DATA ANALYSIS IN MASS SPECTROMETRY BASED METABOLOMICS

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Stanford Mass Spectrometry Users' Meeting

September 26, 2011

Stanford University MASS SPECTROMETRY

Types of Experiments in Metabolomics

targeted		non-targeted
quantitative	semi-quantitative	
• Number of analyzed metabolites is limited by the number of available ***standards***	•Number of analyzed metabolites is limited by the number of available ***library spectra***	•Number of analyzed metabolites is limited by capacity of ***analytical instrumentation***
• ***Absolute quantitation*** of metabolites (nM, mg/mL)	• ***Relative quantitation*** of metabolites (fold)	• ***Relative quantitation*** of metabolites (fold)

Targeted LC/MS metabolomics

- Absolute quantitation of metabolites using triple quadrupole instruments

  SUMS: Quattro Premier (Waters) and TSQ Vantage (Thermo)

- Chemical standards of metabolites are required, internal standards (isotopically labeled metabolites, ^{13}C or ^{2}H) are desired

- Custom methods; amino acid method is available (~30 amino acids)

  SUMS contact: Karolina Krasinska

Untargeted GC/MS based metabolomics

- Agilent 5795 single quadrupole GC/MS
- Metabolomics method based on Kind T. et al 2009, Anal Chem 81(24)
- Identification based on Agilent MSRI library (~700 metabolites, mostly primary metabolism)

For general overview: SUMS 2010 users' meeting workshop presentation

Untargeted GC/MS based metabolomics

- Service began: Summer 2011
- Sample requirements: dry or non-aqueous solvent
- Samples tested: tissue, bacterial cells, urine, plasma
- Metabolites identified: ~30-120
- Metabolites detected: ~200-500
- Data output: AMDIS IDs, non-targeted and pathway analysis is under development

Untargeted LC/MS based metabolomics

Sample prep

Blood Plasma: protein precipitation with 4 v of Acetonitrile, evaporation, reconst in 5 % ACN

LC

Reversed Phase Chromatography

Aqueous Normal Phase Chromatography

MS

Thermo Exactive: +ESI and –ESI, R =50,000
m/z 70-800 (+APCI if necessary)

Data Analysis

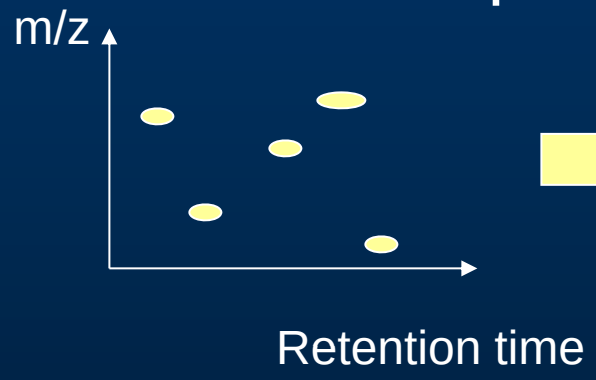
<u>MZmine 2.2</u>
accurate mass database search (HMDB)

Identification

structure elucidation (Orbitrap Velos),
data interpretation (Mass Frontier)

Data Processing Workflow

LC/MS Map



Peak ID (m/z, t_R)	Area
0001	3500
0002	6000
0003	9000
0004	700
0005	1200

	Sample1	Sample2		SampleN
Peak ID	Area	Area		Area
0001	3500	2300		300
0002	6000	7800		5600
0003	9000	5100		9700
0004	700	1300		1200
0005	1200	400		900

Peak annotation and statistical analysis

File Format Conversion

Universal MS data formats:

- netCDF (*.cdf)

  All MS manufacturers provide tools for conversion into netCDF

- XMLs (mzXML, mzML)

  Less common in metabolomics

Data Processing Software

- Proprietary

  MarkerLynx (Waters), SIEVE (Thermo), MarkerView (Sciex), Mass Profiler Professional (Agilent)

- Open Source

  XCMS (http://metlin.scripps.edu/xcms/)

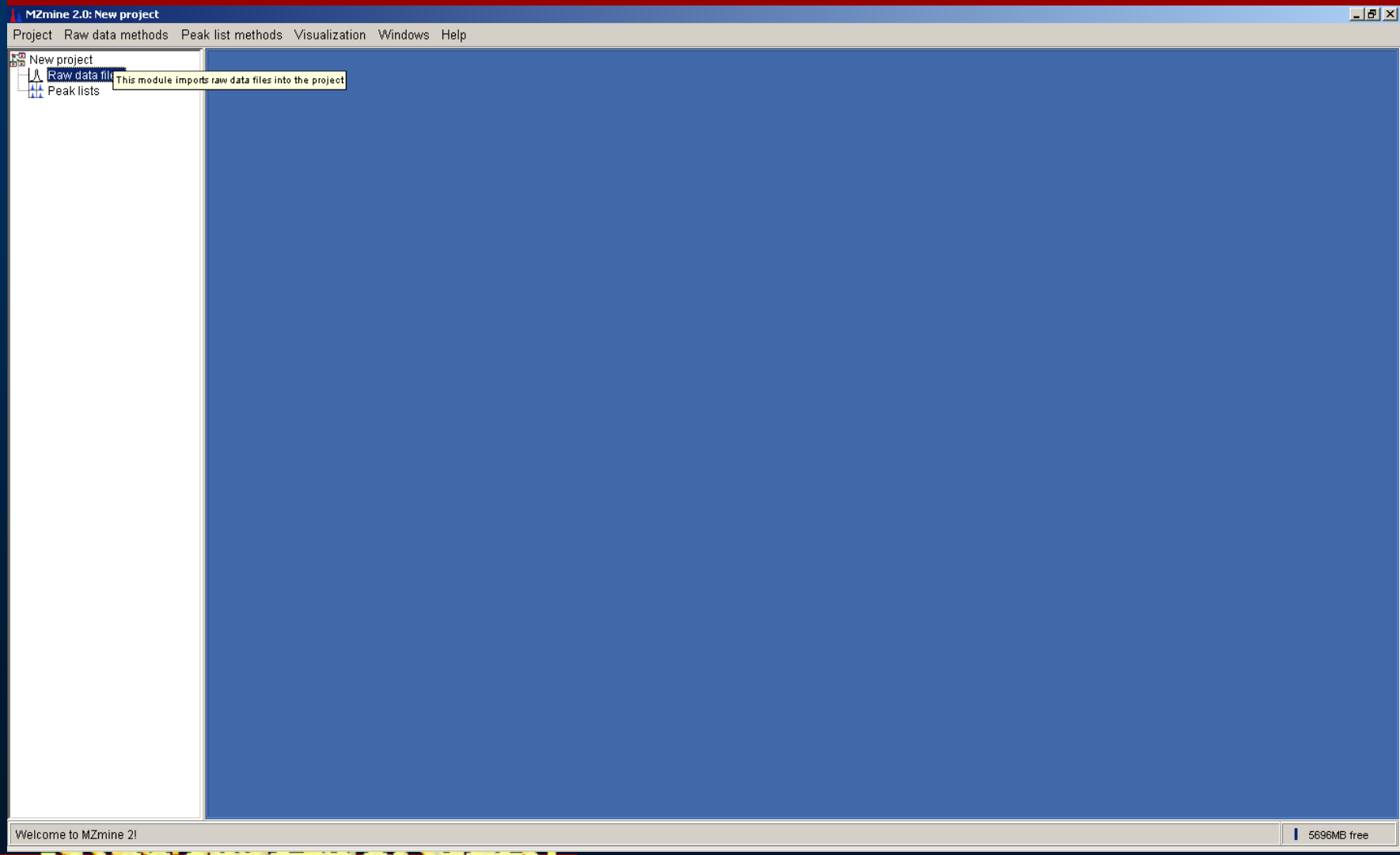
  and

  MZmine (http://mzmine.sourceforge.net/)

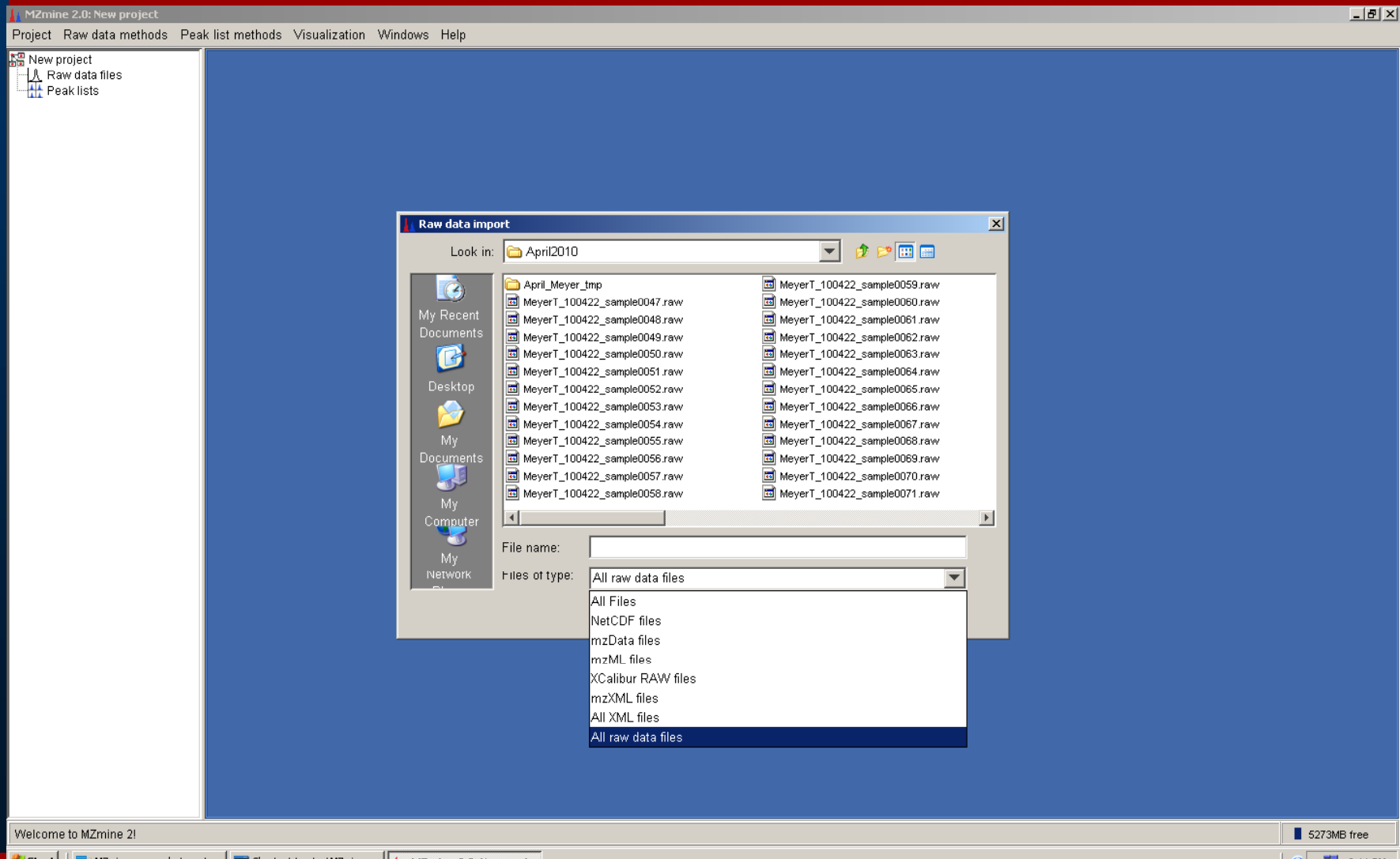
MZmine: Hardware considerations

- Multi-processor systems
- Server CPU are expensive (Xeon, Opteron), Desktop processors are ok (Phenom, Core i7)
- 64-bit OS with Java and CPUs to address more than 4 GB memory
- At least 8 GB memory (high resolution profile data)

MZmine: main window



MZmine: supported file formats



MZmine: use of multiple CPU cores

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

- New project
  - Raw data files
  - Peak lists

Tasks in progress...

