MZmine Tutorial

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About MZmine

MZmine was developed at Okina wa Institute of Science and Technology, Japan and VTT Finland.More recently some development has been sponsored by Syngenta. It is a Java based program and is therefore platform independent. You may download it from the following website however it should be pre-installed on the EBI machines for this tutorial.

http://MZmine.sourceforge.net/

MZmine will import the following filetypes: Net CDF, mzData, mzML, mzXML, Xcalibur Raw files, Agilent CSV files. (For the Thermo Xcalibur files it is necessary either to have the Thermo Xcalibur software installed on the same machine or to have downloaded and installed the free ThermoMSFilereader software to be found at

http://sjsupport.thermofinnigan.com/public/detail.asp?id=586.

The version of MZmine used in the following examples was 2.8

Processing a simple Metabolomics dataset in MZmine

In the example dataset we have an excerpt of a metabolomic study on the ripening of fruits. We have nine samples of two different varieties, Wild-type and non-ripening Mutant plus ten control samples which consist of a large batch of identical fruit extract that are run at every fifth sample. In addition the fruit are sampled everyday from the onset of ripening between 47 and 54 days. (This example datasets is only a small excerpt of a larger replicated study). The data were collected on a Thermo Velos Orbitrap running in ESI+ mode with a UPLC column.

Loading the data

One of the great advantages of MZmine is its interactivity. Firstly we begin by importing the data. *Raw Data Methods/Import*

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100	MU48_917_3	19.mzData 🌆 QC7_917_30.mzData		
Recent	MU48_921_1	9.mzData 🎧 QC8_917_41.mzData		
Items	MU49_917_5	8.mzData 🌆 QC9_917_46.mzData		
1000	MU50_917_3	3.mzData 🌆 WT47_917_13.mzData		
	MU51_917_4	15.mzData 🌆 WT48_917_40.mzData		
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Once the data is imported we can right click on the data file to reveal several display options

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CC3_917_08.mzData		Peaks		Clear OK Cancel		
CC5_917_19 mzData CC2_917_02 mzData				[Carrol] [Carrows	f (Tower)	

The TIC option offers the option of Base peak or TIC and allows you to set various ranges. Clicking OK leads to a high quality spectrum plot. The plot is fully zoomable and interactive, and double clicking a peak leads to its mass spectrum. Clicking and dragging upward or to the left is a gesture which results in zooming back out to maximum zoom. Clicking and dragging downwards or to the right zooms in.



NB: Make sure you take a note of the height of the baseline and the height of the smallest peaks. This will be useful later.

The mass spectrum plot also enables you to see associated ms-ms data. (In this dataset the MS-MS information has been removed).

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Peak detection is a three step process:

- (1) Mass detection
- (2) Chromatogram building
- (3) Peak Deconvolution

Mass Detection

Click on Raw data methods/Peak Detection/Mass detection



In this case we have imported mzData files which are centroided during the conversion from .RAW so the only option is Centroid mode. (If you have imported Thermo .RAW files then the data is continuous and you can use the exact mass, local maxima, recursive threshold or wavelet methods).

The 'Show preview' option allows you to interactively set the threshold for peak detection in the mass dimension. The aim is to detect peaks but not too many noisy features.

Aass detector	Centroid	Noise level 1.0E4
/IS level	1 •	Show preview
/lass list name	masses MS1	
-		OK Cancel Help

ise level 1.0E4	[MUS1 917 45.mzData] scan #1
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After clicking OK MZmine will build the mass list. Depending on the speed of your computer this may take some time. When the mass list is built the icon will show a green tick mark.



