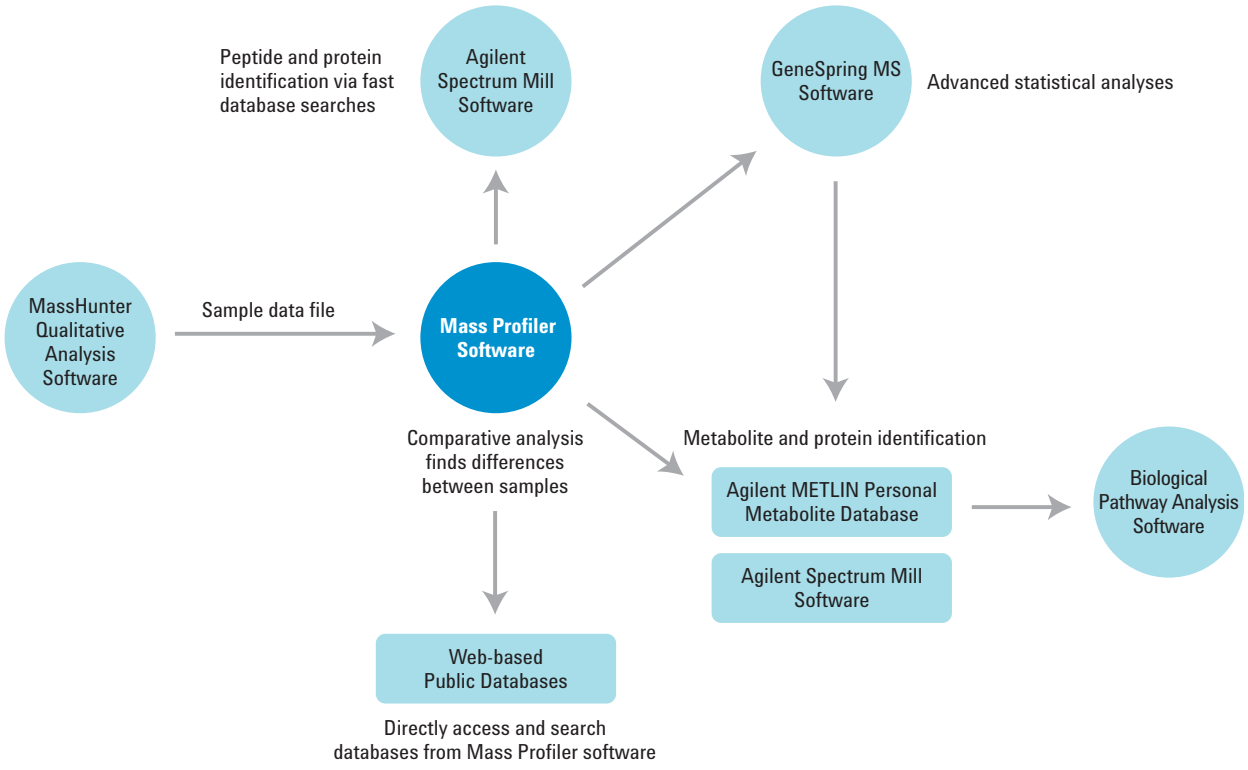
Agilent Mass Profiler Software

Essential Tool Set for Differential Profiling

The Agilent MassHunter Mass Profiler software performs comparative analysis of one or two data sets generated from Agilent time-of-flight (TOF) or quadrupole-TOF (Q-TOF) instruments. The comparative analysis results are displayed in graphical and tabular formats that enable you to quickly identify statistically meaningful differences between features (discrete molecular entities defined by retention time and mass) in the same group or two different groups. The Mass Profiler application can directly query the optional Agilent METLIN Personal Metabolite Database and several web-based public databases, or export results for further analysis.



