4_pH7_pos_01</td><td>Not Infected</td><td>Value 4</td><td>1</td></tr> <tr><td>3-4_pH7_pos_01</td><td>Infected</td><td>Value 4</td><td>2</td></tr> </tbody> </table>			Samples	Infection	Test Group	Test	1-1_pH7_pos_01	Not Infected	Value 1	1	3-1_pH7_pos_01	Infected	Value 1	2	1-2_pH7_pos_01	Not Infected	Value 2	1	3-2_pH7_pos_01	Infected	Value 2	2	1-3_pH7_pos_01	Not Infected	Value 3	1	3-3_pH7_pos_01	Infected	Value 3	2	1-4_pH7_pos_01	Not Infected	Value 4	1	3-4_pH7_pos_01	Infected	Value 4	2
Samples	Infection	Test Group	Test																																			
1-1_pH7_pos_01	Not Infected	Value 1	1																																			
3-1_pH7_pos_01	Infected	Value 1	2																																			
1-2_pH7_pos_01	Not Infected	Value 2	1																																			
3-2_pH7_pos_01	Infected	Value 2	2																																			
1-3_pH7_pos_01	Not Infected	Value 3	1																																			
3-3_pH7_pos_01	Infected	Value 3	2																																			
1-4_pH7_pos_01	Not Infected	Value 4	1																																			
3-4_pH7_pos_01	Infected	Value 4	2																																			
	<p>c Re-order the parameter values by selecting a parameter column, then click the Re-order parameter values  button.</p> <p>d Click one or more values that you want to reorder.</p> <p>e Click the Up  or Down  button to reorder the selected value(s).</p> <p>f Click OK when the order for this parameter is complete.</p>	 <p>The screenshot shows the 'Order Parameter Values' dialog box. It contains a list of parameter values: Value 1, Value 2, Value 3, and Value 4. Value 3 is currently selected. To the right of the list are 'Up' and 'Down' arrow buttons. At the bottom are 'Help', 'OK', and 'Cancel' buttons.</p>																																				
6 OPTIONAL: Saving and importing experiment grouping information in a spreadsheet.	<p>a Save the experiment parameters and parameter values to a .tsv. Click the Save experiment parameters to file button .</p> <p>b Load your experiment parameter grouping values from a .tsv file, instead of using the MPP user interface. Click the Load experiment parameters from file button .</p>	<ul style="list-style-type: none"> An example experiment grouping file that is in the <i>Malaria Demo</i> directory 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. 																																				

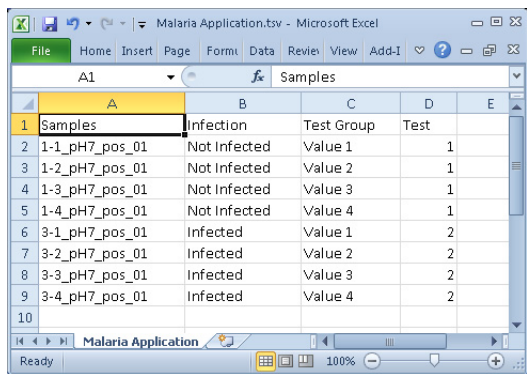
3. Import and Organize your Data

Steps

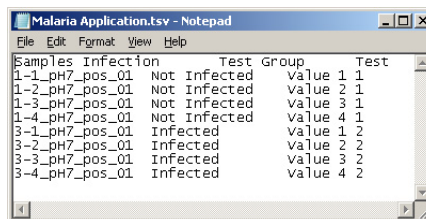
Detailed Instructions

Comments

- c Load your experiment parameter grouping values from a sample file, if applicable, by clicking the **Import parameters from samples** button.
- Creating and editing experiment parameter groupings may be more convenient for you using Microsoft Excel. Save your file as a .tsv file.



Samples	Infection	Test Group	Test
1-1_pH7_pos_01	Not Infected	Value 1	1
1-2_pH7_pos_01	Not Infected	Value 2	1
1-3_pH7_pos_01	Not Infected	Value 3	1
1-4_pH7_pos_01	Not Infected	Value 4	1
3-1_pH7_pos_01	Infected	Value 1	2
3-2_pH7_pos_01	Infected	Value 2	2
3-3_pH7_pos_01	Infected	Value 3	2
3-4_pH7_pos_01	Infected	Value 4	2



```
Malaria Application.tsv - Notepad
File Edit Format View Help
Samples Infection Test Group Test
1-1_pH7_pos_01 Not Infected Value 1 1
1-2_pH7_pos_01 Not Infected value 2 1
1-3_pH7_pos_01 Not Infected value 3 1
1-4_pH7_pos_01 Not Infected value 4 1
3-1_pH7_pos_01 Infected value 1 2
3-2_pH7_pos_01 Infected value 2 2
3-3_pH7_pos_01 Infected value 3 2
3-4_pH7_pos_01 Infected value 4 2
```

Steps	Detailed Instructions	Comments
<p>7 Filter the molecular features by abundance, mass range, number of ions per feature, and charge state in the MS Experiment Creation Wizard (Step 7 of 11).</p>	<p>a Mark the Minimum absolute abundance check box under Abundance filtering.</p> <p>b Type a value of 5000 counts.</p> <p>c Clear the Limit to the largest and Minimum relative abundance check boxes.</p>	<ul style="list-style-type: none"> • The filtering parameters dialog box is unique for each experiment type. More information may be found in the online Help. • MassHunter Qual as the selected data source, 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 (MFE) generated data file the term abundance actually refers to the feature volume. • In a Find by Formula (FbF) generated data file the term abundance actually refers to the feature chromatographic area.
	<p>d Mark the Use all available data check box under Retention time filtering.</p> <p>e Clear the Use all available data check box and type 50.00 for the Min Mass and 1000 for the Max Mass under Mass filtering.</p> <p>f Click the Minimum number of ions button and type 2 under Number of ions.</p> <p>g Click Multiple charge states forbidden under Charge states.</p> <p>h Click Next.</p>	<ul style="list-style-type: none"> • Filtering by maximum mass may improve your 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.

3. Import and Organize your Data

Steps	Detailed Instructions	Comments
<p>8 Align the features across the samples based on tolerances established by retention time and mass in the MS Experiment Creation Wizard (Step 8 of 11).</p>	<p>a Clear the Perform RT correction check box.</p> <p>b Type 0.1 % and 0.15 min for RT Window. A smaller value reduces compound grouping and leads to a larger list of unique compounds.</p> <p>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.</p> <p>d Click Next.</p>	<ul style="list-style-type: none"> • This step is omitted when the experiment type is “identified.” • GC/MS data alignment includes retention time difference and mass spectral match factor. • A large retention time shift may be used to compensate for less than ideal chromatography. • If retention time correction is used, it is recommended to use at least two widely spaced standards, and to use standards that are present in every sample. 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 are above the specified level. • Retention alignment rewrites the retention times in the data file.

Steps	Detailed Instructions	Comments
9 Review the compounds present and absent in each sample in the MS Experiment Creation Wizard (Step 9 of 11) .	<p>a Click the Compound Frequency tab.</p> <p>b Clear the Export for Recursion check box.</p> <p>c Click Next.</p>	<ul style="list-style-type: none"> This step shows 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. The Compound Frequency chart and table report the number of <i>common</i> entities that appear in your samples (i.e., there are 474 entities that appear in all 8 samples and 1283 entities that appear in only 1 sample - "one-hit wonders"). The percent columns show you abundance distribution of the <i>identical</i> entities normalized to the most abundant <i>common</i> entity. If most of the "one-hit wonders" have a low relative abundance your sample data alignment is likely good. If the "one-hit wonders" have a high relative abundance (i.e., in the 30-100% column) then you may need to improve your sample data alignment. In the Mass vs. RT table, replicate samples are expected to have a similar number of compounds present and absent. Use the Back and Next feature to independently assess the effects of your retention time alignment versus compound alignment. It is not recommended to export the compounds for recursion at this step in your experiment. Better results are obtained after the data has been filtered for significance.

