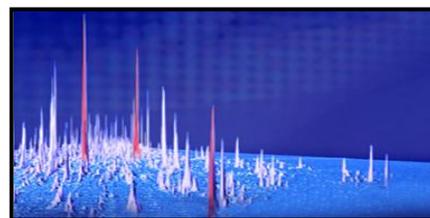
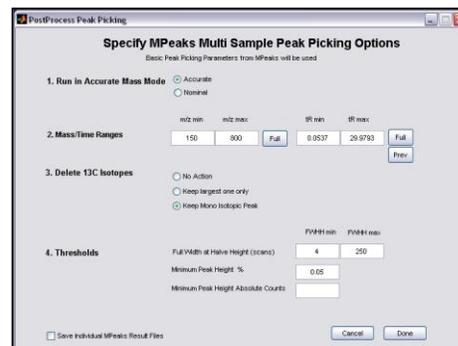


MsCompare™ High Resolution Peak Picking for LC/MS and GC/MS: - A Quick Starting Guide -

Introduction: this quick starting guide teaches you to quickly perform high resolution peak picking for LC/MS and nominal peak picking for GC/MS data. Both techniques have their own peak picking algorithms. Peak Picking, as opposed to Peak Matching, is applied to each individual sample. The results from all samples are grouped / clustered and presented in the final table. Peak Picking can be followed by univariate statistical or multivariate analysis using many of the specialized algorithms from MsCompare. You must be familiar with the MPeaks and MsCompare programs.

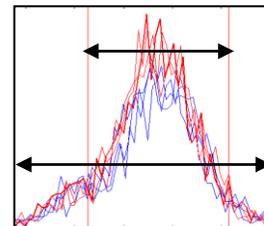


1. **MsCompare Data Loading:** start MsCompare and load or create a project containing all of your samples. Based on a visual analysis, decide if alignment is necessary. If this is the case, see the tutorial documents on Alignment.
2. **Start MPeaks and Run LC/MS Peak Picking:** MsCompare will use the last used LC/MS Peak Picking parameters from MPeaks. Load one of your samples into MPeaks and run Peak Picking using the criteria and sensitivity you need. For more information on Peak Picking, see the Quick Reference Guide: MPeaks High Resolution Peak Picking.
3. **Return to MsCompare and Run LC/MS Peak Picking:** To run High Resolution LC/MS Peak Picking, select from the menu: Peak Picking > LC/MS settings. A GUI is presented with additional options for multi sample peak picking. These settings will be used after peak picking on individual samples is completed. First select the type of analysis to be applied: Accurate or Nominal Mass Mode. Select the time and mass ranges. Next, you will have the option to remove ¹³C Isotope peaks, delete broad peaks and to specify a new relative or absolute peak height threshold.



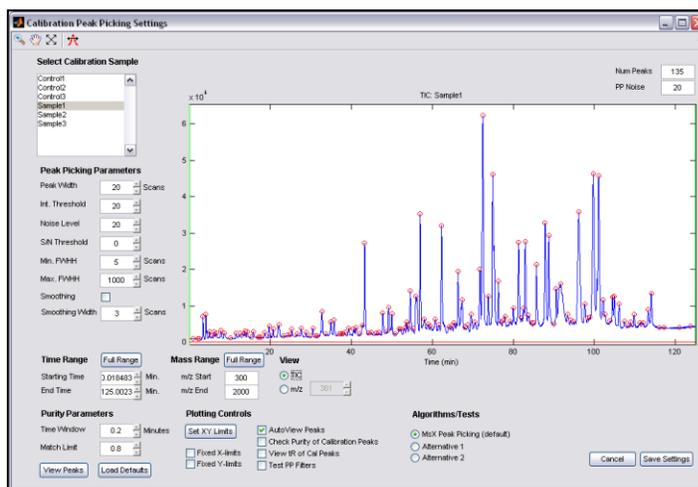
Peak Clustering:

