

# **Hands-on Data Analysis with MZmine 2**

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# About MZmine 2

- LC-MS metabolomics data processing, analysis, and visualization
  
- Supported open data formats
  - mzXML
  - mzML
  - mzData
  - NetCDF

# Workflow

- Raw data import
- Mass detection
- Chromatogram building
- Deconvolution / Feature detection
- Feature alignment
- Feature identification
- Export

# Data import

The screenshot shows the MZmine 2 software interface. The menu bar includes 'MZmine 2', 'Project', 'Raw data methods', 'Peak list methods', 'Visualization', 'Windows', and 'Help'. The 'Raw data methods' menu is open, showing options: 'Raw data import', 'Order raw data files', 'Filtering', and 'Peak detection'. The main workspace is divided into two panes: 'Raw data files' on the left and 'Peak lists' on the right. At the bottom, there is a 'Tasks in progress...' table and a status bar.

Item	Priority	Status	% done
------	----------	--------	--------

[10:56:36 PM]: Processing of task Detecting masses in PosMode\_NR3.mzXML done, status FINISHED

5595MB free

# Data import

MZmine 2.21: MZmine analysis

Raw data files | Peak lists

Open

fromBarnes

Name	Date Modified
Analysis of a set of metabolomics data.docx	Saturday, July 16, 2016 8:28 PM
jms_3780.pdf	Monday, July 4, 2016 12:25 PM
jms_3782.pdf	Monday, July 4, 2016 12:26 PM
Pos_Workshop for upload to Metaboanalyst	Saturday, July 16, 2016 4:01 PM
Pos_Workshop_Metaboanalyst_output	Saturday, July 16, 2016 5:47 PM
Pos_Workshop_XCMS	Saturday, July 16, 2016 5:48 PM
PosMode_IR1.mzXML	Thursday, June 25, 2015 3:50 PM
PosMode_IR2.mzXML	Thursday, June 25, 2015 3:49 PM
PosMode_IR3.mzXML	Thursday, June 25, 2015 3:51 PM
PosMode_NR1.mzXML	Thursday, June 25, 2015 3:52 PM
PosMode_NR2.mzXML	Thursday, June 25, 2015 3:53 PM
PosMode_NR3.mzXML	Thursday, June 25, 2015 3:54 PM
PRESENTATION SLIDES BOOK--MASTER FILE--...	Friday, July 15, 2016 1:57 PM

File Format: All raw data files

Cancel Choose

Tasks in progress... | Item | % done

[10:56:36 PM]: Processing of task Detecting masses in PosMode\_NR3.mzXML done, status FINISHED | 4947MB free

# Mass detection

- Mass detection: detect masses from mass spectra
  - Centroid
  - Exact mass
  - Local maxima
  - Recursive threshold
  - Wavelet transform

# Mass detection

- Mass detection: detect masses from mass spectra
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  - Wavelet transform

# Mass detection

The screenshot shows the MZmine 2.21 software interface. The 'Raw data methods' menu is open, showing options for 'Raw data import', 'Order raw data files', 'Filtering', and 'Peak detection'. The 'Peak detection' sub-menu is also open, listing several methods: 'Mass detection', 'FTMS shoulder peaks filter', 'Chromatogram builder', 'GridMass - 2D peak detection', 'MS/MS peaklist builder', and 'Targeted peak detection'. A tooltip for 'Mass detection' is visible, stating: 'This module detects individual ions in each scan and builds a mass list for each scan.' The interface includes a 'Raw data files' panel on the left with a list of files (PosMode\_IR3.mzXML, PosMode\_IR2.mzXML, PosMode\_NR1.mzXML, PosMode\_IR1.mzXML, PosMode\_NR3.mzXML, PosMode\_NR2.mzXML), a 'Peak lists' panel on the right, and a 'Tasks in progress...' table at the bottom. The table has columns for 'Item', 'Priority', 'Status', and '% done'. A status bar at the bottom shows the time [12:37:51 AM], a task completion message, and a memory usage indicator (5461MB free).

Item	Priority	Status	% done
------	----------	--------	--------

[12:37:51 AM]: Processing of task Updating TIC visualizer of PosMode\_IR2.mzXML done, status FINISHED

5461MB free



# Mass detection

**MZmine 2** MZmine 2.21: MZmine analysis

Raw data files

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

Peak lists

Please set the parameters

Raw data files 6 selected As selected in main window

Scans MS level: 1 Set filters Clear filters

Mass detector  Exact mass

- Centroid
- Local maxima
- Recursive threshold
- Wavelet transform

Mass list name

OK Cancel Help

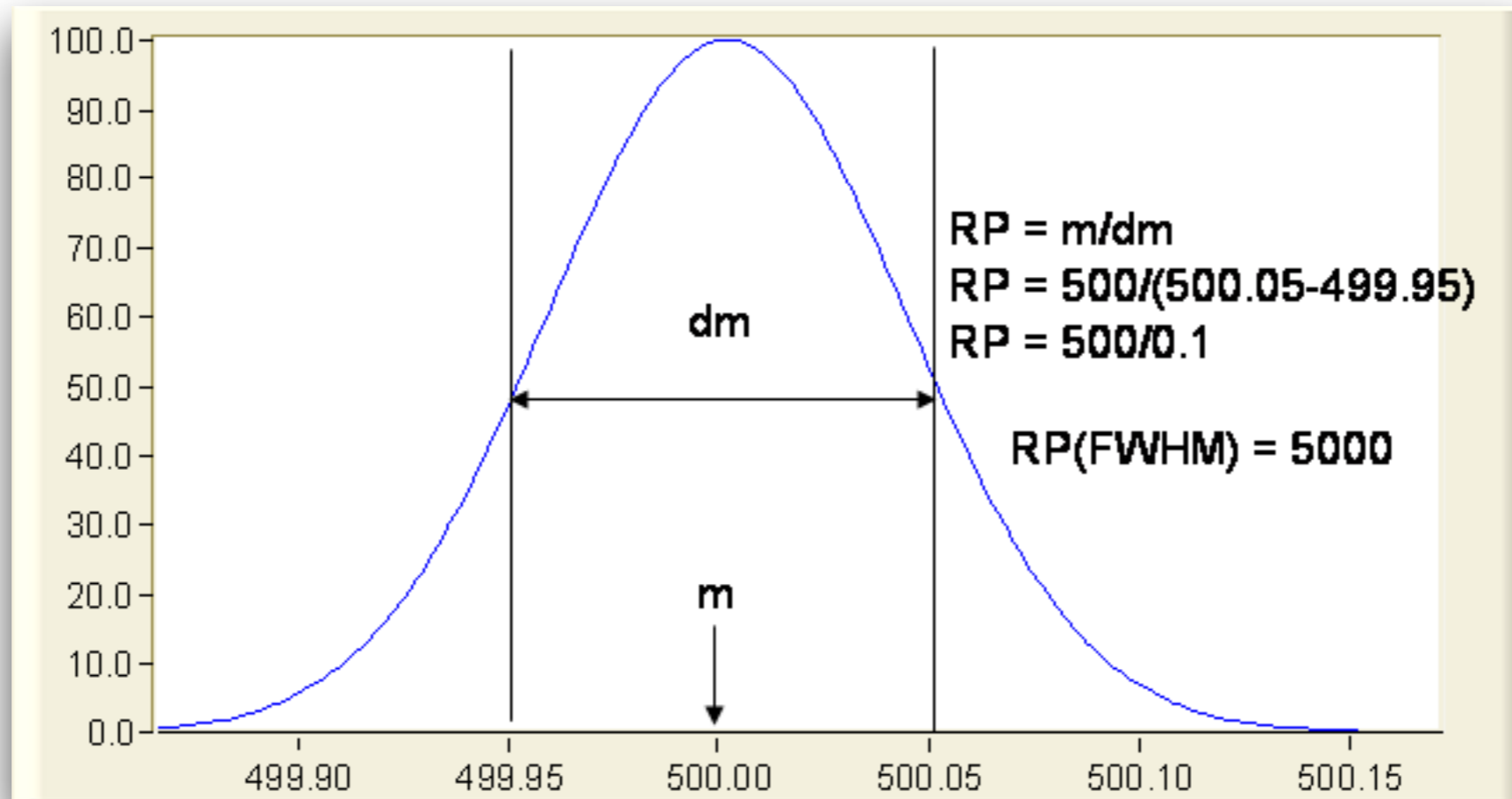
Tasks in progress...

Item	Priority	Status	% done
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[12:37:51 AM]: Processing of task Updating TIC visualizer of PosMode\_IR2.mzXML done, status FINISHED 5078MB free