MS Experiment Creation Wizard (Step 9 of 11)

Sample Summary
From the Entities tab, use merging options to manually merge entities. Spectra of selected entities are displayed to help merging.
Compound Frequency tab displays the frequency of aligned compounds across all the samples.
Mass vs RT tab displays a scatter plot of compounds and spreadsheet has the summary of aligned compounds present or absent in individual samples.

Export For Recursion

Entities | **Compound Frequency** | Mass vs RT | Total number of Aligned Compounds = 4000

Merged	Compound	Mass	Retention Time	Frequency
158.950400	113366568	158.9504	0.134	1
50.158490	313	50.1584	0.133	1
144.950800	10365667	144.9508	0.144	3
114.948800	143	114.9488	0.143	2
232.275180	18133334	232.2751	0.142	6
204.950400	1455	204.9504	0.145	4
131.950800	14365667	131.9508	0.144	3
112.950800	14349398	112.9508	0.145	6
120.044800	147	120.0448	0.147	2
237.950800	10365667	237.9508	0.144	3
288.046100	272	288.0461	0.272	1
69.950800	274	69.9508	0.274	2
220.044700	276	220.0447	0.276	2
95.950800	27613336	95.9508	0.276	3
230.100900	27533998	230.1009	0.275	5

Preview Merged Entity Spectra

Composite Spectra

Legend: Composite Spectra

MS Experiment Creation Wizard (Step 9 of 11)

Sample Summary
From the Entities tab, use merging options to manually merge entities. Spectra of selected entities are displayed to help merging.
Compound Frequency tab displays the frequency of aligned compounds across all the samples.
Mass vs RT tab displays a scatter plot of compounds and spreadsheet has the summary of aligned compounds present or absent in individual samples.

Export For Recursion

Entities | **Compound Frequency** | Mass vs RT | Total number of Aligned Compounds = 4000

Total Samples: 8

Frequency	Number	0-1%	1-3%	3-10%	10-30%	30-100%	Total	Cumulative Total
8	539	472	51	10	5	0	4312	4312
7	257	237	17	2	1	0	1799	6111
6	235	210	7	6	1	0	1350	7461
5	220	208	6	4	1	0	1100	8561
4	207	207	2	2	0	0	1132	9693
3	372	361	5	3	1	0	1116	10809
2	590	569	10	8	2	1	1180	11989
1	1514	1472	30	8	4	0	1514	13503

MS Experiment Creation Wizard (Step 9 of 11)

Sample Summary
From the Entities tab, use merging options to manually merge entities. Spectra of selected entities are displayed to help merging.
Compound Frequency tab displays the frequency of aligned compounds across all the samples.
Mass vs RT tab displays a scatter plot of compounds and spreadsheet has the summary of aligned compounds present or absent in individual samples.

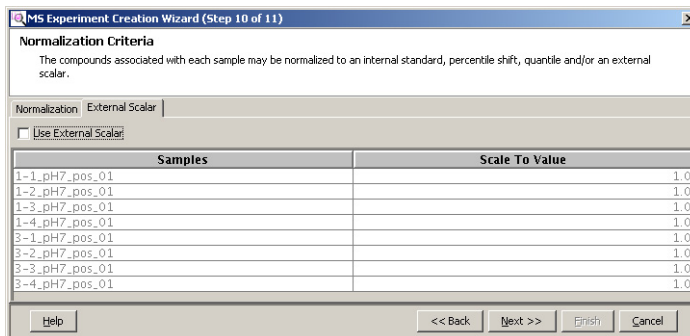
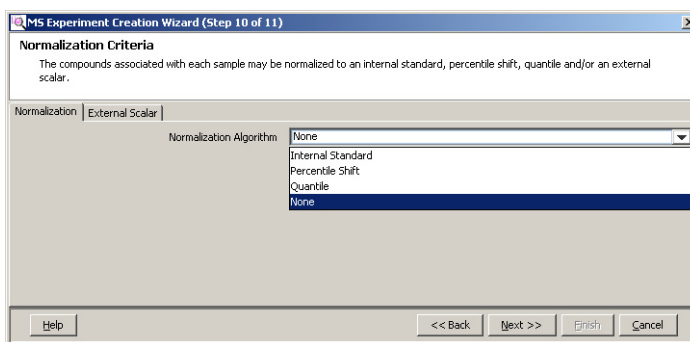
Export For Recursion


Entities | **Compound Frequency** | Mass vs RT | Total number of Aligned Compounds = 4000

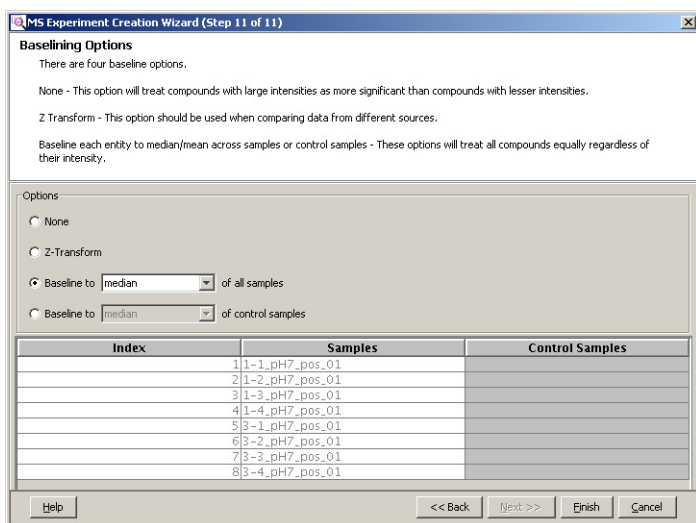
Sample Name	Compounds Present	Compounds Absent
3-3_pH7_pos_01	1767	2333
1-4_pH7_pos_01	1709	2297
1-1_pH7_pos_01	1480	2115
1-2_pH7_pos_01	1478	2522
3-3_pH7_pos_01	1552	2448
3-4_pH7_pos_01	2369	3511
3-2_pH7_pos_01	1502	2498
1-1_pH7_pos_01	1627	2373

3. Import and Organize your Data

Steps	Detailed Instructions	Comments
10	<p>Select whether to normalize the data to reduce the variability caused by sample preparation and instrument response in the MS Experiment Creation Wizard (Step 10 of 11).</p> <p>a Select None for the Normalization Algorithm.</p> <p>b Clear the Use External Scalar check box.</p> <p>c Click Next.</p>	<ul style="list-style-type: none"> You may use normalization and external scalar techniques to reduce the variability in your data that was caused by sample preparation and instrument response.



Steps	Detailed Instructions	Comments
11 Compare the features in each sample to the response of each feature across multiple samples, or the control samples, in the MS Experiment Creation Wizard (Step 11 of 11) .	<p>a Click the Baseline to ___ of all samples button.</p> <p>b Select median for the Baseline to ___ of all samples.</p> <p>c Click the Finish button .</p>	<ul style="list-style-type: none"> There are four baselining options: <ul style="list-style-type: none"> 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. If you selected Analysis: Significance Testing and Fold Change for the Workflow type in the New Experiment dialog box you immediately begin your analysis.



4. Create your Initial Analysis

4. Create your Initial Analysis

The **Analysis: Significance Testing and Fold Change Wizard** (Figure 5) improves the quality of your results and helps you create an initial differential expression from your data. The steps are predetermined and based on the experiment type, experiment grouping, and conditions you entered when creating your project and setting up your experiment. Some steps may be automatically skipped for your experiment.

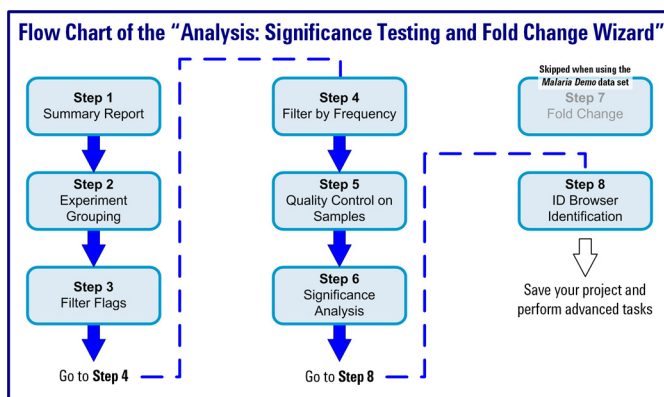


Figure 5 Analysis: Significance Testing and Fold Change Wizard

Steps	Detailed Instructions	Comments
1 Review the summary of your new experiment. Summary Report (Step 1 of 8) .	<p>a Review the Summary Report.</p> <p>b Click and right-click features on the plot, or spreadsheet, to review the data, change the plot view, export selected data, or export the plot to a file.</p> <p>c Click Next.</p>	<ul style="list-style-type: none"> Familiarize yourself with the tools available to you in the summary report view. The Summary Report is displayed as a spreadsheet view when you have more than 30 samples.

