

Metabolomics Software Tools

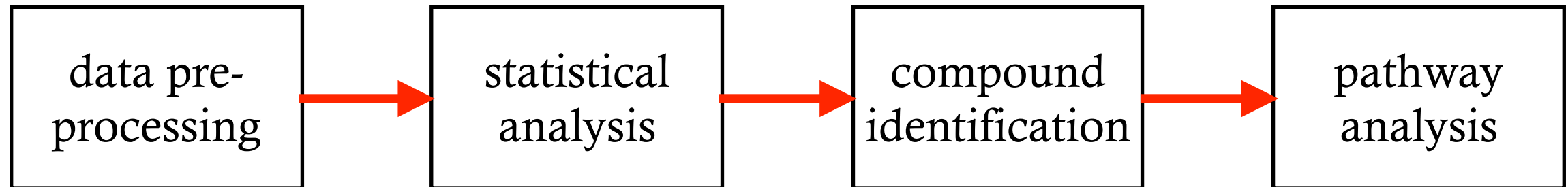
Xiuxia Du, Paul Benton, Stephen Barnes

Outline

- **Introduction**
- **Software Tools for LC-MS metabolomics**
- **Software Tools for GC-MS metabolomics**
- **Software Tools for Statistical Analysis**

Introduction

- **LC-MS data analysis workflow**



- **GC-MS data analysis workflow**



Software Tools for LC-MS

- **Commercial**

- MassHunter — Agilent
 - MetQuest — Thermo Scientific
 - MetWorks — Thermo Scientific
 - Multiple Mass Defect Filter — Thermo Scientific
 - Progenesis QI — Waters
 - XCMS^{plus} — Sciex
-
- and more

Software Tools for LC-MS

- **Free**
 - **Insilicos Viewer**
 - **ProteoWizard**
 - **SeeMS**: interactive viewer for mass spec data files (Windows only)
 - **MSConvert**: convert between various file formats
 - **MZmine 2**: LC-MS data processing
 - **MetaboSearch**: perform mass-based metabolite search simultaneously against four major metabolite databases
 - **MetaboAnalyst**: a web server for metabolomics data analysis
 - and more

Data format

- **Proprietary data formats:** need vendor library functions to access

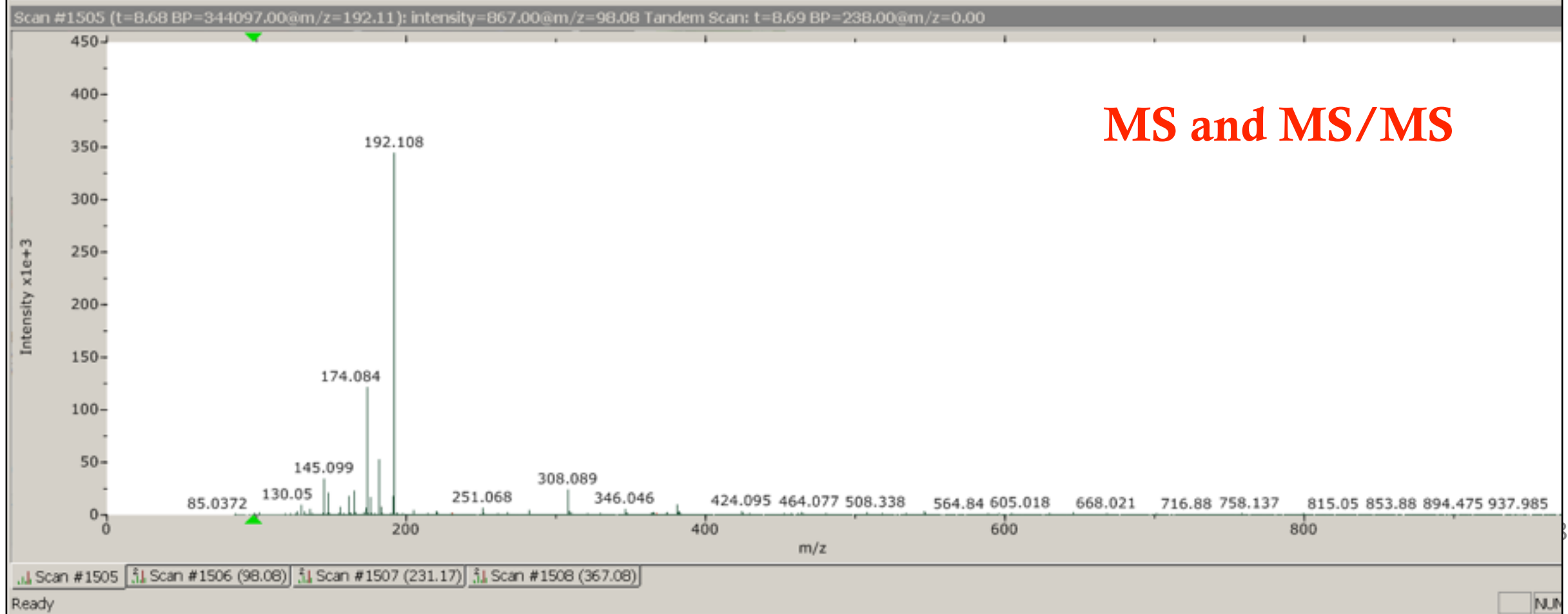
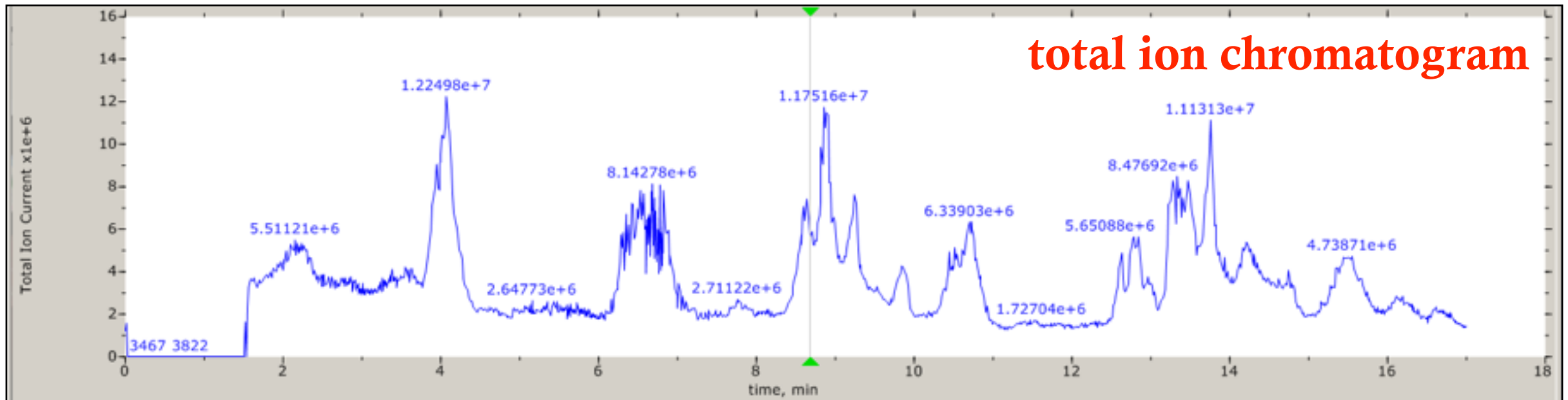
Company	File extension
Agilent	.D
Sciex	.WIFF
Theomo	.RAW
Waters	.RAW
.....	

- **Open data formats:** free library functions to access
 - netCDF
 - mzData
 - mzXML
 - mzML

Insilicos Viewer

Insilicos Viewer

Raw data viewer



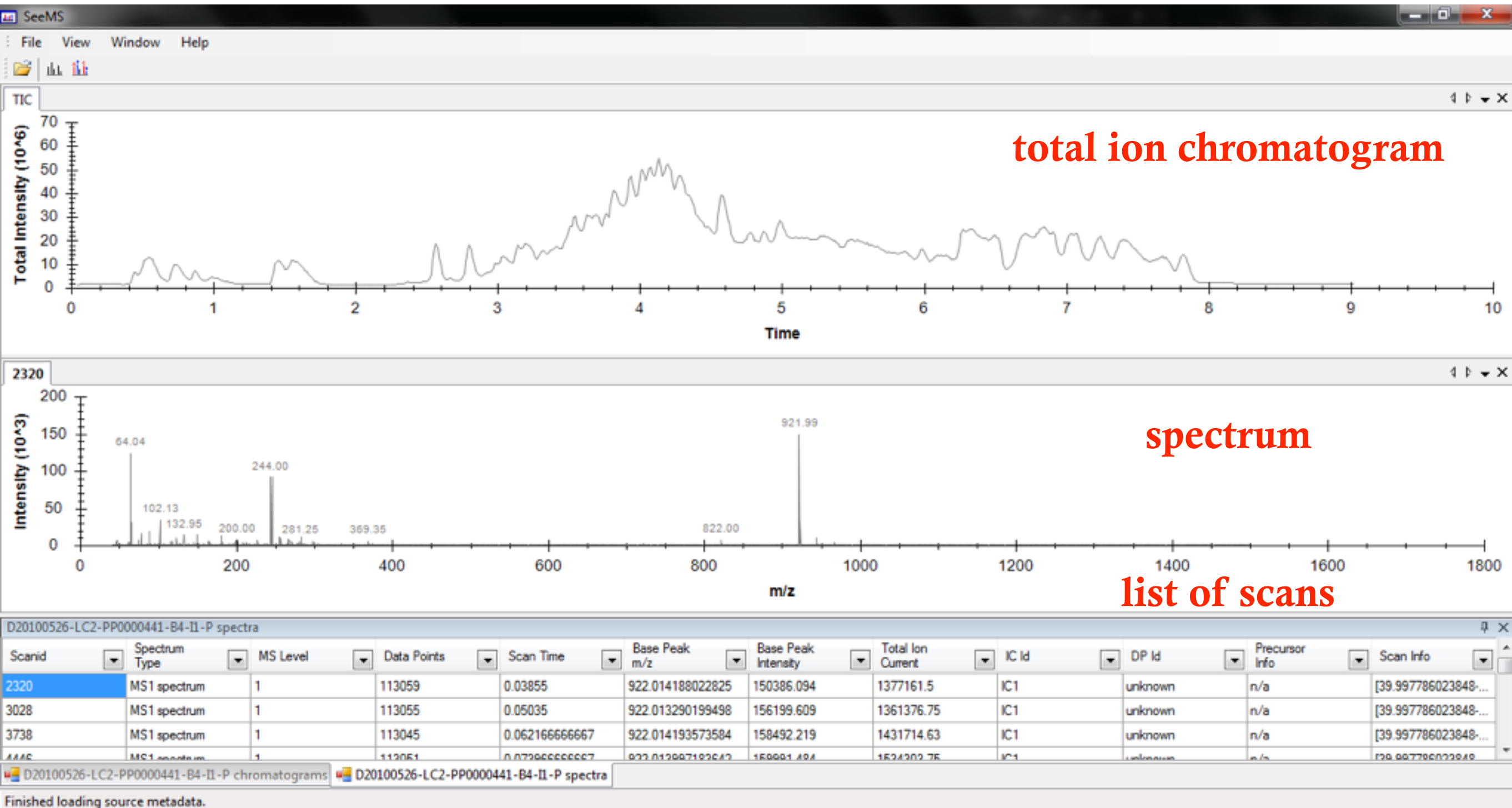
Proteo Wizard

ProteoWizard: SeeMS

- **A data viewer**
- **Can read these open data formats**
 - mzML
 - mzXML

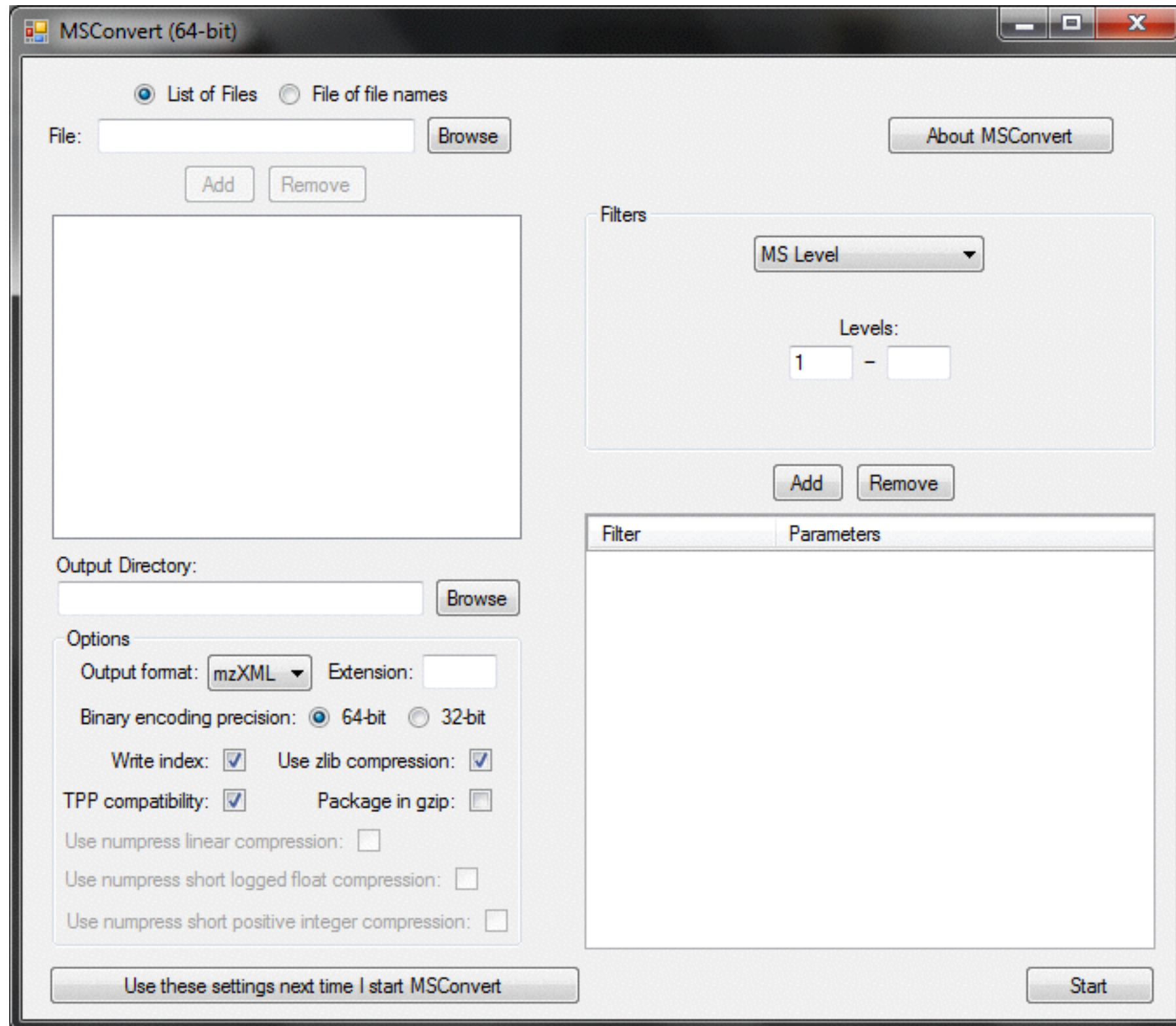
ProteoWizard: SeeMS

Raw data viewer



ProteoWizard: MSConvert

Data format converter



ProteoWizard: MSConvert

- **Supported data formats**
 - Read: open formats, vendor formats
 - Write: open formats
- **Filters and transformation**
 - msLevel
 - Peak picking
 - Zero samples
 - ETD filter
 - Threshold peak filter
 - Charge state predictor
 - Activation
 - Subset

