Agilent Mass Profiler Software

Quick Start Guide

What is Mass Profiler Software?

Mass Profiler operates on the extracted data files (.mhd files) produced by Mass Hunter to let you investigate similarities and differences in features across multiple analyses/samples.

Comparing feature information

A feature is a discrete molecular entity defined by the combination of retention time and mass.

The software aligns and normalizes features from different .mhd files generated by Mass Hunter. It presents the results in both graphic and tabular forms.

From the plot:

- Investigate features using three different plot types: Mass vs Retention Time, Log 2 Ratio vs Retention Time or Log/Log plot.
- Investigate features in all samples or in one sample
- Investigate only features above the visibility threshold
- Investigate features by color (color each feature a different color, or color all features in the same .mhd file the same color, or color all features in the same group the same color)

From the table:

- View all the features for their summary, group statistics and differences between the two groups.
- View individual feature details, including species clusters found in a selected .mhd file and possible compositions for the feature

You can also export most of the data generated by Mass Profiler to an .xls file or to a .txt file for import to GeneSpring.

For a complete list of tasks, see the Mass Profiler online help.

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Getting started with the Mass Profiler software

Install the software

1. Go to the directory on the CD that contains the Mass Profiler setup.exe file.
2. Double-click **setup.exe**.
3. Follow the instructions on each screen of the InstallShield wizard, and click **Next** to move on to the next screen.

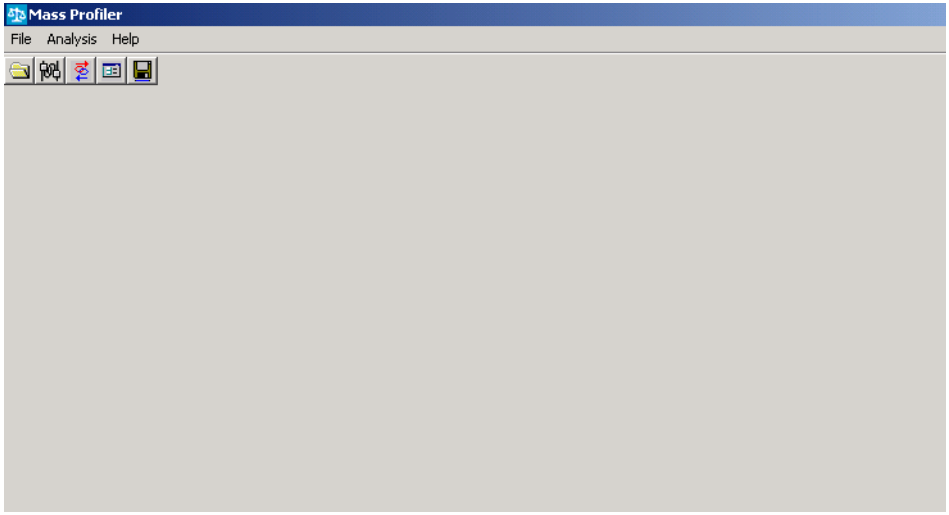
   Accept the default path for the software.
4. Click **Finish** to complete the installation.

Start the software

- Double-click the **Mass Profiler** icon on the desktop, or

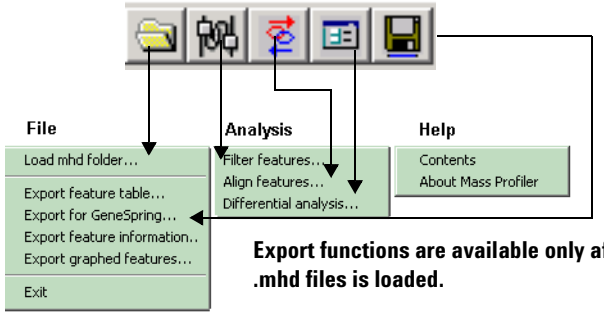
  Select **Start > Programs > Agilent > Mass Profiler** from the desktop.

The system displays the Mass Profiler main window.



Learn how to access Mass Profiler functions

You can use the toolbar or the menus to prepare the data for comparison either before or after you load an .mhd folder:



Export functions are available only after a folder containing .mhd files is loaded.

Learn how to use Mass Profiler

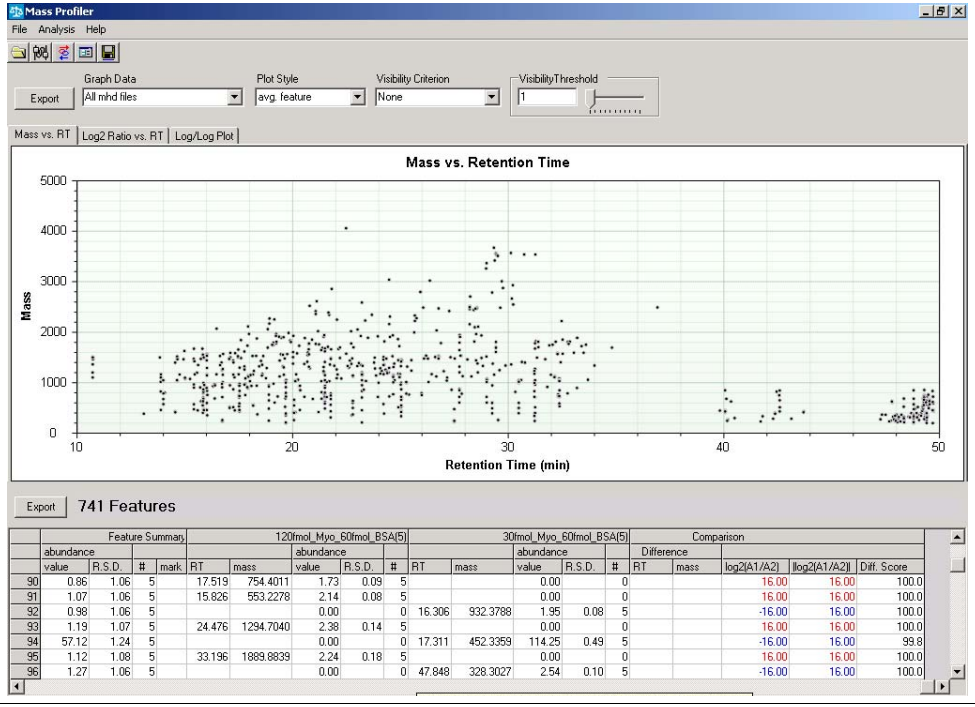
Try these exercises to familiarize yourself with the Mass Profiler application. Try the **Steps** on the left in the exercises on the next pages without the **Detailed Instructions**. If you need more help, follow the detailed instructions.

If you want to do this:	Refer to this exercise:
Take a look at the all the features loaded with the default parameters	"Exercise 1 – Take a quick look at the results" on page 4
Filter, align, normalize or perform differential analysis on feature data	"Exercise 2 – Set up to process feature data" on page 7
Work with plot data to view the differences in features	"Exercise 3 – View Feature Plot" on page 10
Work with feature table data to view differences	"Exercise 4 – View Feature Table" on page 13
View feature species clusters or possible compositions	"Exercise 5 – View more detailed feature information" on page 15
Export Mass Profiler data to GeneSpring	"Exercise 6 – Export Mass Profiler Feature Summary for GeneSpring" on page 17

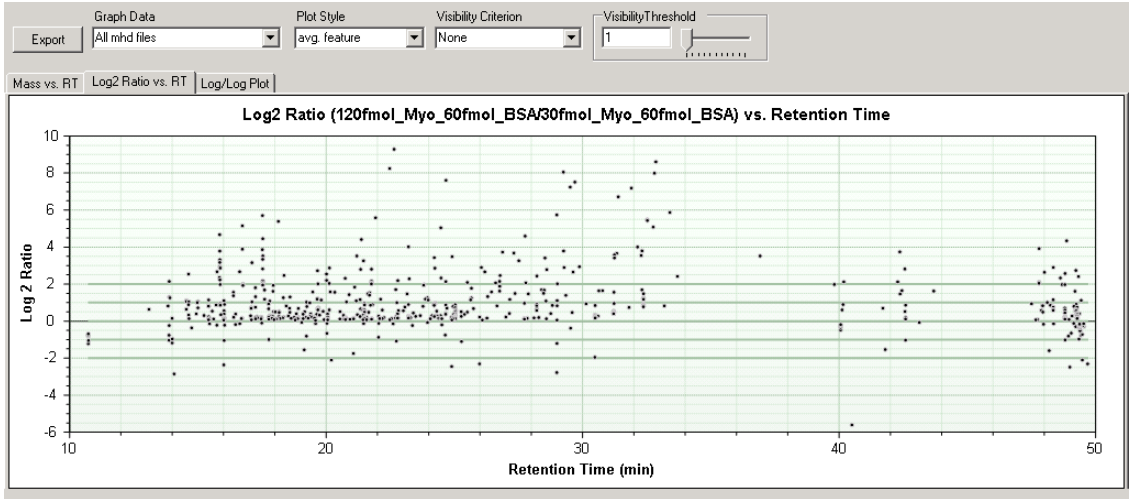
Exercise 1 – Take a quick look at the results

Before you start to change parameters on how to choose, process and display the data, you may want to take a quick look at the results with the default parameters..