Item	Priority	Status	% done
Opening file E:\Aronov\April2010\MeyerT_100422_sample0047.raw	NORMAL	PROCESSING	36%
Opening file E:\Aronov\April2010\MeyerT_100422_sample0048.raw	NORMAL	PROCESSING	36%
Opening file E:\Aronov\April2010\MeyerT_100422_sample0049.raw	NORMAL	PROCESSING	35%
Opening file E:\Aronov\April2010\MeyerT_100422_sample0050.raw	NORMAL	PROCESSING	38%
Opening file E:\Aronov\April2010\MeyerT_100422_sample0051.raw	NORMAL	PROCESSING	37%
Opening file E:\Aronov\April2010\MeyerT_100422_sample0052.raw	NORMAL	PROCESSING	35%

[6:25:38 PM]: Started parsing file E:\Aronov\April2010\MeyerT_100422_sample0050.raw

Start C:\WINDOWS\system32... MZmine 2.0: New proj... Untitled 1 - OpenOffice.... 6:25 PM

MZmine: inspection of raw data

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
MeyerT_100422_sample0052.raw
Peak lists
MeyerT_100422_sample0050.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000
MeyerT_100422_sample0047.raw peak list5000
MeyerT_100422_sample0048.raw peak list5000
MeyerT_100422_sample0051.raw peak list5000
MeyerT_100422_sample0049.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000 resolved
MeyerT_100422_sample0050.raw peak list5000 resolved
MeyerT_100422_sample0047.raw peak list5000 resolved
MeyerT_100422_sample0048.raw peak list5000 resolved
MeyerT_100422_sample0051.raw peak list5000 resolved
MeyerT_100422_sample0049.raw peak list5000 resolved
MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped

Please set parameter values for TIC/XIC visualizer

Raw data files
- [ ] MeyerT_100422_sample0051.raw
- [ ] MeyerT_100422_sample0050.raw
- [x] MeyerT_100422_sample0048.raw
- [ ] MeyerT_100422_sample0047.raw
- [ ] MeyerT_100422_sample0049.raw
- [ ] MeyerT_100422_sample0052.raw

MS level: 1

Plot type: TIC

Retention time: 1:00 - 49:59

m/z range: 212.000 - 213.000 m/z

Selected peaks:

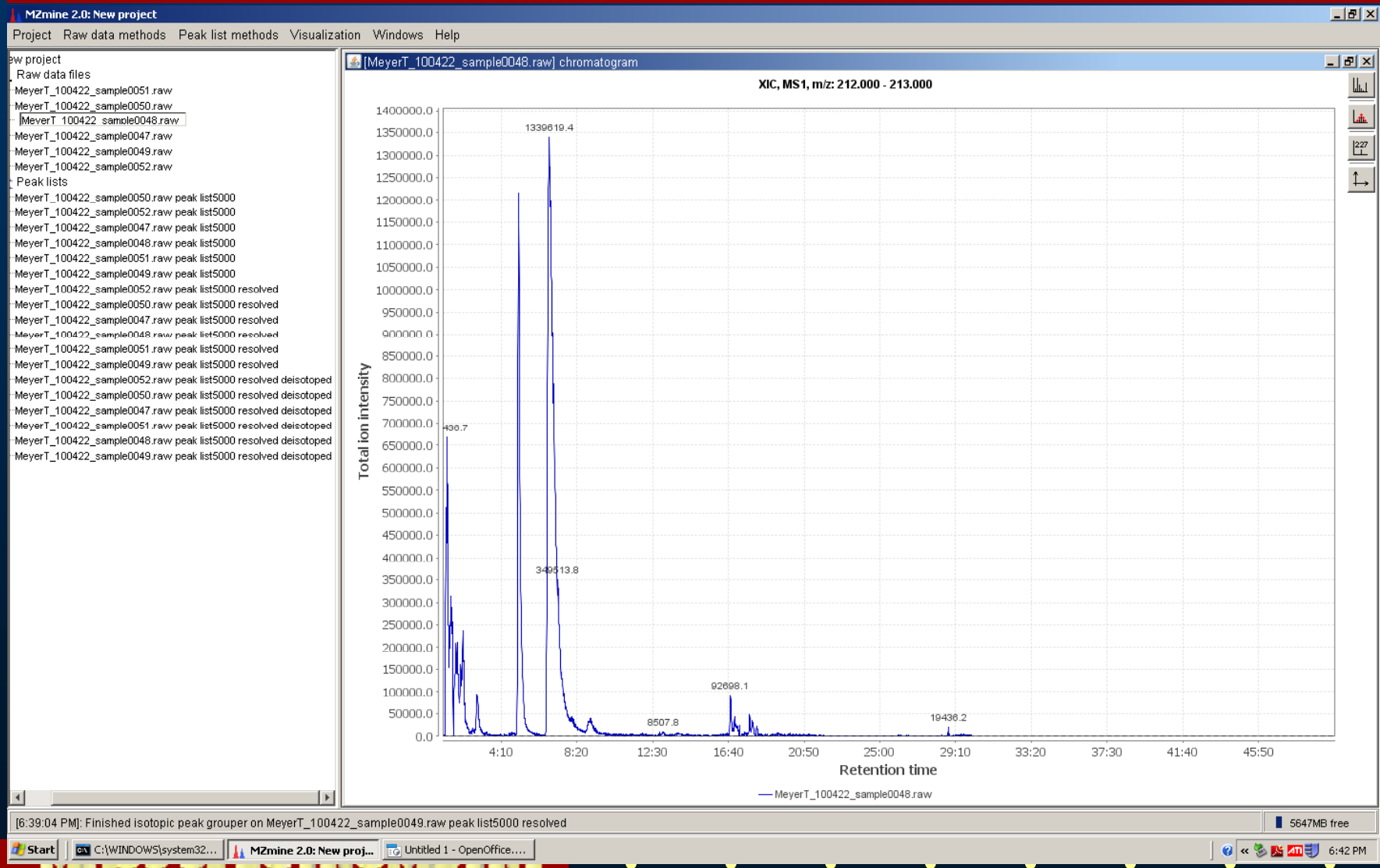
OK Cancel Set automatically Help

[6:39:04 PM]: Finished isotopic peak grouper on MeyerT_100422_sample0049.raw peak list5000 resolved

5350MB free

Start C:\WINDOWS\system32... MZmine 2.0: New proj... Untitled 1 - OpenOffice.... 6:41 PM

MZmine: raw data SIC



MZmine: peak picking

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

- New project
  - Raw data files
    - MeyerT_100422_sample0051.raw
    - MeyerT_100422_sample0050.raw
    - MeyerT_100422_sample0048.raw
    - MeyerT_100422_sample0047.raw
    - MeyerT_100422_sample0049.raw
    - MeyerT_100422_sample0052.raw
  - Peak lists

Please set parameter values for Chromatogram builder

Filename suffix	peak list5000	
Mass detection	Exact mass	Set parameters
Filtering	FTML shoulder peaks filter	Set parameters
Chromatogram construction	Highest data point	Set parameters

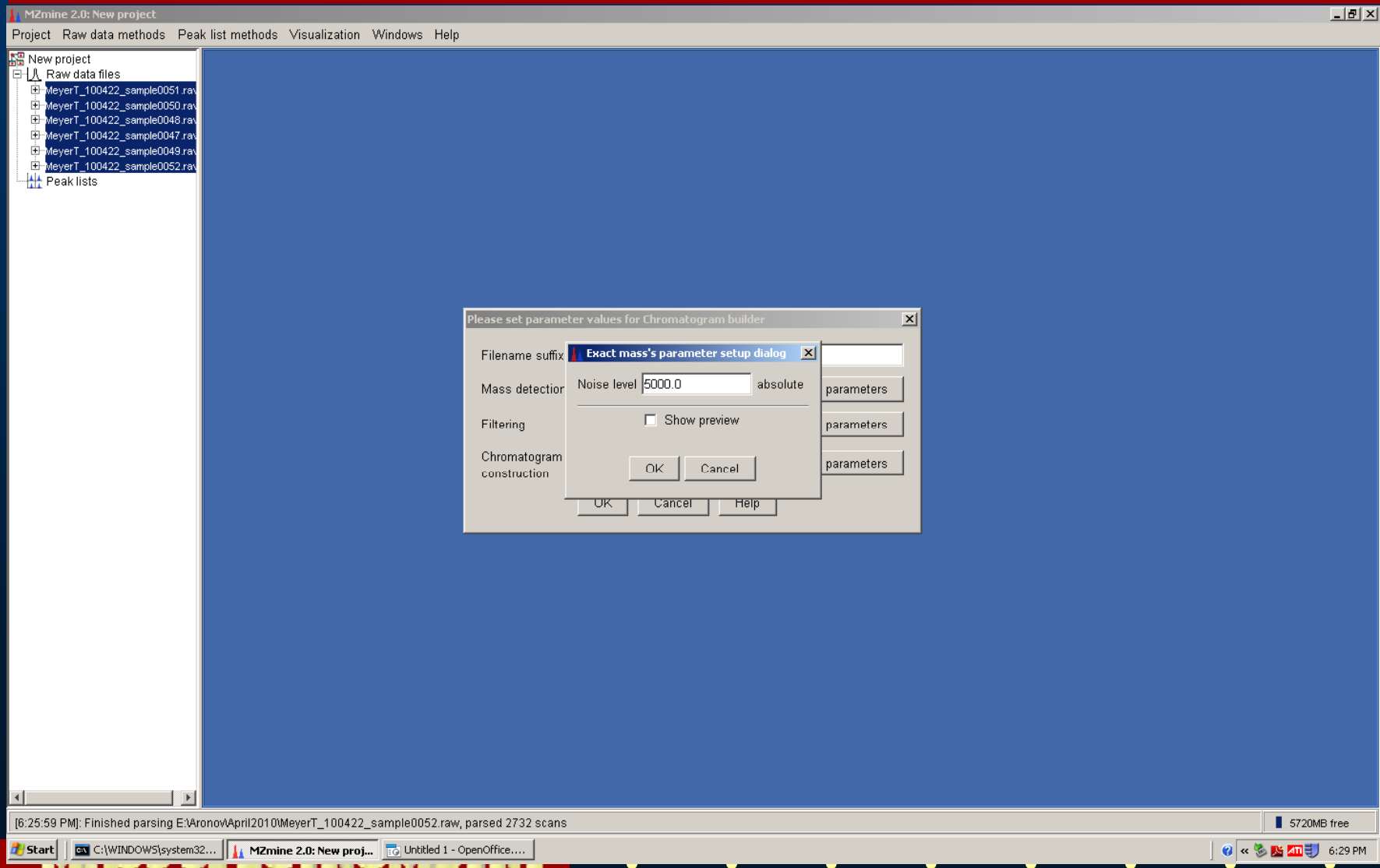
OK Cancel Help

[6:25:59 PM]: Finished parsing E:\Aronov\April2010\MeyerT_100422_sample0052.raw, parsed 2732 scans