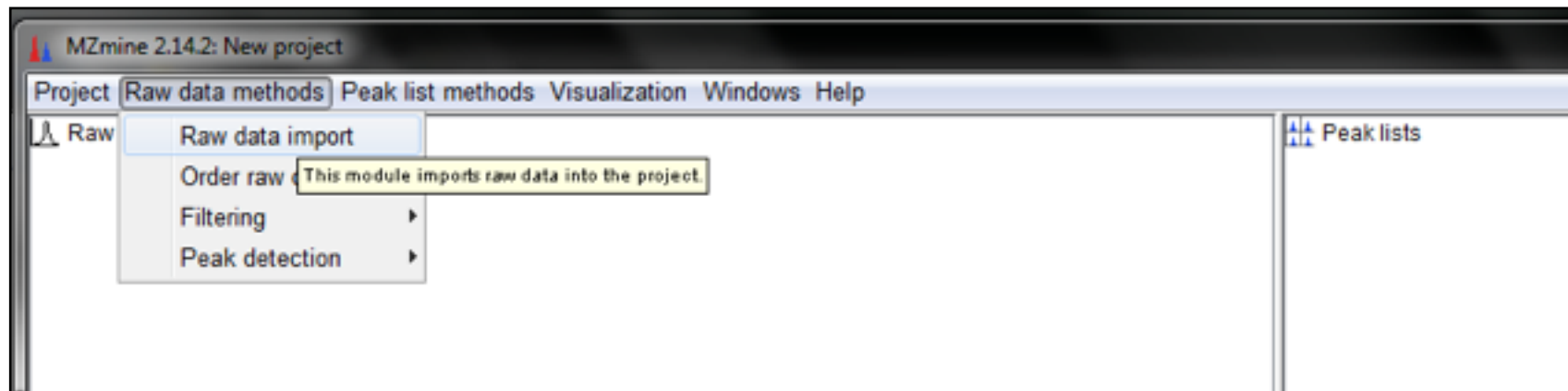
MZmine 2

MZmine 2

- **LC-MS metabolomics data processing, analysis, and visualization**

- **Supported open data formats**
 - NetCDF
 - mzData
 - mzML
 - mzXML

Raw data import

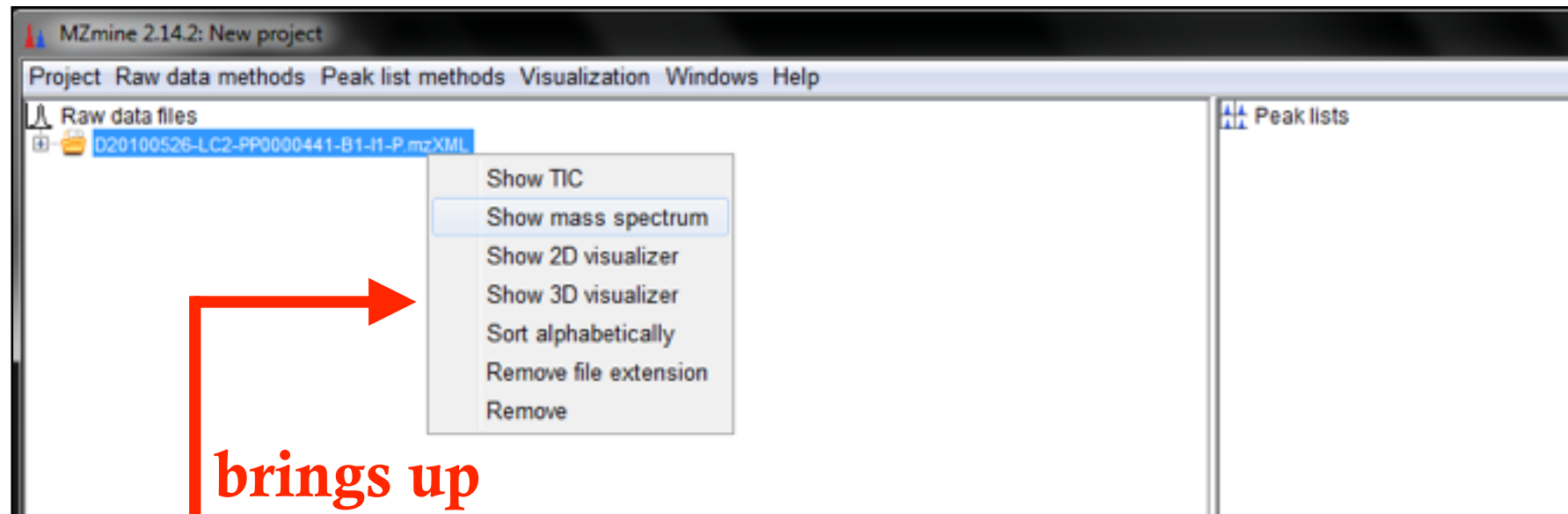


The screenshot shows the MZmine 2.14.2 interface with a 'New project' window. The 'Project' menu is open, and the 'Raw data import' option is highlighted. A tooltip for 'Raw data import' reads: 'This module imports raw data into the project.' Other menu options include 'Order raw', 'Filtering', and 'Peak detection'. The 'Peak lists' panel is visible on the right.

list of scans in raw files

- MS scans in blue
- MS/MS scans in red
- # sequential number
- @ retention time
- MS level
- type of spectrum
 - p = profile
 - c = centroid
 - t = thresholded
- polarity of ionization
 - + = positive
 - - = negative
 - ? = unknown

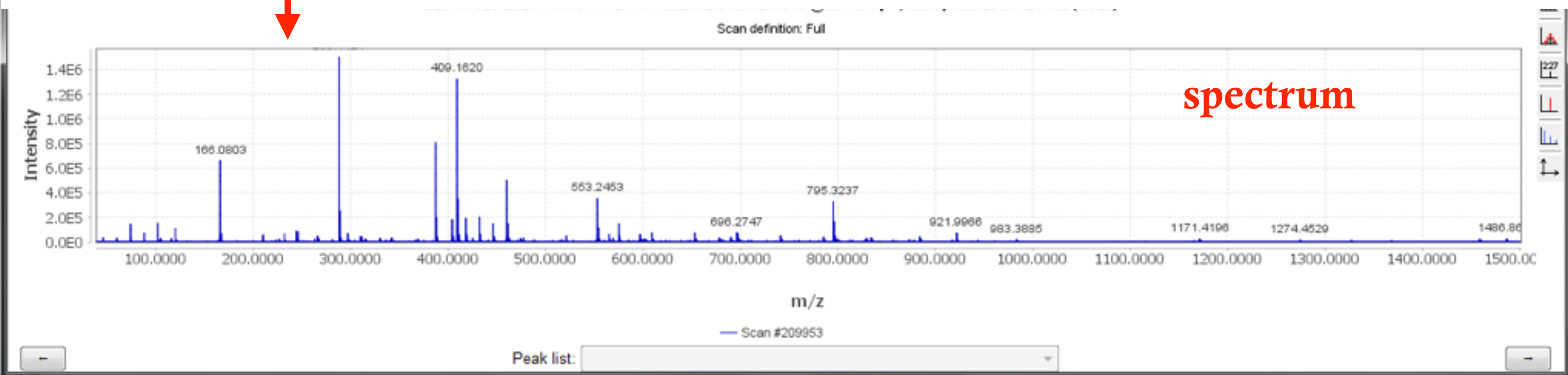
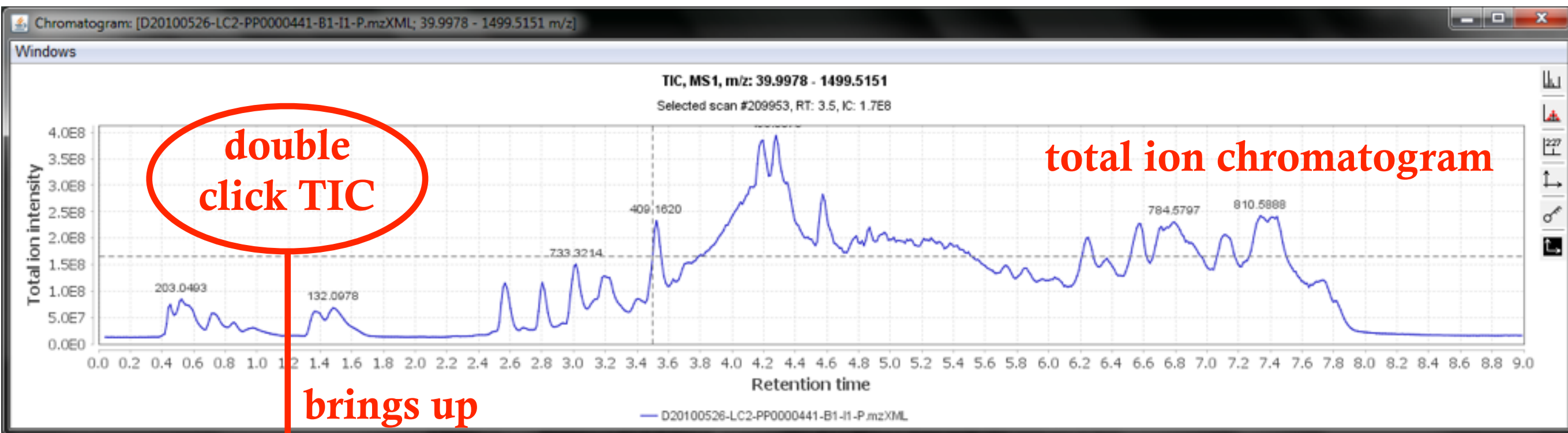
Raw data visualization



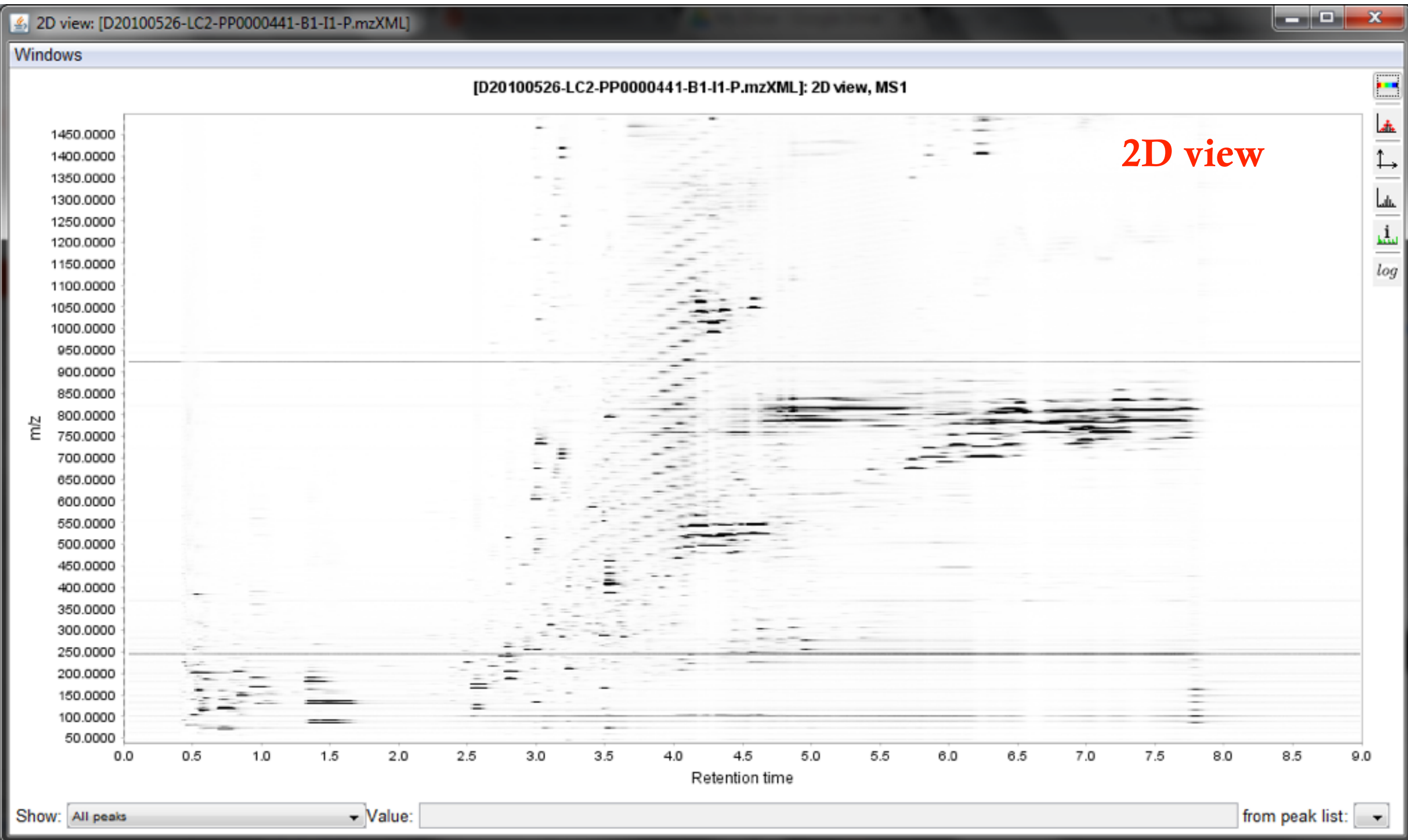
brings up

Right click on the file name

Raw data visualization



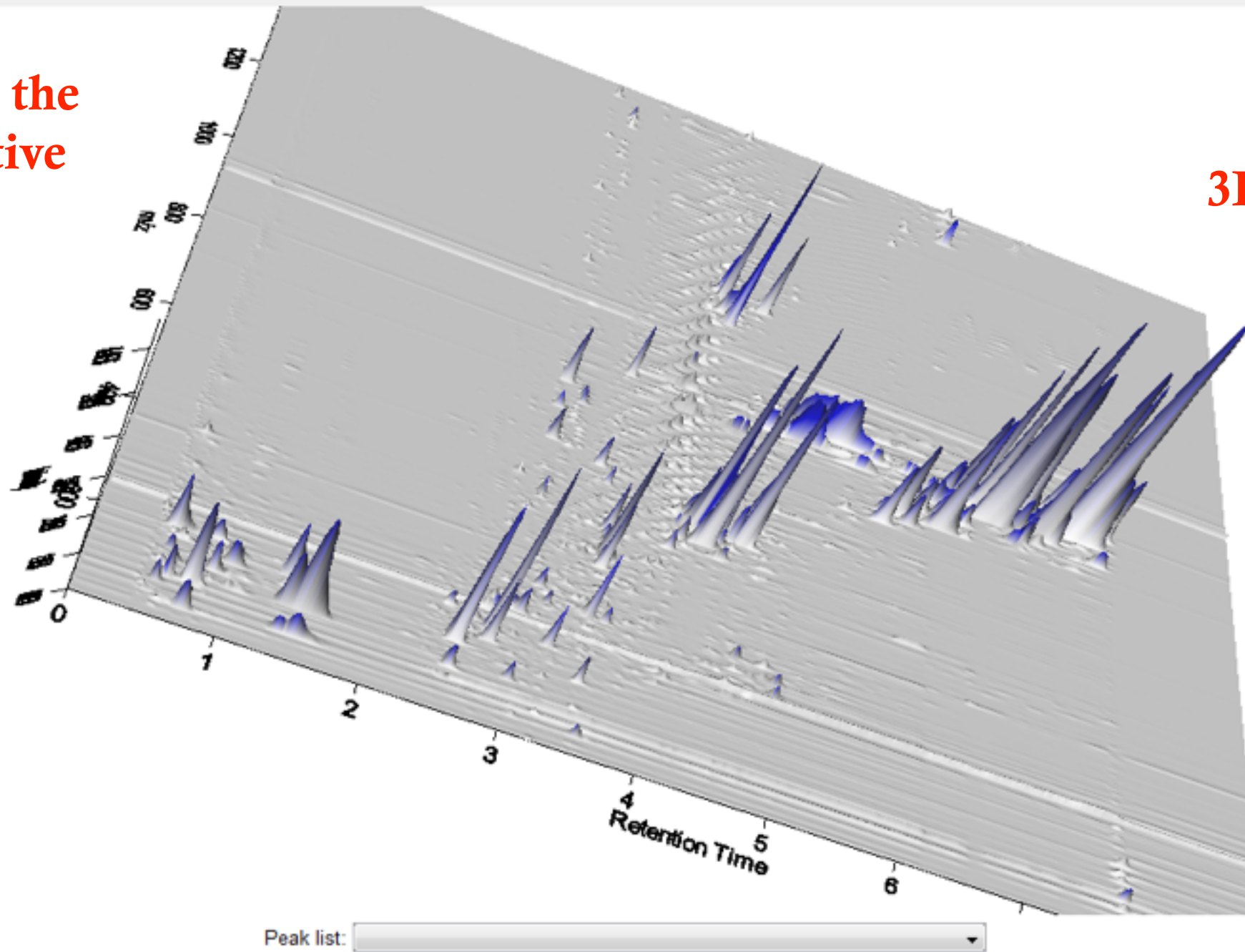
Raw data visualization



Raw data visualization

rotate to find the best perspective

3D view



Data processing

- **Workflow**

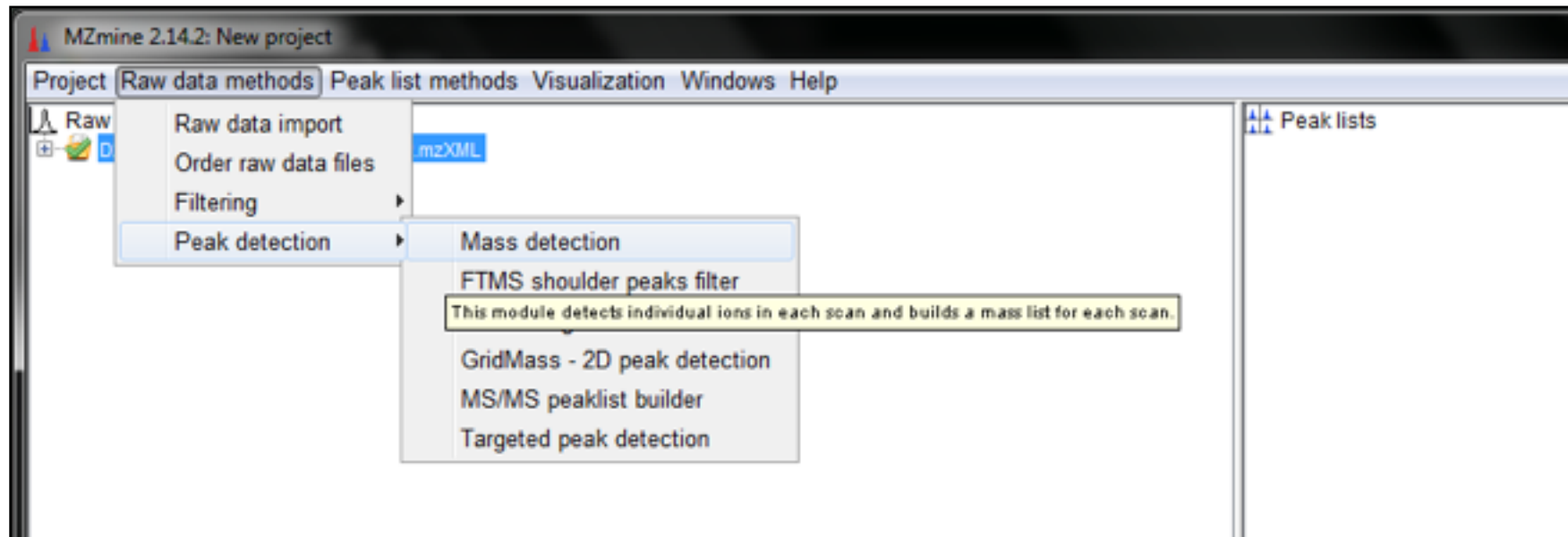
- Raw data import
- (optional) Raw data methods / Filtering
- **Peak detection**
- Isotopic peak grouping
- (optional) **Identification** of fragments, adducts, and peak complexes
- (optional) Normalization of retention time
- **Alignment**
- (optional) **Gap filling**
- (optional) Normalization of peak heights / areas
- (optional) **Identification** using database search, formula prediction, etc.
- **Data analysis and export**

