MassHunter Profinder Software

Quick Start Guide

For Research Use Only. Not for use in diagnostic procedures.

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What is MassHunter Profinder?

Agilent MassHunter Profinder software is a stand-alone program, optimized for batch feature extraction from TOF and Q-TOF based profiling data files and MSD data files. Profinder is also part of the MassHunter VistaFlux software. Feature extraction, combined with chromatographic alignment across multiple data files, is a critical step in the peak finding and data reduction workflow that minimizes the appearance of both false positive and false negative features by “binning” the features in the chromatographic time domain. Profinder reduces your acquired data size and complexity through the removal of redundant and non-specific information by identifying the

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important variables (features) associated with the data. Careful feature extraction yields a smaller data set that is more easily processed without any compromise in the information quality.

Profinder is optimized to not only extract features from large data sets but also provides you with an intuitive user interface to inspect and review each feature across the files associated with your data set. With Profinder you can review and compare extracted ion chromatograms, mass spectral data, and isotopologues associated with each feature. You can save your feature extraction progress to a project to review, adjust, and reprocess at a later time.

The feature files generated by Profinder (CEF files) can be imported into Agilent Mass Profiler Professional (MPP), a chemometrics platform used in any MS-based differential analysis to determine relationships among two or more sample groups and variables. Profinder Archive files (PFA files) containing extracted compound features and sample group information are imported into Omix Premium, a visualization platform, to view your data in combination with biochemical networks, including isotopologue results as part of a metabolic flux analysis.

What's new in B.08.00?

- Isotopologue feature extraction support is added for metabolic flux analysis in MassHunter VistaFlux Software.
- Profinder Archive export provides a single-file transfer of project data to Omix Premium.
- Untargeted and recursive feature extraction is supported for GC/Q-TOF and GC/MSD.
- Targeted All Ions fragment confirmation is supported for LC/TOF, LC/Q-TOF and GC/Q-TOF data files.
- Additional feature extraction algorithms are multithreaded and memory optimized to support large projects and datasets.

Features of Profinder

- Open all of the files associated with your Agilent TOF and Q-TOF MS data set (sample files) in a single selection process.
- Create methods for your feature extraction and chromatographic alignment by following a guided step-by-step wizard for the selected algorithm.
- Choose from five feature extraction algorithms:
  - Batch Molecular Feature Extraction
  - Batch Recursive Feature Extraction (small molecules/peptides)
  - Batch Recursive Feature Extraction (large molecules)
  - Batch Targeted Feature Extraction
  - Batch Isotopologue Extraction
- Extract the features from all of the data files in your data set by running a single algorithm.
- Automatically generate an extracted ion chromatogram (EIC) and extracted compound chromatogram (ECC) for each feature found in each data file.
- Review the chromatographic integration/alignment, mass spectra, and isotopologues using an intuitive graphical interface.
- Curate the features in your data post-analysis.
- Export your extracted features via three file formats:
  - Compound exchange format (CEF) is a file format that is used to exchange data between Agilent MassHunter software. CEF files, for example, are imported into MassHunter Mass Profiler Professional for statistical analysis.
  - Comma separated values (CSV) is an ASCII text file that is readily imported into text editors and spreadsheets.
  - Profinder Archive (PFA) is a binary file format used to transfer your features to Omix Premium for metabolic flux analysis and visualization within the context of biochemical networks.

- Save your feature extraction parameters as Profinder methods.
- Save your feature extraction progress as a Profinder project file and open the project at a later time to review, adjust, and reprocess your data files.

Where is Profinder used in your experiment?

Profinder is used to extract the features from LC/TOF, LC/Q-TOF, GC/Q-TOF, and GC/MSD data files you acquired from your experimental samples. An untargeted differential analysis experiment may include the steps shown below.

1. Prepare for your experiment
2. Acquire your data - Data Acquisition
3. Extract the spectral features - **Profinder**
4. Perform a differential analysis - Mass Profiler Professional
5. Identify features in your data - ID Browser
6. Perform advanced analysis operations - Mass Profiler Professional
7. Visualize your Profinder results within biochemical networks - Omix Premium

Figure 1 shows the steps and Agilent tools that are used in an untargeted differential analysis and visualization in biochemical networks. Some of the feature finding steps performed by Profinder can alternatively be accomplished using a sequence of steps involving MassHunter Qualitative Analysis, DA Reprocessor, and MPP as described in the *Agilent Metabolomics Workflow - Discovery Workflow Guide*.

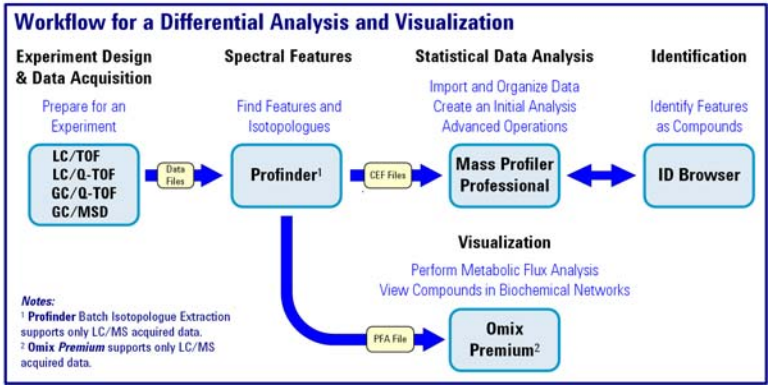


Figure 1 MassHunter Profinder is an optimized feature extraction tool.

Where to Find More Information

Online Help

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

Help Menu Click **Help > Contents** to access the contents of the Profinder Help including wizards, basic tasks, user interface, and reference information.

Click **Help** for information specific to wizards.

Documents

Mass Profiler Professional

- Agilent G3835AA MassHunter Mass Profiler Professional Software - Quick Start Guide
- Agilent G3835AA MassHunter Mass Profiler Professional Software - Familiarization Guide
- Agilent G3835AA MassHunter Mass Profiler Professional Software - Application Guide
- Agilent MassHunter Mass Profiler Professional User Manual

Workflow Guides and Overviews

- MassHunter VistaFlux Software - Workflow Guide
- Agilent Metabolomics Workflow - Discovery Workflow Guide
- Agilent Metabolomics Workflow - Discovery Workflow
- Integrated Biology with Agilent Mass Profiler Professional - Workflow Guide
- Integrated Biology with Agilent Mass Profiler Professional - Workflow Guide Overview
- Class Prediction with Agilent Mass Profiler Professional Workflow Guide

- Class Prediction with Agilent Mass Profiler Professional Workflow Overview

MassHunter Qualitative and Quantitative Analysis

- MassHunter Qualitative Analysis Familiarization Guide for LC/MS
- MassHunter Quantitative Analysis Familiarization Guide

Training

Road Show Slide Presentation Metabolome Analysis - From Sample Prep Through Data Analysis (Metabolomics Road Show, March 2011)

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

Getting Started

How do I get started?

This *Quick Start Guide* helps you install Profinder, review the Profinder user interface, and perform feature extraction using your data.

1. Install Profinder. Follow the instructions in "Profinder Installation" on page 39.
2. Launch Profinder. Double-click the **Profinder** icon located on your desktop, or click **Start > All Programs > Agilent > MassHunter Workstation > Profinder B.08.00**.
3. Review the following three sections in this *Quick Start Guide*:

   "User Interface" on page 9

   "Basic Profinder Workflow" on page 19

   "Feature Extraction Workflow Algorithms" on page 31
4. Review the *Agilent Metabolomics Workflow - Discovery Workflow Guide*. This guide helps you improve your results by covering topics that help you:
   - Prepare for an experiment
   - Explain the parameters related to finding features
   - Perform an initial differential analysis using MPP
5. Extract the features in your samples following the example Basic Profinder Workflow (see "Basic Profinder Workflow" on page 19).

User Interface

A feature may be referred to as a compound, molecular feature, element, or entity during the various steps of analysis using Agilent MassHunter software.

Help and detailed information regarding the various parameters and statistical treatments are available when you press **F1** or click **Help > Contents** from the menu bar.

Main Functional Areas

The main Profinder window consists of three parts: (1) the Menu Bar, (2) the Toolbar, and (3) the Main Window. The main functional areas are shown in Figure 2.