Chromatogram Builder

The Chromatogram builder is found under Raw data methods/Peak detection/chromatogram builder



Click on the *Choose…* button to select the mass list just generated and fill in the parameters as shown (for your own data these will vary)

Aass list	masses M	IS1		Choose
Ain time span (min)	0.017			
Min height	1.0E4			
m/z tolerance	0.0050	m/z or	5.0	ppm
Suffix	chromatog	rams		

You will then see a number of chromatograms listed in the left hand pane



Double clicking on a chromatogram will bring up the results:

Yoject Raw data methods. Peak ist methods. Visu	ualization Window	is Help							
New project	· CONTRACT	53_917_06 mzData chrom	atogrami						
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1 1015_917_11 nuties chromatograme	-				6 - 50 -				

The peak list is comprised of a series of ion chromatograms taken at each point that was detected in the chromatogram builder. Some ion chromatograms may contain more than one peak so a second peak deconvolution stage is required.

Peak Deconvolution

Make sure you highlight the chromatograms in the left hand pane. Click *Peak list methods/Chromatogram deconvolution*. You have a choice between Baseline Cut-off, Noise Amplitude, Savitsky-Golay, Local minimum search [and Wavelets (XCMS) in a future version]

↓ MZmine 2.2: New project					
Project Raw data methods	Peak list methods Vis	sualiz	ation Windows Help		
New project	Peak detection	Þ	Smoothing		
E Raw data files	Gap filling		Chromatogram deconvolution Ctrl+D	le atitus	
Peak lists	Isotopes		Peak shape modeler (experimental)	ientity	
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Here we use *Noise Amplitude* [My personal preference is Wavelets XCMS but it may not be implemented in this demonstration version]

luffix	deconvoluted_NA	Min peak height	1.0E4	
Algorithm	Noise amplitude 🔹	Peak duration range (min)	0.0	- 0.8
Remove original peak list		Amplitude of noise	5.0E4	
OK	Cancel Help		Show pre	New
		OK	Cancel	Help

Fill in the boxes with the appropriate values. One of the recent key improvements to MZmine is the ability to specify a maximum for peak duration. This is very useful for removing some of the artefact peaks caused by column bleed. Here we use 0.8 mins. Another trick used here is to set the amplitude of noise slightly higher than the min peak height. This means that peaks with raised baselines as in the example get detected. The downside is a slight loss in integration accuracy. (The alternative is to baseline correct first - see later, or use a more robust peak picker such as the wavelet option). Peak picking is always a compromise and requires a lot of experimentation for optimal results.

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After the peak deconvolution step MZmine produces a resolved peak list with one peak per row:

WTail S17 43 mcData chromatograma	(In second	13 BIT OF HURDER	and a local second s	Get REE		
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WTS1_817_06 mcDate chrumatogreese deconvoluted_RA	18	88.676 8.1			1	+ 1.204
W148 525 17 mcDate chromatograms deconvoluted SA	14	88.876 3.2				* 1.524
WTS4_917_28 mzDate chromatograme deconvoluted_NA	-					
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GC9_91T_40.mzData chromatograma deconvoluted_NA	1.			10		10.0

We can visualise the peaks using the 3D visualiser plot on the raw data. This is a useful check of the accuracy of peak picking.

Project Raw data	methods Peak list methods Visualization	Windows H	I Please set the paran	neters					No. 1
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0010_917	_36 wcData _51 wcData 66 wcData				OK.	Cancel	Help		





There is also a 2D "gel view" of the data - click Show 2D visualiser.

Right clicking a peak in the peak list From the peak list and selecting (*show... chromatogram quick*) shows the peak and the peak integration in pink. (There is also an option to see the peak in 3D but this appears to be broken in 2.8)



Peak Alignment

In MZmine peak alignment is done after the peaks are picked. To adjust for any slight variation in retention time a retention time normaliser is provided. Click on *Peak list methods /Normalisation /Retention time normaliser*.