# Mass detection

- Exact mass method



# Mass detection

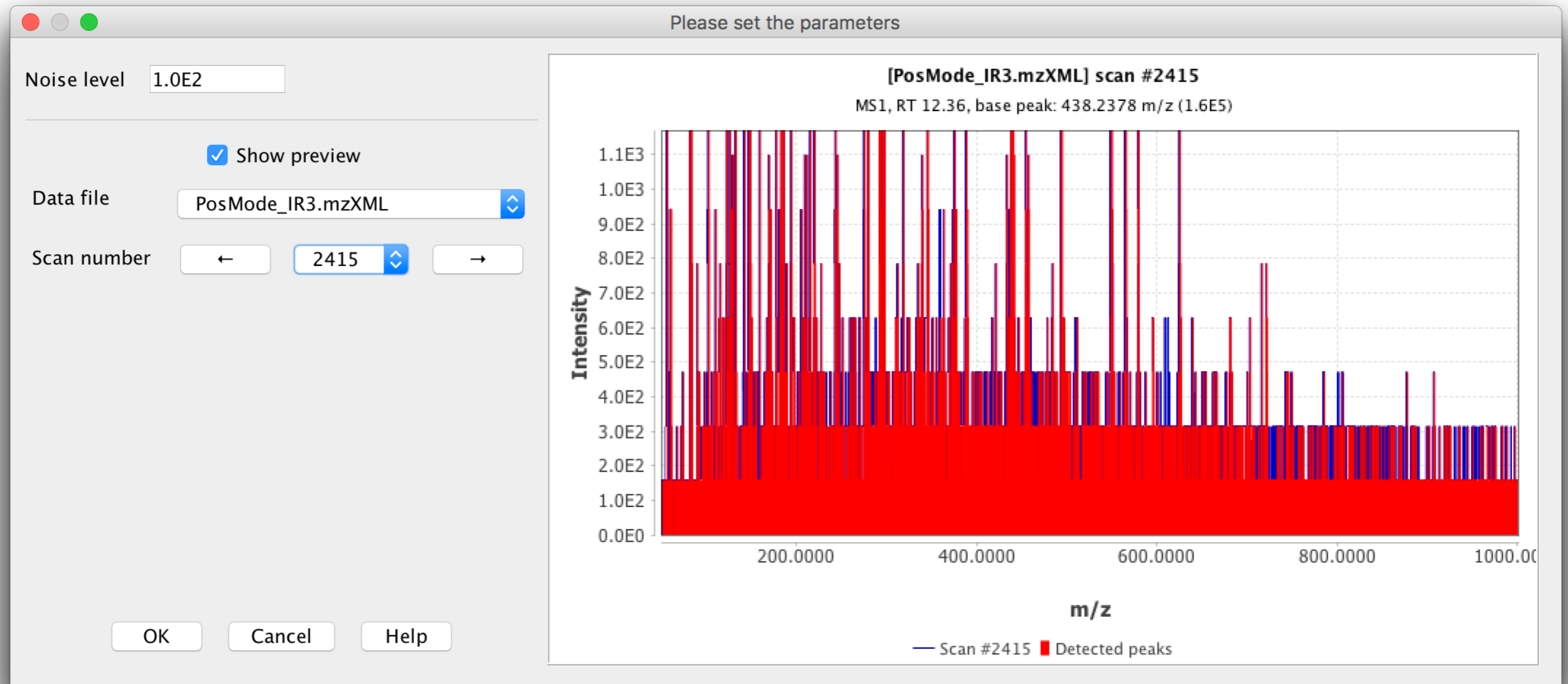
The screenshot shows the MZmine 2.21 interface. The main window displays a list of raw data files under 'Raw data files' and an empty 'Peak lists' section. A dialog box titled 'Please set the parameters' is open, showing settings for 'Raw data files' (6 selected), 'Scans' (MS level: 1), 'Mass detector' (Exact mass selected), and 'Mass list name'. A red arrow points to the '...' button next to the 'Mass detector' dropdown. A large red diagonal watermark reads 'click here to bring up the parameter window'. At the bottom, a task log shows 'Processing of task Updating TIC visualizer of PosMode\_IR2.mzXML done, status FINISHED' and a system tray shows '5078MB free'.

Item	Priority	Status	% done

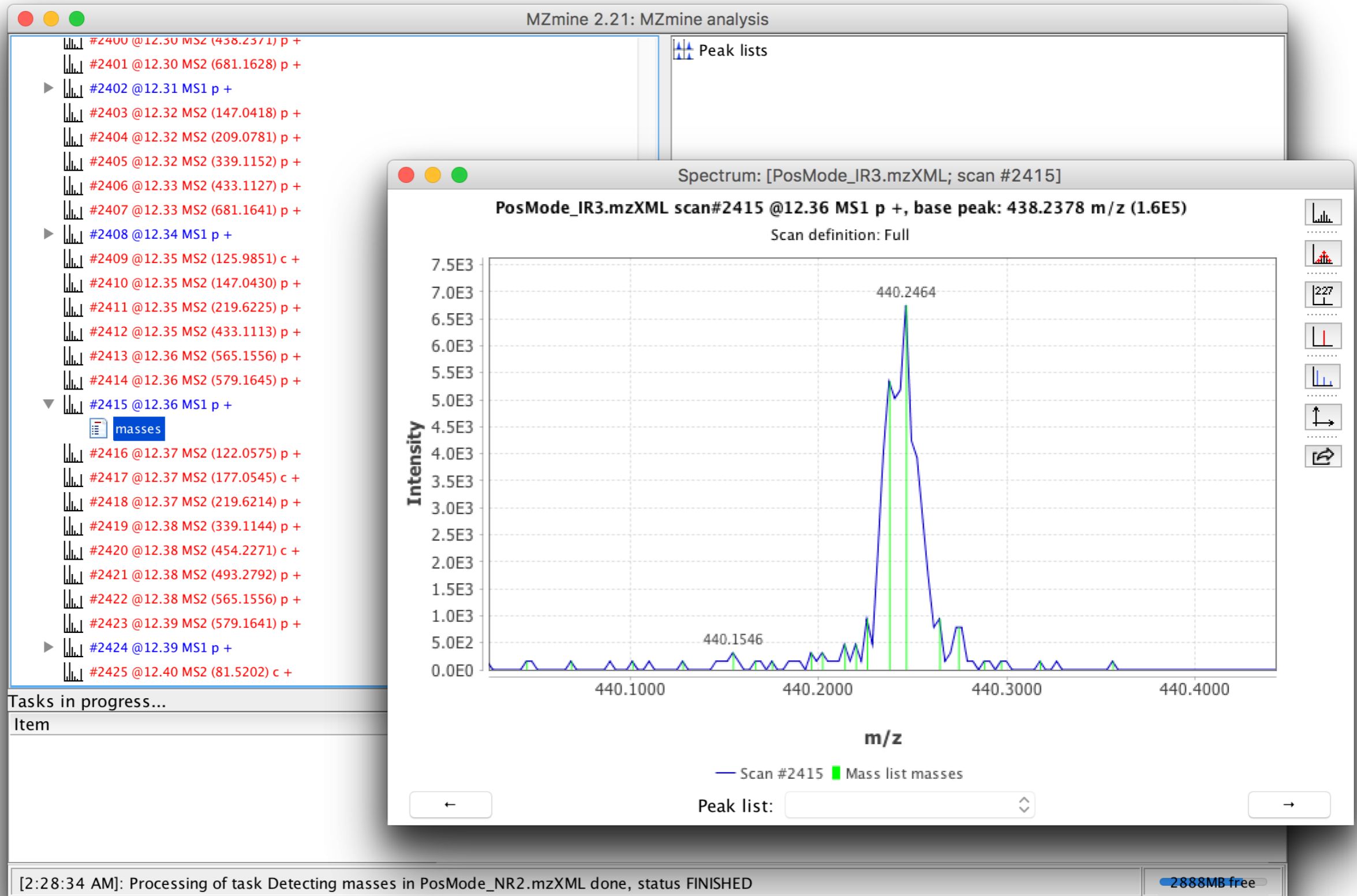
[12:37:51 AM]: Processing of task Updating TIC visualizer of PosMode\_IR2.mzXML done, status FINISHED

5078MB free

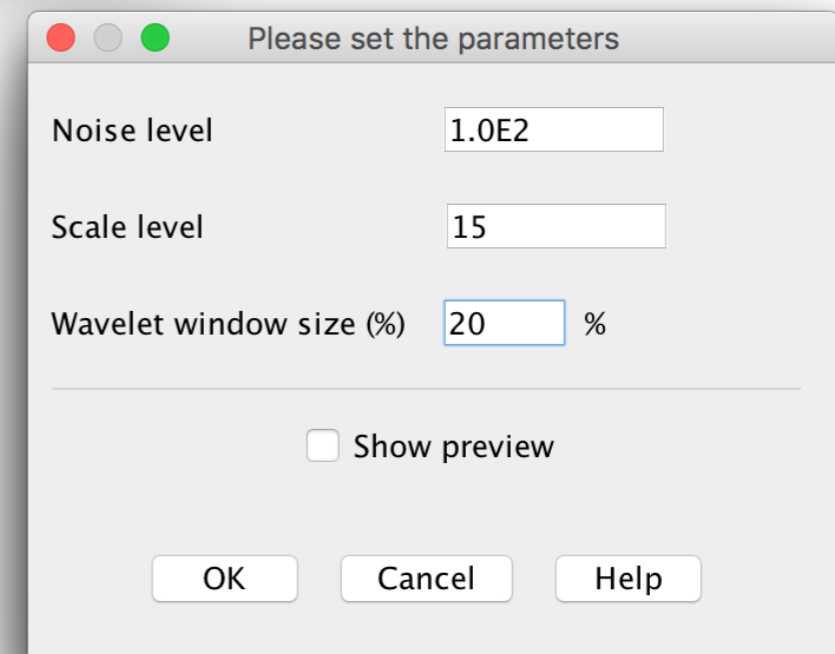
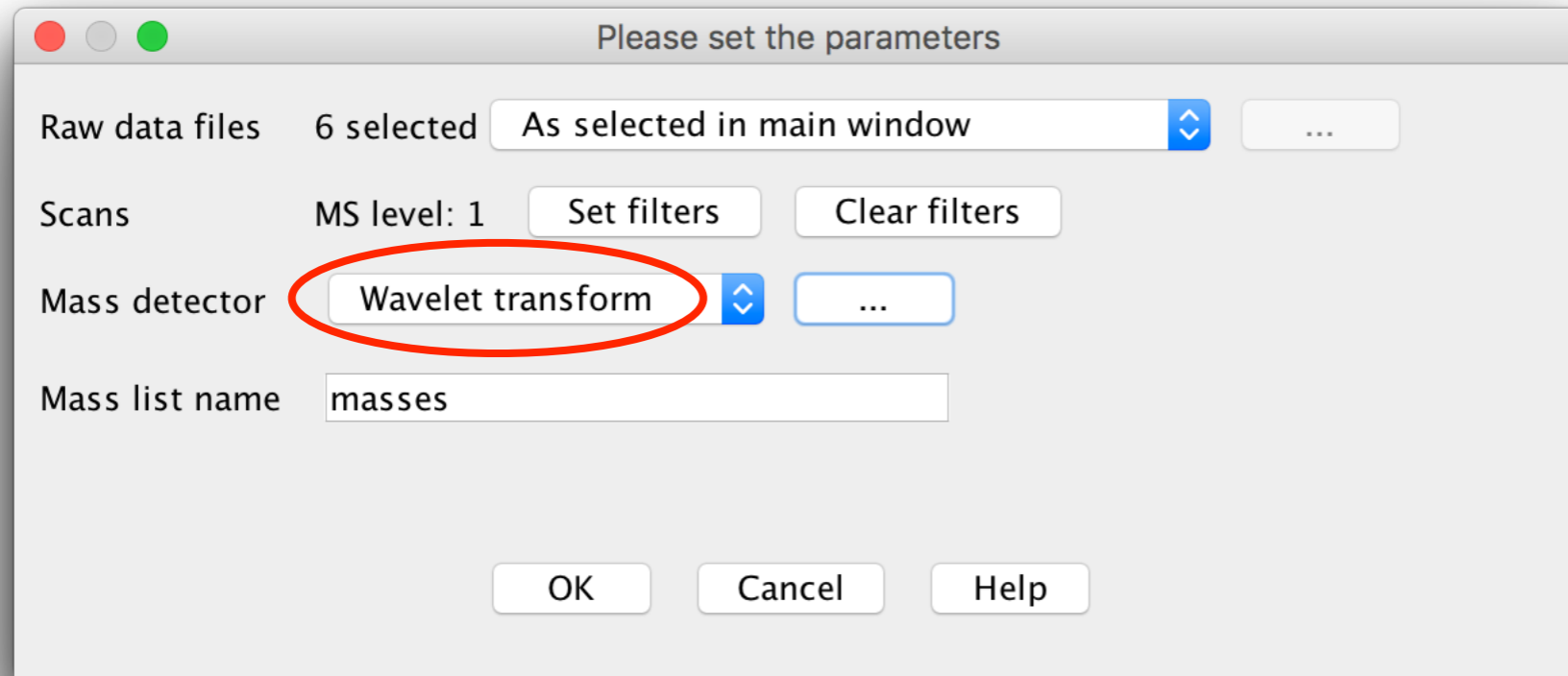
# Mass detection