Peak detection

- **Mass detection**
 - Centroid
 - Exact mass
 - Local maxima
 - Recursive threshold
 - Wavelet transform
- **Chromatogram building**
- **Peak deconvolution**

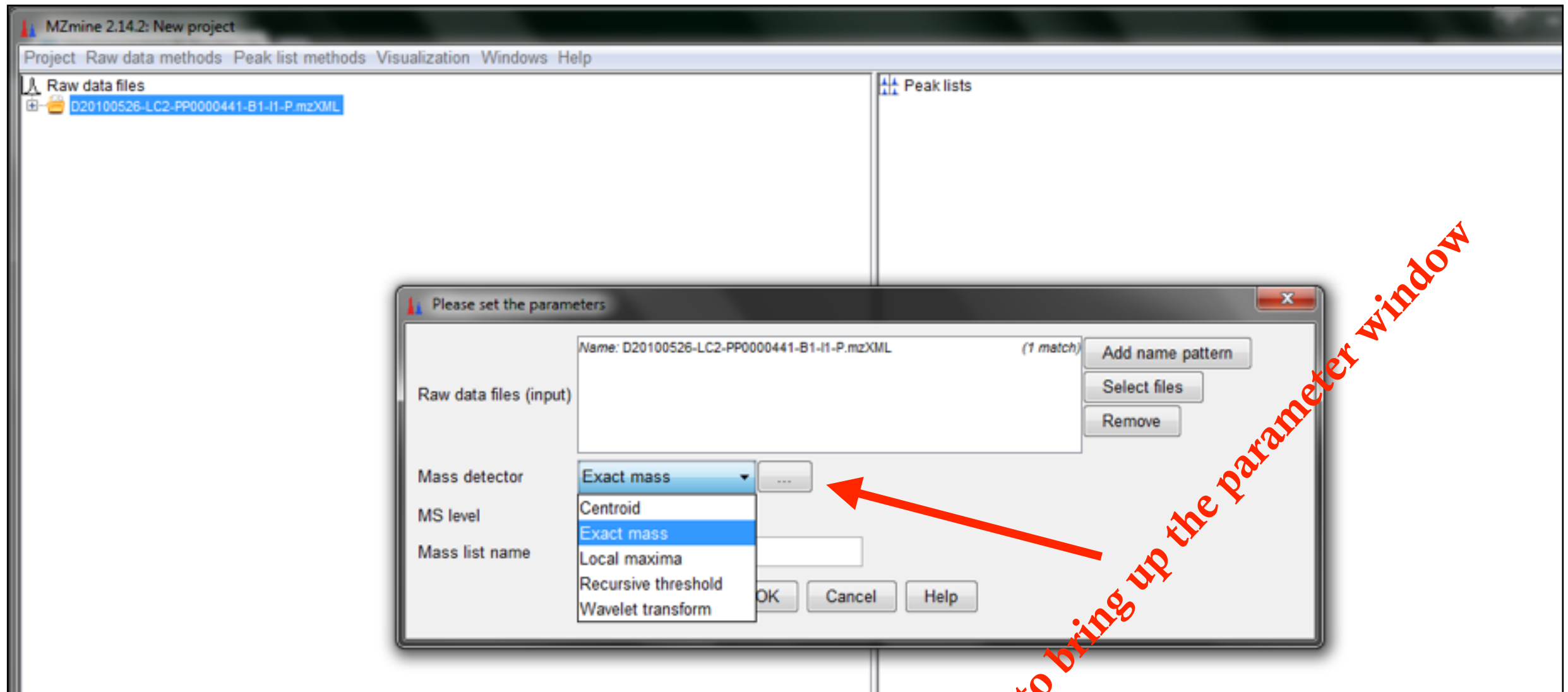
Peak detection

Peak detection procedure



Mass detection

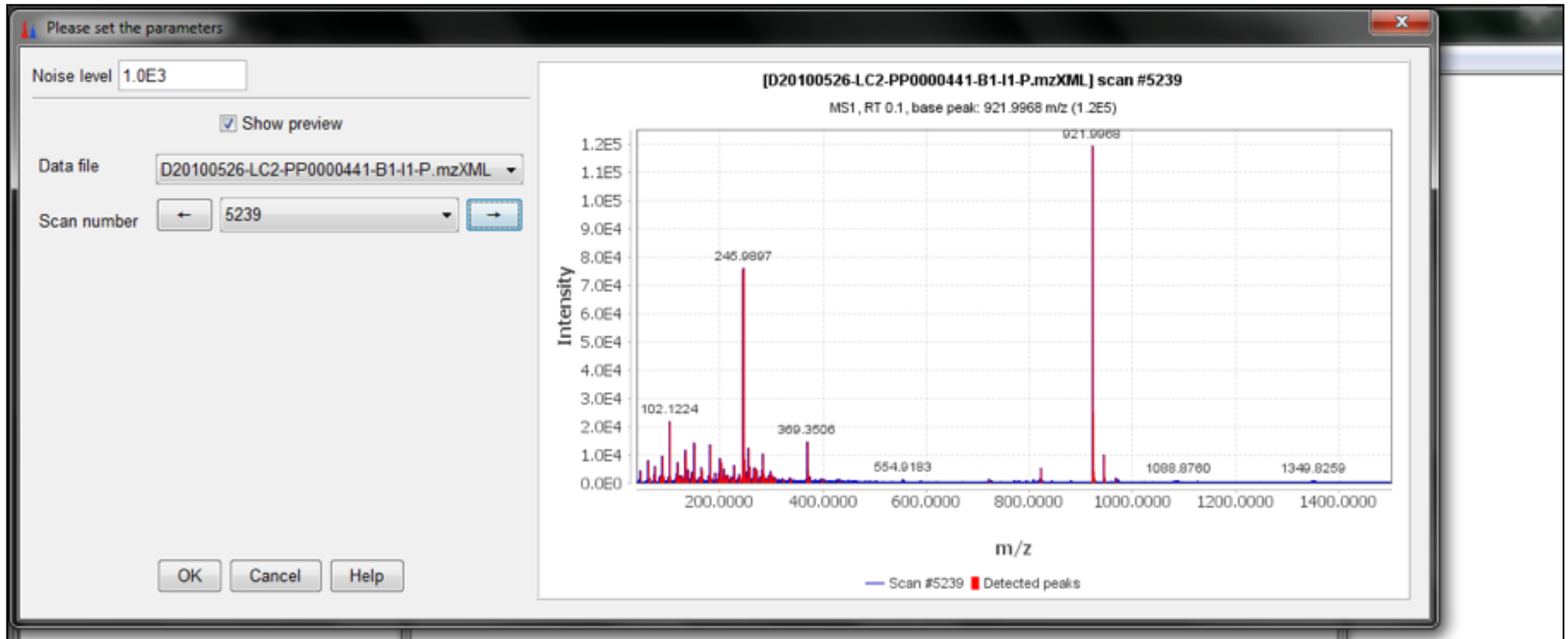
Mass detection options



click here to bring up the parameter window

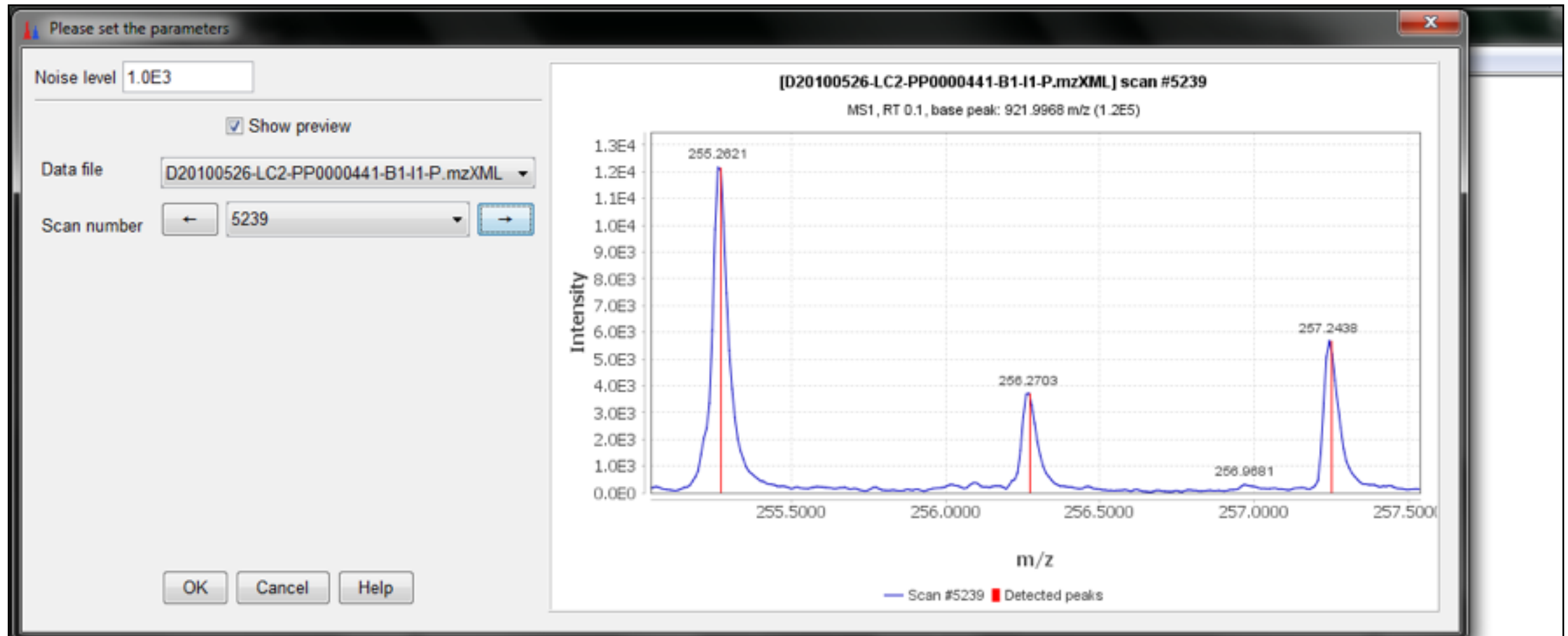
Mass detection

Set mass detection parameter



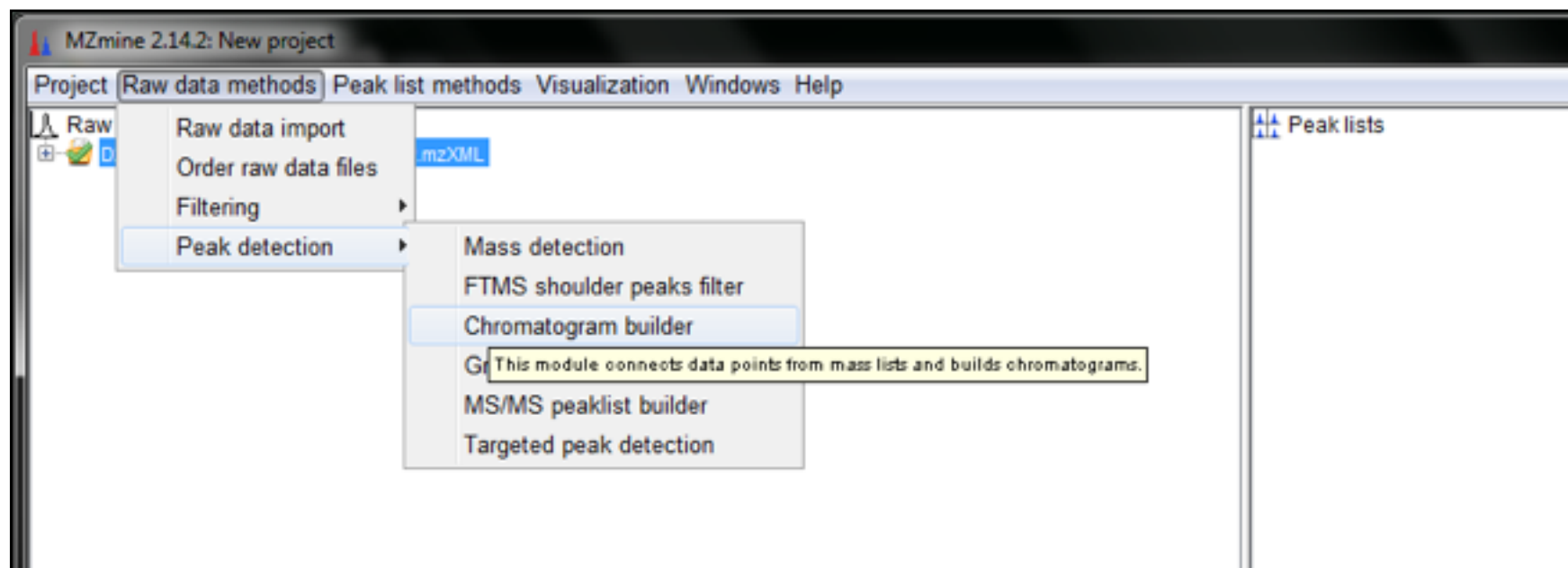
Mass detection

Set mass detection parameter



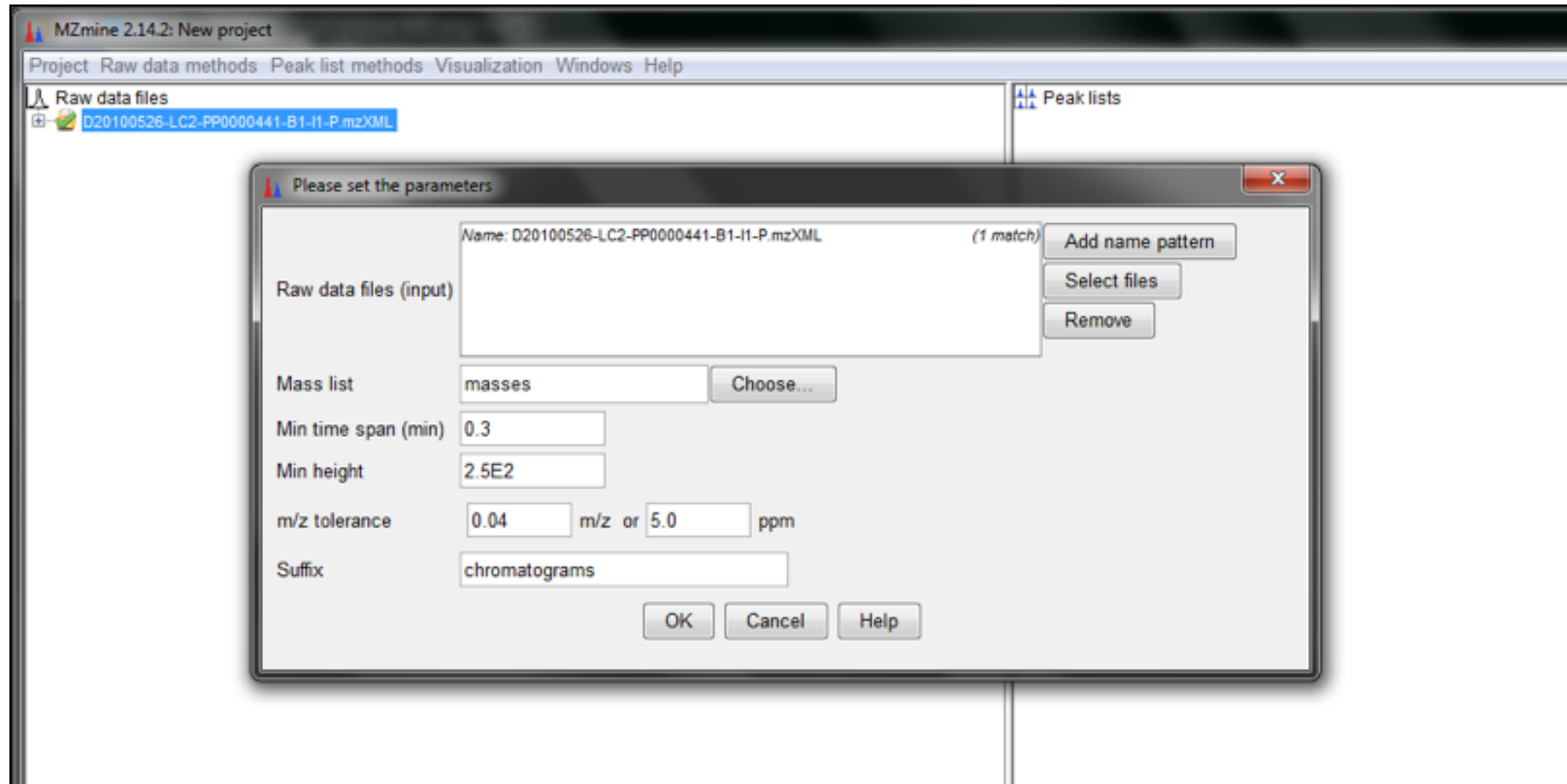
Chromatogram building

Chromatogram builder



Chromatogram building

Chromatogram builder — set parameters



Chromatogram building

After chromatograms are built

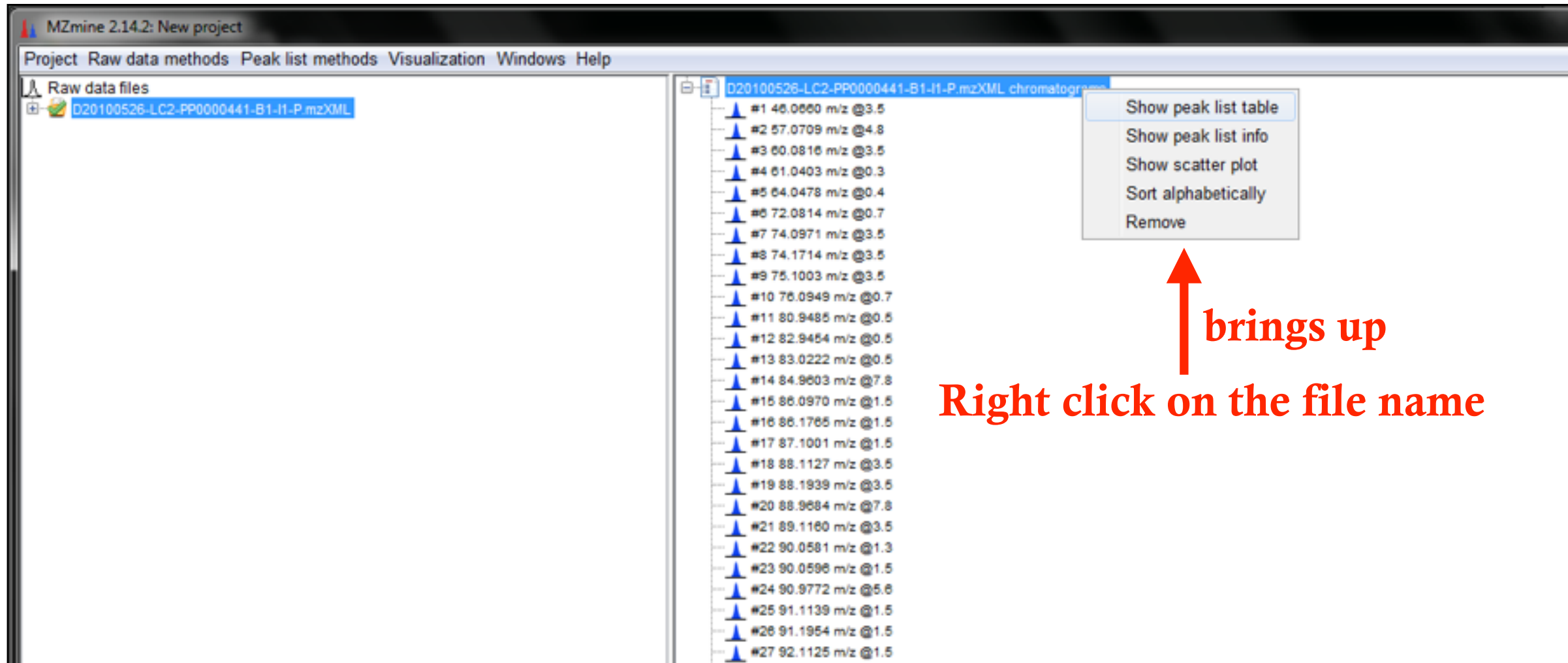
peak list

- # ID
- m/z value
- @ retention time

Peak ID	m/z	Retention Time
#1	46.0660	@3.5
#2	57.0709	@4.8
#3	60.0816	@3.5
#4	61.0403	@0.3
#5	64.0478	@0.4
#6	72.0814	@0.7
#7	74.0971	@3.5
#8	74.1714	@3.5
#9	75.1003	@3.5
#10	76.0949	@0.7
#11	80.9485	@0.5
#12	82.9454	@0.5
#13	83.0222	@0.5
#14	84.9603	@7.8
#15	86.0970	@1.5
#16	86.1765	@1.5
#17	87.1001	@1.5
#18	88.1127	@3.5
#19	88.1939	@3.5
#20	88.9684	@7.8
#21	89.1160	@3.5
#22	90.0581	@1.3
#23	90.0596	@1.5
#24	90.9772	@5.6