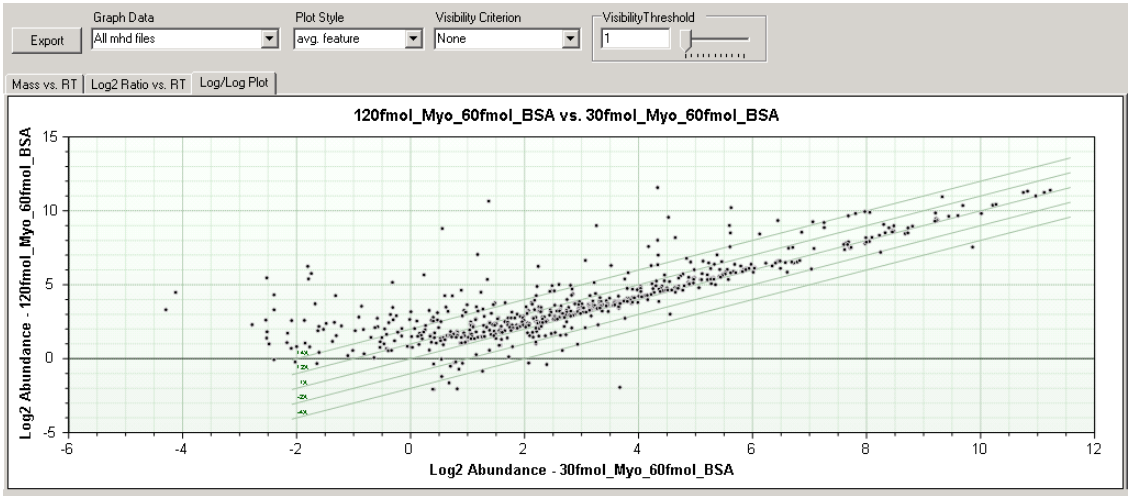
Steps	Detailed Instructions	Comments
1 Load the example loadable folder, **Example_Myoglobin_Spike_into_BSA_mhd** • Copy this example Mass Profiler folder to a directory that only you will use. • Mass Profiler lets you compare feature data in one set of .mhd files or in two sets, but not more.	**a** Select **File > Load mhd folder.** **b** Go to the directory that contains the Mass Profiler example folder. **c** Select the **Example_Myoglobin_Spike_into_BSA_mhd** folder. **d** Click **OK**. 741 average features (mass and RT) averaged over all 10 mhd files appear in the Feature Comparison Window in 3 plots and a table.	• This loadable folder contains two *groups* of data. That is, it contains two subfolders, each with a different set of *.mhd files*. • Group 1 – 5 .mhd files of 120 fmol Myoglobin in 60 fmol BSA digest. • Group 2 – 5 .mhd files of 30 fmol Myoglobin in 60 fmol BSA digest. • For an explanation of why log2 columns are in blue or red in the Feature Table, see Step 1 of Exercise 4 on page 13.



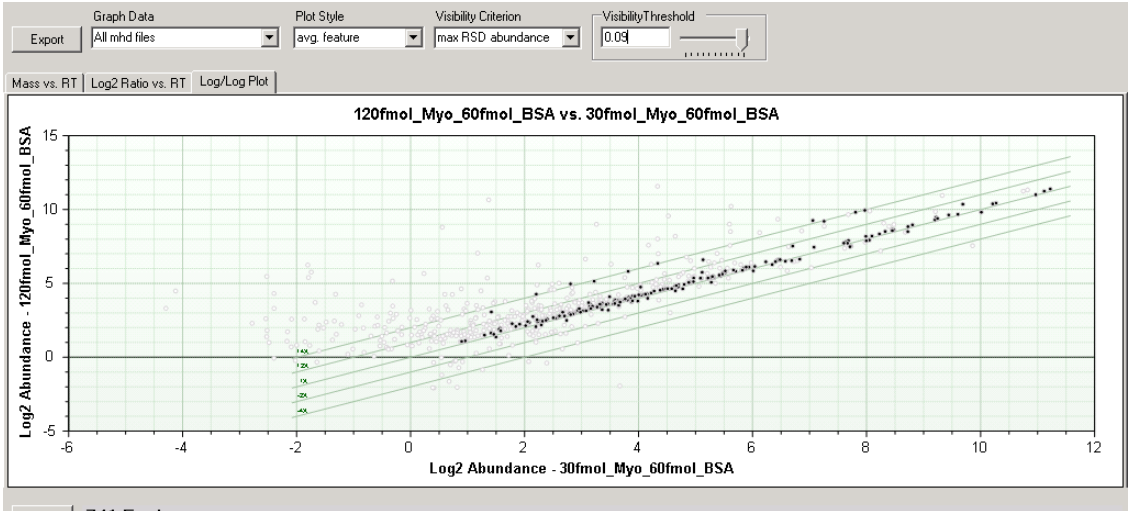
Steps	Detailed Instructions	Comments
2 View the other two plots for a quick overview of the data.	**e** Click the **Log2 Ratio vs RT** tab to view the plot below.	• The lines represent boundaries where the ratio of abundances for the groups are two-fold or four-fold different from an abundance ratio of 1 (log2 ratio = 0).



f Click the **Log/Log Plot** tab.



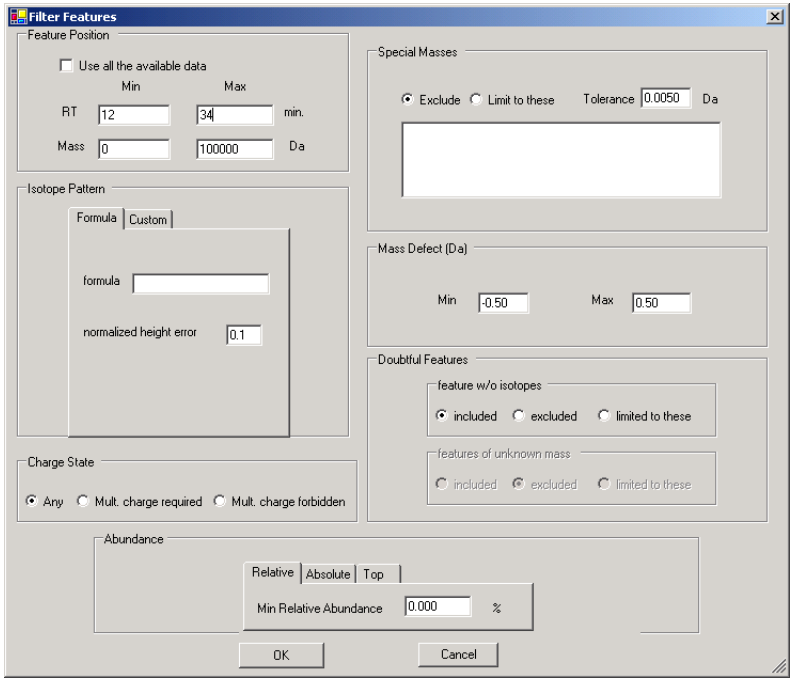
Steps	Detailed Instructions	Comments
3 Compare only features below this visibility threshold: • Visibility Criterion: max RSD abundance • Visibility Threshold: .09.	**1** As the **Visibility Criterion**, select **max RSD abundance**. **2** Enter the **Visibility Threshold** as .09. The plot below shows the data points at .09 threshold. Note that only those features whose max RSD abundance is below the threshold appear in the plot.	• Note that the points along the 1X fold are BSA features and the points along the 4X are myoglobin. • Note that one group of .mhd files contains 4X the concentration of myoglobin spike-in relative to the other group (120 vs 30). • In another exercise, you will gather more information about one of these myoglobin features.



