

# 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.



# **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.

I	Project	Search	View	Tools	Annotations	<u>W</u> indows	Help	
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#### 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





**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).

Selection Mode Zoom Mode Invert Selection Clear Selection		Selection Mode     Zoom Mode     Invert Selection     Clear Selection
Limit To Selection	n	Limit To Selection
Reset Zoom		Reset Zoom
Copy View	Ctrl+C	Copy View Ctrl
Print	Ctrl+P	Export Column to Dataset
Publish	Ctrl+Y	Print Ctrl-
Export As	•	Export As
Properties	Ctrl+R	Irelis
		⊆atView
		Color By Venn
		Properties Ctrl-

**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.





**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 **m** at any time to reduce the memory usage.

Ξ		Status Area	II		Ticker Area		Memory	Ξ
	Displa	aying 941; 9 selected		(y	= -15.05, 3-4_pH7_pos)		86M of 109M	Î



# **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.

Hass Profiler Professional License Activation
Select License Type
Please choose your license type Individual license 👻
Order ID
You should have a valid OrderID to activate Mass Profiler Professional over the web. To get an OrderID, write to the support team at informatics_support@agilent.com.
MassProfiler OrderID
Gene Spring OrderID
Sure Select OrderID
Pathway OrderID
Example: ABC1-2345-6789
Proxy settings
Use proxy
proxy
port
Authenticate
user
password
OK Cancel



#### **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.

	MassProfiler Order ID: N	-4		
	GeneSpring Order ID: D	-0	- 7	
Surrender	Add/Change			Re-activate
	Plogue			
	Plogue			20000
avadis platform	Module	Exp	res Or	-201
avadis platform avadis analytics	module	Exp Exp	res Or res Or	-201 201
avads platform avads analytics GeneSpring expression	module	Exp Exp Exp	res Or res Or res Or	1 -201: 1 -201: 1 -201:
avadis platform avadis analytics GeneSpring expression MassProfiler	Module	Exp Exp Exp Exp	res Or res Or res Or res Or	-2013 -2013 -2013 -2013 -2013 -2013
avadis platform avadis analytics GeneSpring expression MassProfiler Pathway	Ploque	Ε.φ Ε.φ Ε.φ Ε.φ	res Or res Or res Or res Or res Or	-2013 -2013 -2013 -2013 -2013 -2013 -2013 -2013

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.

Confirm	Surrender X
0	This operation allows you to use the application with the same OrderId on another computer. Are you sure you want to surrender the license?
	<u>Yes</u> No

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.

🔊 🕞 🔹 🔹 Control Panel 🔹 🖌	All Control Panel Items • Programs and Features			- 🔯 I	Search Programs	and Features	
e Edit View Iools Help							
Control Panel Home	Uninstall or change a program						
View installed updates	To uninstall a program, select it from the list and then click Uni	nstall, Change, or Repair.					
Turn Windows features on or off							0++
	Organize  Uninstall/Change Name #	- Dubleher	al tostal al	0 m ( -	Version	1-1	8== •
	ArtiveState Konodo Edit 6, 1, 2	ActiveState Software Inc.	7/29/2011	155 MR	6.1.2		
	Adobe AIR	Adobe Systems Inc.			2.5.1.17730		
	Adobe Flash Player 10 Plugin	Adobe Systems Incorporated		6.00 MB			
	Adobe Reader X (10.1.1)	Adobe Systems Incorporated		160 MB			
	Adobe Shockwave Player 11.5	Adobe Systems, Inc.			11.5.9.615		
	Agient MassHunter ID Browser		5/15/2011	54.4 MB			
	Aglient MassHunter Workstation Acquisition Software	Aglent Technologies	8/29/2011	145 MB	5.0.5023.0		
	Aglent MassHunter Workstation Qualitative Analysis B.05.00	Aglent Technologies	9/15/2011	143 MB	1.00.0000		
	🕐 Dell Driver Download Manager	Del Inc.			2.1.0.0		
	ESET Smart Security	ESET, spol. s r.o.	10/1/2011	82.0 MB	5.0.93.0		
	Mass Profiler Pro	Strand Life Sciences Pvt Ltd	10/2/2011		3.2.0.0		
	🕐 Roxio Creator Tools	Roxio	2/4/2011	348 KB			
	Roxio Express Labeler	Roxio	2/4/2011	15.5 MB	2.1.0		
	😁 Roxio Update Manager	Roxio	2/4/2011	2.39 MB	3.0.0		
	System Requirements Lab						