#25	91.1139	@1.5
#26	91.1954	@1.5
#27	92.1125	@1.5
#28	97.1020	@4.8
#29	100.1111	@2.5
#30	101.0665	@2.5

Chromatogram building

To display extracted ion chromatograms

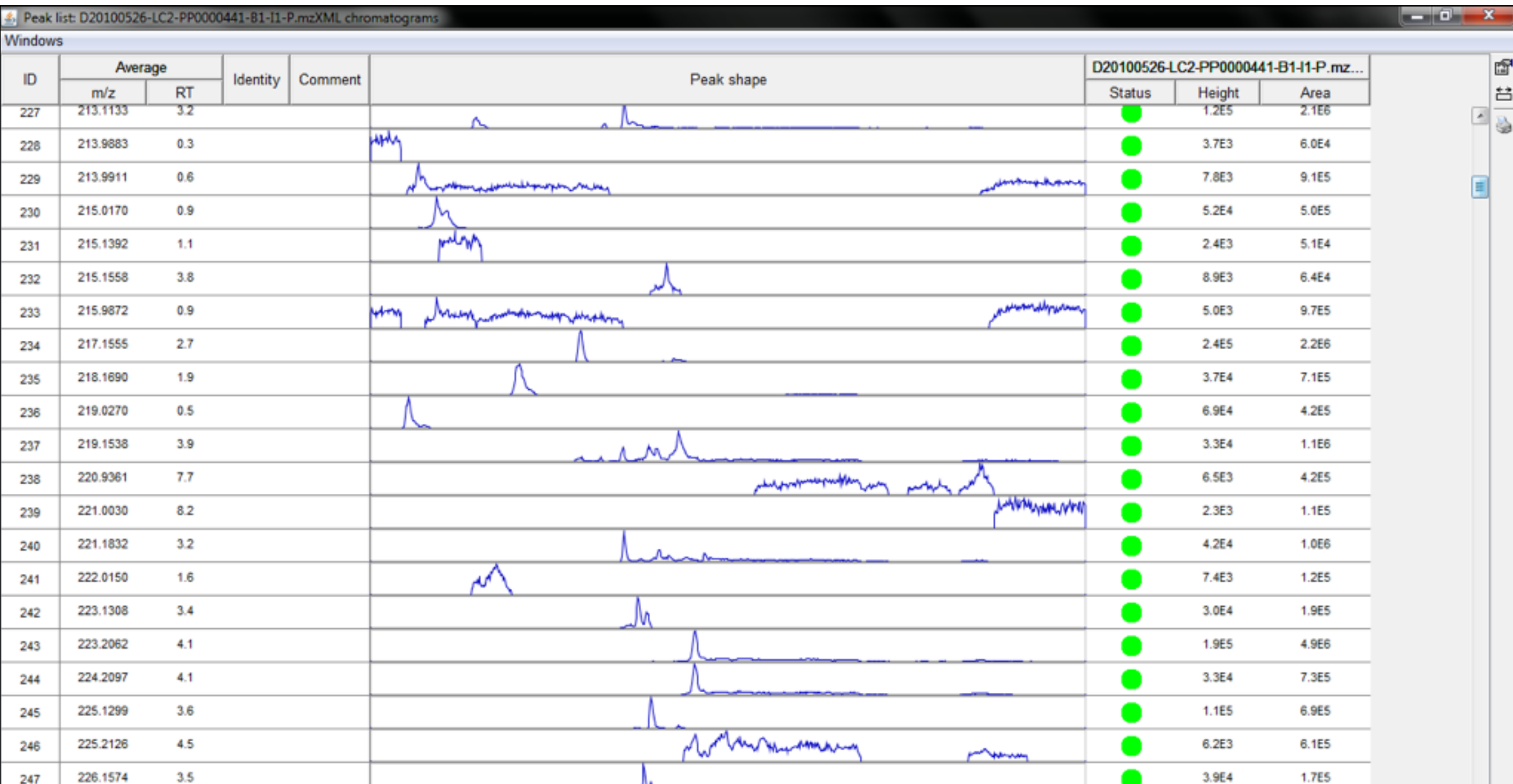


The screenshot shows the MZmine 2.14.2 software interface. The main window displays a list of peaks with their retention times, m/z values, and widths. A context menu is open over the list, showing options: Show peak list table, Show peak list info, Show scatter plot, Sort alphabetically, and Remove. A red arrow points from the text 'Right click on the file name' to the context menu, with the text 'brings up' above it.

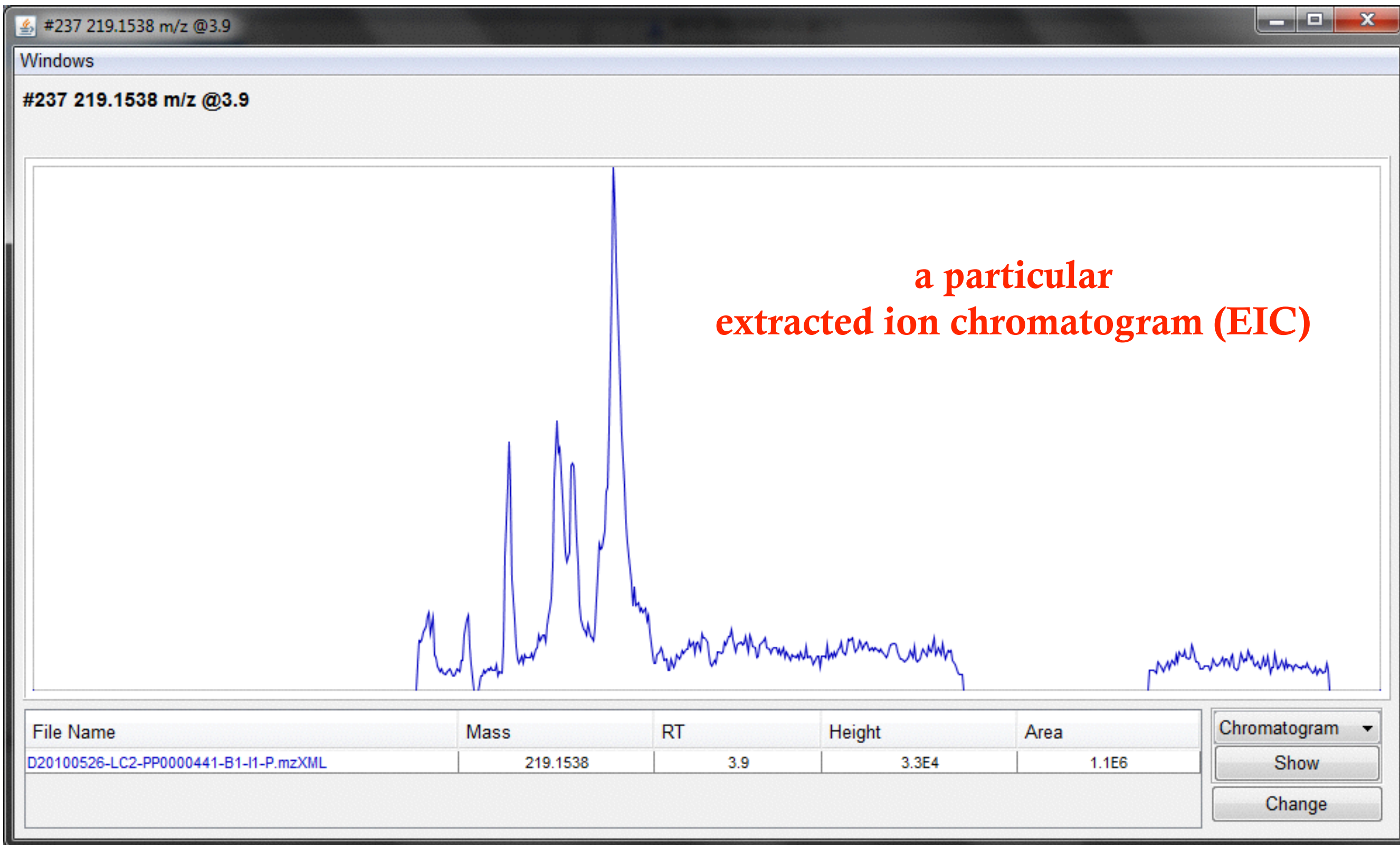
Peak ID	Retention Time (min)	m/z	Width (min)
#1	46.0660	m/z	@3.5
#2	57.0709	m/z	@4.8
#3	60.0816	m/z	@3.5
#4	61.0403	m/z	@0.3
#5	64.0478	m/z	@0.4
#6	72.0814	m/z	@0.7
#7	74.0971	m/z	@3.5
#8	74.1714	m/z	@3.5
#9	75.1003	m/z	@3.5
#10	76.0949	m/z	@0.7
#11	80.9485	m/z	@0.5
#12	82.9454	m/z	@0.5
#13	83.0222	m/z	@0.5
#14	84.9603	m/z	@7.8
#15	86.0970	m/z	@1.5
#16	86.1765	m/z	@1.5
#17	87.1001	m/z	@1.5
#18	88.1127	m/z	@3.5
#19	88.1939	m/z	@3.5
#20	88.9684	m/z	@7.8
#21	89.1160	m/z	@3.5
#22	90.0581	m/z	@1.3
#23	90.0596	m/z	@1.5
#24	90.9772	m/z	@5.6
#25	91.1139	m/z	@1.5
#26	91.1954	m/z	@1.5
#27	92.1125	m/z	@1.5