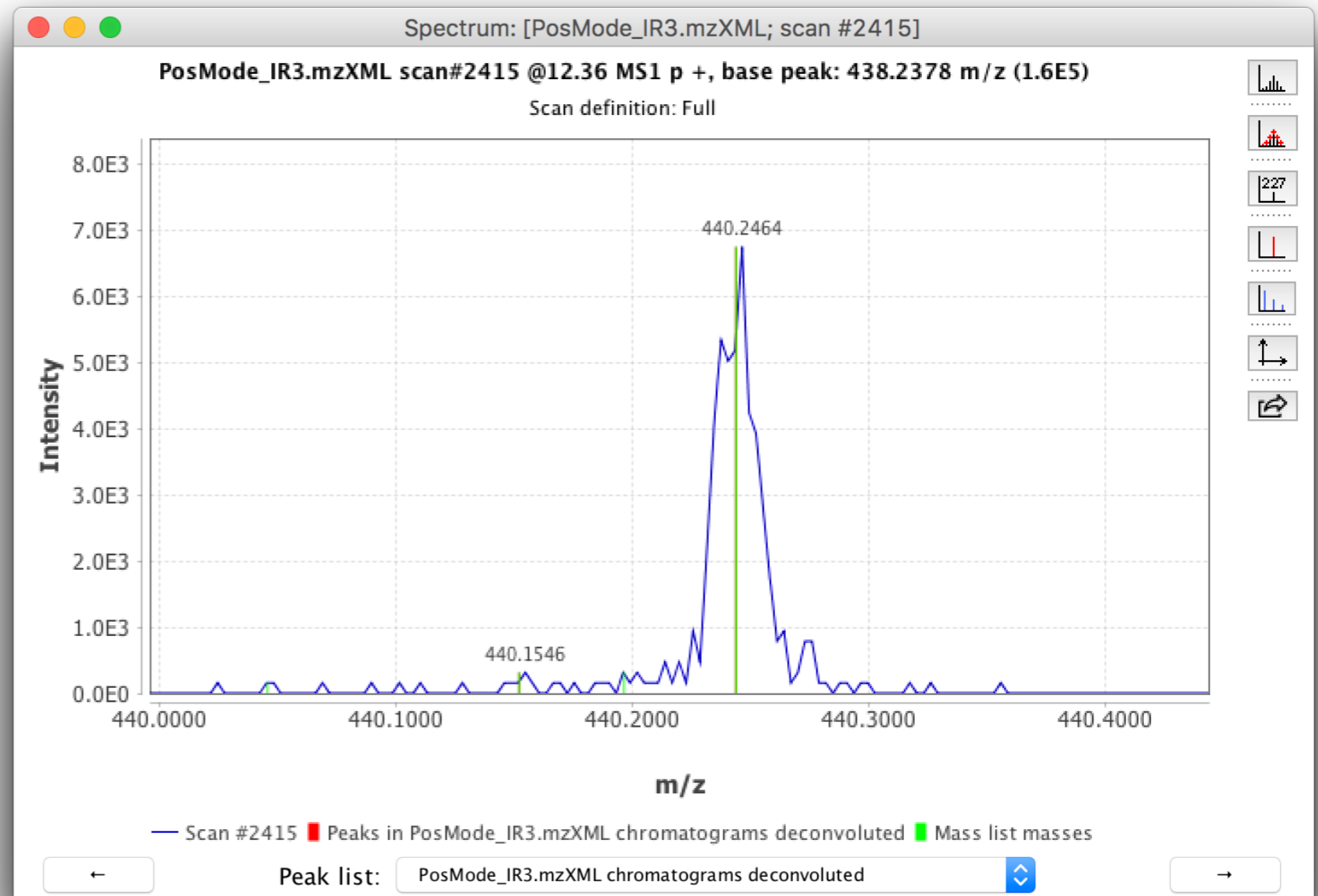
# Mass detection



# Mass detection



# Mass detection



# Chromatogram builder

The screenshot shows the MZmine 2.21 software interface. The main window is titled "MZmine 2.21: MZmine analysis". The menu bar includes "MZmine 2", "Project", "Raw data methods", "Peak list methods", "Visualization", "Windows", and "Help". The "Raw data methods" menu is open, showing options: "Raw data import", "Order raw data files", "Filtering", and "Peak detection". The "Peak detection" sub-menu is also open, listing: "Mass detection", "FTMS shoulder peaks filter", "Chromatogram builder" (highlighted), "GridMass - 2D peak detection", "MS/MS peaklist builder", and "Targeted peak detection".

On the left, a "Raw data files" panel lists several files: PosMode\_IR3.mzXML, PosMode\_IR2.mzXML, PosMode\_NR1.mzXML, PosMode\_IR1.mzXML, PosMode\_NR3.mzXML, and PosMode\_NR2.mzXML. The "Peak lists" panel on the right is currently empty.

At the bottom, a "Tasks in progress..." table is visible:

Item	Priority	Status	% done

The status bar at the bottom left shows a log entry: "[3:04:02 AM]: Processing of task Detecting masses in PosMode\_NR2.mzXML done, status FINISHED". The bottom right corner indicates "3487MB free".



# Chromatogram builder

Please set the parameters

Raw data files    6 selected    As selected in main window    ...

Scans    MS level: 1    Set filters    Clear filters

Mass list    masses    Choose...

Min time span (min)    0.3

Min height    2.5E2

m/z tolerance    0.05    m/z or 15    ppm

Suffix    chromatograms

OK    Cancel    Help

In progress, be patient .....

# Chromatogram builder

MZmine 2.21: MZmine analysis

Raw data files

- ▶ PosMode\_IR3.mzXML
- ▶ PosMode\_IR2.mzXML
- ▶ PosMode\_NR1.mzXML
- ▶ PosMode\_IR1.mzXML
- ▶ PosMode\_NR3.mzXML
- ▶ PosMode\_NR2.mzXML

Peak lists

- ▶ PosMode\_IR2.mzXML chromatograms
- ▶ PosMode\_NR1.mzXML chromatograms
- ▶ PosMode\_IR1.mzXML chromatograms
- ▶ PosMode\_IR3.mzXML chromatograms
- ▶ PosMode\_NR3.mzXML chromatograms
- ▶ PosMode\_NR2.mzXML chromatograms

Tasks in progress...

Item	Priority	Status	% done
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[3:24:49 AM]: Processing of task Detecting chromatograms in PosMode\_NR2.mzXML done, status FINISHED

3903MB free

# Chromatogram builder

The screenshot displays the MZmine 2.21 interface. On the left, under 'Raw data files', several .mzXML files are listed. On the right, under 'Peak lists', a list of peaks is shown for 'PosMode\_IR3.mzXML chromatograms'. A red arrow points from a callout box to peak #9 in the list.

**Raw data files:**

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

**Peak lists:**

- PosMode\_IR2.mzXML chromatograms
- PosMode\_NR1.mzXML chromatograms
- PosMode\_IR1.mzXML chromatograms
- PosMode\_IR3.mzXML chromatograms
  - #1 53.0045 m/z @17.68
  - #2 53.0048 m/z @3.31
  - #3 55.0435 m/z @18.56
  - #4 56.0513 m/z @18.89
  - #5 56.9667 m/z @2.81
  - #6 57.0553 m/z @19.45
  - #7 59.0510 m/z @6.07
  - #8 59.0511 m/z @29.10
  - #9 60.0458 m/z @25.22
  - #10 61.0086 m/z @2.01
  - #11 62.0257 m/z @3.30
  - #12 62.5081 m/z @2.89
  - #13 63.0450 m/z @0.00
  - #14 63.5253 m/z @3.31
  - #15 64.0170 m/z @3.67
  - #16 65.0612 m/z @3.80
  - #17 67.0296 m/z @3.31
  - #18 69.0341 m/z @6.32
  - #19 70.0133 m/z @0.06
  - #20 70.0663 m/z @4.55
  - #21 71.5129 m/z @0.06
  - #22 72.0810 m/z @6.07

**peak list**

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

**Tasks in progress...**

Item	Priority	Status	% done
------	----------	--------	--------

[3:24:49 AM]: Processing of task Detecting chromatograms in PosMode\_NR2.mzXML done, status FINISHED