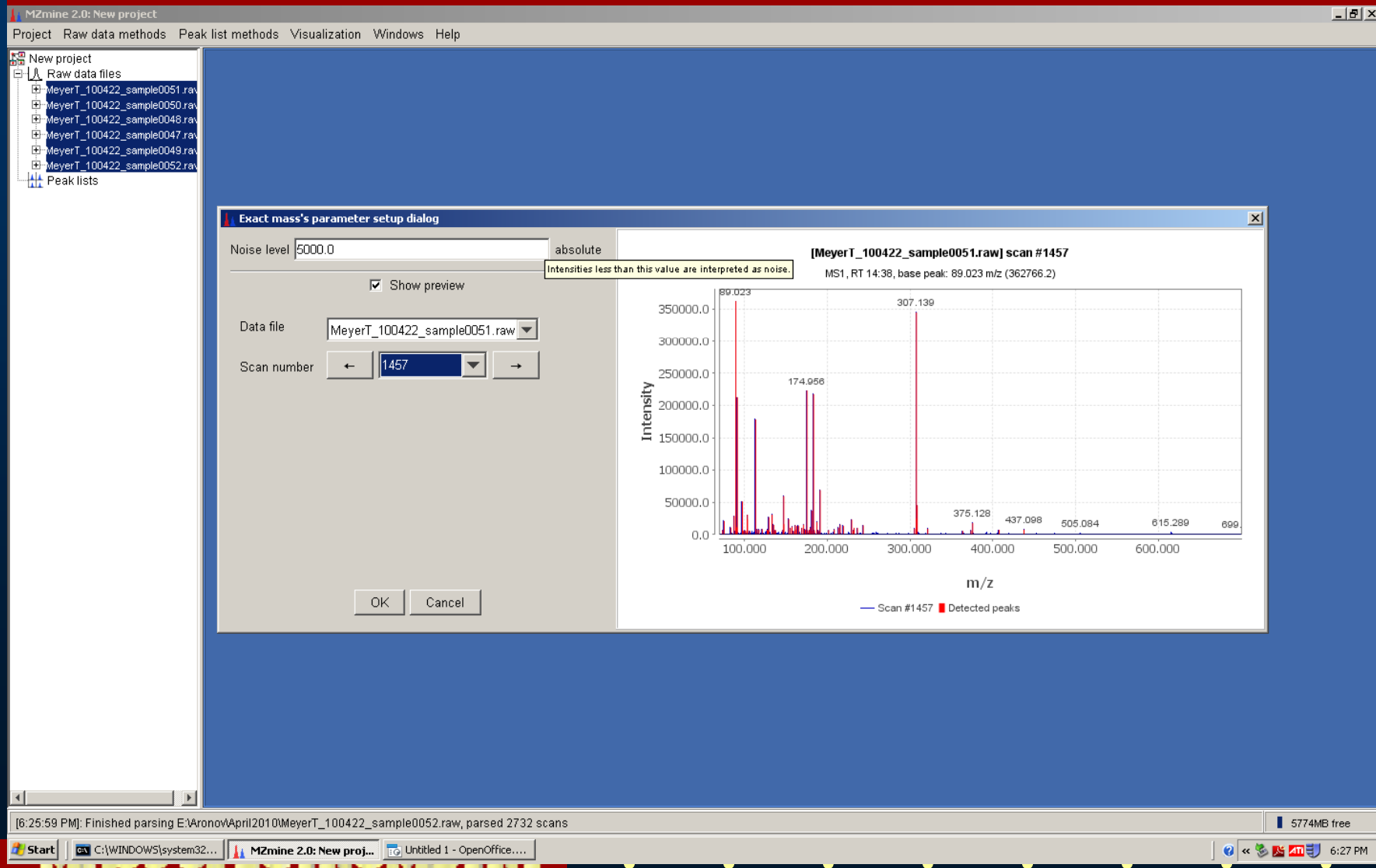
5839MB free

Start C:\WINDOWS\system32... MZmine 2.0: New proj... Untitled 1 - OpenOffice.... 6:26 PM

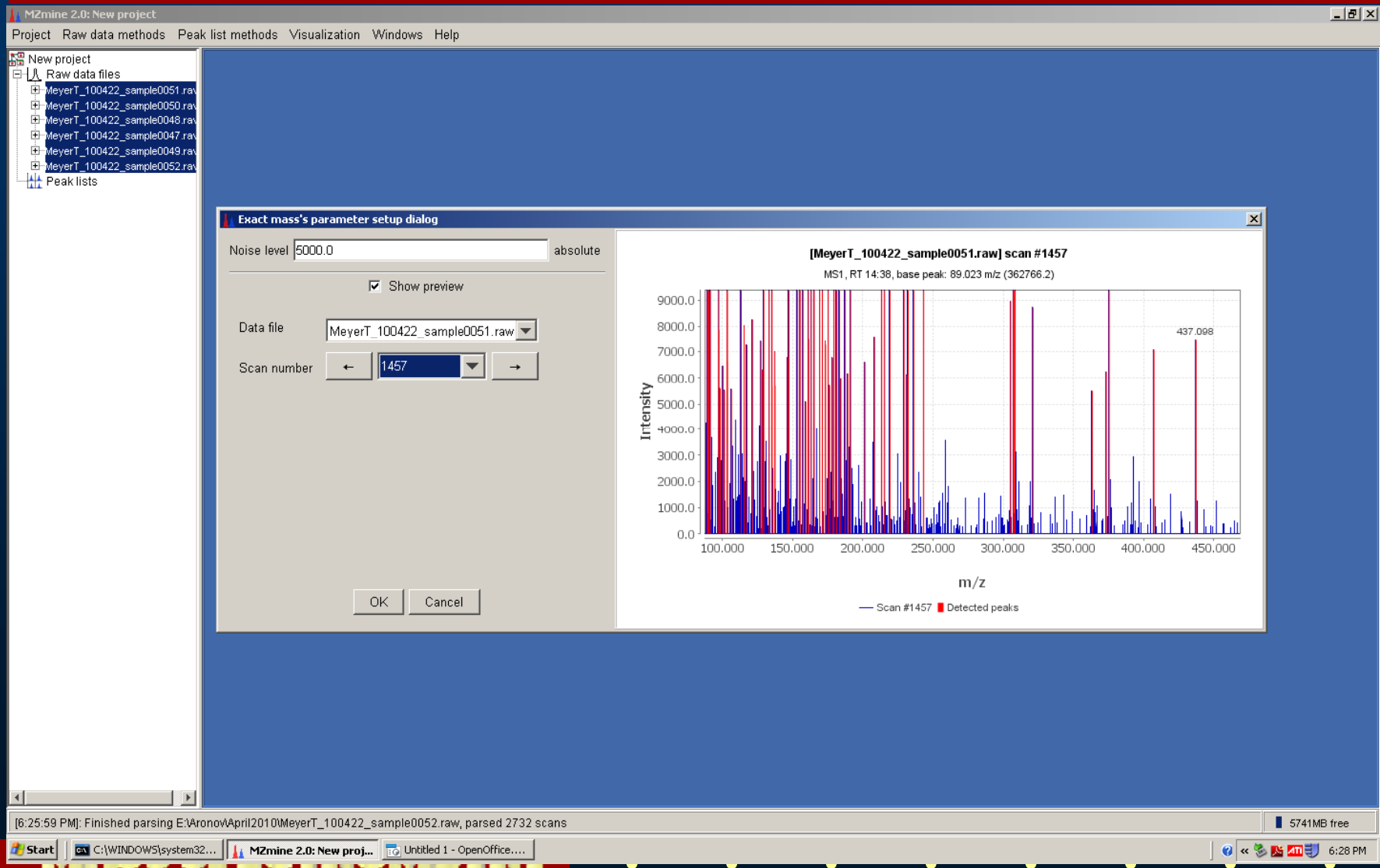
MZmine: preview of parameters



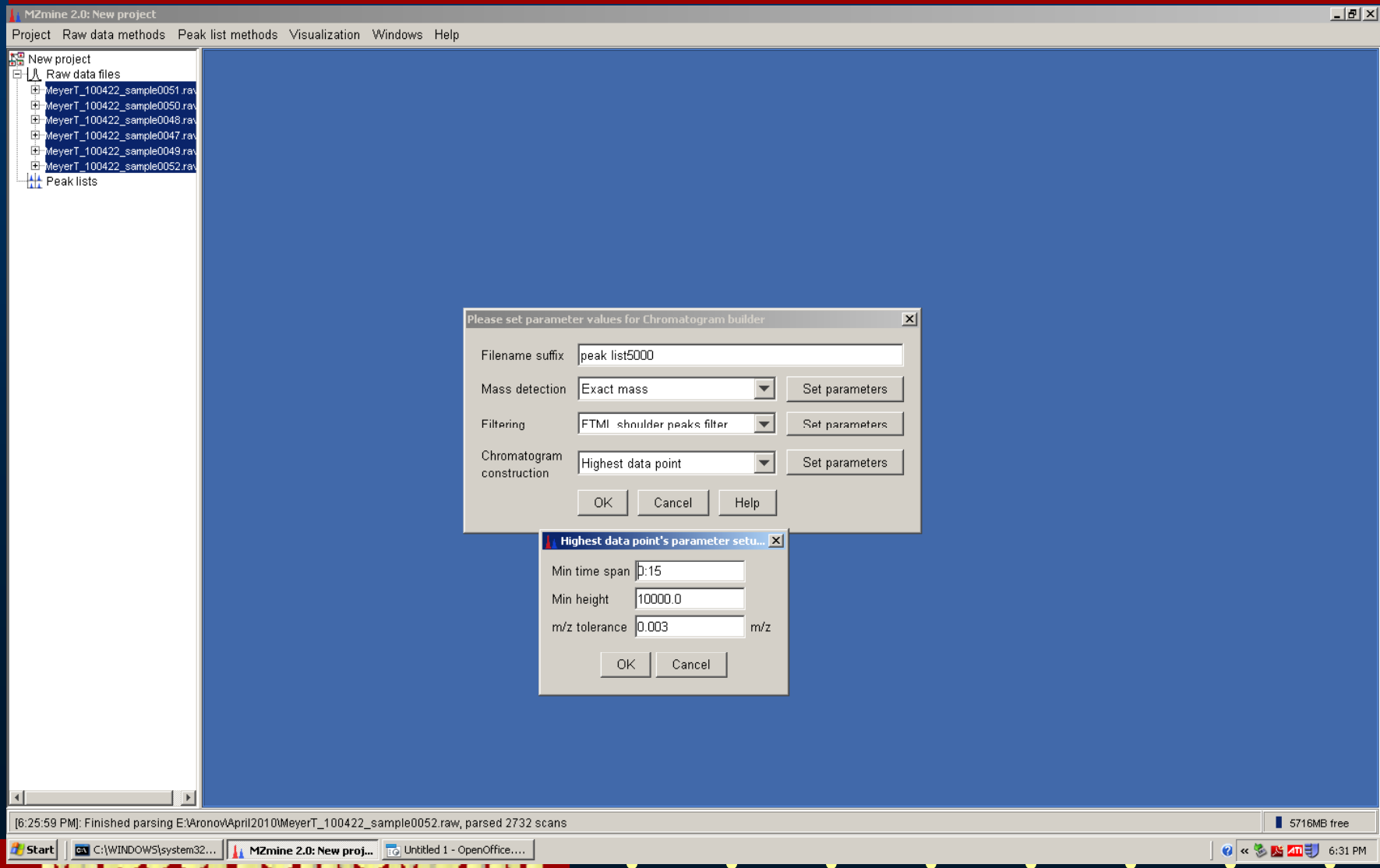
MZmine: estimating the noise level



MZmine: zooming into noise



MZmine: chromatogram construction



MZmine: peak picking results

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

- New project
  - Raw data files
    - MeyerT_100422_sample0051.raw
    - MeyerT_100422_sample0050.raw
    - MeyerT_100422_sample0048.raw
    - MeyerT_100422_sample0047.raw
    - MeyerT_100422_sample0049.raw
    - MeyerT_100422_sample0052.raw
  - Peak lists
    - MeyerT_100422_sample0050.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000
    - MeyerT_100422_sample0047.raw peak list5000
    - MeyerT_100422_sample0048.raw peak list5000
    - MeyerT_100422_sample0051.raw peak list5000
    - MeyerT_100422_sample0049.raw peak list5000