**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.	<ul> <li>This will open the <b>Startup</b> dialog box.</li> </ul>		
<ul> <li>Open a recent project:</li> <li>2 Open the recent project named Malaria in the Startup dialog box.</li> </ul>	<ul> <li>a Click the Open recent project button.</li> <li>b Select Malaria from the Select recent project list.</li> <li>c Click the OK button.</li> </ul>	<ul> <li>Open recent project opens the project and the experiment that was stored in the project.</li> <li>A project is a container for a collection of experiments. A project</li> </ul>		
Startup      Welcome to MassProfiler Pro     Select what you would like to do from the options by     to continue.	elow, then dick on OK	can have multiple experiments on different sample types and organisms.		
Options C Create new project Open existing project Open recent project Select recent project Do not show this dialog again Help	Cancel			

Steps	Detailed Instructions	Comments		
Open an existing project:         3       Open an existing project named Malaria in the Startup dialog box.         Image: Creation Date Modif Malaria       Modif Modif Malaria         Sat May 21 14:1 Sat May       Displaying results from 1 to 1 of 1. Showing page         Help       Help	<ul> <li>a Click the Open existing project button in the Startup dialog box in step 2 on page 12.</li> <li>b Click the OK button.</li> <li>c Select the Malaria project row from the Open Project dialog box.</li> <li>d Click the Open button.</li> </ul>	<ul> <li>Open existing project allows you to open a project that is no longer displayed in the recent project list.</li> <li>Opens the project and the experiment that was stored in the project.</li> <li>When you have a large number of prior projects you may have to use the page navigation buttons in the <b>Open Project</b> dialog box.</li> <li>A selected project will be highlighted by a color based on your PC's system settings.</li> </ul>		

#### 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.

🔍 Create New Project	×	
New Project Details		
Name	New Project	
Notes		>
Help	OK Cancel	

New Project Details		
	Name (Mai	ana Project
	Notes Pro	oject containing the Agilent Malaria Demo

Steps	Detailed Instructions	Comments
	e Click the <b>Open existing experiment</b> button in the <b>Experiment Selection</b> <b>Dialog</b> dialog box.	
Experiment Selection Dialog	×	
An experiment is an organized collection of LC/MS given data source. If you have an experiment you project, please choose "Open existing experiment, experiment with new data or previously imported or	or GC/MS sample data from a wish to use from a previous " You may also create a new lata.	
Choose Experiment		
C Create new experiment     Dpen existing experiment		
Help	OK Cancel	
	f Select the <b>Malaria</b> experiment in the <b>Add Experiment</b> dialog box	<ul> <li>A selected experiment will be shown highlighted using a background color.</li> </ul>

🝳 Add Experiment 🛛 🗙				
Name	<b>Creation Date</b>	Modified Da	Owner	Technology
Malaria LCM	Sat May 21	Sat May 21	gxuser	MassHunter
Displaying result	ts from 1 to 1 of 1	. Showing page	1	() of <b>1</b>
Help			Ad	d Cancel

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 A on your desktop, or click Start > Programs > Agilent > MassProfilerPro > MassProfilerPro.	<ul> <li>This will open the Startup dialog box.</li> </ul>
2 Create a new project in the <b>Startup</b> dialog box.	<ul><li>a Click the Create new project button.</li><li>b Click the OK button.</li></ul>	<ul> <li>Create new project allows you to import an existing project and experiments into the new project.</li> <li>After closing an open project, you</li> </ul>
Startup     Welcome to MassProfiler Pro     Select what you would like to do from the options be     to continue.     Options     © Create new project     © Open existing project     © Open recent project     Select recent project     New Project     Do not show this dialog again     Help	elow, then dick on OK	<ul> <li>(1) Menu bar by clicking         <ul> <li>Project &gt; New Project, or</li> <li>(2) Toolbar by clicking                 the New project button.</li> <li>You will then be prompted to enter                 your project information in the next                 step.</li> </ul> </li> <li>A project is a container for a                 collection of experiments. A project                 can have multiple experiments on                 different sample types and</li> </ul>