3397MB free

# Chromatogram builder

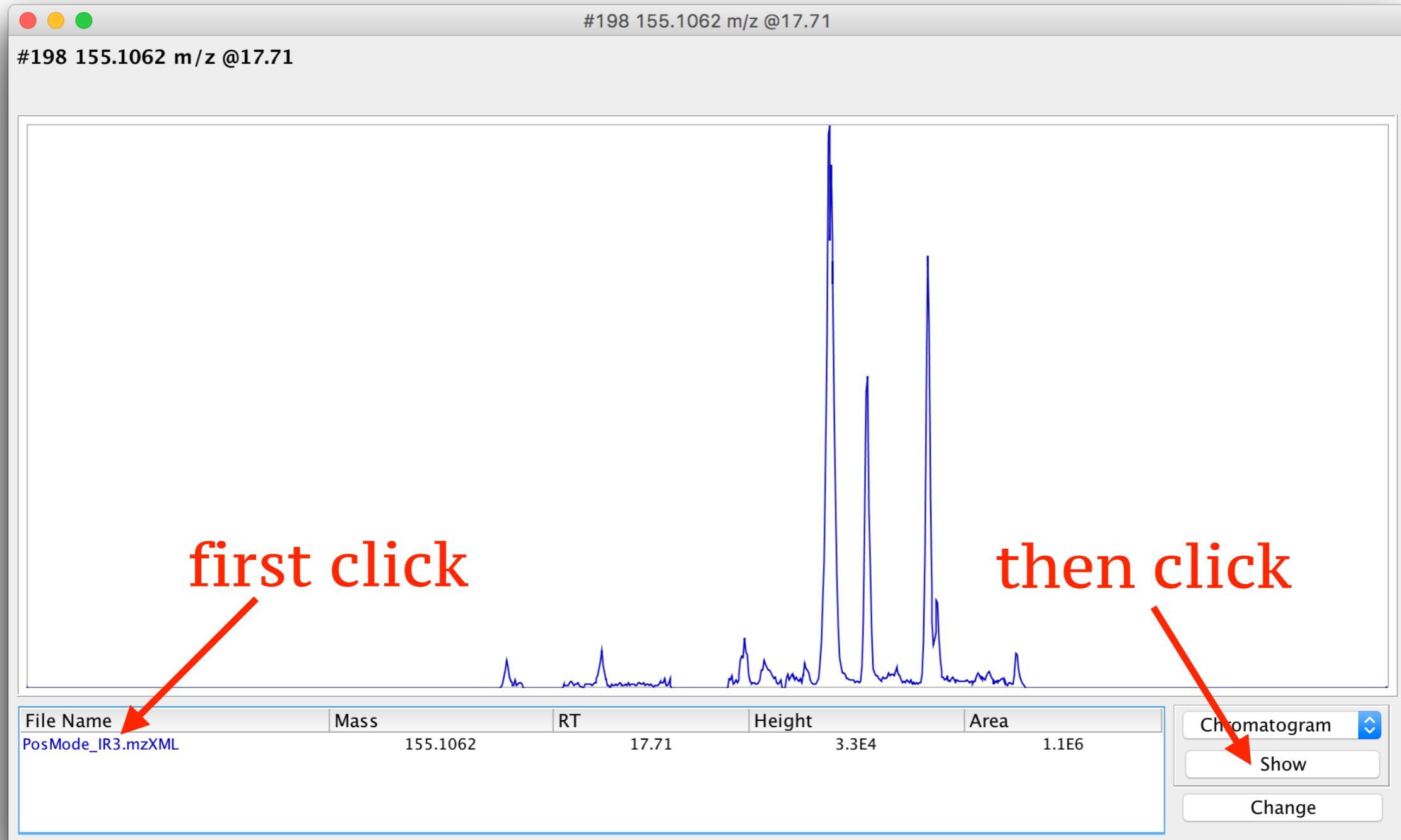
The screenshot shows the MZmine 2.21: MZmine analysis interface. On the left, under 'Raw data files', several files are listed, including PosMode\_IR3.mzXML, PosMode\_IR2.mzXML, PosMode\_NR1.mzXML, PosMode\_IR1.mzXML, PosMode\_NR3.mzXML, and PosMode\_NR2.mzXML. On the right, a list of peaks is displayed, each with a blue triangle icon, a peak number, m/z value, and retention time. The peak #198 (155.1062 m/z @ 17.71) is highlighted with a blue background and circled in red. A red arrow points to this peak with the word 'click' written in red. Below the peak list is a 'Tasks in progress...' section with a table header: Item, Priority, Status, % done. The table is currently empty. At the bottom, a status bar shows the message: '[3:31:18 AM]: Processing of task Updating TIC visualizer of PosMode\_IR3.mzXML done, status FINISHED' and a memory indicator '1584MB free'.

Item	Priority	Status	% done
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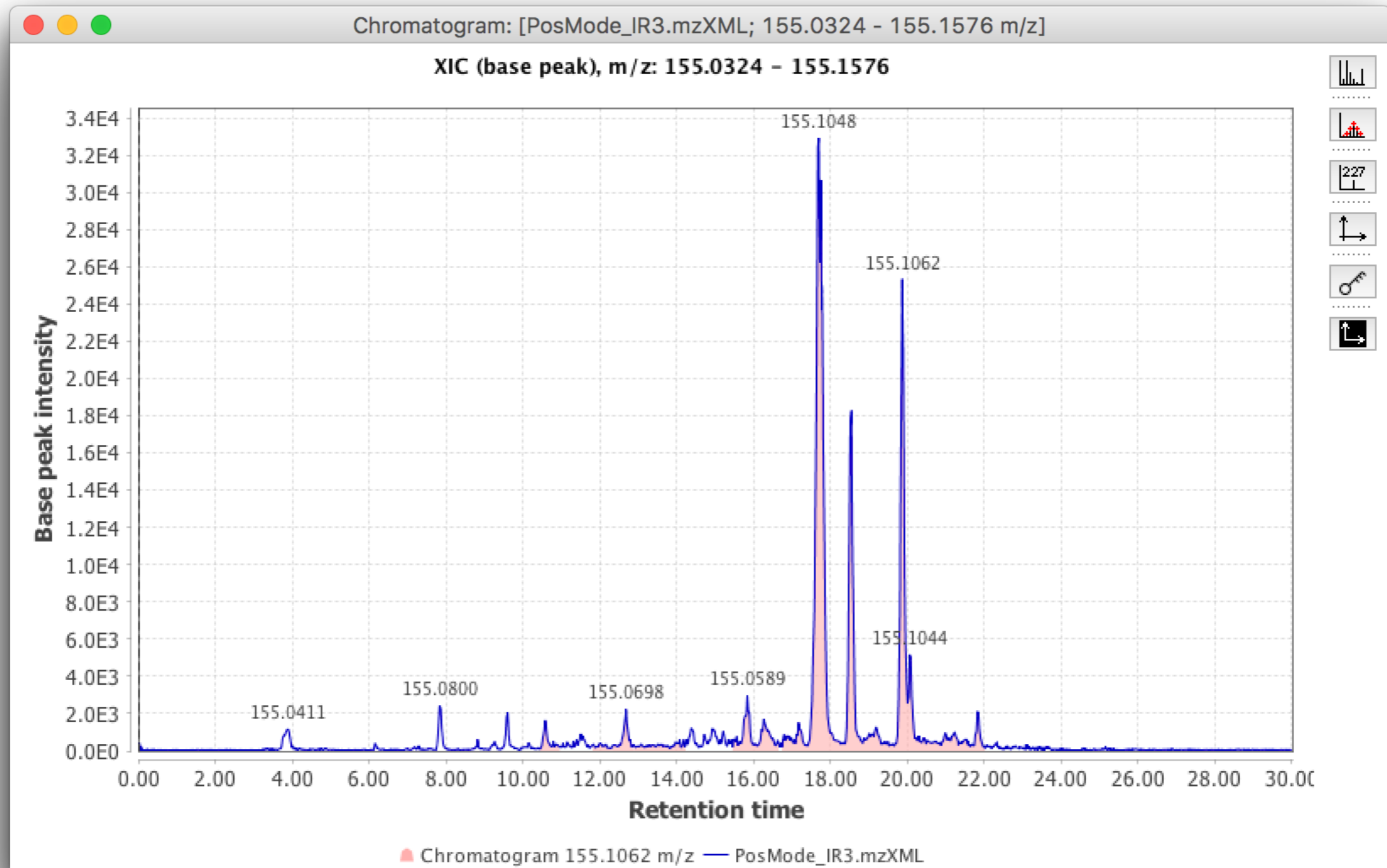
[3:31:18 AM]: Processing of task Updating TIC visualizer of PosMode\_IR3.mzXML done, status FINISHED

1584MB free

# Chromatogram builder



# Chromatogram builder



# Deconvolution

The screenshot shows the MZmine 2 software interface. The 'Peak list methods' menu is open, and 'Chromatogram deconvolution' is selected. The interface includes a file list on the left, a task list at the bottom, and a status bar at the very bottom.

