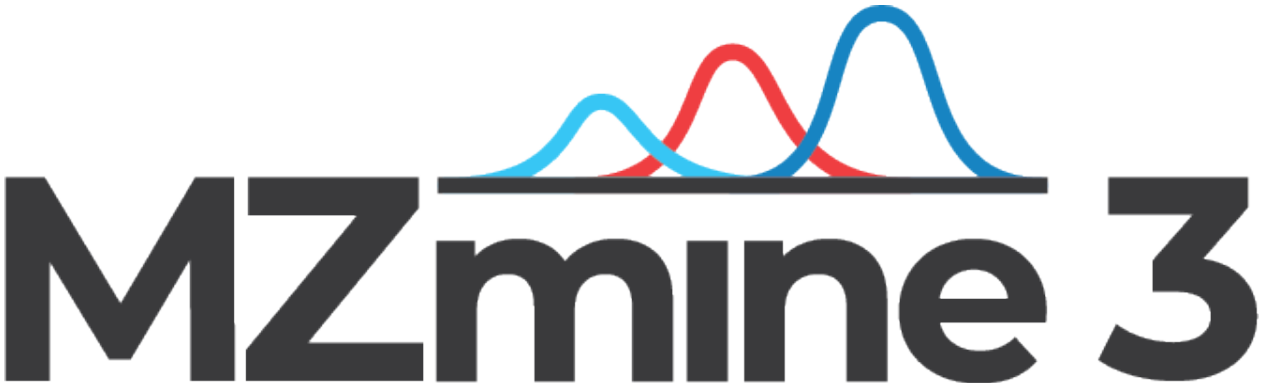


MZmine 3

Documentation

The MZmine Community

Table of contents

1. Welcome to the MZmine 3 wiki!

MZmine 3 is an open-source and platform-independent software for mass spectrometry (MS) data processing and visualization. It enables large-scale metabolomics and lipidomics research by spectral preprocessing, feature detection, and various options for compound identification, including spectral library querying and creation.

Since the introduction of MZmine 2 in 2010, the project has matured into a community-driven, highly collaborative platform and its functions continue to expand based on the users' needs and feedbacks. This has also enabled the tight integration of the MZmine ecosystem with popular third-party software for MS data analysis, such as the SIRIUS suite for *in silico* metabolite annotation, the GNPS platform with Ion Identity Molecular Networking, the MetaboAnalyst web app for univariate and multivariate statistical anlysis, *etc.*

Such a great progress was made possible by the invaluable contribution of many developers from research labs distributed all over the world!

Want to get started with MZmine 3? Check out our getting started page!

1.1 What's new compared to MZmine 2?

MZmine 3 comes with a redesigned and fully customizable GUI based on the JavaFX technology that allow an interactive visualization and validation of results from every processing step.

A completely new data structure provides the flexibility to process any type of mass spectrometry, including LC-MS, GC-MS and MS-imaging. Moreover, MZmine 3 now supports ion mobility, with a dedicated LC-IM-MS data visualization module and feature detection algorithms.

Finally, significant effort was devoted to trace memory issues and bottlenecks, resulting in an unprecendent processing performance and scalability.

COMING SOON! We are implementing the Mass Spec Query Langauge (MassQL) to explore your MS data with human-readable, succinct queries! The project is suppported by the Google Summer of Code program.

1.2 About this documentation

Here you can find documentation for both processing and visualization modules in MZmine 3. Moreover, data processing pipelines for untargeted LC-MS and LC-IMS-MS feature detection are described and general recommendations are given.

COMING SOON! We are currently working on a series of short videotutorials to help get you started with the main features of MZmine 3!

1.3 How to contribute

The MZmine community is always welcoming new developers and contributions! You can contribute by improving existing modules or even adding new featurs in MZmine 3! Please, check out our brief tutorial.

You can also contribute to this wiki and help new users to get started with MZmine 3! See here how to contribute to the documentation.

Last update: April 6, 2022 09:29:09

2. Getting Started

2.1 Download

Download MZmine 3 portable versions or installers from GitHub:

https://github.com/mzmine/mzmine3/releases/latest

2.2 Installation

On Windows and Linux the installers and portable versions should function directly. Windows users might be warned that MZmine is not signed or from a trusted source and have to click run anyways. Before creating your first project, we recommend to set the preferences.

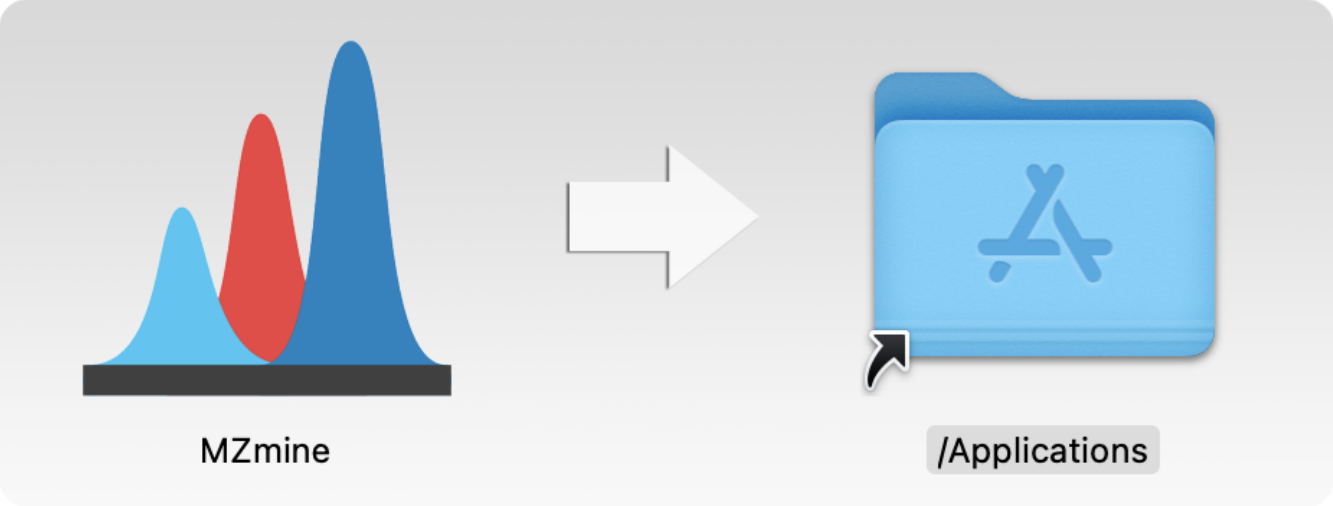
2.2.1 On macOS

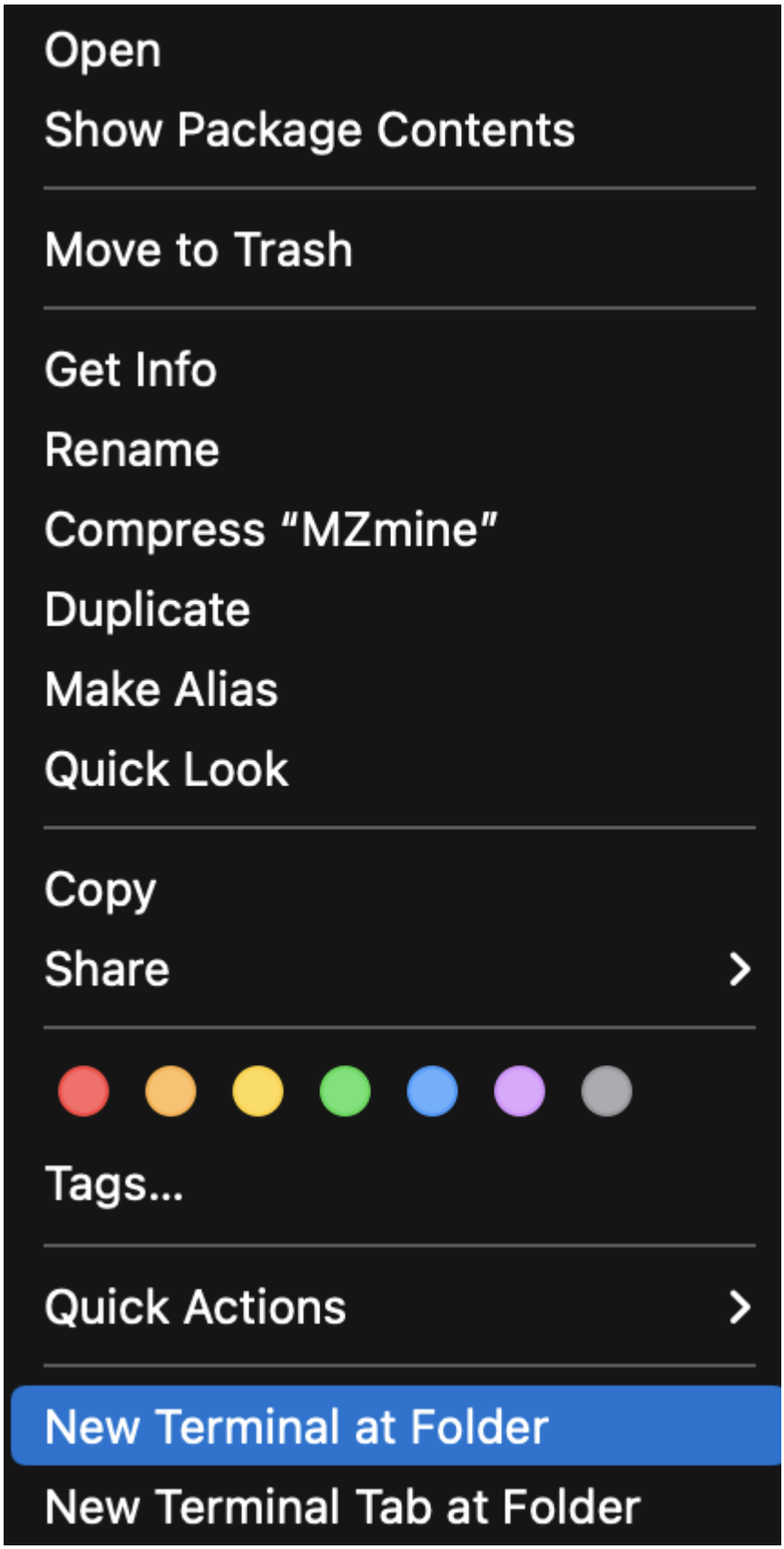
Currently, MZmine 3 lacks a signature for macOS. While we are working on this, user can allow MZmine in the macOS Gatekeeper protection by running the following command in the terminal from the Applications folder.

- Download MZmine and click the MZmine.dmg installer - Drag and drop MZmine into the Applications folder
- Open the Applciations folder, right click (CTRL click) anywhere, e.g., on the MZmine icon, and choose "New Terminal at folder" from the context menu
- Run the provided command to tell macOS to trust the installed version of MZmine. The terminal directory has to be the Applications folder. (Depending on the actual folder use or omit the `../` to jump to the parent directory).
- Approve command with user password
- Start MZmine

```
sudo xattr -cr ../MZmine.app

# if this fails try
sudo xattr -cr MZmine.app
```





The Terminal does not output any log or message.

```
(base) mauriciocaraballo@Mauricios-MacBook-Pro MZmine.app % cd ../
(base) mauriciocaraballo@Mauricios-MacBook-Pro /Applications % sudo xattr -cr MZmine.app
(base) mauriciocaraballo@Mauricios-MacBook-Pro /Applications %
```

Before creating your first project, we recommend to set the preferences.

2.3 Set User Preferences

Before creating your first project, we recommend setting up some things.

1. Set a temporary file directory. Go to *Project → Set preferences → Temporary file directory*. This requires a restart to take effect.

a. We recommend setting the directory to an SSD with enough space for fast processing and visualizations.

b. On Windows, old temporary files are deleted when a new session is started.

2. MZmine 2 projects cannot be imported due to changes in the data structure.
3. MZmine 2 batch files cannot be imported due to parameter optimizations.

You can get familiar with the new GUI here: Main window overview

A quick insight to data processing workflows can be found here: LC-MS workflow or LC-IMS-IMS workflow

You can also check out the new processing wizard under *Processing wizard* in the main menu.

Last update: April 14, 2022 08:25:43

3. Main window overview

The MZmine 3 man window is made up of mainly four important building blocks.

3.1 MS data files and feature lists tab

[1]: The (raw) ms data and feature list tabs. Here you can find your imported data files and processed feature lists. *Hint: you can also import files by dragging & dropping them to the ms data tab.*

3.2 Main content pane

[2]: The main content pane. Visualisations such as a raw data overview or a feature list can be viewed here. This pane can also contain multiple tabs. Every tab can also be opened in a new separate window by right-clicking on the header.

3.3 Main menu

[3]: The main menu. Here you can find methods to import and process your data files and feature lists and visualise the results. Furthermore, projects can be saved and preferences can be set.

3.4 Task overview

[4]: The task overview. Current tasks are displayed and their status and progress are indicated. Tasks can also be canceled by right clicking on a task.

3.5 Page Contributors

SteffenHeu

Last update: April 5, 2022 13:22:07

4. Processing modules

4.1 I/O

4.1.1 **Data import**

LC-MS data

DESCRIPTION

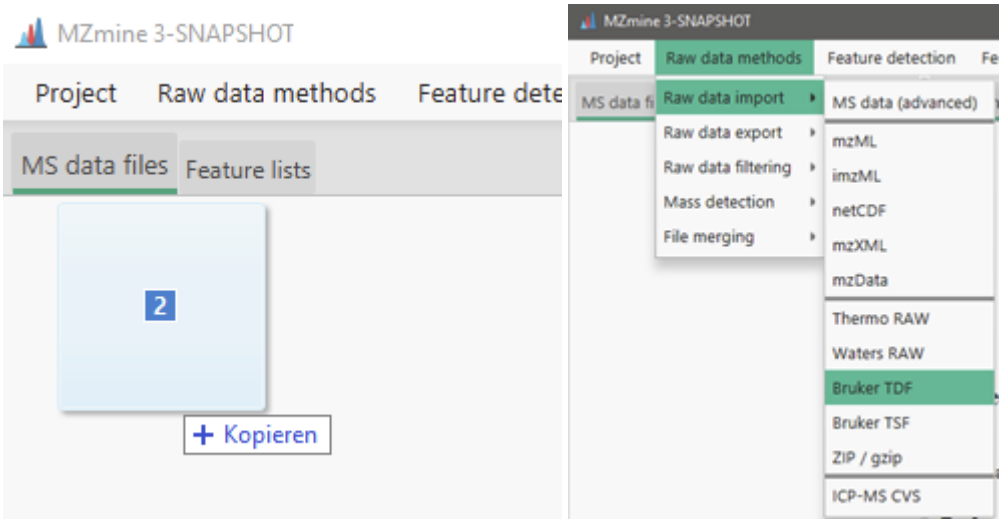
Raw data can be imported via **Raw data methods → Raw data import**.

Note that multiple data files/folders can be dropped into the **Raw data methods → Raw data import → MS data (advanced)** dialog.

If individual modules are used, folder based formats can only imported as one folder at a time.

When using the **MS data (advanced)** dialog, inexperienced users should deactivate the direct mass detection steps, since they alter the raw data on the import. Mass detection is then performed, when the scans are loaded and only peaks above the noise level are imported.

Alternatively, you can simply drag & drop the raw data into the raw data list of the main window.



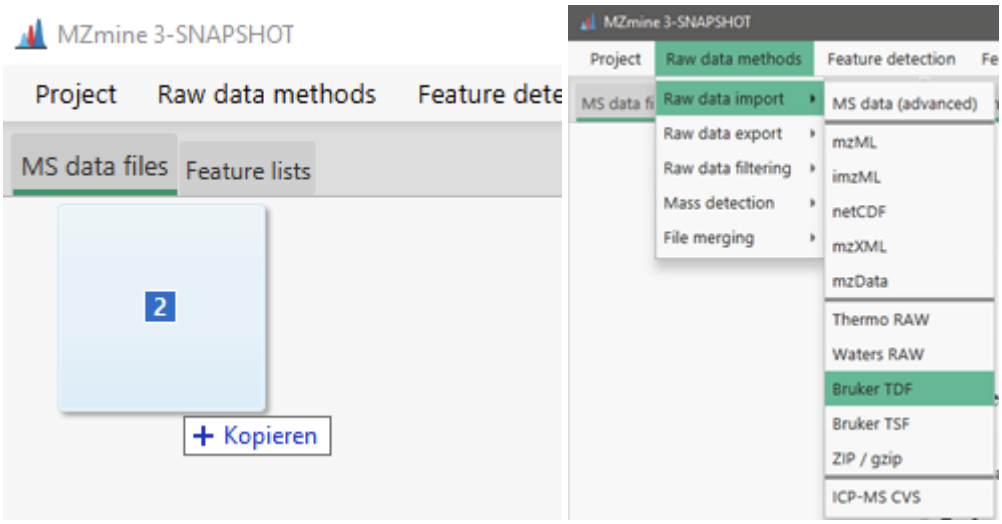
LC-IMS-MS data

As any other data format, ion mobility data can be imported via **Raw data methods → Raw data import**.

Note that multiple .tdf data folders can be dropped into the **MS data (advanced)** dialog. The Bruker TDF import can only select a single folder.

When using the **MS data (advanced)** dialog, inexperienced users should deactivate the direct mass detection steps, since they alter the raw data on the import. Mass detection is then performend, when the scans are loaded and only peaks above the noise level are imported.

Alternatively, you can simply drag & drop the raw data into the raw data list of the main window.



Last update: September 23, 2022 17:08:14

4.1.2 **Data export**

Description

Export scans - Spectra/mass list to mgf, txt, msp and mzML export

This module exports scans or mass lists. MZmine allows several types of data export:

- to *.mzML,
- to *.netCDF,
- spectra/mass list to mgf, txt, msp and mzML export,
- MS(n) trees export.

Parameters

Raw data files

One or more raw data files that contain the scans/mass lists

Optional mass list

If checked, mass lists are exported instead of raw scans

File

The destination

Format

- mgf: MASCOT generic format - useful for SIRIUS
- txt: Plain text format
- msp: NIST search format
- mzML: Open format standard for MS data

Last update: September 23, 2022 17:08:14

4.2 File merging

4.2.1 **Merge raw data files**

Description

Raw data methods → File merging → Raw data file merging

This module merges all raw data files into a new. For example to combine positive and negative scans with MS2, all from different raw data files.

Method parameters

Raw data files

Raw data files the module will take as an input.

Mode

- MERGE PATTERN:

  Merge files based on a grouping identifier which can be a name suffix or prefix. (e.g., Sample_A_1, Sample_B_1: Use AFTER LAST _ to combine these files)
- MERGE SELECTED:

  Merge all selected files to a new

Grouping identifier position

Search for the specific group identifier before the first or after the last marker.

Position marker

The marker that splits the specific group identifier from the rest of the file names.

MS2 marker

If a raw data file has this marker in its name, it will only be used as a source of MS2 (MSn) scans. All MS1 scans of this file will be discarded.

Suffix

Suffix to be added to the new file name.

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4.2.2 Mobility scan merging

Description

Raw data methods → File merging → Mobility scan merging

This module merges mobility scans in each **single ion mobility data file** at the same retention time to a summed frame spectrum.

The merged frame spectrum is used if a *.mzML file is imported. The merged frame spectrum is required to gain access to MZmine's LC-MS functionality.

❗ This step uses the centroided and thresholded data produced by the mass detection step.

⚠️ This step is not required when importing native **Bruker .tdf or .tsf** data from .d folders. When importing native Bruker data, a merged spectrum for the frame is created automatically by the vendor library.

Parameters

Raw data files

Raw data files the module will take as an input.

Noise level

Data points beyond the defined noise level threshold will be ignored.

Merging type

The way to calculate intensities. Intensities can be either averaged, summed, or the maximum value can be chosen.

m/z weighing

Chosen function is used to weigh m/z values by their intensities. The available options are:

- None,
- Linear,
- log10,
- log2,
- square root,
- or cube root.

Scans

Selects the scans that should be included.

m/z tolerance

Maximum allowed difference between two m/z values in order for them to be considered the same.

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4.3 Raw data filtering

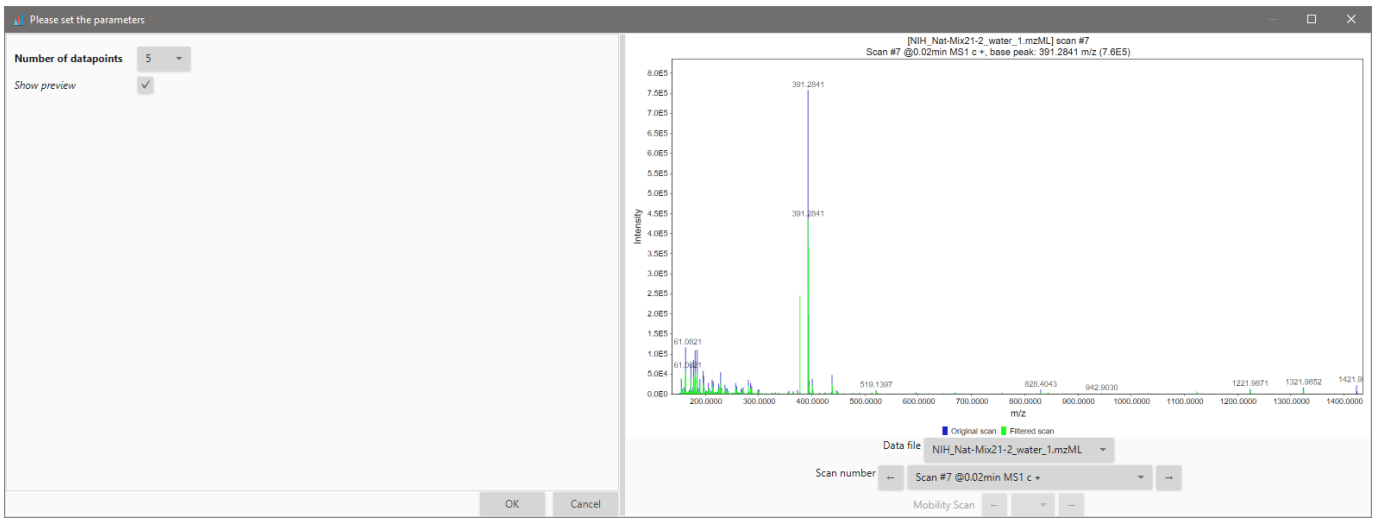
4.3.1 **Scan by scan filtering**

Description

Raw data methods → Raw data filtering → Scan by scan filtering

This module can be used to preprocess data in each scan using various filters.

The preview shows the superposed spectra of the scan before and after the Savitzky-Golay filter is applied.



Filters

MEAN FILTER

For each data point, the filter assigns to it the intensity average of all the datapoints inside the user defined window. The window is centered in the mass value of this data point.

Parameters

Window length

One-sided length of the m/z smoothing window.

SAVITZKY GOLAY FILTER

The Savitzky–Golay smoothing filter was first described in 1964 by Abraham Savitzky and Marcel J. E. Golay.

The Savitzky–Golay method essentially performs a **local polynomial regression (of degree k)** on a series of values (of at least k+1 points which are treated as being equally spaced in the series) to determine the smoothed value for each point.

http://en.wikipedia.org/wiki/Savitzky-Golay_smoothing_filter

Parameters

Number of datapoints

This number can be 5, 7, 9, 11, 13 or 15.

RESAMPLING FILTER

Each scan is divide in m/z bins whose length is defined by the user in the parameters. The mass of the new data point will be in the middle of each m/z bin's space. Its intensity is the average of the intensity of all the data points inside the bin.

Parameters

m/z bin length

The length of m/z bin.

ROUND RESAMPLING FILTER

All data points in each scan is shifted to the nearest rounded integer (**ion number**). The mass of the new data point will be on the rounded value.

If several data points are competing for the same rounded value / mass, the new data point's intensity is the average of all the competing data points.

Unless **"Sum duplicate intensities"** is checked, leading to a single new data point with intensity equal to the sum of the intensities of all the competing data points.

⚠️ If the scan is not centroided, its spectrum is first turned into centroid using a default "local maxima" algorithm, then the same algorithm as described above is applied.

Parameters

Sum duplicate intensities

Sums ions count (intensity) of m/z peaks competing for being rounded at same m/z unit. If unchecked, the intensities are averaged rather than summed.

Remove zero intensity m/z peaks

Clear all scans spectra from m/z peaks with intensity equal to zero.

Last update: September 23, 2022 17:08:14

4.3.2 Crop filter

Description

Raw data methods → Raw data filtering → Crop filter

This module performs cropping of raw data files based on the user-defined parameter range defined by user. This allows user to obtain a new raw data file that contains only the information from the range of interest.

Parameters

Scans

Define scan filtering parameters, which include:

- Scan number,
- Base filtering number,
- Retention time,
- Mobility,
- MS level,
- Scan definition (matching some pattern),
- Polarity,
- and Spectrum type.

m/z

m/z boundary of the cropped region

Suffix

This string is added to filename as suffix

Remove source file after filtering

If checked, only filtered file version is stored.

Last update: September 23, 2022 17:08:14

4.3.3 Baseline correction

Description

Raw data methods → Raw data filtering → Baseline correction

This module performs baseline correction on raw data files. It is designed to **compensate for gradual shifts** in the chromatographic baseline by detecting the baseline and then subtracting it from the raw data intensity values.

The module proceeds as follows for each raw data file passed to it:

- The full range of m/z values present in the raw data is divided into a series of bins of a specified width (see m/z bin width).
- For each bin a chromatogram is constructed from the raw data points whose m/z values fall within the bin. This chromatogram may be either the base peak chromatogram or total ion count (TIC) chromatogram.
- The raw intensity values of each data point in a bin are corrected by subtracting the bin's baseline. Subtraction of baseline intensity values proceeds according to the type of chromatogram used to determine the baseline.

  If the **base peak chromatogram** was used then the corrected intensity values are calculated as follows:

  $$I_{corr}=max(0,I_{orig}-I_{base})$$

  If the **TIC chromatogram** was used then the corrected intensity values are calculated as follows:

  $$I_{corr}=max(0,I_{orig}*(1-I_{base}/I_{max}))$$

  where I_{orig}, I_{base}, I_{max}, and I_{corr} are the original, baseline, maximum and corrected intensity values, respectively, for a given scan and m/z bin.

  If I_{base} is less or equal to zero then no correction is performed, i.e. $I_{corr}=I_{orig}$.
- A new raw data file is generated from the corrected intensity values.

Parameters

Filename suffix

The text to append to the name of the baseline corrected raw data file.

Chromatogram type

TIC: total ion count, i.e. summed intensities per scan, or

Base peak intensity: maximum intensity per scan.

MS-level

MS level to which to apply correction. Select "0" for all levels.

Use m/z bins

Baselines can be calculated and data points corrected per m/z bin or to the entire raw data file. If no binning is performed then a single chromatogram is calculated for the entire raw data file and its baseline used to correct the full data file. No binning is very quick but much less accurate and so is only suitable for fine-tuning the smoothing and asymmetry parameters.

m/z bin width

The width of the m/z bins if binning is performed (see use m/z bins). Smaller bin widths result in longer processing times and greater memory requirements. Avoid values below 0.01.

Correction method

The width of the m/z bins if binning is performed (see use m/z bins). Smaller bin widths result in longer processing times and greater memory requirements. Avoid values below 0.01.

R engine

This option allows you to choose between two Java libraries to communicate with R - RServe or RCaller.

Remove source file after baseline correction

Whether to remove the original raw data file once baseline correction is complete.

Correction methods

More information on correction methods is available in CRAN decription of baseline package

ASYMMETRIC BASELINE CORRECTOR

This corrector estimates a baseline using asymmetric least squares and subtracts it from the data.

Additional parameters

Smoothing

The smoothing factor (>=0), generally ranges from 1E5 to 1E8. The larger this factor is, the smoother the baseline.

Asymmetry

Default value is 1E-3. The weight (0 <= p <= 1) for points above the trend line, whereas 1-p is the weight for points below it. Naturally, p should be small for estimating baselines.

ROLLING BALL CORRECTOR

The corrector estimates a trend based on the **Rolling Ball algorithm**, and subtracts it from the raw data intensity values. (Ideas from **Rolling Ball algorithm for X-ray spectra by M.A.Kneen and H.J. Annegarn**. Variable window width has been left out).

Additional parameters

wm (number of scans)

Width of local window for minimization/maximization (in number of scans).

ws (number of scans) Width of local window for smoothing (in number of scans).

PEAK DETECTION BASELINE CORRECTOR

The corrector estimates a trend based on the Peak Detection algorithm, and subtracts it from the raw data intensity values. Peak detection is done in several steps sorting out real peaks through different criteria. Peaks are removed from spectra and minimums and medians are used to smooth the remaining parts of the spectra. (A translation from **Kevin R. Coombes et al.'s MATLAB code** for detecting peaks and removing baselines).

Additional parameters

left (number of scans)

Smallest window size for peak widths (in number of scans).

right (number of scans)

Largest window size for peak widths (in number of scans).

lwin (number of scans)

Smallest window size for minimums and medians in peak removed spectra (in number of scans).

rwin (number of scans)

Largest window size for minimums and medians in peak removed spectra (in number of scans).

snminimum

Minimum signal to noise ratio for accepting peaks.

mono

Monotonically decreasing baseline if ‘mono’ > 0.

multiplier

Internal window size multiplier.

RUBBER BAND CORRECTOR

The corrector estimates a trend based on the Rubber Band algorithm (which determines a convex envelope for the spectra - underneath side), and subtracts it from the raw data intensity values.

Additional parameters

noise

Ignored if **"auto noise"** is checked. Noise level to be taken into account.

auto noise

Determine noise level automatically (from lower intensity scan).

df Degree of freedom.

spline

Logical indicating whether the baseline should be an interpolating spline through the support points or piecewise linear.

bend factor

Does nothing if equals to zero. Helps fitting better with low **"df"**.

💡 Try starting with value around 5E4.

LOCAL MINIMA + LOESS CORRECTOR

The corrector estimates a trend based on Local Minima + LOESS (smoothed low-percentile intensity), and subtracts it from the raw data intensity values.

Additional parameters

method

"**loess**" (smoothed low-percentile intensity) or "**approx**" (linear interpolation).

bw

The bandwidth to be passed to loess.

breaks

Number of breaks set to m/z values for finding the local minima or points below a certain quantile of intensities; breaks -1 equally spaced intervals on the log(m/z) scale.

break width (number of scans)

Width of a single break. Usually the maximum width (in number of scans) of the largest peak.

⚠️ Overrides **"breaks"** value.

qntl

If 0, find local minima; if >0 find intensities < qntl*100th quantile locally.

Requirements

⚠️ This module relies on the local installation of R statistical computing software and several R packages.

Quick install

The whole thing can be setup as follows by running the following code in R:

```
install.packages(c("Rserve", "ptw", "baseline", "hyperSpec"))
source("http://bioconductor.org/biocLite.R")
biocLite("PROcess")
```

Details

- Rserve (All correctors): provides an interface between MZmine and R. To install Rserve from CRAN packages run R and enter:

  install.packages("Rserve")

- ptw (Asymmetric corrector): parametric time-warping provides the asymmetric least-squares implementation. To install ptw run R and enter:

  install.packages("ptw")

- baseline (RollingBall and PeakDetection correctors): provides a trend based on "Rolling Ball" and "Peak Detection" algorithms implementation. To install baseline run R and enter:

  install.packages("baseline")

- hyperSpec (RubberBand corrector): provides a trend based on "Rubber Band" algorithm (which determines a convex envelope for the spectra) implementation. To install hyperSpec run R and enter:

  install.packages("hyperSpec")

- PROcess (Local Minima + LOESS corrector): provides the local minima search + LOESS (smoothed low-percentile intensity) implementation. To install PROcess run R and enter:

  source("http://bioconductor.org/biocLite.R") biocLite("PROcess")

References

[1] Boelens, H.F.M., Eilers, P.H.C., Hankemeier, T. (2005) "Sign constraints improve the detection of differences between complex spectral data sets: LC-IR as an example", Analytical Chemistry, 77, 7998 – 8007.

[2] Rserve "A TCP/IP server which allows other programs to use facilities of R"

https://rforge.net/Rserve.

TODO Add images for each type of baseline correction

Last update: September 23, 2022 17:08:14

4.3.4 **Align scans (MS1)**

Description

Raw data methods → Raw data filtering → Align scans (MS1)

This module aligns scans for small fluctuations correlating consecutive scans.

Parameters

Suffix

This string is added to filename as suffix.

Horizontal scans

Number of scans to be considered in the correlation (to the left and to the right of the scan being aligned).

Max Vertical Alignment

Maximum number of shifts to be compared. This depends on equipment, normally this should be 1.

Minimum height

Minimum intensity to be considered for align correlation.

If chromatogram height is below this level, it is not used in the correlation calculation.

Correlation in log

Transform intensities to *log* scale before comparing correlation.

Remove previous files

Remove processed files to save memory.

Last update: September 23, 2022 17:08:14

4.3.5 **Scan smoothing (MS1)**

Description

Raw data methods → Raw data filtering → Scan smoothing (MS1)

This module averages intensity values within a time-scan frame.

Parameters

Suffix

This string is added to filename as suffix.

Time (min)

Time span over which intensities will be averaged in the same m/z over scans.

⚠️ The max between this and Scan Span will be used.

Scan span

Number of scan in which intensities will be averaged in the same m/z.

⚠️ The max between this and Time Span will be used.

m/z tolerance

m/z range in which intensities will be averaged.

⚠️ The max between this and m/z points will be used. If both equal 0 no m/z smoothing will be performed.

m/z min points

Number of m/z points used to smooth.

⚠️ The max between this and m/z tol will be used. If both equal 0 no m/z smoothing will be performed.

Min height

Minimum intensity of the highest data point in the chromatogram.

If chromatogram height is below this level, it is not used in the average calculation.

Remove previous files

Remove processed files to save memory.

Last update: September 23, 2022 17:08:14

4.4 Mass detection

4.4.1 **Mass detection**

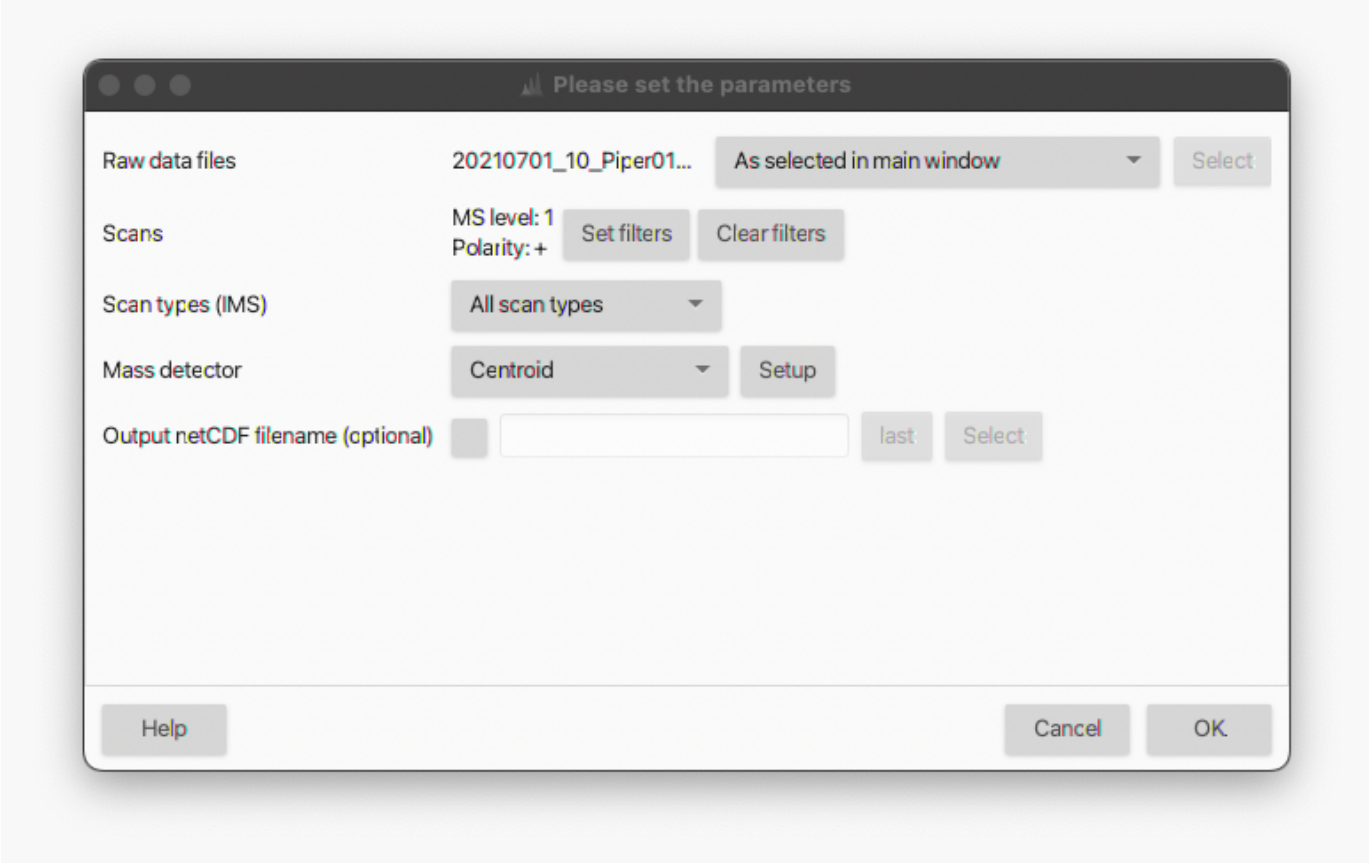
Description

Raw data methods → Mass detection → Mass detection

The mass detection module generates a mass list (*i.e.* list of m/z values and corresponding signal intensities) for each scan, in each raw data file. During the mass detection, profile raw data are centroided and a noise filtering is performed based on a user-defined threshold (see Setting the noise level).

The available algorithms are described here.

Parameters



Raw data files

Select the input raw data files for the mass detection. All the imported data files can be processed in bulk (*i.e. All raw data files*), or a subset can be selected directly from the *MS data files* panel (*i.e. As selected in the main window*) or based on the filename (*i.e. File name pattern*) As an alternative, the files' directory can be also specified (*i.e. Specific raw data files*). Finally, if the *Those created by previous batch step* option is selected, MZmine takes the output of the last processing step as input. This option is only avaialble for batch processing.

Scans

Select (or filter out) the MS scans to be processed. Several filters are available (*Select filters* button). A scan number, RT and mobility range can be set (*i.e Scan number*, *Retention time* and *Mobility* options); only the scans belonging to the defined range(s) will be processed. The *Base Filtering Integer* option allows to prcesses one every-*N* scans. The *Scan definition* field can

be used to filter scans based on the scan's description normally included in the raw file's metadata (*e.g.* FTMS). Scans can also be filtered by *MS level* (*i.e.* 1, 2, ..., *N*), polarity and spectrum type (*i.e.* Centroided, Profile and Thresholded).

Scan types (IMS)

This parameter applies only to IM data and determines if *mobility scans*, *frame scans* or both (*i.e. All scan types*) are processed. For more details about *mobility* and *frame scans*, see here.