Press Done and the settings will be saved. In the next step select: Peak Picking > Run Peak Picking. You will be asked to enter a value for clustering similar peaks from different samples. Check your data and see how much shift exists between some of the typical peaks. Measure the distance in scans or minutes using the Peak Width Measurement Tool (on the Icon Toolbar). Enter the number of scans so that all peak maxima will be in the interval (top arrow). A too small clustering value will create additional split clusters; a too large value might miss small peaks. Press "OK" and the Peak Picking & Clustering will be started. If accurate mass mode was selected you will need to enter the mass accuracy and time window for extracting accurate EIC's. Typical values used are an accuracy of 0.01 Da and a time window of 0.5 minutes (lower arrow), depending on the accuracy of your instrument and the width of your peaks.



Press "OK", and the calculations will begin. At the end the table can be saved directly and will be presented in MsCompare. You will now be ready to apply univariate and multivariate techniques to find peaks that are responsible for discriminating the different groups/classes in your project.

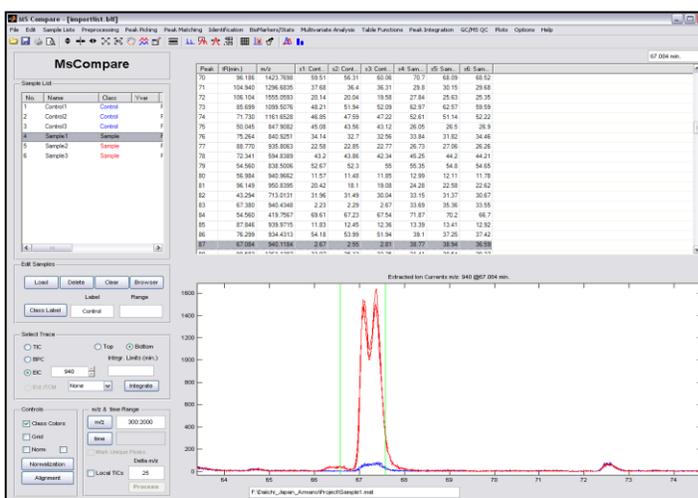
- Run GC/MS Peak Picking:** MsCompare has a special algorithm for GC/MS Peak Picking. Compared to the LC/MS algorithm, this one is slower, but is more accurate when many peaks are present in one and the same mass chromatogram. To start GC/MS Peak Picking select from the menu: Peak Picking > GC/MS Settings. A window will be displayed from which you can select and experiment with the GC/MS peak picking parameters. The peak picking results for the TIC or individual mass traces will be shown and updated immediately. For detailed description of all parameters and the peak picking interface, see the GC/MS - QC Quant Manual.



Press “Save Settings” to store the GC/MS peak picking parameters. Next, from the menu select: Run Peak Picking. You will have to enter a clustering value to group similar peaks from different samples (see previous page). After the GC/MS Peak Picking has been completed, you will be presented with the final table showing clustered peaks for all samples.

You will now be ready to apply univariate and multivariate techniques to find differences between your groups or classes.

- Applying Multivariate Analysis methods to Discriminate between Groups:** From the Menu select Multivariate Analysis to explore the data set. Use **PCA** to get an impression of outlying samples, unsupervised grouping and to find peaks that are responsible for class separation. For two groups you can use **PLS-DA** to apply a supervised analysis. In case of more than two groups use Extended Canonical Variate Analysis (**ECVA**), a combination of PLS and Linear Discriminant Analysis.