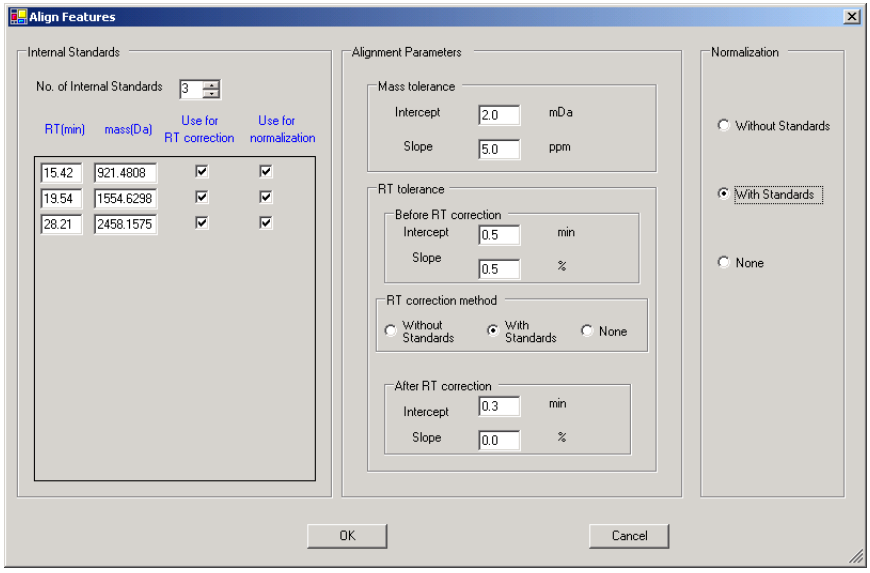
Exercise 2 – Set up to process feature data

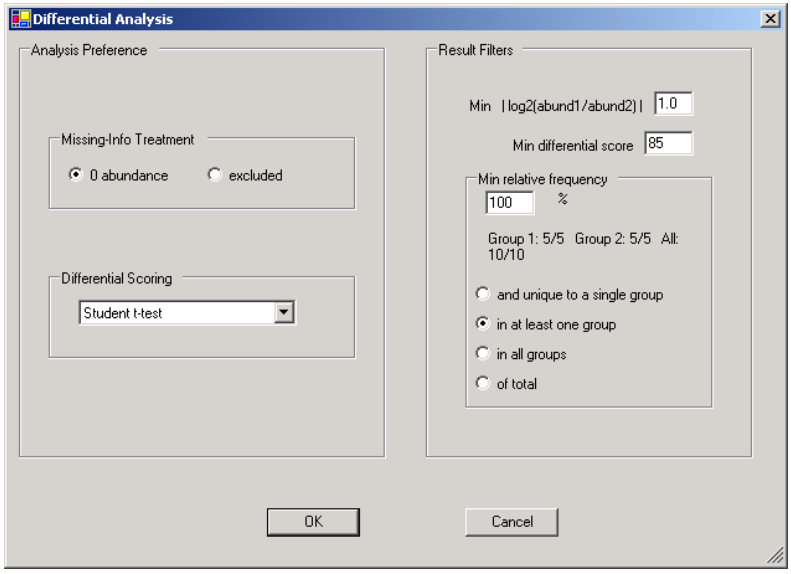
After an initial review of the data, you are now ready to change the default settings to meet your special needs. This exercise helps you prepare the .mhd files so that you can easily study features between different .mhd files.

Steps	Detailed Instructions	Comments
1 Filter the features that are loaded into the application. Change these settings: • RT – 12-34 min.	**a** Select **Analysis > Filter Features**, or Click the **Filter Features** icon. A dialog box appears that is the same one used for Display Filters in Mass Hunter. **b** For **Min RT**, enter 12. **c** For **Max RT**, enter 34.	



Steps	Detailed Instructions	Comments
	d Click **OK**. The Feature Comparison Window now appears with only 602 average features.	• When you click OK, the settings are immediately applied, along with the current settings for aligning features and performing differential analysis.
2 Change the alignment and normalization settings to these values: • Use standards for RT correction and normalization. • Use 3 internal standards with these RTs and masses: RT: 15.42; Mass: 921.4808 RT: 19.54; Mass: 1554.6298 RT: 28.21; Mass: 2458.1575	**a** Select **Analysis > Align features**, or Click the **Align features** icon. **b** For the RT correction method, select **with standards**. **c** For Normalization, select **with standards**. **d** From the **No. of Internal Standards** list, enter or select 3. **e** Make sure that the **Use for RT correction** and **Use for normalization** check boxes are marked.	
	f Click **OK**. The Feature Comparison Window now appears with only 529 features.	• When you click OK, the alignment settings are immediately applied to the feature plots and tables, as are the current filtering and differential analysis settings.

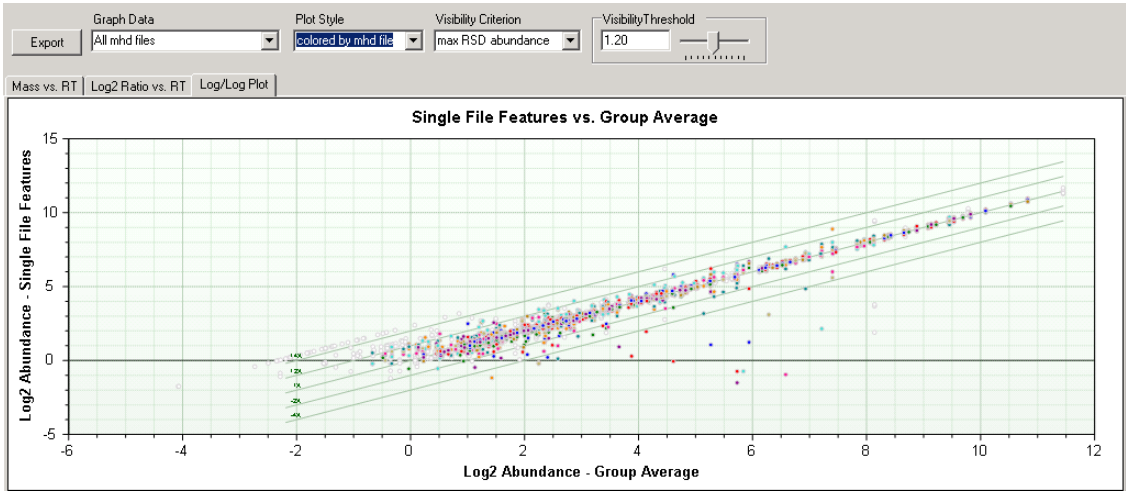


Steps	Detailed Instructions	Comments
3 Change the differential analysis settings to these values: • Min|log2(abund1/abund2)| = 1	**a** Select **Analysis > Differential Analysis...**, or Click the **Differential Analysis** icon. **b** For **Min|log2(abund1/abund2)|**, enter 1.	• Abund1 is the average abundance of a feature in the set1 .mhd files (120 fmol myo). Abund2 is the average abundance of a feature in the set2 .mhd files (30 fmol myo).
		
	c Click **OK**. The Feature Comparison Window now appears with only 265 average features.	• When you click OK, the settings are immediately applied, along with the current settings for filtering and aligning/normalizing features.

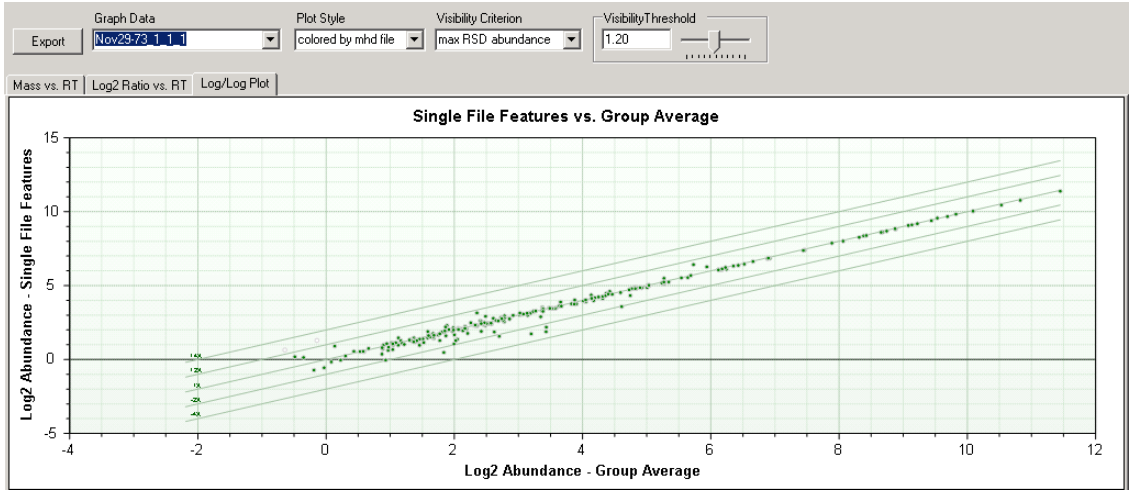
Exercise 3 – View Feature Plot

This exercise shows you how to view features using different plot functions.

Steps	Detailed Instructions	Comments
1 Compare the features in the log/log plot by color. • Reset the Visibility Criterion to max RSD abundance and the threshold to 1.2. • Select to have the features in each .mhd file in a different color.	**a** From the Visibility Criterion list, select **max RSD abundance**. **b** Enter a **Visibility Threshold** of 1.2. **c** From the **Plot Style** list, select **colored by mhd file**. Note that many more data points appear than were on the previous plot with Plot Style Avg. Feature.	• Avg. Feature is one data point—a result of averaging the masses and RTs of ten features over ten .mhd files. • Data points for "Colored by mhd file" represent the same feature in each .mhd file, each of which has a different RT.