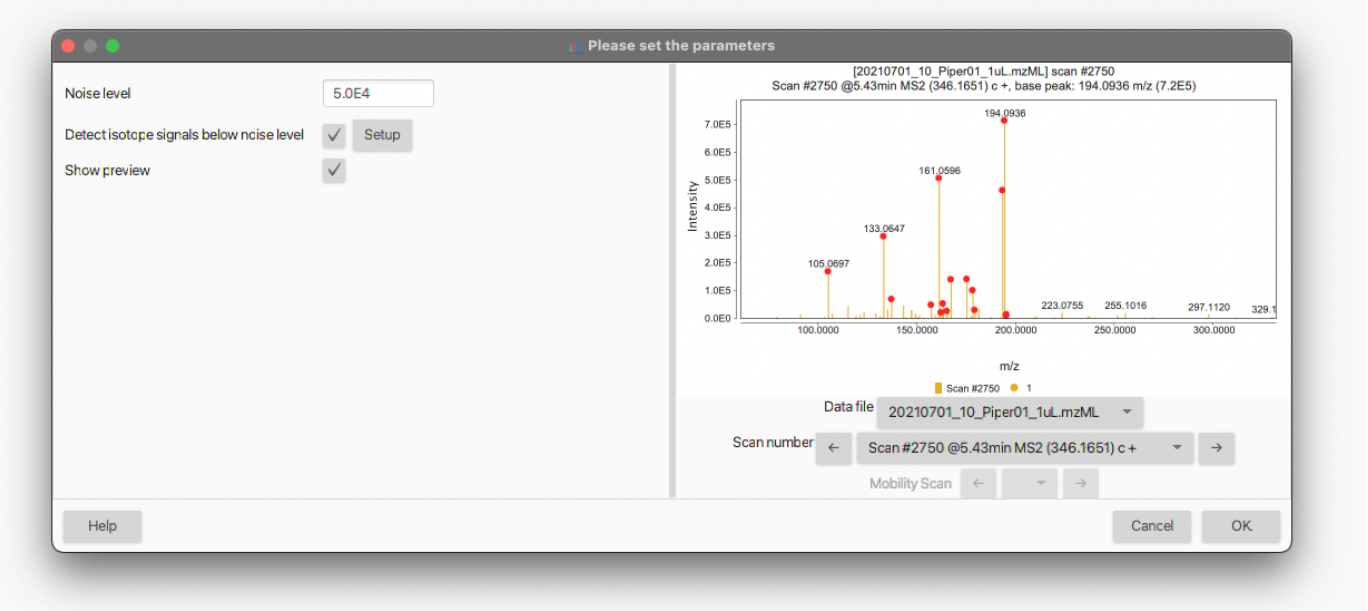
Tip. Since *frame scans* are obtained by merging multiple *mobility scans*, the noise thresholds will likely be different. However, only one noise level can be set per processing step. Therefore, if one wants to run the mass detection for *mobility* and *frame scans* using different noise cutoffs, two module calls are required. As an alternative, mass detection can be performed only on the *mobility scans* by selecteing the appropriate noise level. *Mobility scans* can then be merged into *frame scans* with a dedicated module.

Mass detector

Select the algorithm to be used for the mass detection. Several mass detection algorithms are available and can be selected in the drop-down menu. The choice depends on the raw data characteristics (profile/centroided, mass resolution, etc.). The *Centroid* algorithm must be used for already-centroided data. A step-by-step guide to convert profile into centroided data is provided in the GNPS documentation Other algorithms are available for profile raw data and are described in more details here. The *Exact mass* algorithm is highly recommended for profile HRMS data. When *Auto* is selected, the *Centroid* and *Exact mass* algorithms are used by default for centroided and profile data, respectively.

SETTING THE NOISE LEVEL

All the mass detection algorithms allow to set a threshold for the noise filtering (*i.e. Noise level*) by hitting the *Setup* button next to the *Mass detector* field. A dialog box like the following will open up:



The noise threshold can be entered either in standard or scientific notation. By checking the *Show preview* box, an interactive visualization panel will open to help the user to adjust the noise level (see also How do I determine the noise level in my data?). The red dots denotes the mass signals retained in the mass list according to the set threshold. Different data files and scan numbers can be visualized using the corresponding drop-down menus.

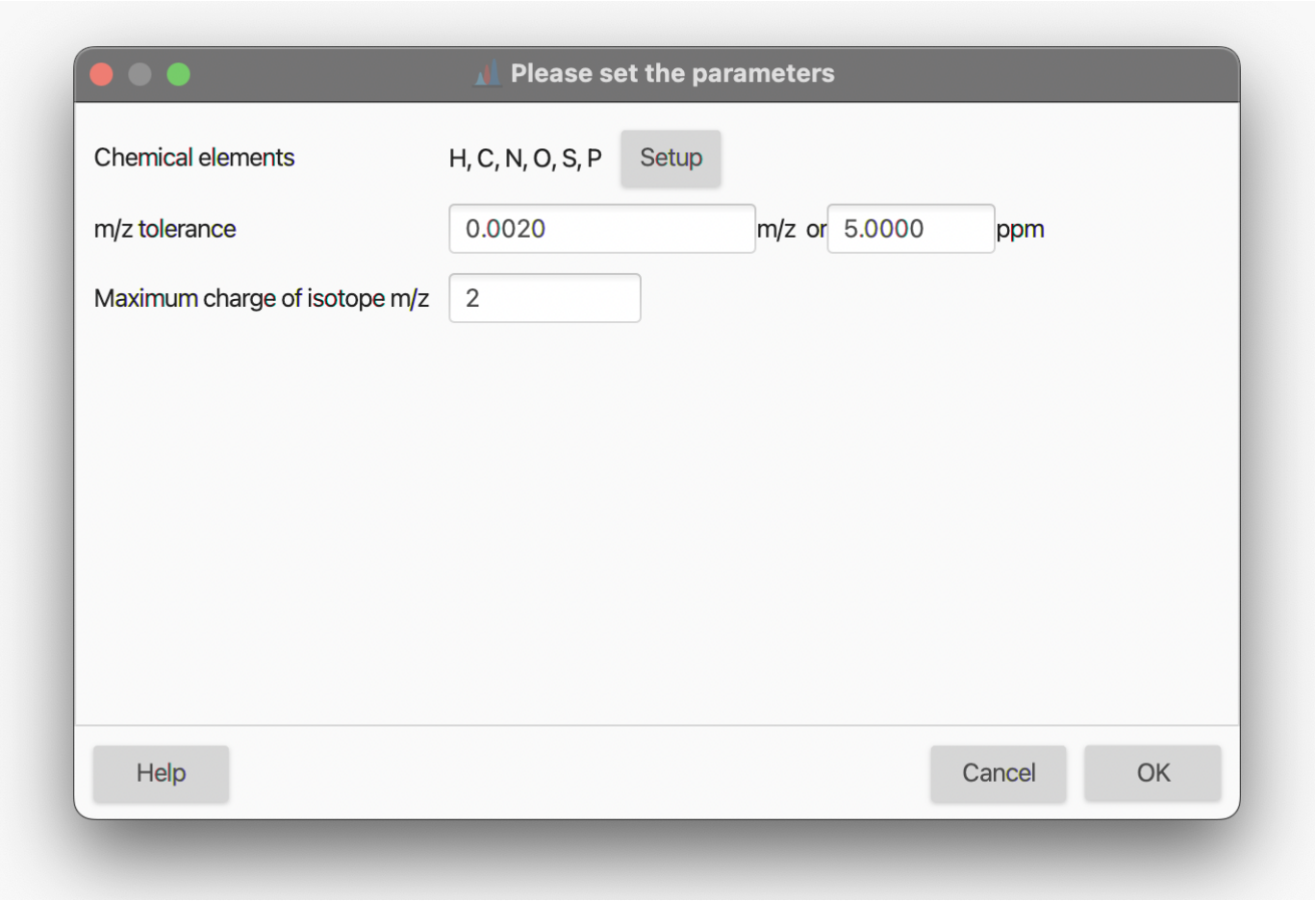
DETECT ISOTOPE SIGNALS BELOW NOISE LEVEL

The *Centroid* and *Exact mass* algorithms provide the option to retain signals that are below the noise level (and would be otherwise discarded), but correspond to isotopes of the detected masses. Theoretical isotopic distributions are calculated for each mass detected in the *mass list* based on the speciefied chemical elements. If a signal below the noise threshold that matches a theoretical isotopic mass is found in the raw data, it will be included in the final mass list.

Tip. In the case of LC-MS data processing, the low-intensity isotope signals included in the final mass list will undergo the whole feature detection workflow (see, for example, LC-MS data processing workflow. Due to the low intensity, these masses often produce LC peaks with poor peak shape during the chromatogram building step and might be discarded if they do not meet

the user-defined parameters (*e.g.* minimum number of data points and intensity, see ADAP chromatogram builder for more details). Therefore, it might be advisable not to use this option during the mass detection, but rather use the Isotope finder module (CREATE DOC)

By ticking the corresponding checkbox and hitting the *Setup* button, the following dialog box opens up:



Chemical elements

Elements considered when generating the isotopic distributions. Select the elements from the periodic table by hitting the *Setup* button.

***m/z* tolerance**

Maximum allowed difference between measured and theoretical isotope *m/z*. It is an intra-scan *m/z* tolerance. The tolerance can be set in *m/z*, ppm or both. Since mass deviations expressed in ppm are dependent on the *m/z* (*e.g.* higher at low *m/z* and lower at high *m/z*), MZmine automatically uses the largest tolerance.

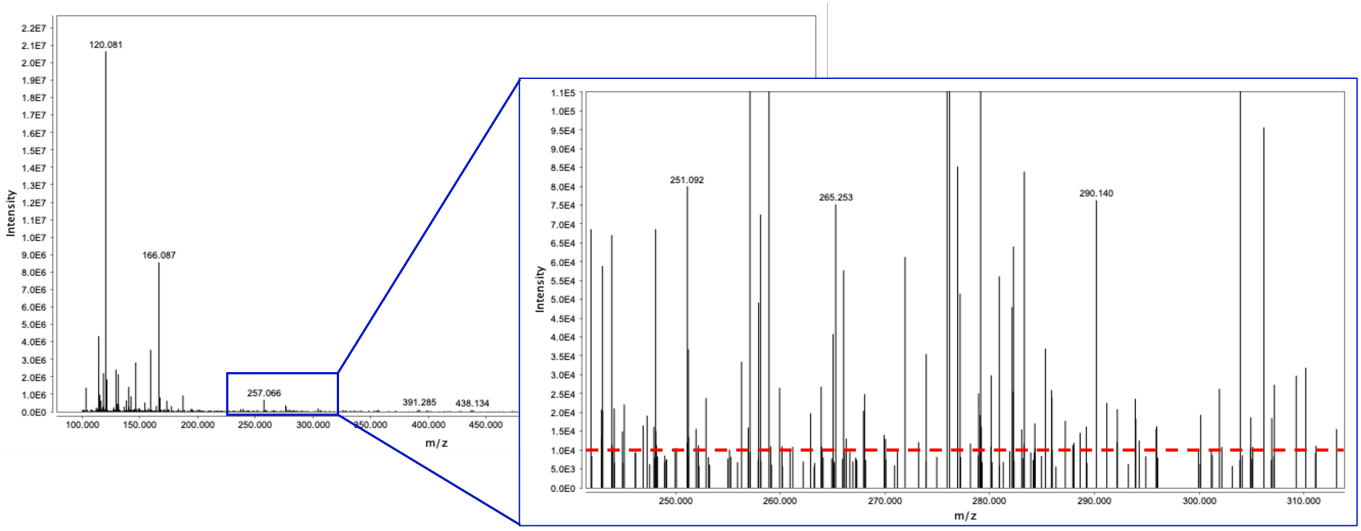
Maximum charge of isotope *m/z*

Maximum allowed charge state of the isotope to be retained in the mass list. Default value is 1.

How do I determine the noise level in my data?

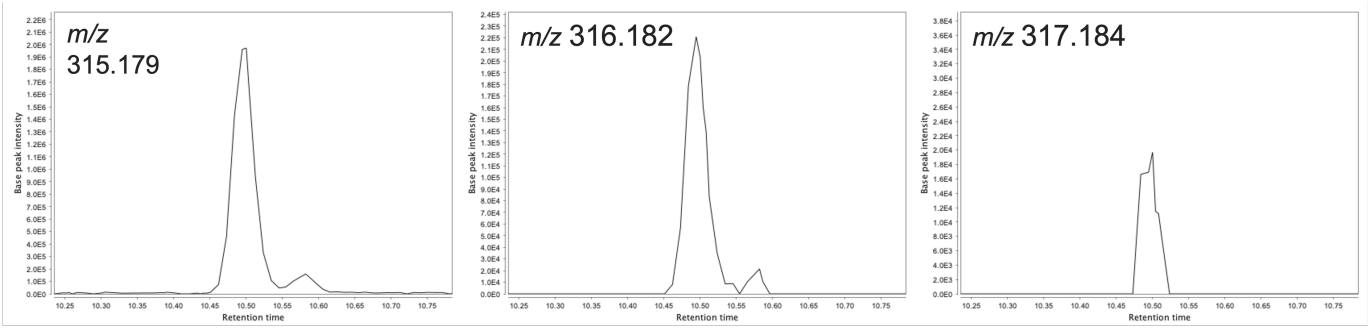
The backgorund noise level largely depends on the mass spectrometer and detector type. For example, Orbitrap instruments normally provides higher signal intensities than TOF devices. To provide some numbers, while 1.0E2 - 1.0E3 could be an appropriate noise level for TOF analyzers, the same would be overly low for Orbitrap instruments (which normally require 1.0E4 - 1.0E5).

The best way to determine the instrumental noise level is undoubtedly by looking at the raw data. The backgorund noise (often referred to as "grass" in technical jargon) is characterized by several signals having the same intensity and no clear pattern among them (see Figure).



Since these signals are produced by electrical and/or mechanical noise, rather than actual ions being detected, they should be excluded from the mass detection and downstream data processing. The red dashed line in the figure corresponds to a hypothetical noise level (1.0E4 in this case) that would filter out most of the "grass"-type noise from the mass detection.

Another way, more releant for the fearure detection, to determine the noise level consists of picking a mid-intensity LC peak and extract the EICs of its 13C isotopes. When the chromatographic peak shape starts to deteriorate, it means we are approaching the instrument detection limit (see Figure).



Such approach can also be useful to determine other parameters in the feature detection such as the **Group intensity threshold** and **Min highest intensity** parameters in the ADAP chromatogram builder module.

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4.4.2 **Mass detection algorithms**

Mass detection can be done with the following six algorithms:

- Centroid
- Factor of the lowest signal
- Exact mass
- Local maxima
- Recursive threshold
- Wavelet transform
- Auto

Centroid

This mass detector is suitable for already centroided data.

Centroid algorithm assumes that each signal above a given noise level is a detected ion.

Factor of the lowest signal

This mass detector is suitable for already centroided data.

It removes all data points below a spectrum's lowest intensity multiplied by a factor.

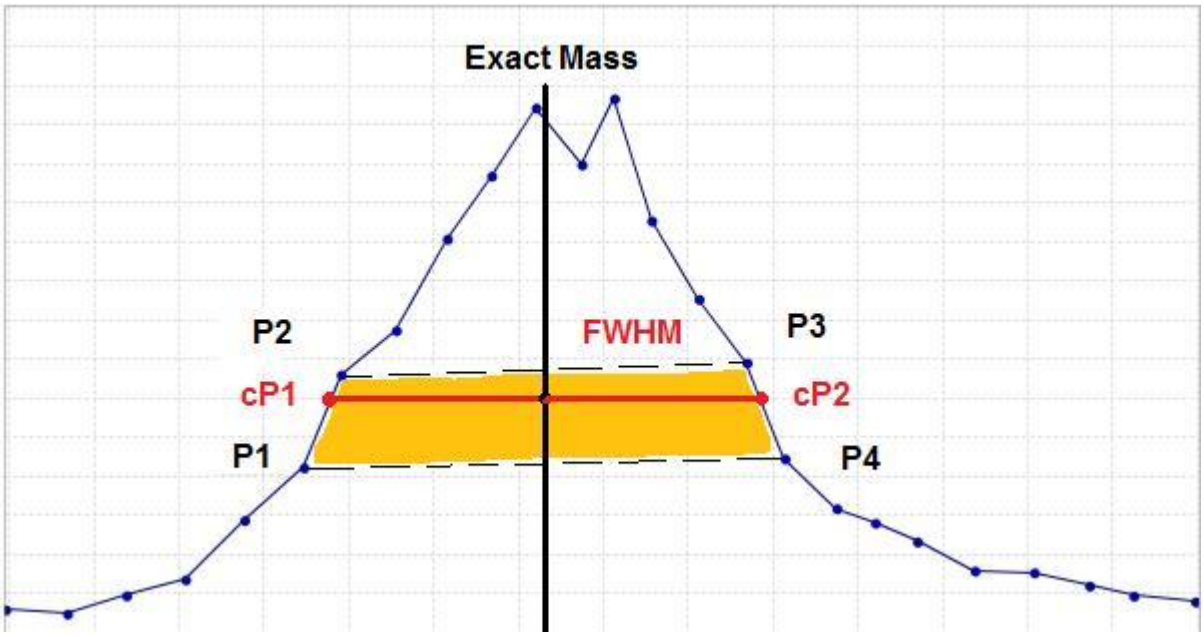
Exact mass

The exact mass algorithm is highly recommended for profile MS data.

This mass detector first searches for all local maxima within the spectrum, which then form candidate ions.

This method calculates the exact mass of a peak using the **FWHM** (full width at half maximum) concept and linear equation (\(y = mx + b\)). FWHM is the difference between the two values of the independent variable at which the dependent variable equals half of its maximum value.

First, the method locates the data points located nearest to the peak center at half of the maximum intensity (P1, P2, P3, P4). With these four points it calculates two points (cP1, cP2) that define the width of the peak. The exact mass is then obtained as the center of the width.



This method is suitable for high-resolution MS data, such as provided by FTMS instruments.

If the continous data is too noisy, one can use recursive threshold algorithm.

Local maxima

This very simple mass detector detects all local maxima within the spectrum, except the signals below the given noise level. The practical usability of this method on real MS data is limited, but it is useful to demonstrate and understand the functionality of mass detection using the preview plot.

Recursive threshold

The algorithm finds all m/z ranges within the given limit in a recursive way.

Initially, it looks at the whole range of data points. If the m/z width of this range is not within given limits, a minimum data point is found and used to split the range in two parts. The same algorithm is then applied recursively on each part. Recursion continues until all m/z ranges fitting into the given width limits are found.

Final m/z values are determined as local maxima of the identified m/z ranges.

This mass detector is suitable for continuous data, which has too much noise for the Exact mass detector to be used, but which shows a consistent width of m/z peaks.

Additional parameters

Min m/z peak width: Minimum acceptable peak width in m/z.

Max m/z peak width: Maximum acceptable peak width in m/z.

Recursive threshold method can be used with the noisy continuous data that shows a consistent width of m/z peaks.

Wavelet transform

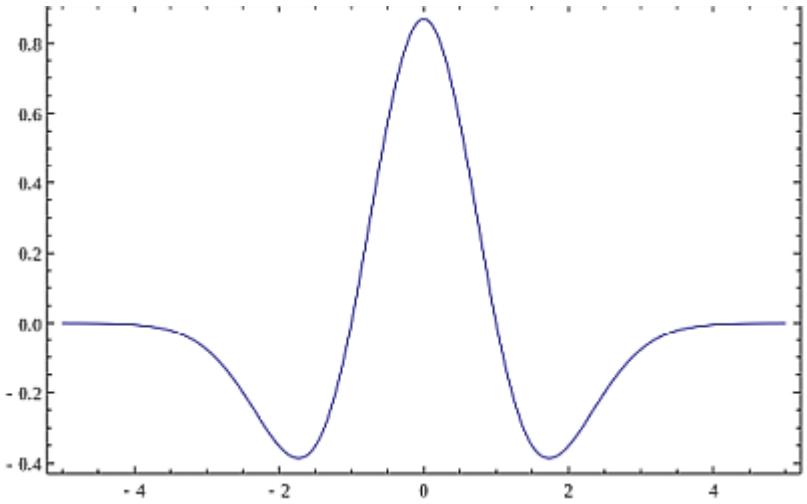
The method uses the **Mexican Hat wavelet model** of the continuous wavelet transform (**CWT**) algorithm.

The search of mass spectrum peaks is executed in three steps. First, the data point intesity is converted into wavelet domain. Second, all the local maxima of the calculated wavelet are found. Finally, m/z peaks (ions) are declared in those points, where the wavelet has a local maximum. The m/z peak is formed with the selected data point (mass and intensity) using the wavelet and all

surrounding data points. The final m/z value of the ion is calculated as an average of m/z values of surrounding data points weighted by their intensity.

Mathematical model

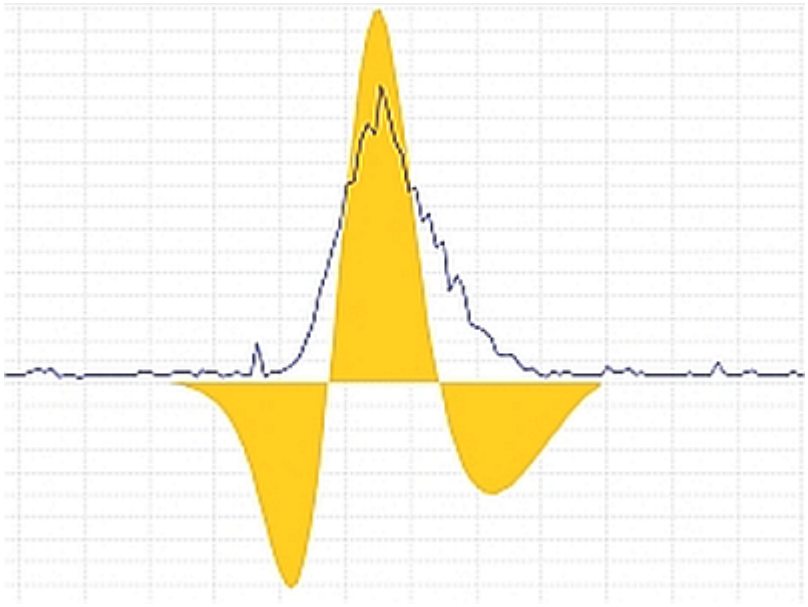
In numerical analysis, the Mexican hat wavelet is the normalized second derivative of a Gaussian function.



$$\psi(t)=\frac{1}{\sqrt{2\pi}\sigma^3} \left(1-\frac{t^2}{\sigma^2} \right)e^{-\frac{t^2}{2\sigma^2}}$$

The parameter t is the intensity of each data point in the curve, and σ corresponds to the standard deviation.

To simplify the process of wavelet calculation, the original function is transformed into two parts, where W_{c} is the wavelet coefficient and y is the intensity of the wavelet at certain point. In the following formula, t corresponds to the Wavelet window size (%) parameter.



$$W_{c}=\left[\frac{2}{\sqrt{3}\pi^\frac{1}{4}}\left(1-t^2\right)e^-\frac{t^2}{2}\right]$$
$$ y=\lim_{LL \to UL}(W_{c}x)$$

The lower (LL) and upper (UL) limits, where the Mexican Hat wavelet is evaluated, are from -5 until 5. The incremental step used in this range is the result of limits range division by 60,000.

Additional parameters

Scale level

Number of wavelet coeficients to use in m/z feature detection. Serves as the scale factor that either dilates or compresses the wavelet signal.

When the scale factor is relatively low, the signal is more contracted, which results in a more detailed resulting graph and more noisy peaks are detected. On the other hand, when the scale factor is high, the signal is stretched out, which means that the resulting graph will be less detailed with a smoothed signal.

Wavelet window size (%) The size of the window used to calculated the wavelet signal. When the size of the window is small, more noisy peaks can be detected. The proper value of this parameter may help to avoid the undesired noise peaks.

The Wavelet transform mass detector is particularly suitable for low-resolution and noisy data.

Auto

Auto mass detector recognizes if the spectrum is of profile or centroided data type and applies centroid or exact mass algorithms correspondingly.

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4.4.3 FTMS shoulder peak filter

Description

⚠️ This module should be used after mass detection step is performed.

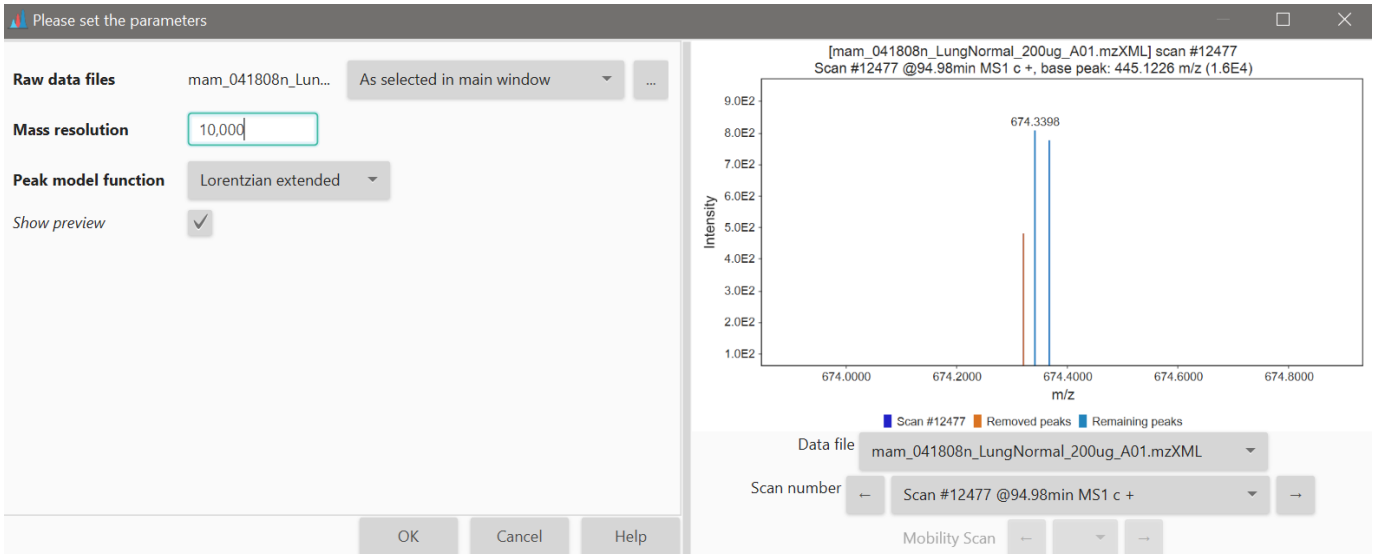
Raw data methods → Mass detection → FTMS shoulder peak filter

Raw data obtained from FTMS (Fourier Transform Mass Spectrometer) instruments often contains false signals around high-intensity m/z peaks, called **"shoulder peaks"**. These signals are residues of the Fourier Transform function and their intensity is usually below 5% of the main (true) m/z peak.

The FTMS shoulder peaks filter attempts to remove these false signals. Ions in the mass lists (generated previously by the Mass detector module) are processed in the order of decreasing intensity. A peak model (shape) is built around each ion peak using given function and resolution, and those m/z peaks which fall below the model are considered to be shoulder peaks and therefore are removed.

The method offers three theoretical peak models.

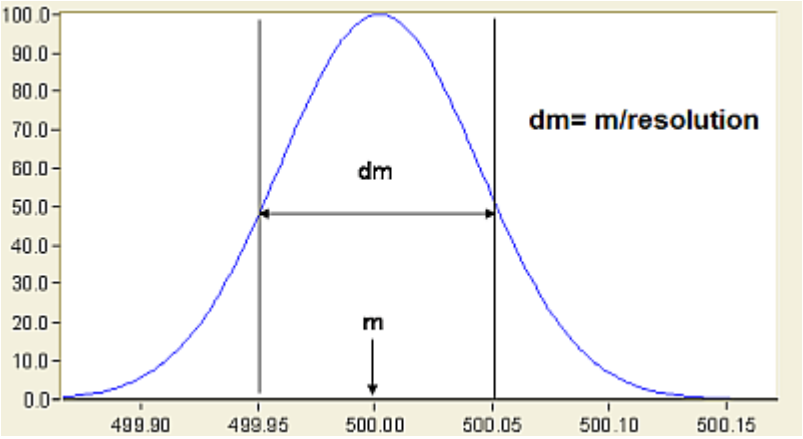
Example of running the shoulder peaks filter on LTQ Orbitrap data:



Parameters

Mass resolution of the data

Defines the width of the model, which should be equal to the estimated resolution of the peaks in the raw data. Mass resolution is the dimensionless ratio of the mass of the peak divided by its width. Peak width is taken as the full width at half maximum intensity (FWHM).



Peak model function

Defines the shape of the model function, as described below. Peaks under the curve of this peak model will be removed.

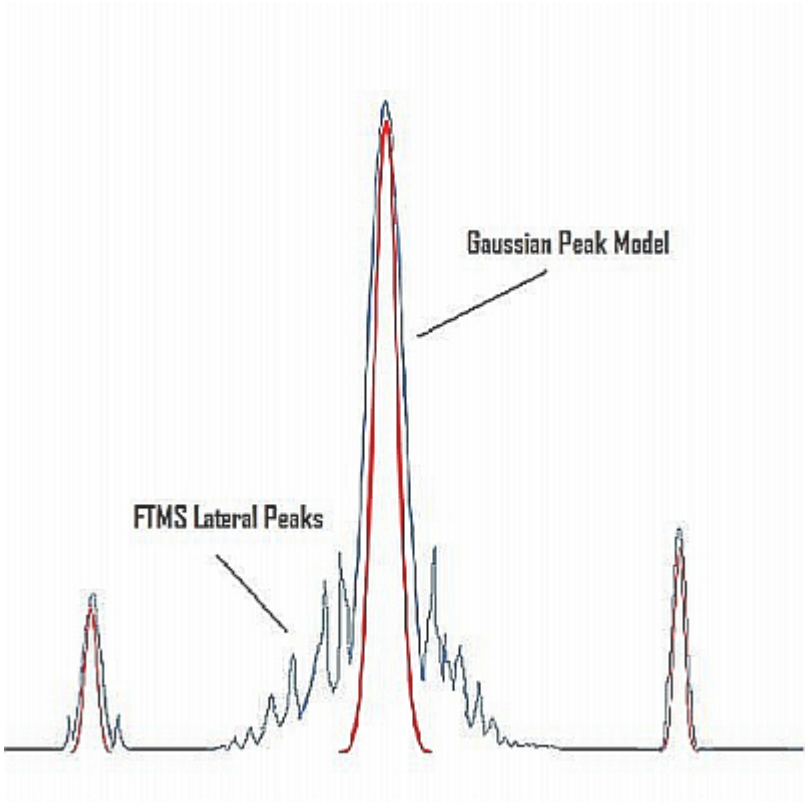
Available peak models

GAUSSIAN PEAK MODEL

The Gaussian peak model is a characteristic symmetric "bell shape curve" that quickly falls off towards plus/minus infinity, described by the following formula.

$$f(x)=ae^{-\frac{(x-b)^2}{2c^2}}$$

The parameter "a" is the height of the curve's peak, "b" is the position of the center of the peak, and "c" controls the width of the "bell".

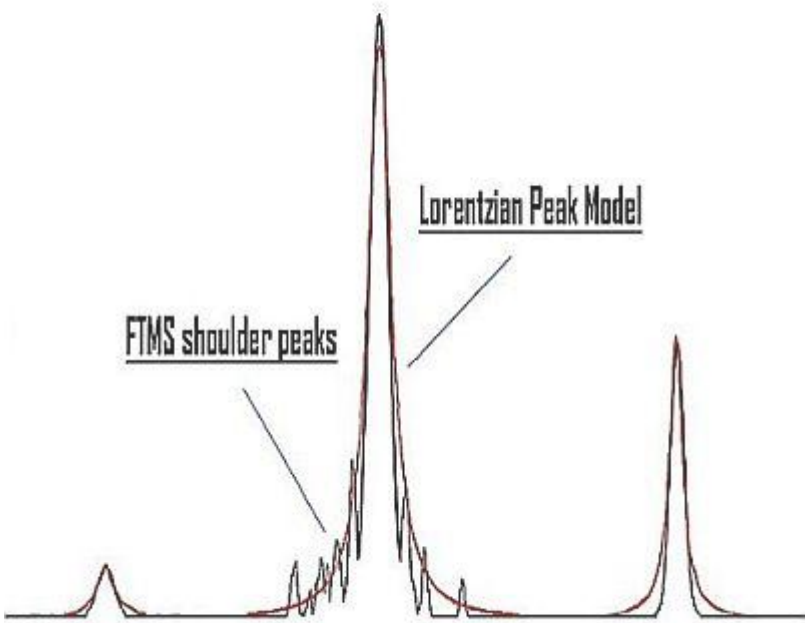


LORENTZIAN PEAK MODEL

The Lorentzian function (Cauchy-Lorentz distribution) is used for this model. The Lorentzian peak model is described by the following formula:

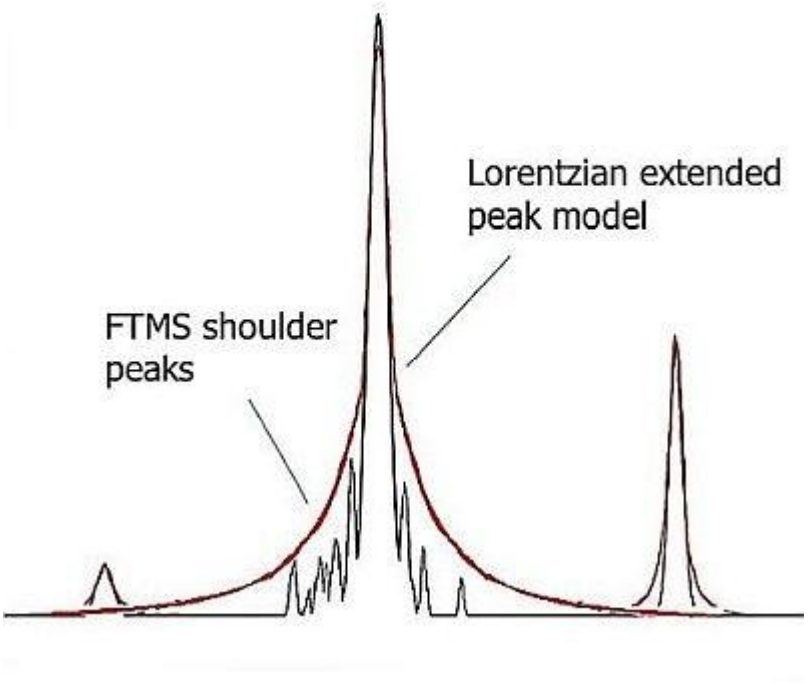
$$f(x;x_0,\gamma,I)=\frac{I}{\biggl[1+\frac{x-x_0}{\gamma}^2\biggr]} =I\biggl[\frac{\gamma^2}{(x-x_0)^2+\gamma^2}\biggr]$$

Where "x0" is the location parameter, specifying the location of the peak of the distribution, and "y" is the scale parameter which specifies the width of the peak.



LORENTZIAN EXTENDED PEAK MODEL

This model uses the same mathematical formula as the Lorentzian peak model, but the lower part of the model (below 5% of the intensity) is extended. The width of the peak below 5% intensity is calculated from another Lorentzian peak with 5% of the resolution of the main peak.



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4.4.4 Mass calibration

Description

⚠️ This module should be used after mass detection step is performed.

Raw data methods → Mass detection → Mass calibration

Mass calibration module estimates the measurement error of each detected mass and calibrates them using reference libraries of ions through three main processes:

1. Peak matching with library of reference and extraction of errors,
2. Estimation of overall mass bias, and
3. Mass calibration of detected masses.

Parameters

Raw data files

The raw datafiles to calibrate. Each selected datafile is calibrated independently in a separate task.

Mass list name

Name of the mass lists to be calibrated. The mass lists must be previously generated for each scan by the Mass detector module.

Intensity threshold

Determines the intensity of the peaks used for matching against the library of ions and subsequent mass calibration. Only mass peaks with intensity above this threshold will be considered for calibration. This is useful to avoid certain noise peaks that could have been picked in the Mass Detection module. To consider all peaks, the Intensity Threshold needs to be set with a value equal or lower than the previously used in the Mass Detection module.

Duplicate Error Filter

Removes duplicate ions with the same m/z value independently of their retention time. If enabled, for a specific detected exact mass present in different scans (not necessarily consecutive), only a single ion with that exact mass value will be considered for calibration. This filter performs for the full list of masses and does not consider RT difference between ions.

Reference Library of ions

Selects the library used for ion matching and determination of mass errors. SCL and UCL libraries are available.

- **SCL-only parameters**

  Standard Calibrant Library file

  File with a list of ion formulas and retention times (xls, xlsx and csv files are supported). This list should contain ions that are expected to be detected in the samples. Files need to contain a first column with the retention time in minutes and a second column with the ion formula strings. Additional columns are optional. Sample standards list file:

  Retention time tolerance (only for SCL)

  Maximum difference in retention time between an actual measured ion and a calibrant to consider a match.

- **UCL-only parameters**

  Ionization mode

  Ionization mode for which an appropriate universal calibrants list is used.

m/z tolerance

Maximum allowed difference in m/z between an actual measured ion and a calibrant to consider a match.

Overall Mass Bias Estimation

Measurement mass errors are calculated based on the matching of detected ions against the library, a distribution of errors is built, and measurement bias is estimated. Because not all ion matches can be considered as correct, the calibration model will automatically identify the high-density mass error range (mass error range with larger number of matches) from the generated distribution of matching errors.

If both parameters (*Primary High-Density Range of Errors size* and *Error range tolerance*) are set to zero, all errors obtained after matching against the reference library of ions are used for calibration of peaks.

- **High-Density Range of Errors**

  Primary High-Density Range of Errors size

  Determine the range (in PPM) containing most mass errors after matching the detected ions with the ion calibration library. Use zero to skip this step, in such case the distribution is split into subranges containing all the errors within the error tolerance and the largest subrange is used.

  Error Range Tolerance

  Maximum distance (in PPM error) between the maximum and minimum thresholds of the Primary High-Density Range of Errors and the consecutive error to allow the extension of the error range. Determines how far the distribution range of errors will be extended to extract the errors used for mass calibration. The module will include any consecutive matched error from the most populated error range found within the established error range tolerance (i.e., 0.1PPM).

  This process continues until the algorithm does not find any consecutive error within the Error Range Tolerance value. Use zero to skip this step and no extension will be computed.

- **Percentile Range of Errors**

  Percentile range

  The module calculates the Interquartile Range (IQR) from the overall distribution of errors to extract those errors to be used for mass calibration of peaks. IQR can be modified (by default determined at 25th and 75th percentiles). In such case, errors distributed below 25th or above 75th percentile will not be considered for mass calibration of peaks.

Mass Calibration method

Method used for mass calibration. Described in more details below.