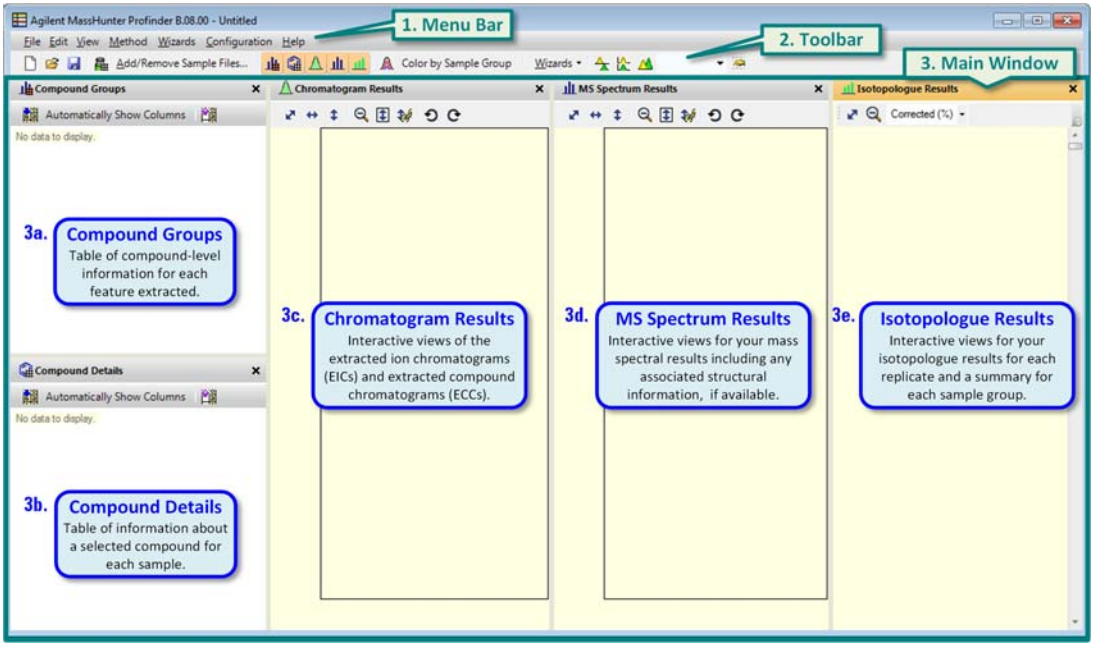


Figure 2 The main functional areas of Profinder as viewed before you begin a project.

1. Menu Bar

The menu bar (Figure 3) provides actions that are used for managing your projects, methods, display, and extracting features.

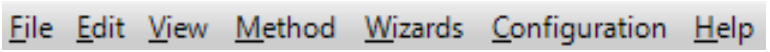


Figure 3 Menu bar

2. Toolbar

The toolbar is located below the menu bar and contains five groups of buttons for commonly performed tasks:

Project New project, Open project, and Save project

Button	Equivalent Command
	**File > New Project**
	**File > Open Project**
	**File > Save Project**

Samples Add sample files to your new or current project.

Button	Equivalent Command
	**File > Add/Remove Sample Files**

Main Window

Display or hide the various tables and results generated by Profinder, so you can increase the available display area for your review.

Button	Equivalent Command
	View > Compound Groups
	View > Compound Details
	View > Chromatogram Results
	View > MS Spectrum Results
	View > Isotopologue Results

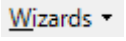
Feature Coloring

Toggle the feature coloring by sample group.

Button	Equivalent Command
	Edit > Color by Sample Group

Extraction

Select the feature selection algorithm you want to review, edit, and apply to your data set.

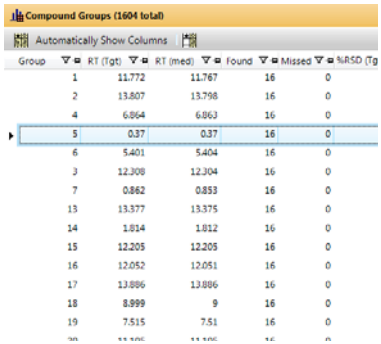
Button	Equivalent Command
	Wizards > Batch Molecular Feature Extraction Wizards > Batch Recursive Feature Extraction (small molecules / peptides) Wizards > Batch Recursive Feature Extraction (large molecules) Wizards > Batch Target Feature Extraction Wizards > Batch Isotopologue Extraction

Results Modes Select the display mode and display options to use in your results windows.

Button	Equivalent Command
	List mode, no equivalent command
	Sample group mode, no equivalent command
	Overlaid mode, no equivalent command
	Maximum number of chromatograms or spectra to display, no equivalent command
	**Configuration > Plot Display Options**

3. Main Window

The main window, see Figure 2 on page 9, is further divided into up to five windows – (3a) Compound Groups, (3b) Compound Details, (3c) Chromatogram Results, (3d) MS Spectrum Results, and (3e) Isotopologue Results that are used to review the results from applying the feature extraction method to your data set. Each window can be floated independently to any location and size on your computer display or arranged to your preference within the main window. The various windows are described in the following pages.

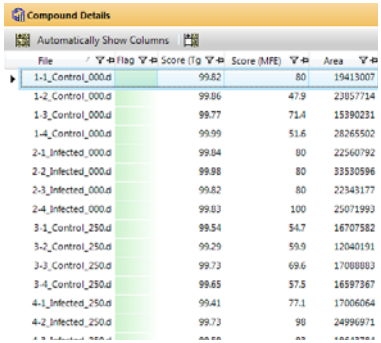


Compound Groups The data presented in Compound Groups is organized as a list of all of your extracted feature data averaged and summarized across all of the data files in your project.

The Compound Groups window shows a table of compound-level information for each feature extracted from at least one data file, if the data was extracted using Batch Molecular Feature Extraction, or for all targeted features, if the data was extracted using Batch Targeted Feature Extraction or Batch Isotopologue Extraction. Semi-quantitative information is shown as the average value for the feature across all of the files where the feature was found.

A *compound group* is a single compound (feature) found in any one or more of the data files in a project. For example, if the first data file in the project yields 35 compounds, then at least 35 compound groups are in the project. If additional unique compounds are found in the other data files, then additional compound groups are created.

Information regarding the available columns are found in the online Help in the topic “Compound Groups Columns.” A list of the available columns is displayed when you right-click within the Compound Groups table, and then click **Add/Remove Columns** (see Figure 4 on page 17).



Compound Details The data presented in Compound Details is organized as a list of the appearance of a selected feature (compound) in all of the data files in your project - *feature information by data file*.

The Compound Details window shows a table of compound-level information for a single feature selected in the Compound Groups window. The quantitative information is shown for the selected feature as it is found in each data file in your project.

Information regarding the available columns are found in the online Help “Compound Details Columns.” A list of the available columns is displayed when you right-click within the Compound Details table, and then click **Add/Remove Columns** (see Figure 4 on page 17).

The *Flags (Tgt)* column, highlighted, provides a feature extraction summary for each isotopologue for the selected compound, for each sample data, file based on warning, or error flags, reported from the Find by Formula algorithm.

? = no errors (qualified)

The isotopologue qualified and was used in the calculation.

M = m/z tolerance (disqualified)

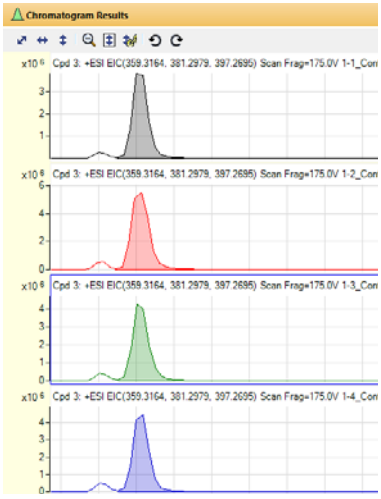
The isotopologue was disqualified for not meeting the m/z tolerance.

T = time correlation (disqualified)

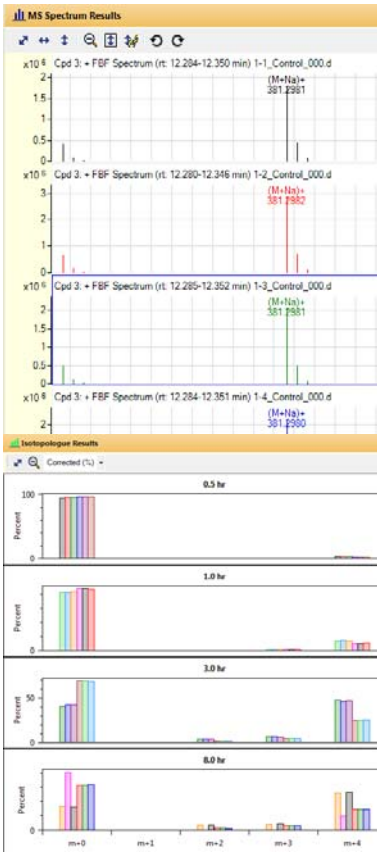
The isotopologue was disqualified for not coeluting.