**Raw data files:**

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

**Peak list methods menu:**

- Order peak lists
- Peak detection
- Gap filling
- Isotopes
- Filtering
- Alignment
- Normalization
- Identification
- Data analysis
- Export/Import

**Chromatogram deconvolution sub-menu:**

- Smoothing
- Chromatogram deconvolution
- Peak shape modeler (experimental)
- Peak extender

**Task list:**

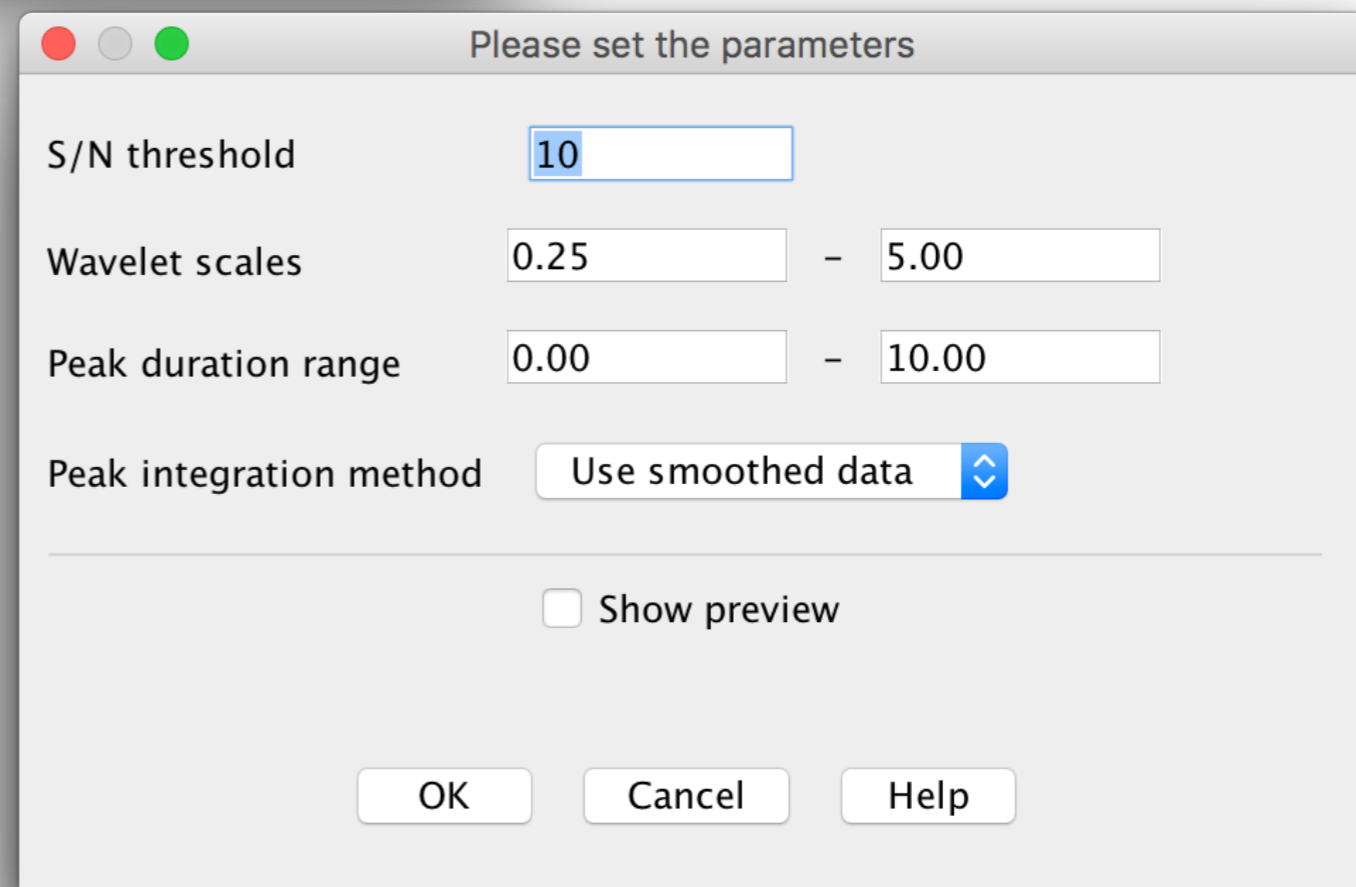
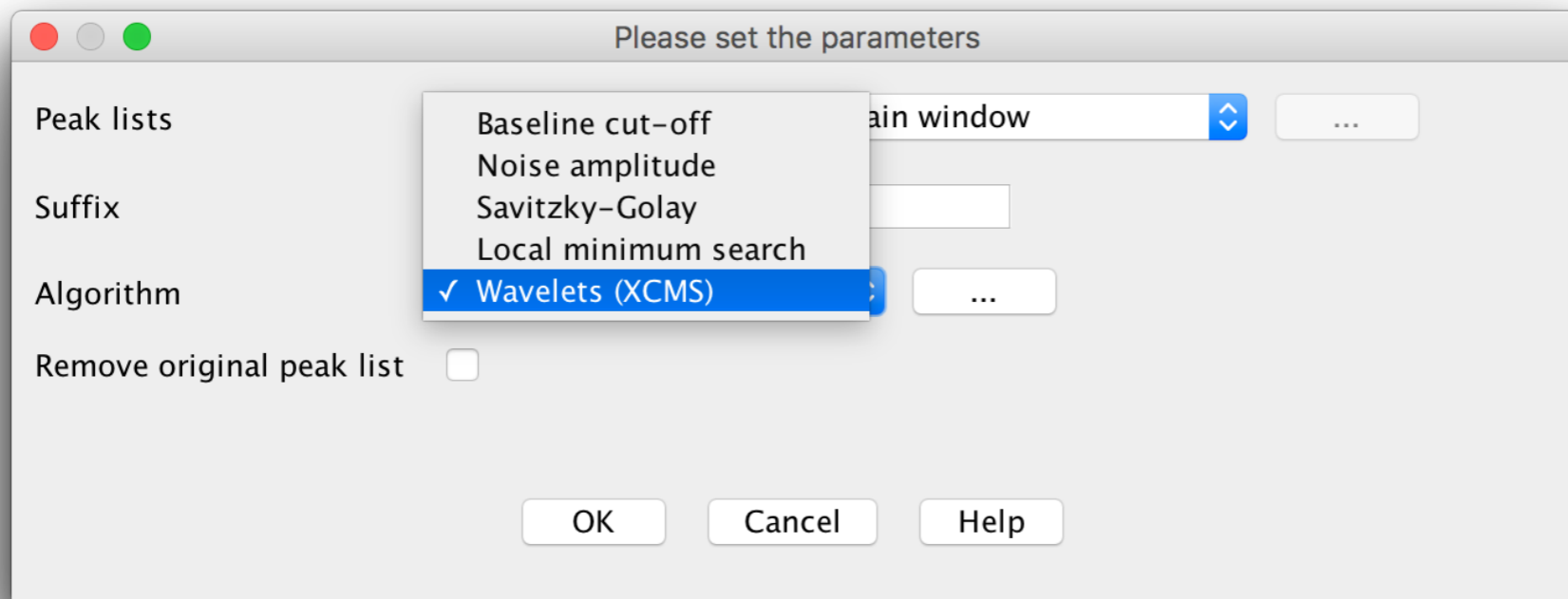
Item	Priority	Status	% done

**Status bar:** [3:38:10 AM]: Processing of task Updating TIC visualizer of PosMode\_IR3.mzXML done, status FINISHED

**System tray:** 3075MB free

separation of individual peaks in the EIC

# Deconvolution





# Deconvolution

MZmine 2.21: MZmine analysis


**Raw data files**

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

**Peak lists**

- PosMode\_IR2.mzXML chromatograms
- PosMode\_NR1.mzXML chromatograms
- PosMode\_IR1.mzXML chromatograms
- PosMode\_IR3.mzXML chromatograms
- PosMode\_NR3.mzXML chromatograms
- PosMode\_NR2.mzXML chromatograms

**Error of task Peak recognition on PosMode\_IR1.mzXML chromatograms**

 'R computing error' during CentWave detection.  
The "Wavelets (XCMS)" requires the "xcms" R package, which couldn't be loaded - is it installed in R?

OK

**Tasks in progress...**

Item	Priority	Status	% done
Peak recognition on PosMode_IR2.mzXML chromatograms	NORMAL	ERROR	0%
Peak recognition on PosMode_NR1.mzXML chromatograms	NORMAL	ERROR	0%
Peak recognition on PosMode_IR1.mzXML chromatograms	NORMAL	ERROR	0%
Peak recognition on PosMode_IR3.mzXML chromatograms	NORMAL	ERROR	0%
Peak recognition on PosMode_NR3.mzXML chromatograms	NORMAL	WAITING	0%
Peak recognition on PosMode_NR2.mzXML chromatograms	NORMAL	WAITING	0%

[3:46:06 AM]: Error of task Peak recognition on PosMode\_IR3.mzXML chromatograms: 'R computing error' during CentWave detection. The "Wavelet... 2141MB free

# Deconvolution

- Install XCMS in R

```
1 source("https://bioconductor.org/biocLite.R")
2 biocLite("xcms") # install the xcms package
3 library(xcms) # load the xcms package|
```

- Start deconvolution again

# Deconvolution

The screenshot shows the MZmine 2.21 interface with two main panels. The left panel, titled 'Raw data files', contains a list of six mzXML files: PosMode\_IR3.mzXML, PosMode\_IR2.mzXML, PosMode\_NR1.mzXML, PosMode\_IR1.mzXML, PosMode\_NR3.mzXML, and PosMode\_NR2.mzXML. The right panel, titled 'Peak lists', contains a list of six corresponding chromatogram files: PosMode\_IR2.mzXML chromatograms, PosMode\_NR1.mzXML chromatograms, PosMode\_IR1.mzXML chromatograms, PosMode\_IR3.mzXML chromatograms, PosMode\_NR3.mzXML chromatograms, and PosMode\_NR2.mzXML chromatograms. A large red text overlay reads 'In progress, be patient .....'. At the bottom, a 'Tasks in progress...' table shows the status of peak recognition tasks for each chromatogram. A status bar at the very bottom indicates the start time and the amount of free memory.