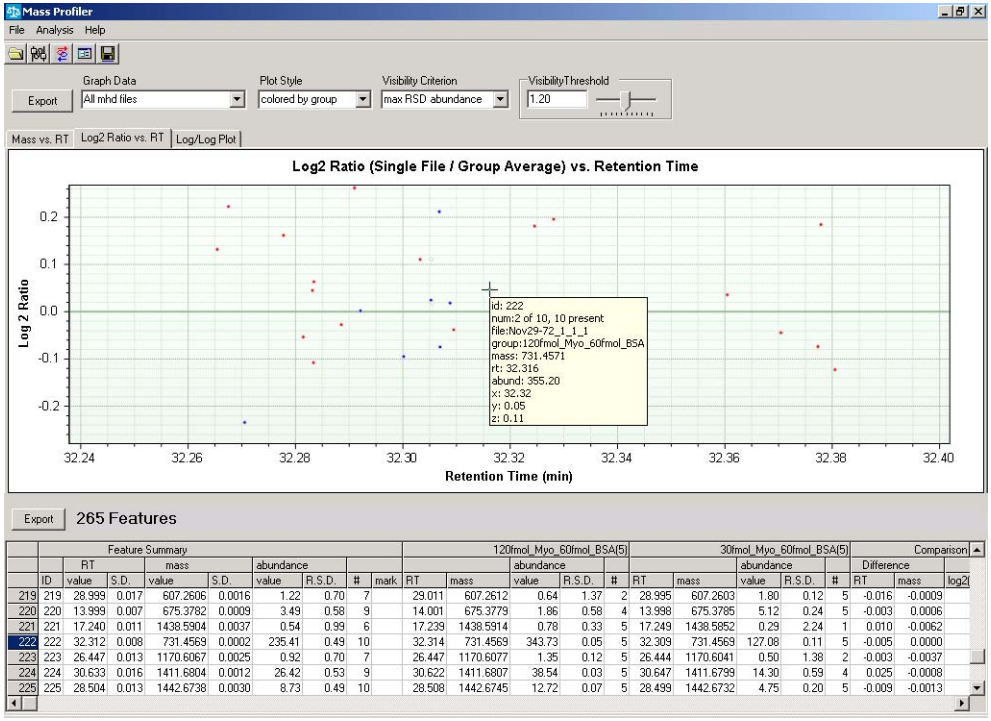


Steps	Detailed Instructions	Comments
2 View the data for the single file, Nov29-73_1_1_1.	**d** From the **Graph Data** list, select **Nov29-73_1_1_1**.	• You should now see just one color. • Note that there are no myoglobin spike-ins for this file.



3 Compare the features in the Log2 Ratio vs Retention Time plot by color for all the .mhd files and find information on feature #222: • Color features by feature. • Color features by group. • Zoom into the RT range of 32.2-32.4. • Find information on mass, RT and abundance for feature #222 in the Feature Plot and Feature Table.	**a** Click the **Log2 Ratio vs RT** tab. **b** From the **Graph Data** list, select **All mhd files**. **c** From the **Plot Style** list, select **colored by feature**. **d** From the **Plot Style** list, select **colored by group**. **e** Click a point around RT 32 that lets you include points between RT 32 and 34. **f** Draw a rectangle whose opposite corner is about RT 34 and release the mouse. **g** Continue to zoom in until you see RT 32.2 to 32.4 on the plot. **h** To find the feature with ID 222 on the plot, pass the cursor over the data points until you see the information in the tooltip for #222. **i** Click the #222 data point to now see that feature highlighted in the Feature Table.	• Note how many more colors are visible when you switch from "colored by mhd file" to "colored by feature". • Note that when you select "colored by group", features are now colored by the group of .mhd files in which they are found. • Group 1 = 120 fmol .mhd files = red Group 2 = 30 fmol .mhd files = blue

Steps	Detailed Instructions	Comments



	Feature Summary									120fmol_Myo_60fmol_BSA(5)					30fmol_Myo_60fmol_BSA(5)					Comparison		
		RT		mass		abundance						abundance					abundance			Difference		
	ID	value	S.D.	value	S.D.	value	R.S.D.	#	mark	RT	mass	value	R.S.D.	#	RT	mass	value	R.S.D.	#	RT	mass	log2
219	219	28.999	0.017	607.2606	0.0016	1.22	0.70	7		29.011	607.2612	0.64	1.37	2	28.995	607.2603	1.80	0.12	5	-0.016	-0.0009	
220	220	13.999	0.007	675.3782	0.0009	3.49	0.58	9		14.001	675.3779	1.86	0.58	4	13.998	675.3785	5.12	0.24	5	-0.003	0.0006	
221	221	17.240	0.011	1438.5904	0.0037	0.54	0.99	6		17.239	1438.5914	0.78	0.33	5	17.249	1438.5852	0.29	2.24	1	0.010	-0.0062	
222	222	32.312	0.008	731.4569	0.0002	235.41	0.49	10		32.314	731.4569	343.73	0.05	5	32.309	731.4569	127.08	0.11	5	-0.005	0.0000	
223	223	26.447	0.013	1170.6067	0.0025	0.92	0.70	7		26.447	1170.6077	1.35	0.12	5	26.444	1170.6041	0.50	1.38	2	-0.003	-0.0037	
224	224	30.633	0.016	1411.6804	0.0012	26.42	0.53	9		30.622	1411.6807	38.54	0.03	5	30.647	1411.6799	14.30	0.59	4	0.025	-0.0008	
225	225	28.504	0.013	1442.6738	0.0030	8.73	0.49	10		28.508	1442.6745	12.72	0.07	5	28.499	1442.6732	4.75	0.20	5	-0.009	-0.0013	

Exercise 4 – View Feature Table

You can also use the feature data in tabular form to investigate features across different .mhd files.

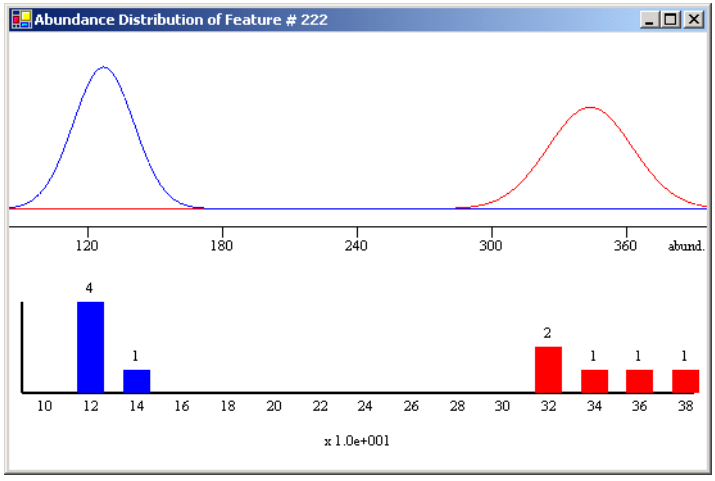
Steps	Detailed Instructions	Comments
1 View the features in the Features Table for feature #222. • See the Reference Help for descriptions of the columns in the Feature Table.	• Scroll the Features Table if necessary..	• The comparison data shows the actual differences in RT, mass, log values and differential score. • If the Group 1 (120-red) abundance is greater than that of Group 2 (30-blue), the log2 ratio for the feature is shown in red. If the Group 2 abundance is greater, then the value is shown in blue.

Export 265 Features

	Feature Summary				120fmol_Myo_60fmol_BSA(5)					30fmol_Myo_60fmol_BSA(5)					Comparison				
	abundance						abundance					abundance			Difference				
	value	R.S.D.	#	mark	RT	mass	value	R.S.D.	#	RT	mass	value	R.S.D.	#	RT	mass	log2(A1/A2)	|log2(A1/A2)|	Diff. Score
219	1.22	0.70	7		29.011	607.2612	0.64	1.37	2	28.995	607.2603	1.80	0.12	5	-0.016	-0.0009	-1.49	1.49	97.9
220	3.49	0.58	9		14.001	675.3779	1.86	0.58	4	13.998	675.3785	5.12	0.24	5	-0.003	0.0006	-1.46	1.46	99.8
221	0.54	0.99	6		17.239	1438.5914	0.78	0.33	5	17.249	1438.5852	0.29	2.24	1	0.010	-0.0062	1.45	1.45	85.2
222	235.41	0.49	10		32.314	731.4569	343.73	0.05	5	32.309	731.4569	127.08	0.11	5	-0.005	0.0000	1.44	1.44	100.0
223	0.92	0.70	7		26.447	1170.6077	1.35	0.12	5	26.444	1170.6041	0.50	1.38	2	-0.003	-0.0037	1.43	1.43	97.2
224	26.42	0.53	9		30.622	1411.6807	38.54	0.03	5	30.647	1411.6799	14.30	0.59	4	0.025	-0.0008	1.43	1.43	100.0
225	8.73	0.49	10		28.508	1442.6745	12.72	0.07	5	28.499	1442.6732	4.75	0.20	5	-0.009	-0.0013	1.42	1.42	100.0

2 Sort data by the RT column, then by the mass column and then by the ID.	**a** Double-click the RT column until you see the features in order of lowest RT to highest RT. **b** Double-click the mass column header until you see the features in order of lowest mass to highest mass. **c** Double-click the ID column to return the table to its original format.	