Workflow Type - Analysis: Significance Testing and Fold Change (Step 1 of 8)

Summary Report

The distribution of normalized intensity values across all samples is displayed in the Profile Plot.

MassHunterQual.IDENTIFIED_UNIDENTIFIED_COMPOUND5 experiment, No. of sample(s): 8

Log₂-Normalized Abundance Values

1-1_pH7_pos_0 1-2_pH7_pos_01: L 1-3_pH7_pos_01: L 1-4_pH7_pos_01: L 3-1_pH7_pos_01: L 3-2_pH7_pos_01: L 3-3_pH7_pos_01: L 3-4_pH7_pos_01: L

All Samples

Help

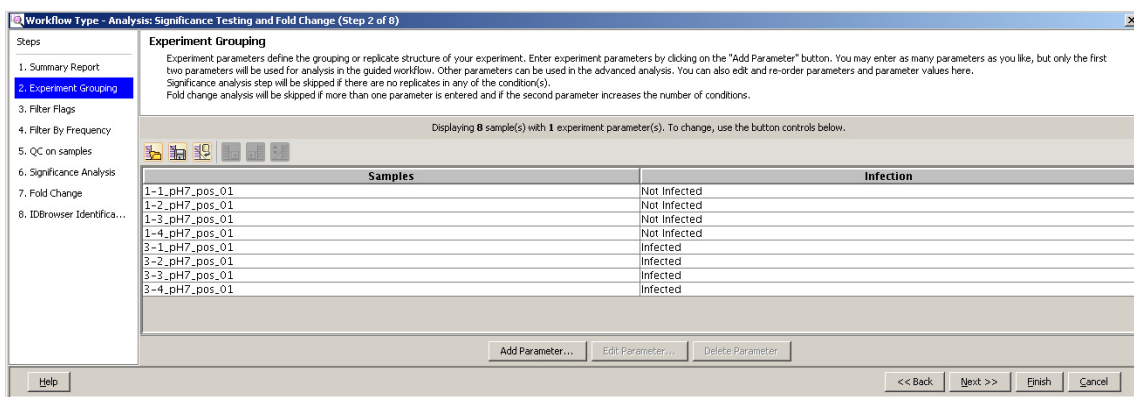
Select All Rows
 Invert Row Selection
 Clear Row Selection
 Limit To Row Selection
 Select Columns
 Invert Column Selection
 Clear Column Selection
 Reset Filters
 Freeze Columns Before
 Unfreeze Columns
 Copy
 Copy View Ctrl+C
 Print Ctrl+P
 Publish
 Export As
 Properties Ctrl+R

Selection Mode
 Zoom Mode
 Invert Selection
 Clear Selection
 Limit To Selection
 Reset Zoom
 Copy View Ctrl+C
 Export Column to Dataset
 Print Ctrl+P
 Export As
 Trellis
 CatView
 Color By Venn
 Properties Ctrl+R

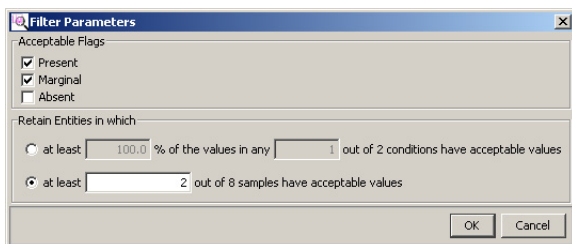
<< Back Next >> Finish Cancel

4. Create your Initial Analysis

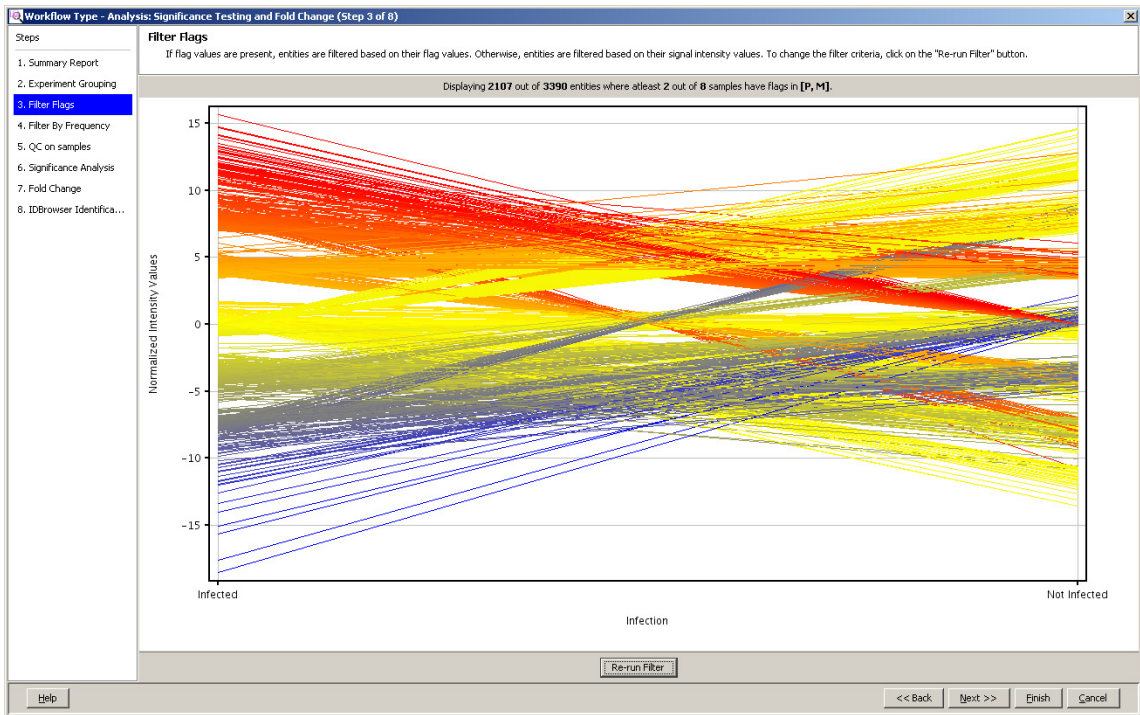
Steps	Detailed Instructions	Comments
2 Define or adjust the sample grouping with respect to the independent variables and the replicate structure of your experiment. Experiment Grouping (Step 2 of 8).	<p>a Click Add Parameter to define or adjust your experiment grouping.</p> <p>b Follow the steps in “Define 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).” on page 18.</p> <p>c Click Next when you have completed your experiment grouping.</p>	<ul style="list-style-type: none"> • Note: In order to proceed to the next step at least one parameter with two parameter values must be assigned. • 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.