- **Arithmetic mean**
- **KNN regression**
- *Nearest neighbors' percentage*

  Percentage of nearest neighbors used for error prediction.

- **OLS regression**
- *Polynomial degree*

  The degree of polynomial trend used, the summand powers of the polynomial will be the OLS regression features. Use 0 for constant component, 1 for linear, 2 for quadratic and so on.

- *Exponential feature*

  When selected, an exponential feature $exp(x/10)$ is included.

- *Logarithmic feature*

  When selected, logarithmic feature $ln(x)$ is included.

REFERENCE LIBRARY OF IONS

First, mass lists (MS1) from raw data files are matched against a reference library and mass measurement errors are calculated.

This module can support two different matching strategies:

- **Standard Calibrant Library (SCL)** (recommended method): file needs to be provided by the user in xls, xlsx or csv format). The file needs to include retention time (RT) information and the ion formula of a collection of ions that are expected to appear in the samples analyzed with a known chromatographic method. Ion formulas format of the SCL will depend on the ionization method used to analyze the samples. See below in the “Parameters” section a detailed description for the library format.

  Using a SCL, the matching of measured peaks the matching of measured peaks against the library is performed using both RT and exact m/z. RT and m/z error tolerances are needed to be defined.

  💡 Note: To ignore RT parameter when using SCL for mass calibration, the parameter can be set with an equal or larger value of the chromatography length (i.e., 30 min).

- **Universal Calibrant Library (UCL)** include a collection of ions often found in mass spectrometry experiments. The module has two different lists in positive (+ve) and negative (-ve) ionization modes to be chosen by the user.

  Universal calibrant lists are based on ***Keller et al. 2008 Analytica Chimica Acta 627:71-81*** and ***Hawkes et al. 2020 Limnology and Oceanography Methods 18:235-258***. MZmine includes both those libraries.

  Matching of detected ions against the UCL library is performed using m/z values alone independently where the ions are appearing along the chromatography and only m/z error tolerance will be needed.

More details on mass calibration method

💡 To estimate mass measurement bias more accurately, we can model the trend exhibited by the error size vs m/z value relation obtained by matching the mass peaks. With the estimation model we can shift/calibrate the mass peaks at different particular m/z values more accurately.

The module supports two main modes for mass calibration:

- Arithmetic mean:

  This method uses the arithmetic mean of the extracted errors of the overall bias estimation.

  Calibration of peaks will be performed globally based on a single overall bias value. This method is especially recommended for datasets with low number of extracted errors (i.e., blank samples).

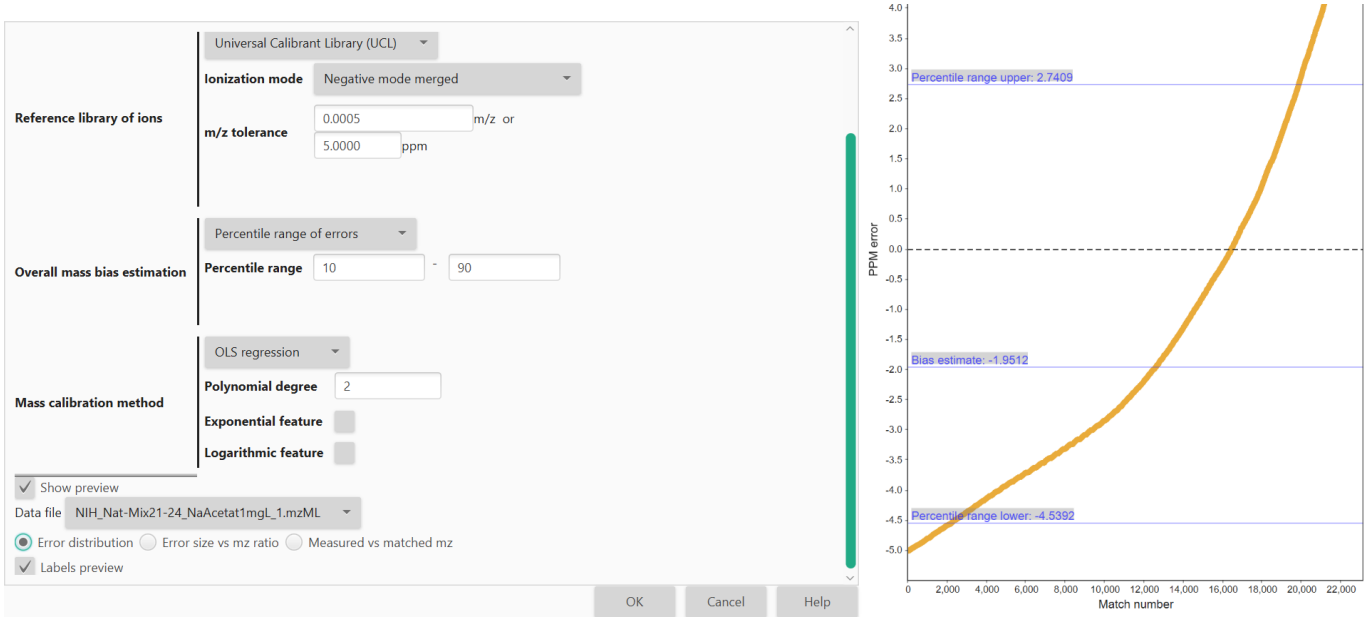
- Regression mode:

  This mode models a trend from the direct relationship of error size (in PPM) vs. measured m/z of detected peaks. Mass lists will be calibrated according to the estimated model. The mass calibration module supports two different methods of regression: **OLS** and **KNN**.

- *OLS (ordinary least squares)* regression minimizes the mean squared error between the predicted trend and the datapoints in the dataset. Available features include power features (polynomial trend), logarithmic feature and exponential feature. **By default**, linear trend is fitted. This mode is suitable for datasets with enough data and exhibit a clear and strong trend (Error vs. m/z).
- *KNN (K-Nearest Neighbors)* regression finds the average value of the K nearest neighbors. In this module, the number of neighbors is defined by a percentage set by the user of all the errors present in the dataset. The K closest neighbors are thus found by the absolute difference of the m/z values within such percentage. Then the arithmetic mean of the neighbors' errors is calculated for each individual error and will serve as an error estimate for a specific m/z. This method is suitable for datasets with enough data and a trend between mass error vs. m/z is not clear. Therefore, KNN regression allows the trend to match the dataset closely without introducing additional assumptions on how the variables are related.

⚠️ Overfitting problems at large m/z values (>800) can occur when modeling the errors with regression as those regions commonly have less matches against the reference libraries of ions. If regression is modelled mainly by matches with small m/z ions (<400), it is recommended to use the arithmetic mean for mass calibration. This also applies to blank samples were the number of matched ions is commonly low and regression can produce overfitting problems.

Examples



`This module was intially created during a GSoC 2020 project with MZmine by Łukasz Fiszer and MZmine team.`

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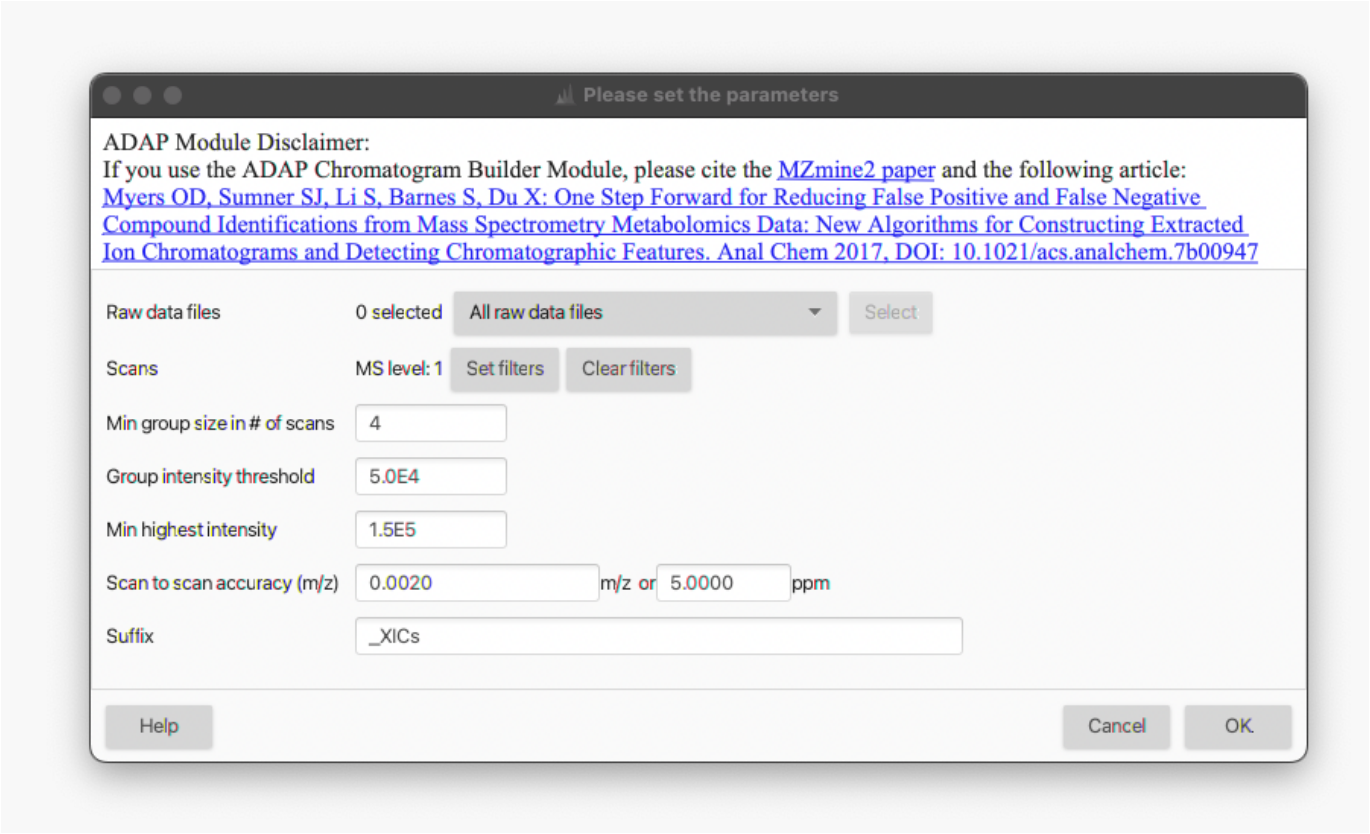
4.5 LC-MS feature detection

4.5.1 **ADAP chromatogram builder**

Description

Feature detection → LC-MS → ADAP chromatogram builder



The *ADAP chromatogram builder* module is one of the LC-MS feature detection algorithms provided by MZmine 3. The module essentially builds an EIC for each *m/z* value that was detected over a minimum number of consecutive scans in the LC-MS run. Each data file is processed individually. The mass list associated to each MS1 scan in a data file (see Mass detection module) are taken as input and a feature list is returned as output. Since a mass list must be available, the *Mass detection* module must be run first.

The *ADAP chromatogram builder* algorithm operates as follows:

- Only MS1 scans are processed.
- All the data points are extracted from all the MS1 scans in a data file and sorted in order of decreasing intensity.
- The processing starts from the most intense data point. Since no EICs have yet been created, a new EIC is initialized and associated to the corresponding *m/z* value.
- The processing proceeds with the second-highest data point. The corresponding *m/z* is checked to determine if it "belongs" to the existing EIC (based on the user-defined tolerance, *i.e.* "Scan to scan accuracy (m/z)" parameter).
- If yes, then the data point is added to the EIC and the EIC-associated *m/z* is updated. Otherwise, a new EIC is initialized.
- The process repeats iteratively until all the data points have been processed and a set of EICs has been created.
- Finally, the EICs are checked according to the user-defined parameters (*i.e.* minimum number of data points and intensity). The EICs matching the requirements are retained in the *feature list*, whereas the rest are discarded.

The so-built EICs can then be resolved into individual features by one of the deconvolution algorithms provided by MZmine 3 (*e.g.* Local minimum resolver module).

Parameters

Raw data files

Select the input raw data files for chromatogram building. Mass lists associated with the data files will be automatically selected. See option descriptions in Mass detection module.

Scans

Select (or filter out) the MS scans to be processed. Although setting the *MS level = 1* is usually sufficient for this module, several filters are available (see option descriptions in Mass detection module). For example, specific RT ranges (*e.g.* dead volume, equilibration time, calibration segments, *etc.*) can be excluded from the processing by setting the corresponding filter.

Min group size in number of scans

Minimum number of consecutive MS1 scans where a *m/z* must be detected with a non-zero intensity in order for the corresponding EICs to be considered valid and retained in the feature list.

This parameter largely depends on the chromatographic system setup (*e.g.* HPLC vs UHPLC) and the acquisition rate (*a.k.a.* MS scan speed) of the mass spectrometer. The best way to optimize this setting is by manually inspecting the raw data and determining the typical minimum number of data points of the LC peaks. Usually, no less than 4-5 should be used.

Group intensity threshold

Minimum signal intensity that the group scans (see previous parameter) must exceed in order for the corresponding EICs to be considered valid and retained in the feature list.

A good starting point for this parameter is 3 times the noise level used in the Mass detection, if the instrumental noise is used as cutoff. See also How do I determine the noise level in my data? for more details.

Min highest intensity

Minimum intensity that the highest point in the EIC must exceed in order for the corresponding trace to be considered valid and retained in the feature list. This parameter mainly depends on the mass spectrometer characteristics (*e.g.* Orbitrap instruments normally provides higher signal intensities than TOF devices) as well as the overall goal of the processing. Overly low intensity thresholds normally leads to a larger number of background signals being retained as features, extending the overall processing time. On the other hand, overly high thresholds may lead to low-intensity features being erroneously discarded.

A good starting point for this parameter is 7-10 times the noise level used in the Mass detection, **if** the instrumental noise is used as cutoff. See also How do I determine the noise level in my data? for more details.

Scan to scan accuracy (m/z)

Maximum allowed difference between an EIC-associated *m/z* and a new data point to be added to the existing EIC trace. It is essentially the maximum allowed mass accuracy deviation between consecutive data points in the EICs. The tolerance can be specified as absolute tolerance (in *m/z*), relative tolerance (in ppm), or both. When both are specified, the tolerance range is calculated using the maximum between the absolute and relative tolerances.

This is an inter-scan *m/z* tolerance, and it depends on the mass accuracy, resolution and stability of the instrument. The best way to optimize this parameter is by manually inspecting the raw data and determining the typical fluctuation of the accurate mass measurement over consecutive scans. A good starting point is 0.002-0.005 *m/z* and 5-10 ppm for Orbitrap instruments, while 0.005 *m/z* and 10-15 ppm can be used for TOF devices.

Suffix

String added to the filename as suffix when creating the corresponding feature list.

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4.5.2 GridMass

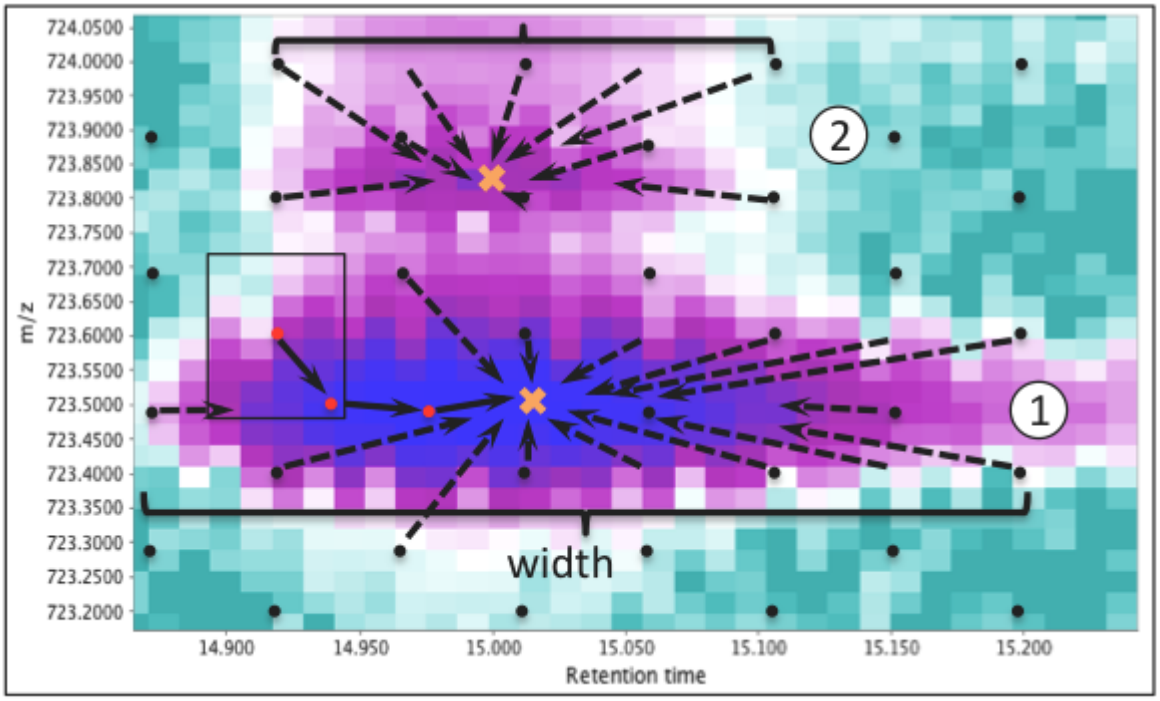
Description

Feature detection → LC-MS → GridMass

GridMass is an algorithm to detect peaks analyzing 2D data. It directly generates a peak list, which can then be operated using the peak list methods.

ALGORITHM

To detect the position and boundaries of masses, the GridMass algorithm first generates a grid of equally spaced probes covering the entire chromatographic area. A representative section is shown in the figure below.



- Each probe (black dots in Figure) explores a rectangular region around it to find a local maximum.
- The probe location is moved to the local maxima to further search for a higher value.
- The procedure is performed until no higher values exist within the exploring rectangle.
- This local maximum is then defined as a **feature**, which contains information of the m/z, the time, and the intensity detected.

A putative trajectory of a probe is shown in the figure. All probes converging to the same feature provide an estimation of its **boundaries**.

Consequently, different features represent different masses.

This procedure is highly sensitive and specific for smooth surfaces. However, given that in real chromatographic data a certain level of noise and artifacts are present, in MZmine additional criteria (such as possibility to choose time windows, additional smoothing, etc.) were implemented.

A summary of the algorithm is as follows:

1. Ignore artifact spectra in time domain. In chromatography, it is typical to find a peak near the injection time, corresponding to metabolites that show no interaction with the column in the particular gradient. While this is not an artifact per se, given the myriad of signals present and the nature of the detector, the resulting peak is a strong source of artifacts that later affect analysis. To avoid this, the user may enter a list of time ranges to be ignored. The controlling parameter is ignore times whose format is time1-time2, time3-time4, ... Alternatively, the user may crop these data before processing, for example using “Raw Data→Filtering→Data Set" and ignore setting this parameter. Therefore, this step is optional.
2. Generate equally spaced probes over the mass-time space.To generate the grid, the parameters used are m/z tolerance and minimum width. The gap in the m/z dimension between probes is set to m/z tolerance multiplied by 2 or minimum to 1e-6 and are intercalated between scans. The gap in the time dimension is calculated by time associated to scans, which is estimated by the minimum width divided by 4 (down to a minimum of 1 scan).
3. Move each probe to corresponding local maximum until convergence.Each probe explores its surroundings (limited by the positions of other probes) to locate the highest intensity value, then after updating its position, it keeps exploring the surrounding until a local maximum is reached. To speed up the procedure, generate only interesting features above a certain level of noise, and limit the number of reported features, only intensities higher than minimum height threshold are considered.
4. Generate features by merging probes with similar 2D positions.Many probes will reach the same maximum that must correspond to the same feature. In addition, experimental chromatographic data is noisy and non-smooth, which may generate local maxima very close to each other. Therefore, probes whose difference in m/z is lower than the m/z tolerance and whose difference in time is lower than minimum width are merged. Then, from all probes reaching the same maximum, the m/z assigned to the feature corresponds to the highest observed intensity. The width of the feature is estimated from the probes with the lowest and highest time. To form the peak and estimate its area, the highest value in each scan is used.
5. Remove features whose width is out of a range.Features having large or very low width are likely to be artifacts. To avoid this, all features out within the range given by the parameters minimum width and maximum width (in minutes) are removed.
6. Remove features of similar mass and high cumulative times.Chemical noise or large blurs are characterized by generating many features of similar mass, similar intensities, and separated by short times. To avoid these artifacts, we merge features whose m/z difference is lower than m/z tolerance and whose intensity ratio (higher/lower) is higher than an intensity similarity ratio parameter. Once merged, the removal implemented in step 5 is performed on merged features.

Parameters

Suffix

Suffix to be added to peak list name.

Minimum height

Only intensities larger than this minimum are considered.

m/z tolerance

Maximum distance in m/z from the expected location of a peak.

Min-max width time (min)

Time range for a peak to be recognized as a 'mass'. The optimal value depends on the chromatography system setup. Check 2D raw data to determine typical time spans.

Smoothing time (min)

Time window used to smooth the signal before detection.

Smoothing m/z

m/z window used to smooth the signal before detection.

False+: Intensity similarity ratio

Ratio between features to be recognized as the same. This is highly useful to detect artifacts.

False+: Ignore times

Ranges of time to be ignored by the method. This can be avoided if the region is previously cleaned using the crop option. Format: timeA-timeB, timeC-timeD,...

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4.5.3 **Targeted feature detection**

Description

Feature detection → LC-MS → Targeted feature detection

This algorithm opens a *.csv file with a list of peaks and searches for each peak in the selected raw data file. The most crucial parameters are m/z tolerance and Retention time tolerance**,* which define the window where the algorithm should find the new peak. It is centered in the m/z average and retention time average of the source peak list. Once the best candidate is found inside the window, its shape in RT direction is also checked.

The *.csv file should have three columns:

- The first column should contain the expected M/Z,
- the second column the expected RT,
- and the third the peak name.

⚠️ Each peak should be in a different row.

Parameters

Name suffix

Suffix to be added to the peak list name.

Peak list file

Path of the csv file containing the list of peaks to be detected.

Field separator

Character(s) used to separate fields in the peak list file.

Ignore first line

Check to ignore the first line of peak list file.

Intensity tolerance

This value sets the maximum allowed deviation from expected shape of a peak in chromatographic direction.

Noise level

The minimum intensity level for a data point to be considered part of a chromatogram. All data points below this intensity level are ignored.

m/z Tolerance

Maximum allowed m/z difference to find the peak

Retention time tolerance

Maximum allowed retention time difference to find the peak

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4.6 LC-IMS-MS feature detection

4.6.1 **IMS Expander**

4.6.2 **Description**

Feature detection → LC-IMS-MS → IMS expander.

The IMS expander searches for data points in mobility scans for existing features.

⚠️ This requires prior chromatogram building (see ADAP Chromatogram builder and resolving in retention time dimension (see Resolving).

PARAMETERS

m/z tolerance

If selected, a tolerance will be applied to the feature's detected m/z while searching for data points in mobility dimension. Otherwise, the accepted m/z range is determined by the feature's m/z distribution in accumulated frame spectra.
Recommended setting: selected, 0.003 m/z and 15 ppm

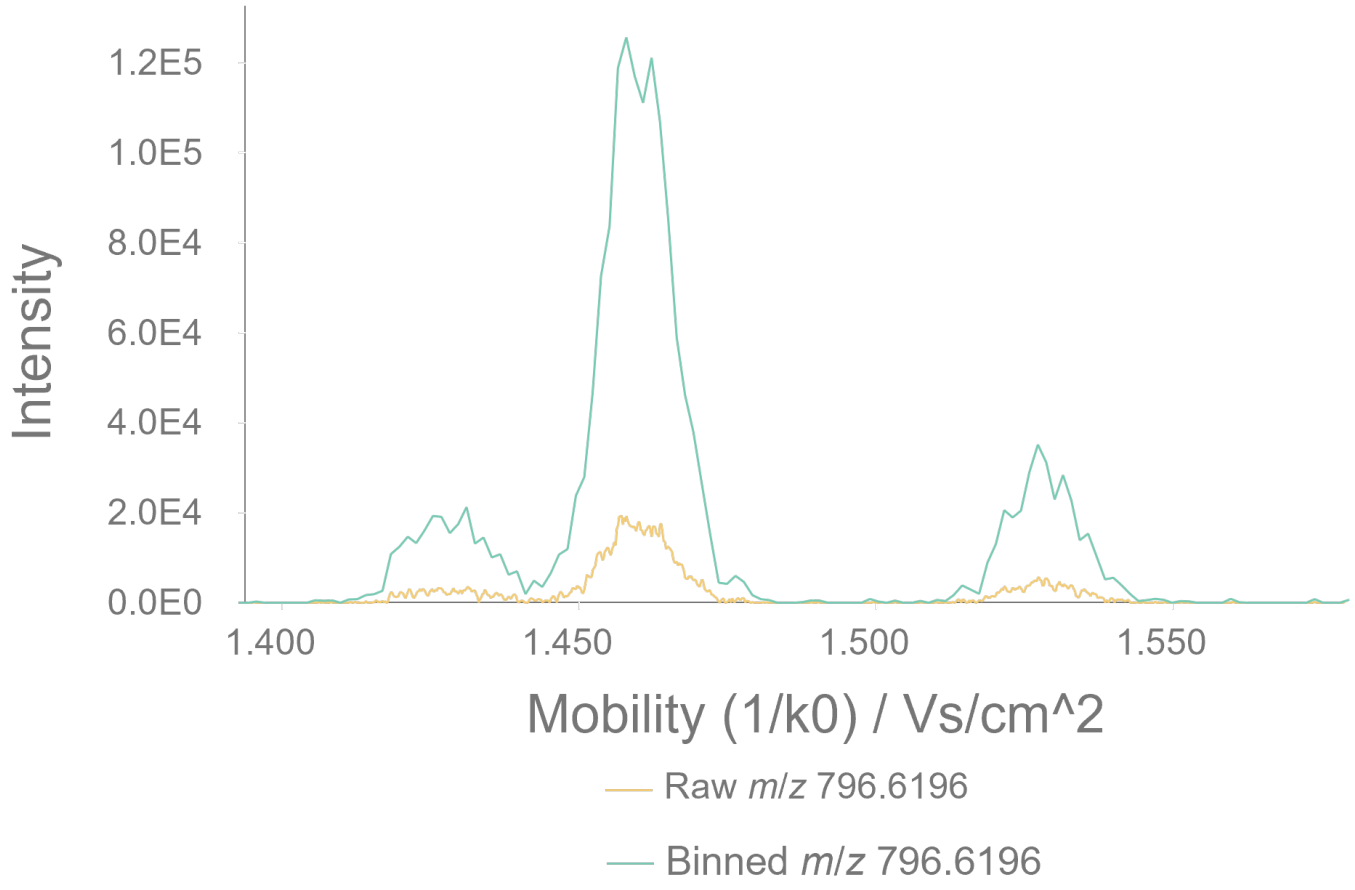
Raw data instead of thresholded

Enables searching in mobility scan raw data instead of the thresholded (=mass detected) data. Only possible for centroid raw data files.

Override default mobility bin width (scans)

If selected, the default number of binned mobility scans can be overriden. Useful for data with high mobility resolution.

BINNED MOBILOGRAM EXAMPLE



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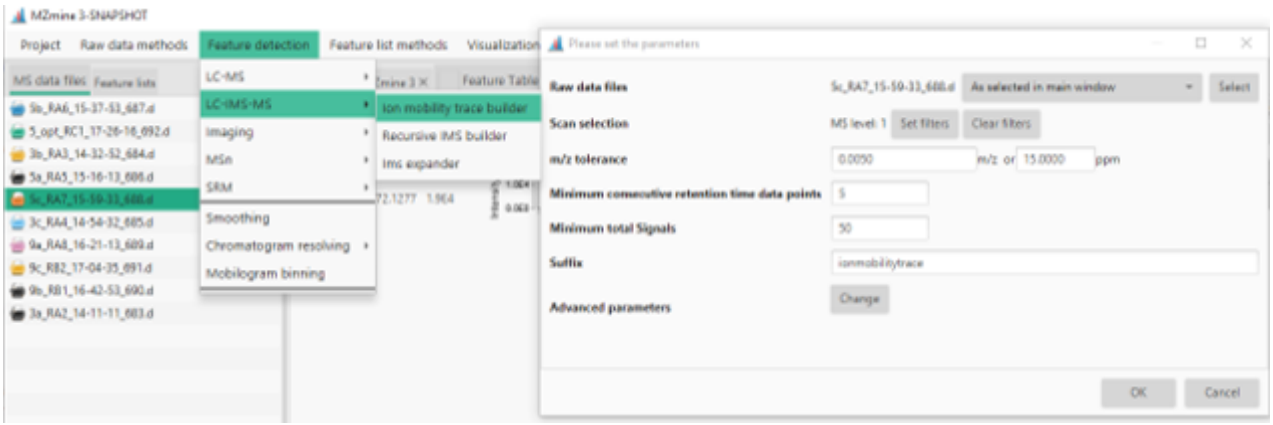
4.6.3 Ion mobility trace builder

Description

Feature detection → LC-IMS-MS → Ion mobility trace builder.

The **Ion mobility trace builder** will build ion mobility traces from the raw data. Alternatively, the **Recursive IMS builder** can be used, which requires less RAM but takes longer.



Parameters

Scan selection

The scan selection parameter specifies the scans that shall be processed for feature detection. Usually, setting the ms level to 1 is sufficient. If a calibration segment is present, it can be cut out via the retention time filter in the scan selection.

m/z tolerance

The **m/z tolerance** specifies the scan-to-scan tolerance for ion mobility traces. This tolerance window may need to be set higher that for classic LC-MS feature detection (e.g. to 0.005 m/z and 15-20 ppm instead of 10 ppm) due to lower intensities therefore less accuracy in individual mobility scans compared to LC-MS scans. Note that the overall accuracy is achieved via LC-IMS-MS is the same due to the higher number of scans.

Minimum consecutive retention time data points

This parameter specifies the number of consecutive detections of the same m/z value in a chromatographic peak (rt dimension only). This means that a single m/z has to be detected in, e.g, 5 frames with an intensity higher than zero. This parameter helps to filter noise. Consecutive detections in the mobility dimension do not affect this parameter. Usually no less than 5 should be set here if the MS1 acquisition rate is sufficient.

Minimum total signals

Specifies the total number of peaks in the mobility dimension in all mobility scans. Every "dot" in an ion mobility trace represents a single datapoint.

Advanced parameters

⚠️ For most applications, these parameters do not need to set/altered.

For high mobility resolved data the mobilograms might become noisy due to a fewer ions reaching the detector at the same time. By default, the number of binned scans is set to cover about 0.0008 Vs/cm^2 per bin. The effect of binning can be seen here. If you are unsure about the nature of your data, you can perform trace building with the standard parameters and apply/preview the binning afterwards via the **Feature detection → Mobilogram binning** module.

Override default TIMS binning width (Vs/cm²)

The binning width in mobility units of the selected raw data file.

Travelling wave binning width (ms)

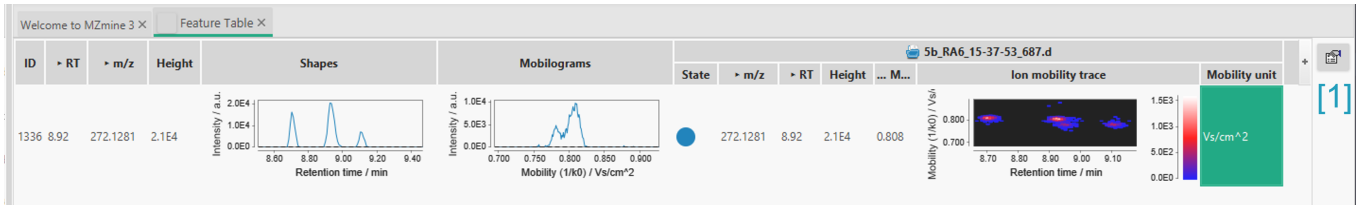
The binning width in mobility units of the selected raw data file.

Drift tube binning width (ms)

The binning width in mobility units of the selected raw data file.

Processing result

After performing ion mobiltiy trace detection, a feature list is created in the feature list tab (see feature lists tab). In the feature table, multiple columns are created. The displayed columns can be set via the button on the right of the feature table ([1]).



The **shapes** displays an EIC of the ion mobility trace (intensities summed in rt dimension). The **mobilograms** column shows a mobilogram for the ion mobility trace (intensities summed in mobility dimension). The shapes and projections can be smoothed and resolved. However, the ion mobility trace is always represented by the raw data and remains unaltered. After resolving, the shapes and mobilograms have to be recalculated from the raw data, which is why the smoothing is lost after resolving.

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4.6.4 Recursive IMS builder

Description

Feature detection → LC-IMS-MS → Recursive IMS builder.

Builds m/z traces for ion mobility spectrometry data.

Parameters

m/z tolerance

The m/z tolerance to build ion traces. The tolerance is specified as a +- tolerance.

m/z 500.000 with a tolerance of 0.01 will allow m/z change from 499.99 to 501.01.

Minimum consecutive retention time data points

The minimum number of consecutive detections in frames (retention time dimension).

Minimum number of data points

The minimum number of consecutive detections in frames (retention time dimension).

Advanced parameters

Allows adjustment of internal binning parameters for mobilograms. The default values are set to 1.

Override default TIMS binning width (Vs/cm²)

The binning width in mobility units of the selected raw data file.

Travelling wave binning width (ms)

The binning width in mobility units of the selected raw data file.

Drift tube binning width (ms)

The binning width in mobility units of the selected raw data file.

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4.7 Smoothing

4.7.1 **Description**

⚠️ This module should be used after feature detection step is performed.

Feature detection → Smoothing

Smoothing is an optional feature that is used depending on the noisiness of the data. Smoothing allows to approximate a peak shape to the "ideal" shape defined by the target function of the used algorithm.

In MZmine 3, two algorithms can be used for smoothing:

- Savitzky-Golay,
- and Loess smoothing.

Savitzky-Golay smoothing

This smoothing method is also implemented in **Raw data methods → Raw data filtering → Scan by scan filtering**. Its brief description is available in that section. For more details see [1].

💡 The benefit of Savitzky–Golay is its efficiency. Due to a fixed user-defined window size and equal spacing, the weights are also fixed. As a result, the local fit need be solved only once.

Loess smoothing

LOESS (locally weighted smoothing) or **LOWESS (Locally Weighted Scatterplot Smoothing)** is a non-parametric method that relies only on a smoothing parameter value and the degree of polynomial without a predefined function. Each polynomial is fitted locally depending on the defined bandwidth.

More details on LOESS smoothing can be found in [2].

💡 Can be slightly slower than the Savitzky-Golay algorithm (due to the repeated local fitting) but also a bit more precise.

4.7.2 References

1. A. Savitzky and M. J. E. Golay, Anal. Chem., 36, 1627 (1964). DOI: 10.1021/ac60214a047
2. William S. Cleveland & Susan J. Devlin (1988) Locally Weighted Regression: An Approach to Regression Analysis by Local Fitting, Journal of the American Statistical Association, 83:403, 596-610, DOI: 10.1080/01621459.1988.10478639

4.7.3 **Parameters**

FEATURE LISTS

Feature lists that the module will take as an input.

SMOOTHING ALGORITHM

Choose if Savitzky-Golay or LOESS smoothing will be used.

Savitzky-Golay additional parameters

Retention time smoothing

Number of data point to smooth in retention time dimension. Defines window size and coefficients used in smoothing.

Mobility smoothing

Number of data point to smooth in mobility dimension.

LOESS additional parameters

Retention time width (scans)

Number of scans to smooth in retention time dimension. Used to calculate a fraction of source points, which is subsequently used to calculate LOESS "bandwidth". "Bandwidth or "smoothing parameter" determines how much of the data is used to fit each local polynomial.

Higher values of smoothing parameter lead to the smoother output but simultaneously to a larger loss of information.

Mobility width (scans)

Number of data point to smooth in mobility dimension. The points about previous parameter apply here as well.

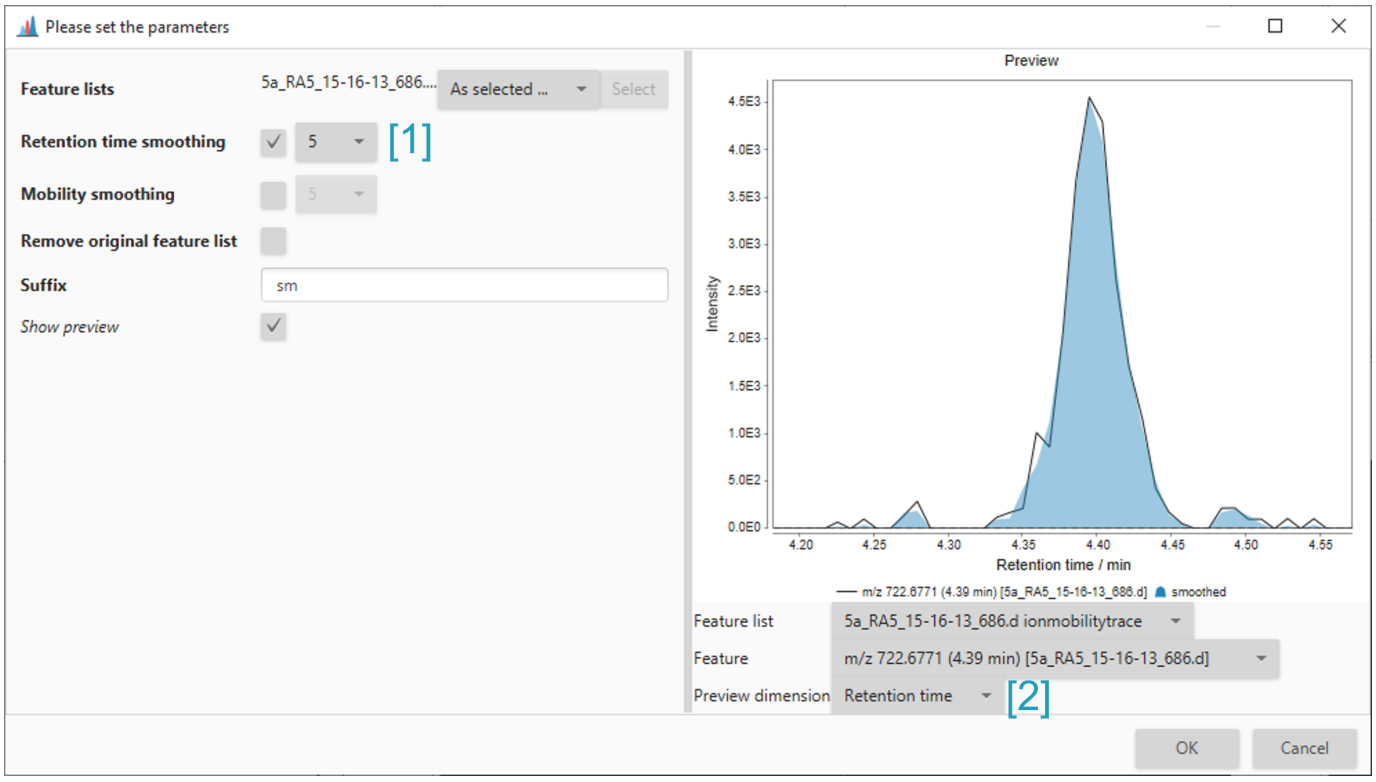
4.7.4 Optimizing smoothing parameters using the preview

Retention time dimension

Smoothing chromatograms is optional. The necessity of smoothing in RT dimension is determined by the noisiness of chromatographic peaks. These can be influenced by the overall spray stability, instrument accumulation times, transfer efficiency and many more.