MeyerT_100422_sample0048.raw peak list5000

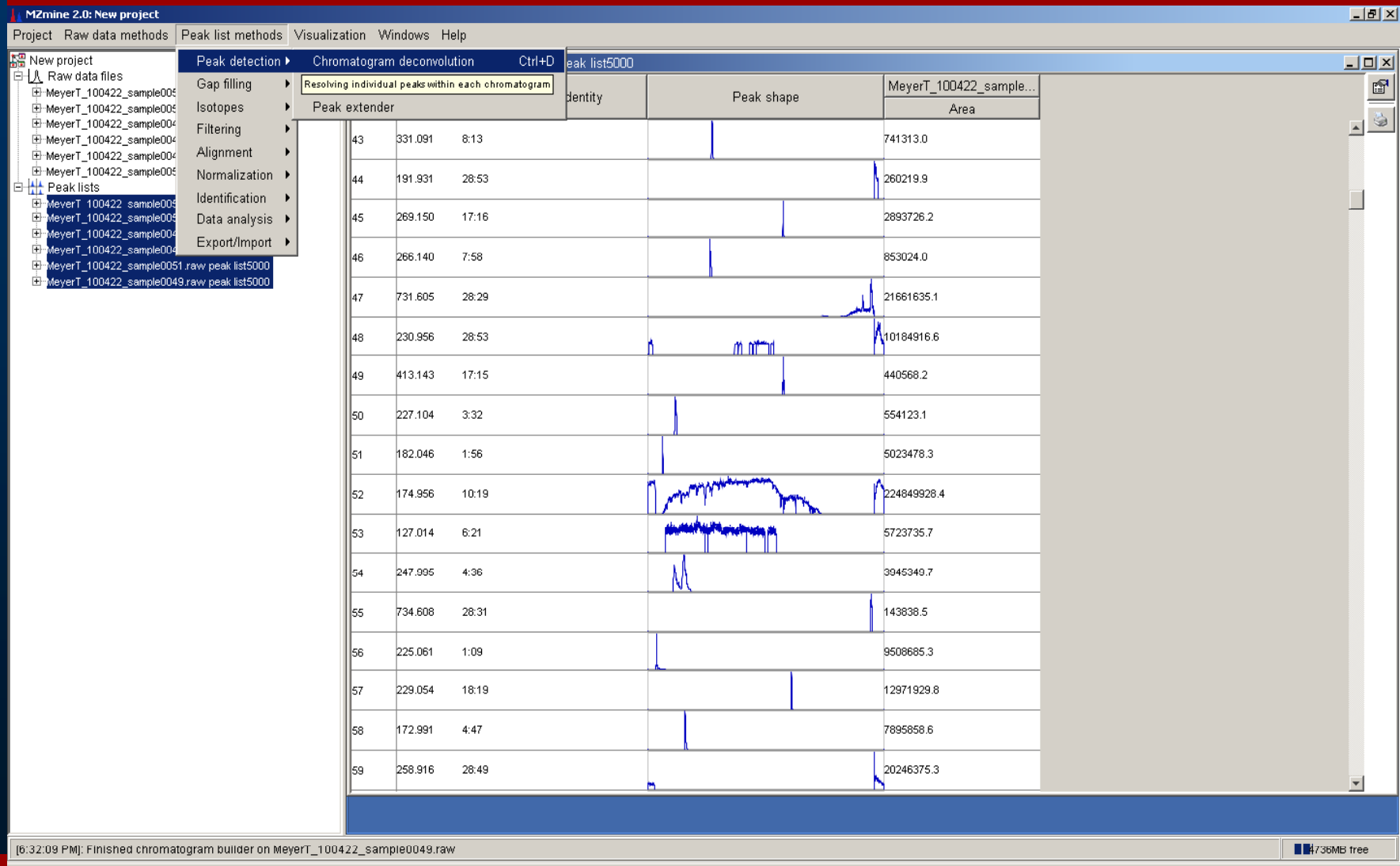
ID	Average		Identity	Peak shape	MeyerT_100422_sample...
	m/z	Ret.time			Area
43	331.091	8:13			741313.0
44	191.931	28:53			260219.9
45	269.150	17:16			2893726.2
46	266.140	7:58			853024.0
47	731.605	28:29			21661635.1
48	230.956	28:53			10184916.6
49	413.143	17:15			440568.2
50	227.104	3:32			554123.1
51	182.046	1:56			5023478.3
52	174.956	10:19			224849928.4
53	127.014	6:21			5723735.7
54	247.995	4:36			3945349.7
55	734.608	28:31			143838.5
56	225.061	1:09			9508685.3
57	229.054	18:19			12971929.8
58	172.991	4:47			7895858.6
59	258.916	28:49			20246375.3

[6:32:09 PM]: Finished chromatogram builder on MeyerT_100422_sample0049.raw

4738MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:32 PM

MZmine: deconvolution



MZmine: deconvolution parameters

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

- New project
  - Raw data files
    - MeyerT_100422_sample0051.raw
    - MeyerT_100422_sample0050.raw
    - MeyerT_100422_sample0048.raw
    - MeyerT_100422_sample0047.raw
    - MeyerT_100422_sample0049.raw
    - MeyerT_100422_sample0052.raw
  - Peak lists
    - MeyerT_100422_sample0050.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000
    - MeyerT_100422_sample0047.raw peak list5000
    - MeyerT_100422_sample0048.raw peak list5000
    - MeyerT_100422_sample0051.raw peak list5000
    - MeyerT_100422_sample0049.raw peak list5000

MeyerT_100422_sample0048.raw peak list5000

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area
43	331.091	8:13			741313.0
44	191.931	28:53			260219.9
45	269.150	17:16			2893726.2
46	266.140	7:58			853024.0
47	731.605	28:29			21661635.1
48	230.956	28:53			10184916.6
49	413.143				
50	227.104				
51	182.046				
52	174.956				
53	127.014	6:21			5723735.7
54	247.995	4:36			3945349.7
55	734.608	28:31			143838.5
56	225.061	1:09			9508685.3
57	229.054	18:19			12971929.8
58	172.991	4:47			7895858.6
59	258.916	28:49			20246375.3

Please select peak resolver

Suffix: resolved

Peak recognition: Local minimum search — Set parameters

Remove original peak list ☐

OK Cancel Help

Local minimum search's parameter setup dialog

Chromatographic threshold	85% %
Search minimum in RT range	0:20
Minimum relative height	5%
Minimum absolute height	10000.0
Min ratio of peak top/edge	2

☐ Show preview of peak building

OK Cancel

[6:32:09 PM]: Finished chromatogram builder on MeyerT_100422_sample0049.raw

716MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:34 PM

MZmine: deconvolution results

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

- New project
  - Raw data files
    - MeyerT_100422_sample0051.raw
    - MeyerT_100422_sample0050.raw
    - MeyerT_100422_sample0048.raw
    - MeyerT_100422_sample0047.raw
    - MeyerT_100422_sample0049.raw
    - MeyerT_100422_sample0052.raw
  - Peak lists
    - MeyerT_100422_sample0050.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000
    - MeyerT_100422_sample0047.raw peak list5000
    - MeyerT_100422_sample0048.raw peak list5000
    - MeyerT_100422_sample0051.raw peak list5000
    - MeyerT_100422_sample0049.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000 resolved
    - MeyerT_100422_sample0050.raw peak list5000 resolved
    - MeyerT_100422_sample0047.raw peak list5000 resolved
    - MeyerT_100422_sample0048.raw peak list5000 resolved
    - MeyerT_100422_sample0051.raw peak list5000 resolved
    - MeyerT_100422_sample0049.raw peak list5000 resolved

MeyerT_100422_sample0048.raw peak list5000 resolved

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area
598	211.098	17:50			1037100.8
84	212.002	5:07			2935889.4
85	212.002	6:49			9822647.9
281	212.020	2:04			394124.2
289	212.839	1:12			1764625.7
290	212.839	1:40			169158.9
237	212.877	1:44			548883.5
19	213.005	6:49			679269.3
416	213.017	1:11			413916.6
31	213.023	17:50			2036443.6
595	213.077	14:54			289948.9
594	213.077	14:11			396467.4
593	213.077	12:21			213881.6
592	213.077	8:02			285060.4
555	213.113	18:02			2934530.7
83	213.998	6:47			289841.7
412	214.874	1:40			291352.8
266	215.033	1:11			89719184.9

[6:34:40 PM]: Finished peak recognition on MeyerT_100422_sample0049.raw peak list5000

27MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:37 PM

MZmine: deisotoping

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- New project
  - Raw data files
    - MeyerT_100422_sample005
    - MeyerT_100422_sample005
    - MeyerT_100422_sample004
    - MeyerT_100422_sample004
    - MeyerT_100422_sample004
    - MeyerT_100422_sample005
  - Peak lists
    - MeyerT_100422_sample005
    - MeyerT_100422_sample005
    - MeyerT_100422_sample004
    - MeyerT_100422_sample004
    - MeyerT_100422_sample0051.raw peak list5000
    - MeyerT_100422_sample0049.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000 resolved
    - MeyerT_100422_sample0050.raw peak list5000 resolved
    - MeyerT_100422_sample0047.raw peak list5000 resolved
    - MeyerT_100422_sample0048.raw peak list5000 resolved
    - MeyerT_100422_sample0051.raw peak list5000 resolved
    - MeyerT_100422_sample0049.raw peak list5000 resolved

Peak list methods:
- Peak detection
- Gap filling
- Isotopes → Isotopic peaks grouper
- Filtering
- Alignment
- Normalization
- Identification
- Data analysis
- Export/Import

Grouping of isotopic peaks into one representative peak

MeyerT_100422_sample0048.raw peak list5000 resolved

	Average		Identity	Peak shape	MeyerT_100422_sample...
		Ret.time			Area
598	211.098	17:50			1037100.8
84	212.002	5:07			2935889.4
85	212.002	6:49			9822647.9
281	212.020	2:04			394124.2
289	212.839	1:12			1764625.7
290	212.839	1:40			169158.9
237	212.877	1:44			548883.5
19	213.005	6:49			679269.3
416	213.017	1:11			413916.6
31	213.023	17:50			2036443.6
595	213.077	14:54			289948.9
594	213.077	14:11			396467.4
593	213.077	12:21			213881.6
592	213.077	8:02			285060.4
555	213.113	18:02			2934530.7
83	213.998	6:47			289841.7
412	214.874	1:40			291352.8
266	215.033	1:11			89719184.9

[6:34:40 PM]: Finished peak recognition on MeyerT_100422_sample0049.raw peak list5000

22MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:37 PM

MZmine: deisotoping parameters

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

- New project
  - Raw data files
    - MeyerT_100422_sample0051.raw
    - MeyerT_100422_sample0050.raw
    - MeyerT_100422_sample0048.raw
    - MeyerT_100422_sample0047.raw
    - MeyerT_100422_sample0049.raw
    - MeyerT_100422_sample0052.raw
  - Peak lists
    - MeyerT_100422_sample0050.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000
    - MeyerT_100422_sample0047.raw peak list5000
    - MeyerT_100422_sample0048.raw peak list5000
    - MeyerT_100422_sample0051.raw peak list5000
    - MeyerT_100422_sample0049.raw peak list5000
    - MeyerT_100422_sample0052.raw peak list5000 resolved
    - MeyerT_100422_sample0050.raw peak list5000 resolved
    - MeyerT_100422_sample0047.raw peak list5000 resolved
    - MeyerT_100422_sample0048.raw peak list5000 resolved
    - MeyerT_100422_sample0051.raw peak list5000 resolved
    - MeyerT_100422_sample0049.raw peak list5000 resolved

MeyerT_100422_sample0048.raw peak list5000 resolved

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area
598	211.098	17:50			1037100.8
84	212.002	5:07			2935889.4
85	212.002	6:49			9822647.9
281	212.020	2:04			394124.2
289	212.839	1:12			1764625.7
290	212.839	1:40			169158.9
237	212.877	1:44			548883.5
19	213.005	6:49			679269.3
416	213.017	1:11			413916.6
31	213.023	17:50			2036443.6
595	213.077	14:54			289948.9
594	213.077	14:11			396467.4
593	213.077	12:21			213881.6
592	213.077	8:02			285060.4
555	213.113	18:02			2934530.7
83	213.998	6:47			289841.7
412	214.874	1:40			291352.8
266	215.033	1:11			89719184.9

Please set parameter values for Isotopic peak...