3 Filter entities from your samples based on the quality of their presence in specified samples and conditions. Filter Flags (Step 3 of 8).	<p>a Review the summary plot.</p> <p>b Click Re-run Filter to enter parameters into the Filter Parameters dialog box.</p> <p>c Mark the Present and Marginal check boxes.</p>	<ul style="list-style-type: none"> • A flag is a term used to denote the quality of an entity within a sample. A flag indicates if the entity was detected in each sample as follows: Present means the entity was detected, Absent means the entity was not detected, and Marginal means the signal for the entity was saturated. • This filter removes irreproducible entities from further consideration by your analysis.
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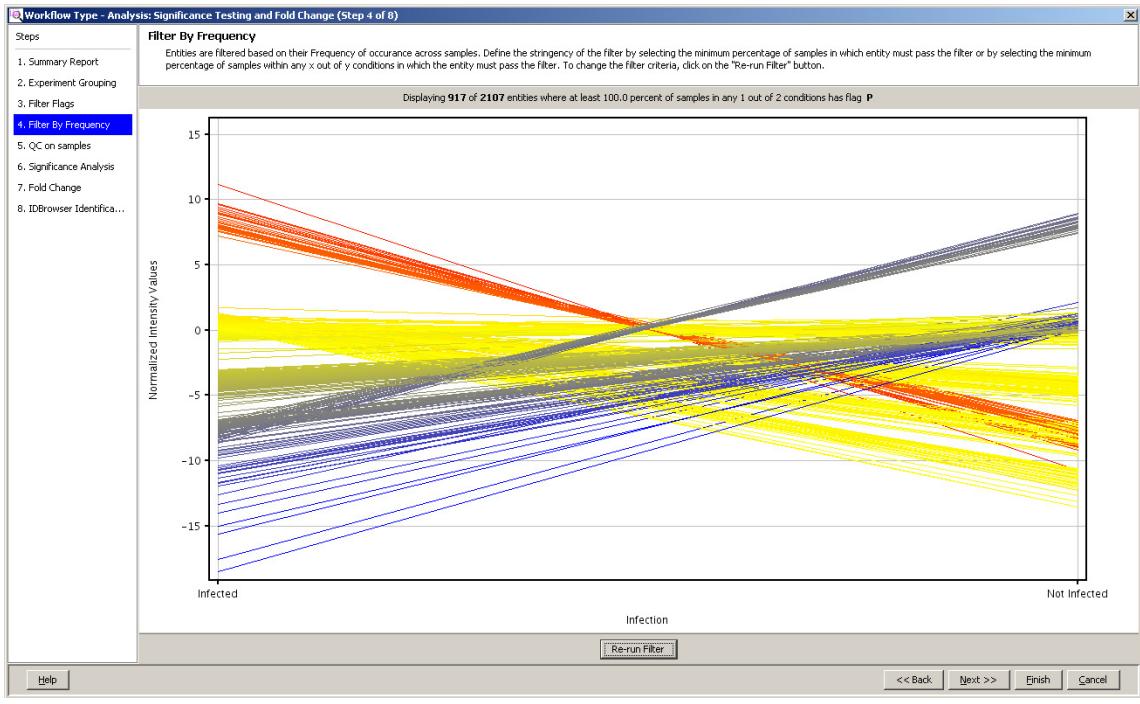
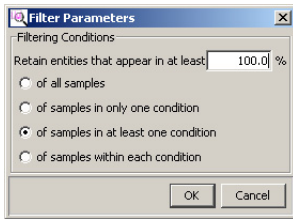


Steps	Detailed Instructions	Comments
	<p>d Clear the Absent check box. This flag is useful when you want to identify missing entities in the sample data.</p> <p>e Click at least ___ out of X samples have acceptable values. The "X" is replaced in your display with the total number of samples in your data set.</p> <p>f Type 2 in the entry box. By setting this parameter to a value of two or more, "one-hit wonders" are filtered.</p> <p>g Click OK.</p> <p>h Review the profile plot. You are encouraged to repeat the Re-run Filter until you obtain the best results for your experiment.</p> <p>i Click Next.</p>	<ul style="list-style-type: none"> The number of entities displayed above the profile plot is expected to decrease as you progress through the workflow. A "one-hit wonder" is an entity that appears in only one sample, is absent from the replicate samples, and does not provide any utility for statistical analysis.



4. Create your Initial Analysis

Steps	Detailed Instructions	Comments
<p>4 Filter the remaining entities in your samples based on their frequency of occurrence among the samples and conditions. Filter by Frequency (Step 4 of 8).</p>	<p>a Review the summary plot.</p> <p>b Click Re-run Filter to enter parameters into the Filter Parameters dialog box.</p> <p>c Type 100 in the Retain entities that appear in at least.</p> <p>d Click of samples in at least one condition.</p> <p>e Click OK.</p> <p>f Review the profile plot. You are encouraged to repeat the Re-run Filter until you obtain the best results for your experiment.</p> <p>g Click Next.</p>	<ul style="list-style-type: none"> Set the minimum % and the applicable condition of samples that an entity must be present to pass the filter: (1) of all samples (conditions are not evaluated), (2) of samples in only one condition (one and only one condition) (3) of samples in at least one condition (one or more conditions), and (4) of samples within each condition (all conditions). For experiments that contain five or fewer replicates, 100% of all samples is recommended. For experiments with a larger number of replicates, the filter frequency percentage may be lowered. A larger % removes more entities.



Steps	Detailed Instructions	Comments
5 Assess the sample quality of your experiment. QC on samples (Step 5 of 8) .	<p>a Review the summary plot.</p> <p>b <i>Highly recommended:</i> Click Back to make adjustments to prior steps in the workflow to improve the results.</p> <p>c Click Next.</p>	<ul style="list-style-type: none"> QC on samples provides you with the first view of the data using a Principle Component Analysis (PCA). PCA allows you to assess the data by viewing a 3D scatter plot of the calculated principle components. You want your samples to form discrete groups in the 3D PCA Scores view based on their parameter assignments.

Workflow Type - Analysis: Significance Testing and Fold Change (Step 5 of 8)

Steps

- Summary Report
- Experiment Grouping
- Filter Flags
- Filter By Frequency
- QC on samples**
- Significance Analysis
- Fold Change
- IDBrowser Identifica...

QC on samples
Sample quality can be assessed by examining the values in the PCA plot and other experiment specific quality plots.

Displaying 8 out of 8 samples retained in the analysis.

Samples	Infection
1-1_pH7_pos_01	Not Infected
1-2_pH7_pos_01	Not Infected
1-3_pH7_pos_01	Not Infected
1-4_pH7_pos_01	Not Infected
3-1_pH7_pos_01	Infected
3-2_pH7_pos_01	Infected
3-3_pH7_pos_01	Infected
3-4_pH7_pos_01	Infected

Legend - 3D PCA Scores

Color by Infection

- Infected
- Not Infected

Description
Algorithm: Principal Components Analysis
Parameters:
Column indices = [1-8]
Pruning option = [numPrincipalComponents, [4]]
Mean centered = true
Scale = true
3-D scores = true
PCA on = Columns

X-Axis: Component 1 (...)
Y-Axis: Component 2 (...)
Z-Axis: Component 3 (...)

Help << Back Next >> Finish Cancel

4. Create your Initial Analysis

Steps

- Assess the differential significance of your samples. **Significance Analysis (Step 6 of 8).**

Detailed Instructions

- Review the summary plot.
- Highly recommended:** Click **Back** to make adjustments to prior steps in the workflow to improve the results.
- Customize the window panes.
- Move the **p-value cut-off** slider(s) or type a value to change the **p-value cut-off** value(s). A larger p-value passes a larger number of entities.

Comments

- The statistical analysis is either a T-test or an Analysis of Variance (ANOVA) based on the samples and experiment grouping.
- The last row of data in the Result Summary spreadsheet shows the number of entities that would be expected to meet the significance analysis by random chance based on the p-value specified in each column heading. If the number of entities expected by chance is much smaller than those based on the corrected p-value, your entities show significance among the parameter values.
- The display of a diagram (Venn Diagram, Fold Change, none, or other plot) depends on your samples and experiment grouping for the analysis.

Workflow Type: Analysis: Significance Testing and Fold Change (Step 6 of 8)

Significance Analysis
Entities are filtered based on their p-values calculated from statistical analysis. To apply the new p-value cut-off, drag the "p-value cut-off" slider or input the new cut-off value in the text box. You will not be able to proceed to the next step if no entities pass the filter.