Chromatogram building

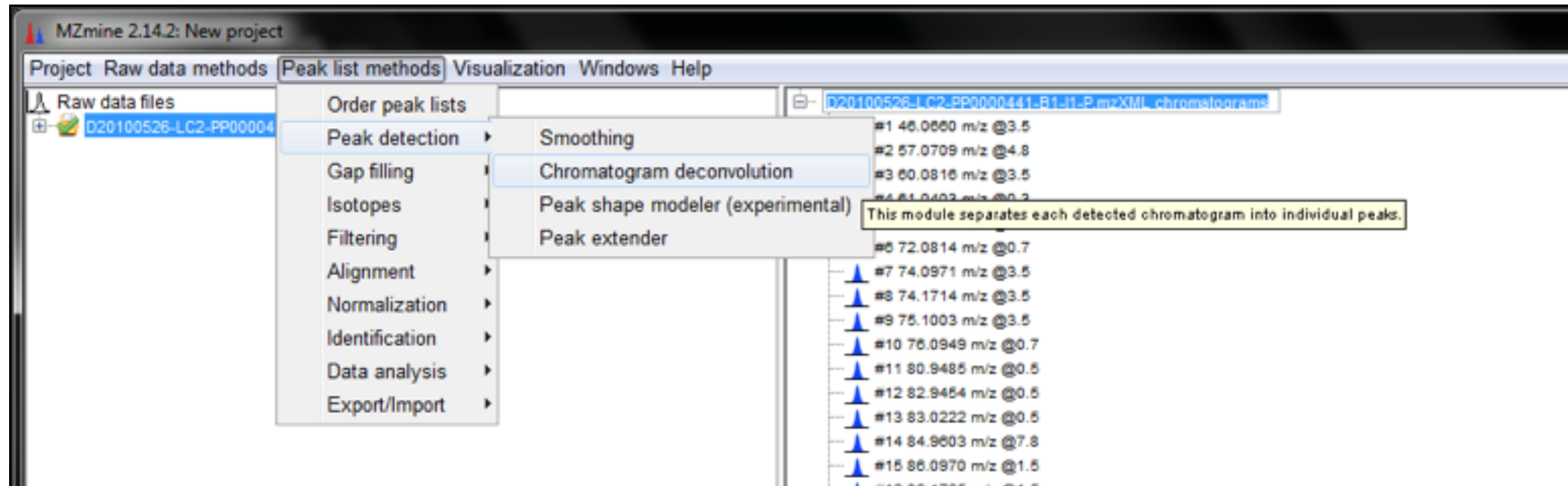
Show chromatogram information



Chromatogram building

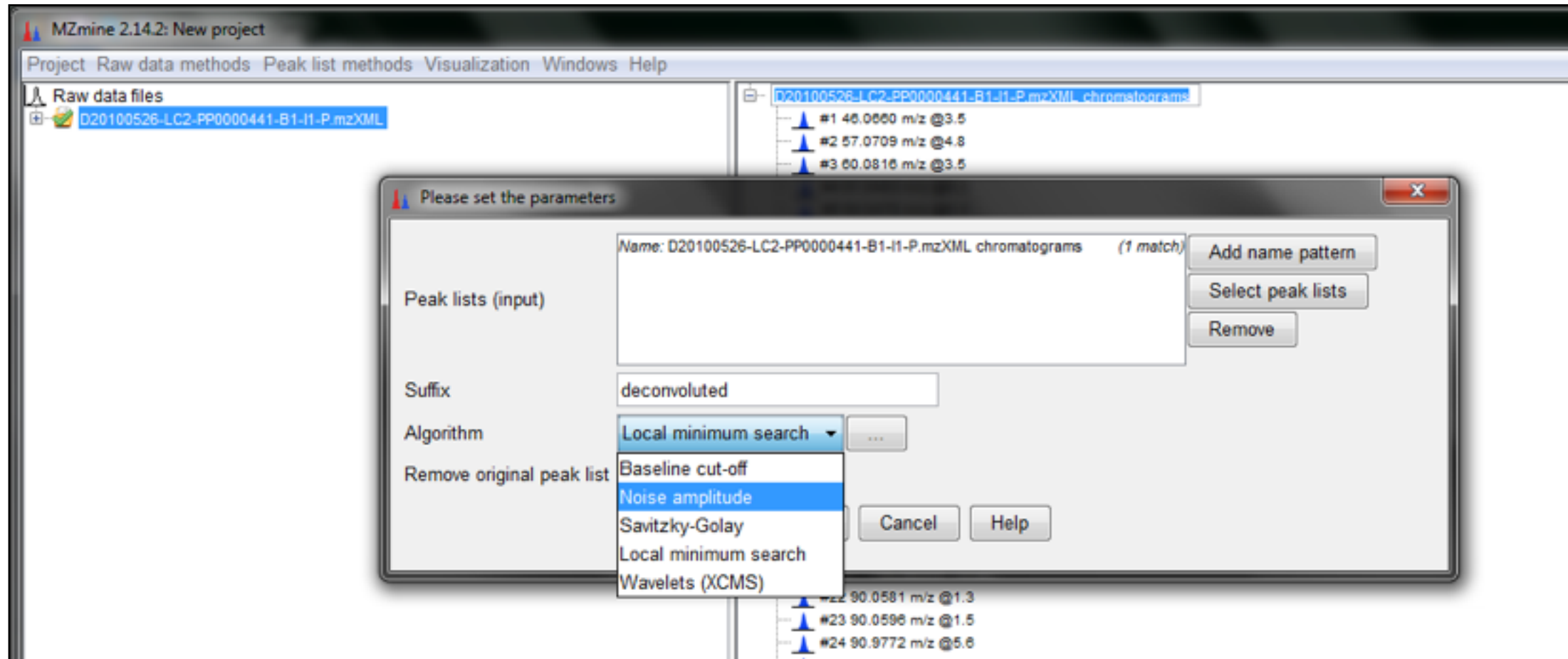


Peak deconvolution



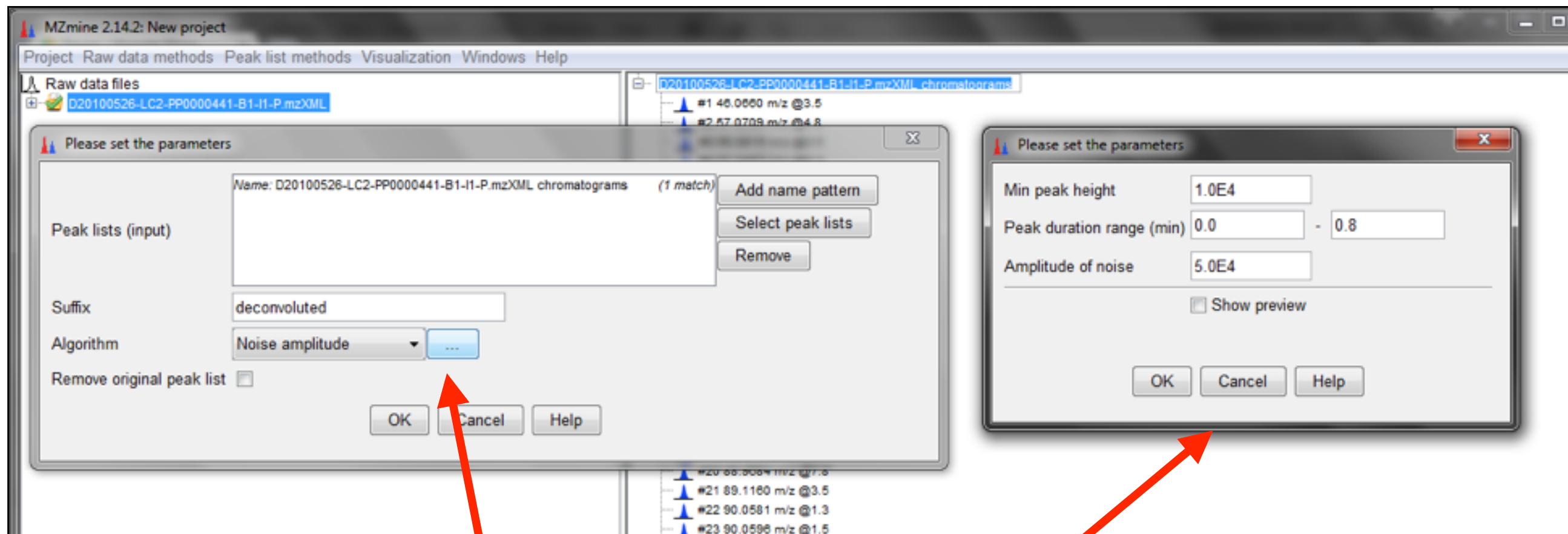
Peak deconvolution

Algorithms



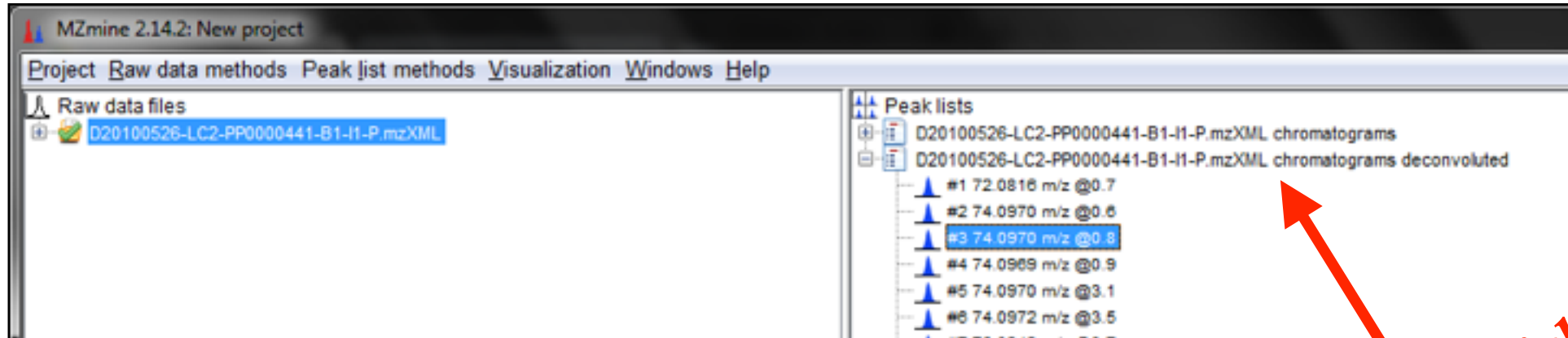
Peak deconvolution

Set parameters



click here ————— to bring up the parameter window

Peak deconvolution



deconvolution finished

Peak deconvolution

Results

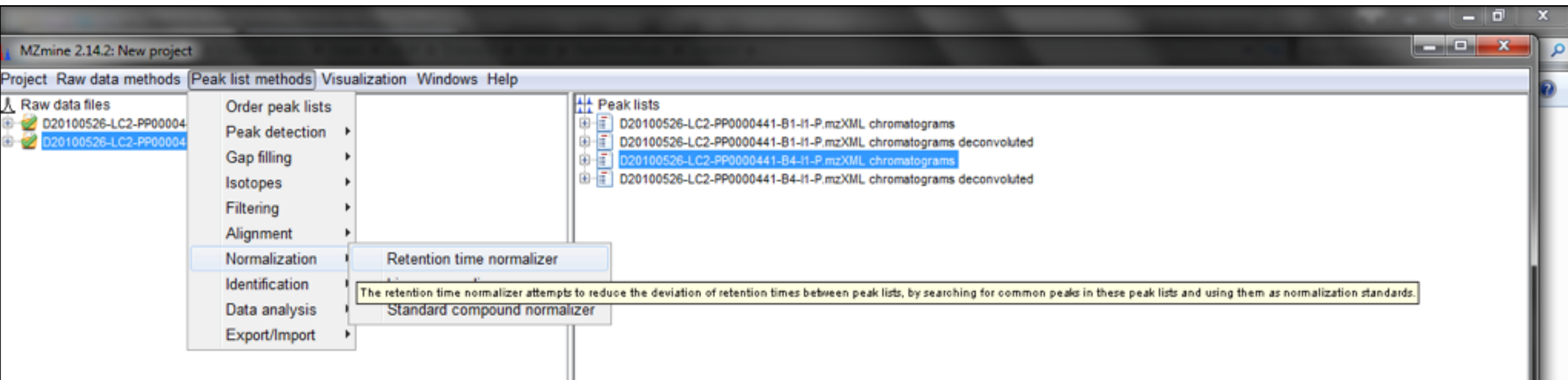
Peak list: D20100526-LC2-PP0000441-B1-I1-P.mzXML chromatograms deconvoluted

Windows

ID	Average		Identity	Comment	Peak shape	D20100526-LC2-PP0000441-B1-I1-P.mz...		
	m/z	RT				Status	Height	Area
85	187.0587	0.6				●	2.9E5	8.5E5
86	191.0183	0.9				●	3.1E5	1.9E6
87	191.1122	1.4				●	4.8E5	2.5E6
88	195.1237	2.7				●	1.2E5	3.1E5
89	203.0536	0.5				●	1.5E6	7.0E6
90	204.1238	0.8				●	5.2E5	2.0E6
91	204.0573	0.5				●	1.1E5	2.9E5
92	204.0638	1.4				●	1.2E5	5.7E5
93	205.0983	2.8				●	4.7E6	1.5E7
94	209.1909	4.1				●	1.6E5	6.5E5
95	210.0769	3.5				●	6.7E4	7.3E4
96	212.0909	3.2				●	1.3E6	3.5E6
97	213.0943	3.2				●	1.2E5	2.6E5
98	215.0168	0.9				●	5.2E4	7.1E4
99	217.1055	2.7				●	2.4E5	9.4E5
100	219.0275	0.5				●	6.9E4	2.0E5
101	223.2070	4.1				●	1.9E5	6.6E5
102	225.1497	3.6				●	1.1E5	2.4E5
103	227.1897	2.5				●	3.6E5	1.1E6
104	227.2382	4.6				●	1.7E5	1.9E6
105	229.1552	0.9				●	5.6E4	3.7E4

Alignment

Retention time normalization



Alignment

Retention time normalization: set parameters

Please set the parameters

Peak lists (input)

Name: D20100526-LC2-PP0000441-B1-I1-P.mzXML chromatograms deconvoluted(

Name: D20100526-LC2-PP0000441-B4-I1-P.mzXML chromatograms deconvoluted(

Add name pattern

Select peak lists

Remove

Name suffix

normalized

m/z tolerance

0.001 m/z or 5.0 ppm

Retention time tolerance

5.0 relative (%)

Minimum standard intensity

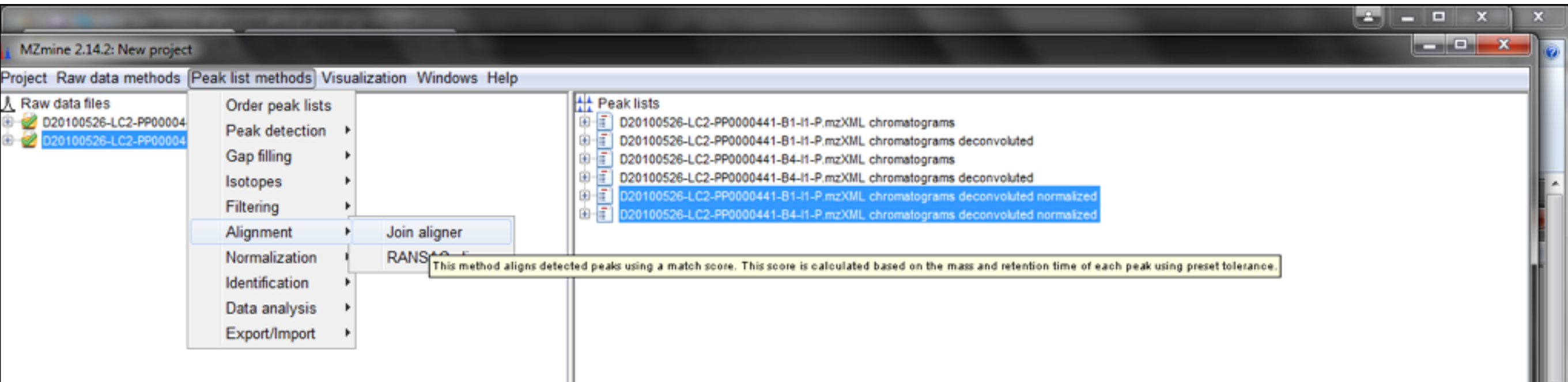
1.0E6

Remove original peak list

OK Cancel Help

Alignment

Join aligner



Alignment

Join aligner: set parameters

The screenshot displays the MZmine 2.14.2 interface with a 'Please set the parameters' dialog box open. The dialog box is titled 'Please set the parameters' and contains the following fields and options:

- Peak lists (input):** A list box containing two entries: 'D20100526-LC2-PP0000441-B1-I1-P.mzXML chromatograms deconvoluted' and 'D20100526-LC2-PP0000441-B4-I1-P.mzXML chromatograms deconvoluted'. Buttons for 'Add name pattern', 'Select peak lists', and 'Remove' are located to the right of the list.
- Peak list name:** A text field containing 'Aligned peak list'.
- m/z tolerance:** A text field with '0.001' and a dropdown menu set to 'm/z or 5.0 ppm'.
- Weight for m/z:** A text field with '20'.
- Retention time tolerance:** A text field with '3.0' and a dropdown menu set to 'relative (%)'.
- Weight for RT:** A text field with '10'.
- Require same charge state:** A checked checkbox.
- Require same ID:** An unchecked checkbox.
- Compare isotope pattern:** A checked checkbox with a 'Setup..' button next to it.

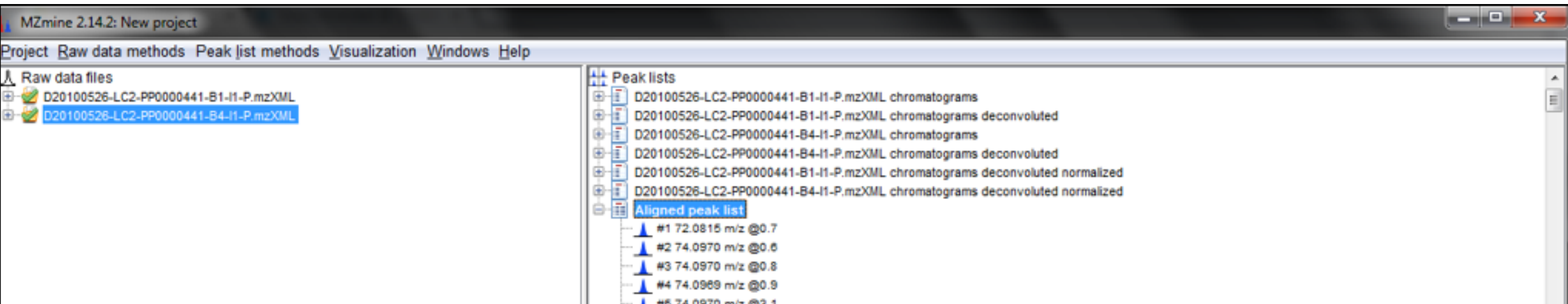
At the bottom of the dialog box are 'OK', 'Cancel', and 'Help' buttons. In the background, another 'Please set the parameters' dialog box is visible, showing the following settings:

- Isotope m/z tolerance:** '0.005' m/z or '5.0' ppm.
- Minimum absolute intensity:** '1E3'.
- Minimum score:** '65' %.

At the bottom of the background dialog box are 'OK', 'Cancel', and 'Help' buttons. The main MZmine window shows a project named 'MZmine 2.14.2: New project' with a menu bar (Project, Raw data methods, Peak list methods, Visualization, Windows, Help) and a tree view of 'Raw data files' and 'Peak lists'.

Alignment

Get aligned peak list



The screenshot displays the MZmine 2.14.2 software interface. The title bar reads "MZmine 2.14.2: New project". The menu bar includes "Project", "Raw data methods", "Peak list methods", "Visualization", "Windows", and "Help".

The interface is divided into two main panels:

- Raw data files:** Located on the left, it shows two files: "D20100526-LC2-PP0000441-B1-I1-P.mzXML" and "D20100526-LC2-PP0000441-B4-I1-P.mzXML". The second file is selected.
- Peak lists:** Located on the right, it shows a list of processing steps for the selected files:
 - D20100526-LC2-PP0000441-B1-I1-P.mzXML chromatograms
 - D20100526-LC2-PP0000441-B1-I1-P.mzXML chromatograms deconvoluted
 - D20100526-LC2-PP0000441-B4-I1-P.mzXML chromatograms
 - D20100526-LC2-PP0000441-B4-I1-P.mzXML chromatograms deconvoluted
 - D20100526-LC2-PP0000441-B1-I1-P.mzXML chromatograms deconvoluted normalized
 - D20100526-LC2-PP0000441-B4-I1-P.mzXML chromatograms deconvoluted normalizedThe "Aligned peak list" is selected and expanded, showing the following data:

Peak ID	m/z	Retention Time (min)
#1	72.0815	0.7
#2	74.0970	0.6
#3	74.0970	0.8
#4	74.0969	0.9
#5	74.0970	3.1

Alignment

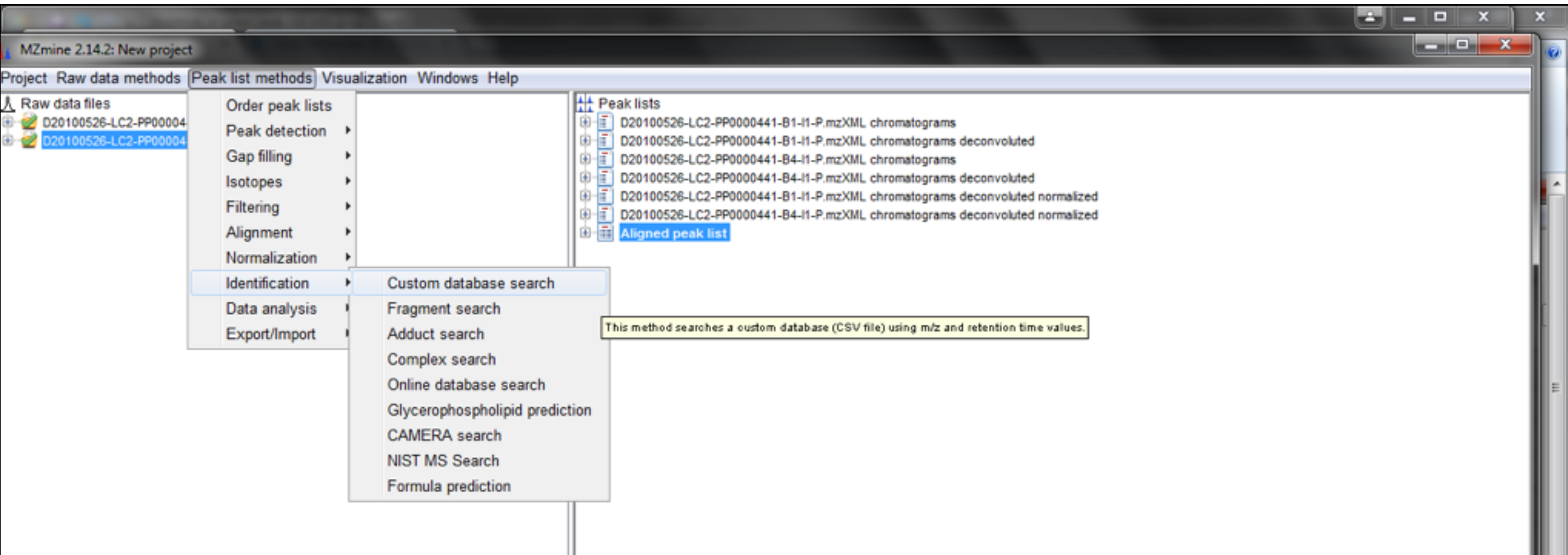
Information on aligned peak list

ID	Average		Identity	Comment	Peak shape	D20100526-LC2-PP0000441-B1-I1-P.mz...			D20100526-LC	
	m/z	RT				Status	Height	Area	Status	
1	72.0815	0.7				●	8.0E5	3.7E6	●	
2	74.0970	0.6				●	1.4E5	8.9E5	●	
3	74.0970	0.8				●	8.2E4	5.4E5	●	
4	74.0969	0.9				●	5.1E4	3.4E4	●	
5	74.0970	3.1				●	1.6E5	5.4E5	●	
6	74.0971	3.4				●	3.6E5	1.6E6	●	
7	76.0948	0.7				●	1.9E5	8.4E5	●	
8	80.9485	0.5				●	1.3E5	6.4E5	●	
9	84.9603	7.8				●	2.3E5	1.2E6	●	
10	86.0972	1.5				●	6.4E5	6.8E6	●	
11	88.1124	2.8				●	5.1E4	7.1E4	●	
12	88.1127	2.9				●	5.2E4	7.2E4	●	
13	88.1127	2.9				●	5.5E4	2.9E5	●	
14	88.1128	3.5				●	9.5E4	2.7E6	●	
15	88.1127	3.7				●	5.4E4	2.2E5	●	