_ = no peak (disqualified)

The isotopologue was disqualified because its EIC did not have enough contiguous non-zero points.



Chromatogram Results Chromatogram Results presents the extracted ion chromatogram (EIC) for each feature and, for isotopologue extraction results, the sum of the EICs for all of the isotopologues. For non-targeted feature extraction, the extracted compound chromatogram (ECC) is displayed for the ions contained in the molecular feature of the feature selected in the Compound Groups window. An EIC/ECC set is displayed for each data file. By default the chromatograms are displayed in an alternating cycle of ten colors to help you review the data for a particular data file as you select different features. **Color by Sample Group** displays the samples in an alternating cycle of colors based on the sample group assignment.



MS Spectrum Results MS Spectrum Results presents the averaged mass spectrum (MS) across the integrated ECC for the feature selected in the Compound Groups window for each data file. For isotopologue extraction results the mass spectra re presented across the isotopologue extraction region. By default the MS is displayed in an alternating cycle of ten colors, matched with the Chromatogram Results, to help you review the MS data for a particular data file as you select different features. **Color by Sample Group** displays the samples in an alternating cycle of colors based on the sample group assignment.

Isotopologue Results Isotopologue Results presents a sequence of charts, or a single chart, depending on the results mode selected from the toolbar (see “Results Modes” on page 12).

List mode displays the isotopologue results for each sample file. The isotopologue charts are arranged in the order of your sample groups with each sample replicate displayed in an alternating cycle of ten colors, matched with the Chromatogram Results and MS Spectrum Results. The coloring can be changed to represent the sample groups by selecting **Color by Sample Group** from the toolbar.

Sample group mode displays the isotopologue results for each sample group. Each sample replicate is displayed within each group chart in an alternating cycle of ten colors, matched with the Chromatogram Results and MS Spectrum Results. The coloring can be changed to represent the sample groups by selecting **Color by Sample Group** from the toolbar.

Overlaid mode displays a single summary chart of the isotopologue results. The summary chart contains the average and standard error for each isotopologue per sample group presented in gray scale. The sample groups can be viewed in color by selecting **Color by Sample Group** from the toolbar.

The order of the appearance of the samples and sample groups is set in the **Add/Remove Sample Files** dialog box. The compound containing the isotopologues is selected in the Compound Groups window.

The chart y-axes can be scaled to raw abundances (Raw), raw abundances normalized to 100% (Raw (%)), natural isotope abundance corrected abundances (Corrected), and natural isotope abundance corrected and normalized to 100% (Corrected (%)).

Raw: The actual abundances of each isotopologue by sample data file, or average abundance of each isotopologue when the data is viewed in the summary chart.

Corrected: The abundance for each isotopologue is corrected to remove the natural isotopic contributions so that the abundance is due to the isotopic enrichment from the qualitative flux analysis.

Raw (%): The actual relative abundances of each isotopologue by sample data file, or average of each isotopologue when the data is viewed in the summary chart.

Corrected (%): The relative abundance for each isotopologue after the abundance for each isotopologue is corrected to remove the natural isotopic contributions so that the abundance is due to the isotopic enrichment from the qualitative flux analysis.

Shortcut menu commands

A right-click in any of the main window shows you a menu of options (Figure 4 on page 17) applicable to where you click and to your current selection. Options available include customize the columns in the table views, copy to the system clipboard, and delete compound groups or individual compounds.

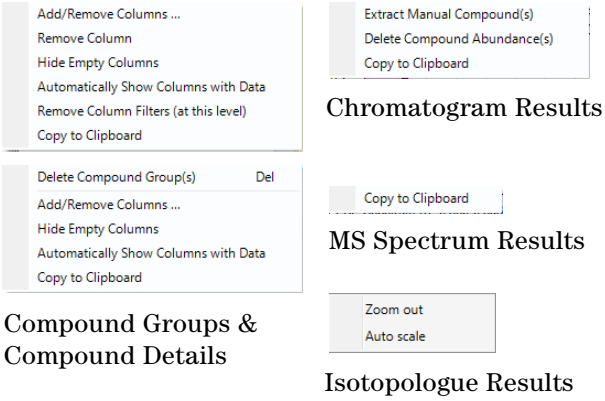


Figure 4 The shortcut menu commands available within the main window automatically adjust based on the active window.

Definitions

Algorithm An algorithm is a set of automated, sequential mathematical tasks performed to find, filter, align, and extract features from your chromatographic/mass spectral data sets.

Feature A feature is synonymous with compound. A feature is referred to interchangeably with compound, molecular feature, element, or entity during the various steps of analysis using Agilent MassHunter software.

Isotopologue Molecules that contain the same molecular formula and structure but differ in their isotopic composition through the substitution of one or more atoms with a different isotope.

Isotopomer Molecules that contain the same molecular formula, structure, and number of isotopes but differ in the location of the isotopes in the molecular structure.

Method A method is a set of parameters that are associated with the feature extraction algorithms used by Profinder. Methods containing the parameters for the algorithms can be saved using unique file names.

Wizard A wizard is a sequence of interactive steps used by Agilent MassHunter software to guide you through the steps necessary to complete an analytical task. Profinder uses a set of wizards to guide you through the parameters associated with each feature extraction algorithm.

Workflow A workflow is an Agilent document or a graphical overview that captures a sequence of steps to guide you through an analytical task. A workflow may cover more than one wizard and may include steps performed by more than one Agilent MassHunter software program.

Help

Online Help and detailed information regarding the various fields and statistical treatments are available by pressing **F1**, clicking 

, or clicking **Help > Contents** from the menu bar.

Unsaved parameter changes

When you make a change to a parameter in Profinder, the software automatically places a change icon  (a blue triangle shape) in the wizard tab and next to the value containing the parameter where you made a change. This icon indicates that you have unsaved parameters changes and helps you remember to save the changes you have made to the method. The original parameter value may be viewed by placing your pointer over the change icon. When you save your method, the change icon(s) disappear.

Basic Profinder Workflow

The basic Profinder workflow guides you through the steps necessary to extract features from your sample files. The steps shown in Figure 5 are described in the following sections:

- "Add sample files to your project" on page 20
- "Edit and run your feature extraction algorithm method" on page 24
- "Export your Profinder features" on page 26
- "Save your Profinder method" on page 29
- "Save your Profinder project" on page 30