Name suffix	deisotoped	
m/z tolerance	0.030	m/z
RT tolerance	0:10	
Monotonic shape	☐	
Maximum charge	3	
Remove original peaklist	☐	

OK Cancel Help

[6:34:40 PM]: Finished peak recognition on MeyerT_100422_sample0049.raw peak list5000

Start C:\WINDOWS\system32... MZmine 2.0: New proj... Untitled 1 - OpenOffice.... 6:38 PM

MZmine: deisotoping results

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

ew project
- Raw data files
  - MeyerT_100422_sample0051.raw
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0049.raw
  - MeyerT_100422_sample0052.raw
- Peak lists
  - MeyerT_100422_sample0050.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000
  - MeyerT_100422_sample0047.raw peak list5000
  - MeyerT_100422_sample0048.raw peak list5000
  - MeyerT_100422_sample0051.raw peak list5000
  - MeyerT_100422_sample0049.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000 resolved
  - MeyerT_100422_sample0050.raw peak list5000 resolved
  - MeyerT_100422_sample0047.raw peak list5000 resolved
  - MeyerT_100422_sample0048.raw peak list5000 resolved
  - MeyerT_100422_sample0051.raw peak list5000 resolved
  - MeyerT_100422_sample0049.raw peak list5000 resolved
  - MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped

MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area
85	212.002	6:49			9822647.9
281	212.020	2:04			394124.2
289	212.839	1:12			1764625.7
290	212.839	1:40			169158.9
237	212.877	1:44			548883.5
416	213.017	1:11			413916.6
31	213.023	17:50			2036443.6
595	213.077	14:54			289948.9
594	213.077	14:11			396467.4
593	213.077	12:21			213881.6
592	213.077	8:02			285060.4
555	213.113	18:02			2934530.7
412	214.874	1:40			291352.8
266	215.033	1:11			89719184.9
189	215.092	14:35			487504.0
188	215.092	10:19			164853.7
190	215.092	16:23			649105.6
349	215.129	18:27			1047291.8

[6:39:04 PM]: Finished isotopic peak grouper on MeyerT_100422_sample0049.raw peak list5000 resolved

41MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:39 PM

MZmine: alignment

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- Peak detection
- Gap filling
- Isotopes
- Filtering
- Alignment
  - Join aligner
  - RANSAC aligner
- Normalization
- Identification
- Data analysis
- Export/Import

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
MeyerT_100422_sample0052.raw
Peak lists
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0052.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0051.raw peak list5000
MeyerT_100422_sample0049.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000 resolved
MeyerT_100422_sample0050.raw peak list5000 resolved
MeyerT_100422_sample0047.raw peak list5000 resolved
MeyerT_100422_sample0048.raw peak list5000 resolved
MeyerT_100422_sample0051.raw peak list5000 resolved
MeyerT_100422_sample0049.raw peak list5000 resolved
MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped

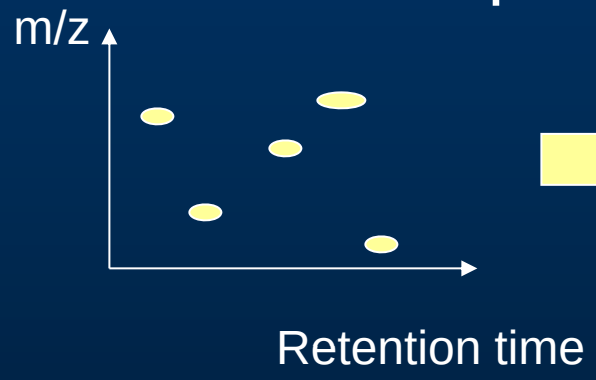
[6:39:04 PM]: Finished isotopic peak grouper on MeyerT_100422_sample0049.raw peak list5000 resolved

5642MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:43 PM

Data Processing Workflow

LC/MS Map



Peak ID (m/z, t_R)	Area
0001	3500
0002	6000
0003	9000
0004	700
0005	1200

	Sample1	Sample2		SampleN
Peak ID	Area	Area		Area
0001	3500	2300		300
0002	6000	7800		5600
0003	9000	5100		9700
0004	700	1300		1200
0005	1200	400		900

Peak annotation and statistical analysis

MZmine: alignment parameters

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
MeyerT_100422_sample0052.raw
Peak lists
MeyerT_100422_sample0050.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000
MeyerT_100422_sample0047.raw peak list5000
MeyerT_100422_sample0048.raw peak list5000
MeyerT_100422_sample0051.raw peak list5000
MeyerT_100422_sample0049.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000 resolved
MeyerT_100422_sample0050.raw peak list5000 resolved
MeyerT_100422_sample0047.raw peak list5000 resolved
MeyerT_100422_sample0048.raw peak list5000 resolved
MeyerT_100422_sample0051.raw peak list5000 resolved
MeyerT_100422_sample0049.raw peak list5000 resolved
MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped

Please set parameter values for Join aligner

Peak list name	test10000-30sec	
m/z tolerance	0.003	m/z
Weight for m/z	10	
Retention time tolerance type	Absolute	
Absolute RT tolerance	0:30	
Relative RT tolerance	15%	%
Weight for RT	10	
Require same charge state	☐	
Require same ID	☐	
Compare isotope pattern	☐	
Isotope pattern score thresold level	65%	%

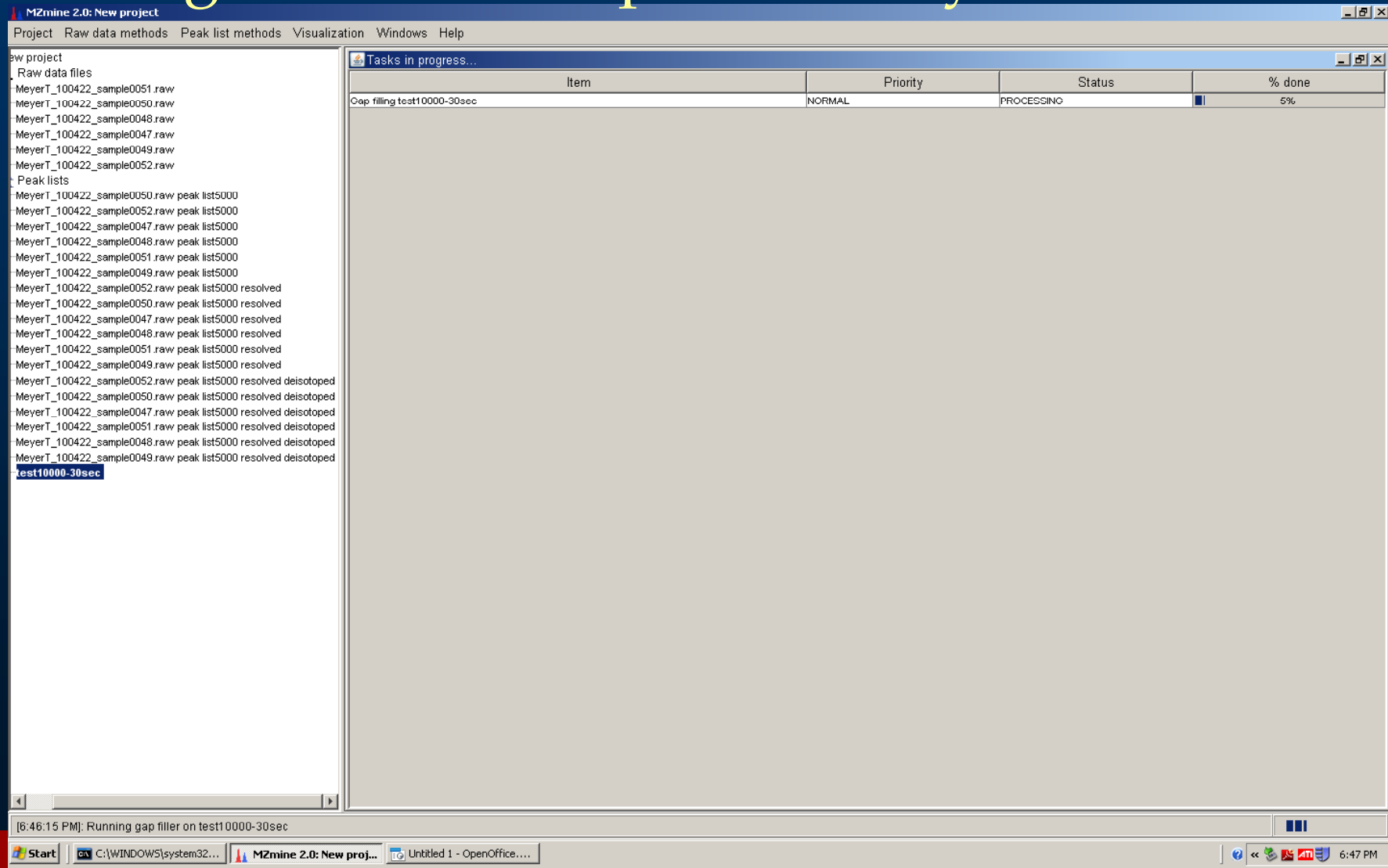
OK Cancel Help

[6:39:04 PM]: Finished isotopic peak grouper on MeyerT_100422_sample0049.raw peak list5000 resolved

5640MB free

Start C:\WINDOWS\system32... MZmine 2.0: New proj... Untitled 1 - OpenOffice.... 6:44 PM

MZmine: alignment is computationally intense



MZmine: alignment results

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

ew project
- Raw data files
  - MeyerT_100422_sample0051.raw
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0049.raw
  - MeyerT_100422_sample0052.raw
- Peak lists
  - MeyerT_100422_sample0050.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000
  - MeyerT_100422_sample0047.raw peak list5000
  - MeyerT_100422_sample0048.raw peak list5000
  - MeyerT_100422_sample0051.raw peak list5000
  - MeyerT_100422_sample0049.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000 resolved
  - MeyerT_100422_sample0050.raw peak list5000 resolved
  - MeyerT_100422_sample0047.raw peak list5000 resolved
  - MeyerT_100422_sample0048.raw peak list5000 resolved
  - MeyerT_100422_sample0051.raw peak list5000 resolved
  - MeyerT_100422_sample0049.raw peak list5000 resolved
  - MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
  - **test10000-30sec**
  - **test10000-30sec gap-filled**
  - **test10000-30sec gap-filled Duplicate peaks filtered**

test10000-30sec

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
413	209.067	1:12				857620.9	
1086	210.511	6:18					
227	210.842	1:39			761332.6	520060.1	479039.3
76	210.842	1:09			5629438.1	4687256.3	4705571.9
675	211.061	16:19					
876	211.098	16:59					
478	211.098	17:52				208353.7	387628.7
499	212.002	6:25					133535483.4
253	212.002	6:54			1103352.8	8909034.6	
731	212.002	5:07					
744	212.002	8:38					
613	212.020	2:50					318786.5
556	212.020	2:07					570211.5
316	212.839	1:39			330264.4	209649.0	189298.2
111	212.839	1:10			2599460.9	2158614.0	2160293.9
250	212.877	1:43			692756.4	586464.8	548998.4
144	213.017	1:11			986734.3	738830.6	
74	213.023	17:45			2296385.7		

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

1MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:51 PM

MZmine: gap filling

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- Peak detection
- Gap filling
  - Peak finder
  - Same RT and m/z range filler
- Isotopes
- Filtering
- Alignment
- Normalization
- Identification
- Data analysis
- Export/Import

ew project
- Raw data files
  - MeyerT_100422_sample0051.raw
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0049.raw
  - MeyerT_100422_sample0052.raw
- Peak lists
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0052.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0051.raw peak list5000
  - MeyerT_100422_sample0049.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000 resolved
  - MeyerT_100422_sample0050.raw peak list5000 resolved
  - MeyerT_100422_sample0047.raw peak list5000 resolved
  - MeyerT_100422_sample0048.raw peak list5000 resolved
  - MeyerT_100422_sample0051.raw peak list5000 resolved
  - MeyerT_100422_sample0049.raw peak list5000 resolved
  - MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
  - **test10000-30sec**
  - **test10000-30sec gap-filled**
  - **test10000-30sec gap-filled Duplicate peaks filtered**

test10000-30sec gap-filled

		time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
76	210.842	1:09			5629438.1	4687256.3	4705571.9
675	211.061	16:42			91418.3	244881.8	521914.3
478	211.098	17:53			157953.5	208353.7	387628.7
876	211.098	17:06			46422.0	60684.0	88497.7
744	212.002	7:52			5484.2	959214.9	8269194.9
253	212.002	6:39			1103352.8	8909034.6	90583223.2
499	212.002	6:39			1007087.4	8740976.3	133535483.4
731	212.002	5:07			696.2	13770.3	1259124.3
556	212.020	2:08			21594.0	10079.5	570211.5
613	212.020	2:15			11784.9	10079.5	318786.5
111	212.839	1:10			2599460.9	2158614.0	2160293.9
316	212.839	1:34			330264.4	209649.0	189298.2
250	212.877	1:43			692756.4	586464.8	548998.4
144	213.017	1:11			986734.3	738830.6	371572.9
74	213.023	17:44			2296385.7	451149.7	153632.8
907	213.077	7:40			251.4	39032.6	20693.7
596	213.077	14:54			6324.0	68865.7	504302.6
917	213.077	14:46			8259.7	68865.7	519639.5