Project Raw data methods	Peak list methods Vi	ualization Windows Help
VT14,317_29 mcDate VT14,317_29 mcDate VT14,317_29 mcDate V151,517_56 mcDate	Peak detection Gap tiling Isotopes Filtering Alignment	
0 0 000 000 000 000 000 000 000 00	Normalization Identification Data analysis	Retartion time normalizer The intention time normalizer The intention time normalizer attempt to referse the deviation of refering taken peak 100, by searching to common peaks in their peak 100 and using them as normalization dandards Standard compound normalizer
 GC10_917_51 m25ets GC5_917_18 m25ets GC3_917_58 m25ets 	Expert/Import	

Name suffix	RT_norma	lized	
m/z tolerance	0.0050	m/z or 5.0	ppm
Retention time tolerance	5.0	relative (%)	
Minimum standard intensity	1.0E6		
Remove original peak list	171		

Next we will combine the peaks using the Peak list methods/Alignment/Join aligner.



The alignment is based upon RT and m/z tolerance. There are options to only merge ions with the same charge state, the same ID or by isotope pattern. We will not use "Require same ID" because we have not identified any compounds yet.

Peak list name	Aligned per	ak list		Isotope m/z tolerance	0.0050	m/z or 5.0	ppm
m/z tolerance	0.0050	m/z or 5.0	ppm	Minimum absolute intensity	1E3		
Weight for m/z	20			Minimum score	65 86		
Retention time tolerance	3.0	relative (%)		winistion score	00 10		
Weight for RT	10			OK	Cancel	Heip	
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	11						
Require same ID	Trend						

You should now have an aligned peak list. Green dots indicate the presence of that peak in the scan. A red dot indicates the peak was not detected. After the identification process we will return to fill in these gaps with baseline levels from the other scans. (Gap-filling may alter the accuracy of the m/z value due to averaging)

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Identification

In order to identify peaks a database of m/z or m/z and Retention times are required. There are two options. One is a custom database compiled on your own instrument based upon the measured masses of the molecular ion and any adducts or fragments. The other option is an online database search based purely on the accurate mass and isotopic pattern matching.

Custom database search

Peak list methods/Custom database search

🗄 🚺 WT47_817_13.mzDati	Peak detection	Alig	ried peak i	int			
UT52_917_20 msDate	Gap Siling	* in	A	verage	Mantha	Comment	
WTS0_917_33.M2Det	Isotopes	•	m'z	Retitime	- opening	COMPANY	
WT48_921_17 HIDAN WT54_917_39 mpDate	Filtering	•	85.029	1.56			
WT49_917_59.mzDell WT91_917_43.mzDell WT91_917_43.mzDell WT91_917_917_43.mzDell	Alignment Normalization	:	85.048	1.82			1
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E GC0_917_41 mcData	Data analysis	The	a method per	eshes a cadots	databane (CS	V tite) using m/p (and selection time values
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In the dialog box select POS_mzRT_database.csv (which will be with the demo datasets).

This is a database we have compiled for our library using our UPLC-MS system in positive ESI+ mode. (NB: Your own data will differ in RT and possibly the ionisation profiles - you will need to compile your own library database relevant to your own system!) Here is an excerpt of our custom database. The first column is KEGG ID, then accurate m/z, Retention time, Identity and Formula. We have also included common adducts and dimers in the list such as [M+K], [M+Na], [2M+H]

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

Database file	34905_40	RT_database.cov	COMO:	
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Field order	E) mitz Retention tim Identity Formula	ne (mini)		
ignore first line				
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Retention time tolerance	5	relative (%)		
0	Can	el Help	1	

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	File name	POS_m	zRT_database csv		16	Select Ne
Network	Films of Israe	AL PLAN	2010 - 100 -		21 1	Cancel

Clicking the Identity tab twice should bring all the identified peaks to the top of the list. Notice that some of the identified component are isotopic internal standards which we use to normalise the data (outside MZmine).

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0C1_017_01 mcData chromatograma deci	80.78	273.076	134	namproin (Mrid)					•	_			1
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201_917_91 noOata chromatograma deci 202_817_03 noOata chromatograma deci ++	M1	198.017	1.30	Tabilita (NPT)		1			+ 2	111	4.005	+ 1.281	ŝ

Adduct search

Under peak list methods/identification there is an adduct search option. Note you can now load or save a customised list of adducts. Set the RT and m/z tolerance and the relative adduct peak height.