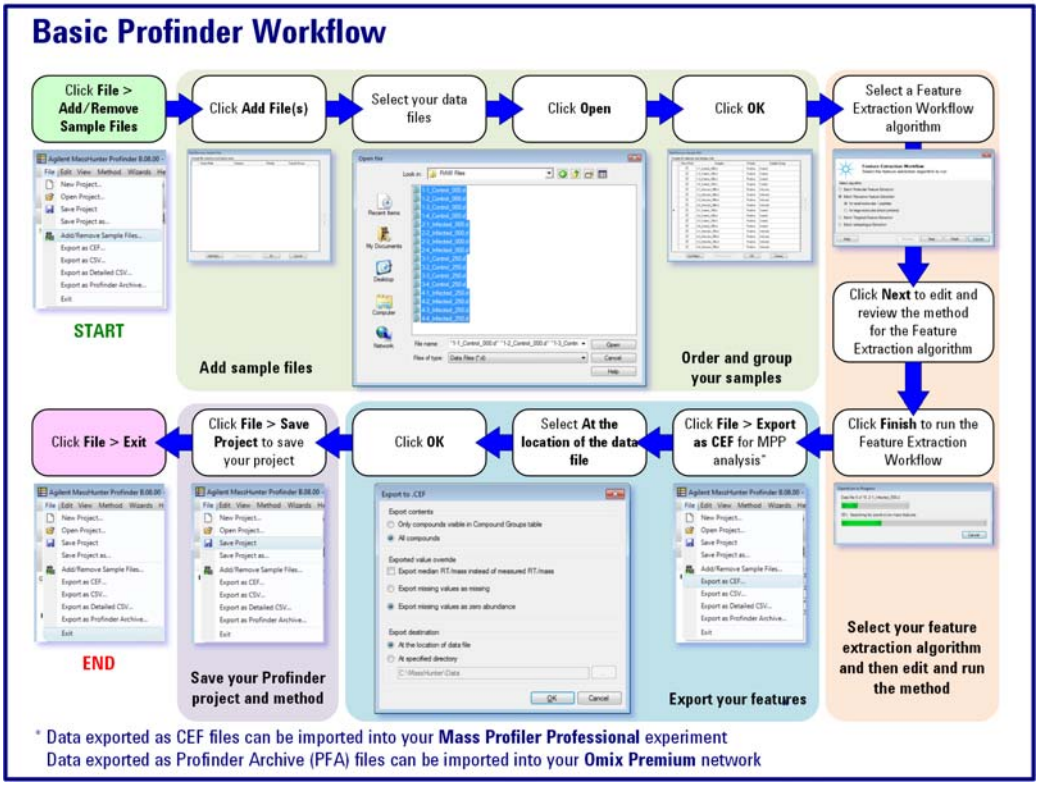
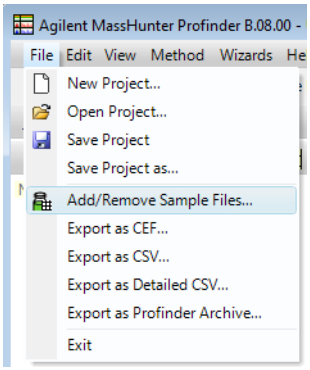
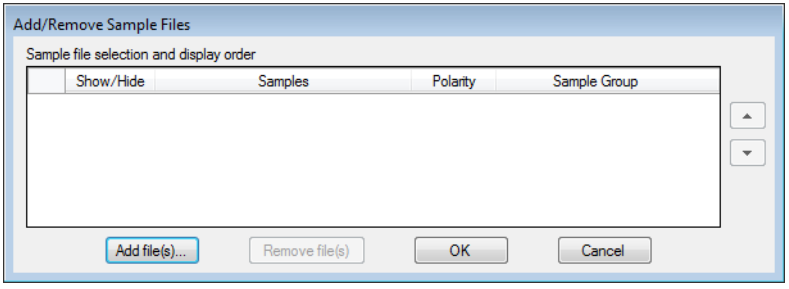


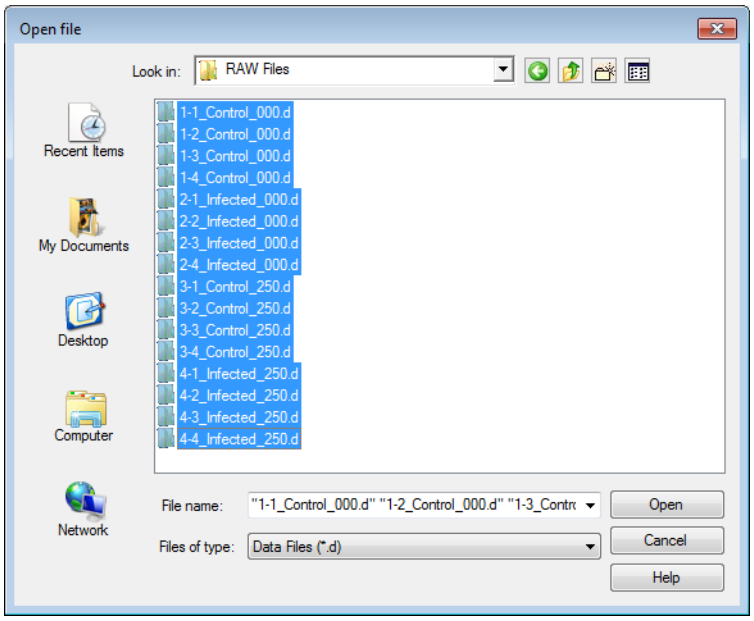
Figure 5 The basic Profinder workflow (see the *MassHunter VistaFlux Software - Workflow Guide* for the Profinder Batch Isotopologue Extraction workflow)

Add sample files to your project

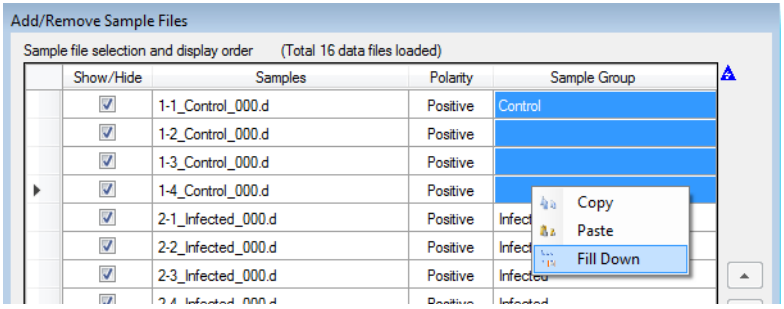
In this task, you launch Profinder, select your sample files, and add the sample files to your Profinder project.

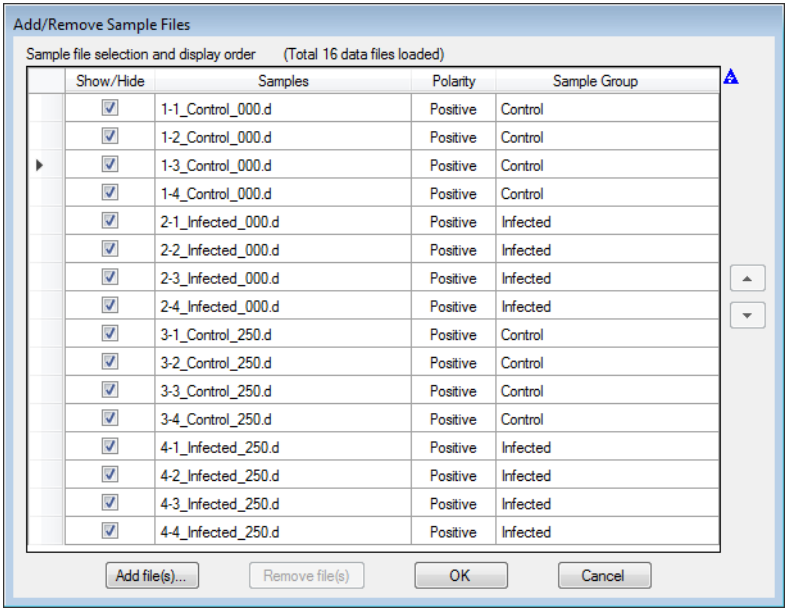
Steps	Detailed Instructions	Comments
1 Start Profinder.	• Click the **Profinder** icon on your desktop, or click **Start > All Programs > Agilent > MassHunter Workstation > Profinder B.08.00**.	• The first time you start Profinder it displays in full screen. Subsequent program launches remember the screen size from your prior session.
2 Begin your Profinder project by adding sample files. 	• Click **File > Add/Remove Sample Files**, or click Add/Remove Sample Files... on the toolbar.	• If you click **File > New Project** after starting Profinder, you are automatically prompted to add sample files. • When you click **Add/Remove Sample Files** after launching Profinder, Profinder starts a new project as if you clicked **File > New Project**.
3 Add files to the **Add Sample Files** dialog box. 	• Click **Add file(s)** to display the **Open file** dialog box.	

Steps	Detailed Instructions	Comments
4 Select all of the sample data files in the **Open file** dialog box.	**a** Browse to the folder containing your sample files. **b** Select all of the sample data files for your Profinder project. **c** Click **Open**.	• Selected sample data files are highlighted in the **Open file** dialog box. • A Profinder project contains all of the sample data files from your experiment - replicates samples for each condition (group) in your experiment. • You can click **Add Sample Files**, to add additional sample data files to your project and rerun feature extraction.



Steps	Detailed Instructions	Comments
5 Enter Sample Group values to the sample files in the **Add Sample Files** dialog box.	**a** Click the data entry cell under the **Sample Group** column, next to the sample file name. **b** Enter the sample group identification text. **c** Repeat for each sample file.	• When a data entry cell is selected it is highlighted using a background color. • When entering **Sample Group** values, the entries must use identical letters, numbers, punctuation, and case in order for the grouping functions to perform properly. • Use the **Copy**, **Paste**, and **Fill Down** shortcuts (click the right mouse button) to help assign **Sample Group** values to each sample.
6 Mark the samples to add to your project.	**a** Mark the samples to add to your project in the **Show/Hide** column. **b** Click **OK**.	• All of the **Samples** in the same project must have the same polarity. If some of the Samples have a Positive **Polarity** and some have a Negative **Polarity** an error message is shown when you click **OK**.





Your new Profinder project is now begun.