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Mass Profiler comparative analysis

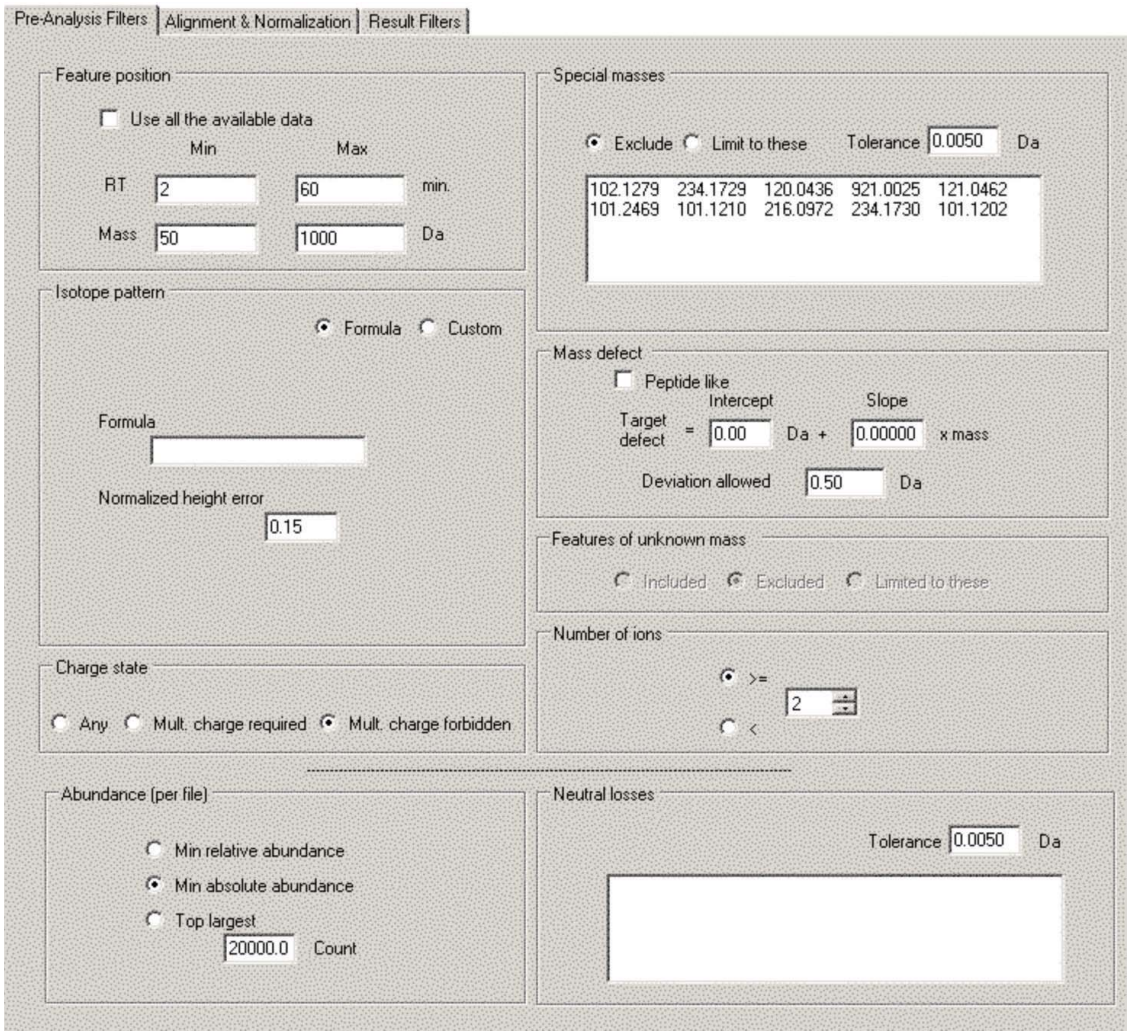
After creating your differential analysis project, you can customize the analysis by editing the parameter settings.

Apply pre-analysis filters to charge state, isotope pattern, abundance level, neutral loss, and mass defect. Specify masses to exclude from the analysis or limit the analysis to specific masses, and set mass and retention time tolerance windows.