RT tolerance	3		celative.	(%) *				
	(i) ge-	-	(71.962 m)			•	AL	
	V (M-	6.00	37.856 mis				Clear	
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	12 (M-	H20	03) 62 800	92		.]	Resot	
n/z tolerance	0.005	3	miz a	5.0	ppr	í.		
Max relative adduct peak height	100	1						
(OK.	í G	Cancel	Help	1			

-	A	verage	1000	1000000	
	m/z	Ret time	identity -	Comment	r'eak shape
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\$121	835.298	8.25	(64+DACN++4) 83 060 mid adduct of 460 198 mid		
5122	533.248	9.25	(52+24/24+1) 83 850 mit adduct of 452 136 mit		
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5106	218.229	8.23	34+24/2014 (\$3.000 mix assuut of 433.171 mix		
1089	407.987	1.17	(51+2+21++) 03.000 mit adduct of 404.120 m/s		
1799	671.228	8.55	(NH2ACHH4) 83.059 mit adduct of 388.150 m/z		
1010	#17 237	8.14	(NI+CACH++C 88 385 mill adduct of 384 178 mill		

There is a similar option for fragment search (based upon MS/MS data). This cannot be used on the current dataset as the MS2 information has been removed in the conversion from RAW to mzData. There is also a method for removing isotopic Peaks *list methods/Isotopes/Isotopic peaks grouper* (not shown).

Online Search

Identification by online searching should be done <u>with caution</u>. We have found that this method often returns many research compounds, drugs and pharmaceuticals which are irrelevant to our plant based studies. For this reason we recommend searching <u>individual peaks</u> using the peak list.

Let's keep things simple and search a single peak:

Right click/Search/Search online database:

	-		1			Lange and		I and a second		-
D.	A	werage	Identity	Comment	Peak shape	MU53_917_06	m2Opt	a MU47_917_1	10 mzData	MUS
-	m/z	Ret time	l.		1 the second	Height	Area -	Height	Area	1. 1
221	104.107	141	1		Show					•
781	148.991	1.52	glubernic acid (MHH)		Search			Search online d	distabase .	
509	150.050	1.44			Export		1	NIST MS Search	ch	
758	147.077	143	glutamina (Mrof)		Identities		1	Predict molecul	lar formula	17
1310	325.114	1.50			Manually de	tensity Plot module free peak				•
230	104.571	1.49	petre (M+H)		Delete selec	ted rows				
864	136,995	1.79	trigonationa (M++1)		Add new rov	v				
492	127.835	1.55			1	. 3 000 2	087			

You are presented with a number of online database options: Let's try a search of KEGG. Set the charge appropriate to the technique (here it is ESI+ so we set +H). Set the m/z tolerance and the isotope pattern filter (as before)

latabase	KEGG Compound Database	T (1990)	Database	KEGG Compound D	atabase
	PubChem Compound Database Human Metabolome Database (HMDB)	-		m/z: 130.050	Charge 1
Neutral mass	Yeast Metabolome Database (YMDB)		Neutral mass	Ionization type:	+Ha * 👻
	METLIN Database	=		Calculated mass:	129.043
Number of results	MassBank Database		Number of results	100	
m/z tolerance	ChemSpider Database PlantCyc Database	-	m/z tolerance	0.0010 m/z or	r 5.0 ppm
Isotope pattern filte	setup.		isotope pattern filte	r 🔽 Setup	
	OK Cancal Hula			OK Cancel	Halp

MZmine will start the search and any hits are displayed in a new window. The isotope pattern and structure may be viewed. If you think the structure is an appropriate match then the identity may be added using the *Add identity* button.





The otheralternative is to search the whole list from the *Peak list methods/Identification/Online* database search menu. WARNING If you do this be prepared for many hours of deleting irrelevant peaks!