The **Feature Extraction Workflow** immediately prompts you to select a feature extraction algorithm and then guides you through the steps to edit and run the feature extraction method.

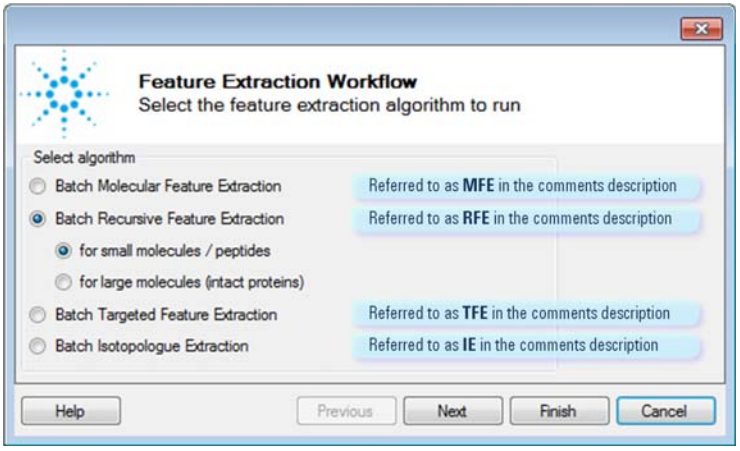
Edit and run your feature extraction algorithm method

Batch feature extraction performed by Profinder is part of a workflow for statistical data analysis, qualitative flux analysis, and data visualization in biochemical networks.

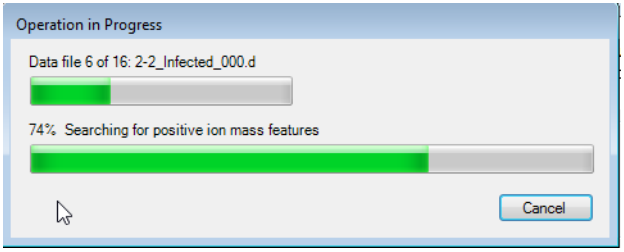
- Batch Molecular Feature Extraction
- Batch Recursive Feature Extraction *[default selection]*
  - for small molecules / peptides *[default selection]*
  - for large molecules (intact proteins)
- Batch Targeted Feature Extraction
- Batch Isotopologue Extraction

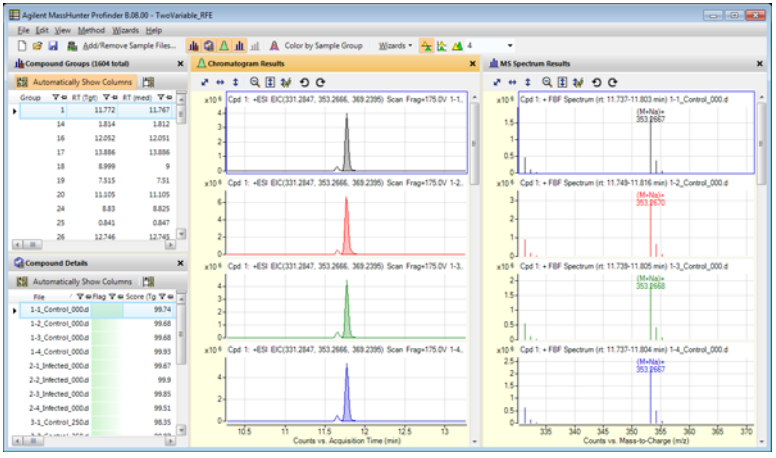
An overview of each extraction algorithm is found in the section "Feature Extraction Workflow Algorithms" on page 31. Batch Isotopologue Extraction is optimized to extract isotopologues for targeted features for qualitative flux analysis.

Steps	Detailed Instructions	Comments
1 Select the feature extraction algorithm to apply to your samples.	**a** Click one of the batch extraction algorithms. **b** Click **Next**.	• **MFE** performs chromatographic deconvolution and aligns the features across the selected sample files using mass and RT. See "Batch Molecular Feature Extraction" on page 31 for the wizard steps. • **RFE** performs MFE and then uses the mass and RT of each feature to automatically perform a targeted feature extraction. See "Batch Recursive Feature Extraction" on page 32 for the wizard steps. • **TFE** uses an input formula source to extract features. See "Batch Targeted Feature Extraction" on page 34 for the wizard steps. • **IE** locates possible isotopologues of target compounds. See "Batch Isotopologue Extraction" on page 35 for the wizard steps.



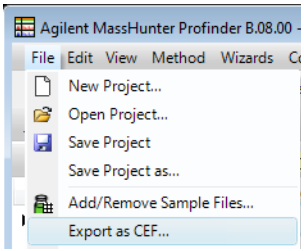
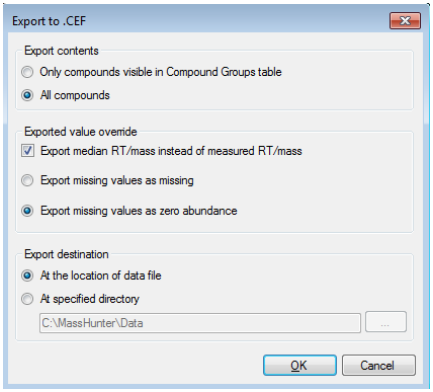
Steps	Detailed Instructions	Comments
2 Review and edit the parameters for the feature extraction algorithm.	**a** Make changes to the parameters on each wizard tab for each step. **b** Click **Next** to go to the next step of the wizard. **c** Click **Previous** to return to an earlier step.	• Click **Help** to activate online Help specific for the current tab in the wizard. • Click **Cancel** to stop editing the algorithm parameters. Any changes made to the algorithm parameters are not saved.
3 Run the feature extraction algorithm.	**a** Click **Finish** to run the extraction algorithm on your sample data. **b** An **Operation in Progress** dialog box is displayed while the extraction process is running.	• The feature extraction process can take a long time. To significantly improve performance, increase the amount of memory and use a faster processor.
4 Review your results.	• Review your results and then export the results and save your method.	• Using the Compound Groups, Compound Details, Chromatogram Results, and MS Spectrum Results windows, you can edit your results: delete a compound group, delete abundance from compound details window or chromatogram results window, and integrate a peak manually. More information is available in the online Help. • The next steps in this *Quick Start Guide* help you export your results and save your method.





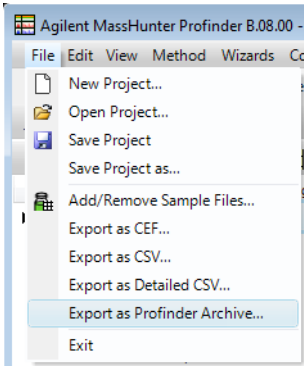
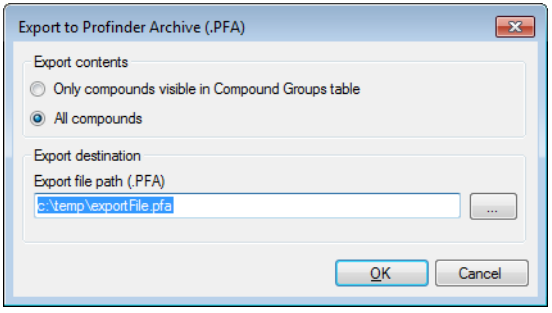
Export your Profinder features

You save the features in your sample data files to a file format called a compound exchange file (CEF) that is used to exchange data between Agilent software. CEF files are used to import your molecular features into Mass Profiler Professional.