1. Summary Report
2. Experiment Grouping
3. Filter Flags
4. Filter by Frequency
5. QC on samples
6. **Significance Analysis**
7. Fold Change
8. Deconvolve Identifications

Test Description: Displaying 81 out of 917 entities satisfying corrected p-value cut-off 0.05.
Selected Test: 1 Test unpaired
p-value calculation: Asymptotic
Multiple Testing Correction: Benjamini-Hochberg

Result Summary:

	P-val	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
FC all	917	106	90	82	78	60
FC > 1.1	761	106	90	82	78	60
FC > 1.5	534	98	86	81	77	60
FC > 2.0	475	81	76	74	71	60
FC > 3.0	66	66	66	67	67	60
Expected by chance	61	5	1	0	0	0

Compound	p	p (Corrected)	FC
649 967790 31575	1.15E-11	4.03E-10	-18910.65
693 380790 32	6.68E-05	1.29E-02	3.08
443 957190 318673	2.89E-04	7.48E-03	2.14
611 577690 32137498	4.65E-05	6.28E-04	2.60
529 974890 3217498	2.01E-04	2.52E-03	2.49
791 957190 321829	2.86E-05	4.07E-04	2.63
633 391790 31725	5.66E-14	1.10E-11	-24984.03
531 942890 31775	6.38E-13	5.33E-11	-131746.46
447 9790 322	1.37E-04	1.79E-03	2.01
791 927690 31879	1.48E-10	3.26E-08	-41751.44
479 935790 3297498	3.35E-03	4.93E-02	-2.18
217 935190 328625	2.34E-03	2.31E-02	-2.00
397 935190 32937903	4.31E-03	3.93E-02	-2.33
593 867890 32750002	4.54E-14	1.10E-11	-101585.82
115 911390 330125	1.58E-03	1.49E-02	-1.46
135 933290 330325	1.43E-03	1.49E-02	-2.23
511 863990 33075	2.24E-15	2.10E-12	-41139.32
974 127490 37195	1.19E-09	1.14E-08	-237029.63
169 166290 3785	8.85E-07	1.11E-05	2.56
242 133790 3815	8.78E-11	5.33E-10	-81776.70
541 060790 419	1.73E-08	2.58E-07	-25514.73
837 933290 4174936	4.70E-10	4.13E-08	-1344.07

Fold Change cut-off: 0.0 Control Group: [Detected]

Workflow Type: Analysis: Significance Testing and Fold Change (Step 6 of 8)

Significance Analysis
Entities are filtered based on their p-values calculated from statistical analysis. To apply the new p-value cut-off, drag the "p-value cut-off" slider or input the new cut-off value in the text box. You will not be able to proceed to the next step if no entities pass the filter.

1. Summary Report
2. Experiment Grouping
3. Filter Flags
4. Filter by Frequency
5. QC on samples
6. **Significance Analysis**
7. Fold Change
8. Deconvolve Identifications

Test Description: Displaying 271 out of 1220 entities satisfying corrected p-value cut-off 0.05.
Selected Test: Dney ANOVA
p-value calculation: Asymptotic
Multiple Testing Correction: Benjamini-Hochberg

Result Summary:

	P-val	P < 0.05	P < 0.02	P < 0.01	P < 0.0050	P < 0.0010
Corrected p-value(Defect)	1220	75	67	61	30	35
Corrected p-value(Defect+Treatment)	1220	99	91	36	30	36
Corrected p-value(Treatment)	1220	236	173	162	144	99
Expected by chance	3	1	0	0	0	0

Compound	p (defect)	p (defect+Treat)	p (Treat)	p (Corrected Defect)	p (Corrected Defect+Treat)	p (Corrected Treat)	SRatio
237 0059	3.74E-01	3.74E-01	1.60E-05	5.83E-01	6.42E-01	2.34E-04	21.245924
1210 0788	4.16E-01	6.11E-01	5.53E-05	5.21E-01	7.68E-01	3.05E-01	0.946214
410 0033	5.17E-01	8.21E-03	1.09E-03	7.16E-01	1.33E-01	8.72E-03	0.04395
375 8233	3.02E-04	1.58E-02	7.94E-05	6.97E-03	1.72E-01	4.23E-02	403.049
959 9567	5.67E-01	6.48E-02	5.98E-03	3.75E-01	4.16E-01	3.43E-01	1.88094
785 9379	1.15E-01	2.38E-04	1.15E-01	2.28E-01	8.62E-03	3.02E-01	53.06321
255 0341	2.02E-03	2.18E-01	2.79E-02	4.94E-02	6.22E-01	1.94E-01	0.7287
633 3917	2.88E-01	2.88E-01	1.23E-05	5.57E-01	6.25E-01	2.11E-04	16.6169
515 9601	1.40E-02	8.94E-03	8.39E-04	9.92E-02	1.15E-01	6.88E-03	157.24
879 9857	6.64E-02	1.07E-04	1.77E-01	5.75E-01	4.23E-01	3.76E-01	75.38801
857 9856	4.93E-14	1.49E-13	8.03E-15	2.23E-12	7.02E-12	2.39E-13	256.327
535 0132	9.59E-01	9.93E-01	1.37E-01	1.09E-01	9.99E-01	9.93E-01	0.074912
433 3582	1.09E-17	4.38E-18	2.28E-17	6.99E-16	3.34E-16	8.61E-16	260.136
531 9461	3.62E-01	3.15E-01	2.01E-05	5.74E-01	6.25E-01	2.71E-04	87.2278
693 9068	5.75E-01	6.87E-01	3.75E-07	4.14E-01	6.17E-01	9.15E-06	1.40974
613 9494	8.10E-02	4.70E-01	6.51E-02	2.81E-01	7.27E-01	4.48E-02	146.798
613 9782	9.01E-01	4.54E-01	5.18E-01	8.11E-01	7.14E-01	1.21E-01	0.69296
529 9749	2.57E-02	2.83E-01	1.01E-06	1.42E-01	6.25E-01	2.33E-01	430.077
871 9638	1.11E-02	4.37E-01	3.01E-01	3.98E-01	1.02E-01	3.04E-01	0.101101
791 9571	2.61E-01	1.12E-03	6.74E-01	5.18E-01	2.84E-02	3.75E-02	0.191515
444 9433	3.18E-01	3.18E-01	3.65E-05	5.66E-01	6.28E-01	2.34E-04	17.2316
447 972	1.72E-01	8.10E-02	2.37E-06	4.24E-01	4.52E-01	5.17E-01	0.080972
372 0081	3.61E-01	3.61E-01	1.54E-05	5.75E-01	6.37E-01	2.32E-04	12.87604

p-value cut-off: 0.05

Steps

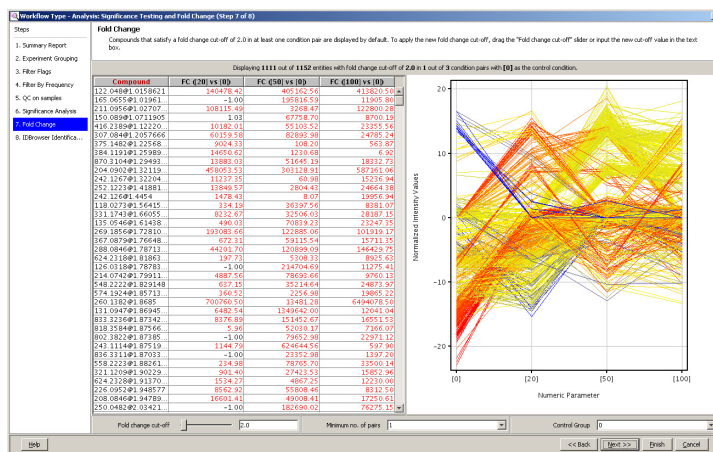
Detailed Instructions

Comments

7 Filter the remaining entities in your samples based on their relative abundance ratios among the samples and conditions. **Fold Change (Step 7 of 8).**

- Review the summary plot.
- Move the **Fold change cut-off** slider or type a value to change the **Fold change cut-off**. The default value is 2.0. A larger cut-off value passes a smaller number of entities through to the final results.
- Select a value for the **Minimum number of pairs** of conditions that must have entities with a fold change greater than the cut-off. The default value is 1.
- Click **Next**.

- The Fold Change workflow step may be automatically skipped depending on your experiment setup (it is skipped using the *Malaria Demo*). If your experiment has a parameter that contains at least three parameter values, the Fold Change step is available.
- Fold change is a signed value that describes how much an entity changes from its initial to its final value. For example, when an entity changes from a value of 60 to a value of 15, the fold change is -4. The quantity experienced a four-fold decrease. Fold change is the ratio of the final value to the initial value.
- Fold change analysis is used to identify entities with abundance ratios, or, for example, differences between a treatment and a control, that are in excess of specified cut-off or threshold value. Fold change is calculated between the conditions where Condition 1 and another condition, Condition 2, are treated as a single group.