Steps	Detailed Instructions	Comments
3 View the abundance distribution graph for feature #222.	**a** In the Features Table, right-click the row number for feature #222. **b** Select **Abundance Distribution**.	• The Abundance Distribution window shows the abundance distribution of feature 222 in the two sets of .mhd files.
4 Mark feature #222 for annotation.	**a** In the Features Table, right-click feature #222, and select **MarkOn/Off.** You see an X in the Mark column next to feature #222.	•



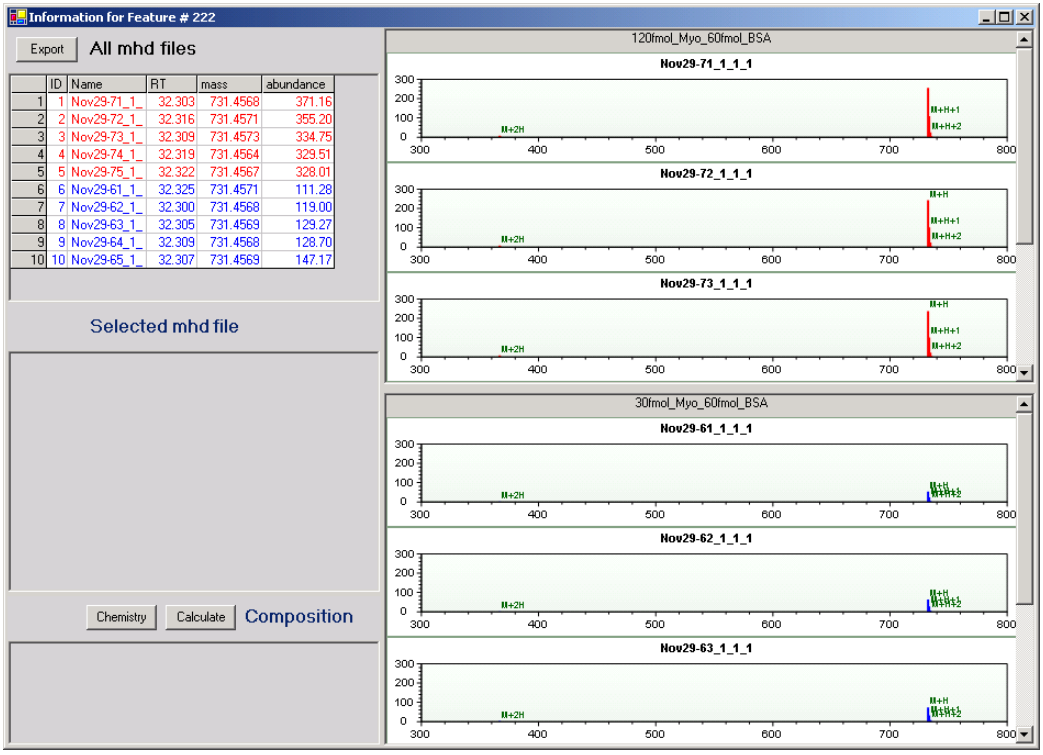
Export 265 Features

	Feature Summary								
		RT		mass		abundance			
	ID	value	S.D.	value	S.D.	value	R.S.D.	#	mark
219	219	28.999	0.017	607.2606	0.0016	1.22	0.70	7	
220	220	13.999	0.007	675.3782	0.0009	3.49	0.58	9	
221	221	17.240	0.011	1438.5904	0.0037	0.54	0.99	6	
222	222	32.312	0.008	731.4569	0.0002	235.41	0.49	10	X
223	223	26.447	0.013	1170.6067	0.0025	0.92	0.70	7	
224	224	30.633	0.016	1411.6804	0.0012	26.42	0.53	9	
225	225	28.504	0.013	1442.6738	0.0030	8.73	0.49	10	

Exercise 5 – View more detailed feature information

This exercise shows you how to access information on species clusters and chemical compositions for individual features. You can see the species clusters for the feature in each of the .mhd files in which it is found and calculate the possible compositions for the feature.

Steps	Detailed Instructions	Comments
1 Display the Feature Information Window for feature #222.	• In the Features Table (or the plot), right-click feature #222, and select **Details** (**Feature Details** in the plot). A listing of the feature data for all the .mhd files containing the feature appears on the left.	• A plot of the species cluster data for the feature for each .mhd file appears on the right.



Steps	Detailed Instructions	Comments
2 View the feature species table for .mhd file Nov29-72_1_1_1.	• Double-click the row number for .mhd file #2. The table listing the species clusters for feature #222 in that .mhd file appears.	

Export Nov29-72_1_1_1

	species	RT	m/z	m0	abundance	wid
1	M	32.316		731.4571	355.20	0.
2	M+2H	32.322	366.7367	731.4588	6.87	0.
3	M+2H+1	32.314	367.2380		3.33	0.
4	M+2H+2	32.296	367.7409		0.50	0.
5						
6	M+H	32.320	732.4643	731.4570	224.22	0.
7	M+H+1	32.320	733.4671		94.66	0.
8	M+H+2	32.320	734.4701		21.24	0.
9	M+H+3	32.318	735.4721		3.82	0.
10	M+H+4	32.321	736.4764		0.57	0.
11						

3 Display the possible compositions for feature #222.	• Click **Calculate**. The possible compositions for the feature appear in a table at the bottom of the window.	• If you need to change the elements selected to calculate the composition, see the *Mass Hunter Quick Start Guide*.

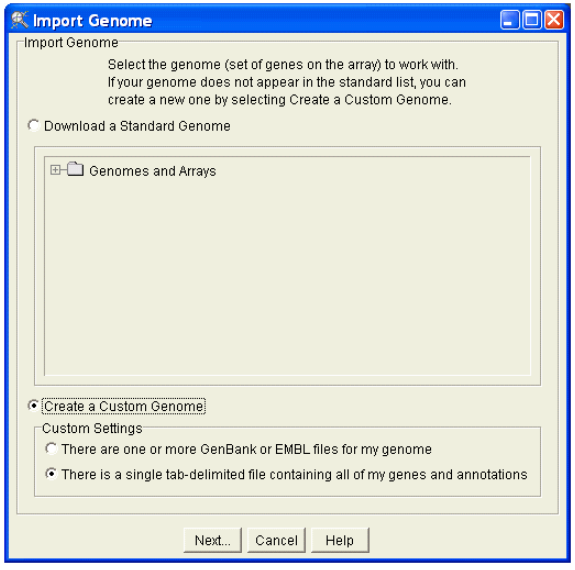
Export Chemistry Calculate Compositions

	chemical form	dm(Da)	dm(ppm)	DBE	score
1	C37H61N7O8	0.0014	1.9	11.0	97
2	C23H53N2O3	0.0032	4.4	9.0	42
3	C31H57N1O7	0.0034	4.6	12.0	78
4	C31H65N5O1	-0.0040	-5.4	2.0	71
5	C39H53N15	0.0041	5.5	21.0	70
6	C41H65NO10	0.0041	5.6	10.0	82
7	C34H65N7O8	0.0047	6.5	6.0	69
8	C27H65N13O	0.0054	7.4	2.0	42
9	C27H57N1O7	0.0059	8.1	8.0	65
10	C35H61N11O	0.0061	8.3	11.0	68
11	C43H57N9O2	0.0067	9.2	20.0	47
12	C28H61N1O7	0.0068	9.3	7.0	43
13	C28H53N21O	0.0072	9.9	13.0	80
14	C30H65N7O1	0.0073	9.9	2.0	68
15	C38H69NO10	0.0074	10.2	5.0	57