Steps	Detailed Instructions	Comments
3 Type descriptive information in the New Project Details in the Create New Project dialog box.	<ul> <li>a Type Malaria Project in Name.</li> <li>b Type Project containing the Agilent Malaria demoin Notes.</li> <li>c Click the OK button.</li> </ul>	<ul> <li>The project name and notes may be viewed and edited at any time using the Project Inspector dialog box by clicking Project&gt;Inspect Project from the menu bar.</li> </ul>
Create New Project  - New Project Details  Name New Project Notes  Help OK Cance	Create Hew Project  New Project Details  Name Malaria Project  Notes Project containing the Agilent t  Help  OK	Malaria Demo]
4 Select the option to create a new experiment in the Choose Experiment in the Experiment Section Dialog dialog box. Section Dialog dialog box.	<ul> <li>a Click the Create new experiment button.</li> <li>b Click the OK button.</li> </ul>	<ul> <li>You may create or add a new experiment from the Menu bar by clicking Project &gt; New Experiment, or Toolbar by clicking the New experiment is button, or the Add experiment is button.</li> <li>You will then be prompted to enter your experiment information in the next step.</li> <li>If you click the Open existing experiment button, you are</li> </ul>
An experiment is an organized collection of LC/MS or GC/ given data source. If you have an experiment you wish t project, please choose 'Open existing experiment. 'You experiment with new data or previously imported data. Choose Experiment Choose Experiment Copen existing experiment Help	MS sample data from a ouse from a previous may also create a new	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.	<ul> <li>a Type the descriptive name Malaria Demo for the experiment in Experiment name. This name may be different from the project name previously entered.</li> <li>b Select Mass Profiler Professional for Analysis type.</li> <li>c Select Unidentified for the Experiment type.</li> <li>d Select Data Import Wizard for Workflow type. Analysis: Significance Testing and Fold Change is covered in another Quick Start Guide.</li> <li>e Type Agilent demonstration data in Experiment notes.</li> <li>f Click the OK button.</li> </ul>	<ul> <li>The selection of experiment type determines how Mass Profiler Professional manages the data.</li> <li>Unidentified is the proper experiment type selection when the compounds have only been identified by their molecular features of neutral mass and retention time.</li> <li>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.</li> </ul>	

New Experiment	<u>^</u>		www.experiment	<u>×</u>
Experiment description			Experiment description	
Enter a name, analysis type, experiment t statistical significance test and fold chang only. "Class Prediction" will guide you thro training data.	ype and a desired workflow type. "Analysis" will guide you through a analysis. "Data Import" will guide you through experiment creation gigh the creation and testing of a prediction model, using imported		Enter a name, analysis type, experiment ty statistical significance test and fold change only. "Class Prediction" will guide you throu training data.	ype and a desired workflow type. "Analysis" will guide you through a analysis. "Data Import" will guide you through experiment creation gh the creation and testing of a prediction model, using imported
Experiment name	New Experiment		Experiment name	My Malaria Demo
Analysis type	Expression		Analysis type	Mass Profiler Professional
Experiment type	Aglient Expression Two Color	>	Experiment type	Unidentified
Workflow type	Analysis: Biological Significance	-	Workflow type	Data Import Wizard
Experiment notes			Experiment notes	Test experiment to recreate the Malaria project supplied in MPP.
	1			
Help	OK Cancel		Help	OK Cancel

- 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.

St	eps	Detailed Instructions	Comments
1	Create a new project.	<ul> <li>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."</li> </ul>	<ul> <li>A project is a container for a collection of experiments. A project can have multiple experiments on different sample types and organisms.</li> </ul>
2	Create a new experiment.	<ul> <li>a Click the Create new experiment button or Project &gt; New Experiment.</li> <li>b Click the OK button.</li> </ul>	
3	Type and select information that guides the experiment creation in the <b>New Experiment</b> dialog.	<ul> <li>a Type the descriptive name of your data, such as My Malaria Demo for the demo data in Experiment name.</li> <li>b Select Mass Profiler Professional for Analysis Type.</li> </ul>	• Table 1 and Table 2 on page 20 show selection and entry options available for the <b>New Experiment</b> dialog box
	Enter a name, analysis type, experiment statistical significance test and fold chan only. "Class Prediction" will guide you thr training data.	: type and a desired workflow type. "Analysis" will guid ge analysis. "Data Import" will guide you through expe ough the creation and testing of a prediction model, u	de you through a eriment creation Ising imported
	Experiment name	e My Malaria Demo	
	Analysis type	e Mass Profiler Professional	
	Experiment type	e Unidentified	<b>_</b>
	Workflow type	e Data Import Wizard	<b></b>
	Experiment note	s Test experiment to recreate the Malaria project s	upplied in MPP.
	Help		OK Cancel