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:53 PM

MZmine: removal of duplicates

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- Peak detection
- Gap filling
- Isotopes
- Filtering
  - Duplicate peak filter
  - Peak list rows filter
- Alignment
- Normalization
- Identification
- Data analysis
- Export/Import

ew project
- Raw data files
  - MeyerT_100422_sample0051.raw
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0049.raw
  - MeyerT_100422_sample0052.raw
- Peak lists
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0052.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0051.raw peak list5000
  - MeyerT_100422_sample0049.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000 resolved
  - MeyerT_100422_sample0050.raw peak list5000 resolved
  - MeyerT_100422_sample0047.raw peak list5000 resolved
  - MeyerT_100422_sample0048.raw peak list5000 resolved
  - MeyerT_100422_sample0051.raw peak list5000 resolved
  - MeyerT_100422_sample0049.raw peak list5000 resolved
  - MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
  - **test10000-30sec**
  - **test10000-30sec gap-filled**
  - **test10000-30sec gap-filled Duplicate peaks filtered**

test10000-30sec gap-filled Duplicate peaks filtered

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
		16:42			91418.3	244881.8	521914.3
478	211.098	17:53			157953.5	208353.7	387628.7
876	211.098	17:06			146422.0	60684.0	88497.7
744	212.002	7:52			5484.2	959214.9	8269194.9
499	212.002	6:39			1007087.4	8740976.3	133535483.4
731	212.002	5:07			696.2	13770.3	1259124.3
556	212.020	2:08			21594.0	10079.5	570211.5
111	212.839	1:10			2599460.9	2158614.0	2160293.9
250	212.877	1:43			692756.4	586464.8	548998.4
144	213.017	1:11			986734.3	738830.6	371572.9
74	213.023	17:44			2296385.7	451149.7	153632.8
907	213.077	7:40			251.4	39032.6	20693.7
917	213.077	14:46			8259.7	68865.7	519639.5
945	213.077	12:07			1125.9	30266.0	39073.6
212	213.113	18:02			503510.6	1162807.6	1918710.3
620	214.005	6:38			401.1	10621.0	693328.5
598	214.837	1:10			327047.0	202366.4	430162.6
303	214.874	1:41			410872.2	347242.2	290299.1

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:54 PM

MZmine: normalization options

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- Peak detection
- Gap filling
- Isotopes
- Filtering
- Alignment
- Normalization
  - Retention time normalizer
  - Linear normalizer
  - Standard compound normalizer
- Identification
- Data analysis
- Export/Import

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
MeyerT_100422_sample0052.raw
Peak lists
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0052.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0051.raw peak list5000
MeyerT_100422_sample0049.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000 resolved
MeyerT_100422_sample0050.raw peak list5000 resolved
MeyerT_100422_sample0047.raw peak list5000 resolved
MeyerT_100422_sample0048.raw peak list5000 resolved
MeyerT_100422_sample0051.raw peak list5000 resolved
MeyerT_100422_sample0049.raw peak list5000 resolved
MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
test10000-30sec
test10000-30sec gap-filled
test10000-30sec gap-filled Duplicate peaks filtered

test10000-30sec gap-filled Duplicate peaks filtered

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
675	211.061	16:42			91418.3	244881.8	521914.3
					157953.5	208353.7	387628.7
					146422.0	60684.0	88497.7
744	212.002	7:52			5484.2	959214.9	8269194.9
499	212.002	6:39			1007087.4	8740976.3	133535483.4
731	212.002	5:07			696.2	13770.3	1259124.3
556	212.020	2:08			21594.0	10079.5	570211.5
111	212.839	1:10			2599460.9	2158614.0	2160293.9
250	212.877	1:43			692756.4	586464.8	548998.4
144	213.017	1:11			986734.3	738830.6	371572.9
74	213.023	17:44			2296385.7	451149.7	153632.8
907	213.077	7:40			251.4	39032.6	20693.7
917	213.077	14:46			8259.7	68865.7	519639.5
945	213.077	12:07			1125.9	30266.0	39073.6
212	213.113	18:02			503510.6	1162807.6	1918710.3
620	214.005	6:38			401.1	10621.0	693328.5
598	214.837	1:10			327047.0	202366.4	430162.6
303	214.874	1:41			410872.2	347242.2	290299.1

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:56 PM

MZmine: identification options

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- Peak detection
- Gap filling
- Isotopes
- Filtering
- Alignment
- Normalization
- Identification
  - Custom database search
  - Fragment search
  - Adduct search
  - Complex search
  - Online database search
  - Mascot MS/MS Ion Search (experimental)
  - Glycerophospholipid prediction
- Data analysis
- Export/Import

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
MeyerT_100422_sample0052.raw
Peak lists
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0052.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0051.raw peak list5000
MeyerT_100422_sample0049.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000 resolved
MeyerT_100422_sample0050.raw peak list5000 resolved
MeyerT_100422_sample0047.raw peak list5000 resolved
MeyerT_100422_sample0048.raw peak list5000 resolved
MeyerT_100422_sample0051.raw peak list5000 resolved
MeyerT_100422_sample0049.raw peak list5000 resolved
MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
test10000-30sec
test10000-30sec gap-filled
test10000-30sec gap-filled Duplicate peaks filtered

test10000-30sec gap-filled Duplicate peaks filtered

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
675	211.061	16:42			91418.3	244881.8	521914.3
478	211.098	17:53			157953.5	208353.7	387628.7
					146422.0	60684.0	88497.7
					5484.2	959214.9	8269194.9
					1007087.4	8740976.3	133535483.4
					696.2	13770.3	1259124.3
556	212.020	2:08			21594.0	10079.5	570211.5
111	212.839	1:10			2599460.9	2158614.0	2160293.9
250	212.877	1:43			692756.4	586464.8	548998.4
144	213.017	1:11			986734.3	738830.6	371572.9
74	213.023	17:44			2296385.7	451149.7	153632.8
907	213.077	7:40			251.4	39032.6	20693.7
917	213.077	14:46			8259.7	68865.7	519639.5
945	213.077	12:07			1125.9	30266.0	39073.6
212	213.113	18:02			503510.6	1162807.6	1918710.3
620	214.005	6:38			401.1	10621.0	693328.5
598	214.837	1:10			327047.0	202366.4	430162.6
303	214.874	1:41			410872.2	347242.2	290299.1

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 6:57 PM

MZmine: accurate mass online search

MZmine 2.0: New project

Project Raw data methods Peak list methods Visualization Windows Help

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
MeyerT_100422_sample0052.raw
Peak lists
MeyerT_100422_sample0050.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000
MeyerT_100422_sample0047.raw peak list5000
MeyerT_100422_sample0048.raw peak list5000
MeyerT_100422_sample0051.raw peak list5000
MeyerT_100422_sample0049.raw peak list5000
MeyerT_100422_sample0052.raw peak list5000 resolved
MeyerT_100422_sample0050.raw peak list5000 resolved
MeyerT_100422_sample0047.raw peak list5000 resolved
MeyerT_100422_sample0048.raw peak list5000 resolved
MeyerT_100422_sample0051.raw peak list5000 resolved
MeyerT_100422_sample0049.raw peak list5000 resolved
MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0047.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
test10000-30sec
test10000-30sec gap-filled
test10000-30sec gap-filled Duplicate peaks filtered

test10000-30sec gap-filled Duplicate peaks filtered

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
675	211.061	16:42			91418.3	244881.8	521914.3
478	211.098	17:53			157953.5	208353.7	387628.7
876	211.098	17:06			146422.0	60684.0	88497.7
744	212.002	7:52			5484.2	959214.9	8269194.9
499	212.002	6:39			[illegible]	8740976.3	133535483.4
731	212.002	5:07			[illegible]	13770.3	1259124.3
556	212.020	2:08			[illegible]	10079.5	570211.5
111	212.839	1:10			[illegible]	2158614.0	2160293.9
250	212.877	1:43			[illegible]	586464.8	548998.4
144	213.017	1:11			[illegible]	738830.6	371572.9
74	213.023	17:4			[illegible]	451149.7	153632.8
907	213.077	7:40			[illegible]	39032.6	20693.7
917	213.077	14:46			8259.7	68865.7	519639.5
945	213.077	12:07			1125.9	30266.0	39073.6
212	213.113	18:02			503510.6	1162807.6	1918710.3
620	214.005	6:38			401.1	10621.0	693328.5
598	214.837	1:10			327047.0	202366.4	430162.6
303	214.874	1:41			410872.2	347242.2	290299.1

Online database search setup dialog

Database	Human Metabolome Database	
Peak m/z	832.052	m/z
Charge	1	
Ionization method	-H	
Neutral mass	1.008	
Number of results	5	
Mass tolerance	0.003	amu
Isotope pattern filter	☑	
Isotope pattern score threshold	65%	%

OK Cancel Help

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

Start C:\WINDOWS\system32... MZmine 2.0: New proj... Untitled 1 - OpenOffice.... 6:58 PM

Metabolomics Databases

- Human Metabolome Database: http://www.hmdb.ca/
- BioCyc: http://biocyc.org/
- Metlin: http://metlin.scripps.edu/
- KEGG: http://www.genome.jp/kegg/
- PubChem (not biology specific): http://pubchem.ncbi.nlm.nih.gov/

MS/MS databases

- Metlin

http://metlin.scripps.edu/

- MassBank (not only metabolites)

http://www.massbank.jp/?lang=en

MZmine: identification results

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

ew project
- Raw data files
  - MeyerT_100422_sample0051.raw
  - MeyerT_100422_sample0050.raw
  - MeyerT_100422_sample0048.raw
  - MeyerT_100422_sample0047.raw
  - MeyerT_100422_sample0049.raw
  - MeyerT_100422_sample0052.raw
- Peak lists
  - MeyerT_100422_sample0050.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000
  - MeyerT_100422_sample0047.raw peak list5000
  - MeyerT_100422_sample0048.raw peak list5000
  - MeyerT_100422_sample0051.raw peak list5000
  - MeyerT_100422_sample0049.raw peak list5000
  - MeyerT_100422_sample0052.raw peak list5000 resolved
  - MeyerT_100422_sample0050.raw peak list5000 resolved
  - MeyerT_100422_sample0047.raw peak list5000 resolved
  - MeyerT_100422_sample0048.raw peak list5000 resolved
  - MeyerT_100422_sample0051.raw peak list5000 resolved
  - MeyerT_100422_sample0049.raw peak list5000 resolved
  - MeyerT_100422_sample0052.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0050.raw peak list5000 resolved deisotoped
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  - MeyerT_100422_sample0051.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0048.raw peak list5000 resolved deisotoped
  - MeyerT_100422_sample0049.raw peak list5000 resolved deisotoped
  - **test10000-30sec**
  - **test10000-30sec gap-filled**
  - **test10000-30sec gap-filled Duplicate peaks filtered**

test10000-30sec gap-filled Duplicate peaks filtered

ID	Average m/z	Average Ret.time	Identity	Peak shape	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area	MeyerT_100422_sample... Area
492	152.995	28:52			45884.8	192838.9	221099.4
493	126.030	1:31			32126.1	507721.4	492430.8
494	336.072	12:40	2,8-Dihydroxyquinoline-be...		37144.7	147997.6	637636.7
495	171.066	12:35	Glycerol 3-phosphate		59186.5	180012.2	1354886.5
496	165.019	7:20	Phthalic acid		88001.4	140632.0	326728.8
497	187.007	9:47			68288.4	274238.9	484650459.0
498	527.214	8:11			7285.6	9794.4	30724061.8
499	212.002	6:39	Indoxyl sulfate		1007087.4	8740976.3	133535483.4
500	259.028	17:25			61049.8	166690.1	20445459.6
501	194.046	3:34	Salicyluric acid		521701.6	698894.2	17642187.4
502	186.077	4:48			972.4	219.2	12932004.5
503	181.992	4:04			68131.2	129617.5	11669523.9
504	170.082	8:24			156.7	540.9	10317342.8
505	279.099	3:11	L-Aspartyl-L-phenylalanine		444752.7	457439.0	5140130.1
506	283.082	13:17	p-Cresol glucuronide		542.5	73398.1	9403095.8
508	132.030	1:11	L-Aspartic acid		337052.7	651580.0	2308775.8
509	241.119	9:03			686472.7	769137.0	4108820.3
510	302.115	11:26	Indoleacetyl glutamine		260674.5	203886.4	4619000.5