[PLEASE DO NOT DO THIS DURING THE DEMO FOR BANDWIDTH REASONS !!]

Gap Filling

We will now try to fill in the gaps where peaks were detected in some scans but not others. There are a number of occasions where a peak may be present but not detected well due to being close to the detection limit in some samples. Gap filling is done by searching the target window where a peak was detected and looking for appropriate peak features in that window. There are two options "Peak Finder" or "Same mz and RT range gap filler". Let's first use the "Peak Finder" option. The gap filled peak list will appear as a new item in the left hand pane.

(±)	Aligned peak	list gap-	filled	-	
6	III			*	
3 28	:07 PM]: Finished	gap-fillin	ig on Aligne	d peak lis	st
		-			
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Ple	and set the purch	incici s			
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lame	suffix	gap-filled			
Name	suffix	gap-filled	%		
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Here is the final gap filled spreadsheet peak list. Filled gaps are shown with a yellow icon. There may still be some gaps in which no evidence for the peak was found.

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USCUS exclusion eteroregies UST230 exclusion eteroregies UST230 exclusion eteroregies	0	A 112	Rathine	Martily +	Connet		Peak shape		ALED BIT	Area	MUH7 917	til euclasu Anna	MUST 917	Al Avea	-
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UT7_35 miClass (hourse)	1.00	387.018	812	unites (Minte)					+ 3,015	1.000	+110	1.000	+ 1088	1.00	
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917_10 ec/Late chromelog	antis.	NUMBER	8.12	antina (Marris)	-				13.800	1100	1.2.20.0	4302	- 1.000	1383	- 2
117_14 regulate strending	294	104.898	8.70				-		41.000	1160	* 140	1.894	+148	3.49	=1
017_31 ectivate movements	-811	1021.041	321	(APP	-	1					1.003	1.102	+ 400	2.87	- 4
1 PT_DL nuDes inverse	adet	101109	1.00	aniset feature		-		1	+ 104	130	+ 1000	1.014	4.004	4 (6)	
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6.817_38.ncfala.chronate	-811	per set	1.00	Autorian (PPH)				1	+245	1.005	+ 1383	1821	+ 2.985	+ 200	+2
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NC1_18 mcDate records	40	110.000	134	Emethylatine MrvE				*	+2.05	2.005	+ 1389	1.000	140	1.000	- 1
R1_20 estinte chromatic	2110	via pri	1.17	Spratta (Srin)		T			× 4.204	210	- 5284	140	+ 100	1104	
UR7_31 milliola chromain		100.000	179	Ingenetive (Aver	-	1			a 1188	1.07	*****	187	+ 4 395	187	
UP7_2K m25da circinatio	Avenue -	275.165	1.79	mperadow (doing					+ 3.665	4.60	+ 1485	3.965	- 1.008	1.80	
all_st.edges.comments =					-	-		1.							

By right clicking on the peak list and *Show/Chromatogram* (*dialog*) and carefully selecting a scan with a detected peak and a scan with a filled peak you can see if the filling has been done in a sensible way. (Right clicking on the chromatogram and selecting *Show /Chromatogram* (*dialog*) brings up all

peaks overlaid but right clicking on an individual peak column and selecting *Show/Chromatogram* (quick) brings up just that peak). In the chromatogram the pink peak is the original and the yellow is the Gap filled by Peak finder. Note: in this case the earlier decision to set the baseline high may be causing a non-optimal integration of larger peaks. Peak picking is always a compromise between detection and accurate peak representation. (From the authors personal experience the XCMS wavelet method seems to be a more robust peak picker in practice).



However the peak finder option can often backfire because it may detect previously removed broad artefact peaks.



This means our carefully removed artefacts are now back with us! An alternative way of looking at this is that we can used this as a way to detect peaks that may be artefacts anyway!