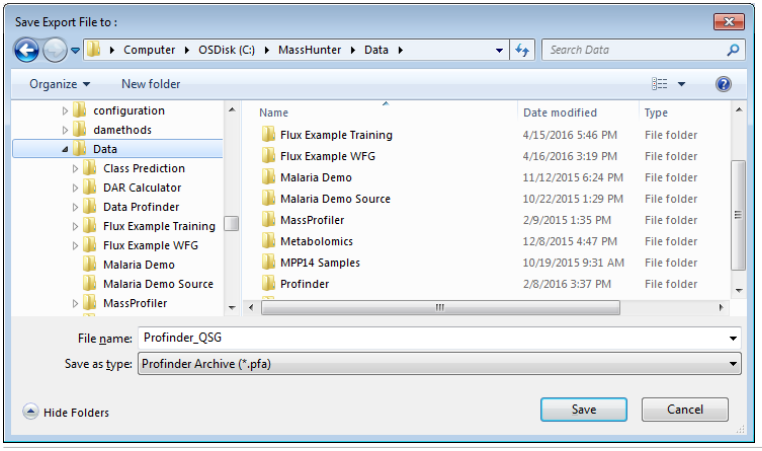
Steps	Detailed Instructions	Comments
1 Select **Export as CEF** from the File menu. 	• Click **File > Export as CEF**.	• Alternate export options include: ◦ **Export as CSV** - average Mass and RT with actual feature abundance for each sample ◦ **Export as Detailed CSV** - average Mass and RT with all actual feature values for each sample ◦ **Export as Profinder Archive** - binary file format used to transfer data to Omix Premium
2 Export your features (compounds) to CEF files. 	**a** Under **Export contents**, click **All compounds**. **b** Under **Exported value override**, mark the **Export median RT/mass instead of measured RT/mass** check box. **c** Under **Exported value override**, click **Export missing values as zero abundance**. **d** Under **Export destination**, click **At the location of data file**. All of the CEF files are placed in the same folder containing the sample data files. **e** Click **OK**.	• The features extracted from your sample data files are saved as CEF files. A separate CEF file is created for each sample data file. • **Export median RT/mass instead of measured RT/mass** replaces the individual mass and RT valves for each feature as it appears in each sample data file with the median values. This aligns the features across the sample data files and removes the need to perform a compound alignment in MPP - the MPP compound alignment values can be set to as small as 0.001 ppm and 0.001 min. • The export option for missing values that you select affects downstream statistics, such as calculation of means. • The number of CEF files created is identical to the number of sample data files in your Profinder project.

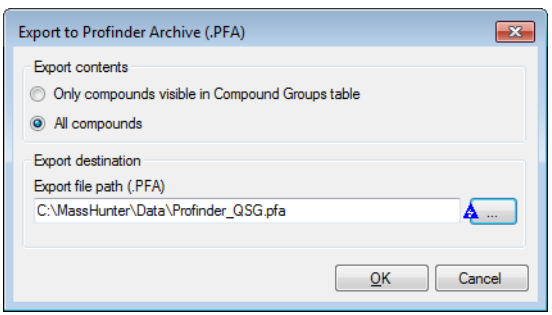
Export your Profinder results for visualization

You save your Profinder results as a Profinder Archive (PFA) file to visualize your results in the context of biochemical networks in Omix Premium.

Steps	Detailed Instructions	Comments
1 Select **Export as Profinder Archive** from the File menu. 	• Click **File > Export as Profinder Archive**.	• Alternate export options include: ◦ **Export as CEF** - features saved to a file format used to exchange data between Agilent software. ◦ **Export as CSV** - average Mass and RT with actual feature abundance for each sample ◦ **Export as Detailed CSV** - average Mass and RT with all actual feature values for each sample
2 Select your export contents and destination. 	**a** Select **All compounds** under the *Export contents heading* in the **Export to Profinder Archive (.PFA)** dialog box. **b** Click the **Select** ... button to select your export destination folder, and enter your file name.	

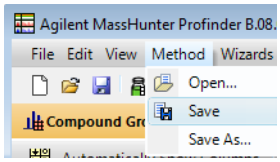
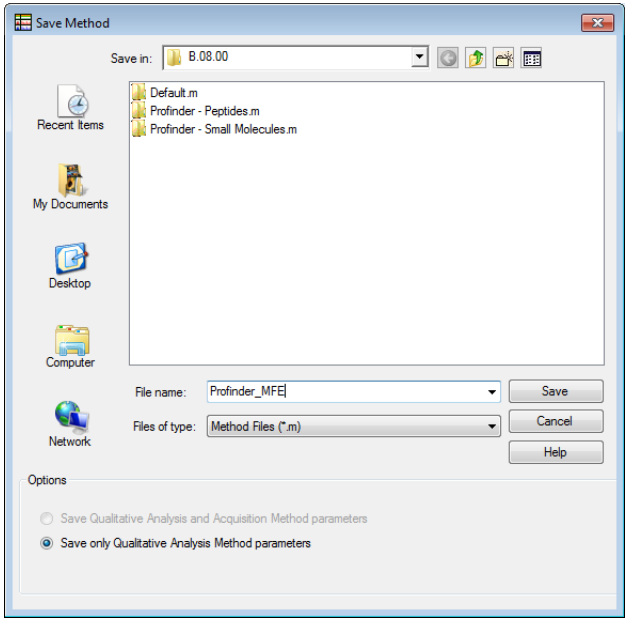
Steps	Detailed Instructions	Comments
3 Navigate to the folder and enter your file name.	**a** Navigate to the folder to save your project in the **Save Export File to** dialog box. **b** Type a name for **File name**. **c** Click **Save** to the **Export to Profinder Archive (.PFA) dialog box**.	• The Profinder Archive is not saved at this time; it is saved from the **Export to Profinder Archive (.PFA) dialog box**.
4 Save your Profinder archive file.	**a** Review your selection, path and file name in the **Export to Profinder Archive (.PFA)** dialog box. **b** Click **OK** to save you Profinder archive.	• Your Profinder Archive (PFA) file is imported into Omix Premium where you can visualize your results in the context of biochemical networks.





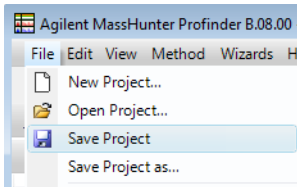
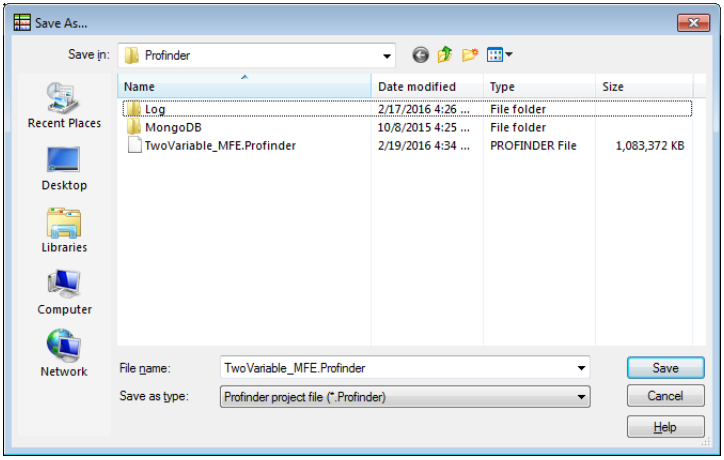
Save your Profinder method

You save your Profinder feature algorithm method using a unique name.

Steps	Detailed Instructions	Comments
1 Select **Save** from the menu bar. 	• Click **Method > Save**.	• If you have not previously saved your method, **Save** is the same as **Save As** and prompts you for a name to save your method.
2 Type the file name and save your method. 	**a** Type the name to use for your method file in the **Save Method** dialog box. **b** Click **Save**.	• The **Save Method** dialog box does not appear when you click **Save** for your method if you previously saved your method. • Click **Method > Save As** if you want to save your method using a new file name. • If acquisition parameters exist in the current method, you can select whether to also save the acquisition method parameters to the new method.

Save your Profinder project

This is an optional step. You can save your Profinder project, method, and the current sample data file extraction results so that you can continue reviewing your results and the extraction method at a later time.

Steps	Detailed Instructions	Comments
1 Select **Save Project** from the menu bar. 	• Click **File > Save Project**.	• If you have not previously saved your project, **Save Project** is the same as **Save Project as** and prompts you for a name to save your project.
2 Type the file name and save your project. 	**a** Type the name to use for your project file in the **Save as** dialog box. **b** Click **Save**.	• The **Save As** dialog box does not appear when you click **Save Project** if you previously saved your project. • Click **File > Save Project as** if you want to save your project using a new file name. • ***Note:*** Profinder project files can be one GB or more in file size. • ***Note:*** Remember to include the original sample data when you share a Profinder project.

Feature Extraction Workflow Algorithms

Five feature extraction algorithms are available in Profinder. Each extraction algorithm is designed to efficiently extract the features (compounds) in your sample data files. The resulting compound groups are imported into your MPP experiment or Omix Premium as part of your qualitative flux analysis.

When you begin a new analysis, start with permissive feature extraction algorithm parameters and review the results for false positive and false negative features. Then adjust the algorithm parameters to reduce the appearance of false positive features without increasing the loss of actual features - false negative features.

Batch Molecular Feature Extraction

Molecular feature extraction (MFE) performs chromatographic deconvolution to find the features in your samples. After finding the features, the features (compounds) are aligned across all of the selected sample files using mass and retention time (RT). After performing MFE, Profinder employs an algorithm to re-assign ion species using cross-file analysis to generate compound groups.