The number of data points to be smoothed in rt dimension can be set at [1] (see image below). Note that the correct preview dimension is selected at [2] (see image below).

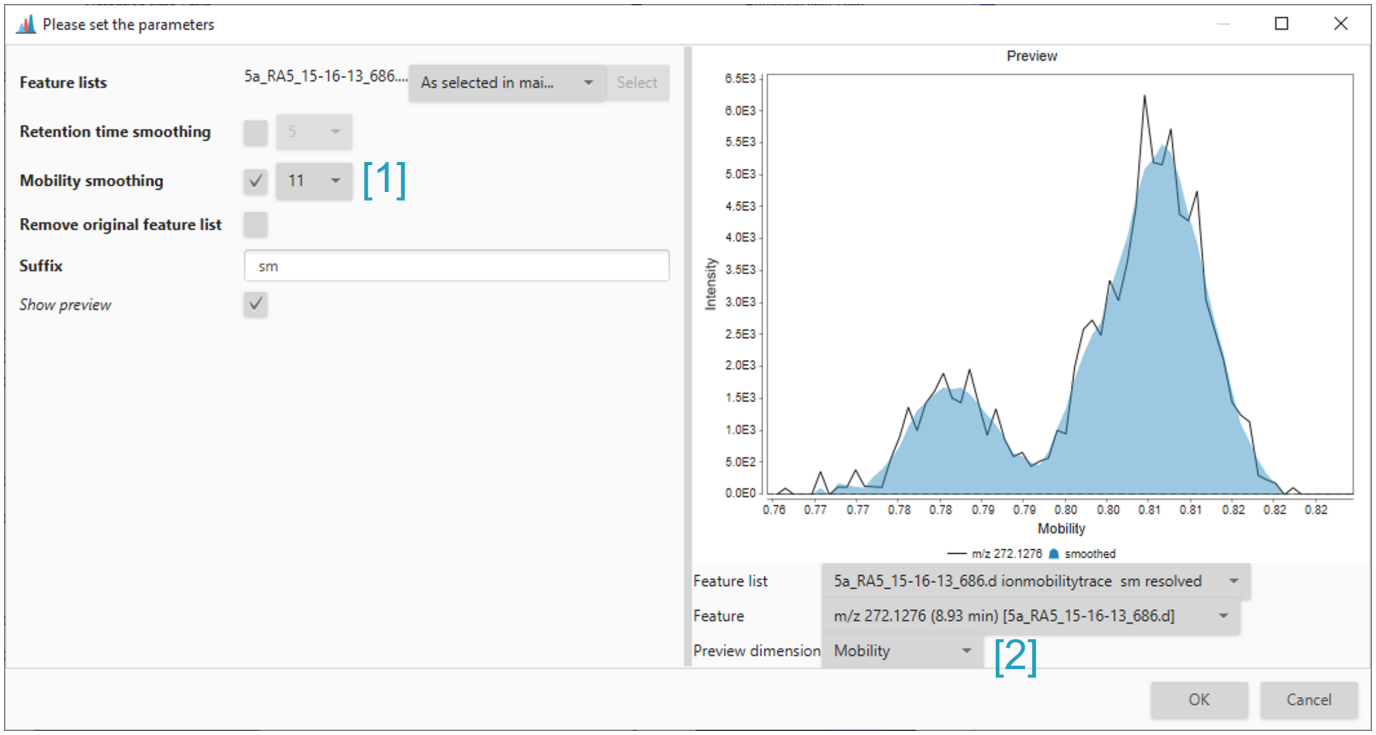
For large batch modes, the **Remove original feature list** parameter should be selected. While parameters are being optimised, this is not recommended, because removing a feature list cannot be undone.



Mobility dimension

After resolving a feature in RT dimension, the mobilograms will be recalculated from the raw data (the resolved ion mobility trace). Therefore, a smoothing step is necessary if the data requires it. The smoothing dialog is opened via **Feature detection → Smoothing**

Select to smooth the mobility dimension [1] and select it as preview dimension [2]. The filter with depends on the number of spectra acquired in the observed mobility range. Usually, a value between 5 and 15 should be appropriate.



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4.8 Resolving

4.8.1 **Local Minimum Resolver**

Description

Feature detection → Chromatogram resolving → Local minimum resolver

During the EICs building, overlapping and partially co-eluting features are retained as single features in the feature list (see, for example, ADAP chromatogram builder). As a local minimum in the EIC trace might correspond to the valley between two adjacent, partially-resolved peaks, the **Local minimum resolver (LMR)** utilizes such minima to split "shoulder" LC peaks into individual features (*i.e.* chromatographic resolving).

The algorithm examines all the data points in the EIC trace starting from the earliest RT. A scan window is set (see **Minimum search range RT/Mobility** parameter) and centered around the examined data point.

A data point is considered a **local minimum** if it is the lowest intense point within the scan window. When a local minumum is found, a set of user-defined intensity and feature duration requirements is checked. If they are fulfilled, the original overlapping peaks are split into new, distinct features.

The **LMR** is particularly suitable for LC-MS data with little noise and nice peak shapes.

With the implementation of ion mobility (IM) support in MZmine 3, this module was expanded and can now be applied over both the RT and IM dimensions (see Resolving the ion mobility dimension).

RESOLVING THE ION MOBILITY DIMENSION

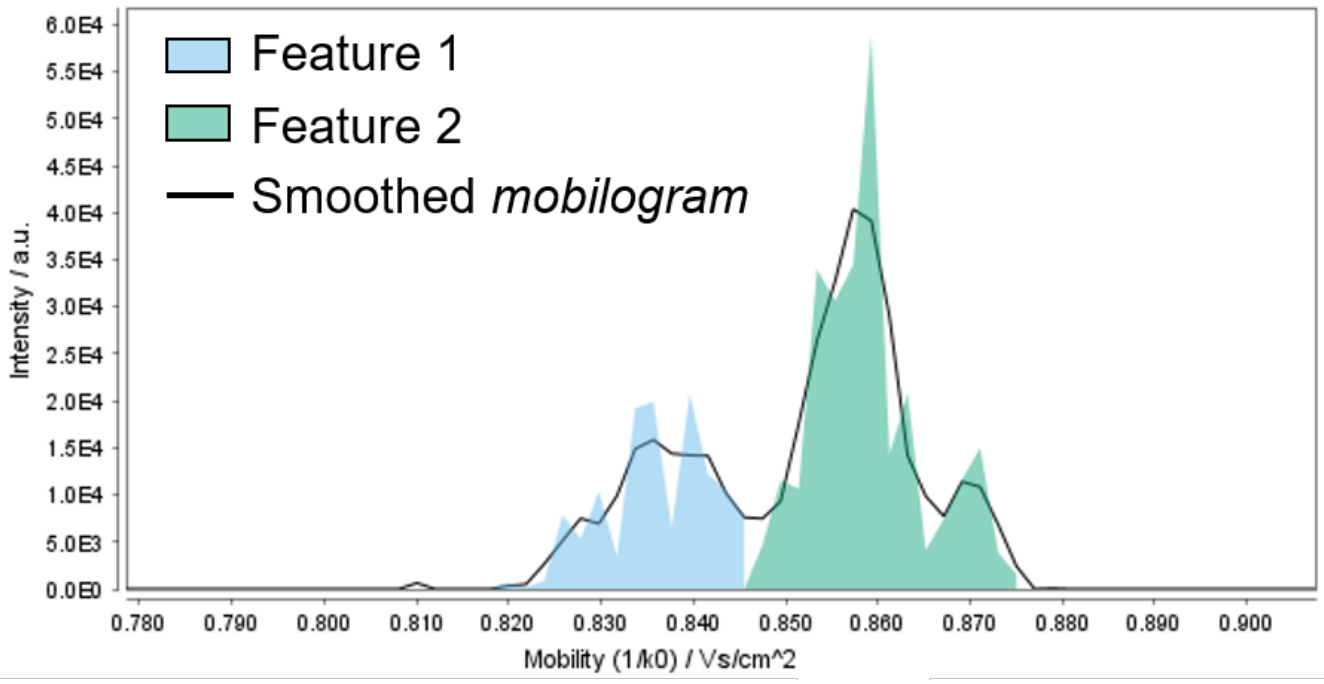
This same module can be used to resolve features co-eluting in the RT dimension, based on their ion mobility. The same concepts apply as in the resolution of the RT dimension.

However, *mobilograms* are examined instead of EIC traces and the same settings used for the RT dimension might not be optimal when resolving IM data. In particular, the following aspects should be born in mind:

1. While *frame scans* are examined over the RT dimension, *mobility scans* are considered over the IM dimension.

   As explained here, *frame scans* are essentially obtained by merging the *mobility scans* acquired over an IM accumulation. Therefore, it might be necessary to adjust parameters like **Minimum absolute height** or **Min ratio of peak top/edge** to account for the lower signal intensity of *mobility scans*.

1. *Mobilograms* are recalculated from raw data, even though a smoothing step was previously applied. Non-smoothed *mobiligrams* tend to be more jagged than regular EIC traces (see Figure). Therefore, some parameters (*e.g.* **Min search range** and **Min ratio of peak top/edge**) should be adjusted accordingly.



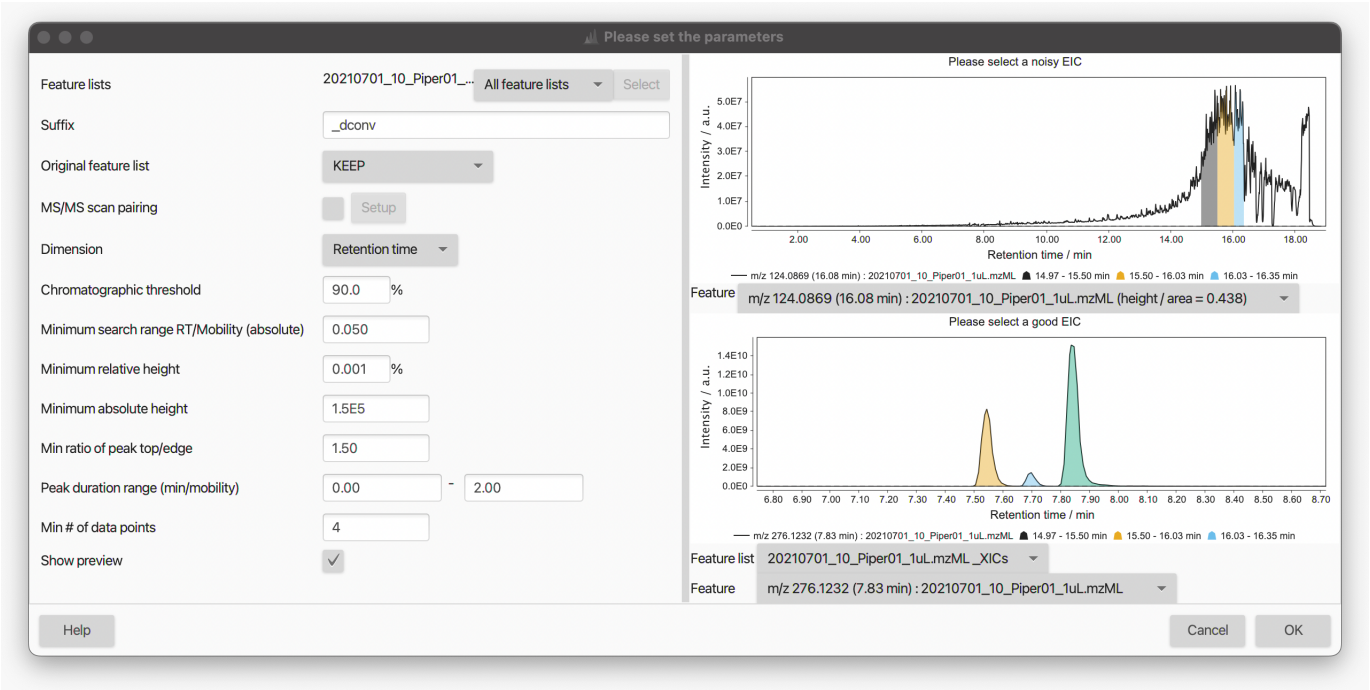
1. *Mobilograms* contain fewer scans (*e.g.* ≈400-1000 per frame, depending on instrument type and acquisition settings), compared to regular EICs (*e.g.* ≈4500 scans for 15 minutes LC run and scan rate of 0.2 seconds). Therefore, a lower **chromatographic threshold** (*e.g.* 80%) is recommended to avoid relevant data points in the mobilogram being discarded.

   On the other hand, a single feature in the IM dimension is normally made up of more data points than regular LC peaks, due to the different timescale the IM separation is performed on (see here for more details).

   Therefore, a higher *Min # of data points* can be set when resolving the ion mobility dimension to filter out noisy features.

1. Different vendors use different units of mobility. For instance, TIMS express ions' mobility as Vs/cm^2, whereas time dispersive IM devices (DTIMS and TWIMS) use the ions' drift time (expressed in milliseconds). TIMS values are numerically smaller than DTIMS or TWIMS; therefore, the **minimum search range** parameter should be adjusted accordingly.

Parameters



Suffix

String added as suffix to when creating the new feature list(s).

Original feature list

Defines the processing.

Standard is to KEEP the original feature list and create a new processed list.

REMOVE saves memory.

PROCESS IN PLACE is an advanced option to process directly in the feature list and reduce memory consumption more - this might come with side effects, apply with caution.

MS/MS scan pairing

Pair MS/MS fragmentation spectra collected in DDA mode to the resolved features. This is optional at this stage as the same can be done later in the pipeline using a separate module. See MS2 scan pairing documentation for more details.

Dimension

Dimension to be resolved. Select *Retention time* or *Mobility* to run the module over the RT or IM dimension, respectively.

Chromatographic threshold

Percentage of data points in the EIC removed before local minima search. This represents an important filter for noisy chromatogram and significantly reduces the precessing time. The algorithm finds the intensity value (threshold) that leaves the specified percentage of data points in the EIC trace below the given value. All such data points are removed.

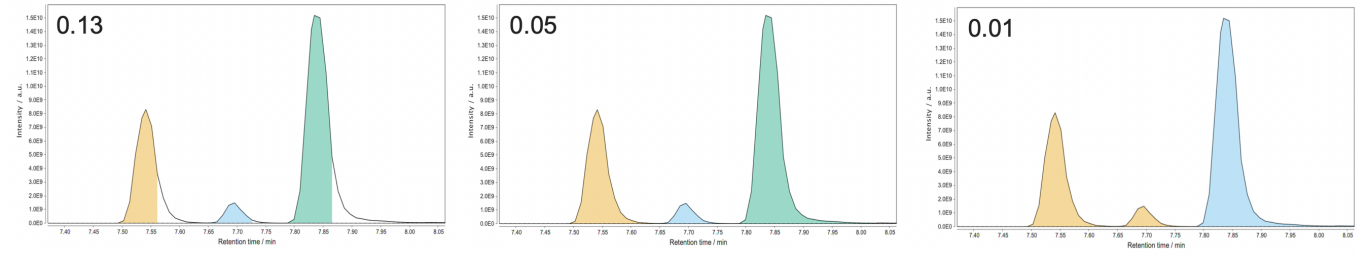
For example, a **Chromatographic threshold** = 50% will discard the lowest-intense 50% data points in the EIC trace.

💡 It must be noted that the algorithm examines the EICs throughout the entire RT range (*i.e.* also the zero data points are considered). Therefore, we recommend to set this value rather high (*e.g.* 90-95%) and lower it only if needed.

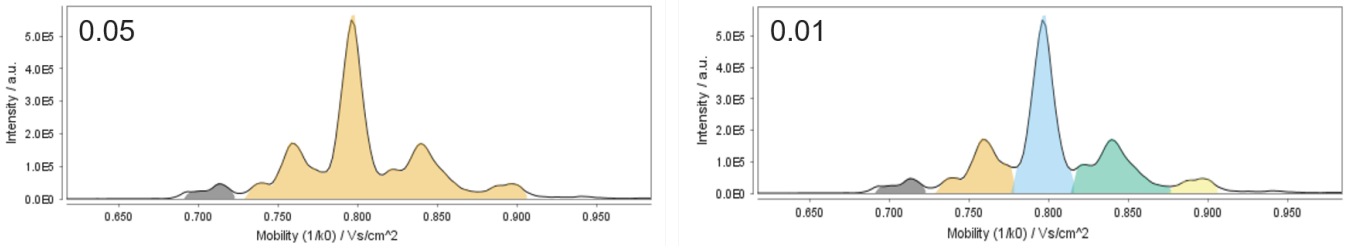
💡 When resolving the ion mobility dimension, we recommend to lower this settings to no more than 80% since mobilograms contains less data points than regular LC traces.

Minimum search range RT/Mobility (absolute)

Size of the RT, or mobility, window examined for local minimum search. An overly narrow search range can cause peak edges to be cut off, whereas a too wide search ranges might lead to an incomplete resolution of narrowly eluting peaks (see example below).



A shorter *Minimum search range* is generally needed when resolving the ion mobility dimension.



Minimum relative height

Minimum relative intensity (with respect to the highest data point in the EIC) a peak needs to reach to be retained as a feature. This parameter can be used in combination with the **Minimum absolute height** setting as to filter the resolved features to be retained.

Many users prefer to rely only on the *Minimum absolute height* as it is more straightforward to set. To do so, set the *Minimum relative height = 0* and the parameter will be ignored.

Modern mass spectrometers provide linear dynamic ranges up to 5 orders of magnitude. If we take an Orbitrap device with a detector saturation around 1.0E10 intensity, a *Minimum relative height* = 0.001 would correspond to a 1.0E5 minimum intensity.

Minimum absolute height

Minimum absolute intensity a peak needs to reach to be retained as a feature. This parameter is very similar to the Min highest intensity settings in the ADAP chromatogram builder module and the same concepts apply.

When resolving the RT dimension, the same value used as Min highest intensity in the EICs building can normally be used here.

While frame scans are examined over the RT dimension, mobility scans are examined over the IM dimension. Therefore, this parameter might need to be adjested accordingly when resolving the ion mobility dimension.

Min ratio of peak top/edge

Minimum ratio between the intensity of the highest (apex) and side (left and right 'edges') points of a peak, to retain it as a feature. The peak edges have to be X times less intense than the peak apex for the feature to be retained.

The purpose of this parameter is to reduce the detection of false local minima when the examined trace (EIC or *mobilogram*) is not smooth. In general, this mainly affects low intensity and not-baseline-resolved signals

This parameter can best be optimized using the *Show preview* option. We recommend values between 1.7 (not baseline separated) and 2 to start the optimisation.

Peak duration range (min/mobility)

Range of acceptable peak length expressed in minutes (RT dimension) or absolute units (mobility dimension). This parameter can be used to filter out noisy features based on their overly short (or long) duration.

Min # of data points

Minimum number of data points a resolved peak needs to have to be considered valid and retained as a feature. This parameter can be used along with the **Peak duration range** setting as peak duration constraint to filter out noisy features.

This parameter is very similar to the Min group size in # of scans settings in the ADAP chromatogram builder module and the same value can normally be used here (usually, no less than 4-5).

A feature in the IM dimension is normally made up of more data points than regular LC peaks. Therefore, a higher *Min # of data points* can be set when resolving the ion mobility dimension to filter out noisy features.

Show preview

By checking this box, an interactive visualization panel will open to help the user to adjust the algorithm parameters. Two EIC traces can be displayed simultaneously in two sub-panels to assess the impact of chosen settings on both "good" and "noisy" EIC traces. The feature list and EIC traces to display can be selected from the corresponding drop-down menus. A noisy EIC can generally be found by sorting the feature table by decreasing area, or by looking at the height/area ratio provided for each feature in the top sub-panel (noisy EIC tend to have low height/area ratios). We recommend optimising the parameters on good EICs and checking the results of these parameters with a noisy EIC.

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4.8.2 **ADAP resolver**

Description

Feature detection → Chromatogram resolving → ADAP resolver

ADAP detects EIC peaks by using the **continuous wavelet transform (CWT)** algorithm. Wavelet coefficients are first calculated as the inner product between the EIC and wavelets at different scales and locations.

Subsequently, peak location and boundaries are determined through a **ridgeline detection** and simple local minima search.

References

1. Du, P., Kibbe W. A., and Lin S. M., Bioinformatics 2006, 22:2059-65.
2. Wee A., Grayden D. B., Zhu Y., Petkovic-Duran K., and Smith D., Electrophoresis 2008, 29:4215-25.

RIDGELINE DETECTION

A real peak in an EIC should create a **local maxima** in the wavelet coefficients at multiple scales. The best scale would create the largest coefficient. In case of the wavelet, it is the scale, for which the wavelet most closely matches the shape of the peak. Scales close to the best scale should also have reasonably similar shapes to the peak and therefore create adjacent maxima between those scales.

Ridgelines are the series of connected local maxima across scales, which are indicative of a real peak.

The applied procedure for detecting the ridgelines is similar to that described by Du et al. [1] and Wee et al. [2] and is as follows:

1. Begin with the coefficients corresponding to the largest wavelet scale.
2. Find the largest coefficient at this scale and initialize a ridgeline.
3. Remove all coefficients that are within half the estimated compact support of the **Ricker wavelet** (2.5 times the current scale).
4. Find the next largest coefficient discounting all removed coefficients and initialize another ridgeline.
5. Repeat steps (3)-(4) until there are no more coefficients remaining for this wavelet scale.
6. Move to the next scale (decrease by one) and repeat (1)-(6). Add new coefficients to an existing ridgeline if they are close in RT.

We define "close" to be a difference in their indices that is less than or equal to the current scale being investigated. 7. After all scales have been processed, ridgelines must have a **length**, i.e., the total number of scales represented in the ridgeline, greater than or equal to 7.

SIGNAL-TO-NOISE THRESHOLD ESTIMATION

Intensity-based

To calculate it, S is chosen to be the maximum intensity between the boundaries of the feature under investigation. Noise, N, is estimated using two different steps. The final estimate of N is the smaller value, which is then used to calculate S/N. Each estimation of the noise attempts to avoid overestimate from the accidental inclusion of other real features that may be close in RT.

Step 1:

1. Set two windows, one on each side of the peak in the EIC. The windows begin at the left and right peak boundaries and end at the peak boundaries plus or minus 2 times **peak width (PW)**, respectively. PW is defined to be the number of scans between the two boundaries of a peak.
2. Calculate the standard deviation of the intensities in the two combined windows and store it as one possible value of the noise.
3. Expand both windows out from the peak by a single scan. The boundaries closest to the peak remain the same. After the first expansion, each window has a length of 2 times PW+1.
4. Calculate and store the standard deviation of the intensities in the combined windows.
5. Repeat steps (3)-(4) until each window has a length of 8 times PW.
6. Incrementally shrink each window by one scan, calculating and storing the standard deviations of the combined windows. The windows are shrunk by moving the boundary closest to the peak toward the boundary furthest from it.
7. Repeat step (6) until the window size is 2 times PW. The final noise estimate is taken to be the smallest stored standard deviation.

Step 2:

1. Same as (1) in step 1.
2. Same as (2) in step 1.
3. Shift each entire window away from the feature by one scan; the window lengths do not change.
4. Repeat steps (2)-(3) until each window's boundary furthest from the feature is 8 times PW from the closest boundary of that feature.
5. The final noise is taken to be the smallest stored standard deviation.

Wavelet coefficients-based

The magnitude of the wavelet coefficient alone is not sufficient for determining if a feature is real due to its strong dependence on the intensities of the data points used in calculations.

To ensure that low-intensity features can be reliably detected and that poorly shaped peaks can be reliably filtered out, the largest coefficient, \(Cmax\), for a given feature is taken and divide it by the area, \(A\), under the curve between the two boundaries of the peak.

Then the area is calculated using a trapezoidal method so that \(A\) is exactly the area under the curve created by connecting adjacent data points with straight lines.

The result is a measure for which large values correspond to the features similar in shape to the wavelet.

One important property of \(Cmax/A\), is that intermittent dips in the intensity can increase the value due to the reduced area. This is beneficial for finding messy low-intensity features but can also be problematic if the area is so small it results in the detection of a feature with a very bad shape.

Parameters

Suffix

This string is added to feature list name as suffix

Original feature list

Defines the processing.

Standard is to KEEP the original feature list and create a new processed list.

REMOVE saves memory.

PROCESS IN PLACE is an advanced option to process directly in the feature list and reduce memory consumption more - this might come with side effects, apply with caution.

MS/MS scan pairing

Set MS/MS scan pairing parameters. For more details see MS2 scan pairing

Dimension

Select the dimension to be resolve - either retention time, or mobility.

S/N threshold

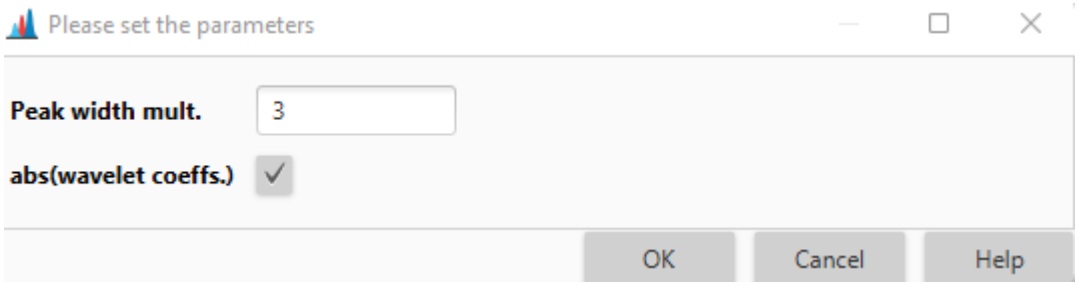
The signal (S) to noise (N) ratio, S/N.

S/N estimator

There two options for S/N estimator:

- **Wavelet coefficient**

  If this parameter is chosen the calculations follow the algorithm described here



  This uses two parameters:

- **Peak width mult.** determines window size
- **abs(wavelet coeffs.)** determines if the absolute values of coefficient are used
- **Intensity window SN**

If this parameter is chosen the calculations follow the algorithm described here.

Min feature height

The smallest intensity a peak can have and be considered a real feature.

Coefficient/area threshold

The best coefficient (the largest inner product of wavelet with peak in ridgeline) divided by the area under the curve of the feature

Peak duration range

The acceptable range of peak widths. Features with widths outside this range will be rejected.

RT wavelet range

The range of wavelet scales used to build matrix of coefficients. Scales are expressed as RT values (minutes) and correspond to the range of wavelet scales that will be applied to the chromatogram. Choose a range that is similar to the range of feature widths expected to be found from the data.

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4.8.3 Baseline resolver

Description

Feature detection → Chromatogram resolving → Baseline resolver

A very simple method that can be used to demonstrate the functionality of chromatogram resolving.

- First, the algorithm removes the lowest part of the chromatogram below a **Baseline level** specified by the user.
- Remaining peaks above the baseline level are recognized if they fulfill the height and duration requirements.

Parameters

Suffix

This string is added to feature list name as suffix

Original feature list

Defines the processing.

Standard is to KEEP the original feature list and create a new processed list.

REMOVE saves memory.

PROCESS IN PLACE is an advanced option to process directly in the feature list and reduce memory consumption more - this might come with side effects, apply with caution.

MS/MS scan pairing

Set MS/MS scan pairing parameters. For more details see MS2 scan pairing

Min peak height

Minimum acceptable feature height (absolute intensity)

Peak duration range

Range of acceptable feature durations

Baseline level

Level below which all data points of the chromatogram are removed (absolute intensity)

Min # of data points

Minimum number of data points on a feature

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4.8.4 CentWave Resolver

Description

Feature detection → Chromatogram resolving → CentWave resolver

This method uses wavelets (from xcms library) to detect features within a chromatogram. A series of wavelets of different scales is convolved within the chromatogram. **Local maxima** in the convolution results determine the locations of possible peaks.

When these candidate feature locations co-occur at multiple scales then the scale with the strongest response indicates **peak width**. Given the candidate feature locations and scales, features can then be reconstructed from the original chromatogram.

Full details of the algorithm are published in Tautenhahn et al. [1].

REQUIREMENTS

The Wavelets detector employs **Bioconductor's XCMS package for R** [2]. Therefore, you must have R v2.15 or later installed. To install the XCMS package, run R and issue the following commands:

```
source("http://bioconductor.org/biocLite.R")
biocLite("xcms")
```

To run R from MZmine the Rserve package [3] must be installed in R, so also run the following R command:

```
install.packages("Rserve")
```

References

1. Ralf Tautenhahn, Christoph Böttcher, and Steffen Neumann "Highly sensitive feature detection for high resolution LC/MS" BMC Bioinformatics 2008, 9:504
2. Bioconductor XCMS "LC/MS and GC/MS Data Analysis" http://www.bioconductor.org/packages/release/bioc/html/xcms.html.
3. Rserve "A TCP/IP server which allows other programs to use facilities of R" https://rforge.net/Rserve/.

Parameters

Suffix

This string is added to feature list name as suffix

Original feature list

Defines the processing.

Standard is to KEEP the original feature list and create a new processed list.

REMOVE saves memory.

PROCESS IN PLACE is an advanced option to process directly in the feature list and reduce memory consumption more - this might come with side effects, apply with caution.

MS/MS scan pairing

Set MS/MS scan pairing parameters. For more details see MS2 scan pairing

S/N Threshold

Features with a signal-to-noise ratio less than the threshold will be rejected.

The S:N ratio is defined as

\[(max - baseline) / sd\]

where max is the maximum feature intensity, baseline is the estimated baseline value, and sd is the standard deviation of local chromatographic noise.

Peak duration range

The acceptable range of feature widths. Features with widths outside this range will be rejected.

Peak integration method

Type of data used during feature reconstruction.

When reconstructing a feature from the chromatogram, gradient descent is used. This can be performed on the raw peak data or a smoothed version of it. Smoothed data is obtained through mexican hat filtering.

Using the unfiltered data is more accurate but can be susceptible to noise. The smooth data provide less exact results but are more robust in the presence of noise.

R engine

The R engine to be used for communicating with R. RServe might provide you with better performance.

Min # of data points

Minimum number of data points on a feature.

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4.8.5 Noise amplitude resolver

Description

Feature detection → Chromatogram resolving → Noise amplitude resolver

This method is suitable for chromatograms with significant background noise of varying intensities. It works in a similar way to the Baseline cut-off method, but sets the baseline level individually for each chromatogram, depending on the amplitude of signal noise.

The baseline level is calculated as follows:

- The intensity range of the chromatogram is divided into bins of the user-specified size (the Noise amplitude parameter)
- The bin with the highest number of data points is found. This bin represents the intensity level of the noise signal.
- The baseline level is set to the intensity of the bin with the most data points

Parameters

Suffix

This string is added to feature list name as suffix

Original feature list

Defines the processing.

Standard is to KEEP the original feature list and create a new processed list.

REMOVE saves memory.

PROCESS IN PLACE is an advanced option to process directly in the feature list and reduce memory consumption more - this might come with side effects, apply with caution.

MS/MS scan pairing

Set MS/MS scan pairing parameters. For more details see MS2 scan pairing

Min peak height

Minimum acceptable height (intensity) for a feature

Peak duration range

Range of acceptable feature durations

Amplitude of noise

This value is the intensity amplitude of the signal in the noise region

Min # of data points

Minimum number of data points on a feature.

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4.8.6 Savitzky-Golay resolver

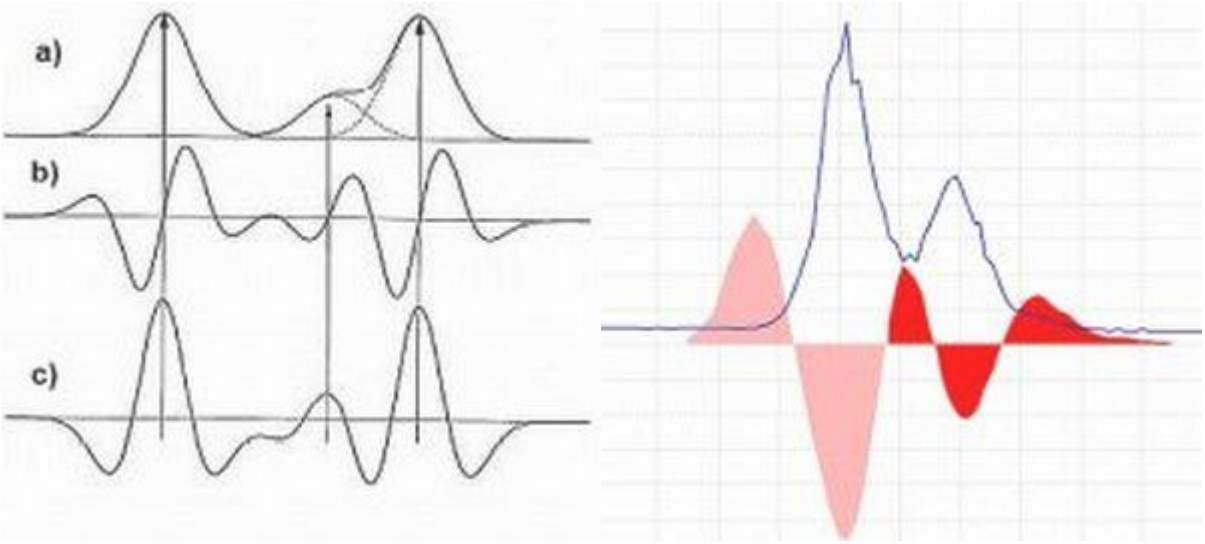
Description

Feature detection → Chromatogram resolving → Savitzky Golay resolver

This method uses the **Savitzky-Golay polynomial** [1] to calculate the smoothed second-derivative of the chromatogram's intensities.

The following figure (left) presents the shape of a) a Gaussian peak, b) the first derivative, and c) the second derivative.

The figure on the right side shows how the signal (blue line) may be divided into individual chromatographic peaks by observing the second derivative.



References

1. A. Savitzky and M. J. E. Golay, Anal. Chem., 36, 1627 (1964). DOI: 10.1021/ac60214a047

Parameters

Suffix

This string is added to feature list name as suffix

Original feature list

Defines the processing. Standard is to KEEP the original feature list and create a new processed list.

REMOVE saves memory.

PROCESS IN PLACE is an advanced option to process directly in the feature list and reduce memory consumption more - this might come with side effects, apply with caution.

MS/MS scan pairing

Set MS/MS scan pairing parameters. For more details see MS2 scan pairing

Min peak height

Minimum acceptable feature height (absolute intensity)

Peak duration range

Range of acceptable feature durations

Derivative threshold level

Minimum acceptable intensity in the second derivative for feature recognition

Min # of data points

Minimum number of data points on a feature.

Last update: September 23, 2022 17:08:14

4.9 Spectral deconvolution (GC)

4.9.1 **Spectral deconvolution: Hierarchical Clustering**

🚧 This module is being updated, which might affect its functionality

Description

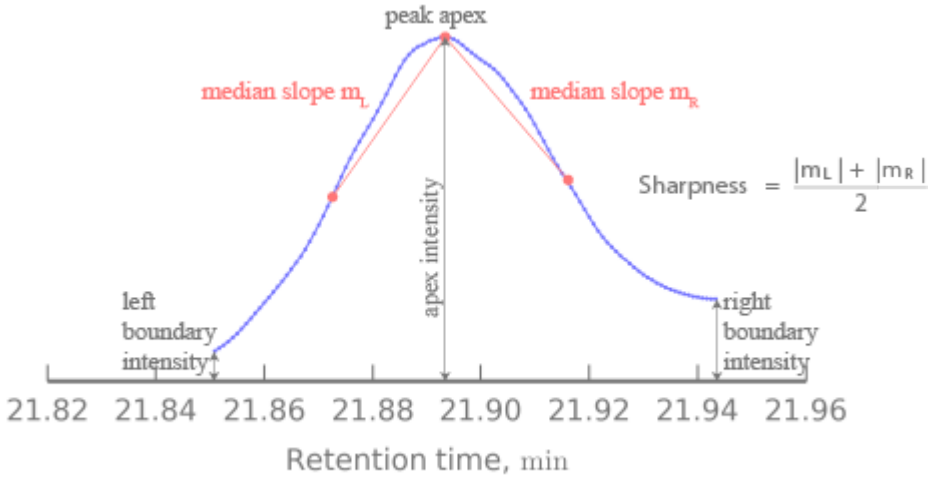
Feature list methods → Spectral deconvolution (GC) → Hierarchical clustering

This ADAP-based method finds analytes by combining similar features into clusters and using their intensities to construct fragmentation mass spectra.

The following steps are performed:

- All EIC peaks are clustered based on proximity of their retention times. The user can specify minimum distance between clusters, minimum number of peaks forming a cluster, and minimum cluster intensity. If preview is selected, the top right plot displays the result of the clustering with dots corresponding to EIC peaks and colors corresponding to different clusters.
- In each cluster, EIC peaks are filtered based on their sharpness and on their elution profiles. All EIC peaks with sharpness below minimum sharpness are filtered out.
- If "**Find shared peaks**" is selected, then shared peaks are filtered out as well. A user can specify minimum edge-to-height ratio and minimum delta-to-height ratio that are used in determining shared peaks.
- EIC peaks that have passed the filtering step, are clustered based on their elution profiles. The user can specify shape-similarity tolerance: small tolerance corresponds to large number of clusters, while large tolerance corresponds to a small number of clusters. If a preview is selected, the result of the clustering is shown on the bottom-right plot.
- Each cluster corresponds to one analyte. Among all EIC peaks in the cluster, a model peak is chosen to represent the elution profile of the analyte.
- If **Choise of Model Peak based on Sharpness** is selected, then the EIC peak with the highest sharpness in the cluster is selected to be a model peak.
- If **Choice of Model Peak based on Intensity** is selected, then the EIC peak of the highest intensity is selected to be a model peak.
- If **Choice of Model Peak based on M/z value** is selected, then the EIC peak with the highest m/z value in the cluster is selected to be a model peak.
- In order to build fragmentation spectra for analytes, each EIC peak is decomposed into a linear combination of the model peaks with the weighting coefficients obtained by solving an optimization problem.
- These coefficients and m/z value of the EIC peak contribute to the fragmentation spectra of the corresponding analytes.

METHOD DEFINITIONS



Sharpness

In order to find sharpness, the medians of the slopes of the lines connecting the peak apex to its other data points are calculated on each side of the peak apex. The sharpness is defined as the average of the two medians.