### Steps Detailed Instructions

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

Dialog Box Field	Your Choices	Comments
Experiment name	<none></none>	Edit field to describe this experiment
Analysis type	Mass Profiler Professional <other choices="" depending="" ids="" on="" order=""></other>	"Mass Profiler Professional" must be selected
Experiment type	Combined (Identified and Unidentified) Identified Unidentified	<see next="" table=""></see>
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	Entries identified by Compound (column C), Formula (column D),
		*.XLSX	CASID (column E)
		*.CSV	
		*.TXT	
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	Entries NOT identified by Compound (column C), Formula
		*.XLSX	(column D), CASID (column E)
		*.CSV	
		*.TXT	
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	Targets and components discovered by AMDIS
		*.ELU	
	Generic	*.XLS	Combination of entries identified by and not identified by
		*.XLSX	Compound (column C), Formula (column D), CASID (column E)
		*.CSV	
		*.TXT	

Comments

Steps	Detailed Instructions	Comments
	<ul> <li>c Select your Experiment type, such as Unidentified for the demo data.</li> <li>d Select Data Import Wizard for Workflow type.</li> <li>• NOTE: The same import steps apply if you select Analysis: Significance Testing and Fold Change.</li> <li>e Type information relevant to your experiment in Experiment notes, such as Test experiment to recreate the Malaria project supplied in MPP data.</li> <li>f Click the OK button.</li> </ul>	<ul> <li>Unidentified is the proper selection when the compounds have only been identified by their molecular features of neutral mass and retention time.</li> <li>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.</li> </ul>
<ul> <li>Select the data source in the MS Experiment Creation Wizard (Step 1 of 11).</li> </ul>	<ul> <li>a Click the description that matches the source of your data.</li> <li>If you are using the sample Malaria data set click MassHunter Qual.</li> <li>If you are using your own data set, click the source of the sample files.</li> <li>b Select the organism represented by your data.</li> <li>c Click the Next &gt;&gt; button.</li> </ul>	<ul> <li>The available data sources will depend on your selection of Experiment type in the previous step shown in Table 2.</li> <li>Selection of an Organism is important if you plan to use pathways.</li> <li>To control your progress through the wizard dialog boxes:</li> <li>Click the Next Selection</li> </ul>
MS Experiment Creation Wizard (Step 1 of 11)		button to go to the pext step
Select Data Source Choose the data sources that will be used for the experiment		Click the << <b>Back</b> << <b>Back</b>
MassHunter Quant     MassHunter Qual     MassHunter Qual     MassHunter Qual     MassHunter ICP-MS     Chemstation     AMDIS     Generic  Organism None Homo sapiens Mus musculus Rattus norvegicus Anopheles gambiae Arabidopsis thaliana Bacillus subtils Bos taurus		<ul> <li>and make modifications to your settings and previous entries.</li> <li>Click the Cancel <u>Cancel</u> button to end the MS Experiment Creation without saving. You may then restart creating a new project and experiment.</li> </ul>
Help	<< Back Next >> Einish Cancel	

Steps	Detailed Instructions	Comments
<ul> <li>Select the sample data to import in the MS Experiment Creation Wizard (Step 2 of 11).</li> </ul>	a Click the Select Data Files button.	• The file type you need to select will depend on the data source you selected in the <b>MS Experiment</b> <b>Creation Wizard (Step 1 of 11)</b> .
HS Experiment Creation Wizard (Step 2 of 11)           Select Data to Import           Data may be imported from files or previous experiments           Type         S	ielected files and samples	<ul> <li>See Table 2 on page 20 for a comprehensive list of data sources you may select from based on your experiment type.</li> </ul>
Select Data Files Select S	amples Remove           Remove           < <back< td="">         Next &gt;&gt;</back<>	
	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
	<ul> <li>d Click the sample molecular feature data files to import into the experiment. The example Malaria data files are:</li> <li>1-1_pH7_pos_01.cef</li> <li>1-2_pH7_pos_01.cef</li> <li>1-3_pH7_pos_01.cef</li> <li>3-1_pH7_pos_01.cef</li> <li>3-2_pH7_pos_01.cef</li> <li>3-2_pH7_pos_01.cef</li> <li>3-3_pH7_pos_01.cef</li> <li>3-4_pH7_pos_01.cef</li> </ul>	<ul> <li>The Open dialog box uses conventional buttons and icons that allow you to navigate and view your file system.</li> <li>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.</li> <li>You may select discontinuous, individual files with a Ctrl-click on any file.</li> </ul>
Computer		
File game:         los_01.cef" "3-3_pH7_pos_01.cef" "3-4           Network         Files of type:         MassHunterQual - Unidentified (".CEF)	pH7_pos_01.cef	