4. Create your Initial Analysis

Steps	Detailed Instructions	Comments
8	<p>Export the significant entities in your experiment for identification.</p> <p>ID Browser Identification (Step 8 of 8).</p> <ol style="list-style-type: none"> Review the summary plot. <i>Highly recommended:</i> Click Back to make adjustments to prior steps in the workflow to improve the results. Click IDBrowser Identification to export your entity list to Agilent MassHunter ID Browser. ID Browser is started and automatically prompts you to set up your identification method parameters. 	<ul style="list-style-type: none"> Processing your entities with ID Browser performs the following automatically: save the selected entity list into a CEF file format, open Agilent MassHunter ID Browser, and import the saved CEF file for identification. Once identification is completed, ID Browser returns an identified CEF file. This CEF file is imported into the MPP experiment and annotations are automatically updated.

The screenshot shows the 'ID Browser Identification' window. The main area contains a table with the following columns: Compound, p, FC, Regulation, FC (obs), FC, and Log FC. The table lists various compounds and their associated p-values, fold changes, and log fold changes. The 'Regulation' column indicates the direction of change (up or down). The 'FC (obs)' column shows the observed fold change, and the 'FC' column shows the expected fold change. The 'Log FC' column shows the log of the fold change.

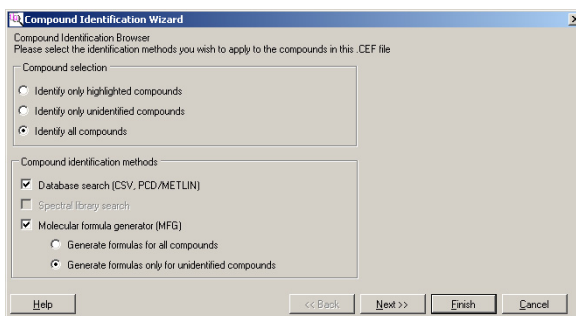
Compound	p	FC	Regulation	FC (obs)	FC	Log FC
649.063700.11775	4.03E-10	1.15E-11	down	16.00	-1.8916.66	-14.21
693.280780.52	1.29E-03	9.68E-05	up	3.00	1.08	1.62
431.557180.118515	3.48E-03	2.89E-04	up	2.15	1.15	1.31
611.977480.12117498	6.28E-04	4.61E-05	up	2.69	2.69	1.38
729.074800.12117498	2.52E-03	2.01E-04	up	2.49	2.49	1.32
791.927180.121875	4.07E-04	2.98E-05	up	2.63	2.63	1.39
631.991180.11725	3.30E-11	5.66E-14	down	16.00	-11.4984.03	-15.09
531.042180.11725	5.31E-11	6.19E-11	down	16.00	-21.1746.46	-16.88
447.97240.122	1.79E-03	1.37E-04	up	2.04	2.04	1.00
792.92760.11875	3.26E-09	1.48E-10	down	16.00	-49.1795.03	-18.25
479.919780.12762498	4.63E-02	5.10E-03	down	2.38	-2.18	-1.13
117.913780.128615	2.31E-02	2.24E-03	down	2.00	-1.00	-1.00
107.916180.12917503	9.91E-02	4.31E-03	down	2.93	-2.93	-1.55
593.867880.12750002	1.30E-11	4.54E-14	down	16.00	-10.1586.82	-16.63
135.912180.130115	1.63E-02	1.58E-03	down	3.16	-1.16	-1.66
135.932180.130115	1.49E-02	1.43E-03	down	2.11	-2.21	-1.14
531.863880.13075	5.10E-12	2.89E-15	down	16.00	-49.3379.33	-15.80
974.377280.57175	1.94E-08	1.10E-09	down	16.00	-12.7095.63	-16.96
169.168380.11785	1.13E-05	7.65E-07	up	2.54	2.54	1.35
242.1159780.1815	3.33E-10	8.58E-12	down	16.00	-81.1736.20	-18.32
541.060780.919	3.58E-07	1.75E-08	down	16.00	-29.554.73	-14.64
427.023980.45174988	4.13E-09	2.07E-10	down	16.00	-23.5596.20	-17.85
122.044180.46225	4.40E-11	4.49E-13	down	16.00	-23.0706.83	-17.82
431.110780.55525	6.52E-09	3.83E-10	down	16.00	-27.617.08	-14.93
343.084980.58325	7.44E-10	2.43E-11	down	16.00	-21.8809.95	-17.74
374.560180.62724996	2.25E-02	2.05E-03	up	2.27	2.27	1.18
623.001280.6505	1.02E-09	3.16E-11	down	16.00	-49.977.84	-15.60
107.03780.11825004	2.26E-10	7.26E-12	down	16.00	-23.9513.75	-17.74
545.932880.73424995	1.72E-09	6.76E-11	down	16.00	-42.292.68	-15.17
850.780180.74450004	1.76E-11	9.57E-14	down	16.00	-20.1232.66	-18.15
265.959180.74725	2.36E-10	5.76E-12	down	16.00	-38.5816.50	-21.87
184.82980.75125	2.38E-10	5.41E-12	down	16.00	-39.768.23	-18.23
559.07680.929	9.78E-10	3.13E-11	down	16.00	-49.40.76	-15.59
427.023980.938	1.02E-09	3.67E-11	down	16.00	-49.040.84	-18.90
663.108780.118475	4.72E-09	2.52E-10	down	16.00	-31.5413.03	-18.17
122.04480.106625	5.43E-05	3.90E-06	down	4.10	4.10	2.11
135.92780.66825	2.18E-10	4.29E-12	down	16.00	-54.844.88	-15.97
221.038585.62210003	9.15E-11	1.40E-12	down	16.00	-33.049.86	-15.01
246.078485.6115	7.71E-09	1.11E-10	down	16.00	-21.8993.02	-16.88
104.002180.2475	4.67E-11	4.27E-13	down	12.00	-12.623.91	18.21

Steps

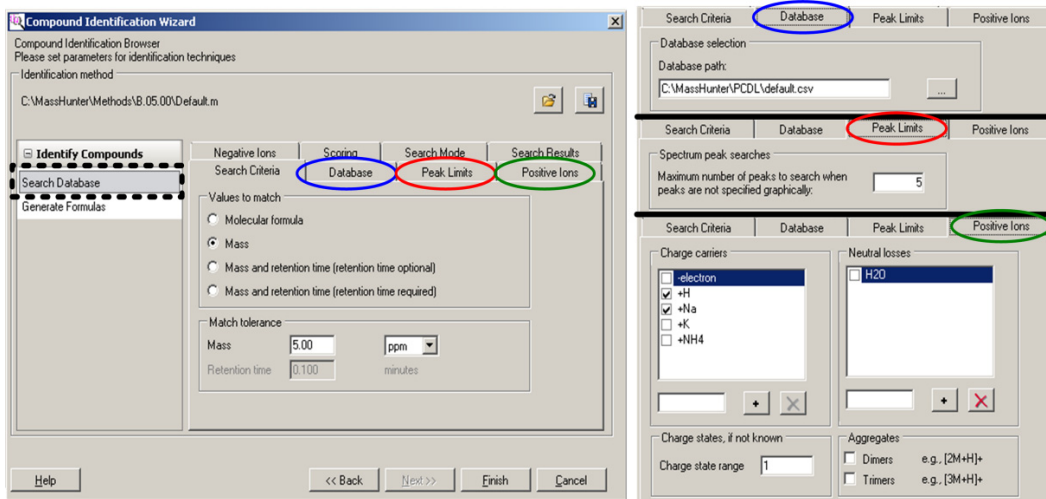
Detailed Instructions

Comments

- d Select the compounds to identify and mark the identification method for your experiment in the **Compound Identification Wizard** dialog box.
- e Click **Next**.



- f Setup the parameters and values for your database search.