Shared peak

EIC peak is considered to be shared (i.e. produced by two co-eluting analytes) if at least one of the following conditions is satisfied:

- its elution profile has several local maxima
- its left boundary intensity divided by the apex intensity exceeds minimum edge-to-height ratio
- its right boundary intensity divided by the apex intensity exceeds minimum edge-to-height ratio
- the absolute difference between its boundary intensities divided by the apex intensity exceeds by minimum delta-to-height ratio

References

1. Pluskal, T., Castillo, S., Villar-Briones, A. & Oresic, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* (2010). DOI: 10.1186/1471-2105-11-395
2. Smirnov A, Jia W, Walker D, Jones D, Du X: ADAP-GC 3.2: Graphical Software Tool for Efficient Spectral Deconvolution of Gas Cromatography—High-Resolution Mass Spectrometry Metabolomics Data. J. Proteome Res 2017, DOI: 10.1021/acs.jproteome.7b00633

Parameters

Min cluster distance (min)

Minimum distance between any two retention-time clusters.

Min cluster size

Minimum number of peaks in a cluster.

Min cluster intensity

Minimum intensity of the highest peak in a cluster.

Find shared peaks

If selected, shared peaks are determined and do not participate in the second clustering.

Min edge-to-height ratio

Minimum value of a boundary intensity divided by the apex intensity of EIC peak that is considered to be shared.

Min delta-to-height ratio

Minimum value of the absolute difference of the boundary intensities divided by the apex intensity of EIC peak that is considered to be shared.

Min sharpness

Minimum sharpness of EIC peak that can participate in the second clustering.

Shape-similarity tolerance (0..90)

Tolerance is used in the second clustering based on the similarity of peaks' elution profiles: small tolerance corresponds to large number of clusters; large tolerance corresponds to a small number of clusters.

Choice of Model Peak based on

In each cluster, a model peak is chosen.

- If Sharpness is used, then EIC peak with the highest sharpness in the cluster is chosen to be a model peak.
- If Intensity is used, then EIC peak of the highest intensity in the cluster is chosen to be a model peak.
- If M/z value is used, then EIC peak with the highest m/z value in the cluster is chosen to be a model peak.

Exclude m/z-values

Optional parameter

Optionally, the user can specify a list of deprecated m/z values such that EIC peaks with those m/z value could not be chosen as model peaks. It is possible to specify single m/z values as well as ranges of m/z values. For example: 1-50, 73, 100.

Suffix

String to add to feature list name as a suffix.

Remove original feature list

If checked, original feature list will be removed.

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4.9.2 Spectral deconvolution: Multivariate Curve Resolution

🚧 This module is being updated, which might affect its functionality

Description

Feature list methods → Spectral deconvolution (GC) → Multivariate Curve Resolution

This **ADAP-based method** performs Spectral Deconvolution of detected peaks. It finds components (compounds, analytes, etc.) and determines their model peaks and fragmentation mass spectra.

The spectral deconvolution uses both constructed chromatograms and detected peaks. The feature list of constructed chromatograms is specified by selecting Specific peak lists for parameter Chromatograms, clicking on the ellipsis button, and choosing one or more lists with chromatograms in the popup window. The list of detected peaks is specified by selecting Specific peak lists for parameter Peaks, clicking on the ellipsis button, and choosing one or more lists with detected peaks in the popup window.

The Spectral Deconvolution consists of **two steps**:

1. Entire retention time interval is split into deconvolution windows so that
2. Peaks produced by the same component or by coeluting components belong to the same deconvolution window,
3. Number of peaks in deconvolution window is significantly smaller than the total number of peaks.

The deconvolution windows are displayed in the top plot of the preview, where small dash lines denote peaks in the (retention time, m/z)-plane, and peaks belonging to one deconvolution window have the same color. The vertical sequences of peaks usually mark the presence of one or several compounds, so it is important that those peaks are assigned to the same deconvolution window, i.e. they have the same color on the plot. The deconvolution windows are controlled by the **Deconvolution window width** parameter.

⚠️ If deconvolution windows contain too many peaks, it will significantly slow down the spectral deconvolution computations, so the deconvolution windows should be as short (in the retention time domain) as possible. 2. The algorithm infers the number of components in each deconvolution window and construct their model peaks and fragmentation spectra. The inferred number of components is controlled by three parameters: **Retention time tolerance (min)**, **Minimum Number of Peaks**, and **Adjust Apex Ret Times**.

References

1. Pluskal, T., Castillo, S., Villar-Briones, A. & Oresic, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* (2010). DOI: 10.1186/1471-2105-11-395
2. Smirnov A, Jia W, Walker D, Jones D, Du X: ADAP-GC 3.2: Graphical Software Tool for Efficient Spectral Deconvolution of Gas Cromatography—High-Resolution Mass Spectrometry Metabolomics Data. J. Proteome Res 2017, DOI: 10.1021/acs.jproteome.7b00633
3. Aleksandr Smirnov, Yunping Qiu, Wei Jia, Douglas I. Walker, Dean P. Jones, Xiuxia Du. ADAP-GC 4.0: Application of Clustering-Assisted Multivariate Curve Resolution to Spectral Deconvolution of Gas Chromatography–Mass Spectrometry Metabolomics Data. Analytical Chemistry 2019, 91 (14) , 9069-9077. 10.1021/acs.analchem.9b01424

Parameters

Deconvolution window width (min)

The algorithm will produce deconvolution windows so that their width (in retention time domain) does not exceed the value of this parameter.

Retention time tolerance (min)

The smallest time-gap between any two components.

Minimum Number of Peaks

The smallest number of detected peaks to form a component.

Adjust Apex Ret Times

If **false**, then the retention time of each detected peak is determined by the retention time of its highest data point. If **true**, then the retention time of each detected peak is determined by fitting a parabola into the top half of that peak.

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4.10 CCS Calibration and calculation

4.10.1 Description

The modules described in this section can be found in **Feature list methods → Processing → Internal reference CCS calibration / External CCS calibration / Calculate CCS values**.

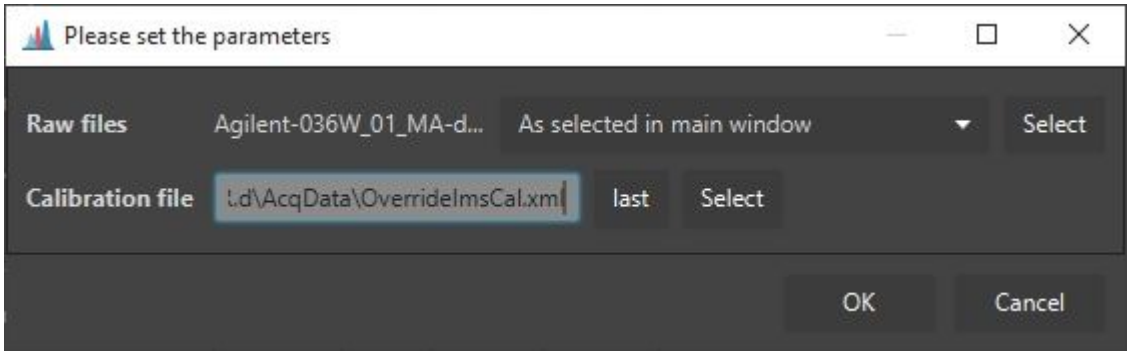
Accurate determination of CCS values requires a valid CCS calibration and molecule charge states to be detected. A CCS calibration can be either imported or created from internal reference.

- **timsTOF** raw data can be recalibrated using data analysis and imported in MZmine. The recalibrated data will be used by default. (see Calculating CCS values)
- **mzML** raw data requires the determination of a calibration function from the raw data (e.g. as detected features) or as import from an external file. (see importing an external CCS calibration and refernce CCS calibration)

4.10.2 Importing an external CCS calibration

Agilent calibration data can be imported from the "OverrideImsCal.xml" file in the Agilent raw data folder. Waters calibration data can be imported from the "mob_cal.csv" file in the Waters raw data folder. The "_extern.inf" file is also required, but will be read automatically when the "mob_cal.csv" is selected.

The calibration import is accessed via **Feature list methods -> Processing -> External CCS Calibration**. Then select the calibration "OverrideImsCal.xml"/"mob_cal.csv" from the raw data folder, and select the raw data files the calibration should be applied to.

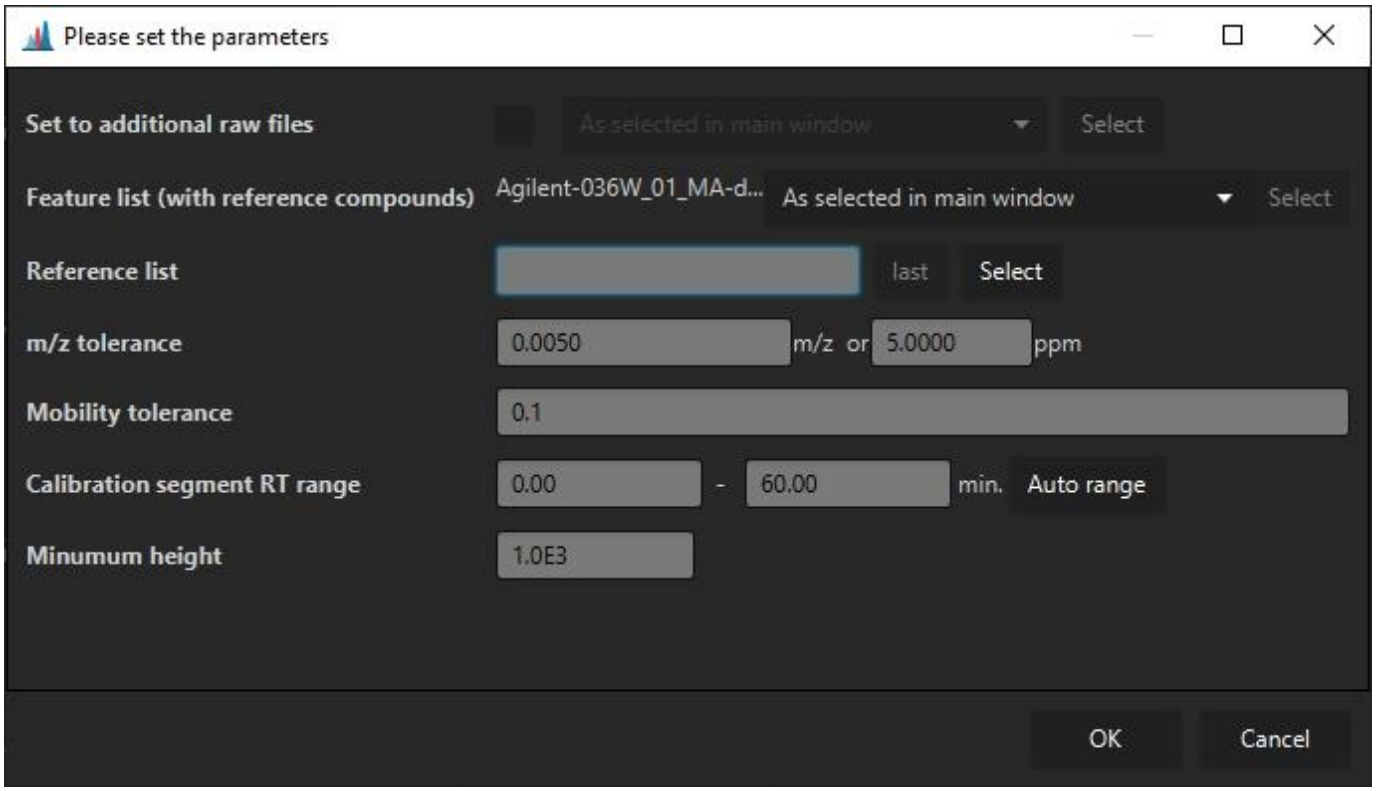


4.10.3 Reference CSS calibration

If a mobility calibrant is infused during an HPLC run of every sample, a CCS calibration can be calculated on a per-raw file basis. (Common procedure on Bruker devices) Otherwise, a single run can be used to calibrate multiple files.

Please note that this is currently only supported for TIMS and DTIMS data.

The calibration module can be accessed via **Feature list methods -> Processing -> Internal reference calibration**.



Parameters

SET TO ADDITIONAL RAW FILES

If a calibration calculated from a single feature list shall be applied to multiple other raw files, the raw files can be selected here. This requires only a single raw file to be selected.

FEATURE LIST (WITH REFERENCE COMPOUNDS)

Specifies (a) feature list(s) that contains the reference compounds. If multiple feature lists are selected, every feature list will be searched for reference compounds, and the calibration will be used for the raw data files in the particular feature list. This means that no raw data file may be selected. (Cannot set multiple calibrations to a single raw file)

If a single feature list is selected, the calibration may be applied to additional raw data files via the **Set to additional raw files** parameter.

REFERENCE LIST

Specifies a ".csv" reference list of for CCS calibrant ions. Must contain the columns "mz", "mobility", "ccs", "charge". Columns must be separated by ";". The ion mode may be specified via the charge of the ion, e.g., as 1 or -1. Only the correct polarity will be used to calculate the calibration.

M/Z TOLERANCE

The m/z tolerance for the reference compounds.

MOBILITY TOLERANCE

The mobility tolerance to detect the reference compounds.

CALIBRATION SEGMENT RT RANGE

Specifies the rt range that shall be searched for calibrant ions. Usually either the beginning or end of a HPLC run.

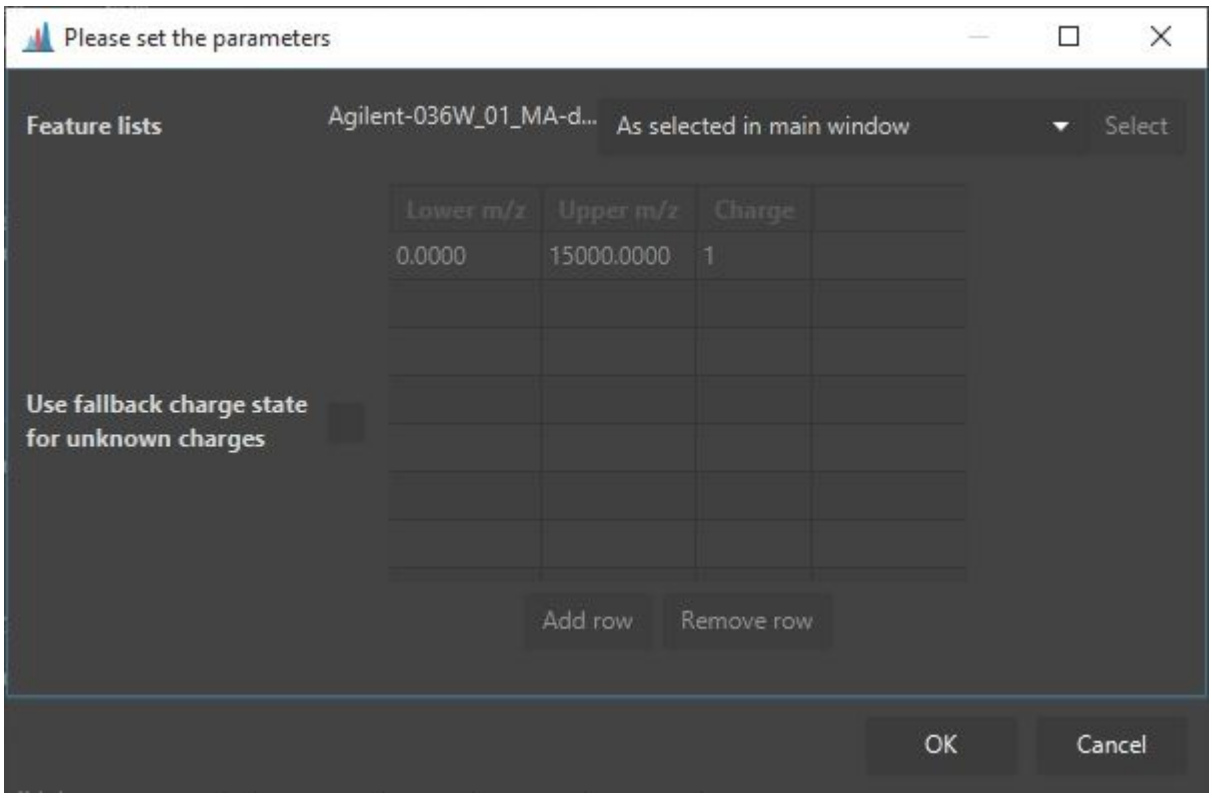
MINIMUM HEIGHT

A minimum intensity for reference compounds to be used as calibrant signals for determination of the calibration.

4.10.4 Calculating CCS values

After a calibration as been set (Agilent/Waters/Bruker mzML) (Bruker tdf works out-of-the-box) CCS values can be calculated via **Feature list methods -> Processing -> Calculate CCS values**.

Here, a default charge state may be set, in case it could not be determined. Otherwise, the charge state determined via the isotope pattern will be used.



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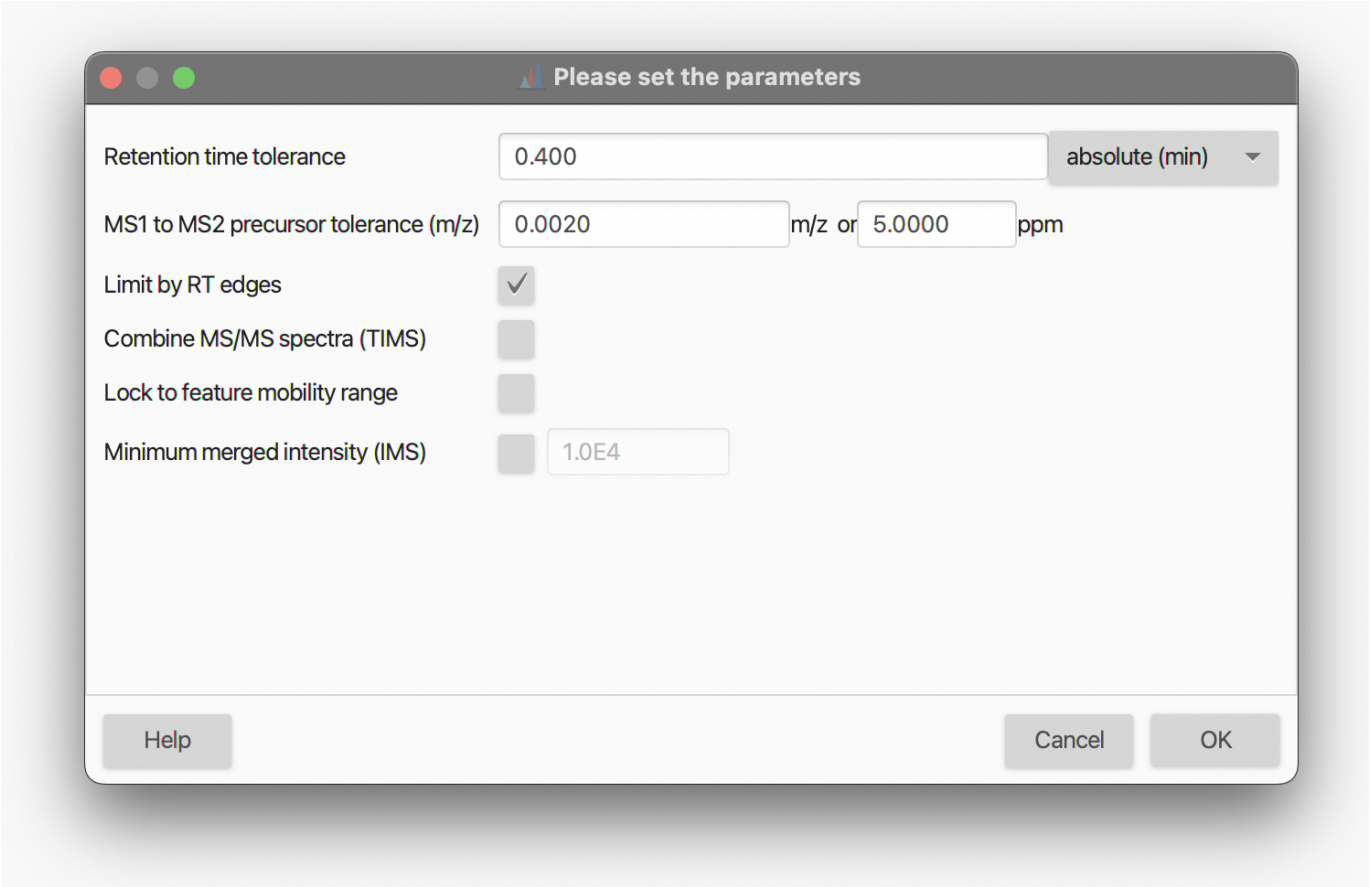
4.11 MS2 Scan Pairing

4.11.1 Description

Feature list methods → Processing → Assign MS2 to feature

This module allows to pair MS2 scans with features. It assigns all MS2 scans within range to all features in chosen feature list.

4.11.2 Parameters



RETENTION TIME TOLERANCE

The maximum offset between the highest point of the chromatographic peak and the time the MS2 was acquired.

MS1 TO MS2 PRECURSOR TOLERANCE (M/Z)

Describes the tolerance between the precursor ion in a MS1 scan and the precursor m/z assigned to the MS2 scan.

LIMIT BY RT EDGES

Use the feature's edges (retention time) as a filter.

COMBINE MS/MS SPECTRA (TIMS)

If checked, all MS/MS spectra assigned to a feature will be merged into a single spectrum.

LOCK TO FEATURE MOBILITY RANGE

If checked, only mobility scans from the mobility range of the feature will be merged.

⚠️ This is usually not needed. However, if isomers/isobars elute at the same retention time and are close in mobility, the MS/MS window might be larger than the peak in mobility dimension and thus cause chimeric MS/MS spectra.

MINIMUM MERGED INTENSITY

If an ion mobility spectrometry (IMS) feature is processed, this parameter can be used to filter low abundant peaks in the MS/MS spectrum, since multiple MS/MS mobility scans need to be merged together.

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4.12 Isotope filtering

4.12.1 ^{13}C isotope filter

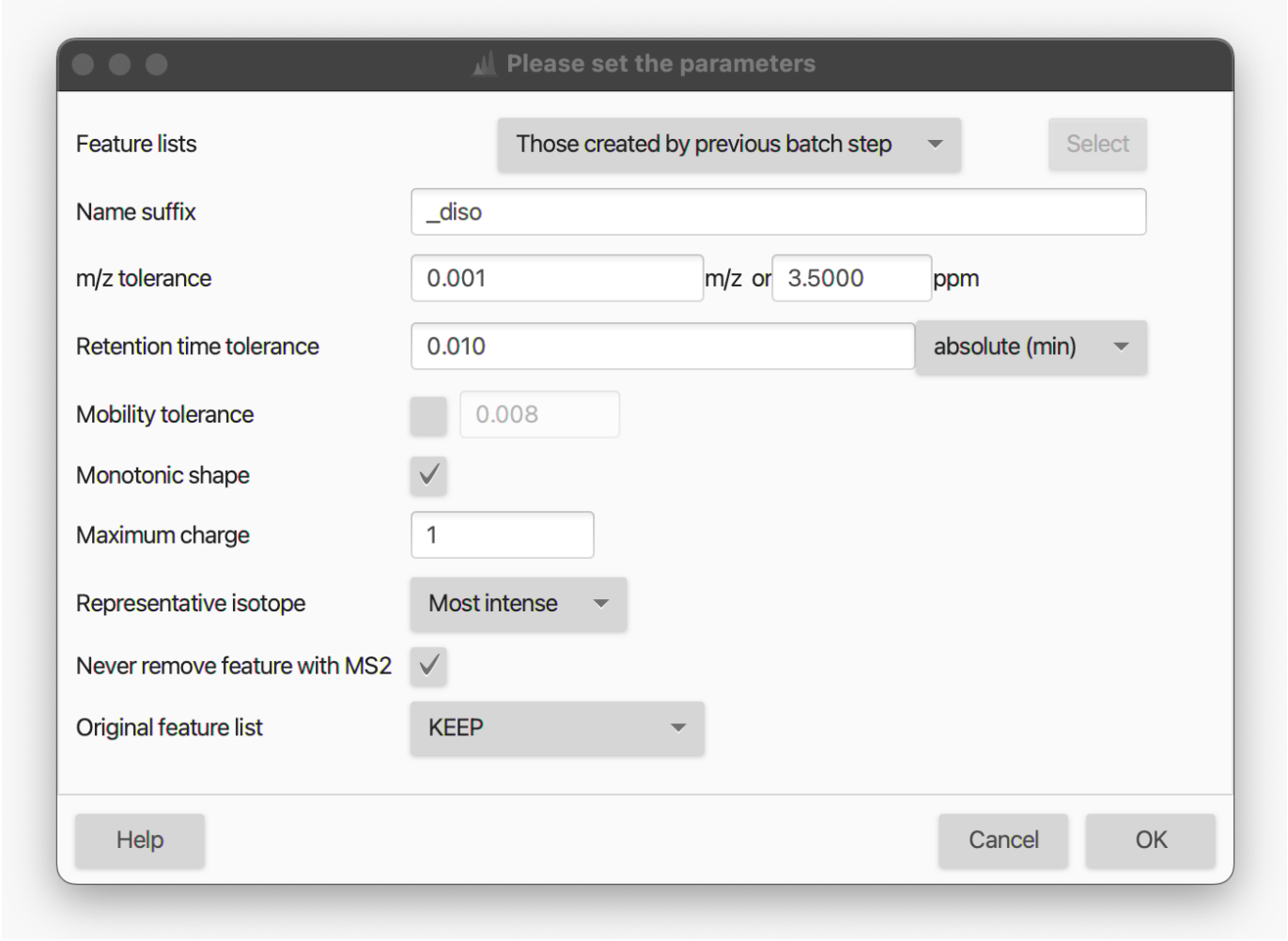
Description

Feature list methods → Isotopes → 13C isotope filter (formerly: isotope grouper)

MZmine carries out the feature detection steps of *chromatogram building* and subsequent *resolving* considering **all** the signals stored in the *mass lists*. As a consequence, signals generated by isotopologues of the same chemical entity (see *isotopic pattern*) are detected as distinct features and included in the *feature lists*, representing redundant information for the downstream data analysis. This issue ordinarily occurs for C-containing molecules, where the ^{13}C isotope signals can be easily detected (^{13}C/^{12}C ≈ 1.1%).

The *13C isotope filter* module (formerly called *Isotope grouper*) aims at filtering out the features corresponding to the ^{13}C isotopes of the same analyte. The algorithm consider each feature individually and checks for the presence of potential ^{13}C-related peak(s) in the *feature lists*. When an isotope pattern meeting the user-defined tolerances (*e.g. m/z*, RT) and requirements (*e.g. monotonic shape*) is found, the information is saved, and only the feature corresponding to the *e.g.* most intense isotope is retained in the *feature list*. It must be noted that ^{13}C peaks are searched within the *feature list*, and not in the raw data.

Parameters



Name suffix

String added as suffix when creating the new feature list(s).

m/z tolerance

Maximum allowed difference between the measured and the predicted *m/z* of the (potential) ^{13}C isotope to be grouped as isotopologues. The tolerance can be specified as absolute tolerance (in *m/z*), relative tolerance (in ppm), or both. When both are specified, the tolerance range is calculated using the maximum between the absolute and relative tolerances.

We recommend to set a failry strict *m/z* tolerance to reduce the risk of discarding false ^{13}C isotopes.

Retention time tolerance

Maximum allowed RT difference between the feature and its (potential) ^{13}C isotope to be grouped as isotopologues.

Isotopologues should exhibit identical chromatographic behaviour and thus produce overlapping LC peak shapes. Therefore, a strict RT tolerance can be used to reduce the risk of discarding false ^{13}C isotopes.

Mobility tolerance

If enabled and the mobility dimension was recorded, potential ^{13}C isotopes will be grouped as isotopologues only if their mobility difference is within the defined tolerance.

The same principle seen for the RT tolerance apply to the IM dimension. Isotopologues should exhibit identical IM separation; therefore, a strict mobility tolerance can be used to reduce the risk of discarding false ^{13}C isotopes.

Monotonic shape

If true, a monotonically decreasing trend of the isotope pattern (typical of the ^{13}C isotope pattern of small molecules) is required for the filtering.

Maximum charge

Maximum charge state considered to predict the ^{13}C isotopes' *m/z*. If a value > 1 is set, the charge state with the maximum number of detected isotope features will be used for the filtering.

Never remove feature with MS2

If checked, potential ^{13}C-related features will not be discarded if associated to a MS2 spectrum.

Original feature lists

Keep or remove the input feature list(s). The *PROCESS IN PLACE* option directly filter the input feature list and performs better in terms of memory usage; therefore, it is recommended over *REMOVE*, when available.

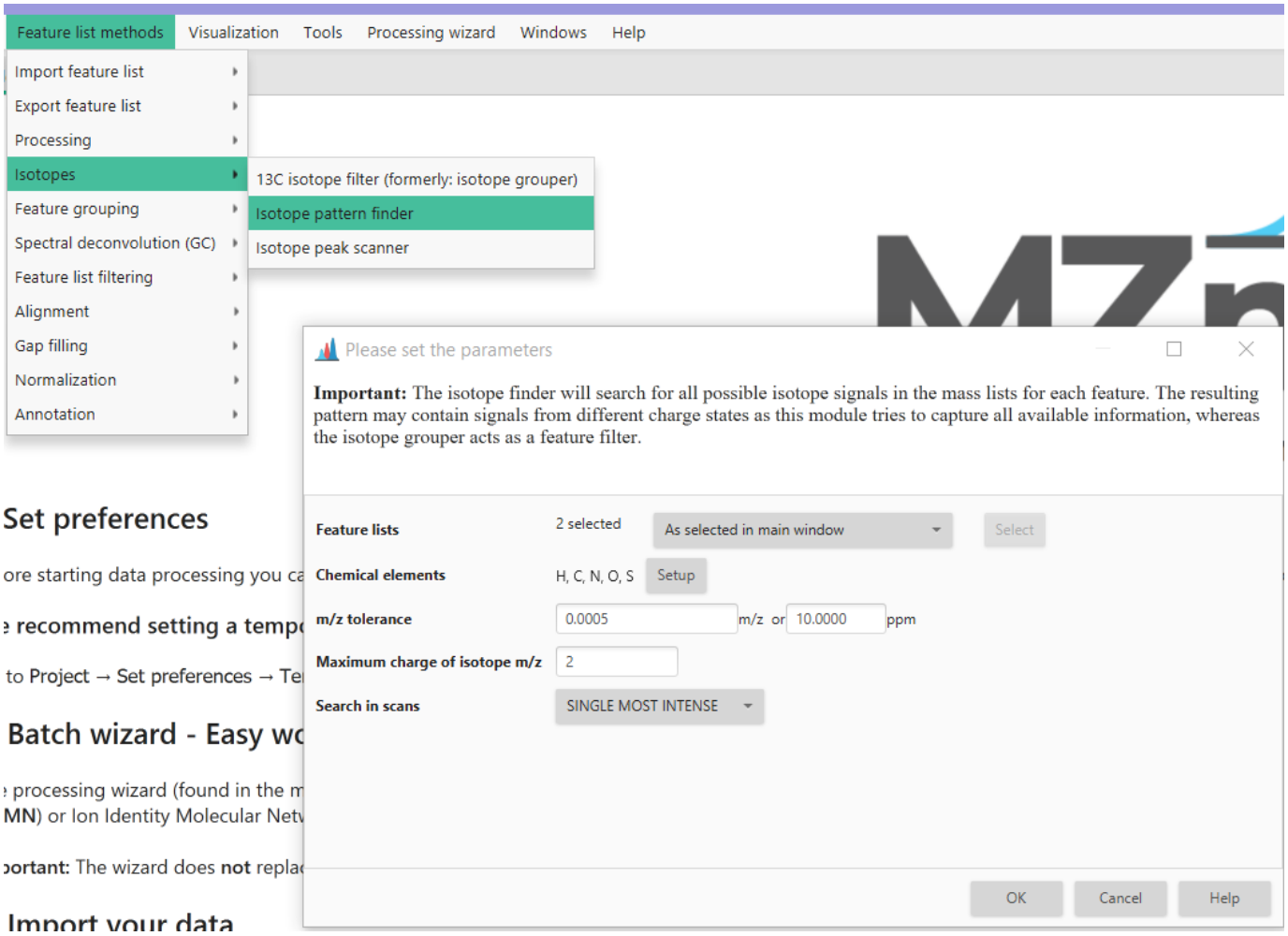
Last update: September 23, 2022 17:08:14

4.12.2 **Isotope pattern finder**

Description

Feature list methods → Isotopes → Isotope pattern finder.

The module searches isotope patterns for each feature in selected feature lists by going back to the mass spectra. Starting from the feature m/z the algorithm will first backtrack any possible preceding isotope signals using a list of delta masses created from elements, their stable isotopes, and an m/z tolerance. For example, a -2 signal might be detected when searching for Br isotopes. In a second step, all picked potential isotope m/z values are used to search next isotope (with higher m/z). This is done for each charge state.



PARAMETERS

Chemical elements

All stable isotopes of the chosen elements are used to create a list of mass differences to search. Signals with this mass difference (m/z difference with different charge states) are then considered as potential isotope signals.

m/z tolerance

Maximum allowed difference between two features' m/z values in order for them to be considered the same. The value is specified both as absolute tolerance (in m/z) and relative tolerance (in ppm). The tolerance range is calculated using maximum of the absolute and relative tolerances.

Maximum charge of isotope m/z

Maximum possible charge of isotope m/z distributions. All present m/z values obtained by dividing isotope masses with 1,2 ...,maxCharge values will be considered. The default value is 1, but insert an integer greater than 1 if you want to consider ions of higher charge states.

Search in scans

Currently, the supported option is "Single most intense", which means the search for isotopes will happen in the single most intense MS scan of each feature.

Last update: September 28, 2022 21:23:56

4.12.3 Isotope peak scanner

Description

Feature list methods → Isotopes → Isotope peak scanner

This module can scan acquired MS-Data for an isotope pattern of a specified **element combination**. The element combination is given as a string parameter (e.g. "Cl3").

The isotope pattern of this element combination will be calculated and compared to your MS-Data. The result is a new feature list containing just the isotope features. The features will have an **isotope rating**, comparing the found features to the calculated isotope pattern giving the user the option to manually evaluate the results.

Additionally, the features will also be assigned to an isotope combination. Furthermore, the isotope features will be registered as an isotope pattern in MZmine so modules like Sum formula prediction can use the results to compare them to the calculated isotope pattern of predicted sum formulas.

In the resulting feature table, the monoisotopic mass (lowest) will be referred to as "Parent".

ISOTOPE RATING

Rating is calculated using the following formulas:

$$R_{m/z,k}=\frac{m/z_{child}}{m/z_{parent}+\Delta{M_k}}$$
$$\frac{I_{parent}}{A_{parent}}=\frac{I_k}{A_k}$$
$$I_{exp.,l}=\frac{I_{parent}}{A_{parent}}\cdot{A_K}$$
$$R_{I,k}=\frac{I_{exp.,k}}{I_k}=\frac{I_{parent}}{A_{parent}}\cdot\frac{A_k}{I_k}$$

where R is the rating, k - the number of an isotope peak, exp. - the calculated intensities, parent - the isotope peak with the lowest mass, child describes all other isotope peaks.

If any rating is bigger than 1, for example if m/z_(parent) + ΔM is smaller than m/z_(child), then it will be adjusted by 1/R to be comparable.

If intensity shall be checked as well, m/z and intensity rating will be multiplied resulting in the final rating.

Parameters

Feature lists

The feature list(s) that shall be analyzed.

m/z tolerance

m/z window around the expected isotope features to scan for isotope peaks.

Check RT

If chosen, compares RT of feature to that of a parent. Based on the following parameter of **Retention time tolerance** feature is either filtered out or preserved.

⚠️ Invoking this parameter might not make sense for imaging or direct infusion, but is critical for chromatographic data.

Retention time tolerance

Maximum allowed difference between two retention time values

Chemical formula

Element (combination) whose isotope pattern to be searched for. Please enter the two letter Symbol of the elements. (e.g. \"Gd\", \"Cl2Br\")

Auto carbon

If activated, Isotope peak scanner will calculate isotope patterns with variable numbers of carbon specified in Setup. The pattern with the best fitting number of carbon atoms will be chosen for every detected pattern.

💡 This will greatly increase computation time but helps with unknown-compound-identification.

⚠️ Please note that 13C isotope peaks might overlap with hetero atom isotope peaks depending on the resolution of your MS. This influences intensity ratios and will yield **wrong results**, since this prediction is based on intensity ratios of isotope peaks.

⚠️ This option yeilds no exact results, but might give a good hint about data.

Charge

Amount and polarity(e.g.: [M]+=+1 / [M]-=-1).

⚠️ This is important for multiply charged molecules because the m/z offset between isotope peaks will halve for molecules with a charge of two.

Min. pattern intensity

The minimum normalized intensity of a peak in the final calculated isotope pattern. Depends on the sensitivity of your MS. This differs from minimum abundance. Min = 0.0, Max = 0.99999.

Merge width(m/z)

This will be used to merge peaks in the calculated isotope pattern if they overlap in the spectrum. Specify in m/z, this depends on the resolution of your mass spectrometer.

Minimum height

Minimum peak height to be considered as an isotope peak.

⚠️ Setting this parameter is crucial if you use the **Calculate accurate average** parameter. (see below)

Check intensity ratios

Compare intensity of peaks to the calculated abundance of the isotope pattern.

💡 It's recommended to check this parameter for more accurate results.

⚠️ However, when processing fragment data, and it's unknown how much of an isotope pattern remains charged it might be reasonable to uncheck this. (e.g.: Fragmenting a Cl8-isotope-pattern-molecule -> If "Element pattern" = Cl4 this module will recognize everything with Cl4 or more Cl. However, this will lead to a messy result feature list)

Minimum rating

Minimum rating to be considered as an isotope peak. min = 0.0, max = 1.0

Rating type

Method to calculate the rating with.

- **Highest Intensity** is the standard method and faster.
- **Temporary average** is slower method but could be more accurate for more intense peaks.

Calculate accurate average

This method will use averaged intensities over all mass lists in which ALL relevant masses were detected in. This will only be done for features that match the defined rating-calculation with the given rating.

This will scan all mass lists for the peak closest to the identified isotope peak in the feature list and average the intensity.

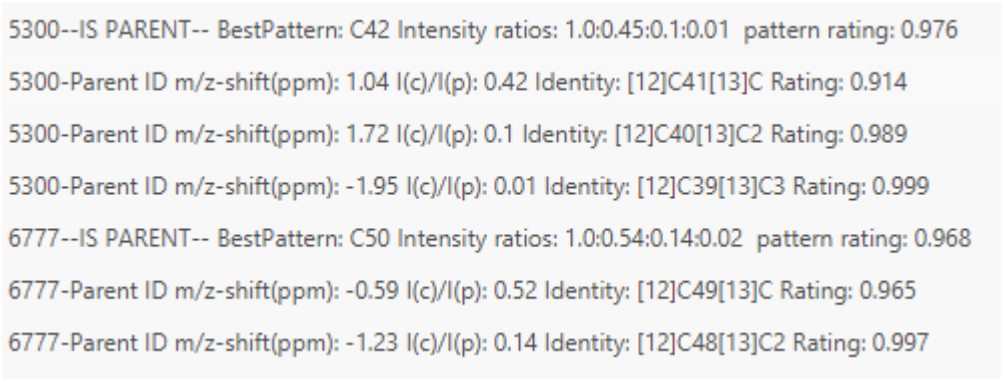
If there are no scans that match all criteria avg rating will be -1.0.

