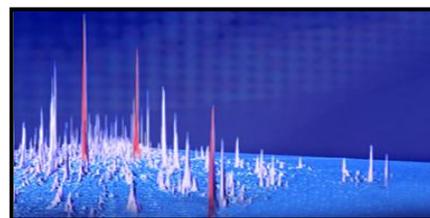
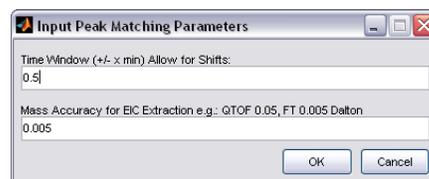
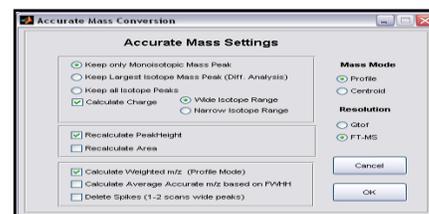


MsCompare™ High Resolution Peak Matching: - A Quick Starting Guide -

Introduction: this quick starting guide teaches you to quickly perform high resolution targeted analysis on a large number of samples. The results from Peak Matching can be followed by statistical or multivariate analysis using many of the specialized algorithms from the MsCompare module. You must be familiar with the MPeaks and MsCompare Programs. Peak Matching, as opposed to Peak Picking, uses one set of reference ions from a single sample. This set of ions will be used to calculate results for a large group of samples with or without alignment. A good procedure to create a proper reference sample is to pool equal aliquots from all samples. These samples are optimal for Quality Control and for Peak Picking as all ions from all samples will be present.

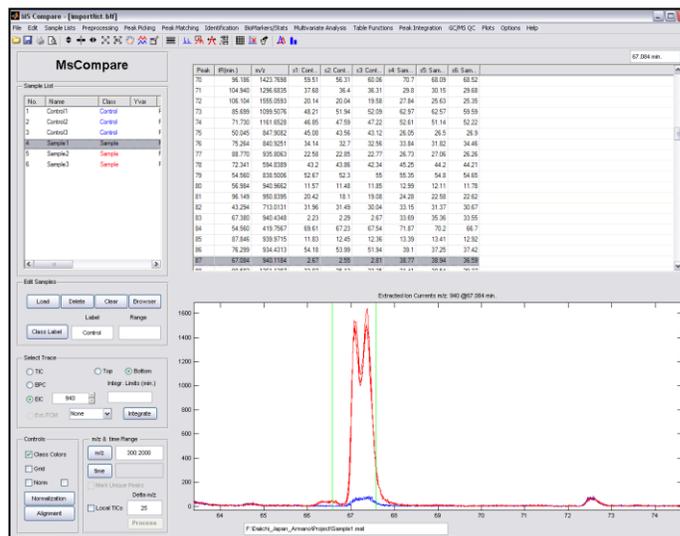


1. **MsCompare Data Loading:** start MsCompare and load or create a project containing all of your samples. Based on a visual analysis, decide if alignment is necessary. If this is the case, see the tutorial documents on Alignment.
2. **Select a Reference Sample for Peak Picking:** based on previous knowledge or using MsCompare, select the reference sample to be used for Peak Picking in MPeaks.
3. **Start MPeaks and Run Peak Picking:** load the selected sample into MPeaks and decide on the minimum Peak width in scans, the type of Noise algorithm and the relative size of the peaks that you want to detect. Experiment with these basic parameters. Press the **Run** button. If you are satisfied with the peak list, the nominal masses have to be converted to accurate m/z values in the next step. For more details on Peak Picking, see the Quick Reference Guide: MPeaks High Resolution Peak Picking.
4. **Conversion to Accurate Mass List:** in MPeaks, select from the menu: Accurate Mass > Accurate Mass Conversion. Use the proper settings for conversion. Depending on the application you may want to keep only the monoisotopic peaks or the largest (deleting the ^{13}C isotopes). When finished, save the result table using an appropriate comment.
5. **Return to MsCompare:** you are now ready to use the high resolution list to apply Peak Matching on all samples loaded into MsCompare. With the reference sample active, select from the menu: Peak Matching. If alignment was applied to the samples you will be asked if the results should be calculated on aligned samples or on the raw data. The list of Peak Picking tables will be presented. Select the table you want to use. Next you will be asked to set the time window to extract accurate EIC traces. Select some of the peaks in MsCompare to guide you with this decision. Decide on the allowed shift and mass accuracy.