Green: present
Red: absent

Peak identification

Options



Peak identification

By searching custom databases

1	ID	m/z	Retention	Identity	Formula
28	C00041	90.05496	1.45	alanine [M+H]	C3H7NO2
29	na	161.0921	1.71	alanine-alanine [M+H]	C6H12N2O3
30	C01551	159.0513	1.62	allantoin [M+H]	C4H6N4O3
31	C06464	181.0707	1.52	altrose [M+H]	C6H12O6
32	C00216 C00259	151.0601	1.62	arabinose [M+H]	C5H10O5
33	C01112	231.0264	1.56	arabinose 5 phosphate [M+H]	C5H11O8P
34	C00532	153.0758	1.51	arabitol [M+H]	C5H12O5
35	C00792	175.119	1.37	arginine [M+H]	C6H14N4O2
36	C00049	134.0448	1.45	aspartic acid [M+H]	C4H7NO4
37	C00099	90.05496	1.38	beta-alanine [M+H]	C3H7NO2
38	C02512	115.0502	1.48	beta-cyano-l-alanine [M+H]	C4H6N2O2
39	C00719	118.0863	1.57	trimethylglycine [M+H]	C5H11NO2
40	C00308	177.0982	1.34	canavanine [M+H]	C5H12N4O3
41	C09773	363.1286	5.98	catalpol [M+H]	C15H22O10
42	C00185	343.1235	1.77	cellobiose [M+H]	C12H22O11
43	C01484	209.0961	10.94	chalcone [M+H]	C15H12O
44	C00852	355.1024	9.1	chlorogenic acid [M+H]	C16H18O9

an example custom database

Peak identification

By adduct search: set parameters

Please set the parameters

Peak lists (input)

RT tolerance

Adducts

<input checked="" type="checkbox"/> [M+Na-H] 21.9825 m/z	<input type="button" value="All"/> <input type="button" value="Clear"/> <input type="button" value="Add..."/> <input type="button" value="Import..."/> <input type="button" value="Export..."/> <input type="button" value="Reset"/>
<input checked="" type="checkbox"/> [M+K-H] 37.9559 m/z	
<input checked="" type="checkbox"/> [M+Mg-2H] 21.9694 m/z	
<input checked="" type="checkbox"/> [M+NH3] 17.0265 m/z	
<input checked="" type="checkbox"/> [M+H3PO4] 97.9769 m/z	
<input checked="" type="checkbox"/> [M+H2SO4] 97.9674 m/z	
<input checked="" type="checkbox"/> [M+H2CO3] 62.0004 m/z	
<input type="checkbox"/> [(Deuterium)]glycerol 5.0000 m/z	

m/z tolerance m/z or ppm

Max relative adduct peak height %

Peak identification

Adduct search results

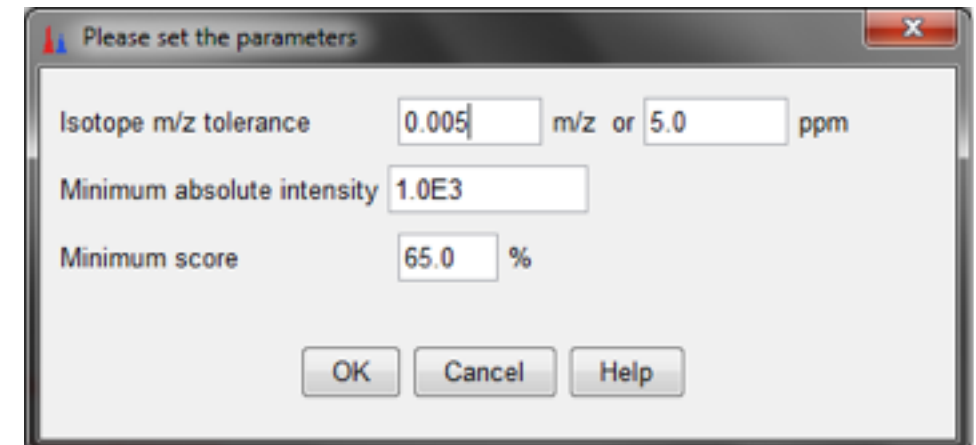
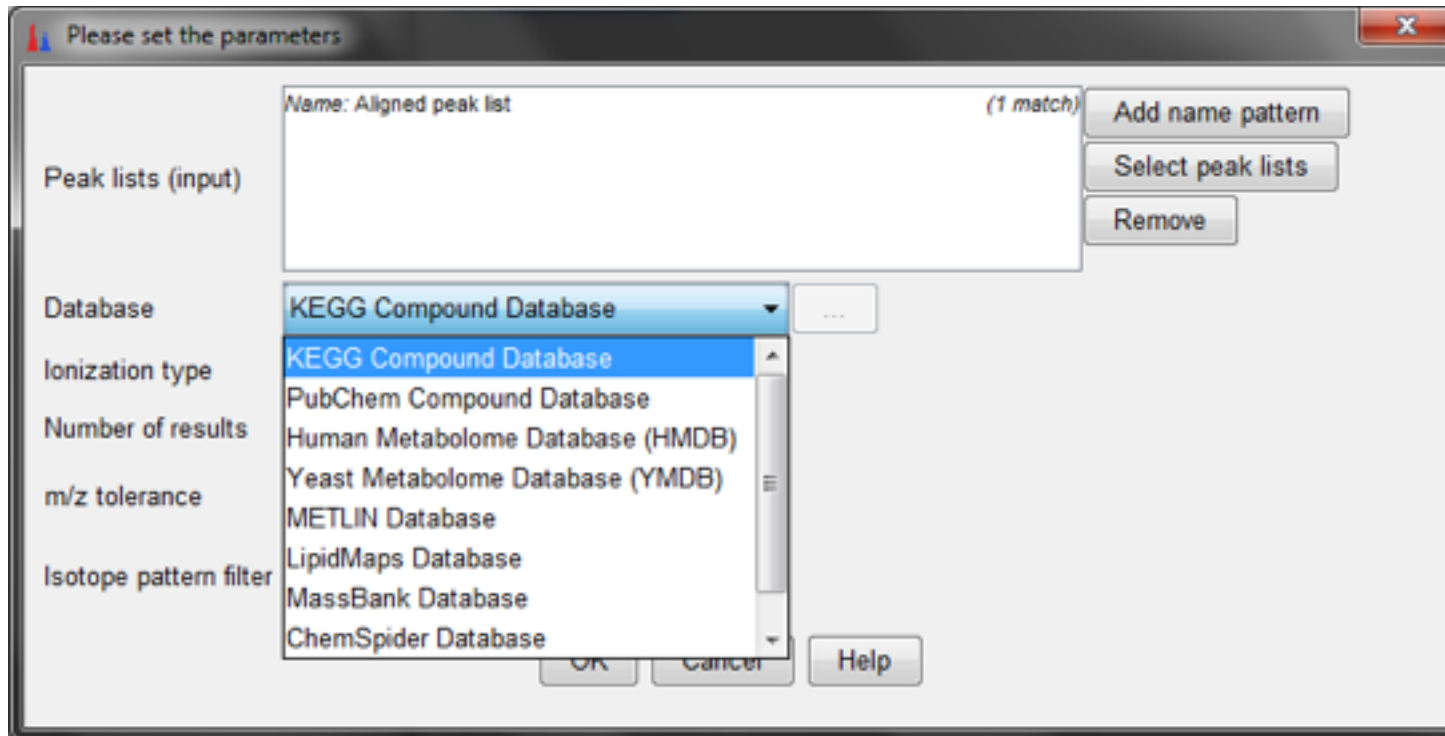
Peak list: Aligned peak list

Windows

ID	Average		Identity	Comment	Peak shape	D20100526-LC2-PP0000441-B1-I1-P.mz...			D20100526-LC2-PP0000441-B4-I1-P.mz...		
	m/z	RT				Status	Height	Area	Status	Height	Area
41	121.0879	0.6				●	1.7E5	4.5E5	●		
42	123.1037	0.7				●	6.1E5	2.7E6	●	7.9E5	3.8E6
43	128.1082	2.6				●	4.0E5	1.5E6	●	5.4E5	1.6E6
44	130.0865	0.8				●	1.2E5	3.8E5	●	1.6E5	5.2E5
45	130.0506	1.0				●	2.2E5	1.6E6	●	3.1E5	1.7E6
46	132.1024	1.5				●	2.5E6	2.8E7	●		
47	132.2032	1.5				●	1.8E5	1.7E6	●		
48	133.1057	1.5				●	1.9E5	6.2E5	●	1.6E5	2.2E5
49	133.0617	3.0				●	5.4E5	1.3E6	●	9.2E5	2.4E6
50	134.0212	7.8				●	1.9E5	8.8E5	●	7.9E5	3.3E6
51	136.0486	0.6	[M+Na-H] 21.9825 m/z adduct of 114.0669 m/z			●	1.7E5	3.3E5	●	2.2E5	4.9E5
52	137.1190	1.5				●	1.2E5	1.1E6	●		
53	138.0530	0.6	[M+Na-H] 21.9825 m/z adduct of 116.0713 m/z			●	7.6E4	1.8E5	●	2.1E5	5.7E5
54	138.0492	0.9				●	5.6E4	3.7E4	●	6.8E4	1.2E5
55	138.1228	1.5				●	1.8E6	2.0E7	●		
56	138.2275	1.4				●	6.1E4	2.0E5	●		
57	138.2274	1.5				●	1.2E5	6.9E5	●		
58	140.0689	0.5				●	1.3E5	2.8E5	●	1.7E5	3.3E5
59	140.0686	0.7	[M+Na-H] 21.9825 m/z adduct of 118.0866 m/z			●	2.5E5	1.0E6	●	3.5E5	1.4E6
60	144.1027	6				●	3.0E5	8.4E5	●	5.7E5	1.5E6
61	145.0855	0.7	[M+Na-H] 21.9825 m/z adduct of 123.1037 m/z			●	6.3E4	1.6E5	●		

Peak identification

By online database search



Peak identification

Online database search results

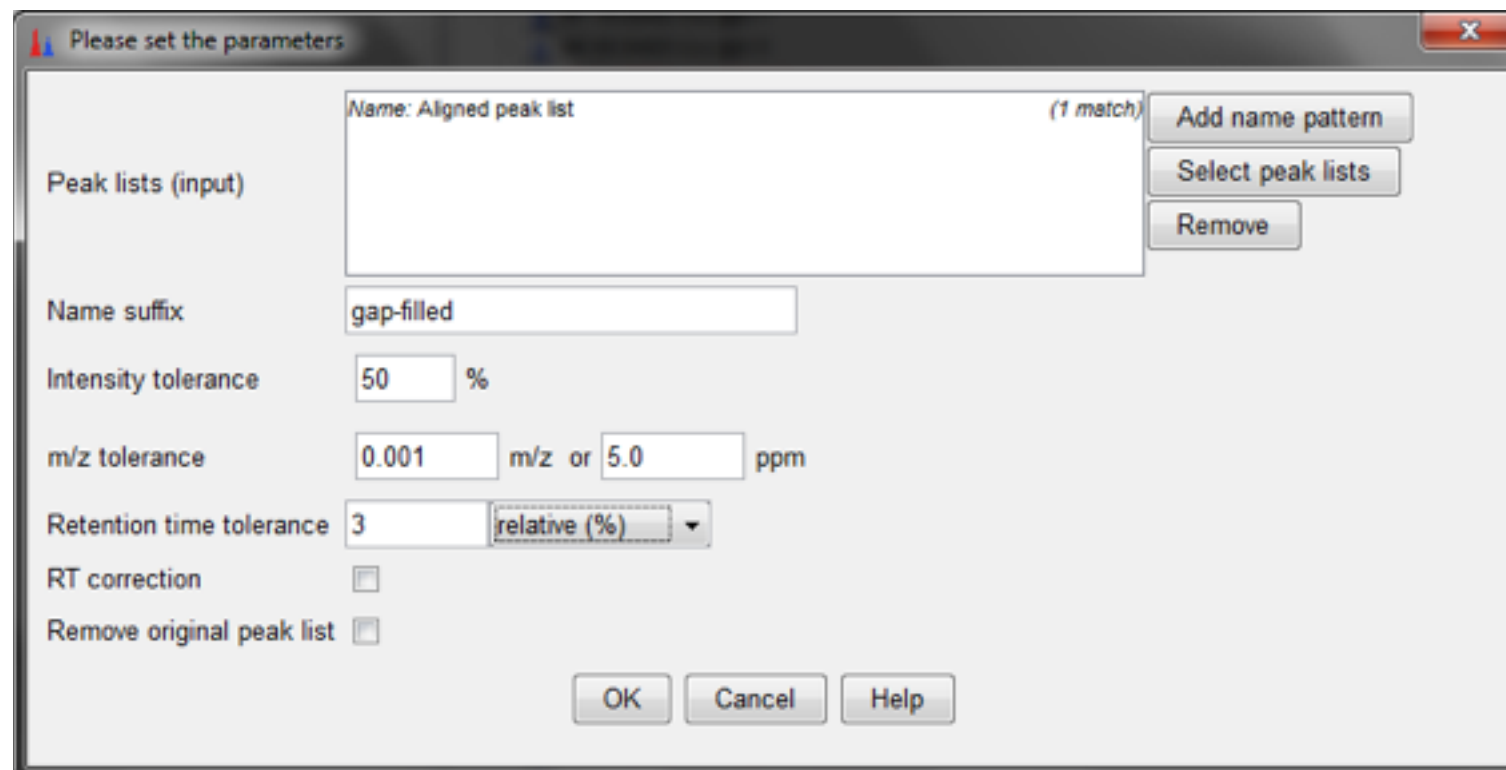
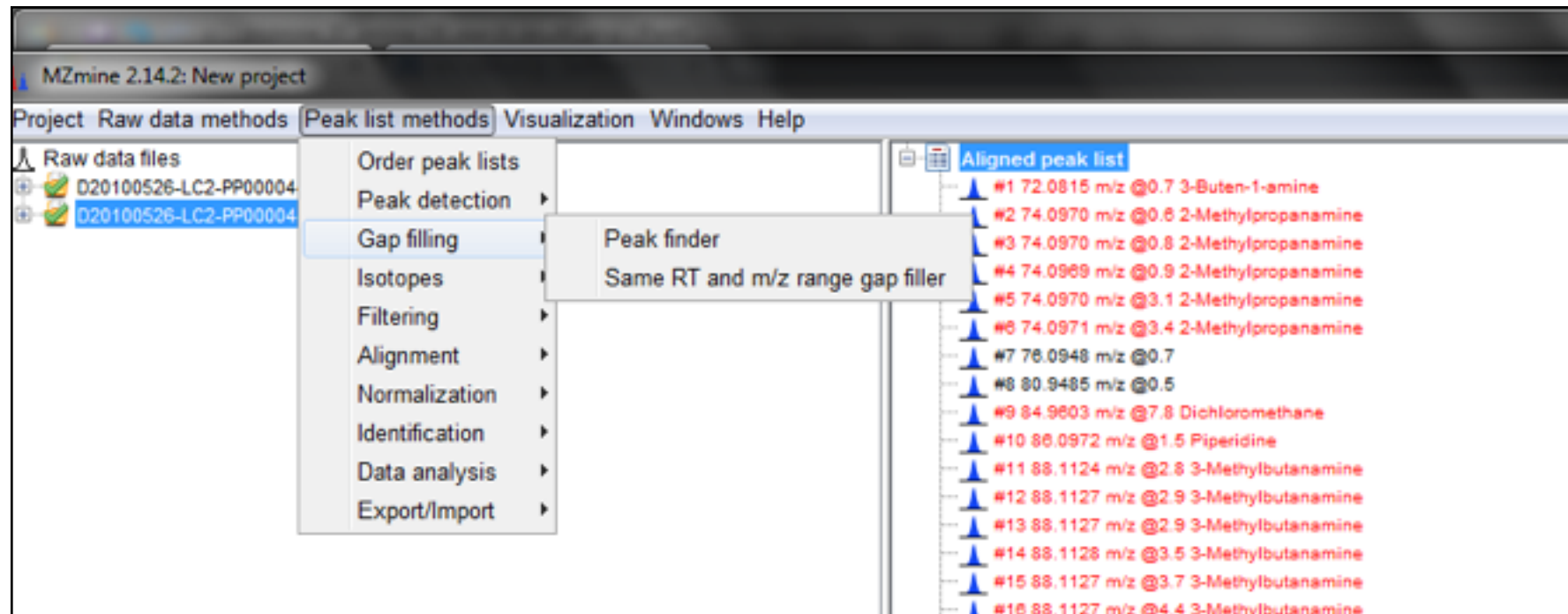
Peak list: Aligned peak list