⚠️ Make sure the mass list is contained in the feature list.

Name suffix

Suffix to be added to feature list name. If "auto" then the module will itself create a suffix.

Results



This figure shows an example of the result peak list produced by Isotope peak scanner. It features the detected isotope peaks, a detected m/z ppm-offset, expected (@monoisotopic mass) and detected intensity ratios, the isotope composition, the rating, and (if specified) the average rating.

Troubleshooting

Error: I'm using "Calculate accurate average" but the average rating is always -1.0!

Solution: All isotope features have been detected in the peak list. But they are not in the same mass lists at the same time which makes them incomparable since isotope features should be detected simultaneously, or they might me less intense than the specified "Minimum height".

Error: I'm not getting any results, although I'm sure a specific element is in the scan!

Solution 1: Are you sure every isotope has been detected? How sensitive is you MS? Try to increase values for minimum abundance or minimum pattern intensity. You might need low minimum abundance but high minimum intensity, because a peak of a specific isotope composition might not been detected due to low relative intensity in the pattern. Check our preview function!

Solution 2: Another solution might be changing the merge width. Check how good the resolution of your MS data is and adjust the merge width to that. If (several) isotope compositions overlap, the intensities have to be merged. You can see a preview in the preview window!

Last update: September 23, 2022 17:08:14

4.13 Feature list filtering

4.13.1 **Duplicate feature filter**

Description

Feature list method → Feature list filtering → Duplicate feature filter.

This filter can help eliminate misaligned feature list rows after the gap-filling process.

It has three different **modes**:

- **Old average (the old filter)**:

  Keeps only the feature list row with the maximum average area. Compares rows with the average m/z and RT.

- **New average**:

  Compares rows with the average m/z and RT and creates a consensus row.Two peaks are considered duplicates when their average m/z and retention time differences are lower than the tolerances set by the user.

  When two (or more) duplicates are found, a **consensus row** is created with the lowest row ID of all duplicates. For this consensus row, all DETECTED features are favored over ESTIMATED (gap-filled) and ESTIMATED are favored over UNKNOWN. Furthermore, if there are only ESTIMATED features in a raw data file, the highest is chosen.

- **Single feature**:

  Compares rows on a raw data file basis. Marks rows as duplicates if they share one feature within the RT and m/z tolerance in the same raw data file. Creates a consensus row.

Parameters

Name suffix

This is the suffix to identify the new aligned peak list.

Filter mode

User can choose one of three modes: old average, new average, and single feature.

m/z tolerance

Maximum m/z difference between duplicate peaks.

RT tolerance

Maximum retention time difference between duplicate peaks.

Require same identification

If the checkbox is selected duplicate peaks must have the same identification.

Original feature list

Can be either processed in place of, kept or replaced.

Last update: September 23, 2022 17:08:14

4.13.2 Feature list rows filter

Description

Feature list methods → Feature list filtering → Feature list rows filter

This filter deletes all rows in a selected peak list that do not meet requirements defined by the user.

A range of different requirements can be set, such as the minimum number of features in the row, the minimum number of features in an isotope pattern, peak duration etc.

When an aligned peak list, i.e. multiple peaks per row, is filtered then the average of each row's peak duration, m/z and retention time values are used to filter the row.

Parameters

Name suffix

Suffix to be added to feature list name.

Minimum features in a row (abs or %)

Minimum number of features detected in a row required to not remove it. Values < 1 will be interpreted as %.

Minimum features in an isotope pattern

Minimum number of features in a row's isotope pattern required to not remove it.

Validate 13C isotope pattern

If ticked, searches for a +1 13C signal (considering possible charge states) within estimated range of carbon atoms. Uses 13C isotope filter.

m/z

Range of acceptable (average) m/z values in a row required to not remove it.

Retention time

Range of acceptable (average) retention times in minutes.

Features duration range

Range of acceptable (average) feature durations in a row required not to remove it.

Chromatographic FWHM

Range of permissible FWHM in a row required not to remove it.

Charge

Range of Charge in a row required not to remove it.

⚠️ Please, run isotopic peaks grouper prior to using this.

Kendrick mass defect

Filter features in a Kendrick mass defect (KMD) range. For more details see Kendrick mass defect.

If KMD is used, following parameters can be changed in the setup.

- **Kendrick mass defect** Permissible range of a Kendrick mass defect per row
- **Kendrick mass base** Enter a sum formula for a Kendrick mass base, e.g. "CH2"
- **Shift** Enter a shift for shift dependent KMD filtering
- **Charge** Enter a charge for charge dependent KMD filtering
- **Divisor** Enter a divisor for fractional base unit dependent KMD filtering
- **Use Remainder of Kendrick mass** Use Remainder of Kendrick mass (RKM) instead of Kendrick mass defect (KMD)

Parameter

Paremeter defining the group of each sample.

Only identified?

If the checkbox is selected, only identified compounds will be retained.

Text in identity

Only rows that contain this text in their peak identity field will be retained.

Text in comment

Only rows that contain this text in their comment field will be retained.

Keep or remove rows

User can select to either keep or remove the rows that match the defined criteria.

Feature with MS2 scan

If checked, only features that have MS2 scan will be kept.

Never remove feature with MS2

If checked, all rows with MS2 are retained without applying any further filters on them.

Reset the feature number ID

If checked, row IDs will be reset.

Mass defect

Mass defect as a feature filter can be used for selective detection of compounds of interest, and the values accepted are 0.314-0.5 or 0.90-0.15.

Original feature list

It can be either processed in place, kept or removed.

Last update: September 23, 2022 17:14:34

4.13.3 **Feature filter**

Description

Feature list methods → Feature list filtering → Feature filter

This module deletes features in a selected feature list that do not meet the requirements defined by the user. The filter is performed separately on each raw data file in the peak list.

Parameters

Name suffix

Suffix to be added to feature list name.

Duration

Peaks with a duration outside the entered range will be removed.

Area

Peaks with an area outside the entered range will be removed.

Height

Peaks with a height outside the entered range will be removed.

 # data points

Peaks with fewer data points than the entered range will be removed.

FWHM

Peaks with a FWHM outside the entered range will be removed.

Tailing factor

Peaks with a tailing factor outside the entered range will be removed.

Asymmetry factor

Peaks with an asymmetry factor outside the entered range will be removed.

Keep only features with MS/MS scans

Peaks without any MS/MS scans will be removed.

Remove source feature list after filtering

If the checkbox is selected, the source feature list will be removed, and the filtered version will remain.

Last update: September 23, 2022 17:08:14

4.13.4 Peak comparison rows filter

Description

Feature list methods → Feature list filtering → Peak comparison rows filter

This method removes certain rows from an aligned feature list based on peak comparisons in two columns.

4.13.5 Parameters

Name suffix

Suffix to be added to feature list name.

1st peak column to compare (zero indexed)

Index of second column for comparison, e.g. "0".

2nd peak column to compare (zero indexed)

Index of second column for comparison,e.g. "1".

Fold change range : log2(peak1/peak2)

Return peaks with a fold change within this range.

m/z difference range : peak1 to peak2 (ppm)

Return peaks with a m/z difference within this range.

RT difference range : peak1 to peak2 (min)

Return peaks with an RT difference within this range.

Remove source feature list after filtering

If checked, the original feature list will be removed leaving only the filtered version.

Last update: September 23, 2022 17:08:14

4.13.6 **Neutral loss filter**

Description

Feature list methods → Feature list filtering → Neutral loss filter

This module can scan acquired MS data for neutral losses.

The result is a new feature list containing only features and their neutral-loss equivalents.

The results will be displayed in the following manner:

- The feature with higher mass without the neutral loss will be named **"Parent"**. The description will contain the ID of the corresponding feature with the neutral loss.
- The feature with lower mass will be named **"Child"**. The description of the child feature will contain the ID of the parent feature and a ppm-shift relative to the calculated mass.

Parameters

m/z tolerance

m/z window size around the expected features.

Check RT

Specify whether the algorithm should compare RT to those of a parent in found peaks or not.

⚠ We recommend to not use it for direct infusion.

If evaluating chromatographic data we recommend to use this parameter.

Retention time tolerance

Tolerance range of the retention time.

Minimum height

Minimium height of a feature to be considered a parent or child.

Neutral loss (m/z)

m/z ratio of the neutral loss to be detected. If the text box of "Molecule" is not left blank, this parameter will be ignored.

Molecule

Element combination/Molecule of the neutral loss (e.g. HI) This module will calculate the mass of the given molecule and ignore the input in the "Neutral loss" text box.

Last update: September 23, 2022 17:08:14

4.13.7 **m/z mobility region extraction**

Description

Feature list methods → Feature list filtering → mobility-m/z region filtering

Extracts subregions of interest from m/z-mobility regions.

Parameters

Region

Regions to extract.

Mobility/CCS

Defines if mobility or mz shall be used for extraction.

Suffix

The suffix of newly created feature lists.

Last update: September 23, 2022 17:08:14

4.13.8 **Feature list blank subtraction**

Description

⚠️ This module needs aligned feature lists as an input. This aligned feature list should contain features from blank/control.

Feature list methods → Feature list filtering → Feature list blank subtraction

Subtracts the features appearing in (procedural) blank measurements feature list from an aligned feature list.

Parameters

Minimum # of detection in blanks

Specifies in how many of the blank files a peak has to be detected.

Fold change increase

Specifies a percentage of increase of the intensity of a feature. If the intensity in the list to be filtered increases more than the given percentage to the blank, it will not be deleted from the feature list.

Suffix

The suffix for the new feature list.

Last update: September 23, 2022 17:08:14

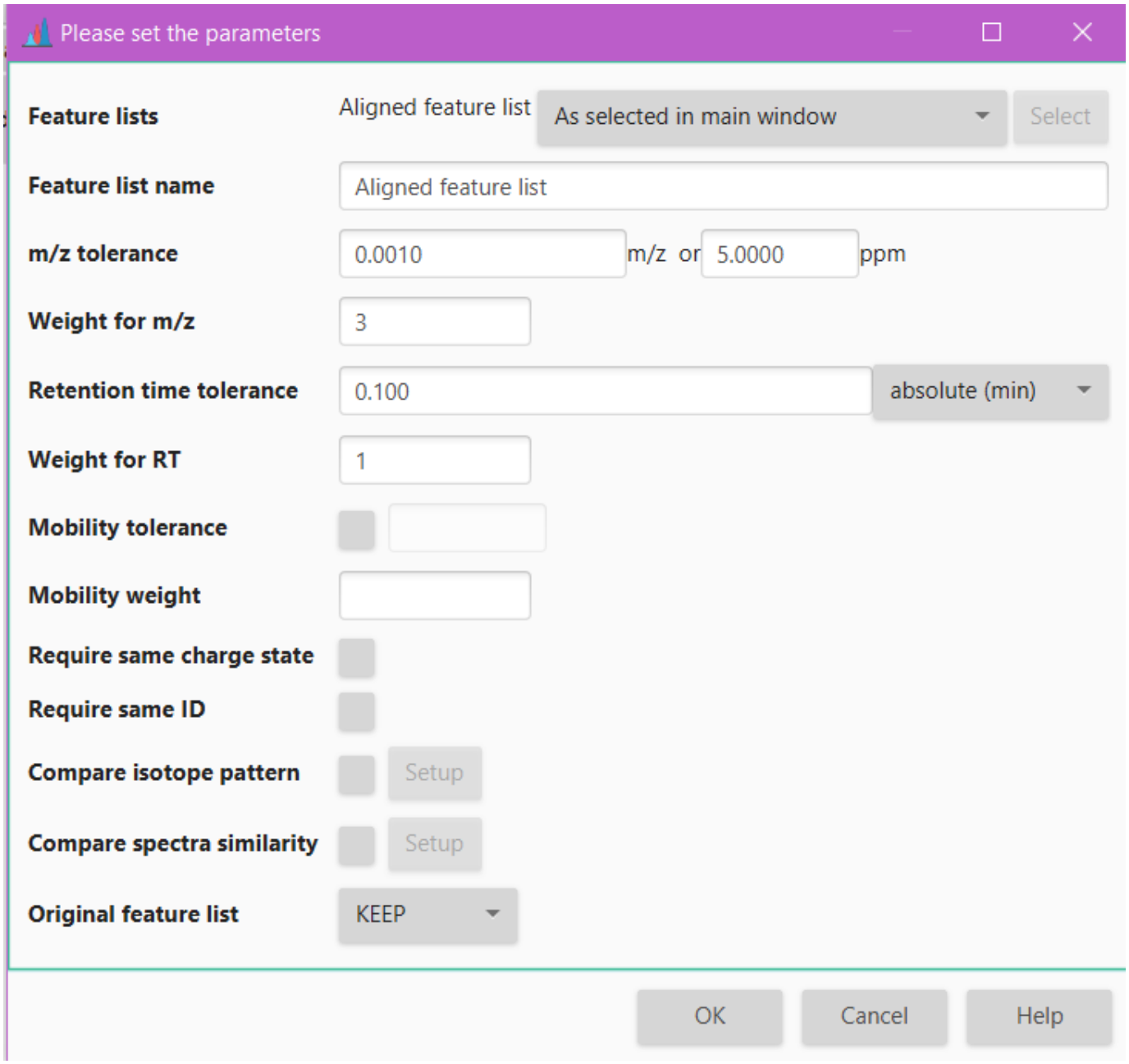
4.14 Alignment

4.14.1 **Join aligner**

Description

Feature list methods → Alignment → Join aligner

This method aligns detected peaks in different samples through a **match score**. This score is calculated based on the mass and retention time of each peak and ranges of tolerance specified in the parameter setup dialog.



ALGORITHM

Input

The peak alignment algorithm uses:

1. A **master list of peaks** (L) against which every new **sample** (Sj) will be matched.

   When aligning peaks from multiple samples, the master list is initially set to the first sample. Subsequently, it becomes a combination of samples aligned this far.

   Master list contains the samples as the columns and the matching peaks as the rows.

2. For every row i in L, a two-dimensional window (a window size is selected by the user), called **Alignment window (AW)**, defines the ranges of m/z and RT.

   The window is centered around the average of m/z and RT of all the individual peaks in the row.

3. A score function is used to compute the similarity of peaks between L and the new sample Sj inside the alignment window.

   The **score function** computes the similarity based on the similarities in m/z, retention time, and (optionally) on identification, and isotope patterns between the peaks to be matched.

   The score is calculated as follows:

   \[score = \frac{1 - MZ_{difference}}{MZ_{tolerance}} × MZ_{weight} + \frac{1 - RT_{difference}}{RT_ {tolerance}} × RT_{weight}\]

Steps

The algorithm works as follows:

- It iterates through the rows of L.
- For each row, it looks for peaks within the alignment window in Sj that has to be aligned with L.
- A score is calculated for each possible match
- The pair getting the best score will be aligned.

Parameters

Feature list name

Name of the new aligned peak list.

m/z tolerance

Maximum allowed difference between two m/z values in order for them to be considered the same and thus the peaks aligned.

The value is specified both as absolute tolerance (in m/z) and relative tolerance (in ppm).

The tolerance range is calculated using maximum of the absolute and relative tolerances for possible peaks to be aligned.

Weight for m/z

This is the assigned weight for m/z difference at the moment of match score calculation between peak rows, as can be seen in the aforementioned formula. Only in cases where there is a perfect match of m/z values, the score receives the complete m/z weight. Generally, higher weight is given to m/z values than to RT values.

Retention time tolerance

Maximum allowed difference between two retention times in order for them to be considered the same and thus peaks aligned. Maximum RT difference can be defined either using absolute or relative value.

Weight for RT

This is the assigned weight for RT difference at the moment of match score calculation between peak rows. Only in cases where there is a perfect match of RT values, the score receives the complete RT weight.

Mobility tolerance

In case of IM data,the user can determine the mobility tolerance. If checked, this parameter specifies the tolerance range for matching the mobility values.

Mobility weight

Score for perfectly matching mobility values. Only taken into account if "Mobility tolerance" is activated. Furthermore, score calculation that is mentioned in the **Algorithm** is then modified to account for the mobility as well. Mobility tolerance and weight are accounted for in the same manner as m/z and RT parameters.

Require same charge state

Optional parameter

If checked, only rows having same charge can be aligned.

Require same ID

Optional parameter

If checked, only rows having same compound identities (or no identities) can be aligned.

Compare isotope pattern

Optional parameter

If both peaks represent an isotope pattern, checking this box will add isotope pattern score to the match score calculation. Additionally, the user can set up **isotope m/z tolerance** which defines what isotopes would be considered same when comparing two isotopic patterns, **minimum absolute intensity** below which isotopes will be ignored and **minimum score** % between isotope patterns that has to be satisfied in order for the match to not be discarded.

Compare spectra similarity

Optional parameter

Compare MS1 or MS2 scans similarity. Select the m/z tolerance, MS level and spectra similarity algorithm. Only features meeting this criteria will be aligned. See compare spectra similarity for additional information.

Original feature list

The user can choose to either KEEP the original feature list and generate a new processed one, or REMOVE the original feature list with the processed one. Generally, you would keep the original feature list, but opting for REMOVE will save memory.

Last update: September 23, 2022 17:08:14

4.14.2 **Merge lists**

Description

Feature list methods → Alignment → Merge lists

This method merges feature lists by appending all rows into a new list.

⚠️ Perform alignment before to align all features from comparable samples and use this method to merge feature lists that should not be aligned: e.g., positive and negative mode data.

Parameters

Feature lists

Feature lists this module will take as an input

Feature list name

Resulting feature list name

Last update: September 23, 2022 17:08:14

4.14.3 RANSAC peak list aligner

Description

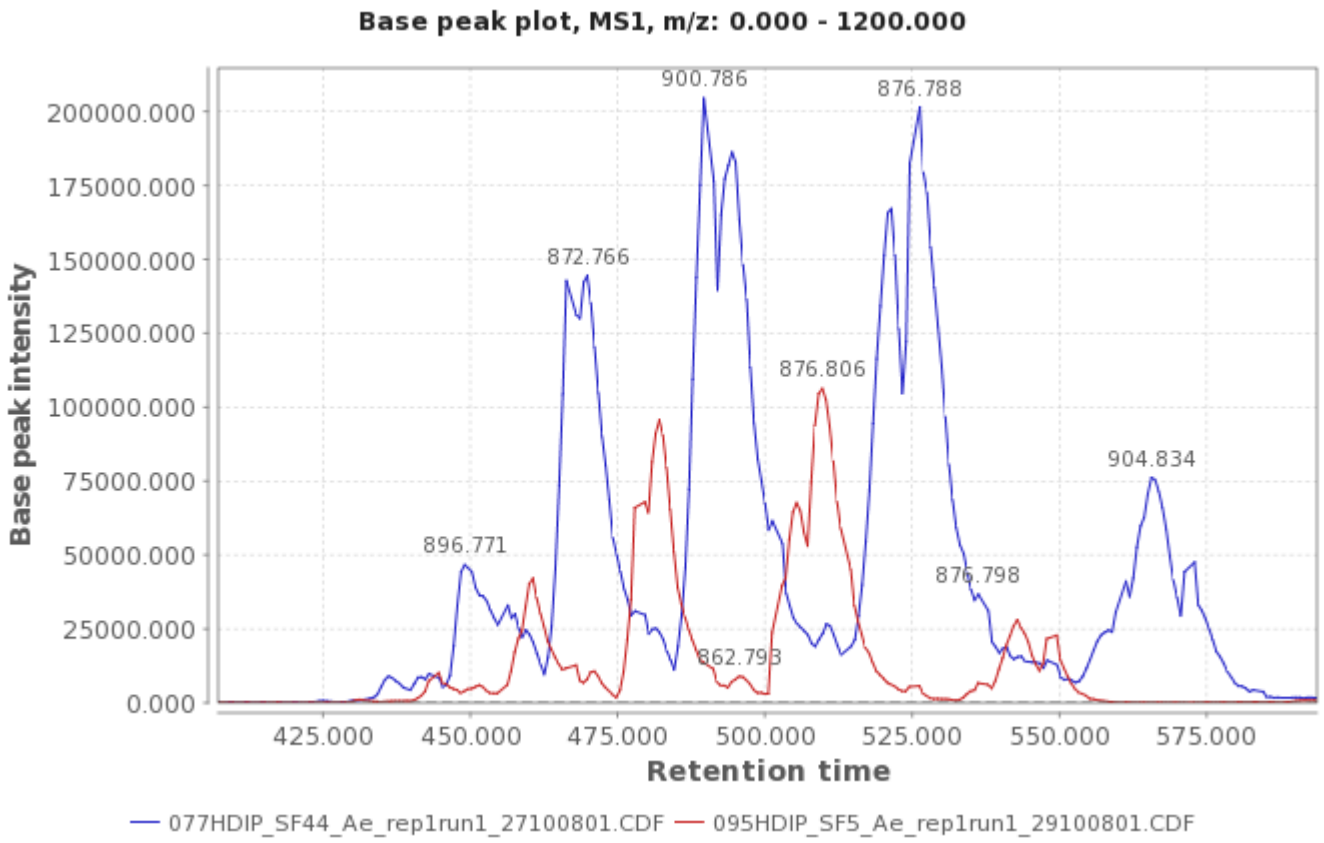
Feature list methods → Alignment → RANSAC aligner

This method is an extension of the **Join aligner** method.

The alignment of each sample is done against a **master peak list**, which is taken from the first sample in the first round and from the average of all aligned peak lists in every round.

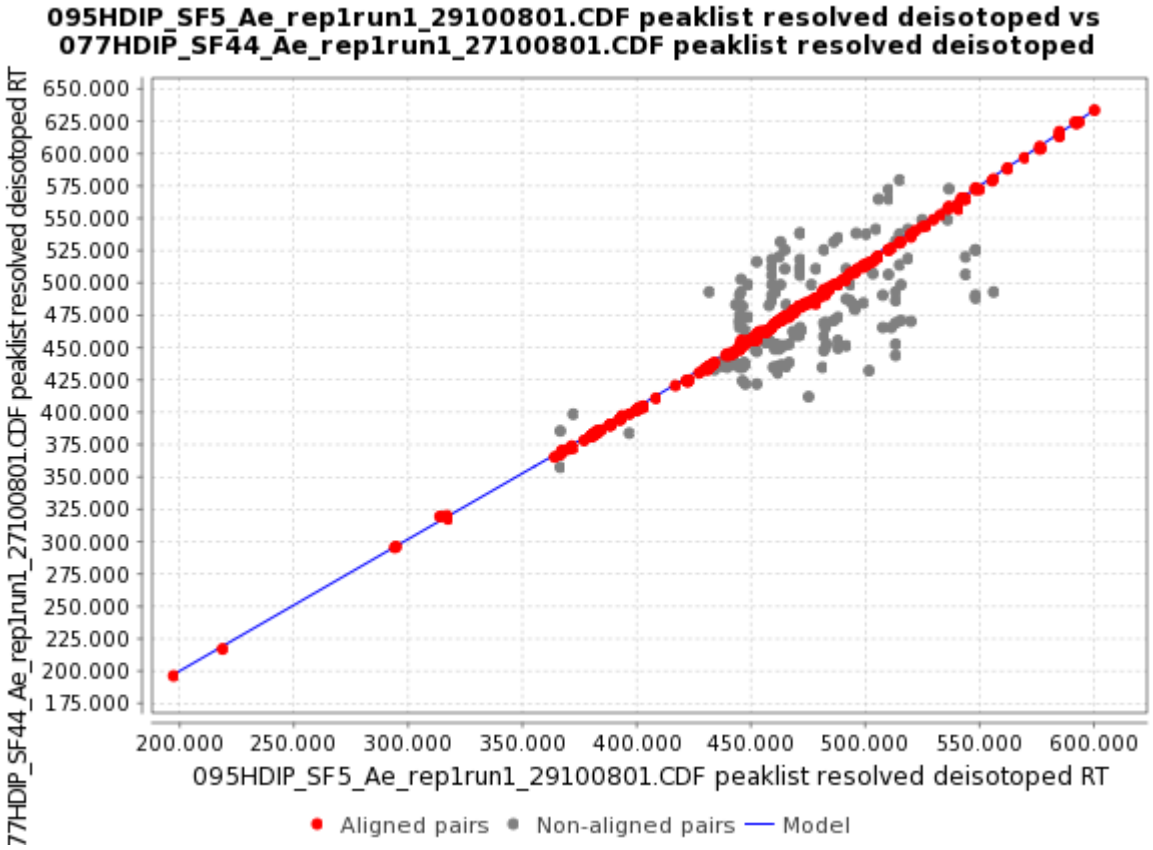
It corrects any linear or non-linear deviation in the retention time of the chromatograms by creating a model of this deviation.

This picture shows an example of two samples with a non-linear deviation in the retention time:



The **"deviation" model** for the retention time is created by taking some corresponding points from the peak list of two samples using the RANSAC algorithm (http://en.wikipedia.org/wiki/RANSAC) and using a non-linear regression method to fit the model.

This picture shows a preview of the model with the red dots representing the aligned peaks taken using RANSAC algorithm, and the blue line represents the fitted model using a non-linear regression.



Using this model, the algorithm can predict the shift in the retention time along all the peak list and use the match score function, used also in **Join Align** algorithm, to match the peaks.

This score is calculated based on the mass and retention time of each peak and ranges of tolerance stipulated in the parameter setup dialog.

Parameters

Feature list name

The name of the new aligned feature list.

m/z tolerance

This value sets the range, in terms of m/z, to verify for possible peak rows to be aligned. Maximum allowed m/z difference.

RT tolerance

This value sets the range, in terms of retention time, to create the model using RANSAC and non-linear regression algorithm. Maximum allowed retention time difference.

RT tolerance after correction

This value sets the range, in terms of retention time, to verify for possible peak rows to be aligned. Maximum allowed retention time difference.

RANSAC Iterations

Maximum number of iterations allowed in the algorithm to find the right model consistent in all the pairs of aligned peaks.

When the value is 0, the number of iterations (k) will be estimate automatically.

Minimum Number of Points

% of points required to consider the model valid (d).

Threshold value

Threshold value (minutes) for determining when a data point fits a model (t).

Linear model

This option should be selected only if the model has to be linear.

⚠️ Please, remember that when the shift in the retention time between the peaks in the samples is not constant the model shape is nonlinear, and this parameter should not be selected.

Recommendations for setting optimal parameters

The three first parameters (m/z tolerance, RT tolerance after the correction and RT tolerance) define **2 bi-dimensional windows** with the same "altitude" (m/z tolerance) and different "longitude" (RT tolerances).

The first window (m/z tolerance - RT tolerance after the correction) sets the space where the matching peak should be present, and **the second window** (m/z tolerance - RT tolerance) sets the total space where RANSAC algorithm will be applied.

- So, **"RT tolerance"** should be as big as the maximum deviation in the retention time along all the chromatogram, and **"RT tolerance after the correction"** can be more flexible and depends on the complexity of the data.

If the data contains few peaks and the separation is good, the window can be bigger than "RT tolerance" window. It will improve the recall without including mistakes. This parameter should not change too much the final results.

- RANSAC is a non-deterministic algorithm, and the probability to find a good result increases with the **number of iterations**. If the user sets "0 iterations" into the parameter "RANSAC iterations" the algorithm will automatically set the optimum number of iterations depending on the number of data points.

  ⚠️ In the case that there is a big number of data points it is better to limit this parameter even though the result could be non-optimal. The preview module can help in setting this parameter.
- The parameter **"Minimum number of points"** should be an estimation of the proportion of the data points inside the model. It is important not to get models composed by few data points which do not correspond to the real model. All the models which contain less proportion of data points won't be taken into account by RANSAC algorithm.
- **Threshold** value represents the width of the model and depends on the nature of the data. If this parameter is too big, it can lead to deviation of the model.

  The preview module can help to set the optimal value.
- The choice of **model** depends on whether the deviation in the retention time can be considered linear or not in the data.

If the deviation in the retention time is linear, a simple linear regression will be used to fit the model.

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4.14.4 Hierachical aligner GC (or Hierarchical Clustering aligner)

Description

Feature list methods → Alignment → Hierachical aligner (GC)

This method aligns detected features in different samples through a **match score**. This score is calculated based on the mass spectrum and retention time of each peak and ranges of tolerance stipulated in the parameters setup dialog.

GENERAL ALGORITHM

This module uses the principles of agglomerative / hierarchical clustering approaches. The general principle behind those algorithms is described below.

Given a set of N items to be clustered, and an N*N distance (or similarity) matrix, the basic process of hierarchical clustering* (defined by S.C. Johnson in 1967 - https://home.deib.polimi.it/matteucc/Clustering/tutorial_html/hierarchical.html#johnson) is this:

1. Start by assigning each item to a cluster, so that if you have **N items**, you now have **N clusters**, each containing just one item. Let the **distances (similarities)** between the clusters the same as the distances (similarities) between the items they contain.
2. Find **the closest (most similar) pair of clusters** and merge them into a single cluster, so that now you have one cluster less.
3. Compute distances (similarities) between the new cluster and each of the old clusters.
4. Repeat steps 2 and 3 until all items are clustered into **a single cluster of size N.** (*)

Step 3 can be done in different ways based on **linkage criteria**:

- In **single-linkage** clustering (also called the connectedness or minimum method), we consider the distance between one cluster and another cluster to be equal to the shortest distance from any member of one cluster to any member of the other cluster. If the data consist of similarities, we consider the similarity between one cluster and another cluster to be equal to the greatest similarity from any member of one cluster to any member of the other cluster.
- In **complete-linkage** clustering (also called the diameter or maximum method), we consider the distance between one cluster and another cluster to be equal to the greatest distance from any member of one cluster to any member of the other cluster.
- In **average-linkage** clustering, we consider the distance between one cluster and another cluster to be equal to the average distance from any member of one cluster to any member of the other cluster.

  A variation on average-link clustering is the **UCLUS method** of R. D'Andrade (1978) - https://home.deib.polimi.it/matteucc/Clustering/tutorial_html/hierarchical.html#dandrade - which uses the median distance, which is much more outlier-proof than the average distance.

All implemented types of hierarchical clustering are called **agglomerative** because they merge clusters iteratively. There is also a **divisive hierarchical clustering**, which does the reverse by starting with all objects in one cluster and subdividing them into smaller pieces. Divisive methods are not generally available, and have rarely been applied.

Of course there is no point in having all the N items grouped in a single cluster. But, once you have got the complete hierarchical tree, if you want to change number of clusters to \(k\), you can just cut the \(k-1\) longest links.

Available linkage criteria

Several linkage criteria are available:

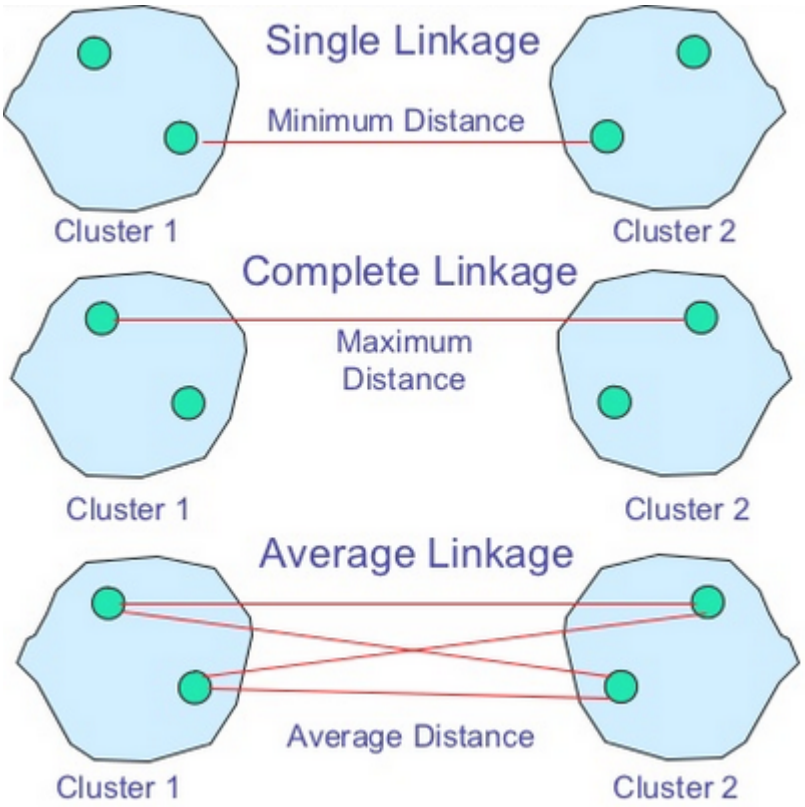
- Single
- Complete
- Average

A linkage criteria determines the distance between sets of observations as a function of the pairwise distances between observations.

Some commonly used linkage criteria between two sets of observations \(A\) and \(B\) are:

Name	Description
Maximum or complete-linkage clustering	\(max \{ d(a,b):a \in A, b \in B \}\)
Minimum or single-linkage clustering	\(min \{ d(a,b):a \in A, b \in B \}\)
Mean or average-linkage clustering, or UPGMA	\(\frac{1}{\lvert {A} \rvert \lvert {B} \rvert} \sum_{a \in A} \sum_{b \in B} d(a,b)\)

where \(d\) is the chosen metric.



MZMINE GC: SPECIFIC CONSIDERATIONS

To obtain a **distance matrix** between all pairs of observations:

1. A similarity score based on **chemical likelihood only (chemSimScore)** is computed.

   Calculation of the dot product is based on work by Stein and Scott (1994), which is the most popular approach for spectrum similarity measure.

   Between two mass spectra \(I_{t_j}\) and \(I_{r_i}\) of two peaks \(t_j\) and \(r_i\) similarity is calculated as follows:

   \[S(t_j,r_i)=dot(I_{t_j},I_{r_i})=<I_{t_j},I_{r_i}>/ (\lvert I_{t_j} \rvert \lvert I_{r_i} \rvert)\]
2. Then a similarity score based on **RT likelihood only (rtScore)** is computed.

   Retention time score is normalized relatively to the RT window tolerance provided by the user

   \[rtScore=\frac{1.0 d-rtDiff}{rtMaxDiff}\]
3. A **combined weighted (or mixture) score** is determined based on a mixture of the above two scores

   The final mixture score

   \[score = (chemSimScore * mzWeight) + (rtScore * rtWeight))\]

A **final square matrix** is generated by comparing all the scores, for all the pairs of peaks.

GETTING CLUSTERS FROM ROOTED BINARY TREE

The algorithm methods described above all lead to a **unique binary tree** where all items are clustered into a single cluster of **size N (number of leafs)**.

One more step is required to get the very final clusters list. A **"cutoff"** based on two criterion allows to split the single cluster into appropriate subgroups:

- A cluster cannot contain more than one leaf per sample
- A cluster cannot contain two leafs for which the distance (or mixture similarity score) is too low

Method parameters

Clustering strategy

What strategy will be used for the clustering algorithm decision-making.

Feature list name

Name of the new aligned peak list.

m/z tolerance

This value sets the range, in terms of m/z, for possible peaks to be aligned. Maximum allowed m/z difference.

Weight for m/z

The assigned weight for m/z difference at the moment of match score calculation between feature rows. In case of perfectly matching m/z values the score receives the complete weight.

Retention time tolerance

Maximum allowed difference between two RT values.

Weight for RT

The assigned weight for RT difference at the moment of match score calculation between peak rows. In case of perfectly matching RT values the score receives the complete weight.

Export dendrogram as TXT/CDT

If checked, exports the resulting dendrogram to the given *txt file.

The dendrogram can be then browsed using common applications such as TreeView https://sourceforge.net/projects/jtreeview/.

Dendrogram output text filename

Name of the resulting TXT file to write the clustering resulting dendrogram to. If the file already exists, it will be overwritten.

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4.14.5 **ADAP Aligner (GC)**

Description

Feature list methods → Alignment → ADAP aligner (GC)

This alignment algorithm has been developed as part of ADAP-GC v1.0, Automatic Data Analysis Pipeline for processing GC-MS metabolomics data.

ADAP Aligner aligns features based on their mass spectra and retention time similarity. Unlike **Join Aligner** (which aligns peaks across all samples, using their m/z and retention time similarity), ADAP Aligner uses mass spectra and retention time to detect similar features in each sample and align them together. In fact, this algorithm is similar to **Hierarchical Aligner (GC)**, but it uses a different clustering method.

Similarity between two features \(f1\) and \(f2\) is calculated by the following score:

\[S(f1, f2) = w*S_{time}(f1, f2) + (1 - w)*S_{spec}(f1, f2)\]

where \(S_{time}(f1, f2)\) is the relative retention time difference between two features, \(S_{spec}(f1, f2)\) is the spectrum similarity between two features.

For more details, see reference [1].

REQUIREMENTS

ADAP Aligner requires mass spectra to be constructed prior to the alignment (e.g. using Spectral Deconvolution).

A typical workflow can be as following:

- "Raw data methods → Raw data import" to import raw data files
- "Raw data methods → Mass detection" to detect masses in the raw data
- "Feature detection → ADAP Chromatogram builder" to build extracted-ion chromatograms
- "Feature detection → Chromatogram resolving → ADAP Resolver" to detect peaks (features) in each chromatogram
- "Feature list methods → Spectral deconvolution (GC) → Multivariate Curve Resolution" to combine the detected peaks (features) into analytes and builds pure fragmentation mass spectra for each analyte
- "Feature list methods → Alignment → ADAP Aligner (GC)" to align the analytes produced by the previous step
- "Feature list methods → Export feature list → MSP file (ADAP)" to export fragmentation mass spectra into MSP format

References

1. Jiang, W.; Qiu, Y.; Ni, Y.; Su, M.; Jia, W.; Du, X.: An automated data analysis pipeline for GC-TOF-MS metabonomics studies. Journal of proteome research 2010, 9 (11), 5974-81. DOI: 10.1021/pr1007703

Parameters

Min confidence (between 0 and 1)

A fraction of the total number of samples. An aligned feature must be detected at least in several samples. This parameter determines the minimum number of samples where a feature must be detected. The default value is 0.7, so an aligned feature must be observed at least in 70% of all samples.

Retention time tolerance

The maximum allowed retention time difference between aligned features from different samples.

m/z tolerance

The maximum m/z difference, when two peaks from different mass spectra are considered equal.

Score threshold (between 0 and 1)

The minimum value of the similarity function \(S(f1, f2)\) required for features to be aligned together. The default value is 0.75.

Score weight (between 0 and 1)

The weight \(w\) that is used in the similarity function \(S(f1, f2)\). The default value is 0.1.

Retention time similarity

A method used for calculating the retention time similarity. The retention time difference (fast) is preferred method.

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4.14.6 **LC-Image Aligner**

Description

Feature list methods → Alignment → LC-Image-Aligner

Aligns LC and imaging measurements based on m/z and mobility.Images are aligned to all LC features that match, only the best match is retained.

Parameters

Feature lists

Select at least two feature lists. The image feature list(s) are aligned to a single (pre-aligned) LC feature list.

m/z tolerance

The file-to-file tolerance for two features.

m/z weight

Maximum score for a perfectly matching m/z. Default value is 1.