Exercise 6 – Export Mass Profiler Feature Summary for GeneSpring

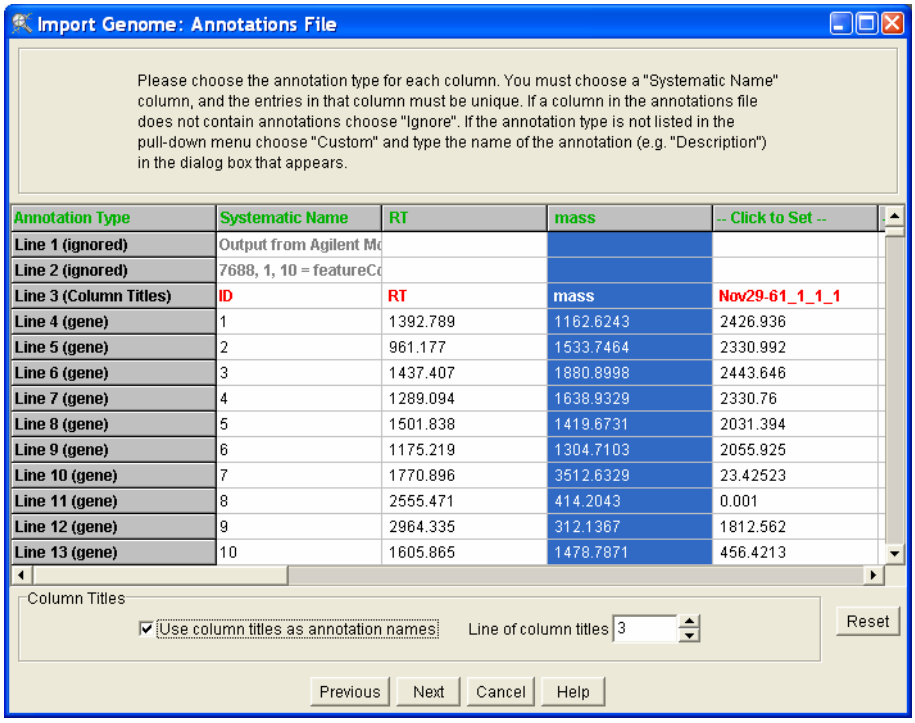
In addition to exporting almost all the tables, graphics and text information from Mass Profiler to an Excel file or .txt file for future use, you can also export the Feature Summary information in the Feature Table to a .txt file and upload the file to GeneSpring. You may want to do this to take advantage of GeneSpring's advanced filtering, normalization and differential analysis techniques or the capacity to compare more than two groups of features. You will, however, have to align the MS-produced features first with Mass Profiler before uploading the .txt file into GeneSpring.

This exercise shows you how to export the Feature Summary to a .txt file and how to use the information in GeneSpring.

Steps	Detailed Instructions	Comments
1 Export the Feature Summary Table to a .txt file. • Call it "Myoglobin-Spikein.txt".	**a** Select **File > Export for GeneSpring**. **b** Enter `Myoglobin-Spikein`, and click **Save**.	
2 In GeneSpring create a custom genome from Mass Profiler output file. • Make sure headers for annotation file say Systematic Name, RT and Mass. • Save the new genome as "Myoglobin Spike-in experiment".	**a** Click the **GeneSpring** icon to open the application.  **b** Select **File > Import Genome**. 	

Steps	Detailed Instructions	Comments
	c Make sure that **Create a Custom Genome** is selected. **d** Make sure that **There is a single tab-delimited file containing all of my genes and annotations** is selected. **e** Click **Next**. **f** Select the Mass Profiler output file, **Myoglobin-Spikein.txt**, and click **Open**. The Import Genome: Annotations File dialog box appears. **g** In the **Line of column titles text** field, use the up arrow to select 3, or simply type in the number 3. Column Titles are indicated with red bold type. **h** Click on the **Click to Set** column header and change this to **Systematic Name**. **i** Right-click the second column header, and select **RT** from the list. **j** Right-click the third column header, and select **mass** from the list.	

Steps	Detailed Instructions	Comments



k Click **Next**.

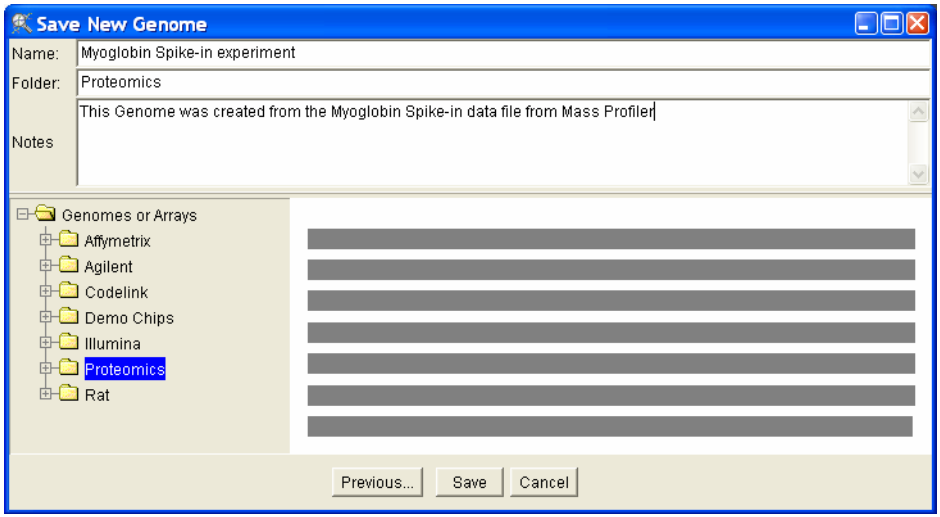
l When the warning appears, click **Yes** to continue.

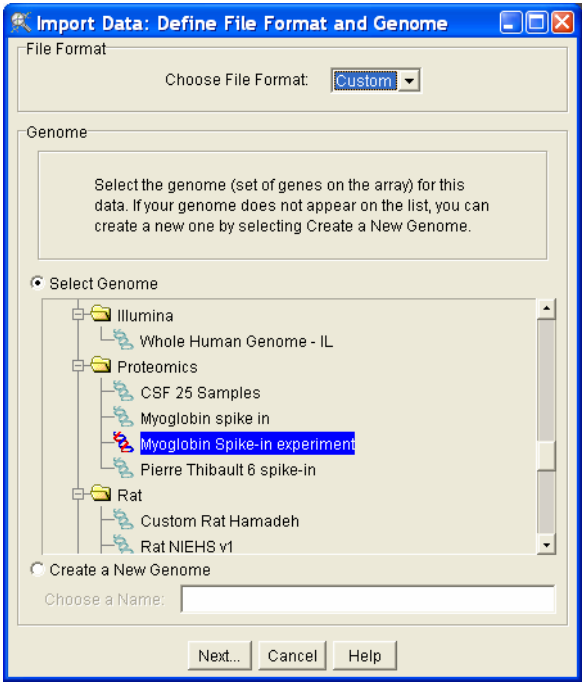
m Ignore the next window, and click **Next**.

n Add any links you want (optional), and click **Next**.

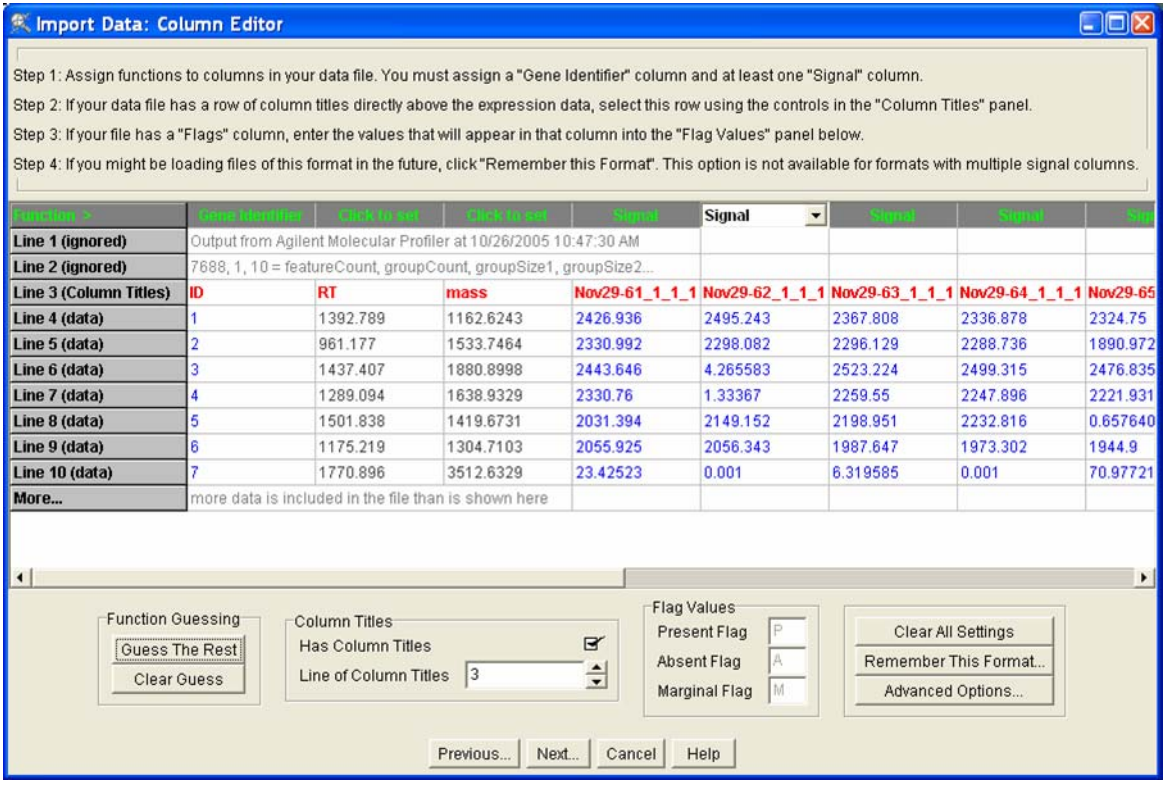
o In the Save New Genome window, enter the **Name** as **Myoglobin Spike-in experiment**.

p Enter the **Folder** and **Notes** that you want.