4. Create your Initial Analysis

Steps

Detailed Instructions

Comments

Compound Identification Wizard

Compound Identification Browser
Please set parameters for identification techniques

Identification method
C:\MassHunter\Methods\B.05.00.Default.m

Identify Compounds

Search Database
Generate Formulas

Search Criteria Database Peak Limits Positive Ions

Negative Ions Scoring Search Mode Search Results

Charge carriers

- +electron
- +H
- +Cl
- +Br
- +HCOO
- +CH3COO
- +CF3COO

Neutral losses

- H2O

Charge states, if not known

Charge state range: 1

Aggregates

- Dimers e.g., [2M-H]
- Trimers e.g., [3M-H]

Help << Back Next >> Finish Cancel

Negative Ions Scoring Search Mode Search Results

Contribution to overall score

Mass score: 100.00

Isotope abundance score: 60.00

Isotope spacing score: 50.00

Retention time score: 100.00

Expected data variation

MS mass: 2.0 mDa + 5.6 ppm

MS isotope abundance: 7.5 %

MS/MS mass: 5.0 mDa + 7.5 ppm

Retention time: 0.115 min

Negative Ions Scoring Search Mode Search Results

Ion search mode

Which database entries should be examined when searching masses from simple ions?

- Neutral entries
- Cation or anion entries

(This choice is not applicable to CSV databases.)

Negative Ions Scoring Search Mode Search Results

Search Results

Limit to the best

10 hits

Steps

Detailed Instructions

Comments

Compound Identification Wizard
Compound Identification Browser
Please set parameters for identification techniques
Identification method
C:\MassHunter\Methods\B.05.00\Default.m

Identify Compounds

Search Database
Generate Formulas

Allowed Species: **Limits** Charge State Scoring

Charge carrier to be assumed if not known:
Positive ions: H Negative ions: H
MS ion electron state: even electron

Elements and limits:

Element	Minimum	Maximum
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

Help << Back Next >> Finish Cancel

Allowed Species | Limits | Charge State | Scoring

Limits on input masses
Maximum neutral mass for which formulas should be calculated: 750.0000

Limits on results
 Minimum overall score: 35.0000
 Maximum MS mass error: 7.5000 ppm
 Require DBE from: 0.0 to 50.0
 Maximum number of hits: 5

Allowed Species | Limits | Charge State | Scoring

Isotope grouping
Peak spacing tolerance: 0.0025 m/z, plus 7.0 ppm
Isotope model: Common organic molecules

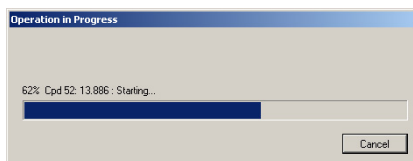
Charge state
 Limit assigned charge states to a maximum of: 2
 Treat ions with unassigned charge as singly-charged

Allowed Species | Limits | Charge State | Scoring

Contribution to overall score
 Mass score: 100.00
 Isotope abundance score: 50.00
 Isotope spacing score: 50.00
 Retention time score: 100.00

Expected data variation
 MS mass: 2.0 mDa + 5.6 ppm
 MS isotope abundance: 7.5 %
 MS/MS mass: 5.0 mDa + 7.5 ppm
 Retention time: 0.115 min

- g** Click **Finish** when you have the method set up for your experiment. ID Browser automatically begins identifying your entities and shows a progress bar.



4. Create your Initial Analysis

Steps

Detailed Instructions

Comments

- h Review and make adjustments to the entity identifications as necessary using the ID Browser interface.
- i Click **Save and Return** to export your entity list back to your experiment in MPP. You are automatically returned to the MPP user interface.

m/z	Abund	Abund % (Nom)	Z	Sat
650.9758	5250		1	
651.9863	1315		1	
652.9739	1672		1	
1317.9713	652		1	

Cpd	Label	Name	Formula	Score	Mass	Avg Mass	Std Dev	Mass (DB)
1	Cpd 1: C19 H18 N6 O10 S5...		C19 H18 N6 O10...	77.57	643.9639			
2	Cpd 2: C23 H10 N4 O22; 0.3...		C23 H10 N4 O22	66.93	693.9802			
3	Cpd 3: C14 H6 N6 O5 S3; 0...		C14 H6 N6 O5 S3	74.59	433.9564			
4	Cpd 4: C24 H8 N2 O18; 0.321		C24 H8 N2 O18	66.68	611.9779			
5	Cpd 5: C13 H22 O12 S5; 0.3...		C13 H22 O12 S5	80	529.9713			
6	Cpd 6: 0.322				791.9571			
7	Cpd 7: C20 H15 Cl N4 O16...		C20 H15 Cl N4...	76.85	633.9889			
8	Cpd 8: C14 H16 N2 O10 S5...		C14 H16 N2 O1...	79.72	531.9396			
9	Cpd 9: C14 H12 N2 O9 S3; 0...		C14 H12 N2 O9...	77.01	447.9638			
10	Cpd 10: 0.319				793.9276			

- k Review your identified entity list in the ID Browser Identification results. The molecular formula now replaces the mass and retention time for identified entities in the compound column.
 - l Click **Finish** when you have completed the ID Browser Identification.
- The **Analysis: Significance Testing and Fold Change** workflow is now complete and you are immediately returned to the main MPP interface.

Steps

Detailed Instructions

Comments

Workflow Type - Analysis: Significance Testing and Fold Change (Step 8 of 8)

Steps

- Summary Report
- Experiment Grouping
- Filter Flags
- Filter By Frequency
- QC on samples
- Significance Analysis
- Fold Change
- IDBrowser Identification**

IDBrowser Identification

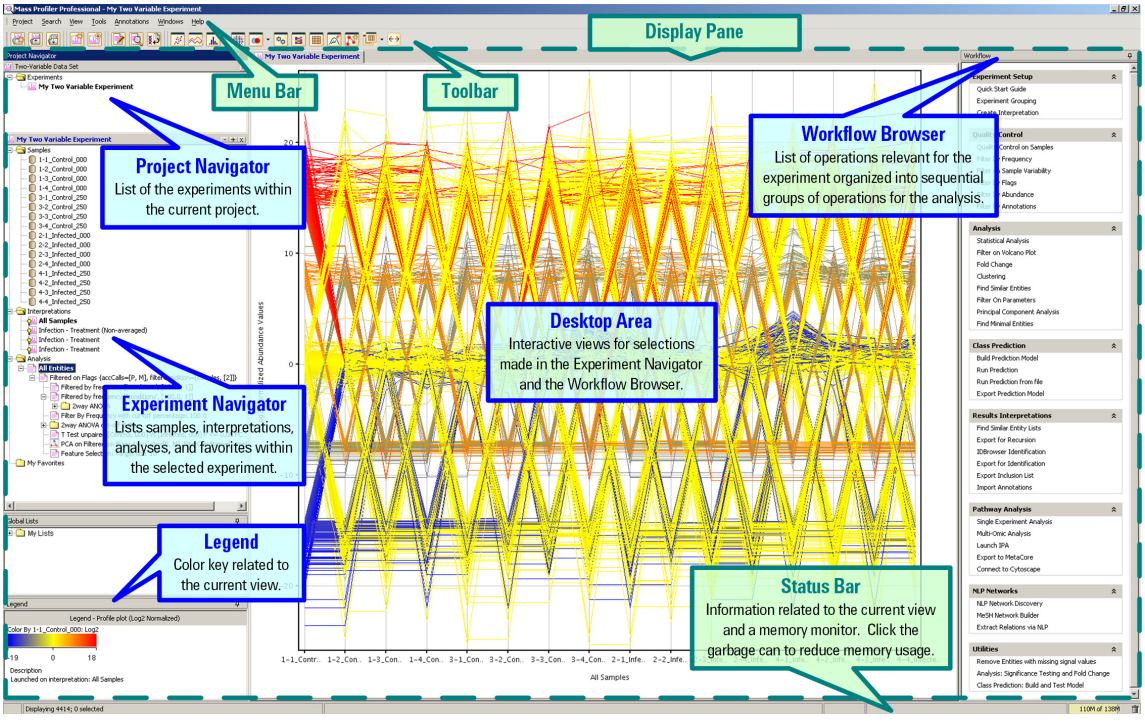
To identify the Entities that passed the fold change cut-off with IDBrowser click on the "IDBrowser Identification" button.