- 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
HS Experiment Cr Select Data to In Data may be impor Type	f Click the Next >> button.  reation Wizard (Step 2 of 11)  mpot  reation Mizard (Step 2 of 11)  mpot  reation files on previous experiments  Selected files and samples  1-1_pH7_pos_01.cef  1-2_pH7_pos_01.cef  1-3_pH7_pos_01.cef  3-3_pH7_pos_01.cef  3-3_pH7_pos_01.cef  3-3_pH7_pos_01.cef  3-4_pH7_pos_01.cef  3-4_pH7_pos_01.c	<ul> <li>You can review and make changes to your selection during the next step before finalizing the experiment creation.</li> <li>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.</li> </ul>
Review ar that will b <b>Experime</b> <b>5 of 11)</b> .	<ul> <li>a Click one or more samples to want to reorder.</li> <li>b Click the Up of or Down of to reorder the selected samples.</li> <li>c Repeat the reordering action as necessary to obtain your</li> <li>d Mark the sample names tha imported into your experime</li> <li>e Click the Next &gt;&gt; button.</li> </ul>	<ul> <li>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.</li> <li>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.</li> </ul>
Deselect the sample them up or down. Select V V V V V V V V V	Jets that need not be imported. To re-order the samples, select the samples and use the appropriate buttons to move.           Same sample order will be used everywhere in experiment.           I-2_pht7_pos_01           I-3_pht7_pos_01           I-4_pht7_pos_01           3-1_pht7_pos_01           3-2_pht7_pos_01           3-3_pht7_pos_01           3-3_pht7_pos_01           3-4_pht7_pos_01	<ul> <li>You may Ctrl-Click any sample name to select multiple samples.</li> <li>Click the <b>Restore</b> <i>C</i> button at any time after any sample reorder to return the sample order to your starting point when this step was begun.</li> </ul>

<< Back Next >> Finish Cancel

Help

Steps	Detailed Instructions	Comments
Steps         Image: Contract State Structure of your experiment in the structure of your experiment in the MS Experiment Creation Wizard (Step 6 of 11).         Image: Creation Wizard Step 6 of 11).         I	<ul> <li>a Click the Add Parameter button.</li> <li>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.</li> </ul>	<ul> <li>Comments</li> <li>NOTE: Grouping at this time is optional. You may add grouping or change your grouping at any time.</li> <li>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.</li> <li>The attribute values within an independent variable are referred to as parameter values. Samples with the same parameter values. Samples with the same parameter values within a parameter name are treated as replicates.</li> <li>Parameter Type options:</li> <li>Select Non-Numeric if the grouping is not a quantitative value.</li> </ul>
Cladd/Edit Experiment Parameter       X         Grouping of Samples       Samples with the same parameter values are treated as replicate samples. To sample samples their parameter values, select the samples and click on the "Assign Values" button, and enter the value for the group. Set the parameter values as numbers.       Parameter same infection         Parameter type       Non-Numeric       Y         Samples 01       Parameter Values       Parameter Values         12.ptf / pos.01       13.ptf / pos.01       14.ptf / pos.01         32.ptf / pos.01       33.ptf / pos.01       33.ptf / pos.01         33.ptf / pos.01       33.ptf / pos.01       33.ptf / pos.01         Assign Value       Clear       Methy	<ul> <li>c Click your replicate Samples that share the same first parameter value in your data. For example:</li> <li>1-1_pH7_pos_01</li> <li>1-2_pH7_pos_01</li> <li>1-3_pH7_pos_01</li> <li>1-4_pH7_pos_01</li> <li>d Select the Parameter type for your grouping. Non-Numeric is selected for the example Malaria data set.</li> <li>e Click the Assign Value button.</li> </ul>	<ul> <li>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.</li> <li>Entry of numerical parameter values for a numeric parameter type will allow some data plots to be scaled by the parameter value.</li> <li>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.</li> <li>You may select discontinuous, individual files with a Ctrl-click on any file.</li> </ul>