Item	Priority	Status	% done
Peak recognition on PosMode_IR2.mzXML chromatograms	NORMAL	PROCESSING	6%
Peak recognition on PosMode_NR1.mzXML chromatograms	NORMAL	PROCESSING	6%
Peak recognition on PosMode_IR1.mzXML chromatograms	NORMAL	PROCESSING	6%
Peak recognition on PosMode_IR3.mzXML chromatograms	NORMAL	PROCESSING	6%
Peak recognition on PosMode_NR3.mzXML chromatograms	NORMAL	WAITING	0%
Peak recognition on PosMode_NR2.mzXML chromatograms	NORMAL	WAITING	0%

[3:58:11 AM]: Started peak deconvolution on PosMode\_IR3.mzXML chromatograms

4571MB free

# Deconvolution

MZmine 2.21: MZmine analysis

Raw data files

- ▶ PosMode\_IR3.mzXML
- ▶ PosMode\_IR2.mzXML
- ▶ PosMode\_NR1.mzXML
- ▶ PosMode\_IR1.mzXML
- ▶ PosMode\_NR3.mzXML
- ▶ PosMode\_NR2.mzXML

Peak lists

- ▶ PosMode\_IR2.mzXML chromatograms
- ▶ PosMode\_NR1.mzXML chromatograms
- ▶ PosMode\_IR1.mzXML chromatograms
- ▶ PosMode\_IR3.mzXML chromatograms
- ▶ PosMode\_NR3.mzXML chromatograms
- ▶ PosMode\_NR2.mzXML chromatograms
- ▶ PosMode\_IR2.mzXML chromatograms deconvoluted
- ▶ PosMode\_IR1.mzXML chromatograms deconvoluted
- ▶ PosMode\_NR1.mzXML chromatograms deconvoluted
- ▶ PosMode\_IR3.mzXML chromatograms deconvoluted
- ▶ PosMode\_NR3.mzXML chromatograms deconvoluted
- ▶ PosMode\_NR2.mzXML chromatograms deconvoluted

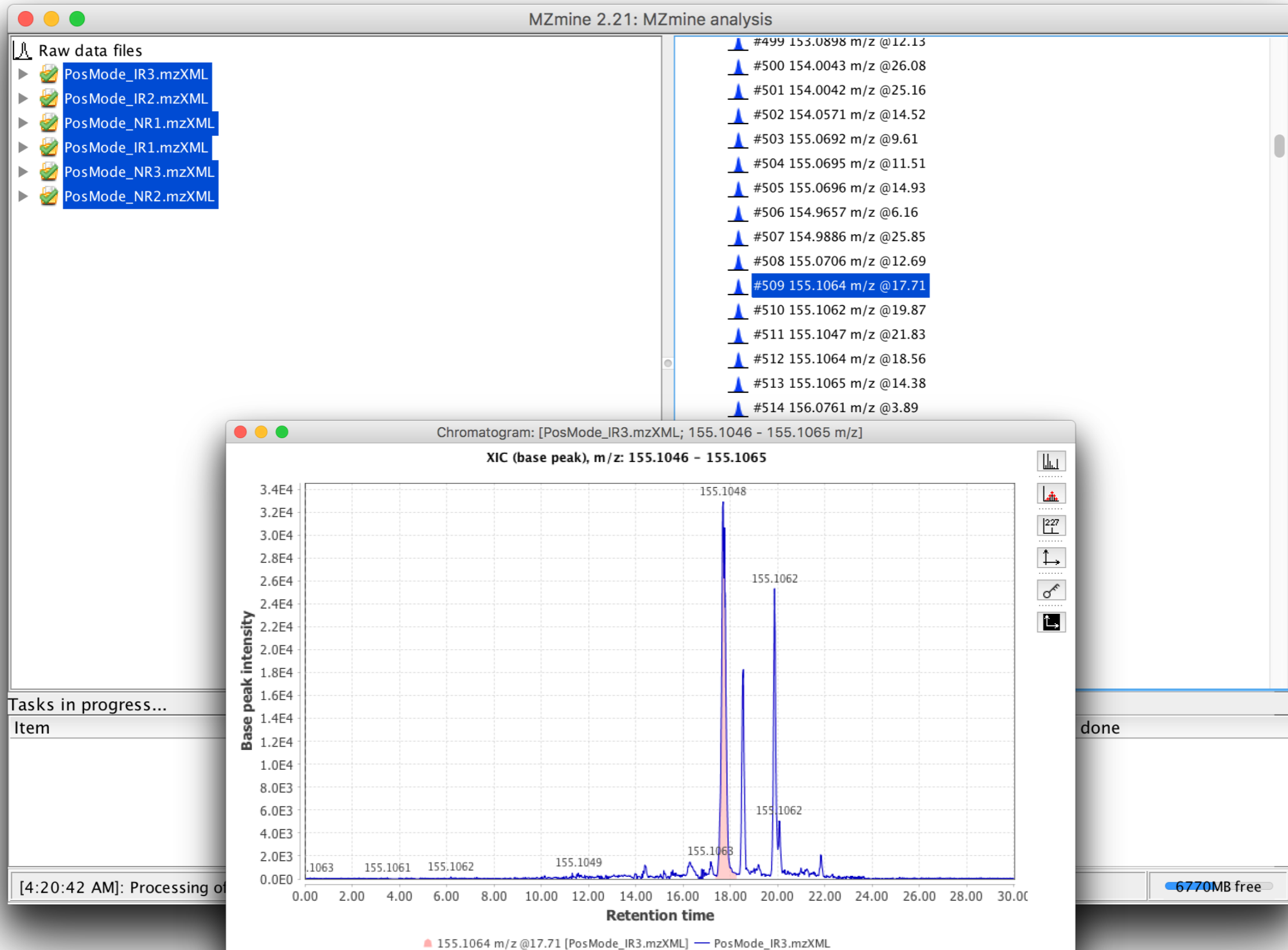
Tasks in progress...

Item	Priority	Status	% done
------	----------	--------	--------

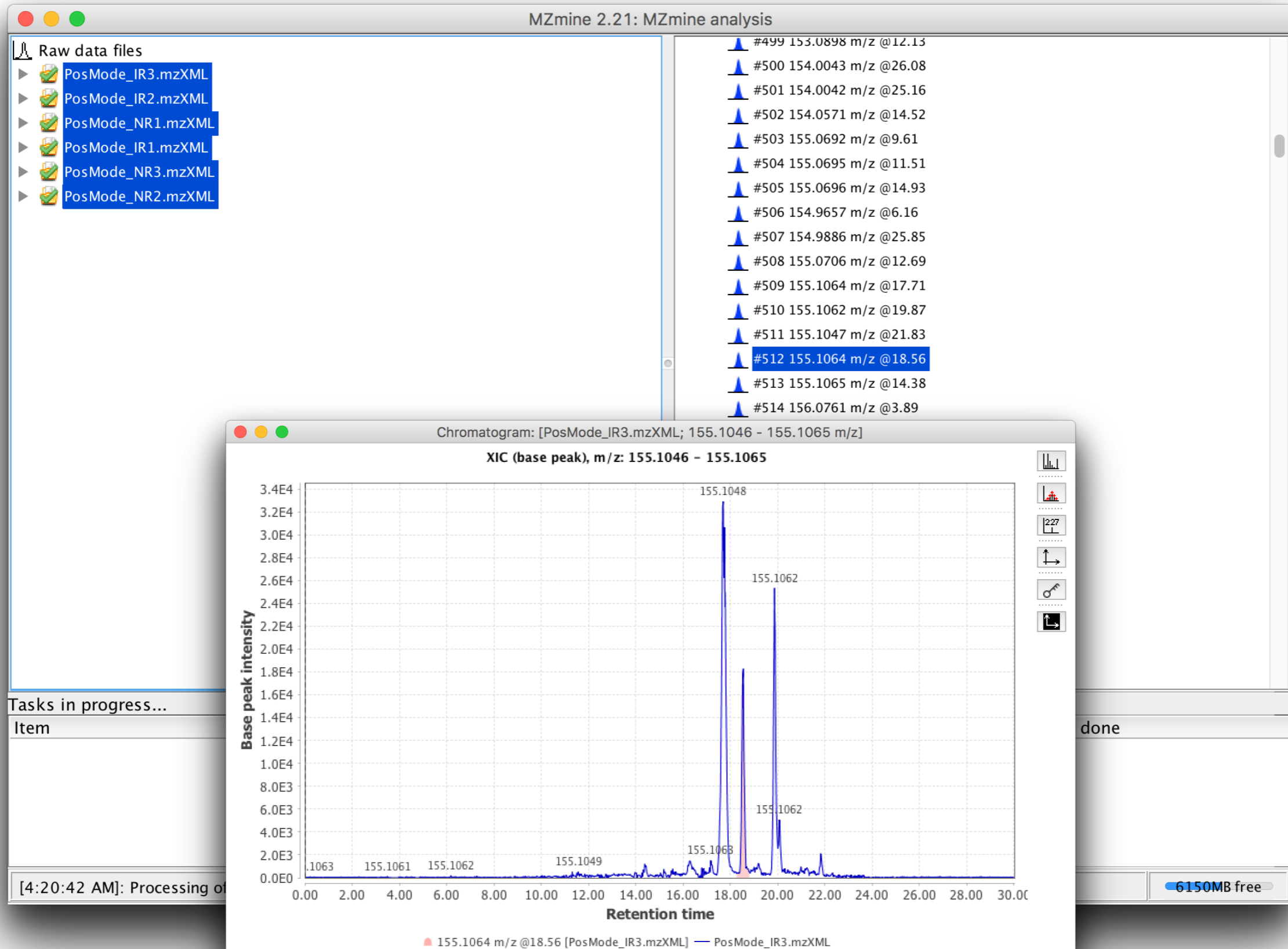
[4:11:54 AM]: Processing of task Peak recognition on PosMode\_NR2.mzXML chromatograms done, status FINISHED