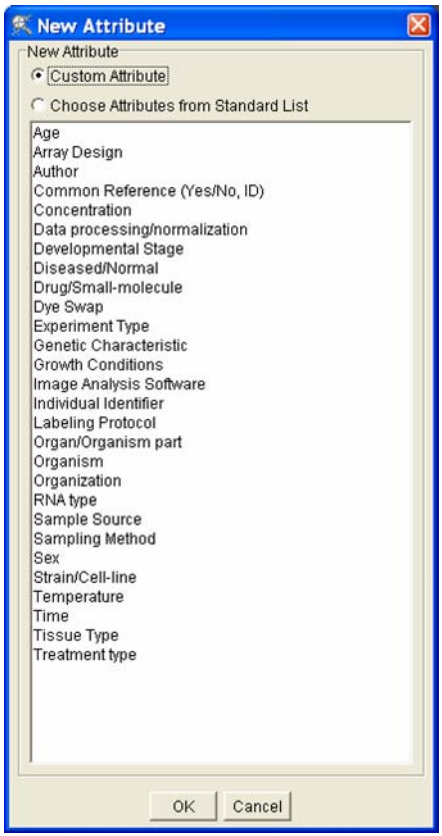
Steps	Detailed Instructions	Comments
		
	q Click **Save**. The New Genome Checklist appears. **r** Click **Close**. The new genome appears in the main window with no data in it.	• The New Genome Checklist lets you add annotations to your genome. • You must now load the Mass Profiler output file again.

Steps	Detailed Instructions	Comments
3 Populate the Myogobin Spike-in experiment genome with Mass Profiler data and define the data format. • Select a custom data format. • Assign the first column to be the Gene Identifier column. • Assign the sample (.mhd file) abundance columns as Signal columns.	**a** Click the **Myoglobin-Spikein.txt** file and drag it to the main GeneSpring window containing the blank genome. **b** For **Choose File Format**, click the down arrow and select **Custom** from the list. **c** Select **Myoglobin Spike-in experiment** from the genome directory.  **d** Click **Next**. The Import Data: Column Editor appears. **e** Click the **Click to Set** column header for the ID column, and select **Gene Identifier.** **f** Click the **Click to Set** column header for the first abundance column (.mhd file name), and select **Signal**. **g** Repeat step f for the second sample.	•

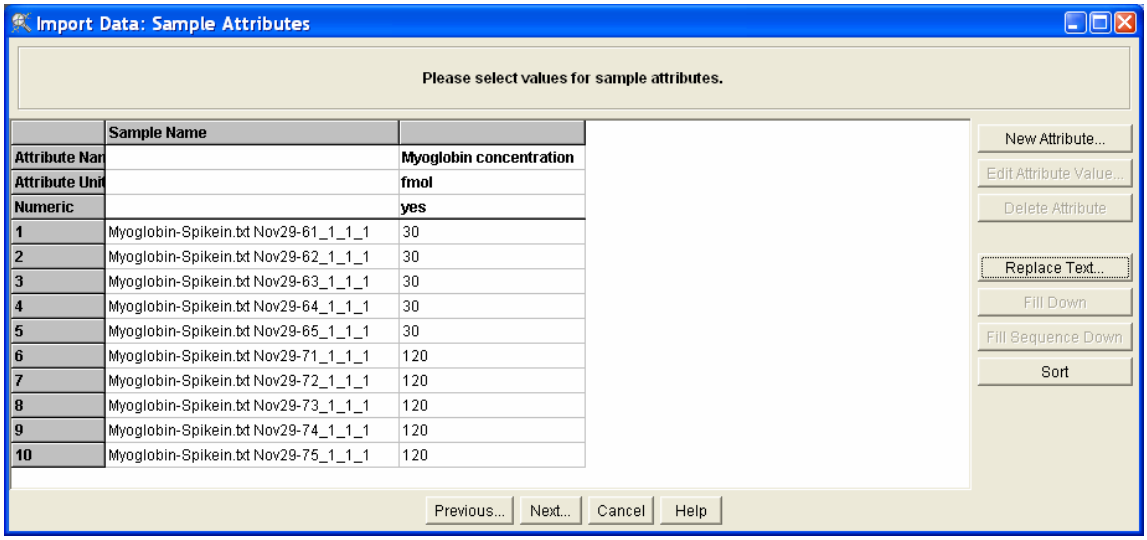
Steps	Detailed Instructions	Comments
	h Click **Guess The Rest** to assign **Signal** as the header for the rest of the .mhd file abundance columns.	



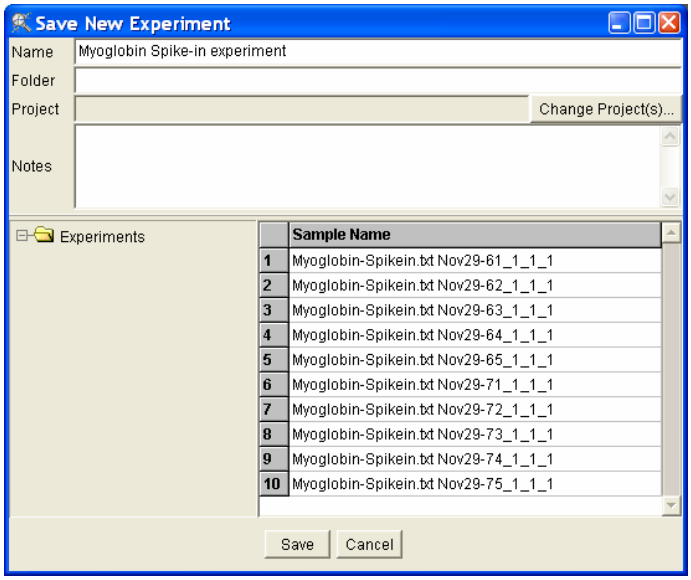
	i Click **Next**. **j** Ignore the Selected Files window, and click **Next**. The Import Data: Sample Attributes window appears.	

Steps	Detailed Instructions	Comments
4 Annotate the samples with their concentrations. • Add an attribute (column) called Myoglobin concentration. • Delete all other columns except for Sample Name.	**a** Click **New Attribute**.  **b** Select **Custom Attribute**, if necessary, and click **OK**. A new empty column is added. **c** Fill in the new column with Myoglobin Concentration and individual concentrations for each sample in fmol. **d** Highlight each of the other columns in turn, and click **Delete Attribute** until all columns are deleted except Sample.	

Steps	Detailed Instructions	Comments

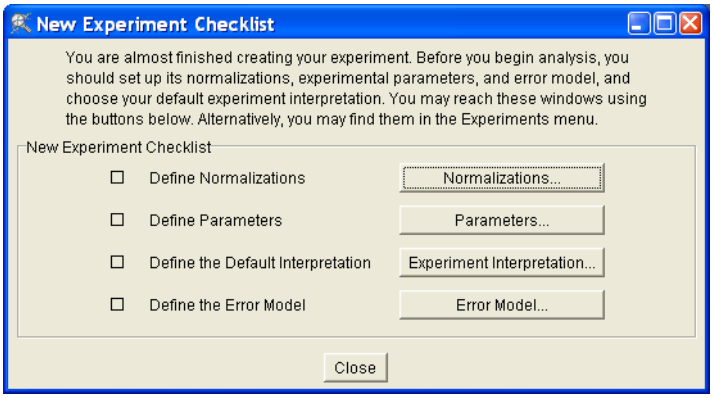


e Click **Next**.
The Import Data: Create Experiment message appears.

Steps	Detailed Instructions	Comments
5 Create a new experiment called "Myoglobin Spike-in experiment". • Add a new myoglobin concentration parameter to the experiment parameters. • Change the interpretation settings and save the interpretation to a file names 30 vs 120: – Sample: Do Not Display – Myoglobin Concentration: Continuous	**a** Click **Yes** to create a new experiment with the ten samples you just annotated. The Save New Experiment dialog box appears. **b** Type in the Name of the experiment, **Myoglobin Spike-in experiment**.  **c** Click **Save**.	