The wizard workflow is shown in Figure 6.

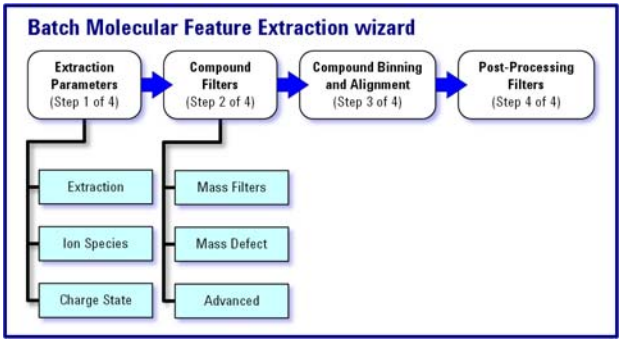


Figure 6 Four steps are presented in the Batch Molecular Feature Extraction wizard.

Batch Recursive Feature Extraction

Recursive Feature Extraction (RFE) performs MFE, as described above, and then uses mass and retention time of the results to perform a targeted feature extraction referred to as Find by Ion (FbI). Using the untargeted MFE results for a targeted FbI feature extraction is referred to as *recursive feature extraction*.

After calculating the median mass, median RT, and composite spectrum from the aligned features found using MFE, FbI uses the median values to perform a targeted extraction to improve the reliability in finding the features in your data. This recursive feature finding, combined with replicate samples for each of your experiment conditions, improves the statistical accuracy of your analysis and reduces the potential for obtaining a false positive or false negative answer to your hypothesis.

The recursive feature extraction workflow performed by Profinder is similar to that outlined in the *Agilent Metabolomics Workflow - Discovery Workflow Guide*. The difference is that the feature extraction process happens in a single step, in the same application, without moving CEF files back and forth between MassHunter Qualitative Analysis, DA Reprocessor, and Mass Profiler Professional as illustrated in Figure 1 on page 5. For a comparison of feature finding between Profinder and MassHunter Qualitative Analysis, refer to *Class Prediction with Agilent Mass Profiler Professional Workflow Guide*.

The wizard workflow for small molecules is shown in Figure 7 on page 33.

The wizard workflow for large molecules is shown in Figure 8 on page 33.

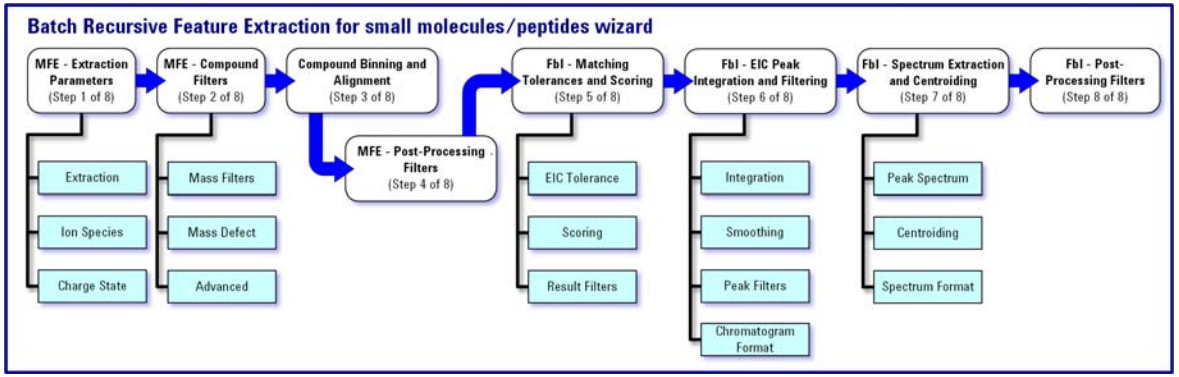


Figure 7 Eight steps are presented in the Batch Recursive Feature Extraction for small molecules/peptides wizard.

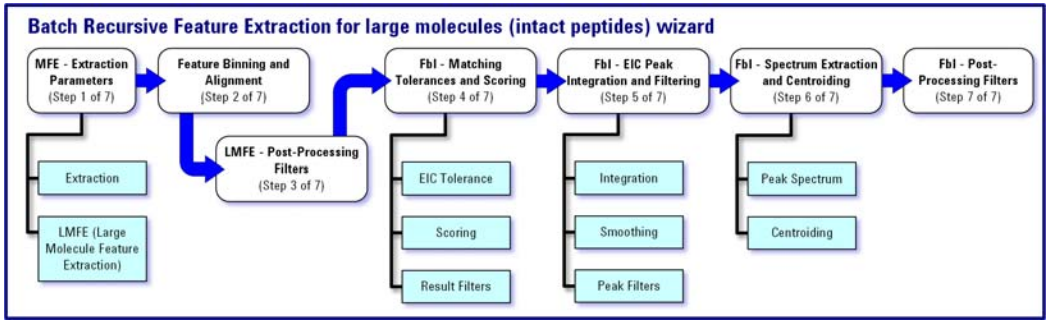


Figure 8 Seven steps are presented in the Batch Recursive Feature Extraction for large molecules (intact peptides) wizard.

Batch Targeted Feature Extraction

Targeted feature extraction (TFE) uses an input formula source, CEF file, or database containing molecular formulas, mass, and/or retention time information to extract features from your data using a process referred to as Find Compounds by Formula. After feature extraction is performed, compound alignment produces a result such that one compound target can only have one compound extracted per data file.

Find Compounds by Formula typically uses molecular formula information, like a PCDL, to calculate the ions and isotope patterns derived from the formula as the basis to find features in the sample data file. When the input molecular features contain mass and retention time, as found in CEF file, instead of just the molecular formula, TFE calculates reasonable isotope patterns and uses these patterns with retention time tolerances to find the target features in the sample data files. When the input molecular features are filtered from a find process that was previously untargeted, the molecular features found using Find Compounds by Formula is also referred to as recursive finding.

The wizard workflow is shown in Figure 9.

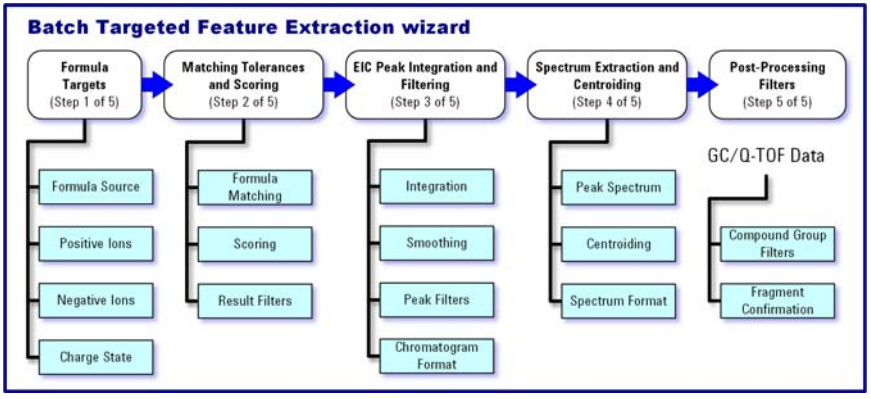


Figure 9 Five steps are presented in the Batch Targeted Feature Extraction wizard.

Batch Isotopologue Extraction

Batch isotopologue extraction supports only LC/MS acquired data.Unlike the other batch feature extraction wizards, target retention times are required for this workflow.

Isotopologue extraction (IE) uses an input CSV file or compound database file, PCD/PCDL, containing the target feature molecular formulas, mass, and/or retention time information, and anticipates that the target compound may have undergone some degree of isotope labeling, to extract features from your data using a process referred to as Find Compounds by Formula. After feature extraction is performed, the extraction algorithm determines which of the possible isotopologues are actually present, measures the raw abundances of the isotopologues, and corrects the isotopologues abundances for the natural occurrence of the unlabeled ions.

See the *MassHunter VistaFlux Software - Quick Start Guide* and the *MassHunter VistaFlux Software - Workflow Guide* for additional information.

Isotopes, Isotopomers, Isotopologues, and Mass Spectra

Isotopologues are molecules that contain the same molecular formula and structure but differ in their isotopic composition through the substitution of one or more atoms with a different isotope. The exact location of the isotope in the molecule, while important chemically, is not important in flux analysis, just the number of isotopes in the molecule. Isotopologues can be identified using single-stage MS.

Isotopomers are molecules that contain the same molecular formula, structure, and number of isotopes but differ in the location of the isotopes in the molecular structure. Isotopomers can be identified using advanced MS/MS techniques.