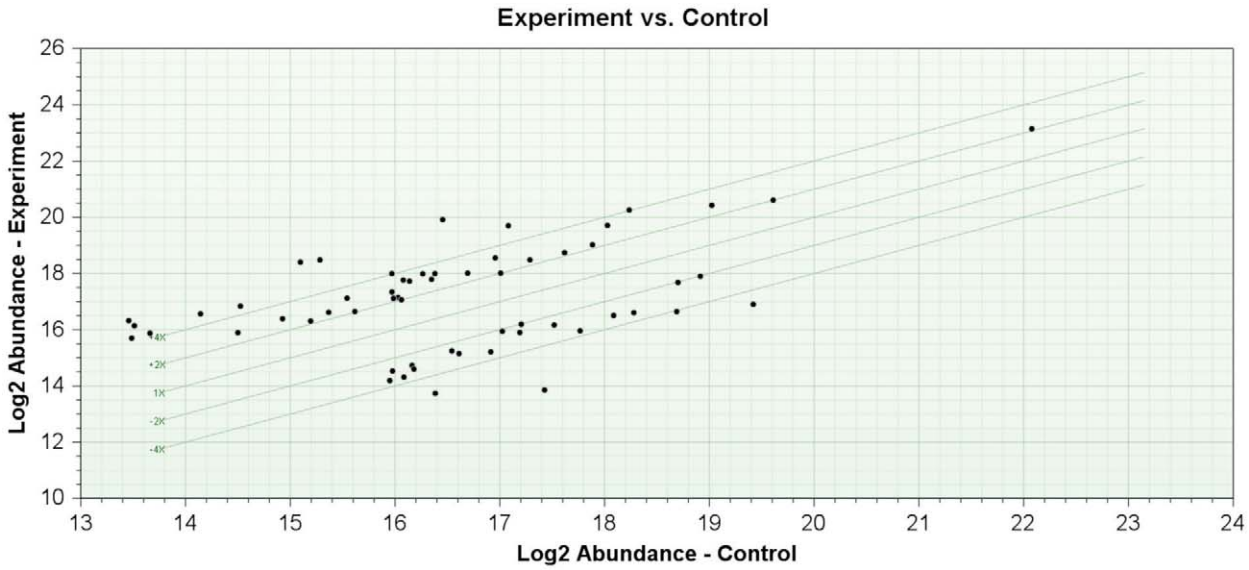
Align and normalize by specifying internal standards to normalize mass abundance values and correct for retention time shifts in the raw data. The software uses information about user-specified internal standards to normalize abundance values and adjust for retention time shifts across all of the data sets.

Apply filters to show only results of interest in the Feature Summary table and graphical plots. Set thresholds for abundance ratios, differential score, and minimum relative frequency. Filter results based on expression fold change, relative frequencies, or minimum differential score.

For further statistical analyses, export a feature summary to the GeneSpring MS software that provides advanced statistical tools.



Set pre-analysis filters to choose particular data to analyze.



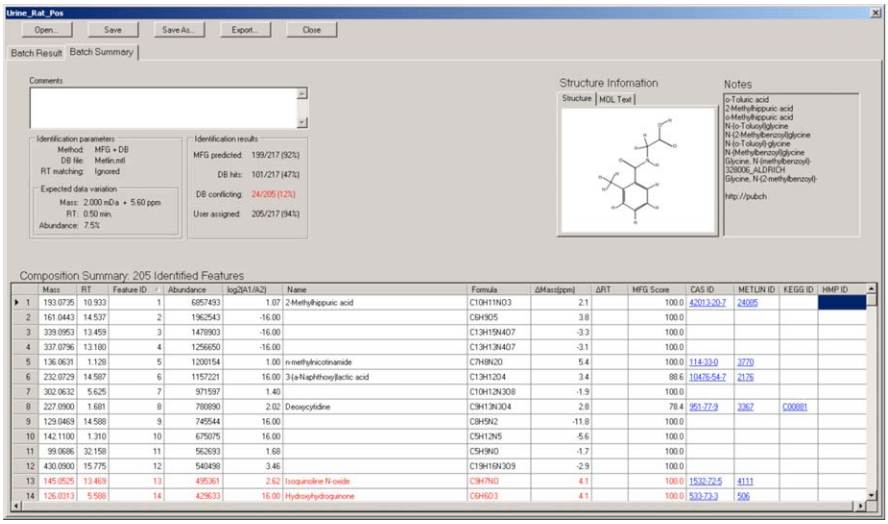
The Log/Log plot of fold changes helps you quickly determine up- or down-regulated features in the samples. Fold change lines in the Log/Log plot represent the constant ratio of abundances between experiment and control groups. In this example, only features with two-fold or greater abundance ratios are displayed.