<ul> <li>f Type the value the Assign Value occord</li> <li>g Click the OK bit h Click your repl share the sam value in your do samples and dch on the 'Asson Values' botton, and enter the value for the granetter values are treated as replace samples are dick on the 'Asson values' botton, and enter the value for the granetter values are interest or are the samples are infected</li> <li>3-1_pH7_point</li> <li>3-2_pH7_point</li> <li>3-4_pH7_point</li> <li>3-4</li></ul>	ions Comments
individual or m	IonsCommentsfor your first grouping in ue dialog box. For the at the first value typed is red.In this example the samples will be assigned a value representing the Infection Parameter name.red.The highlighted samples will be assigned the value typed in the Assign Value dialog box.rata. For example: os_01 os_01 os_01The highlighted samples will be assigned a value representing the Infection Parameter name.os_01 os_01 ameter type for yourIn this example the samples will be assigned a value representing the Infection Parameter name.os_01 os_01 ameter type for yourIn this example the samples will be assigned a value representing the Infection Parameter name.os_01 os_01 ameter type for yourIn this example the samples will be assigned a value representing the Infection Parameter name.os_01 os_01 ameter type for yourIn this example the samples will be assigned a value representing the Infection Parameter name.os_01 os_01 ameter type for yourInfection Parameter name.os_01 
individual or m	ue assignments for
necessary to n	ultiple samples as
changes.	nake corrections or
p Click the <b>OK</b> by	utton when the grouping
for this parame	eter is complete.

Steps			etailed Inst
Add/Edit Experiment Pa Grouping of Samples Samples with the same pa samples. To assign replace the samples and dick on the value for the group. Set the the parameter values as n	rameter rameter values are treated as replicate te samples their parameter values, select re 'Assign Values' button, and enter the he parameter type to 'numeric' to interpret umbers.	q	Repeat the your data l independe • Click th • Repeat have as
Parameter name	Infection		type, ar
Parameter type	Non-Numeric	-	
Samples	Parameter Values		Before cor
1-1_pH7_pos_01	Not Infected		f - 11
1-2_pH7_pos_01	Not Infected	_	tollowing (
1-4 pH7 pos 01	Not Infected		RECOMM
3-1_pH7_pos_01	Infected		
3-2_pH7_pos_01	Infected		parameter
3-3_pH7_pos_01	Infected	_	on nage 28
Assig	n Value		importing informatio page 29. T advanced
Help	OK Cano	el	parameter

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#### **Detailed Instructions**

- 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.

O MS Experiment Creation Wizard (Step 6 of 11)		×		
Experiment Grouping				
Experiment parameters define the grouping or replicate structure of "Add Parameter" button. You may enter as many parameters as you the guided workflow. Other parameters can be used in the advance values here.	your experiment. Enter experiment parameters by clicking on the like, but only the first two parameters will be used for analysis in analysis. You can also edit and re-order parameters and parameter			
Displaying <b>8</b> sample(s) with <b>1</b> experiment parame	ter(s). To change, use the button controls below.			
Spreadsheet		<b>џ</b>		
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			
Add Parameter Edit Parameter Delete Parameter				
Help	<< Back Next >> Einish Gancel			

•	You may review and edit the
	parameters and parameter value
	assignments.

Comments

- 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 <ul> <li>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.</li> </ul>		
8 OPTIONAL: Re-ordering parameter values in the <b>Spreadsheet</b> .			ameter	<ul> <li>a Click any one value in the parameter column to select the whole parameter column.</li> <li>b Re-order the parameter columns by selecting a parameter column and then clicking on the Left or Right button.</li> </ul>					
<b>Q</b> M	5 Experiment Creatior	n Wizard (Step 6 of 11)			×	1	•	when the parameter column is	
Exp	eriment Grouping							selected the column will be	
	Experiment parameters d 'Add Parameter" button, the guided workflow. Oth values here.	efine the grouping or replicate structur You may enter as many parameters as er parameters can be used in the adva	e of your experiment. you like, but only the nced analysis. You car	Enter experiment parameter first two parameters will be u n also edit and re-order para	s by clicking on the ised for analysis in neters and parameter			highlighted.	
	Display	ying 8 sample(s) with 3 experiment par	ameter(s). To change,	use the button controls belo	w.				
Sprea	dsheet	A.12			- Ļ				
<u>b</u>		<u>1</u>							
1 1	Samples	Infection	Test	Group	Test				
1-1.	pH7_p0s_01 pH7_pos_01	Not Infected	Value 1	01					
1-3	pH7_pos_01	Not Infected	Value 3	01					
1-4	pH7_pos_01	Not Infected	Value 1	01					
3-1.	pH7_p0s_01 pH7_pos_01	Infected	Value 3	02					
3-3	pH7_pos_01	Infected	Value 3	02					
3-4	pH7_pos_01	Infected	Value 2	02					
<u> </u>		Add Parameter Edit	Parameter De	lete Parameter					
	Help			<back next="">&gt;</back>	Enish <u>C</u> ancel	-			