Identify Entities with IDBrowser

Compound	p (Corr)	p	Regulation	FC (abs)	FC	Log FC
C19 H18 N6 O10 S5	4.03E-10	1.19E-11	down	16.00	-18910.66	-14.21
C23 H10 N4 O22	1.29E-03	9.68E-05	up	3.08	3.08	1.62
C14 H6 N6 O5 S3	3.48E-03	2.89E-04	up	2.16	2.16	1.11
C24 H8 N2 O18	6.28E-04	4.65E-05	up	2.60	2.60	1.38
C13 H23 O12 S5	2.52E-03	2.01E-04	up	2.49	2.49	1.32
791.957160.321875	4.07E-04	2.98E-05	down	2.63	2.63	1.39
C20 H15 C1 N4 O16 S	1.30E-11	5.66E-14	down	16.00	-34984.03	-15.09
C14 H16 N2 O10 S5	5.31E-11	6.38E-13	down	16.00	-121746.46	-16.89
C14 H12 N2 O9 S3	1.79E-03	1.37E-04	up	2.01	2.01	1.00
793.927690.31875	3.26E-09	1.48E-10	down	16.00	-41750.41	-15.35
C12 H8 N4 O9 S4	4.63E-02	5.30E-03	down	2.18	-2.18	-1.13
C3 H6 O5 S3	2.31E-02	2.34E-03	down	2.00	-2.00	-1.00
C10 H3 C1 O15	3.91E-02	4.31E-03	down	2.93	-2.93	-1.55
C16 H7 C1 N4 O11 S4	1.30E-11	4.54E-14	down	16.00	-101585.82	-16.63
C6 H8 N2 O3 S5	1.61E-02	1.58E-03	down	3.16	-3.16	-1.66
135.933290.330125	1.49E-02	1.43E-03	down	2.21	-2.21	-1.14
C12 H5 C1 N4 O9 S4	2.10E-12	2.28E-15	down	16.00	-43339.32	-15.40
974.377290.37175	1.94E-08	1.19E-09	down	16.00	-127095.63	-16.96
C13 H23 N9 O2 S	1.11E-05	7.85E-07	up	2.56	2.56	1.36
C13 H14 N4 O	3.33E-10	8.58E-12	down	16.00	-81736.20	-16.32
C16 H23 N5 O10 S3	2.58E-07	1.75E-08	down	16.00	-25534.73	-14.64
C18 H9 N3 O10	4.13E-09	2.07E-10	down	16.00	-235596.20	-17.85
C4 H10 O2 S	4.60E-11	4.28E-13	down	16.00	-232076.61	-17.82
C19 H13 N11 O	6.22E-09	3.53E-10	down	16.00	-27617.06	-14.75
C14 H13 N7 O2 S	7.44E-10	2.43E-11	down	16.00	-218809.95	-17.74
274.560290.62724996	2.25E-02	2.26E-03	up	2.27	2.27	1.18
C19 H17 N3 O17 S2	1.02E-09	3.56E-11	down	16.00	-49757.84	-15.60
C6 H5 N O	3.29E-10	7.90E-12	down	16.00	-214511.75	-17.71
C16 H6 N2 O18 S	1.72E-09	6.76E-11	down	16.00	-42292.68	-15.37
850.788390.74450004	1.76E-11	9.57E-14	down	16.00	-291330.66	-18.15
C5 H6 N4 O3 S3	2.36E-10	5.26E-12	down	16.00	-3825816.50	-21.87
C17 H9 C12 N O8 S5	2.36E-10	5.41E-12	down	16.00	-307682.91	-18.23
C23 H17 N3 O14	9.78E-10	3.31E-11	down	16.00	-49340.76	-15.59
C18 H9 N3 O10 + 0.936	1.02E-09	3.67E-11	down	16.00	-490408.84	-18.90
C28 H25 N O18	4.72E-09	2.52E-10	down	16.00	-315417.03	-18.27
C6 H6 N2 O	5.41E-05	3.90E-06	down	4.30	-4.30	-2.11
156.524792.66825	2.18E-10	4.29E-12	down	16.00	-54384.98	-15.73
C5 H11 N5 O S2	9.15E-11	1.40E-12	down	16.00	-33049.86	-15.01
C8 H14 N4 O3 S	2.71E-09	1.15E-10	down	16.00	-128892.02	-16.98
C11 H14 O2	4.60E-11	4.62E-13	down	16.00	43697.04	16.41

Help << Back Next >> Finish Cancel

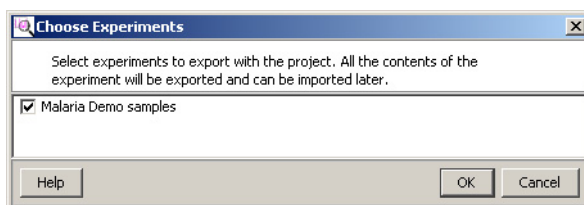
4. Create your Initial Analysis

Steps	Detailed Instructions	Comments
 <p>Project Navigator List of the experiments within the current project.</p> <p>Experiment Navigator Lists samples, interpretations, analyses, and favorites within the selected experiment.</p> <p>Legend Color key related to the current view.</p> <p>Menu Bar</p> <p>Toolbar</p>	<p>Desktop Area Interactive views for selections made in the Experiment Navigator and the Workflow Browser.</p>	<p>Display Pane</p> <p>Workflow Browser List of operations relevant for the experiment organized into sequential groups of operations for the analysis.</p> <p>Status Bar Information related to the current view and a memory monitor. Click the garbage can to reduce memory usage.</p>

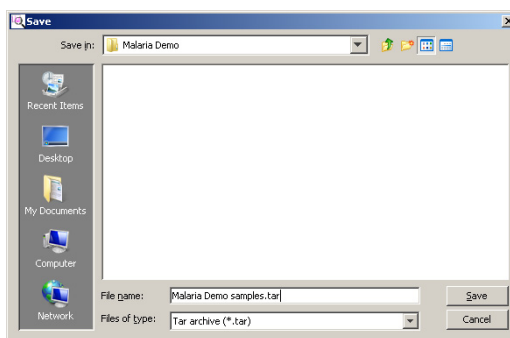
5. Save your project

Save your current analysis as a TAR file for archiving, restoration of any future analysis to the current results, sharing the data with a collaborator, or sharing the data with Agilent customer support.

Steps	Detailed Instructions	Comments
1 Export your project to a TAR file.	<p>a Click Project > Export Project.</p> <p>b Mark the check box next to the experiment you wish to save</p> <p>c Click OK.</p>	<ul style="list-style-type: none"> You have completed creating your project and analyzing an experiment. It is recommended to archive your progress by exporting your project.

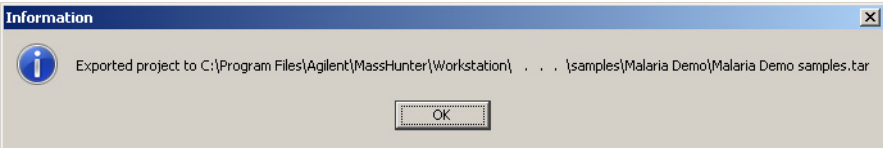


- d** Select or create the file folder.
- e** Type the File name.
- f** Click **Save**.



- g** Click **OK**.

5. Save your project

Steps	Detailed Instructions	Comments
 An information dialog box with a blue title bar labeled "Information" and a close button (X) in the top right corner. The main area contains an information icon (i) and the text "Exported project to C:\Program Files\Agilent\MassHunter\Workstation\ . . . \samples\Malaria Demo\Malaria Demo samples.tar". At the bottom center is an "OK" button. <p>Exported project to C:\Program Files\Agilent\MassHunter\Workstation\ . . . \samples\Malaria Demo\Malaria Demo samples.tar</p>		

6. Perform Advanced Operations

The operations available in the Workflow Browser provide the tools necessary for analyzing features from your mass spectrometry data depending upon the need and aim of the analysis, the experiment design, and the focus of the study. This helps you create different interpretations to carry out the analysis based on the different filtering, normalization, and standard statistical methods.

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In this book

*The Agilent G3835AA
MassHunter Mass Profiler
Professional Software -
Application Guide* presents
additional detail of the
software interface and helps
you use MPP with your data.

This guide applies to MassHunter
Mass Profiler Professional
Software 13.0 and higher until
superseded.

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