Note: Profinder finds and extracts isotopologues in your sample data; it does not find or extract isotopomers.

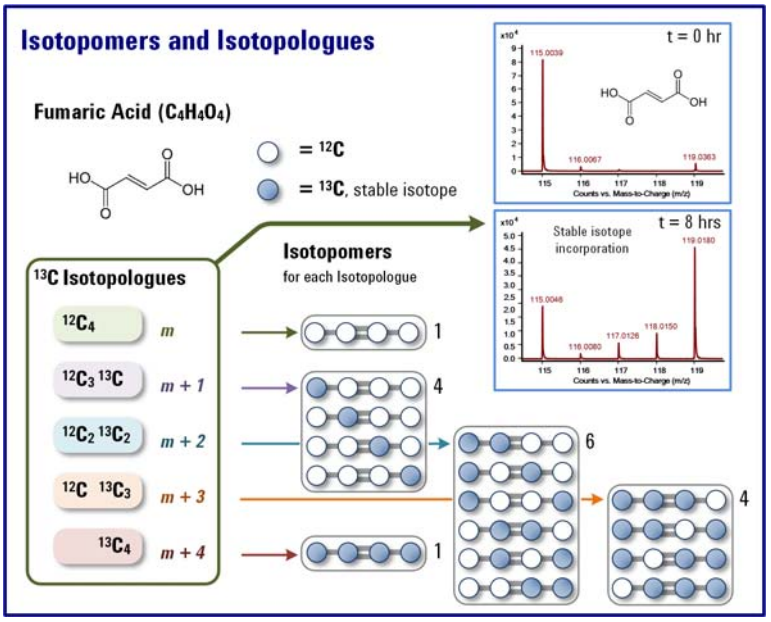


Figure 10 An illustration of how two stable carbon isotopes in a four-carbon molecule relate to isotopomers and isotopologues. Isotopologues are viewed in mass spectra during flux analyses.

A simple four carbon molecule, fumaric acid ($C_4H_4O_4$), is used to explain the relationship of isotopes to isotopomers, isotopologues, and mass spectra. The most abundant isotope of carbon is ^{12}C. However, ^{13}C, also stable, is not nearly as naturally abundant as ^{12}C; ^{13}C has a natural occurrence of 1.1% of ^{12}C. For simplicity, naturally occurring ^{13}C is considered to be negligible; therefore, the mass of the naturally occurring four-^{12}C molecule is m (represented as $^{12}C_4$), and there are no positional differences among the isotopes of the carbon atoms.

When a single ^{13}C atom is substituted for one ^{12}C atom, four locations are possible where the ^{13}C atom can be placed (isotopomers as shown in Figure 10), and each isotopomer has a mass of m + 1 ($^{12}C_3\,^{13}C$). When two of the ^{12}C atoms are replaced with ^{13}C atoms, six isotopomers are possible, and each of the doubly substituted molecules has a mass of m + 2 ($^{12}C_2\,^{13}C_2$). When three of the ^{12}C atoms are replaced with ^{13}C

atoms, four isotopomers are possible, and each isotopomer has a mass of m + 3 ($^{12}C\ ^{13}C_3$). Finally, when all four of the ^{12}C atoms are replaced with ^{13}C atoms, only a single arrangement with a mass of m + 4 ($^{13}C_4$) exists. The five different masses m, m + 1, m + 2, m + 3, and m + 4 represent the masses of the five isotopologues visible in the resulting mass spectra.

Isotopologue mining

Profinder performs isotopologue mining in two stages, an initial screening followed by refinement. The initial screening stage extracts isotopologue EICs around the target retention time range and then evaluates peak mass spectral data to find ions that match the predicted list of possible isotopologues. The refinement stage uses a self-optimizing peak finder to refine the *m/z* assignment from the profile data and then re-extracts the EICs using the new isotopologue *m/z* values, refines the start and end retention time bounds on the newly extracted EICs, and then reports both EIC peak area and summed isotopologue peak heights as the compound abundances.

The wizard workflow is shown in Figure 11.

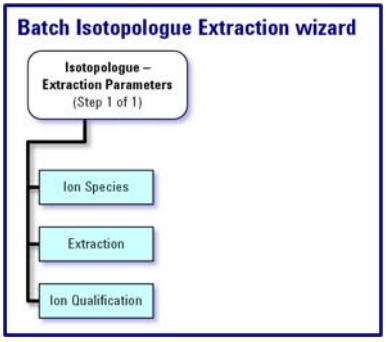


Figure 11 One step is presented in the Batch Isotopologue Extraction wizard.

Monitoring your computer while running Profinder

You can monitor your computer's resources, especially memory, while running Profinder. Right-click on the computer taskbar and then click **Start Task Manager** to launch the Windows Task Manager. Select the **Performance** tab to monitor your CPU and memory usage.

For additional details regarding your computer's memory usage click **Resource Monitor** and select the **Memory** tab. See Figure 12. If your memory usage approaches the amount of physical RAM, adding additional RAM increases performance.

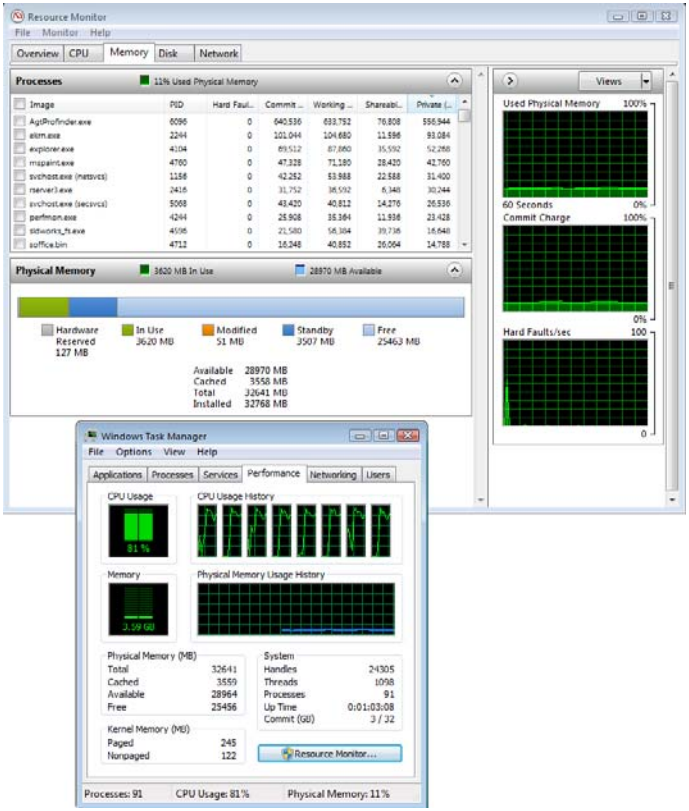


Figure 12 Monitoring the system and memory resources on your computer.

Profinder Installation

Standalone MassHunter Profinder Profinder is installed from a Setup Wizard, which you run from the main installation program. If you have a prior version, uninstall Profinder before installing this newer version (see "Uninstall a prior version" on page 39).

Right-click **ProfinderSetup.exe**, and then click **Run as administrator**.

Install Profinder on the highest performing PC you have available to reduce the time it takes to extract the features from your sample files. Profinder requires a PC running Windows 7 (64-bit) with at least 8GB of RAM and at least 30GB of available disk space.

Note: A PC with 16GB or more of RAM and a solid-state drive will see significantly improved Profinder performance and reduction in the time it takes to extract features from large data sets.

As part of MassHunter VistaFlux Software Profinder is also part of the MassHunter VistaFlux Software suite; for Profinder installation instructions refer to the software installation instructions found in the *MassHunter VistaFlux Software - Quick Start Guide*.

Uninstall a prior version If you have a prior version of Profinder installed, you must delete the prior version before installing this new version.

1. Click **Start > Control Panel**.
2. Click **Programs and Features**.
3. Click **Agilent MassHunter Workstation Profinder Software**.
4. Click **Uninstall/Change** to uninstall Profinder.

www.agilent.com

In this book

The *MassHunter Profinder Software Quick Start Guide* presents the first steps to use the MassHunter Profinder Software. Profinder is part of the MassHunter VistaFlux software.

This Quick Start Guide applies to MassHunter Profinder B.08.00 and later until superseded, as part of the MassHunter VistaFlux Software.

For Research Use Only. Not for use in diagnostic procedures.

Revision A, May 2016

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Agilent Technologies