Windows

ID	Average		Identity	Comment	Peak shape	D20100526-LC2-PP0000441-B1-I1-P.mz...			D20100526-LC2-PP0000441-B4-I1-P.mz...		
	m/z	RT				Status	Height	Area	Status	Height	Area
43	128.1082	2.6				●	4.0E5	1.5E6	●	5.4E5	1.6E6
44	130.0865	0.8	L-Pipecolate			●	1.2E5	3.8E5	●	1.6E5	5.2E5
45	130.0508	1.0	4-Oxoproline			●	2.2E5	1.6E6	●	3.1E5	1.7E6
46	132.1024	1.5	L-Leucine			●	2.5E6	2.8E7	●		
47	132.2032	1.5				●	1.8E5	1.7E6	●		
48	133.1057	1.5				●	1.9E5	6.2E5	●	1.6E5	2.2E5
49	133.0617	3.0	L-Asparagine			●	5.4E5	1.3E6	●	9.2E5	2.4E6
50	134.0212	7.8				●	1.9E5	8.8E5	●	7.9E5	3.3E6
51	136.0480	0.6	[M+Na-H] 21.9825 m/z adduct of 114.0669 m/z			●	1.7E5	3.3E5	●	2.2E5	4.9E5
52	137.1190	1.5				●	1.2E5	1.1E6	●		
53	138.0530	0.6	[M+Na-H] 21.9825 m/z adduct of 116.0713 m/z			●	7.6E4	1.8E5	●	2.1E5	5.7E5
54	138.0492	0.9				●	5.6E4	3.7E4	●	6.8E4	1.2E5
55	138.1228	1.5				●	1.8E6	2.0E7	●		
56	138.2275	1.4				●	6.1E4	2.0E5	●		
57	138.2274	1.5				●	1.2E5	6.9E5	●		
58	140.0689	0.5				●	1.3E5	2.8E5	●	1.7E5	3.3E5
59	140.0686	0.7	[M+Na-H] 21.9825 m/z adduct of 118.0866 m/z			●	2.5E5	1.0E6	●	3.5E5	1.4E6
60	144.1027	0.6	(E)-4-(Trimethylammonio)but-2-enoate			●	3.0E5	8.4E5	●	5.7E5	1.5E6

Gap filling

Options and parameters



Gap filling

Results and visualization options

Peak list: Aligned peak list

Windows

ID	Average		Identity	Comment	Peak shape	D20100526-LC2-PP0000441-B1-I1-P.mz...			D20100526-LC2-PP0000441-B4-I1-P.mz...		
	m/z	RT				Status	Height	Area	Status	Height	Area
64	150.0589	0.9	L-Methionine			●	8.3E5	2.6E6	●	1.1E6	3.4E6
65	152.0325	1.0	[M+Na-H] 21.9825 m/z adduct of 130.0506 m/z			●	9.6E4	6.9E5	●	1.9E5	6.4E5
66	154.0843	1.5	[M+Na-H] 21.9825 m/z adduct of 132.1024 m/z			●	7.9E4	4.4E5	●	6.0E5	1.6E6
67	155.0757	0.9				●	4.5E5	1.5E6	●	6.0E5	1.6E6
68	157.0735	0.8				●	5.8E4	3.7E4	●	8.7E4	2.4E5
69	160.1043	1.5	[M+Na-H] 21.9825 m/z adduct of 138.1228 m/z			●	5.4E4	1.5E5	●	8.7E4	2.4E5
70	162.1133	0.5	L-Carnitine			●	3.8E5	1.1E6	●	3.7E5	1.1E6
71	165.0549	1.3	Phenylpyruvate			●	1.9E5	6.9E5	●	1.9E5	6.4E5
72	165.0350	7.8				●	2.8E5	9.1E5	●	2.8E5	9.1E5
73	166.0870	2.6	L-Phenylalanine			●	3.9E5	1.2E7	●	3.8E6	1.3E7
74	166.0875	3.5				●	7.8E5	2.5E6	●	7.8E5	2.5E6
75	167.0905	2.6				●	4.4E5	1.4E6	●	4.4E5	1.4E6
76	167.0905	3.5				●	8.1E4	2.6E5	●	8.1E4	2.6E5
77	169.0363	1.0	Urate			●	6.2E5	3.6E6	●	6.2E5	3.6E6
78	174.1140	2.6				●	3.0E5	1.1E6	●	3.7E5	1.1E6
79	175.1170	2.6				●	3.1E6	1.2E7	●	3.8E6	1.3E7
80	181.0729	2.7	Theophylline			●	1.0E5	1.7E5	●	1.0E5	1.7E5
81	182.0823	1.3				●	1.5E6	7.9E6	●	1.5E6	7.9E6
82	183.0851	1.3				●	1.6E5	7.2E5	●	1.6E5	7.2E5
83	183.1630	0.6				●	9.0E4	5.0E5	●	9.0E4	5.0E5

- Show
 - XIC (base peak) (quick)
 - XIC (dialog)
 - Mass spectrum
 - Peak in 2D
 - Peak in 3D
 - MS/MS
 - Isotope pattern
 - Peak row summary
- Search
- Export
- Identities
- Plot using Intensity Plot module
- Manually define peak
- Delete selected row(s)
- Add new row

Gap filling

Set visualization parameters

Please set the parameters

Raw data files (input)

Name: D20100526-LC2-PP0000441-B1-I1-P.mzXML (1 match)
Name: D20100526-LC2-PP0000441-B4-I1-P.mzXML (1 match)

Add name pattern
Select files
Remove

MS level: 1

Plot type: Base peak intensity

Retention time: 0.0 - 9.0 Auto range

m/z: 162.1123 - 162.1138 Auto range From formula

Peaks

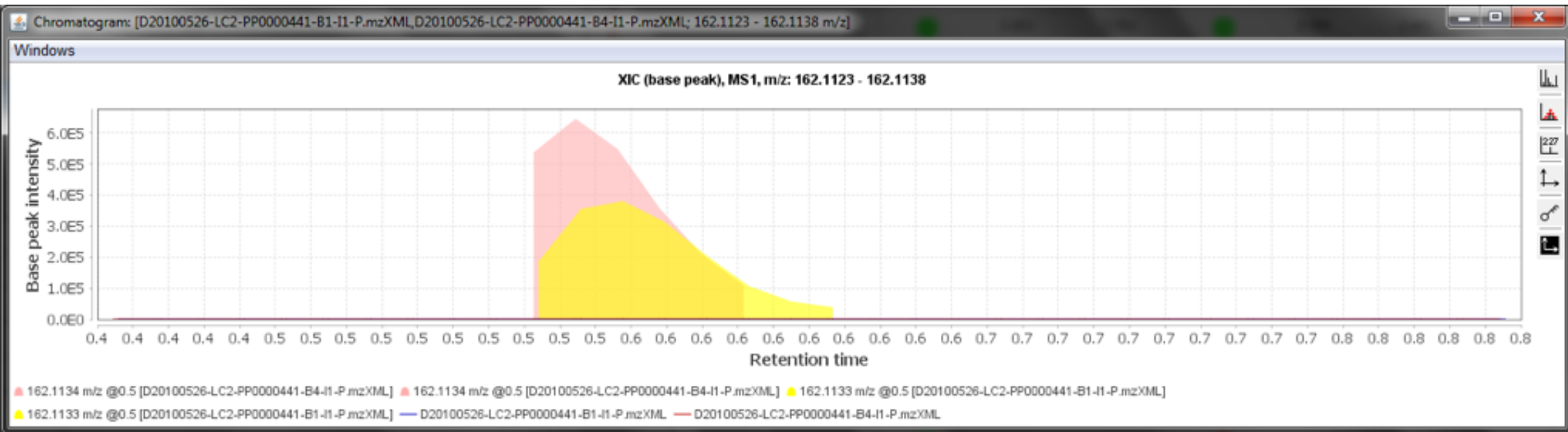
162.1134 m/z @0.5 [D20100526-LC2-PP0000441-B4-I1-P.mzXML]
 162.1133 m/z @0.5 [D20100526-LC2-PP0000441-B1-I1-P.mzXML]

All
Clear

OK Cancel Help

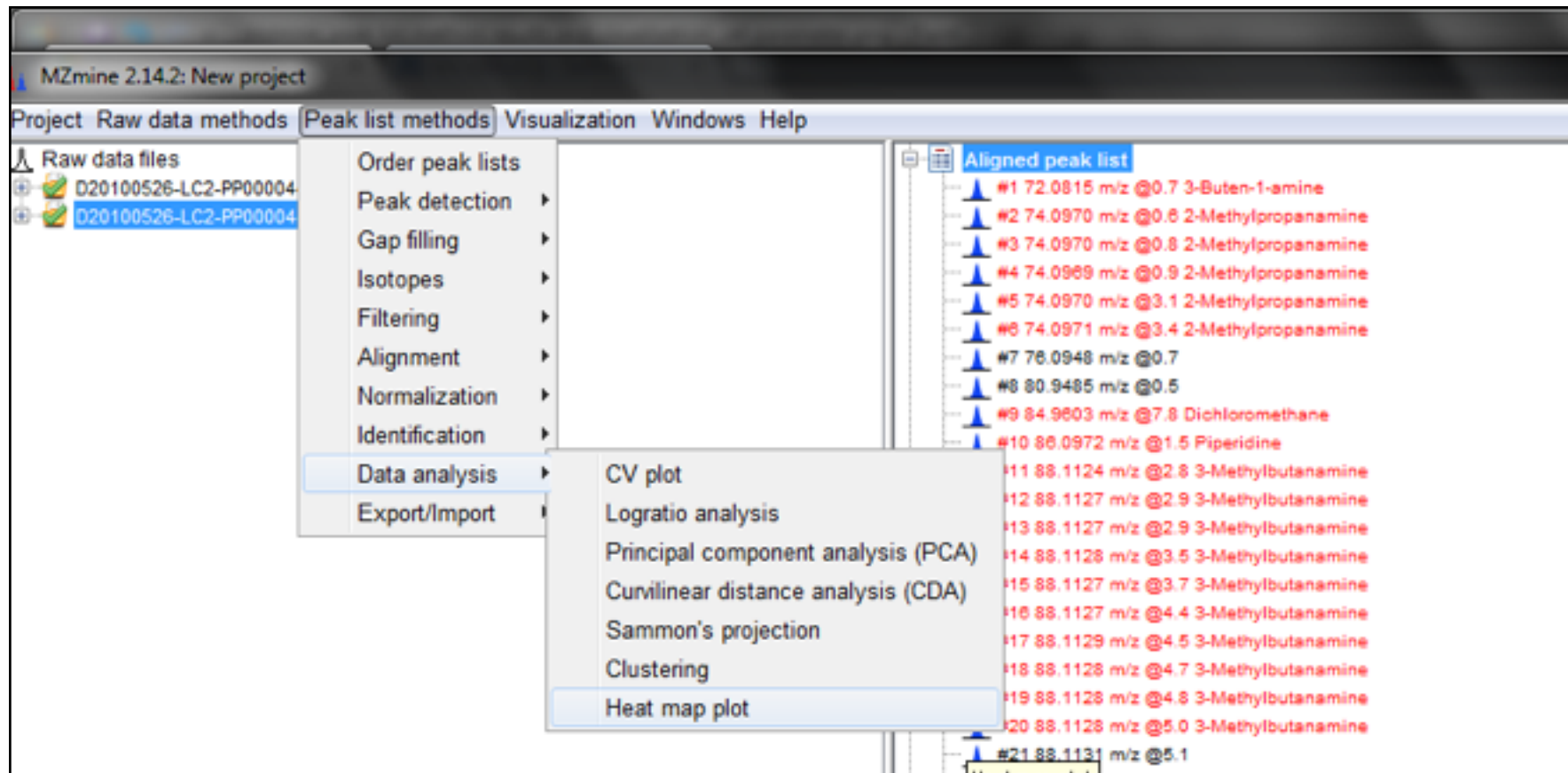
Gap filling

Visualization



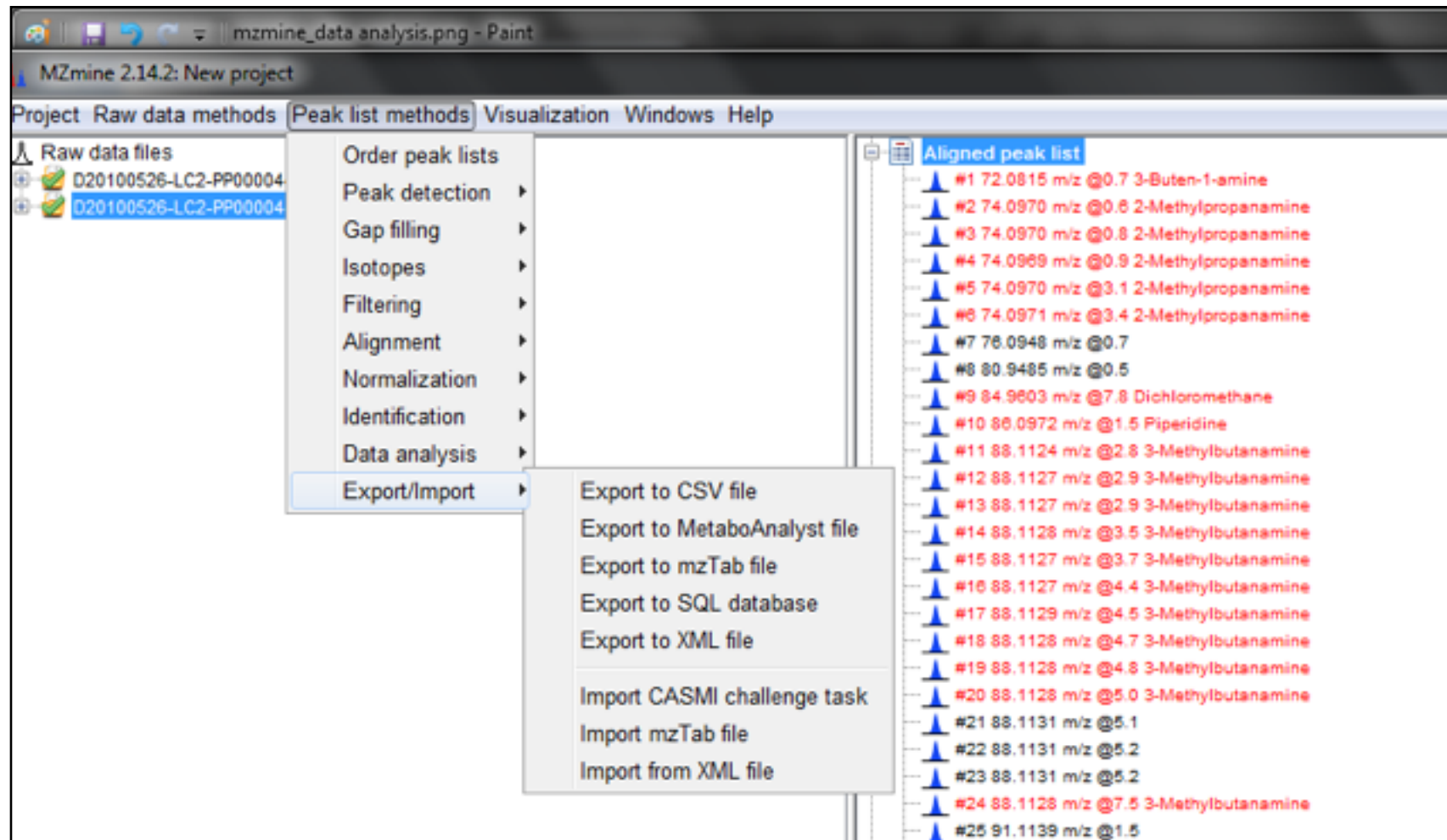
Data analysis

Options



Data export

Options



Data export

Parameters

Please set the parameters

Peak lists (input) Name: Aligned peak list (1 match) Add name pattern
Select peak lists
Remove

Filename

Field separator

Export common elements

- Export row ID
- Export row m/z
- Export row retention time
- Export row comment
- Export row number of detected peaks

All
Clear

Export identity elements

- All identity elements
- ID
- Identification method
- Molecular formula
- Name
- URL

All
Clear

Export data file elements

- Export peak status
- Export peak m/z
- Export peak retention time
- Export peak height
- Export peak area
- Export peak charge
- Export peak duration time