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	****			-		+ 1.000	1.98	6.2.99	1.00	
	(8-11)	10				4-189	4.92	4170	1.01	
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а.	10.14*					4.9365	110	1100	10	
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٠	10.001	**		1		++++++++	144	+144	180	
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18	10.07	94			n	****	1.80	1.0.04	100	
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-	10.00	8.0			-	0.0100	1.00	+1.01	+01	
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-	819	1116			0	4.1.65	1.00	4-1-884	100	
1					100 C					

The other alternative for gap filling is the "Same RT and m/z range gap filler" this limits the gap fill to features within the original detected peak window. This results in much cleaner results.

Peak detectio	n ⊧d_NA	ŝ.	•
Gap filling		Peak finder	
Isotopes		Same RT and m/z	range gap filler
Filtering	P D_NA		
ap-filled SameR1n	nz		
0.0050 m/z	or 5.0	ppm	
0			
	Gap filling Isotopes Filtering eters ap-filled SameRTm 0.0050 m/z	Gap filling Isotopes Filtering to Annual ters ap-filled SameRTmz 0.0050 m/z or 5.0	Gap filling Peak finder Isotopes Same RT and m/z Filtering JANA

	Au	erage		- Announcements			MU47 91	7 10 mzData	MU48 917	39
ID	m/z	Ret time	identity -	Comment		Peak shape	Heig	Area	Height	T
5771	131 190	12								
5772	136.687	11.6					÷ 5.1E4	1.365	5.264	
5772	136.087	11.6					× 5.1E4	9.5E4	6.364	
5774	136 087	11.7				1	a 5.7E4	1 865	<mark>=</mark> 5.064	
5775	136 087	13.0					5.6E4	4.7E5	<mark>-</mark> 5.864	4
577E	136.055	6.9					•			
777	138.055	9.0					< 6.9E3	4.683	= 8.4E3	-
778	139.988	10,1					•		2.165	1
779	141.959	3.7					4.2EA	0.0E4	- 4.1E4	÷
784	146.980	14.0					6.4E4	1.9E5	4.6E4	1
785	147,072	1.4					# 4.7E4	6.584	6.054	
791	158.961	0.3					6 5.5E4	2.565	5.4E4	2
793	159.605	6.5					8 2.963	2.063	-7.4E3	
5795	t77.007	4.8			1		<u>≈</u> 6.9E3	4.0E4	• 1.263	£
179E	154.905	12.2					<mark>≪</mark> 9.8E4	8.7E5	6.464	t
199	154.905	12.4					• 7.184	1.265	3.954	-
900	154.986	12.6					7.754	3.665	4.654	1
108	104 305	13.8					1 7.7E4	3.365	4.554	1

If we look at the same example as above we can now see the detected peak is now cut rather than detected in full. In such cases the hope is that the peak cut-off is applied consistently across all peaks to preserve relative quantitation. Again a compromise is made.



Base peak plot, MS1, m/z: 160.0292 - 160.0298

net.sf.mzmine.modules.peaklistmethods.gapfilling.samerange.SameRangePeak@ff8e75c _ 160.0295 m/z @2.28 [QC1_917_01.mzData] 160.0295 m/z @2.28 [QC1_917_01.mzData] — QC1_917_01.mzData — VVT48_917_40.mzData

Looking again at one of the artefact peaks notice the gap filled peak is now defined by the mz tolerance and RT tolerance. The gap filled is broader than the original peak due to the RT window which is defined by all the peaks in the row. The variation in the retention time across the row is a function of the earlier tolerances used in both RT Normalisation and Join Align. This demonstrates the need to be careful when setting up the parameters from the very beginning.



Despite the limitations of gap filling it is far preferable to have some estimate of baseline levels than to report the value as missing for later statistical analysis.

Export of results



The final exported data is displayed in excel. At this stage it is probable that the data will be processed further in a commercial data analysis package but as we will see in later examples there are some possibilities to use open source tools to analyse metabolomics data.