6925MB free

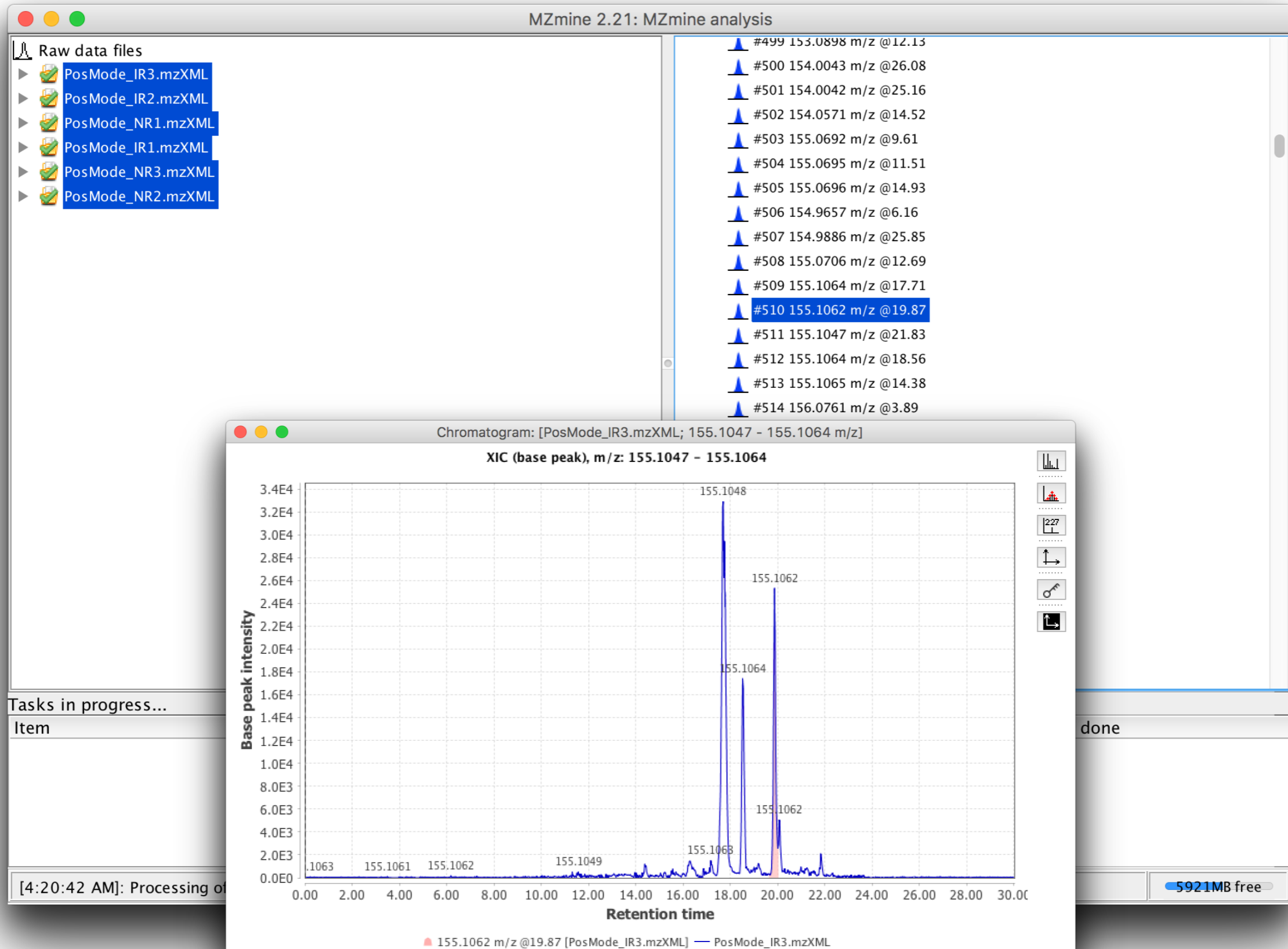
# Deconvolution



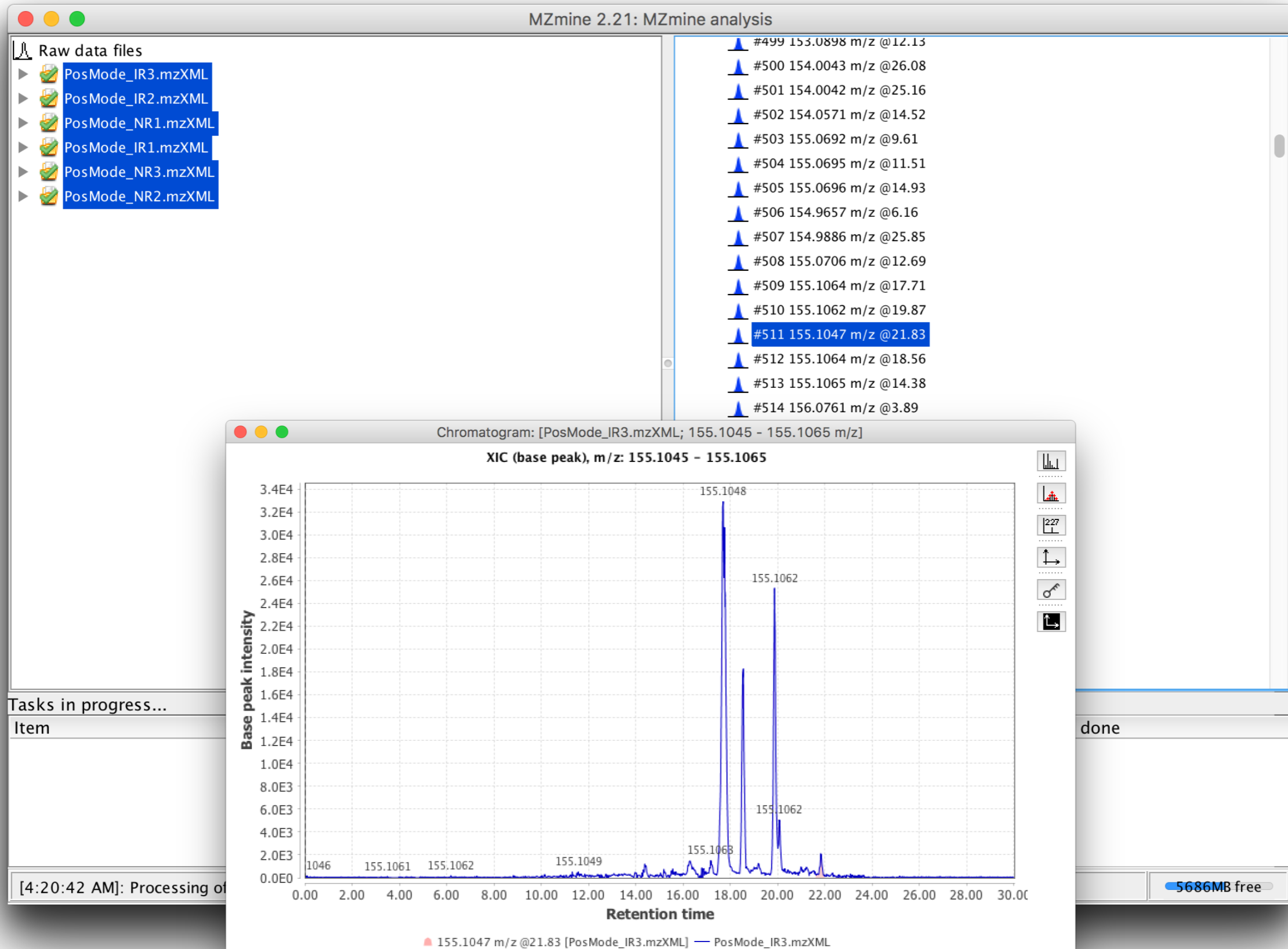
# Deconvolution



# Deconvolution



# Deconvolution





# RT normalizer

The screenshot shows the MZmine 2 software interface. The 'Peak list methods' menu is open, highlighting the 'Retention time normalizer' option. A tooltip for this option explains its function: 'The retention time normalizer attempts to reduce the deviation of retention times between peak lists, by searching for common peaks in these peak lists and using them as normalization standards.' The main window displays a list of peak lists and chromatograms, including 'PosMode\_IR1.mzXML chromatograms deconvoluted' through 'PosMode\_NR2.mzXML chromatograms deconvoluted'. The bottom status bar shows a task progress table and a system tray with a free space indicator.

Item	Priority	Status	% done
Tasks in progress...			

[4:20:42 AM]: Processing of task Updating TIC visualizer of PosMode\_IR3.mzXML done, status FINISHED

3517MB free

# RT normalizer

Please set the parameters

Peak lists 6 selected As selected in main window

Name suffix normalized

m/z tolerance 0.005 m/z or 5.0 ppm

Retention time tolerance 5.0 relative (%)

Minimum standard intensity 1.0E6

Remove original peak list

OK Cancel Help

# RT normalizer

MZmine 2.21: MZmine analysis

Raw data files

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

Peak lists

- PosMode\_IR2.mzXML chromatograms
- PosMode\_NR1.mzXML chromatograms
- PosMode\_IR1.mzXML chromatograms
- PosMode\_IR3.mzXML chromatograms
- PosMode\_NR3.mzXML chromatograms
- PosMode\_NR2.mzXML chromatograms
- PosMode\_IR2.mzXML chromatograms deconvoluted
- PosMode\_IR1.mzXML chromatograms deconvoluted
- PosMode\_NR1.mzXML chromatograms deconvoluted
- PosMode\_IR3.mzXML chromatograms deconvoluted
- PosMode\_NR3.mzXML chromatograms deconvoluted
- PosMode\_NR2.mzXML chromatograms deconvoluted
- PosMode\_IR2.mzXML chromatograms deconvoluted normalized
- PosMode\_IR1.mzXML chromatograms deconvoluted normalized
- PosMode\_NR1.mzXML chromatograms deconvoluted normalized
- PosMode\_IR3.mzXML chromatograms deconvoluted normalized
- PosMode\_NR3.mzXML chromatograms deconvoluted normalized
- PosMode\_NR2.mzXML chromatograms deconvoluted normalized

Tasks in progress...

Item	Priority	Status	% done
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[4:48:21 AM]: Processing of task Retention time normalization of 6 peak lists done, status FINISHED

7008MB free

# Feature alignment

The screenshot shows the MZmine 2 software interface. The 'Peak list methods' menu is open, with 'Alignment' selected. The 'RANSAC aligner' option is highlighted in the sub-menu. The main window displays a list of peak lists, including 'PosMode\_IR2.mzXML chromatograms deconvoluted normalized'. The status bar at the bottom indicates that processing of task Retention time normalization of 6 peak lists is done, status FINISHED, and 6702MB free.

Tasks in progress...

Item	Priority	Status	% done
------	----------	--------	--------

[4:48:21 AM]: Processing of task Retention time normalization of 6 peak lists done, status FINISHED

6702MB free

RANdom SAMple Consensus aligner

# Feature alignment

Please set the parameters

Peak lists 6 selected As selected in main window ...

Peak list name Aligned peak list

m/z tolerance 0.001 m/z or 5.0 ppm

Weight for m/z 20

Retention time tolerance 3 relative (%)

Weight for RT 10

Require same charge state

Require same ID

Compare isotope pattern  Setup..