Group 1 Group 2

Expression: Both Up Down 217 Features

	Feature summary									Experiment(4)					Control(4)					Comparison				
	ID	RT	SD	Mass	SD	Abundance	RSD	Freq.	Mark	RT	Mass	Abundance	RSD	Freq.	RT	Mass	Abundance	RSD	Freq.	RT	Mass	Log2(A1/A2)	|Log2(A1/A2)|	Diff. Score
1	1	10.933	0.018	193.0735	0.0001	6857493	0.38	8		10.922	193.0735	9290860	0.06	4	10.945	193.0735	4424126	0.03	4	0.022	-0.0001	1.07	1.07	99.9997
2	2	14.537	0.011	161.0443	0.0002	1962543	1.07	4				0		0	14.537	161.0443	3925086	0.02	4			-16.00	16.00	100.0000
3	3	13.459	0.010	339.0953	0.0001	1478903	1.07	4				0		0	13.459	339.0953	2957806	0.04	4			-16.00	16.00	100.0000
4	4	13.180	0.010	337.0796	0.0001	1256650	1.07	4				0		0	13.180	337.0796	2513300	0.03	4			-16.00	16.00	100.0000
5	5	1.128	0.004	136.0631	0.0001	1200154	0.36	8		1.131	136.0630	1600728	0.03	4	1.125	136.0631	799580	0.03	4	-0.006	0.0000	1.00	1.00	100.0000
6	6	14.587	0.009	232.0729	0.0001	1157221	1.08	4		14.587	232.0729	2314441	0.10	4			0		0			16.00	16.00	99.9999
7	7	5.625	0.024	302.0632	0.0002	971597	0.48	8		5.609	302.0631	1410536	0.04	4	5.642	302.0634	532659	0.03	4	0.033	0.0003	1.40	1.40	100.0000
8	8	1.681	0.005	227.0900	0.0001	780890	0.75	8		1.681	227.0900	1253252	0.36	4	1.682	227.0900	308527	0.31	4	0.000	0.0000	2.02	2.02	99.3825
9	9	14.588	0.009	129.0469	0.0001	745544	1.08	4		14.588	129.0469	1491088	0.09	4			0		0			16.00	16.00	100.0000
10	10	1.310	0.005	142.1100	0.0000	675075	1.07	4		1.310	142.1100	1350150	0.07	4			0		0			16.00	16.00	100.0000
11	11	32.158	0.024	99.0686	0.0000	562693	0.72	6		32.147	99.0686	858214	0.28	4	32.180	99.0686	267172	1.15	2	0.033	0.0001	1.68	1.68	97.7119
12	12	15.775	0.008	430.0900	0.0002	540498	0.90	8		15.773	430.0899	991194	0.08	4	15.776	430.0900	89802	0.03	4	0.003	0.0001	3.46	3.46	100.0000

The Mass Profiler software applies a student's t-test to the differential analysis to check for statistical significance. In the Feature Summary table, the comparison data shows the retention times and mass differences between features from the two groups. If group 1 abundance > group 2 abundance, the feature is upregulated and the feature $\log_2$ ratio is displayed in red. If group 2 abundance > group 1 abundance, the feature is downregulated and the feature $\log_2$ ratio is displayed in blue. The differential score is a number between 0 and 100 that represents the relative confidence that the difference in intensity of a feature between the two groups is real. Two methods are available for calculating the differential score, a student's t-test-based method or a support vector machine method.



Agilent METLIN Personal Metabolite Database batch search results include the molecular formulas proposed by the molecular formula generation algorithm, and the MFG probability score.

Identify metabolites and proteins

The Mass Profiler software directly interfaces with several web-based public databases and the optional Agilent METLIN Personal Metabolite Database, enabling you to conveniently launch a search for compound identification from the Mass Profiler application.

For metabolites, save or export METLIN database hits, including molecular formula generation (MFG) results. Export search hits from the METLIN database to a biological pathway analysis application to gain insight about cellular and molecular functions of compounds in the context of functional pathways. For proteins, you can export from the Mass Profiler software a mass list that can be uploaded to the Spectrum Mill software for peptide and protein identification through fast database searches.

Conveniently manage projects, methods, and data

- Create and save projects that specify the group(s) and data files for comparative analysis.
- Save user-specified pre-analysis filters, internal standards, alignment parameters, and results filters to a method that can be applied to user-selected projects. Edit a method to conveniently create a new method.
- Generate inputs to the GeneSpring MS or Spectrum Mill applications from the Feature Summary table or a graphical plot.
- Create a target inclusion list for Agilent Q-TOF MS/MS analysis.
- Export batch summary results .csv files from the METLIN to a biological pathway application to explore relevant biological and chemical information.

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