Next you will be asked if small shoulder peaks should be eliminated. Press “Yes” to delete these peaks. Quantitation can be based on Peak Height or Peak Area. In general choose to calculate both. If shifts between chromatograms are severe and many peaks are present in the same EIC, the accurate determination of peak areas might be more difficult.

When the calculations are done, you will be asked to save the table. After saving, the table will be presented showing results for all samples and all peaks. You can click on any peak in the table to plot nominal EIC traces (zoomed) for all samples. The traces will be plotted in the color used for grouping. To plot accurate EIC traces press the accurate EIC icon on the right. It will take more time to plot the results. Results can be plotted in overlay or stacked.



6. **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.

7. **Applying Univariate Statistical methods to find discriminating Peaks for two Groups:** For two groups, select Biomarker/Stats and decide what 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 Fisher Discriminant test etc. MsCompare 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 the overview and peaks of no importance can be deleted from the main table.

Peak No.	m/z	IR (min.)	Avg. Int. %	Ratio	t-value	p-value	Unique	Weighted Uniq.	Selectivity	% Up-Regulated	Fisher
38	1402.2258	88.73	14.24	1.189	5.71	0.0047	8.6	296	1	100	3.29
39	1124.5192	99.78	23.63	0.901	3.08	0.0369	-5.2	-293	-1	0	-1.78
40	1423.269	96.15	29.05	1.17	3.69	0.0211	7.8	599	1	100	2.13
41	1274.1069	82.94	18.47	1.026	3.19	0.0331	1.3	80	1	100	1.84
42	1401.7219	88.73	17.06	1.193	10.78	0.0004	8.8	366	1	100	6.22
43	426.2151	49.19	30.25	11.272	284.5	0	83.7	3713	1	100	164.25
44	1072.0481	74.91	13.6	1.096	4.49	0.0109	4.6	162	1	100	2.59
45	1260.6312	90.58	14.28	0.984	6.28	0.0033	-6.1	-241	-1	0	-3.63
46	645.7947	47.79	20.39	1.049	2.89	0.0446	2.3	159	1	100	1.67
47	628.3206	58.97	12.78	1.043	5.37	0.0058	2.1	81	1	100	3.1
48	973.5335	91.47	43.74	1.273	2.31	0.082	12	1419	1	100	1.33
49	1496.7795	104.5	9.7	0.873	3.88	0.0179	-6.8	-152	-1	0	-2.24
50	830.4195	97.61	17.34	0.88	6.45	0.003	-6.4	-340	-1	0	-3.72
51	934.4651	88.73	23.57	1.122	5.88	0.0042	5.7	420	1	100	3.39
52	1423.7698	96.19	25.7	1.151	7.38	0.0018	7	524	1	100	4.26
53	1296.6835	104.94	11.11	0.819	14.95	0.0001	-10	-346	-1	0	-8.63
54	1555.0593	106.1	9.77	1.279	7.89	0.0014	12.2	318	1	100	4.55
55	1099.5076	85.7	22.95	1.204	6.6	0.0027	9.3	548	1	100	3.81

8. **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 to MsCompare and determine how well separation is.

9. **Using Multivariate Analysis on the most Significant Peaks:** The methods under 7 and 8 can be used to reduce to original table to contain the most important peaks only. After that you can use Multivariate techniques for presentation purposes.

Full Document References:

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