- 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 <b>Spreadsheet</b> .	<ul> <li>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.</li> <li>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 parameter grouping may also be found in some sample files by clicking the Import parameters from samples Sutton.</li> </ul>	<ul> <li>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"</li> <li>The .tsv file is organized using tab separated values (tsv) that may be created, edited, and viewed using Microsoft Excel or Notepad.</li> <li>Creating and editing experiment parameter groupings may be more convenient for you using Excel.</li> </ul>		

	🚽 🤊 -	(°4 ×   ∓ - MA	ALARIA EXPERIM	IENT PARAME	TERS (to be	loaded fro	m file).tsv -	Microso	ft E	- •	8
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		A	В		С	D	E		F	G	
1	Samples		Group								
2	1-1_pH7_	_pos_01	Uninfected								
3	1-2_pH7_	_pos_01	Uninfected								=
4	1-3_pH7_	_pos_01	Uninfected								
5	1-4_pH7_	_pos_01	Uninfected								
6	3-1_pH7_	_pos_01	Infected								-
7	3-2_pH7_	_pos_01	Infected								
8	3-3_pH7_	_pos_01	Infected								
9	3-4_pH7_	_pos_01	Infected								
10											
11											
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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 3-4_pH7_pos	<u> </u>
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Steps	Detailed Instructions	Comments
10 Select and enter the data filter parameters in the MS Experiment Creation Wizard (Step 7 of 11).	<ul> <li>a Mark the Minimum absolute abundance check box under the Abundance filtering heading.</li> <li>• NOTE: Example values are for the Malaria data set.</li> <li>b Type a value of 5000 counts.</li> <li>c Clear the Limit to the largest and Minimum relative abundance check boxes.</li> <li>d Mark the Use all available data check box under the Retention time filtering heading.</li> <li>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.</li> <li>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.</li> <li>g Click the Multiple charge states forbidden button under the Charge states heading.</li> <li>h Click the Next &gt;&gt; button.</li> </ul>	<ul> <li>The filtering parameters dialog box will be unique for each experiment type. More information may be found in the online help.</li> <li>MassHunter Qualitative Analysis data used in this example presents the most active fields.</li> <li>Filtering during the data import process may be used to reject low-intensity data or restrict the range of data.</li> <li>In a find by molecular feature generated data file the term abundance actually refers to the feature volume.</li> <li>In a find by formula generated data file the term abundance actually refers to the feature chromatographic area.</li> <li>Filtering by maximum mass may improve the statistical analysis by rejecting masses that are not significant to the experiment. This is especially relevant to</li> </ul>
HS Experiment Creation Wizard (Step 7 of 11) Filtering Filtering during the data import process may be used to reject low inter there are several filtering options that may be applied: Filter by Frequency memory and the synthese of Model long.   Abundance filtering   Ø Minimum absolute abundance   State   Iminimum relative abundance   %   Retention time filtering   Ø Unimum absolute abundance   %   Retention time filtering   Ø Use all available data   Max RT (15:42)   Number of lons   C Single ion compounds only	Al charge states permitted  Althore states permitted  Autopic charge states permitted  Autopic charge states forbidden  Autopic charge states  A	<ul> <li>metabolomic samples.</li> <li>The filter parameters may be cleared to preserve the prior filtering that was used to generate the feature data file.</li> <li>Filtering works with both GC/MS and LC/MS data.</li> <li>After data is imported, several additional filtering options may be applied: Abundance, Retention Time, Mass, Flags, Number of ions, Mass and Minimum Quality Score.</li> </ul>

Steps	Detailed Instructions	Comments
<ul> <li>Select and enter the retention time and mass alignment parameters in the MS Experiment Creation Wizard (Step 8 of 11).</li> </ul>	<ul> <li>a Clear the Perform RT correction check box under the Retention Time correction heading.</li> <li>NOTE: Example values are for the Malaria data set.</li> <li>b Type 0.1% and 0.15 min for RT Window under the Compound alignment heading.</li> <li>Smaller RT Window values result in reduced compound grouping among the samples leading to a larger list of unique compounds in the experiment</li> <li>c Type 5.0 ppm and 2.0 mDa for Mass Window.</li> <li>It is not recommended to set the mass window less than 2.0 mDa for higher masses.</li> <li>d Click the Next &gt;&gt; button.</li> </ul>	<ul> <li>The alignment parameters dialog box will be unique for each experiment type. More information may be found in the online help.</li> <li>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.</li> <li>A larger retention time shift may be used to compensate for less than ideal chromatography.</li> <li>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</li> </ul>
Its Experiment Creation Wizard (step & of 11)         Alignment Parameters         Unidentified compounds from different samples are aligned or grouped to be rance window and the mass spectral similanity as determined by a similarity as determined by as	Ogether if their retention times are within the specified inple dot product calculation above the specified level.         D.5         2.0         mOs         mOs         0.15         2.0         mOs	<ul> <li>standards the correction is based on a piecewise linear fit</li> <li>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.</li> <li>The alignment methodologies for the data types are explained in Section 2.1.5 in the Mass Profiler Professional User Manual.</li> <li>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.</li> </ul>