OK Cancel Help

# Feature alignment

MZmine 2.21: MZmine analysis

Raw data files

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

Peak lists

- PosMode\_IR2.mzXML chromatograms
- PosMode\_NR1.mzXML chromatograms
- PosMode\_IR1.mzXML chromatograms
- PosMode\_IR3.mzXML chromatograms
- PosMode\_NR3.mzXML chromatograms
- PosMode\_NR2.mzXML chromatograms
- PosMode\_IR2.mzXML chromatograms deconvoluted
- PosMode\_IR1.mzXML chromatograms deconvoluted
- PosMode\_NR1.mzXML chromatograms deconvoluted
- PosMode\_IR3.mzXML chromatograms deconvoluted
- PosMode\_NR3.mzXML chromatograms deconvoluted
- PosMode\_NR2.mzXML chromatograms deconvoluted
- PosMode\_IR2.mzXML chromatograms deconvoluted normalized
- PosMode\_IR1.mzXML chromatograms deconvoluted normalized
- PosMode\_NR1.mzXML chromatograms deconvoluted normalized
- PosMode\_IR3.mzXML chromatograms deconvoluted normalized
- PosMode\_NR3.mzXML chromatograms deconvoluted normalized
- PosMode\_NR2.mzXML chromatograms deconvoluted normalized
- Aligned peak list

Tasks in progress...

Item	Priority	Status	% done
------	----------	--------	--------

[9:18:40 AM]: Processing of task Join aligner, Aligned peak list (6 peak lists) done, status FINISHED

7090MB free

# Feature alignment

The screenshot displays the MZmine 2.21 software interface. The main window is titled "MZmine 2.21: MZmine analysis" and is divided into two panes: "Raw data files" and "Peak lists".

**Raw data files pane:** Shows a list of raw data files under the "Raw data files" folder. The files are:

- PosMode\_IR3.mzXML
- PosMode\_IR2.mzXML
- PosMode\_NR1.mzXML
- PosMode\_IR1.mzXML
- PosMode\_NR3.mzXML
- PosMode\_NR2.mzXML

**Peak lists pane:** Shows a list of peak lists under the "Peak lists" folder. The files are:

- PosMode\_IR2.mzXML chromatograms
- PosMode\_NR1.mzXML chromatograms
- PosMode\_IR1.mzXML chromatograms
- PosMode\_IR3.mzXML chromatograms
- PosMode\_NR3.mzXML chromatograms
- PosMode\_NR2.mzXML chromatograms
- PosMode\_IR2.mzXML chromatograms deconvoluted
- PosMode\_IR1.mzXML chromatograms deconvoluted
- PosMode\_NR1.mzXML chromatograms deconvoluted
- PosMode\_IR3.mzXML chromatograms deconvoluted
- PosMode\_NR3.mzXML chromatograms deconvoluted
- PosMode\_NR2.mzXML chromatograms deconvoluted
- PosMode\_IR2.mzXML chromatograms deconvoluted normalized
- PosMode\_IR1.mzXML chromatograms deconvoluted normalized
- PosMode\_NR1.mzXML chromatograms deconvoluted normalized
- PosMode\_IR3.mzXML chromatograms deconvoluted normalized
- PosMode\_NR3.mzXML chromatograms deconvoluted normalized
- PosMode\_NR2.mzXML chromatograms deconvoluted normalized
- Aligned peak list**

The "Aligned peak list" contains the following entries:

- #1 53.0042 m/z @1.98
- #2 53.0042 m/z @1.75
- #3 53.0042 m/z @2.31
- #4 53.0204 m/z @3.34
- #5 53.0043 m/z @8.72
- #6 53.0043 m/z @10.04
- #7 53.0044 m/z @10.55

**Tasks in progress pane:** Shows a table with columns: Item, Priority, Status, % done.

Item	Priority	Status	% done

**System tray:** Shows the time [9:18:40 AM] and the status bar: [9:18:40 AM]: Processing of task Join aligner, Aligned peak list (6 peak lists) done, status FINISHED. Memory usage: 6901MB free.

# Feature alignment

Peak list: Aligned peak list

ID	Average		Identity	Comment	Peak shape	PosMode_IR2.mzXML			PosMode_IR1.mzXML			PosM
	m/z	RT				Status	Height	Area	Status	Height	Area	
1	53.0042	1.98				●	3.8E2	1.4E3	●			●
2	53.0042	1.75				●	2.0E2	2.0E3	●			●
3	53.0042	2.31				●	2.1E2	1.0E3	●			●
4	53.0204	3.34				●	1.0E3	7.3E3	●	4.0E2	4.6E3	●
5	53.0043	8.72				●	9.5E2	1.0E4	●			●
6	53.0043	10.04				●	4.1E2	6.2E3	●			●
7	53.0044	10.55				●	2.3E2	3.2E3	●			●
8	53.0043	17.71				●	5.3E2	1.0E4	●	3.6E2	4.3E3	●
9	53.0042	19.89				●	6.7E2	8.0E3	●			●
10	55.0438	20.74				●	3.6E2	4.3E3	●			●
11	55.0438	21.23				●	3.8E2	7.3E3	●			●
12	56.0515	25.52				●	7.8E3	2.4E5	●	5.1E3	1.7E5	●
13	56.0515	26.36				●	7.1E3	2.4E5	●	4.1E3	9.1E4	●
14	56.9662	29.78				●	6.6E2	1.2E4	●	6.6E2	6.5E3	●
15	56.9665	28.21				●	5.5E2	6.5E4	●	6.4E2	5.3E3	●
16	56.9662	29.26				●	5.7E2	7.4E3	●	6.7E2	6.8E3	●
17	57.0542	25.42				●	3.1E2	3.8E3	●			●
18	57.0546	26.10				●	2.5E2	3.6E3	●			●
19	57.0542	25.88				●	2.8E2	3.5E3	●			●



# Result export

Please set the parameters

Peak lists      Aligned peak list    As selected in main window    ▾    ...

Filename      peaks\_table    ...

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

All    Clear

Export data file elements

- Peak status
- Peak m/z
- Peak RT
- Peak RT start
- Peak RT end
- Peak duration time
- Peak height

All    Clear

Export all IDs for peak     

Identification separator      ;

OK    Cancel    Help

# Result export

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	row ID	row m/z	row retentio	row commer	row number	All identity e	PosMode_IR	PosMode_IR	PosMode_IR	PosMode_IR	PosMode_IR	PosMode_IR	PosMode_IR	PosMode_IR	PosMode_IR
2	1	53.0042038	1.98293333		1		DETECTED	53.0042038	1.98293333	1.9295	2.04505	0.11555	383	1379.252	
3	2	53.004158	1.75446667		1		DETECTED	53.004158	1.75446667	1.63758333	1.85568333	0.2181	201	2049.6675	
4	3	53.0042038	2.30685		1		DETECTED	53.0042038	2.30685	2.24583333	2.34756667	0.10173333	214	1031.6775	
5	4	53.0203787	3.33863889		3		DETECTED	53.0206013	3.34095	3.28993333	3.51928333	0.22935	1020	7301.794	
6	5	53.0043354	8.72179167		2		DETECTED	53.0043449	8.73858333	8.40756667	8.8201	0.41253333	954	10215.055	
7	6	53.0043316	10.0399833		1		DETECTED	53.0043316	10.0399833	9.86405	10.2838667	0.41981667	406	6207.643	
8	7	53.0044365	10.5459		1		DETECTED	53.0044365	10.5459	10.2838667	10.6338167	0.34995	233	3247.371	
9	8	53.0042953	17.7075		3		DETECTED	53.004261	17.7008333	17.29	18.0461667	0.75616667	525	10351.96	
10	9	53.0041841	19.887		3		DETECTED	53.0042915	19.9138333	19.7115	20.1091667	0.39766667	670	8034.66	
11	10	55.0437565	20.7395		1		DETECTED	55.0437565	20.7395	20.6188333	20.9051667	0.28633333	362	4338.465	
12	11	55.0437927	21.2336667		1		DETECTED	55.0437927	21.2336667	21.179	21.7198333	0.54083333	381	7322.64	
13	12	56.0514822	25.515		4		DETECTED	56.051548	25.4006667	24.9853333	25.8103333	0.825	7816	238067.625	
14	13	56.0514851	26.3572917		4		DETECTED	56.0515633	26.0685	26.0145	26.7928333	0.77833333	7131	236129.215	
15	14	56.9662342	29.78125		4		DETECTED	56.9661369	29.7656667	29.5681667	29.9826667	0.4145	664	12432.35	
16	15	56.9665318	28.2060556		3		DETECTED	56.9661484	28.0778333	25.4333333	28.5543333	3.121	553	65493.62	
17	16	56.9661662	29.2618889		3		DETECTED	56.9660683	29.258	29.1181667	29.401	0.28283333	569	7394.27	
18	17	57.0541906	25.419		2		DETECTED	57.0545273	25.5416667	25.5416667	25.8103333	0.26866667	312	3778.68	
19	18	57.0545883	26.0981667		1		DETECTED	57.0545883	26.0981667	26.0746667	26.3835	0.30883333	252	3618.15	
20	19	57.0541687	25.8750833		2		DETECTED	57.0545616	25.9335	25.8103333	26.0746667	0.26433333	277	3502.81	
21	20	58.9992798	3.3313		5		DETECTED	58.9993916	3.30768333	3.16815	3.48596667	0.31781667	792	9304.4065	
22	21	59.0504074	0.08391667		1		DETECTED	59.0504074	0.08391667	0.00415	0.12348333	0.11933333	1644	9120.509	
23	22	59.0503419	29.6788889		3		DETECTED	59.0503998	29.8088333	29.7291667	29.8483333	0.11916667	1042	6156.54	
24	23	59.0505339	28.4990556		3		DETECTED	59.0504341	28.1441667	28.0538333	28.4215	0.36766667	989	16501.39	
25	24	59.0506624	27.4636111		3		DETECTED	59.0504875	27.6018333	27.243	27.6621667	0.41916667	538	9133.91	
26	25	59.0509605	27.2702778		3		DETECTED	59.0504494	27.1616667	26.968	27.243	0.275	365	3552.01	
27	26	59.0507402	29.0935		2		DETECTED	59.0504169	29.0911667	28.8696667	29.4281667	0.5585	1169	28211.93	
28	27	59.0505924	29.5109167		2		DETECTED	59.0504074	29.4528333	29.4281667	29.7291667	0.301	1025	14973	

# What is next?

- Univariate and multivariate statistical analysis
- Determine discriminating metabolites
- Metabolite identification

**Thank you!**