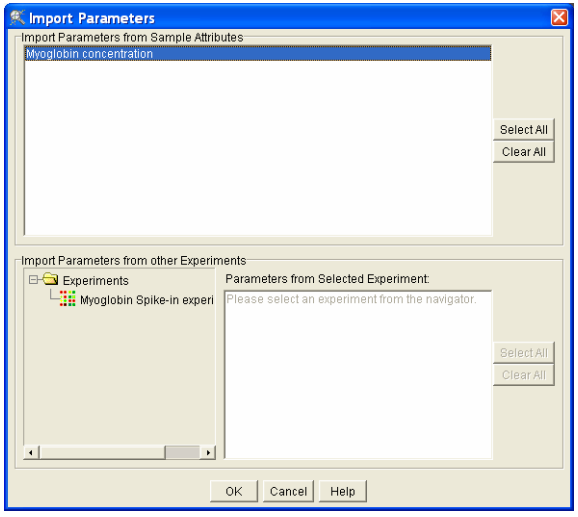
Steps	Detailed Instructions	Comments

The New Experiment Checklist dialog box appears.



d To add parameters, click **Parameters**. The Experiment Parameters dialog box appears.

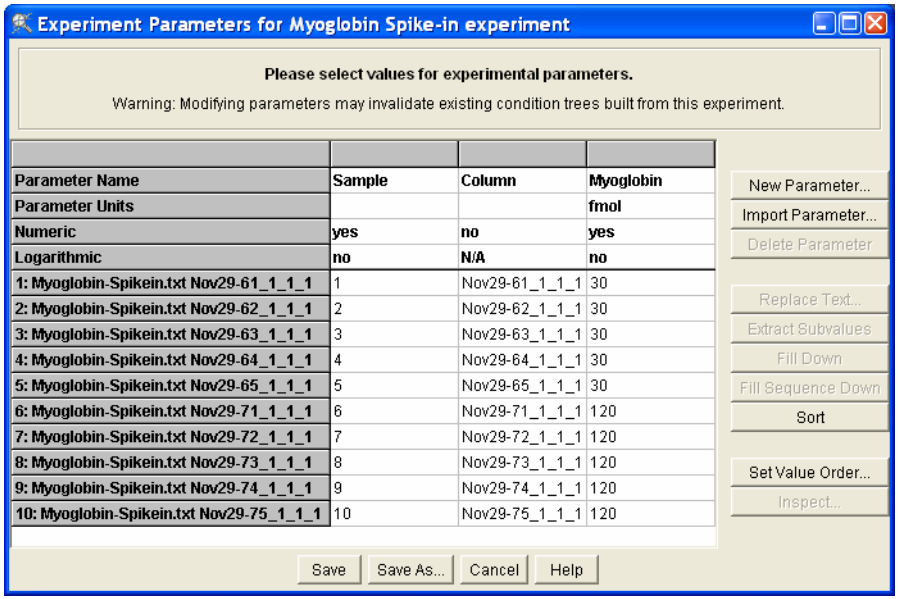
e Click **Import Parameter...**.



f Make sure that Myoglobin concentration is selected, and click **OK**.

Steps	Detailed Instructions	Comments

g Select the **File Name** column, and click **Delete Parameter**.
The Experiment Parameters table is now complete.



h Click **Save**.
The New Experiment Checklist appears again.
The **Define Parameters** check box is automatically marked, and the new settings are saved.

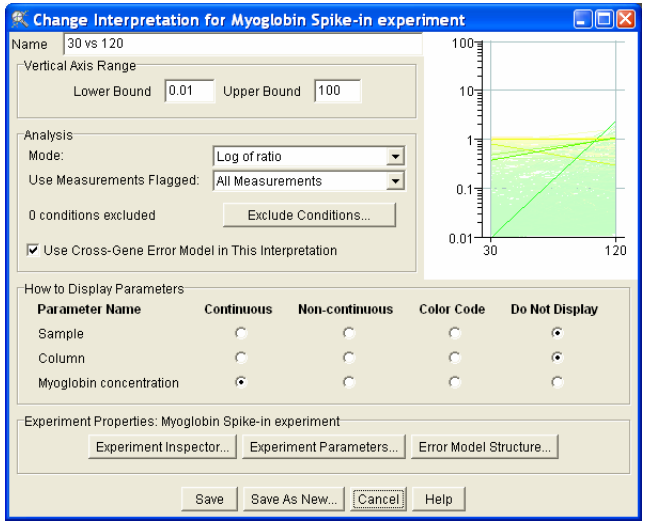
i Click **Experiment Interpretation**.
The Default interpretation for the Change Interpretation dialog box appears.

j Change the Name to **30 vs 120**.

k Click **Save As...** Enter the name `30 vs 120`. Click **OK**.

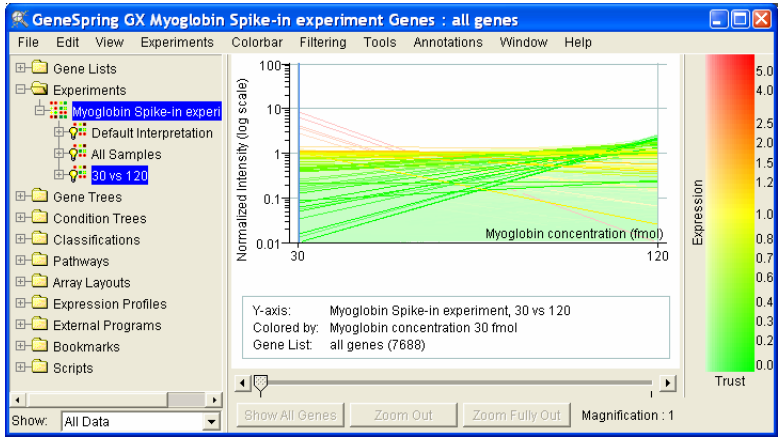
l For the Display Parameters, select **Do Not Display** for Sample and **Continuous** for Myoglobin concentration.

Steps	Detailed Instructions	Comments



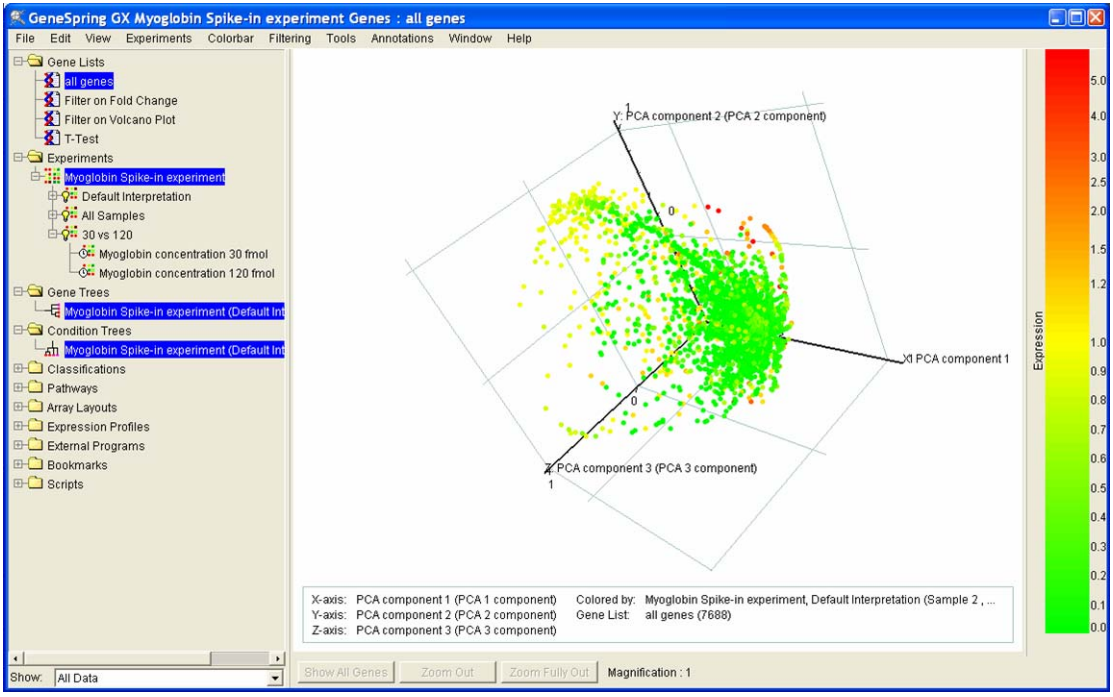
m In the New Experiment Checklist, click **Close**.

n In the GeneSpring main navigator, select **Experiments > Myoglobin Spike-in experiment > 30 vs 120** to see the result of changing the interpretation.



Other GeneSpring analyses available for Mass Profiler data:

- Scatter plot
- Volcano plot
- Fold change filter and plot
- ANOVA
- Venn Diagrams
- Clustering
- Hieriarchical Trees
- Principle Least Squares - Discriminant Analysis (PLS-DA)
- Principle Component Analysis (PCA) – See plot below.



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In this guide

This Quick Start Guide includes an overview of the Mass Profiler software, quick reference information to get started using the software, and a set of tutorials to learn how to use the software.

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