[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

4856MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 7:24 PM

Monoisotopic vs Average Mass

Two stable isotopes important in biochemistry

Carbon-12 (100 %) and Carbon-13 (~1.1 %)

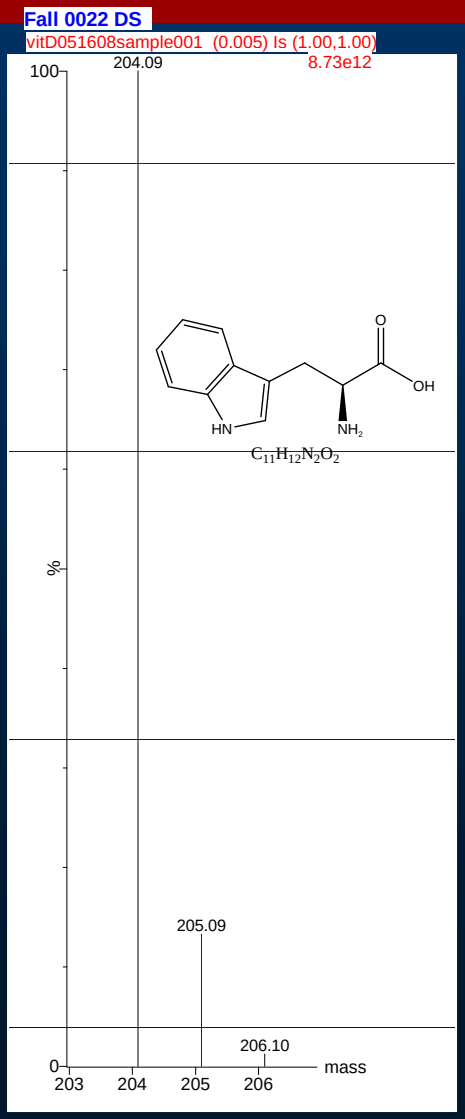
Sulfur-32 (100 %) and Sulfur-34 (4.4 %)

Tryptophan statistically can contain:

- no carbon-13 (M): 204.09 Da (100 %)
- one carbon-13 (M+1): 205.09 Da (11.9 %)
- two carbons-13 (M+2): 206.09 Da (1.4 %)

These are <u>monoisotopic</u> masses

Average mass = (204.09 *100 + 205.09*11.9 + 206.09*1.4)/113.2 = 204.22 (molecular weight, g/mol)



Elemental composition from accurate mass

^{1}H	1.0078 u
^{12}C	12.0000 u
^{14}N	14.0031 u
^{16}O	15.9949 u

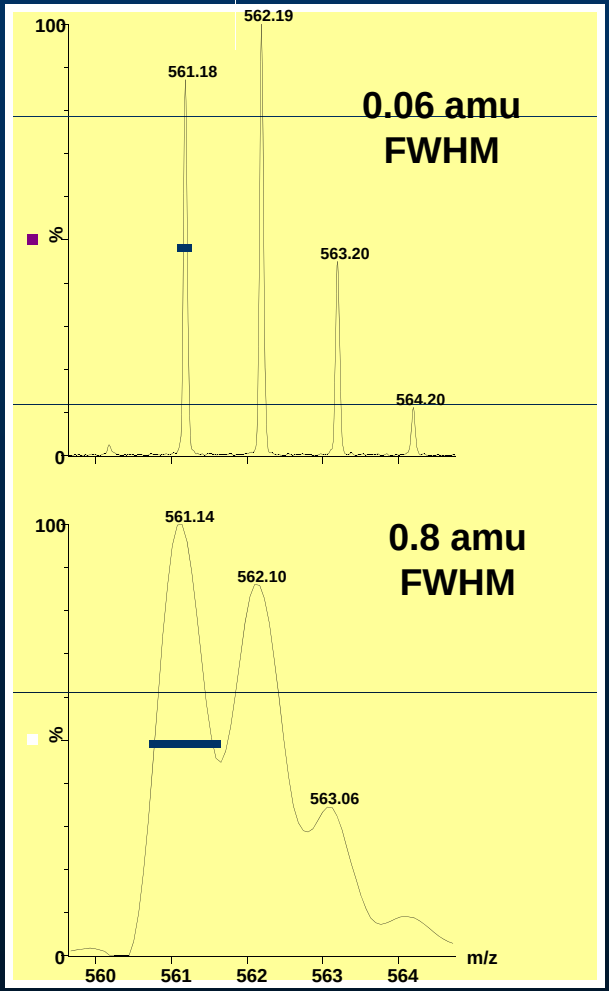
What is 28 u?

N_2 (2 x 14 u), CO (12 u + 16 u) or C_2H_4 (2 x 12 u + 4 x 1 u)?

What is 28.0313 u? [high accuracy]

C_2H_4 (2 x 12.0000 u + 4 x 1.0078 u)

High Resolution



High Resolution: R = 561/0.06 ~ 9,000

TOF: 7,000-50,000
Orbitrap: 10^4-10^5
FT ICR: 10^5-10^6

Nominal Mass Resolution (<1000)
R = 561/0.8 ~ 700

Accurate mass measurement is possible without high resolution. High resolution improves precision of mass measurement.

Mass of an electron becomes important at high accuracies

Even Electron (EE) Ions
Typically generated by chemical ionization techniques and electrospray

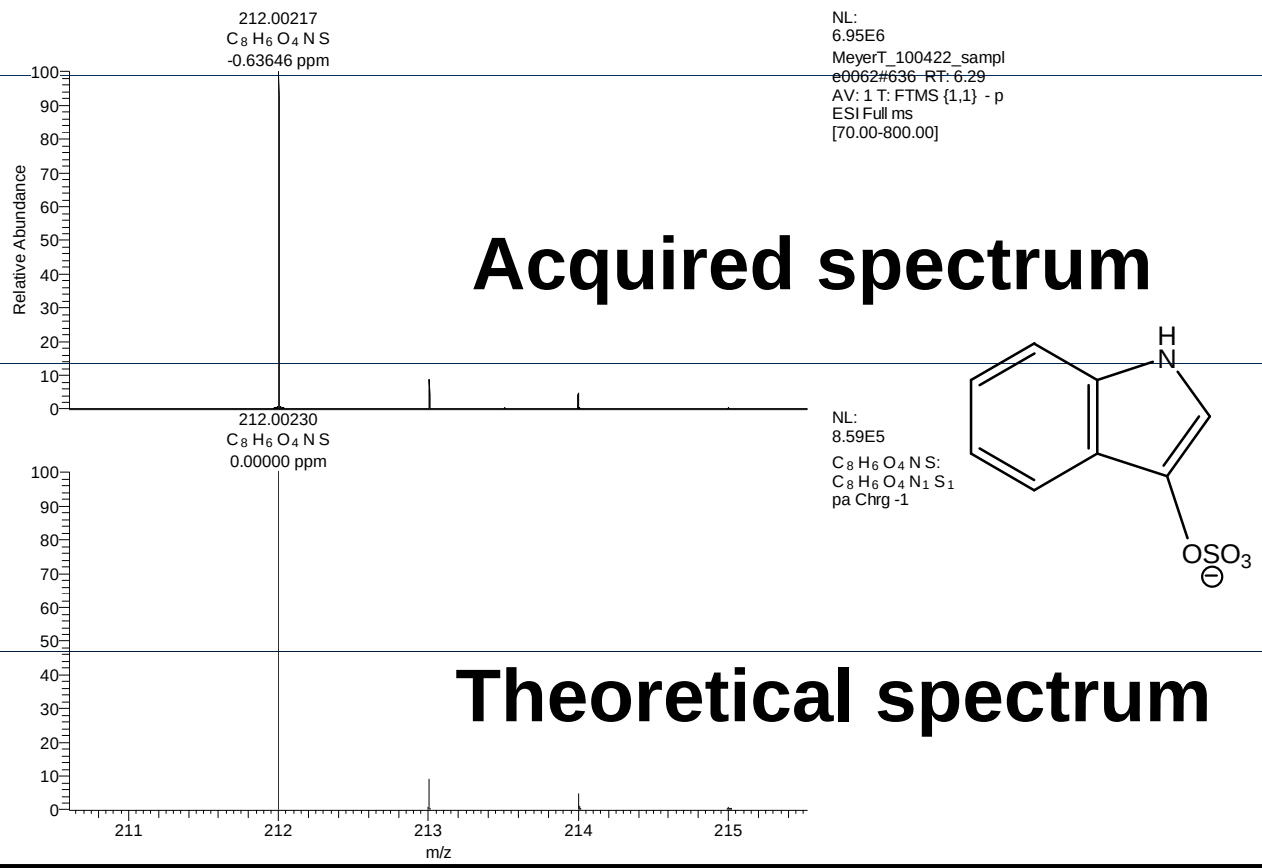
$C_{11}H_{12}N_2O_2 \longrightarrow C_{11}H_{13}N_2^{\oplus}O_2$ **205. 09715 Da (true mass)**

$C_{11}H_{13}N_2O_2$ **205. 09770 Da (2.6 ppm error)**

Orbitrap can achieve < 1 ppm accuracy

Identification based on accurate mass

Matching accurate mass and isotopic peak ratio



Error = -0.00013 Da/212.0023 Da * 1000,000 = 0.6 parts per million (ppm)

MZmine: data export options

MZmine 2.0: New project

Project | Raw data methods | Peak list methods | Visualization | Windows | Help

- Peak detection
- Gap filling
- Isotopes
- Filtering
- Alignment
- Normalization
- Identification
- Data analysis
- Export/Import
  - Export to CSV file Ctrl+C
  - Export to XML file Ctrl+X
  - Import from XML file Ctrl+I

ew project
Raw data files
MeyerT_100422_sample0051.raw
MeyerT_100422_sample0050.raw
MeyerT_100422_sample0048.raw
MeyerT_100422_sample0047.raw
MeyerT_100422_sample0049.raw
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[6:49:34 PM]: Finished duplicate peak filter on test10000-30sec gap-filled

808MB free

Start | C:\WINDOWS\system32... | MZmine 2.0: New proj... | Untitled 1 - OpenOffice.... | 7:24 PM