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).	<ul> <li>a Clear the Export for Recursion check box.</li> <li>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</li> </ul>	<ul> <li>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.</li> <li>It is useful to click the &lt;&lt; Back</li> </ul>		

following steps.

- 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.





#### **Detailed Instructions**

13 View and review the compounds present and absent in each sample in the MS Experiment Creation Wizard (Step 10 of 11).

Steps

- **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.

Comments

🍳 MS Experi	ment Creation	Wizard (Step 10 of 11)			×	
Normalization Criteria The compounds associated with each sample may be normalized to an internal standard, percentile shift, quantile and/or an external scalar.						
Normalization	External Scalar					
Use Extern	nal Scalar					
	S	amples		Scale To Value		
1-1_pH7_pd	os_01		Í		1.0	
1-2_pH7_pc	os_01				1.0	
1-3_pH7_pd	os_01				1.0	
1-4_pH7_pd	os_01				1.0	
3-1_pH7_pd	os_01				1.0	
3-2_pH7_pd	os_01				1.0	
3-3_pH7_pd	os_01				1.0	
3-4_pH7_pd	os_01				1.0	
Help				<< Back	Einish <u>C</u> ancel	

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	<ul> <li>a Click the Baseline to of all samples button.</li> <li>b Select median for the Baseline to of all samples.</li> <li>c Click the Finish button Enish .</li> </ul>	<ul> <li>There are four baselining options:</li> <li>None: Recommended if only a few features in the samples exist.</li> <li>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 dat set from recursion.</li> <li>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 o reducing the weight of very large</li> </ul>	
MS Experiment Creation Wizard (Step 11 of 11) Baselining Options There are four baseline options. None - This option will treat compounds with large intensities as more of 2 Transform - This option should be used when comparing data from d Baseline each entity to median/mean across samples or control sample     for intensity.  Options  Options  C Inone  C Z-Transform  G Baseline to median  of all samples  G Baseline to median  of control samples	ignificant than compounds with lesser intensities.         (fferent sources.         s - These options will treat all compounds equally regardless of		
Index         Sample           11-1_PH7_pos_01         2           21-2_PH7_pos_01         3           31-3_PH7_pos_01         4	es Control Samples	on later statistical analyses.	
5]3-1,pH7,pos,01 6]3-2,pH7,pos,01 7]3-3,pH7,pos,01 8]3-4,pH7,pos,01		samples: The abundance for each compound is normalized to its selected statistical abundance	
Assign Value	clear       < <back< td="">     Mext&gt;&gt;       Enish     Cancel</back<>	across just the samples selected as the control samples. This has the effect of weighting the compound features to a known value that is	

population while reducing the effect of large and small compound

features.

Steps	Detailed Instructions	Comments	
<b>15</b> Export your project. This is the end of the Data Import Wizard.	<ul> <li>a Your MPP screen view will appear with a profile plot of your data.</li> <li>b It is recommended that you export your project by clicking Project &gt; Export Project.</li> <li>• The project may be loaded by clicking Project &gt; Import Project after you close an open project or when you start a new session of MPP.</li> </ul>	<ul> <li>Your project is now ready to begin your analysis.</li> <li>Exporting your project allows you to quickly return to the "saved" point in your analysis. You may export frequently.</li> <li>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.</li> </ul>	



# 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 <b>Analysis:</b> <b>Significance Testing and Fold</b> <b>Change</b> wizard.	<ul> <li>a Obtain a copy of the "Analysis: Significance Testing and Fold Change Quick Start Guide" (G3835-90003).</li> <li>b Click Analysis: Significance Testing and Fold Change under the Utilities section of the Workflow Browser.</li> <li>c Follow the steps presented in the quick start guide.</li> </ul>	<ul> <li>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.</li> </ul>	
2	Customize your analysis using operations available within the <b>Workflow Browser</b> .		<ul> <li>Operations available within the Workflow Browser allow you to customize your experiment.</li> </ul>	

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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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