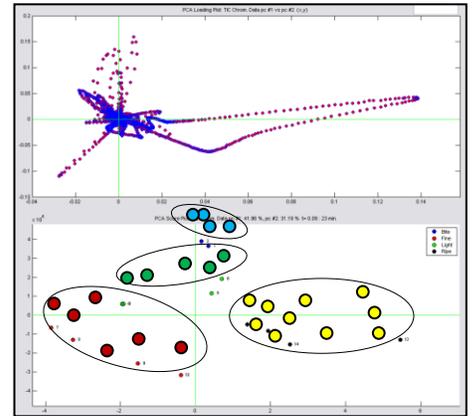


- Applying Univariate Statistical methods to find discriminating Peaks for two Groups:** For two groups, select Biomarker/Stats and decide what kind of peaks to search for; up-regulated or down-regulated. Some of the algorithms (using peak ratios) need to know which group is expected to be up-regulated. MsCompare has a large number of powerful statistical test (and graphics) to find discriminating peaks. These tests include: t-test, ratio analysis, uniqueness test, full selectivity, percent up-regulated and the Fisher Discriminant test. MsCompare also has a new feature: Create a Multi Test Statistical Overview. It will calculate all statistics for all peaks and present an overview of the most discriminating peaks. The peaks can be directly plotted from this overview and peaks of no importance can be deleted from the main table.

Statistics Results Overview (Median): Class Sample/Control

Peak No.	m/z	IR (min.)	Avg. Int %	Ratio	t-value	p-value	Unique	Weighted Uniq.	Selectivity	% Up-Regulated	Fisher	
38	51	1402.2258	88.73	14.24	1.189	5.71	0.0047	8.6	296	1	100	3.29
39	52	1124.5192	99.78	23.63	0.901	3.08	0.0369	-5.2	-293	-1	0	-1.78
40	53	1423.269	96.15	29.05	1.17	3.69	0.0211	7.8	599	1	100	2.13
41	54	1274.1069	82.94	18.47	1.026	3.19	0.0331	1.3	80	1	100	1.84
42	58	1401.7219	88.73	17.06	1.193	10.78	0.0004	8.8	366	1	100	6.22
43	59	426.2151	49.19	30.25	11.272	284.5	0	83.7	3713	1	100	164.25
44	60	1072.0481	74.91	13.6	1.096	4.49	0.0109	4.6	162	1	100	2.59
45	61	1260.6312	90.58	14.28	0.894	6.28	0.0033	-6.1	-241	-1	0	-3.63
46	63	645.7947	47.79	20.39	1.049	2.89	0.0446	2.3	159	1	100	1.67
47	65	628.5286	56.97	12.78	1.043	5.37	0.0059	2.1	81	1	100	3.4
48	66	973.5335	91.47	43.74	1.273	2.31	0.082	12	1419	1	100	1.33
49	67	1496.7795	104.5	9.7	0.873	3.88	0.0179	-6.8	-152	-1	0	-2.24
50	68	830.4195	97.61	17.34	0.88	6.45	0.003	-6.4	-340	-1	0	-3.72
51	69	934.4651	88.73	23.57	1.122	5.88	0.0042	5.7	420	1	100	3.39
52	70	1423.7698	96.19	25.7	1.151	7.38	0.0018	7	524	1	100	4.26
53	71	1286.6835	104.94	11.11	0.819	14.95	0.0001	-10	-346	-1	0	-8.63
54	72	1555.0593	106.1	9.77	1.279	7.89	0.0014	12.2	318	1	100	4.55
55	73	1099.5076	85.7	22.95	1.204	6.6	0.0027	9.3	548	1	100	3.81

7. **Applying Univariate Statistical methods to find discriminating Peaks for more than Groups:** To create an overview of the most discriminating peaks for more than two classes select: BioMarkers/Stats > Multi Class Pairwise Ratio Analysis or Pairwise Fisher Discriminant Analysis. Both statistics will be presented for combination of classes (1-2, 1-3, 2-3 etc.). The overview is interactive. You can directly plot the peaks in MsCompare and determine how well separation is between your groups.
8. **Using Multivariate Analysis on the most Significant Peaks:** The methods under 7 and 8 can be used to reduce to original table so that it will contain the most important peaks only. After that you can use the Multivariate techniques for presentation purposes.



Full Document References:

1. MsX - Differential Analysis for two samples 2011
2. MsCompare - Univariate and Multivariate Methods for Class Discrimination
3. MsX - High Resolution Peak Matching using MsCompare