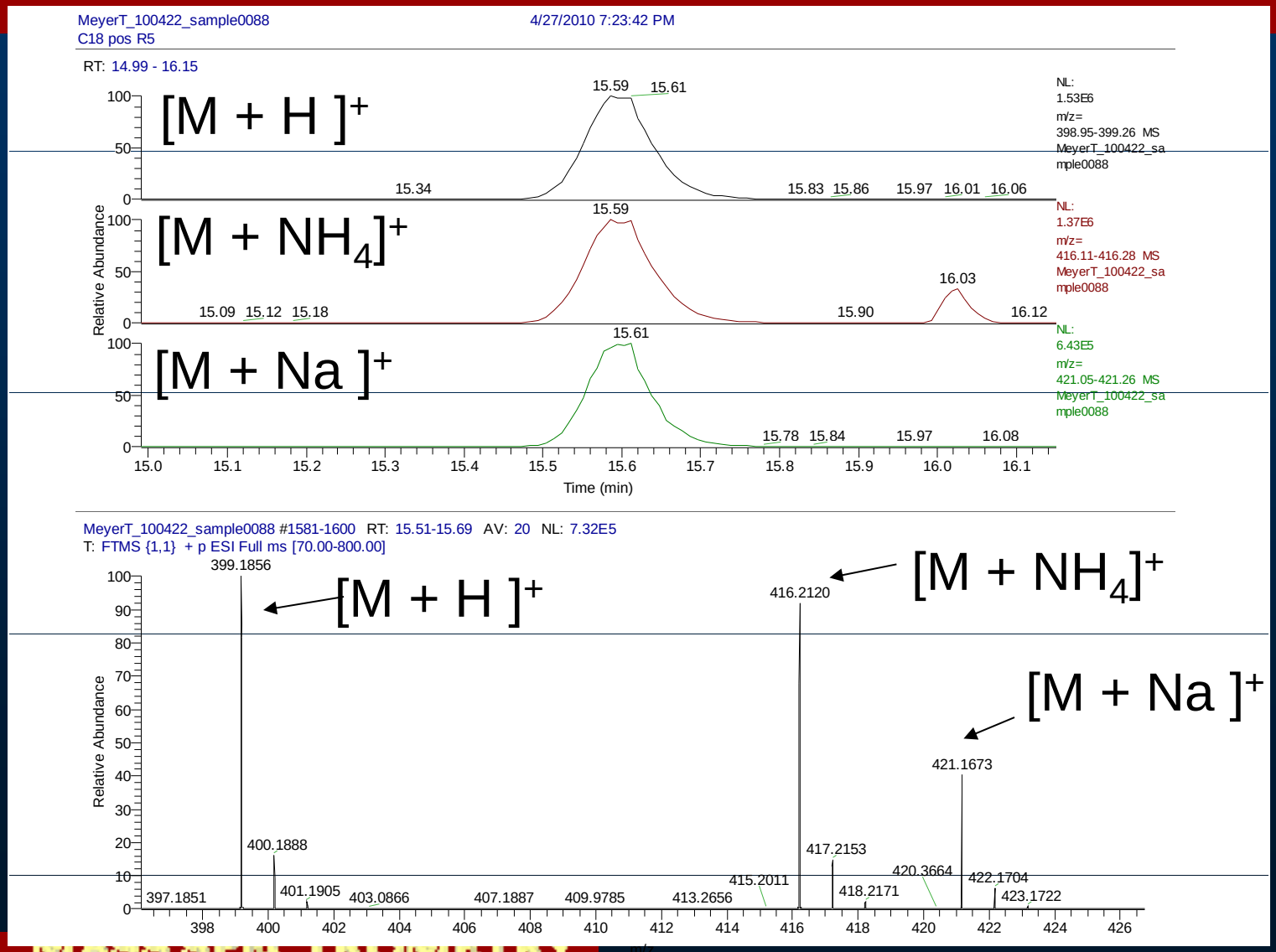
Manual Revision of Metabolomics Data

Many software tools can't remove some artifacts: unusual adducts, fragments

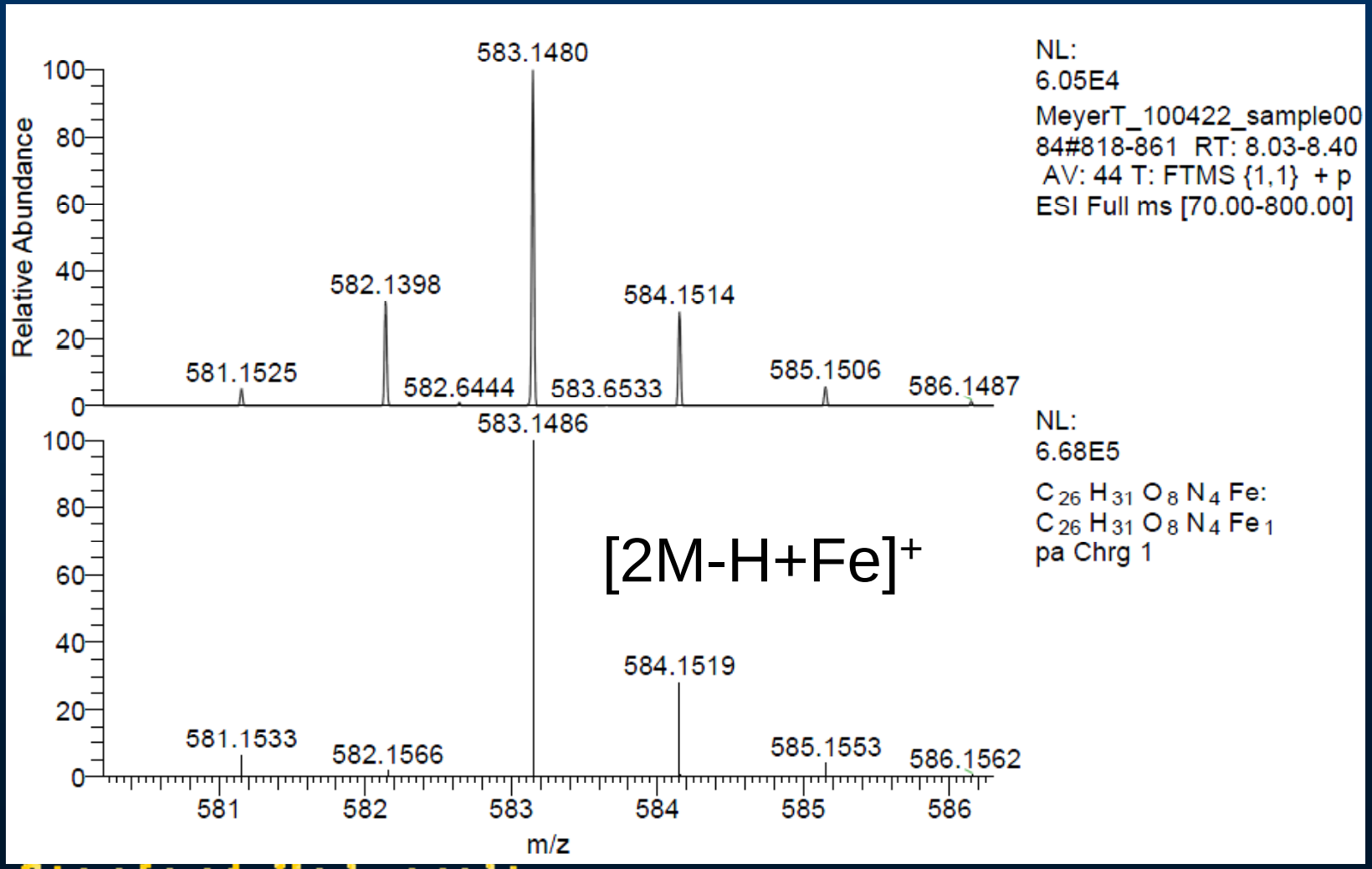
A majority of detected features may be isotopes, adducts, fragments and/or impurities

Baran R et al 2010, Anal Chem

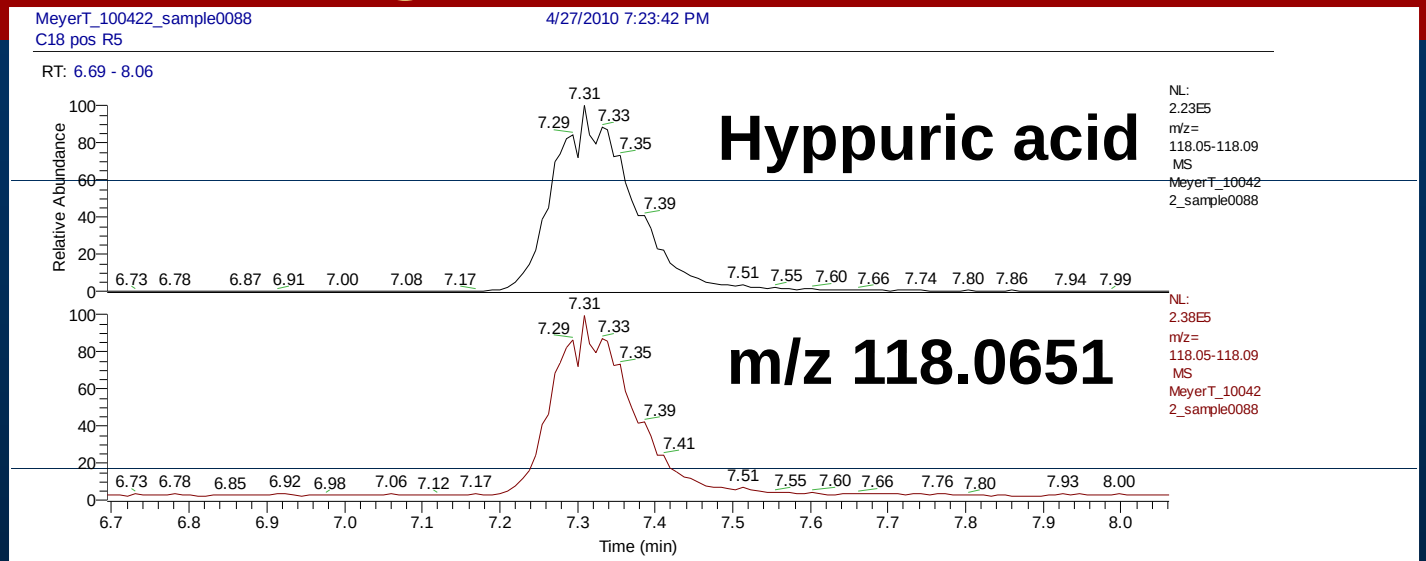
ESI-MS Adducts

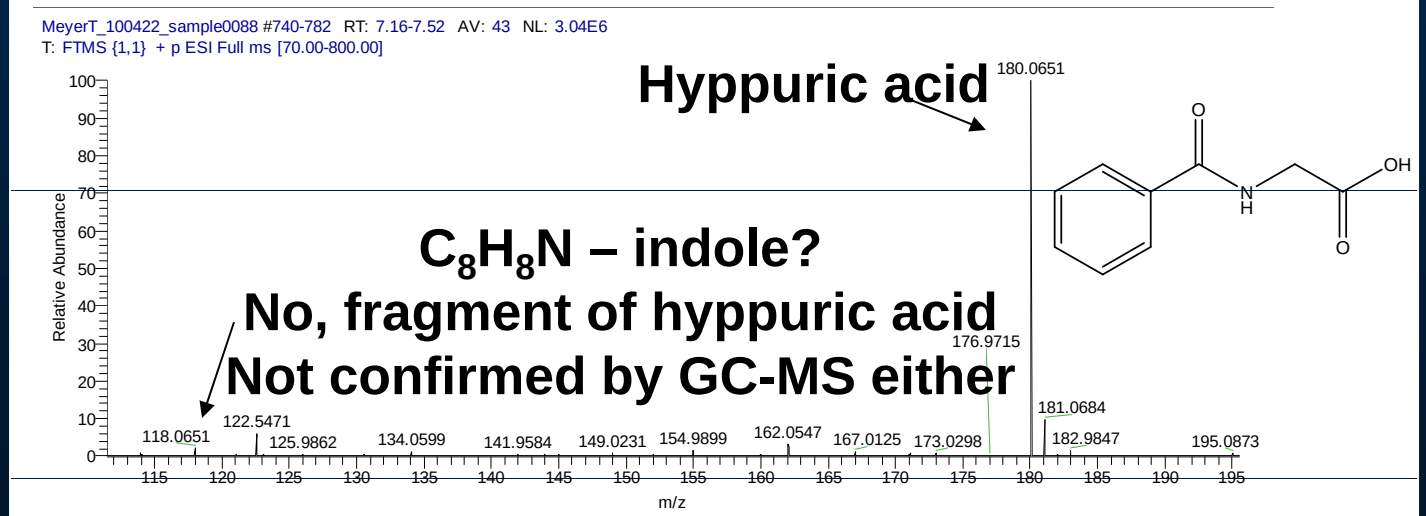


Unusual LC-ESI-MS Adducts



Fragments in LC-MS





Conclusions

- Open source MZmine software is capable to perform automated peak extraction, alignment and annotation from high resolution LC/MS metabolomics and proteomics data

- Manual curation of the results is requires to rule out fragments, some adducts and isotopes

Acknowledgements

- Allis Chien
- Theresa McLaughlin
- Karolina Krasinska
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- Anna Okumu

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