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Batch analysis

MZmine contains a Batch mode tool which allows a chain of processes to be set up which is a very useful feature with large datasets as the processing can be set to run overnight in unattended operation. The output of the previous operation is fed to the next operation.

Another useful feature is that once the parameters for a particular operation have been set up MZmine remembers the last used settings so we can apply the peak picking we developed above to every sample in a study.

The MZmine Batch command is to be found under the Project menu. The screenshot below shows a typical sequence.

	Load	Save		
	Raw data imp	port	-	Configure
	Chromatogram	m builder		Remove
Batch queue	Chromatogram deconvolution Retention time normalizer		=	Clear
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	Export to C	SV file		Add

A recently added feature is the ability to save or load batch sequences.

As an exercise try adding the steps you went through above to a new batch sequence.

Baseline Correction

Another new feature is baseline correction. This is applied to the raw data before peak detection. It is found under *Raw data methods/Baseline correction*.



The baseline correction dialog box has two main options, the Smoothing and Asymmetry factors. Try playing with different settings of these factors and comparing the TIC plots before and after (NB: Ensure the "Remove source file after baseline correction" is switched OFF

Filename suffix	baseline-corrected	1.107 04.00 04.001 04.001
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OK	Cancel Help	1.00 2.00 3.00 4.00 5.00 5.00 7.00 6.00 5.00 10.00 12.
Towns of the second sec		NOSE INT IR-NOSE NOSE INT AD ADDRESS NOSE INTERNAL

Initial data analysis in MZmine

Limited data analysis tools are included in MZmine. In order to use them it is necessary to 'Set Sample Parameters' under the project menu. You can define as many parameters as you wish.

In the example data we have an excerpt of a metabolomic study on the ripening of fruits. We have nine samples of two different varieties, Wild-type and non-ripening Mutant plus ten control samples which consist of a large batch of identical fruit extract that are run at every fifth sample. In addition the fruit are sampled everyday from the onset of ripening between 47 and 54 days. (This example datasets is only a small excerpt of a larger replicated study).

We now set a new experimental parameter called Type with the values "Wildtype", "Mutant" and "Control"

Second and a second	
Add experimental parameter	
Name Type	
Numerical values	
Set of values	
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MZmine has a number of data analysis options, *Coefficient of variation (CV) analysis, Log Ratio Analysis, Principal Component Analysis, Curvilinear Distance Analysis, Sammon's projection,* and *clustering.* The most useful of these options are described below:

Coefficient of variation analysis	Select files for analysis	
calculates the coefficient of variation of each peak and displays the result as a	MU51_917_45.raw 0C10_917_51.raw MU52_917_21.raw 0C1_917_51.raw MU53_917_06.raw 0C2_917_02.raw MU54_917_26.raw 0C2_917_02.raw MU54_917_26.raw 0C2_917_02.raw	
colour coded plot. <i>(Ensure you have</i> your final peak list highlighted)	WT48_917_40 raw CC_917_19 raw WT48_921_17.raw CC_917_19 raw WT49_917_39 raw CC_917_19 raw WT50_917_32 raw CC_917_30 raw WT51_917_33 raw CC_917_34 raw WT52_917_30 raw CC_917_44 raw WT52_917_30 raw CC_917_44 raw WT52_917_30 raw CC_917_44 raw WT52_917_30 raw CC_917_44 raw	
In this case we have selected the 10 control samples. The graph shows that	WT54_917_29.raw Peak measuring approach height area OK Cancel	Help
some unexpectedly high variation.		





Other features in MZmine

There are many more features in MZmine, including some support for ms/ms data and formula prediction. More features are being added all the time, recent developments in this area include links to the NIST MS Search program to allow the use of MZmine for GC-MS data.

Get Involved !

Please join the community! Not just for programmers - testers and document authors always appreciated!

Developers Mailing List:

http://sourceforge.net/mailarchive/forum.php?forum_name=MZmine-devel

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