Mobility tolerance

Optional parameter

The file-to-file mobility tolerance. If the files don't contain mobility information, this parameter will be ignored. Default value is 0.01.

Mobility weight

Maximum score for a perfectly matching mobility. Default value is 1.

Feature list name

The name of the new feature list. Use {lc} to use the name of the input (LC/DI) feature list.

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4.15 Gap filling

4.15.1 **Peak finder**

Description

It is a recommended gap-filling algorithm.

Feature list methods → Gap filling → Peak finder.

Some chromatographic features in an aligned feature list may not be detected in every sample for several reasons, such as:

- feature shape constraints in the resolver or later feature filters
- co-eluting features that are not baseline separated might be resolved in one sample but kept unsplit in another
- misalignment due to shifts in m/z, retention time, or ion mobility within feature lists from different samples (or batches). Might originate from inaccurate mass calibration, etc.

All of these reasons can result in undesirable gaps (missing values) in the aligned feature table. Those gaps are not limited to smaller signals but can also affect abundant features. To account for this problem, the user can use the Peak finder module as a secondary, informed feature finding step. The algorithm searches for signals within the original centroided mass spectra. This algorithm fills the gaps in the feature list according to the user parameters, with the most crucial being **m/z tolerance** and **RT tolerance**. These two tolerances define the window where the algorithm should find the new feature. In the feature table, gap-filled features are marked with a grey color as the feature state.

Parameters

Name suffix

Suffix to be added to the peak list name.

Intensity tolerance

Maximum allowed deviation from the expected peak shape in chromatographic direction.

m/z tolerance

m/z range which will be applied when searching for the possible feature in the raw data.

Retention time tolerance

Retention time range when searching for the possible feature in the raw data.

Minimum data points

Feature will be used for gap filling only if it satisfies the set minimum number of data points.

Usually a lower number of data points is used compared to the primary feature finding workflow with the resolvers.

Original feature list

User can either keep, remove, or process in place of the original feature list. The latter two increase memory efficiency and throughput while users might want to keep the original feature list as a reference.

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4.15.2 Same m/z and RT range gap filler

Description

Feature list methods → Gap filling → Same m/z and RT range gap filler.

This method fills in gaps in each peak list row by using the same m/z and retention time range as other peaks in the row. The m/z and retention time defines where the new peaks will be sought based on the ranges of the rest of the peaks in the same row. The minimum value of these ranges is the minimum value in the range of all the peaks in the row and the same happens with the maximum value. User-specified tolerance is added to the m/z range. The new peak is constructed using the highest data point of each scan within the determined m/z and retention time ranges.

Parameters

Name suffix

Suffix to be added to the peak list name.

m/z tolerance

Tolerance, which is added to the m/z range of other peaks in the peak list row.

Original feature list

The user can either select to keep or remove the original feature list.

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4.16 Normalization

4.16.1 **Retention time calibration**

Description

Feature list methods → Normalization → Retention time calibration

The retention time normalizer attempts to reduce the deviation of retention times between feature lists, by searching for common features in these feature lists and using them as normalization standards.

Ions present in all given feature lists (according to given m/z, RT tolerance and minimum intensity) in exactly one instance are considered as standards. Retention times of the standards are then averaged and equalized in all samples, and retention times of all other features are adjusted according to the retention times of neighboring standard features.

⚠️ The method requires multiple feature lists of different samples, processed by deconvolution (for example, Local minimum resolver) but prior to alignment.

Parameters

Name suffix

Suffix to be added to a processed feature list name

m/z tolerance

Maximum allowed m/z difference for two values to be considered the same

Retention time tolerance

Maximum allowed difference between two retention time values

Minimum standard intensity

Minimum height of a feature to be selected as normalization standard

Original feature list

If REMOVE option is selected, the original feature list is removed, allowing to save memory.

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4.16.2 Linear normalizer

Description

Feature list methods → Normalization → Linear normalizer

Linear normalizer divides the height (or area) of each feature in the feature list by a normalization factor, chosen according to the "Normalization type" parameter.

Each column of the feature list is normalized separately. In other words, normalization factor is determined independently for each raw data file.

NORMALIZATION FACTORS

Different normalization factors can be applied:

1. **Average intensity**

   Average height (or area) of all peaks in the column is calculated and used as the normalization factor
2. **Average squared intensity**

   Same as Average intensity, but values are squared before calculating the average
3. **Maximum peak intensity**

   Maximum height (or area) in the peak list column is used as the normalization factor
4. **Total raw signal**

   Sum of the height (or area) of all peaks in the peak list column is used as the normalization factor

Parameters

Name suffix

Suffix to be added to a processed feature list name

Normalization type

Selection of the normalization factor. Available options:

- Average intensity
- Average squared intensity
- Maximum peak intensity
- Total raw signal

Feature measurement type

Selection of either feature height or feature area, which will be used to calculate the normalization factors

Original feature list

If REMOVE option is selected, the original feature list is removed, allowing to save memory.

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4.16.3 **Standard compound normalizer**

Description

Feature list methods → Normalization → Standard compound normalizer

The purpose of this module is to reduce the deviation between samples caused by different detection efficiency.

Internal standard peaks must be present in the detected samples. User can select one or multiple internal standard peaks, which must be present in all raw data files. Then peak height (or area) of each peak is normalized by either the **nearest standard** or a **weighted contribution** of all standards.

In case a weighted contribution is used, the contributions of all standards are weighted by distance. The distance of the standard peak to the peak being normalized is calculated as

\[distance = MZvsRT_{Balance} * MZ_{difference} + RT_{difference}\]

where \(MZvsRT_{Balance}\) is a multiplier of m/z difference set by **m/z vs RT balance** parameter

⚠️ Feature list must be aligned prior to normalization.

Parameters

Name suffix

Suffix to be added to a processed feature list name

Normalization type

Normalize intensities using either only one (nearest) standard or using a weighted contribution of all selected standards, weighted by distance.

Feature measurement type

Selection of either feature height or feature area, which will be used to calculate the normalization factors

m/z vs RT balance

Used in distance measuring as a multiplier of m/z difference.

Standard compounds

List of features for choosing the normalization standards

Original peak list

If selected, the original peak list is automatically removed

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4.17 Precursor mass search

4.17.1 **Local compound database search**

Description

Feature list methods → Annotation → Search precursor mass → Local compound database (CSV) search

This method assigns identity to peaks according to their m/z and retention time values.

The user has to provide a database of m/z values and retention times in ***.csv format** (see below).

DATABASE FILE

Database file has to be provided in ***.csv format** (Comma-Separated Values). Such files can be exported from a spreadsheet software such as MS Excel, or edited manually using a text editor.

The following examples shows the structure of the database file:

```
ID,m/z,Retention time (min),Identity,Formula
1,175.121,24.5,Arginine,C6H14N4O2
2,133.063,11.9,Asparagine,C4H8N2O3
3,134.047,11.7,Aspartate,C4H7NO4
```

⚠️ If the m/z value or Retention time value in the CSV file is 0, then the value is considered as a wild card. E.g, the following item will match all peaks of 174.121 m/z without considering the retention time:

```
1,175.121,0,Arginine,C6H14N4O2
```

The available fields in a library file include:

Field name	Field description
neutral mass	Neutral mass
mz	Precursor m/z
rt	Retention time
formula	Formula
smiles	SMILES
name	Compound name
CCS	CCS, $Å^2$
mobility	Ion mobility
comment	Text comment
adduct	Information on adduct
PubChemID	Compound ID in PubChem database

Parameters

Database file

Name of file that contains information for peak identification.

Field separator

Character(s) used to separate fields in the database file.

Columns

Columns that will be imported from the library file. The choice of columns depends on the availability of mobility data, information about adducts, and presence of PubChemID.

m/z tolerance

Maximum allowed m/z difference to set an identification to a peak.

Retention time tolerance

Maximum allowed retention time difference to set an identification to a peak.

Mobility time tolerance

Maximum allowed tolerance between two mobility values.

CCS tolerance (%)

Maximum allowed difference (in percents) between two CCS values.

Use adducts

If chosen, m/z values for multiple adducts will be calculated and matched against feature list.

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4.17.2 Precursor search in local spectral MS/MS library

Description

Feature list methods → Annotation → Search precursor mass → Precursor search in spectral libraries

This module uses a **local spectral MS/MS library** to search for putative precursor ions in a feature list.

Supported formats:

- MoNA *.json,
- NIST *.msp,
- GNPS *.json (internal library submission format),
- and JCAMP-DX *.jdx.

Parameters

Spectral libraries file (MS/MS)

Name of the library file of the supported format.

Precursor m/z tolerance

Matches the average row m/z against the precursor m/z of the spectral library entry

Retention time tolerance

Optional parameter, should only be used if the DB entry has a retention time

---

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4.17.3 Online compound database search

🚧 This module has known bugs and is being updated, which might affect its functionality

Description

Feature list methods → Annotation → Search precursor mass → Online compound database search

This module allows identification of peaks or whole peak lists using an on-line compound database. Databases are queried for the calculated neutral mass of the peak and matching compounds are returned.

💡 If a user is interested in more comprehensive online compound database search, they can export their data to Sirius software.

Parameters

Database

On-line database to search (list of the available databases).

Ionization type

Type of ionization that produced the peak subjected to identification.

Number of results

Limit for the number of results to be retrieved from the on-line database.

m/z tolerance

Maximum allowed m/z difference to set an identification to a peak.

Isotope pattern filter

If selected, only results which fit the required isotope pattern similarity score will be returned.

CURRENTLY SUPPORTED DATABASES

Supported databases are listed below. Support for other databases may be implemented as additional plugins.

- **KEGG**

  KEGG database (http://www.genome.jp/kegg/) contains metabolites and other biomolecules present in natural metabolic pathways.

- **PubChem**

  PubChem database (http://pubchem.ncbi.nlm.nih.gov/) contains millions of chemical compound structures.

- **HMDB**

  The Human Metabolome Database (HMDB) (http://www.hmdb.ca/) contains over 7,000 known metabolites found in human body.

- **YMDB**

  The Yeast Metabolome Database (YMDB) (http://www.ymdb.ca) is a manually curated database of small molecule metabolites found in or produced by Saccharomyces cerevisiae (also known as Baker's yeast and Brewer's yeast).

- **LipidMaps**

  LipidMaps Structure Database (LMSD) (https://www.lipidmaps.org/databases/lmsd) is a database of structures and annotations of biologically relevant lipids, containing over 47000 different lipids.

- **MassBank.eu**

  MassBank (https://massbank.eu/MassBank/) is an open source mass spectral library for the identification of small chemical molecules of metabolomics, exposomics and environmental relevance. The majority of MassBank contents now features high-resolution mass spectrometry data.

- **ChemSpider**

  The ChemSpider database (http://www.chemspider.com/) contains over 67 million compounds. To search ChemSpider you must provide a "Security key" from your ChemSpider API account. If you don't have an account, please register at https://developer.rsc.org.

- **MetaCyc**

  MetaCyc (https://metacyc.org/) is a curated database of experimentally elucidated metabolic pathways from all biological domains. MetaCyc currently contains 2937 pathways, 17,780 reactions and 18,124 metabolites.

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4.18 Spectra search

4.18.1 **Spectral library search**

Description

Feature list methods → Annotation → Search spectra → Spectral library search or with a right click on one or multiple selected feature rows **Search → Spectral library search**

The spectral library search module can be performed on feature lists, individual features (contained in feature list rows), or single scans.

Depending on the **MS level** (MS1 or MS2), all corresponding query scans (e.g., extracted from the rows) will be matched against selected spectral libraries that were previously imported.

Preferred ways to import libraries are in this order:

- together with spectral data files in the advanced data import:

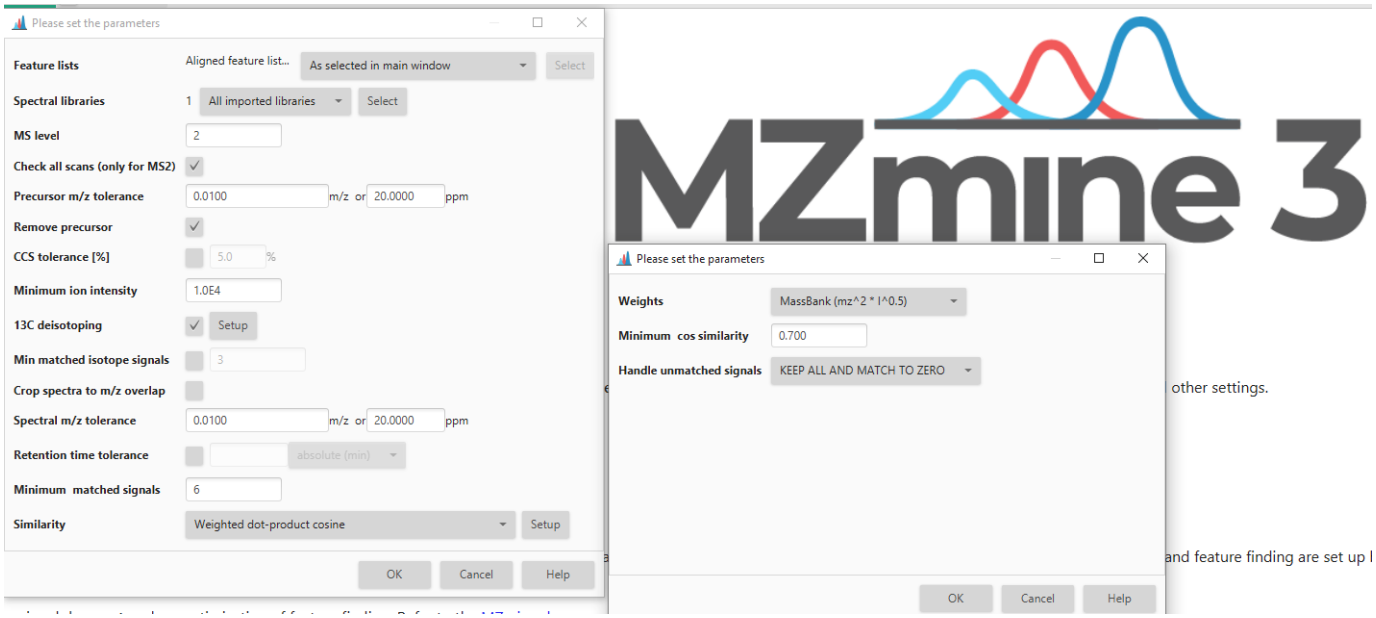
  Raw data methods → Raw data import → MS data (advanced)
- drag and drop into the main window
- with a dedicated import module:

  Raw data methods → Raw data import → Spectral library import or from **Feature list methods → Annotation → Search spectra → Import spectral libraries.**

SUPPORTED LIBRARY FORMATS

- *.json: MassBank of North America (MoNA) (download)
- *.json: The Global Natural Product Social Molecular Networking (GNPS) (format from the spectral DB submission module)
- *.mgf: GNPS (download)
- *.msp: MoNA
- *.msp: National Institute of Standards and Technology (NIST)
- *.jdx: JCAMP-DX

Parameters



Spectral libraries

The spectral libraries of interest need to be imported before applying spectral library search. Either uses all imported spectral libraries or only the selected libraries.

MS level

Choose the MS level of the scans that should be compared with the library.

Set the MS level to "1" to compare MS^1 spectra, e.g., from GC-EI-MS or MALDI-imaging MS^1 data.

Use "2" or higher for fragmentation scans.

⚠️ In case of issues with the scan selection, check how the actual scan numbers are reported in the data files and in MZmine's raw data overview and scan list.

Check all scans (only for MS2)

This option enables the comparison of all MS2 scans.

⚠️ Otherwise, only the most intense MS^2 scan (the highest TIC) is used for the matching.

⚠️ It does not apply to MS1 scans.

Precursor m/z tolerance

This option is only used for MS level > 1. Here, the library entries are filtered by their precursor m/z reducing the number of spectral-pairs to match.

The absolute (in m/z) and relative (in ppm) m/z tolerance can be set. The **maximum tolerance** for each precursor is applied.

Considering that the precursor isolation window is often far greater than the resolution or accuracy of the MS scan, this parameter is often set to higher m/z tolerances.

Another aspect is the used library, which might contain uncalibrated reference spectra from lower resolution instruments.

Remove precursor

⚠️ Can be selected only for MS level > 1.

Depending on the fragmentation method, e.g., collision induced dissociation (CID) or higher-energy collisional dissociation (HCD), the precursor can be detected with different intensities resulting in varying cosine similarities during the library matching.

Therefore, this option enables the removal of the precursor signal within the precursor m/z tolerance (parameter above) prior to the matching.

Spectral m/z tolerance

This m/z tolerance is used to pair signals in the query and library scans. It can be set in absolute (in m/z) and relative (in ppm) m/z tolerance, whereas the maximum tolerance for each m/z value is applied. It must be kept in mind, which mass resolutions are achieved within the experimental spectra and within the spectral library.

CCS tolerance

The collision cross-section (CCS) tolerance can be used in a similar way as the retention time tolerance.

Accordingly, the CCS value of a query will be compared with the library entries and the maximum tolerance can be set in %.

⚠️ If the query or library entry was analyzed without ion mobility (no CCS values), no spectrum will be matched.

Retention time tolerance

The maximum allowed retention time difference when comparing the query and library scan. It can be set in absolute (min or sec) or relative (%) values.

💡 This option is useful for in-house libraries or standardized libraries that follow the same acquisition protocol with the same set-up, e.g., column, instrument, and method).

Crop spectra to m/z overlap

If query and library scans were acquired with different methods, e.g., mass range, fragmentation energy or mode, it can be helpful to crop the spectra to their overlapping m/z range (+ m/z tolerance). This is done by using the maximum m/z range where both spectra contain signals.

⚠️ However, this method will boost false matches and needs strict manual interpretation.

Minimum ion intensity

Signals in the query scan below the minimum ion intensity will be filtered from the mass lists and are not taken into account during the library matching. Absolute values.

^{13}C deisotoping

Removes ^{13}C isotope signals from the mass list using the following parameters:

- **m/z tolerance**: Maximum allowed difference between the measured and predicted isotope m/z values. The absolute (in m/z) and relative (in ppm) m/z tolerance can be set, whereas the maximum tolerance for each m/z value is applied.
- **Monotonic shape**: If enabled, the monotonically decreasing height of isotope pattern is required.
- **Maximum charge**: The maximum charge that will be considered for detecting the isotope pattern. For singly charged ions, the ^{13}C isotope will be expected +1 whereas for doubly charged ions it will be +0.5 (+1 m/z divided by the charge 2).

Min matched isotope signals

This option is only useful if the query AND library entries contain isotope patterns (e.g., in MS^1 or with wider precursor isolation windows).

The minimum number of matched signals of ^{13}C isotopes.

⚠️ It cannot be applied when ^{13}C deisotoping is enabled.

Min matched signals

The query mass list and spectral library entry must contain at least this number of matched (paired) m/z values (+- m/z tolerance).

Common parameters include 4 signals for smaller molecules and 6 for more confident matches.

⚠️ This parameter must be set carefully to not exclude compounds that show less fragmentation, when using a higher number of matched signals.

⚠️ Choosing a lower number of matched signals can result in spurious library hits.

Similarity

Several algorithms can be applied to calculate the similarity of the query and library scans and to filter the resulting library matches. The available algorithms are:

- Weighted dot-product cosine,
- Composite dot-product identity (similar to NIST search).

More details are available here.

💡 The **weighted dot-product cosine** similarity is used for comparing MS^2 data, whereas the **composite dot-product identity (similar to NIST search)** considers the relative intensity of neighboring signals and is, therefore, applied to MS^1 spectra from GC-EI-MS.

Last update: September 23, 2022 17:08:14

4.18.2 **NIST MS search**

Description

Feature list methods → Annotation → Search spectra → NIST MS Search

or, for an individual row in a feature table

highlight the row, right-click on the selection and choose **Search → NIST MS Search** from the pop-up menu.

This module allows searching spectra against spectral libraries using the **NIST MS Search program**, which accepts spectra as input to its searches.

The MS level may be specified to limit a search to MS/MS fragment spectra, clustered spectra produced from any Spectral Deconvolution module, or MS1 precursors ions. The spectra will be searched using the default library search parameters in the NIST MS Search program.

To adjust these parameters: open the program, adjust the library search parameters, and save the configuration to a ***.ini** file.

Automation must be enabled in the library search options to enable automatic searching. Be sure all appropriate libraries are included before starting a search.

Repeated MS/MS spectra may be merged between multiple data files using the Merge MS/MS (experimental) module. The input Mass list filters the fragment ions by intensity, and repeatable signals are assessed via cosine dot product.

REQUIREMENTS

This module relies on the installed NIST MS Search software, which is currently **only available for Microsoft Windows**.

Parameters

NIST MS Search directory

Full path to the directory containing the NIST MS Search executable (**nistms$.exe**).

MS level

MS spectra level for searching.

Use MS level = 1 to search for MS1 spectra or ADAP-GC clustered spectra produced from Spectral Deconvolution modules.

Min cosine similarity

The minimum cosine similarity score (dot product) for identification.

Merge MS/MS (experimental)

Optional parameter.

Merge multiple high-quality MS/MS spectra into consensus feature instead of using the most intense one.

More details are available here.

Integer m/z

Optional parameter.

Merging mode for fractional m/z to unit mass. Converts accurate mass m/z measurements to low-resolution integer values.

Available options are Sum or Maximum.

Spectrum Import

Options for import, can be by either **Overwrite** (overwriting previous spectra) or **Append** (appending new ones).

Last update: September 22, 2022 14:31:57

4.18.3 Chemical formula prediction

Description

Feature list methods → Annotation → Search spectra → Chemical formula prediction

This module attempts to calculate all possible molecular formulas for every peak in the peak list, using given elemental and heuristic constraints.

For a detailed description of the functionality and the embedded algorithms, please see the publication [1].

References

1. Pluskal T. et al, Highly Accurate Chemical Formula Prediction Tool Utilizing High-Resolution Mass Spectra, MS/MS Fragmentation, Heuristic Rules, and Isotope Pattern Matching. Anal Chem (2012), 84(10):4396-403. DOI: 10.1021/ac3000418.
2. Kind and Fiehn, Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. BMC Bioinformatics (2007). DOI: 10.1186/1471-2105-8-105.

Parameters

Charge

The neutral mass is calculated from the peak m/z value, its charge and type of ionization adduct.

Ionization type

The neutral mass is calculated from the peak m/z value, its charge and type of ionization adduct.

Sorting

Optional parameter

If this option is chosen, the user-defined sorting is applied to all selected feature lists.

Max best formulas per feature

This parameter defines the maximum number of formulas to be added to a single feature.

m/z tolerance

Tolerance of the neutral mass for searching the formula.

Elements

Elements allowed in the formula and their minimum and maximum counts.

Element count heuristics

Optional parameter

Selection of heuristic restrictions on element counts. These heuristics check the formula's element counts as defined in [2].

Available parameters

These include:

- **H/C ratio**

  Ratio of hydrogen to carbon atoms, with available value range 0.1 <= H/C ratio <= 6.

- **NOPS/C ratios**

  Ratio of nitrogen to carbon atoms, with available valueN/C ratio <= 4, Ratio of oxygen to carbon atoms O/C ratio <= 3, Ratio of phosphorus to carbon atoms P/C ratio <= 2, Ratio of sulphur to carbon atoms S/C ratio <= 3

- **Multiple element counts**

  If this parameter is chosen, then several following rules apply:

- if number of N/O/P/S atoms all > 1 then N< 10, O < 20, P < 4, S < 3
- if number of N/O/P atoms all > 3 then N < 11, O < 22, P < 6
- if number of O/P/S atoms all > 1 then O < 14, P < 3, S < 3
- if number of P/S/N atoms all > 1 then P < 3, S < 3, N < 4
- if number of N/O/S atoms all > 6 then N < 19 O < 14 S < 8

RDBE restrictions

Optional parameter

Selection of restrictions on RDBE (rings double bonds) values. The **Ring Double Bond Equivalents (RDBE)** value estimates the number of rings and unsaturated bonds in a molecule. It can be calculated from a chemical formula using the following general equation:

\[RDBE=1+\frac{1}{2}(\sum_{i}n_i(\nu_i-2))\]

where \(n_i\) is the number of atoms and \(\nu_i\) the formal valence of the element i.

Theoretically, each ring or a double bond increases the RDBE value by 1, while each triple bond increases the value by 2.

⚠️ This equation can only be used for formulas composed of elements with a well-defined formal valence.

A number of exceptions to the RDBE rule are known, however, the RDBE value still provides a useful indicator regarding the validity of a molecular formula.

Available parameters

These include:

- **RDBE range**

  Range of allowed RDBE (Range or Double Bonds Equivalents) value.

  [2] recommended the RDBE upper limit of 40 for common chemical compounds. The authors also stated that RDBE should not be negative, although certain exceptions may occur when formal valence states are exceeded.

- **RDBE must be an integer**

  Only integer values are allowed for RDBE. This condition is a natural implication of the principle of valence balance, which states that the number of atoms with odd valence must be even. Such assumption is valid for all neutral, non-radical molecules.

Isotope pattern score

Optional parameter

If selected, only results that fit the required isotope pattern similarity score will be returned.

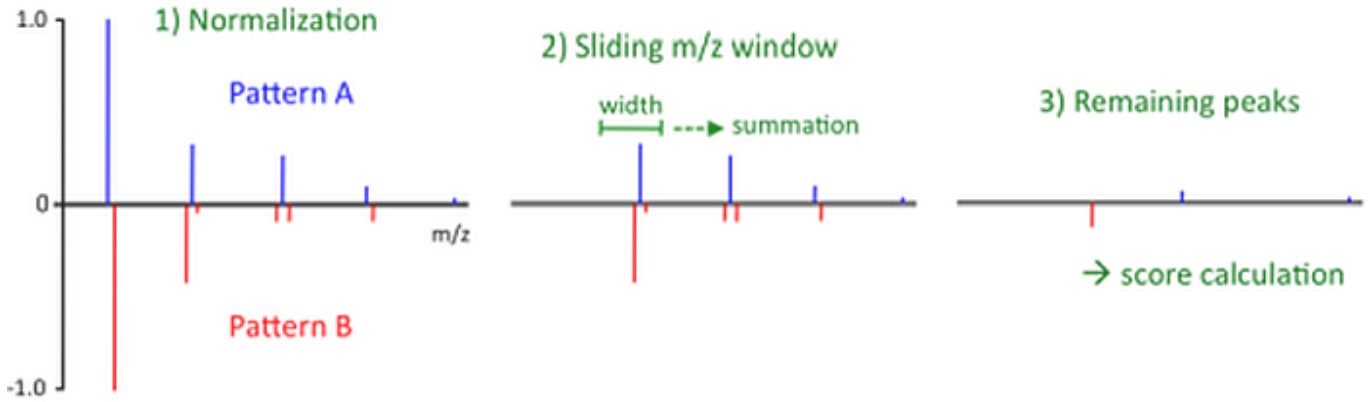
Several modules in MZmine offer the option the compare the isotope patterns of peaks and assign a score (percentage) of similarity to them.

Until MZmine version 2.2, the CDK (Chemistry Development Kit) library was used to perform this operation. An improved algorithm, introduced in MZmine 3, is described below. The **similarity of two isotope patterns** is determined as follows:

- Both isotope patterns are normalized (such that highest isotope in each pattern has the intensity of 1.0) and merged into a single spectrum. In this single spectrum all isotopes from the first pattern have a positive intensity, while the isotopes of the second pattern have negative intensity.
- A sliding window of user-defined width ("Isotope m/z tolerance" parameter) is moved over the whole m/z range, from the lowest m/z to the highest. Each pair of isotope peaks fitting within the window is added together, forming a single peak with the m/z value of the higher m/z of the pair.
- The final similarity score is calculated from the remaining peaks as

$$score_{isotopes}=\prod_{i}(1-\mid{I_i}\mid)$$

where I_i is the intensity of remaining peak i.



A trivial observation is that for two identical isotope patterns the similarity score will be 100%, while for two completely different patterns 0% score is returned.

Only a single parameter is required for the evaluation of the algorithm, defining the width of the sliding window.

It should be noted, though, that the optimal value of width of the sliding window parameter might be different from the commonly perceived “mass accuracy” of the instrument as mass resolving power and preprocessing of the data must be considered. For example, even if the mass accuracy of the major isotopes may be less than 0.001 m/z, the mass difference between minor isotopes may be significantly higher.

Additional parameters

These include:

- **Isotope m/z tolerance**

  m/z tolerance which defines what isotopes would be considered same when comparing two isotopic patterns.

  This tolerance needs to be higher than general m/z precision of the data, because some small isotopes may overlap with the sides of bigger isotopic peaks.

- **Minimum absolute intensity**

  Minimum absolute intensity of the isotopes to be compared. Isotopes below this intensity will be ignored.

- **Minimum score**

  If the score between isotope pattern is lower, the match will be discarded.

MS/MS filter

Optional parameter

Use MS/MS pattern for candidate formula evaluation.

In tandem mass spectrometry (MS/MS), during fragmentation, part of the original ion is detached, and the mass of the detached part is called the **neutral loss**.

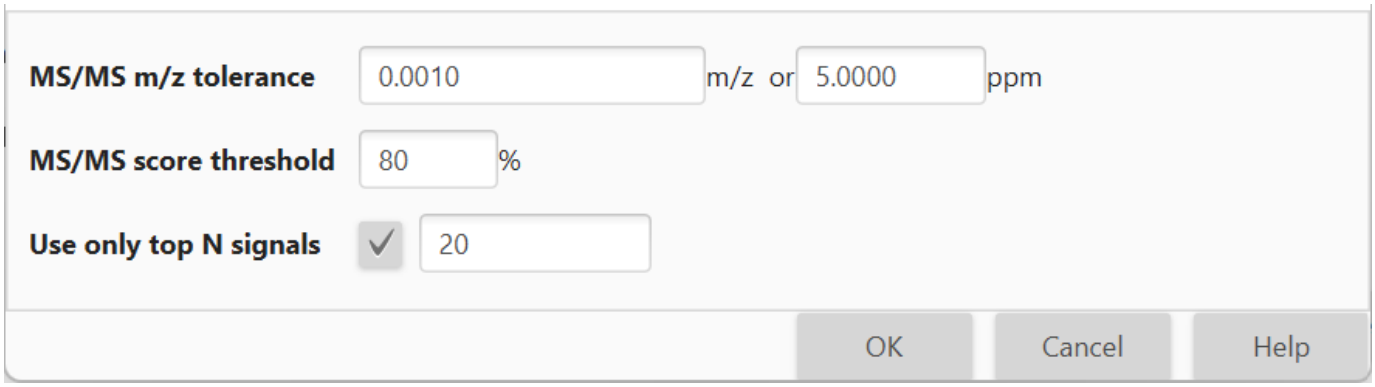
The neutral loss represents a fragment of the original molecule, so the chemical formula of such fragment must be a subset of the chemical formula of the precursor.

When searching for the ion's chemical formula, each candidate formula may therefore be evaluated using the ion's MS/MS spectrum using the **algorithm** described below.

1. Mass list must be provided for the MS/MS spectrum of the ion of interest (see the Mass detection modules). It is assumed that all items in the mass list represent true fragment ions and noise has been removed.
2. If the mass list contains any isotopes, remove them from the list. Isotopes are defined as ions with mass approximately 1 Da higher than another ion on the list, which has higher intensity.
3. Calculate neutral losses for all the ions on the list by subtracting the fragment ion mass from the precursor mass.
4. Try to generate a chemical formula for each neutral loss using the elements and maximum counts of formula F, within the user-defined mass tolerance. Small neutral losses (less than 5 Da) are ignored.
5. If at least one formula can be found, the neutral loss is considered as interpreted.
6. The evaluation score is calculated for each candidate formula F as described belows:

$$score_{MS/MS}=\frac{n_{found}}{n_{total}}$$

where n_{found} is the number of ions for which the neutral loss could be interpreted, and n_{total} is the total number of considered fragment ions.



Available parameters

- **MS/MS m/z tolerance**

  Tolerance of the m/z value to search (+/- range).

- **MS/MS score threshold**

  If the score is lower than the threshold, a match is discarded.

- **Use only top N signals**

  Use only N most abundant signals for scoring. This option speeds up the search.

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4.18.4 **Lipid Annotation**

Description

Feature list methods → Annotation → Search spectra → Lipid annotation

This module contains methods to search for lipids in the feature lists. Potential lipids will be annotated according to their accurate mass on MS^1 level.

If MS/MS data is available, an identification on fatty acid residue level is also possible. MS/MS rules were derived from various sources [2-4] or from MS/MS experiments performed in the Hayen lab (University of Münster, Germany)

⚠️ If you use the Lipid Annotation Module, please cite the MZmine paper and the articles from the references section.

References

1. Korf, A., Jeck, V., Schmid, R., Helmer, P. O., & Hayen, H. (2019). Lipid Species Annotation at Double Bond Position Level with Custom Databases by Extension of the MZmine Open-Source Software Package. Analytical chemistry, 91(8), 5098-5105. DOI: 10.1021/acs.analchem.8b05493
2. LipidBlast. Kind, T., Liu, K. H., Lee, D. Y., DeFelice, B., Meissen, J. K., & Fiehn, O. (2013). LipidBlast in silico tandem mass spectrometry database for lipid identification. Nature methods, 10(8), 755. 10.1038/nmeth.2551
3. MoNA https://mona.fiehnlab.ucdavis.edu/
4. LipidMatch. Koelmel, J. P. et al. (2017). LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data. BMC bioinformatics, 18(1), 331. DOI: 10.1186/s12859-017-1744-3

Parameters

Lipid classes

Selection of lipid classes to consider for annotation based on the backbone.

Number of carbon in chains

Set the number of carbon atoms in chains.

Number of double bonds

Set the number of double bonds in chains.

m/z tolerance MS1 level

Enter m/z tolerance for exact mass database matching on MS1 level

Search for lipid class-specific fragments in MS/MS spectra

Choose if you want to search for lipid class specific fragments in the MS/MS spectra.

To see which lipid class has a MS/MS library check out the database table.

Search for custom lipid class

If chosen, the user can add their own custom class that will be used for further search. This feature allows the user to build any possible lipid, based on the already implemented lipids. This also allows the annotation of lipid derivatization products. Entered modifications can be exported and/or imported using the buttons on the right side.

Show database

By clicking the button "Show database" at the bottom of the window, the user can browse through a database table which holds the information of the created lipid database.

Results description

Peaks will be annotated as potential lipids by setting its peak identity. Always check for multiple assignments and compare the status with the database table and Kendrick plots! The comment holds information on the utilized ionization method, mass accuracy and MS/MS annotation. An MS/MS annotation will be added if MS/MS data was acquired and fragmentation information is listed in the database. More MS/MS data will be added in the future.

Last update: September 23, 2022 17:08:14

4.18.5 **MS2 Similarity Search**

Description

Feature list methods → Annotation → Search spectra → MS2 similarity search

Ions arising from compounds with similar chemical structures often give similar fragmentation patterns (MS2 spectra). Therefore, calculating the similarity between MS2 spectra is a useful approach for the discovery of structurally similar compounds.

This module calculates the similarity between **centroided MS2 spectra** associated with two feature lists. These two feature lists can be the same feature list, or different feature lists.

The module outputs the result of the search, the MS2 similarity comparisons of feature list (1) with feature list (2), into the "Identity" column of feature list (1).

In practice, you can consider the features in feature lists (1) as "bait", which is used to "fish" for MS2 similarity from feature list (2). Feature list (1) or feature list (2) can be a single peak + MS2 spectra, or an entire experiment.

MS2 SIMILARITY SCORE

The similarity metric used is as follows:

- For all MS2 spectra in feature list (1) **"MS2 spectra A"**, and feature list (2) **"MS2 spectra B"**, iterate over all ions in MS2 spectra A (**ion "i"**), and over all ions in MS2 spectra B (**ion "j"**).
- For a given ion, only include this ion in following calculations if its intensity is greater than the **minimum ion intensity parameter**.
- Compare the m/z values of ions "i" and "j". If these m/z values are within the range specified by the **m/z tolerance** parameter, consider these ions identical, and therefore "matched".
- If two ions match, roughly score the match by multiplying the intensity of ions "i" and "j". Save that as the **"ion match subscore"**.
- Repeat this for every ion i and j in MS2 spectra A and MS2 spectra B, and report the sum of the ion match subscores as the total **"spectral match score"**.
- If this spectral match score is greater than the **minimum spectral match score** parameter, annotate the "Identity" column of feature list (1) with the matched ions, and the total spectral match score of the MS2 similarity calculation.

INTERPRETING THE RESULTS

The score from of a MS2 similarity match should not be taken as an absolute measure, as it depends on the instrument reported intensity value (which is an uncalibrated and relative measure).

That being said, as the reported score increases when the intensity of the matched ion is higher, it is useful as a quick metric to find the matches between the most intense MS2 spectra, and potentially the most reliable compounds.

⚠️ It is worth mentioning that the link between the MS2 spectra, and the presumed precursor ion in the feature list is somewhat tentative. As the isolation window of the quadrapoles typically used for selection of the precursor ion in MS2 fragmentation analysis is typically around ~1 m/z unit, ions from the MS2 fragmentation spectra from an abundant compound with a long chromatographic tail will often show up in the MS2 values of unrelated compounds, but whose precursor isolation window picks up ions from the original compound.

An experimental Python script which converts the MS2 similarity relationships exported from MZmine2 in CSV format into GraphML format suitable for viewing in the freely available **graph manipulation software Cytoscape** is available on Github (https://github.com/photocyte/ms2_graph).

Parameters

Feature list (1)

A single feature list with features that have associated centroided MS2 spectra. Results from the module are output into the identifications column of Feature list (1)

Feature list (2)

A single feature list, which has centroided MS2 spectra (in the masslist). This can be the same as Feature list (1), or a different peaklist / experiment.

For best results Feature list (2) should have MS data with the same polarity as Feature list (1), and close range of m/z values close (e.g. LC/MS data obtained on the same day, or m/z calibrated between the two feature lists).

m/z tolerance

Maximum allowed m/z difference between two ions to be considered identical, and therefore "matched".

Minimum MS2 ion intensity

Minimum ion intensity to consider in MS2 comparison. Ions of the MS2 spectra below this threshold will be ignored.

- This parameter depends on your instrument, but 1e5 is a reasonable value.
- Set to 0 to use all ions.

Minimum ion(s) matched per MS2 comparison

Minimum number of matched ions needed in a given MS2 similarity comparison. Otherwise, that spectral match will not be reported.

- This depends on the compounds being compared, but roughly speaking 2-5 is a reasonable number. For complex spectra values of 10-20+ may be reasonable.
- Set to 0 to report all matches.

Minimum spectral match score to report

Minimum spectral match score threshold, below which spectral matches will not be reported.