All
Clear

OK Cancel Help

Data export

csv format

1	row reten	row com	row num	All identit	ID	Identifica	Molecular Name	URL	D2010052	D20100526-LC2-PP0000441	D20100526-LC2-P	D2010052	D2010052	D2010052	D2010052	D2010052	D2010052	D2010052	D2010052
2	0.724		2	C12244	KEGG sear	C4H9N	3-Buten-1	http://ww	DETECTED	72.08156586	0.723997543	800532	3677302	0	0.11805	DETECTED	72.08146	0.7	
3	0.61775		1	C02787	KEGG sear	C4H11N	2-Methyl	http://ww	DETECTED	74.0969696	0.61775	135578	894695.8	0	0.141667	UNKNOW	0		
4	0.806632		1	C02787	KEGG sear	C4H11N	2-Methyl	http://ww	DETECTED	74.09701538	0.806632212	81876	540370.3	0	0.141667	UNKNOW	0		
5	0.912881		1	C02787	KEGG sear	C4H11N	2-Methyl	http://ww	DETECTED	74.09690475	0.912880769	50849	33958.16	0	0.0118	UNKNOW	0		
6	3.065793		1	C02787	KEGG sear	C4H11N	2-Methyl	http://ww	DETECTED	74.09701157	3.065792701	164087	541801.6	0	0.082633	UNKNOW	0		
7	3.44419		2	C02787	KEGG sear	C4H11N	2-Methyl	http://ww	DETECTED	74.09715652	3.494018719	362028	1621563	0	0.177083	DETECTED	74.09711	3.3	
8	0.724		2						DETECTED	76.09487915	0.723997543	188653	844518.9	0	0.10625	DETECTED	76.09476	0.7	
9	0.458367		2						DETECTED	80.94852448	0.464983333	132814	639803.8	0	0.11805	DETECTED	80.9485	0.	
10	7.813333		2	C02271	KEGG sear	CH2Cl2	Dichlorom	http://ww	DETECTED	84.96022797	7.796366667	227123	1180269	0	0.12985	DETECTED	84.96029	.	
11	1.491406		1	C01746	KEGG sear	C5H11N	Piperidine	http://ww	DETECTED	86.09718323	1.49140625	635862	6786687	0	0.295133	UNKNOW	0		
12	2.837807		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11236572	2.837806972	51294	70746.81	0	0.023617	UNKNOW	0		
13	2.873806		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11269379	2.87380561	51532	71944.87	0	0.023617	UNKNOW	0		
14	2.945803		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11274719	2.945802887	55102	293767.2	0	0.09445	UNKNOW	0		
15	3.494019		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11279297	3.494018719	94520	2704280	0	0.672917	UNKNOW	0		
16	3.725024		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11273193	3.725024455	54012	219970.6	0	0.070833	UNKNOW	0		
17	4.383776		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11273956	4.383775926	51651	71837	0	0.023617	UNKNOW	0		
18	4.52545		2	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.1129303	4.525448148	64166	485431.6	0	0.141667	DETECTED	88.11287	4.5	
19	4.735547		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11283875	4.735546894	56805	412476.8	0	0.129867	UNKNOW	0		
20	4.823239		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.1128006	4.823239386	54623	216883.8	0	0.070833	UNKNOW	0		
21	4.998642		1	C02640	KEGG sear	C5H13N	3-Methyl	http://ww	DETECTED	88.11277771	4.998642059	58821	579818.9	0	0.177083	UNKNOW	0		
22	5.111397		1						DETECTED	88.1131134	5.11139713	52386	35898.43	0	0.0118	UNKNOW	0		
23	5.161505		1						DETECTED	88.11308289	5.161504599	52567	145161.7	0	0.047233	UNKNOW	0		
24	5.311612		1						DETECTED	88.11308289	5.311612068	50014	24892.4	0	0.011817	UNKNOW	0		

MetaboSearch

MetaboSearch

Input data format

	A	B	C
1	mz	rt	
2	62.98193	31.44898	
3	69.06956	50.27389	
4	70.06484	29.59302	
5	72.0804	34.29358	
6	73.08333	34.24128	
7	73.53049	23.18216	
8	80.94842	27.18991	
9	82.01386	23.07363	
10	82.53558	23.18671	
11	82.94562	27.18991	
12	83.01783	23.4163	

MetaboSearch

GUI

CUSTOMIZED DATABASES

URL	Description	Check
http://www.hmdb.ca/search/spectra?type=ms_search	HMDB database	<input checked="" type="checkbox"/>
http://metlin.scripps.edu/metabo_batch_list.php	Metlin database	<input checked="" type="checkbox"/>
http://mmcd.nmr.fam.wisc.edu/	MMCD database	<input checked="" type="checkbox"/>
http://www.lipidmaps.org/downloads/index.html	LipidMaps database	<input checked="" type="checkbox"/>

INPUT

Positive/Negative Mode: Positive Negative

MW Tolerance in ppm: 10

Input Mass Data

137.112264043691
168.10127646677
371.228274186181
450.322087352445
496.34252837084

C:\Documents and Settings\lbz37\Desktop\DemoData\Inputs\Demo 1 I...

OUTPUT

The directory of the result is: C:\Documents and Settings\lbz37\Desktop\tempforsearch
Searching databases, please wait...
Grasped Metlin is coming...

QueryID	Name	Formula	Mass	Kegg	CID
5	PC(16:0/0:0)[U] / PC(16:0/0:0)[rac]			C24H50NO7P	495
2	4-(1-hydroxy-2-(methylamino)ethyl)phenol			C9H13NO2	167
2	PYRITHYLDIONE		C9H13NO2	167.094628656	
2	METARAMINOL	C9H13NO2	167.094628656	C07146	Not
2	4-				
2	o-				
2	Phenylephrine	C9H13NO2	167.094629	C07441	Not
2	Methyldopamine		C9H13NO2	167.094629	
2	p-Hydroxynorpseudoephedrine	C9H13NO2	167.094629		
2	3-Methoxytyramine		C9H13NO2	167.094629	
4	N-(3α;12α-dihydroxy-5β-cholan-24-oyl)-glycine			C26	
4	Chenodeoxyglycocholate		C26H43NO5	449.314096	C05

STATUS

Searching Lipid Maps, please wait.....

Progress has been completed 80%

80%

MetaboSearch

Steps

The screenshot displays the MetaboSearch Tool interface with four numbered steps highlighted in yellow:

- 1 Select the parameters**: Located in the INPUT section, showing radio buttons for Positive and Negative modes, and a MW Tolerance in ppm set to 10.
- 2 Paste the m/z values or load data from a local file**: Located in the INPUT section, showing a list of m/z values (137.112264043691, 168.10127646677, 371.228274186181, 450.322087352445, 496.34252837084) and a file path: C:\Documents and Settings\lbz37\Desktop\DemoData\Inputs\Demo 1 I...
- 3 Search the databases**: Located in the STATUS section, showing the progress of searching Lipid Maps (80% completed).
- 4 Export results**: Located in the OUTPUT section, showing a table of search results and an "Export to Local" button.

CUSTOMIZED DATABASES

URL	Description	Check
http://www.hmdb.ca/search/spectra?type=ms_search	HMDB database	<input checked="" type="checkbox"/>
http://metlin.scripps.edu/metabo_batch_list.php	Metlin database	<input checked="" type="checkbox"/>
http://mmcd.nmr.fam.wisc.edu/	MMCD database	<input checked="" type="checkbox"/>
http://www.lipidmaps.org/downloads/index.html	LipidMaps database	<input checked="" type="checkbox"/>

INPUT

Positive/Negative Mode: Positive Negative

MW Tolerance in ppm: 10

Input Mass Data

137.112264043691
168.10127646677
371.228274186181
450.322087352445
496.34252837084

C:\Documents and Settings\lbz37\Desktop\DemoData\Inputs\Demo 1 I...

Browse Submit

OUTPUT

The directory of the result is: C:\Documents and Settings\lbz37\Desktop\tempforsearch
Searching databases, please wait...
Grasped Metlin is coming...

QueryID	Name	Formula	Mass	Kegg	CID
5	PC(16:0/0:0)[U] / PC(16:0/0:0)[rac]			C24H50NO7P	495
2	4-(1-hydroxy-2-(methylamino)ethyl)phenol			C9H13NO2	167
2	PYRITHYLDIONE		C9H13NO2	167.094628656	
2	METARAMINOL	C9H13NO2	167.094628656	C07146	Not
2	4-Methoxytyramine		C9H13NO2	167.094628665	
2	o-Methyldopamine (3-methoxytyramine)		C9H13NO2	167.094629	167
2	Phenylephrine	C9H13NO2	167.094629	C07441	Not
2	Methyldopamine		C9H13NO2	167.094629	
2	p-Hydroxynorpseudoephedrine		C9H13NO2	167.094629	
2	3-Methoxytyramine		C9H13NO2	167.094629	
4	N-(3 α ,12 α -dihydroxy-5 β -cholane-24-oyl)-glycine			C26	
4	Chenodeoxyglycocholate		C26H43NO5	449.314096	C05

Export to Local

STATUS

Searching Lipid Maps, please wait.....

Progress has been completed 80%

80%

Software tools for GC-MS Metabolomics

Software Tools for GC-MS

- **Commercial**

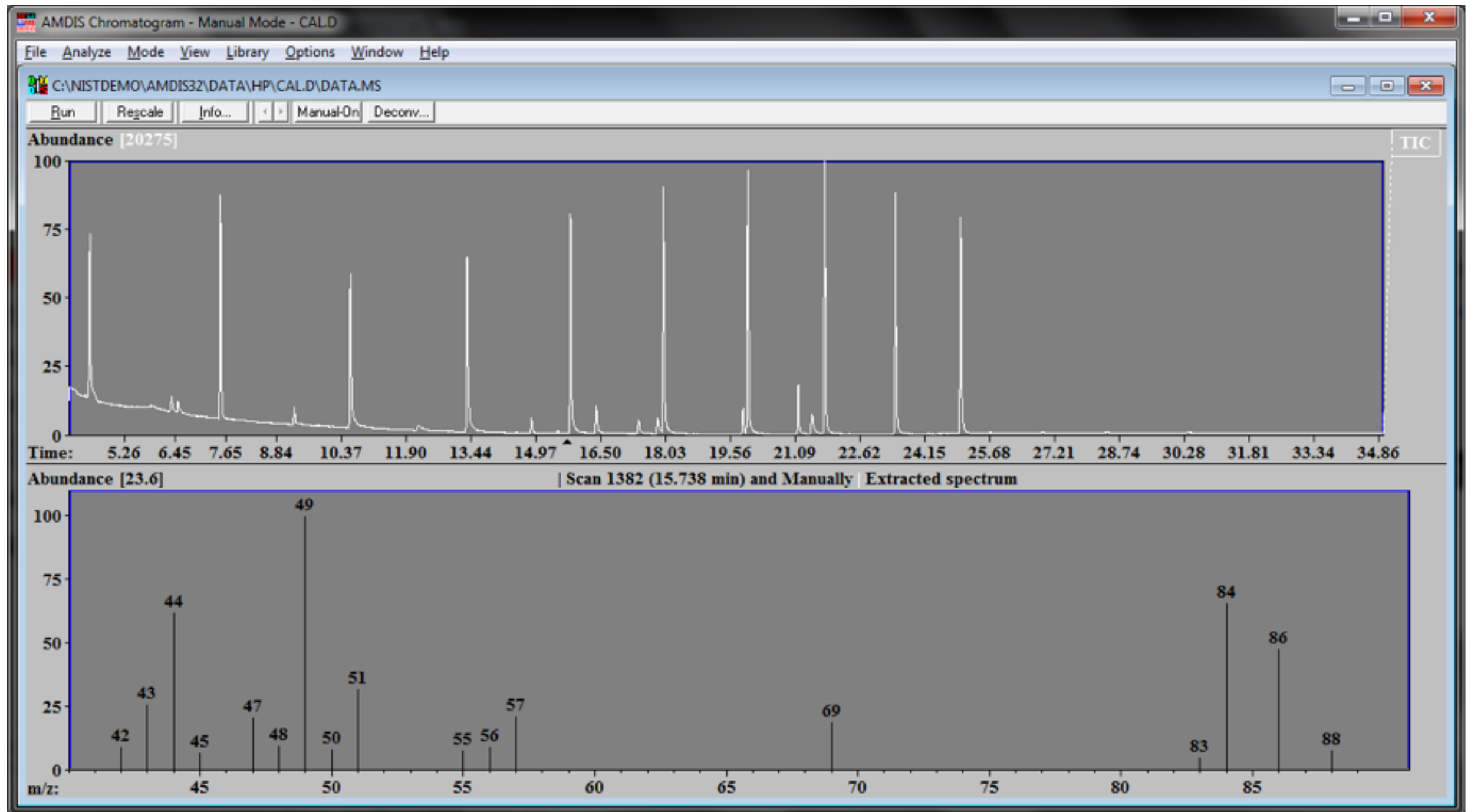
- ChromaTOF[®] — LECO
- MassHunter — Agilent

- **Free**

- **AMDIS:** Automated Mass Spectral Deconvolution and Identification System
- **NIST MS Search**
- Tagfinder

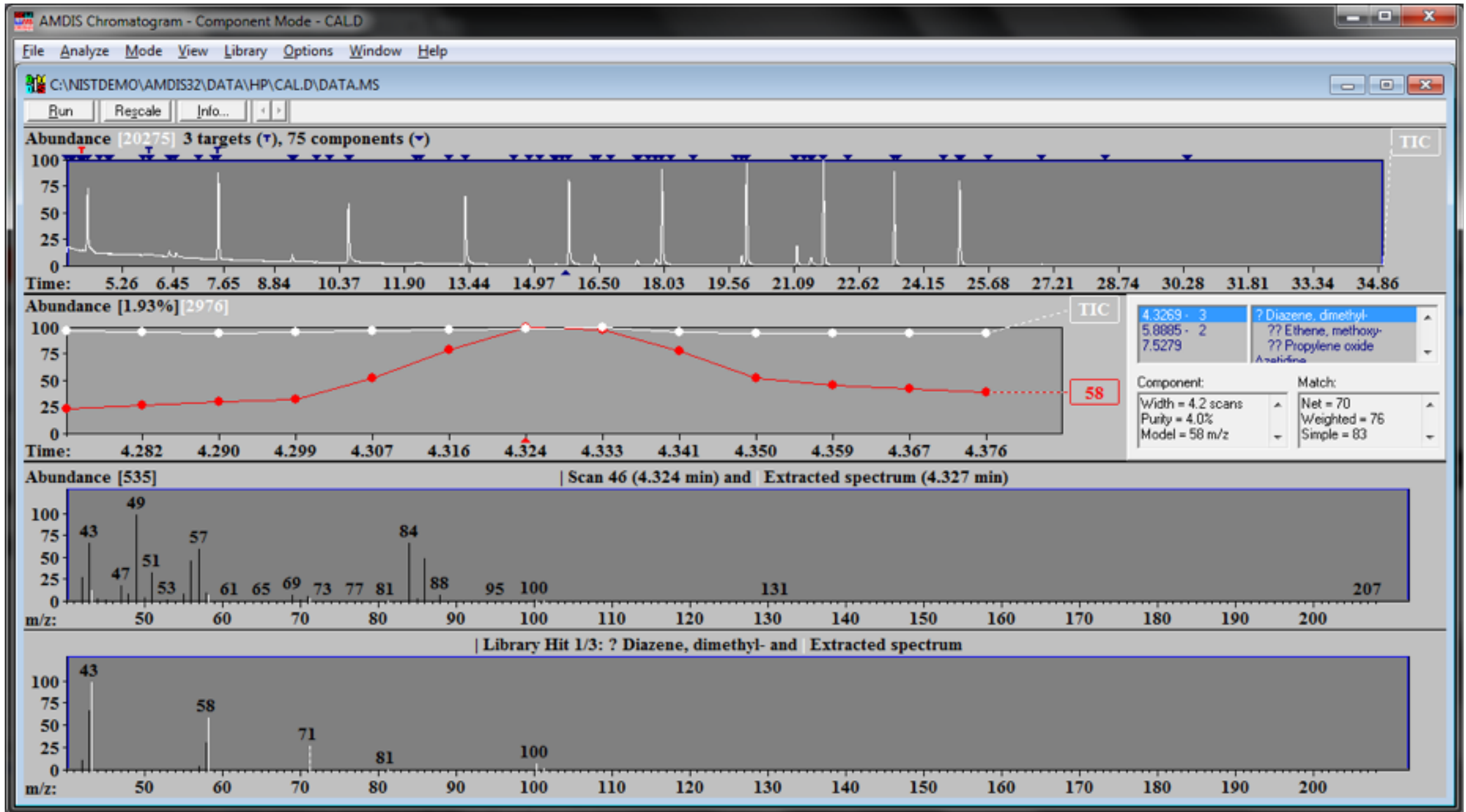
AMDIS

Raw data visualization



AMDIS

Result visualization



NIST MS Search

GUI

The screenshot displays the NIST MS Search 2.0 interface. The main window shows a search for a component at scan 46 (4.327 min). The search results are displayed in a table, and the top result is Acetone. The interface includes a search bar, a list of results, a mass spectrum plot, and a chemical structure viewer.

Search Results Table:

#	Lib.	Match	R.Match	Prob. (%)	Name
1	M	845	929	65.7	Acet...
2	M	823	905	25.9	Ethe...
3	M	774	774	5.33	1-Pro...
4	M	733	806	1.21	Propy...
5	M	719	790	0.76	Butane
6	M	713	713	0.59	Pent...
7	M	682	682	0.16	2-He...
8	M	679	679	0.14	Aceti...
9	M	672	672	0.11	Meth...
10	M	637	637	0.02	2-He...
11	M	612	673	0.00	Etha...
12	M	599	599	0.00	2-He...
13	M	598	658	0.00	Isobu...
14	M	590	649	0.00	2-He...

Mass Spectrum Plot (Search):

Y-axis: 0 to 100. X-axis: 40 to 110. Major peaks at m/z 43 (100%), 58 (~60%), and 100 (~10%).

Mass Spectrum Plot (Hit):

Y-axis: 0 to 100. X-axis: 40 to 110. Major peaks at m/z 43 (100%), 58 (~30%), and 100 (~10%).

Chemical Structure:

Top result: Octadecanoic acid (SMILES: CCCCCCCCCCCCCCCCCC(=O)O)

Hit result: Acetone (SMILES: CC(=O)C)

Search Parameters:

1. Component at scan 46 (4.327 min) [M] (Model = +58u) in C:\NISTDEMO\AMDIS32\DA

mainlib: 2378 total spectra

Head to Tail MF=845 RMF=929

Software tools for Statistical Analysis

Stats and Machine Learning

- **Commercial**

- SIMCA
- Mass Profiler Professional (MPP) — Agilent
- and more

- **Free**

- **MetaboAnalyst**
- R

MetaboAnalyst



Other software tools

- **COMSPARI**
 - **COM**parison of **SP**ectral **A**nd **R**etention **I**nformation
- **MathDAMP**
 - **M**athematica package for **D**ifferential **A**nalysis of **M**etabolite **P**rofiles
- **MSFACTs**
 - **M**etabolomics **S**pectral **F**ormating, **A**lignment and **C**onversion **T**ools

and many more

Thank You!