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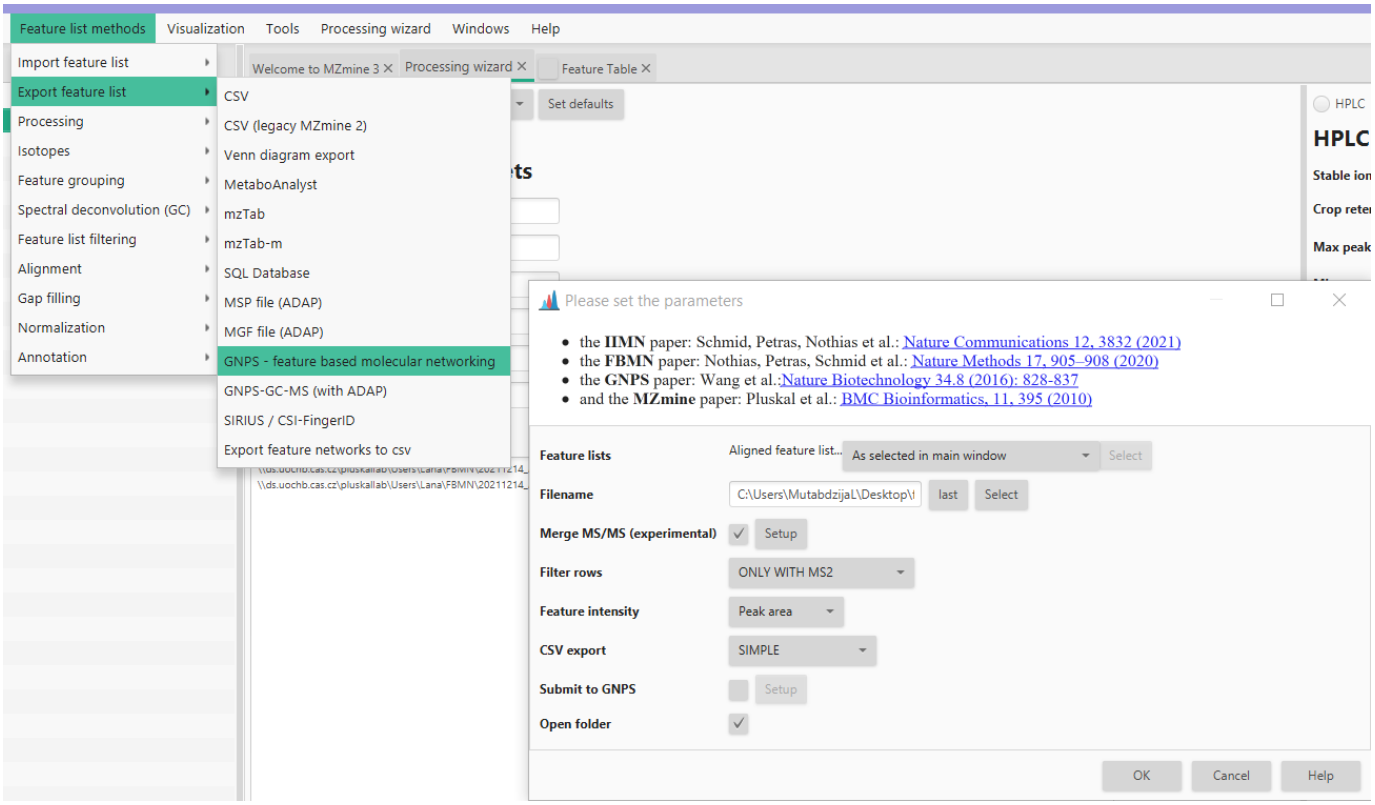
4.19 GNPS-FBMN/IIMN export

4.19.1 **Description**

Feature list methods → Export feature lists → GNPS - feature based molecular networking.

This module connects MZmine feature finding results to the GNPS workflows for Feature-based Molecular Networking (FBMN) and Ion Identity Molecular Networking (IIMN).



Using this module, the user can export the feature list needed for the manual submission to GNPS' feature based molecular networking (GNPS FBMN) or directly submit the job to the GNPS platform from MZmine. In both cases, two files are created:

1. Quantification table (CSV file) which contains the features and their associated information (e.g., average m/z, retention time, and each feature's area or height).
2. MS/MS spectral summary (.MGF file) which contains one representative MS/MS spectrum for each row in the feature list.
3. A supplementary edges file with related ion identities (if ion identity networking was performed).

4.19.2 **References**

IIMN: Schmid R., Petras D., Nothias LF, et al. Ion Identity Molecular Networking for mass spectrometry-based metabolomics in the GNPS Environment. Nat. Comm. 12, 3832 (2021).

FBMN: Nothias, L.-F., Petras, D., Schmid, R. et al. Feature-based molecular networking in the GNPS analysis environment. Nat. Methods 17, 905–908 (2020).

GNPS: Wang, M. et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. Nat. Biotechnol. 34, 828–837 (2016).

Parameters

FILENAME

Name to be given to the output files (.MGF and .CSV). In this field, the user can either write the path where they want to save the file, or click "select", navigate into the desired output folder, write the output name in the "file name" field and click save. Once that is done, the path should be visible in the Filename field in the GNPS export module.

MERGE MS/MS (EXPERIMENTAL)

If checked, high quality MS/MS spectra that correspond to one feature are merged, instead of exporting only the most intense MS/MS spectrum. See MS/MS merger for additional information.

FILTER ROWS

In the final output files, the user can select to export all the rows without any filters applied, rows only with MS/MS spectra, rows with MS/MS and Ion Identity (it gives MS/MS and the adduct information) and rows with MS/MS or Ion Identity. Normally, for FBMN you want to retain features with MS/MS spectra.

FEATURE INTENSITY

The user can either select peak area or peak height which will then be displayed in the quantification table.

CSV EXPORT

The user can choose between **simple**, **comprehensive**, or **all**. Difference is in the amount of information that is present in the quantification table. Simple resembles the legacy format from the MZmine 2 export. Both options can be used for FBMN in GNPS other tools might rely on the simple MZmine 2 style output.

SUBMIT TO GNPS

This option allows any user to directly submit FBMN/IIMN jobs to GNPS. The password and user name are optional and are sent without encryption (until the server has moved to its final location with https). The input files uploaded to GNPS with the "Submit to GNPS" option are not saved on your GNPS user account. These files are deleted on monthly basis, which prevent future cloning of the job and retrieval of the files. Use the "standard" interface of FBMN for persistant jobs and more options. Or log into your GNPS account and click on **Clone to latest version** for a job submitted via direct interface.

OPEN FOLDER

Opens the export folder.

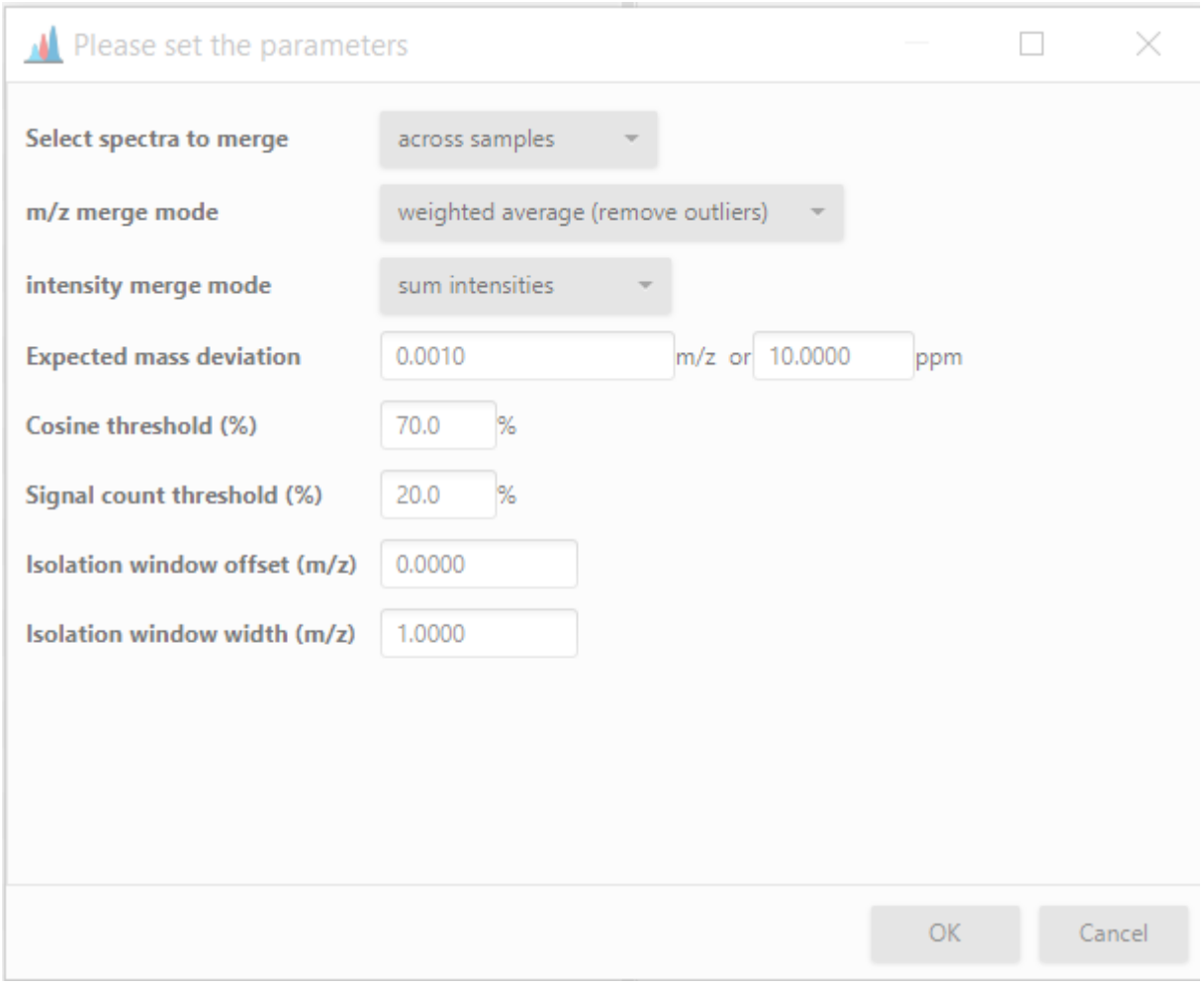
Last update: September 23, 2022 17:08:14

4.20 Other parameters

4.20.1 Merge MS/MS (experimental)

This option is available in the GNPS FBMN export and the SIRIUS export. If checked, high quality MS/MS spectra that correspond to one feature are merged, instead of exporting only the most intense MS/MS spectrum.

PARAMETERS



Select spectra to merge

The users can select to merge the MS/MS spectra:

1. **Across samples**, which will merge all MS/MS spectra that belong to the same feature, and as such is the most convenient option.
2. **Same sample**, which will merge MS/MS spectra for the same feature within one sample, and can be used if the user is not confident about the alignment algorithm.
3. **Consecutive scans**, which will merge MS/MS spectra if they are triggered in a row.

m/z merge mode

This option allows you to select the way to merge the fragments' m/z values associated with a similar precursor value. "Most intense" will always pick the m/z of the best feature, which is a very safe and conservative option. However, "weighted average (remove outliers)" will often yield better result.

Intensity merge mode

Options on how to merge the intensity values of features from different spectra with similar mass.

- **Sum intensities** is a convenient option that will increase the intensities of feature that occur consistently in many fragment scans. However, this will make intensities between merged and unmerged spectra incomparable.
- Use **max intensity** to preserve intensity values

Expected mass deviation

Expected mass deviation between different spectra of the same feature of your measurement in ppm (parts per million) or Da(larger value is used). We reccommend to use a rather large values, e.g. 10ppm for Orbitrap, 15 ppm for Q-ToF, 100 ppm for QQQ.

Cosine treshold

Treshold of cosine similarity between spectra that needs to be met in order for two spectra to be merged. In case they have different collision energies, cosine treshold should be set to 0, since different collision energies will result in different fragmentation pattern.

Signal count treshold

After merging the spectra, signals that occur in less than the user specified % of the merged spectra will be removed.

Isolation window offset (m/z)

Isolation window offset from the precursor m/z.

Isolation window width (m/z)

Width of the isolation window (left and right).

Last update: April 14, 2022 00:14:12

4.20.2 Spectra similarity

Spectral m/z tolerance

Spectral m/z tolerance is used to match all signals between spectra of two compared raw files.

MS level

MS level of scans that should be compared. It can be 1 for MS1 or 2 for MS2 level.

Compare spectra similarity

1. **Weighted dot-product cosine** - used to determine the similarity between two spectra (usually library and query spectra).This option is used for MS2 level.
2. **Composite dot-product identity** - used to determine the similarity between two spectra (usually library and query spectra). Especially useful for very reproducible generation of spectra (GC-EI-MS). Takes into account the relative intensities of neighbouring signals in the two spectra. This option is used for MS1 level.

Additional setup of spectra similarity comparison enables modification of the following parameters:

Weights

Weights for the m/z and intensity values. Usually, MassBank is used, in which higher m/z values contribute more to the cosine similarity calculation.

Minimum cos similarity

Minimum cosine similarity for a match between compared spectra.

Handle unmatched signals

Usually, **keep all and match to zero** is used, which will take all signals into account, and the unmatched ones will decrease the cosine similarity.

Last update: September 7, 2022 15:48:07

5. Visualization modules

5.1 Visualization modules

MZmine provides a rangle of interactive visualization tools for analysis of raw and processed data. JFreeChart library is used for majority of plots. Most of the generated plots are interactive.

The following **functions** are available:

- Use the + or - key on the keyboard to zoom in or out.
- Scroll with the mouse to zoom in or out.
- Drag the mouse from left to right to select the area to zoom in.
- Drag the mouse from right to left to zoom out to the default view.
- Single click on the y-axis to auto set the intensity to auto height.
- Double click on the y- or x-axis to reset the zoom to default.

In the right part of the plot there is a toolbar. Its functionality is also included in a pop-up menu, which appears when you make right click on the plot area.

Last update: September 23, 2022 17:08:14

5.2 MS data visualisation

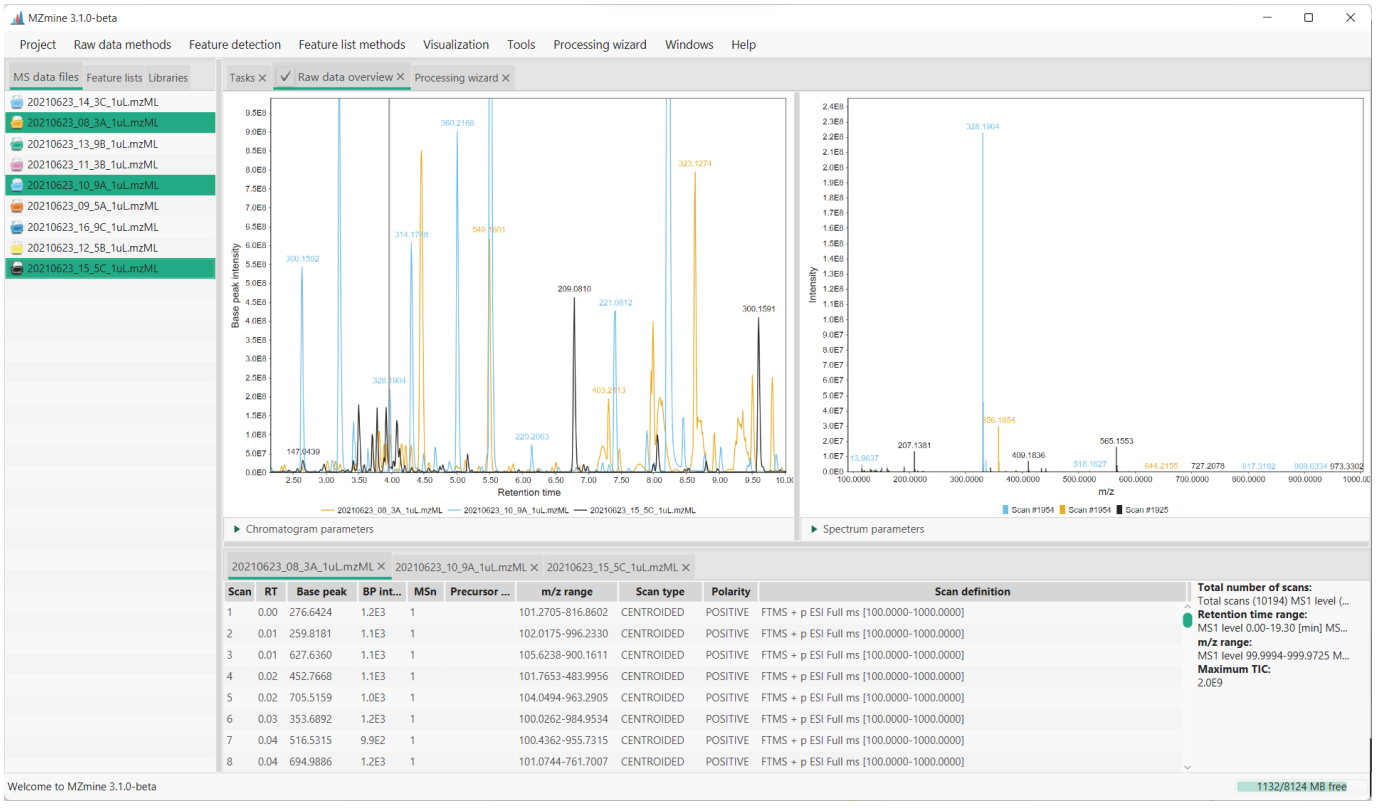
5.2.1 **Raw data overview (LC-MS)**

Description

Visualization → Raw data overview, or it can be accesed by **double-click of left mouse button**, or by clicking the right mouse button on **MS data files** table and choosing **Show raw data overview**

Raw data overview allows user to explore both chromatogram and MS views across all the selected files. By double-clicking on the raw file of interest in the "MS data files" tab, **Raw data overview** tab will open in the main content pane. Raw data overview can either display single or multiple overlaid chromatograms, depending on how many raw files are selected. Additionally, it can be accessed through: Visualization → Raw data overview.

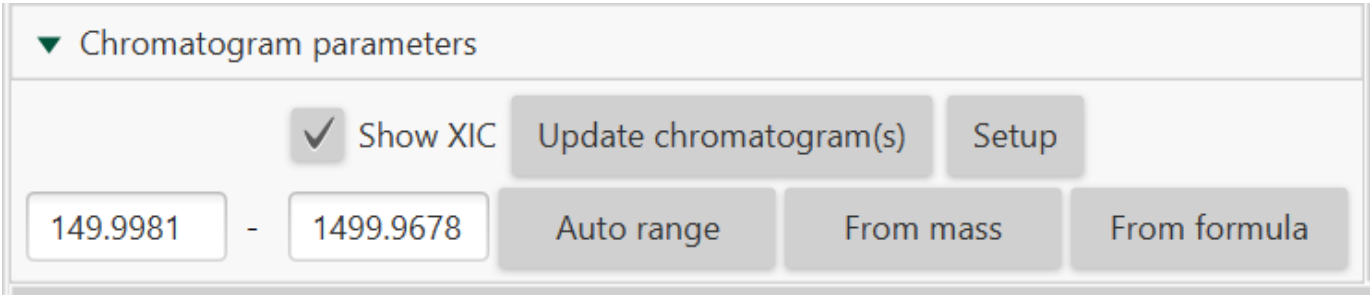
The view consists of three panes - chromatogram representation (on the left), mass spectrum (on the right), and table with tabs containing additional information about raw data files.



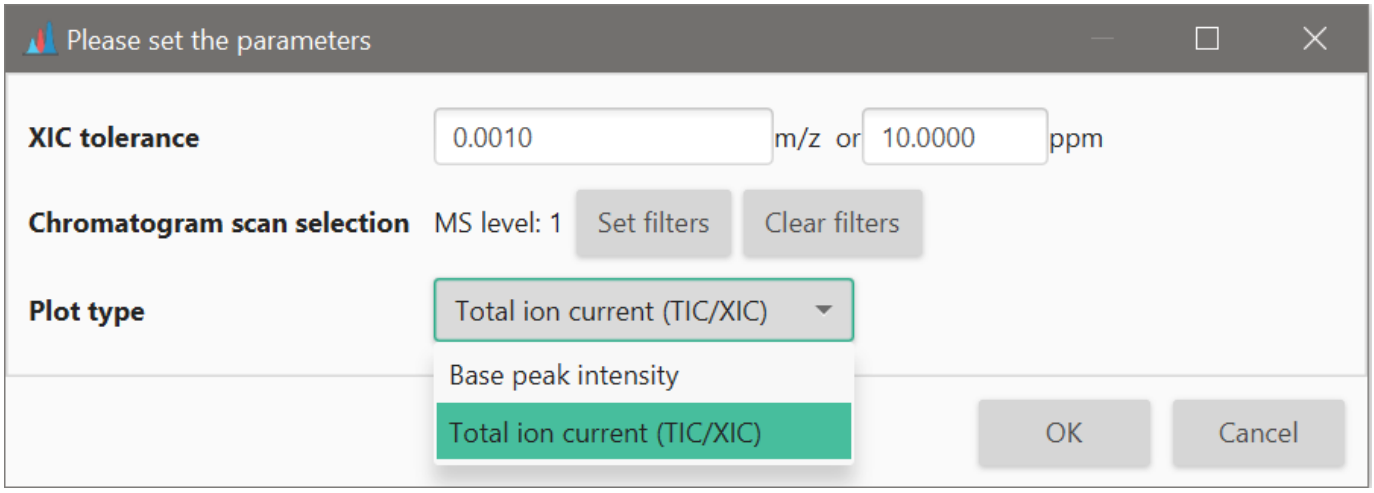
Chromatogram parameters

Show XIC

To display an extracted ion chromatogram (XIC), the user can tick the "Show XIC" box. There are several options for defining the m/z range - from mass, from formula and auto range.



"Setup" button allows to choose the appropriate plot type. **Base peak intensity** plot only shows the signal of the most intense mass peak in each MS spectrum, while the **Total ion current** plot shows the summed signal intensity of all masses at any one retention time point.



Spectrum parameters

When masses are detected, it is possible to display them on the spectrum by ticking an option "Show mass list".

Page Contributors

Olena Mokshyna (91.18%), lalalana5 (8.82%)

Last update: September 23, 2022 17:08:14

5.2.2 Additional tools

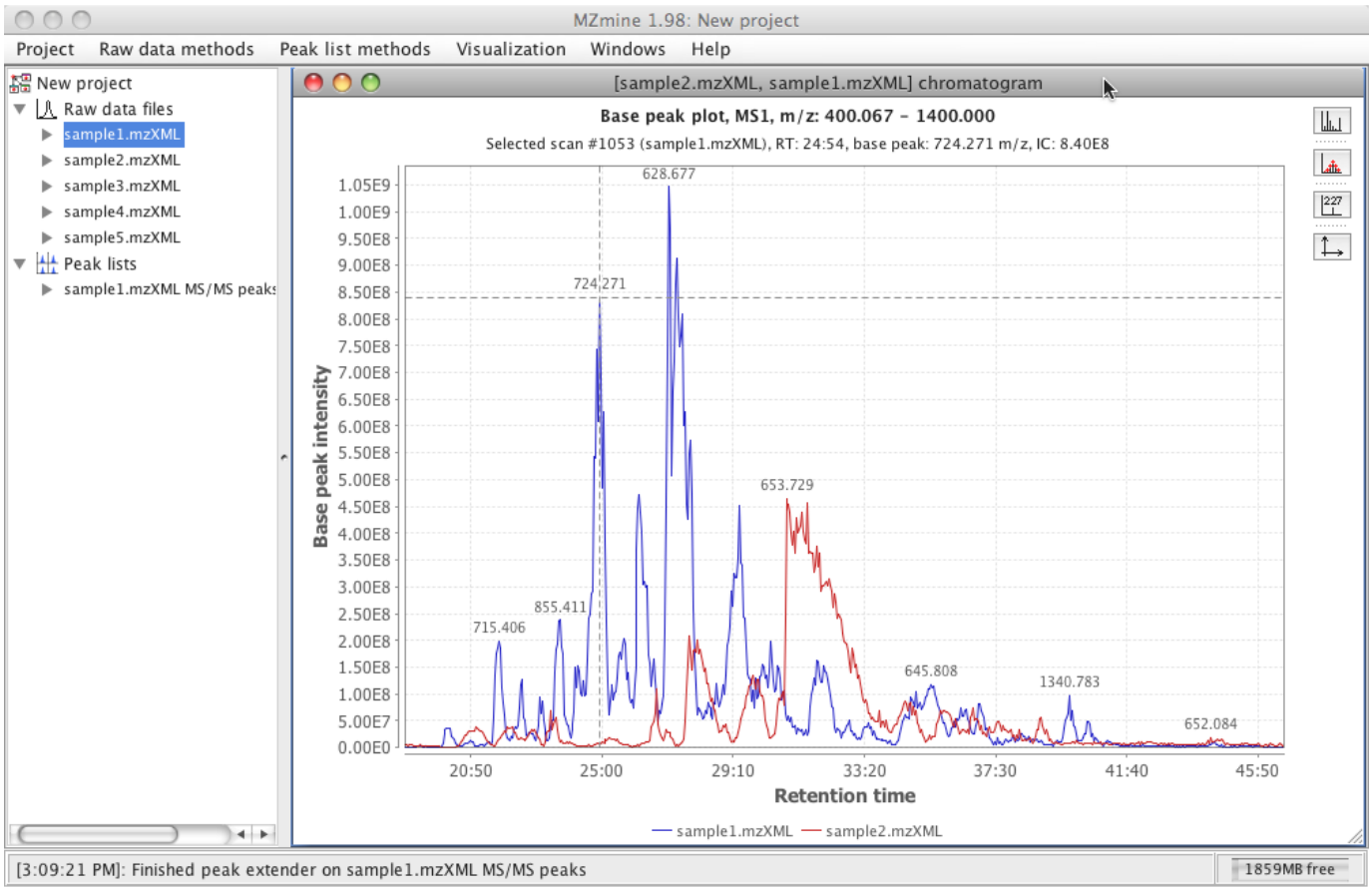
Chromatogram plot

DESCRIPTION

Visualization → Chromatogram plot

There is a possibility to display chromatographic peaks outside of raw data overview. This standalone two-dimensional plot visualizes TIC/XIC data. All the chromatograms are displayed in the same plot.

The x-axis corresponds to retention time and the y-axis is the intensity level of the signal.



PARAMETERS

Raw data files

List of raw data files to display in the TIC visualizer.

MS level

Scan level (MS1,MS2,... ,MSn) to display in the plot.

Plot type

TIC or base peak

Retention time

Retention time (x-axis) range.

m/z range

Range of m/z values. If this range does not include the entire scan m/z range, the resulting visualizer is XIC type.

Selected peaks

List of chromatographic peaks to display in the TIC visualizer. This option is available only if a peak list related to the selected raw data file exists in the current project.

MS spectrum

Visualization → MS spectrum

Displays all the ions from a selected scan. Can be used to explore mass spectrum outside of raw data overview. **Only one** raw file can be chosen.

It shows a plot of two dimensions, where X axis corresponds to m/z value and Y axis is the intensity of the ion signal.

PARAMETERS

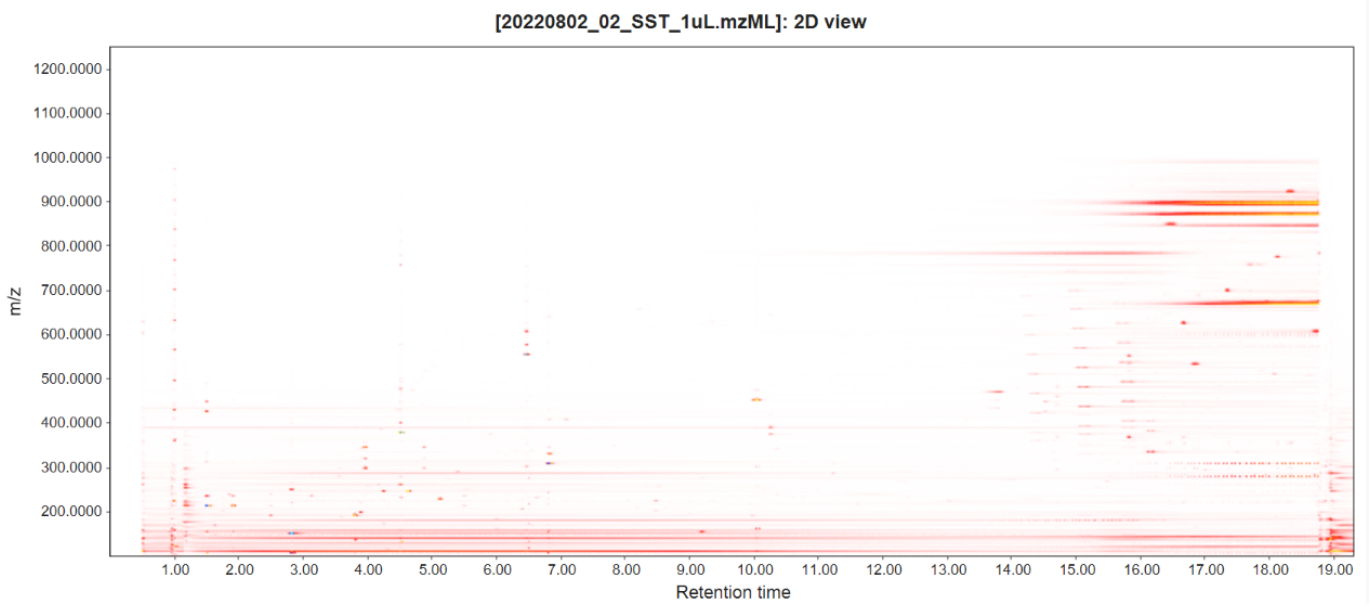
Scan number

Choose the scan to visualize

2D visualizer

Visualization → 2D plot

This tool displays a plot of two dimensions, where X axis corresponds to retention time and Y axis is the m/z value. This visualization of spots in the plot corresponds with the intensity of the data in that region.



User can define features from the feature list to be displayed on the plot.

PARAMETERS

Type of data

This plot can use either resampled data as input (faster), or raw data (slower).

Scans

Here user can set up the level (MS1,MS2,... ,MSn), polarity, retention time, and the other parameters of the scans to be used.

m/z

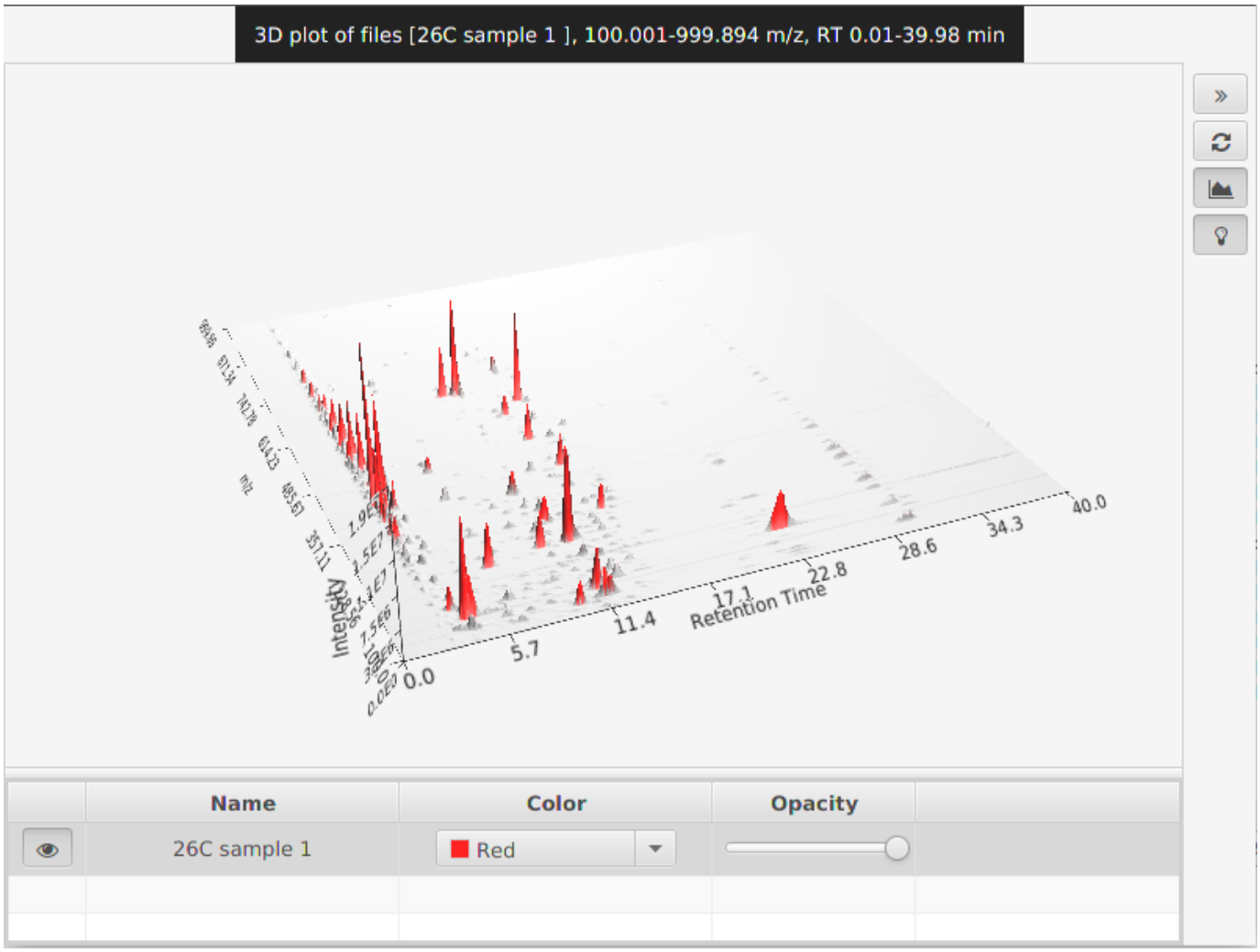
Defines range of m/z values.

3D visualizer

DESCRIPTION

Visualization → 3D plot

This tool presents a three dimensional plot where X axis represents the retention time, Y axis the m/z value and Z axis the intensity of the signal. This plot is the collection of all the information from the raw data in a graphical representation.



PARAMETERS

Scans

Here user can set up the level (MS1,MS2,... ,MSn), polarity, retention time, and the other parameters of the scans to be used.

m/z range

Range of m/z values.

Features

Features that will be taken as an input.

Retention time resolution

Number of data points on retention time axis.

m/z resolution

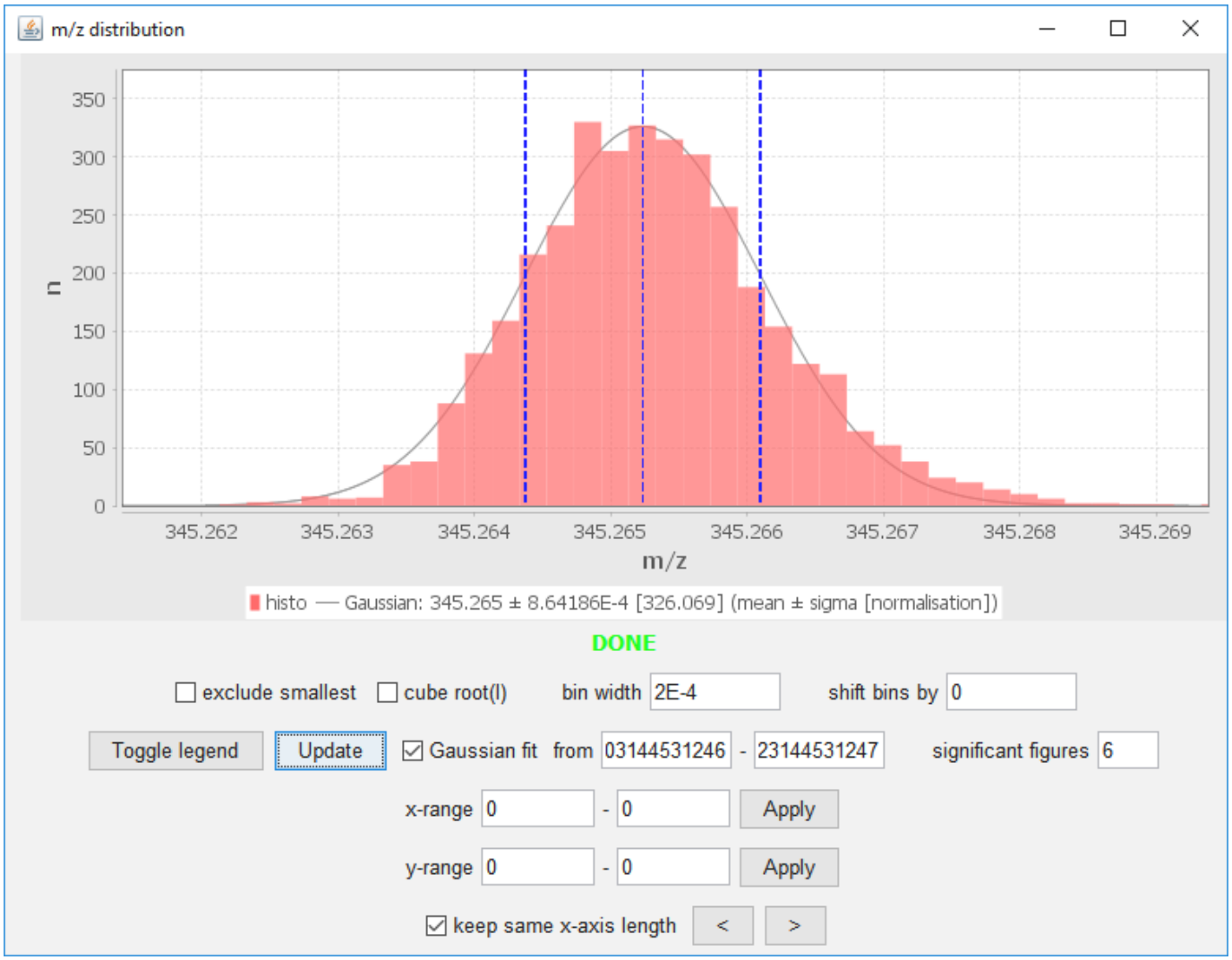
Number of data points on m/z axis.

Scan histogram

DESCRIPTION

Visualization → Scan histogram

This module creates m/z distribution histograms of all m/z values in mass lists across specified scans. The binning width, in which the m/z values are counted, can be changed dynamically. The number of scans that contain a specific m/z value (bin) are plotted.



https://youtu.be/31hwc74vUjA

PARAMETERS

Scans

Here user can set up the level (MS1,MS2,... ,MSn), polarity, retention time, and the other parameters of the scans to be used.

m/z

Limit the range of the histogram (can improve performance).

Signal intensity range

Optional parameter

Allows to limit signal intensities (can improve performance).

Mass defect

Optional parameter

Filters for mass defects in the signals.

Type

Type of the histogram to be created. Available options:

- m/z,
- Intensity,
- Intensity (noise recalibrated),
- Mass defect.

Bin width

The binning width to count m/z value occurence in scans.

Use mobility scans

If the input data has ion mobility dimension, this data can be used instead of the data from the summed frames.

Scan inject time analysis

MS(n) spectra tree

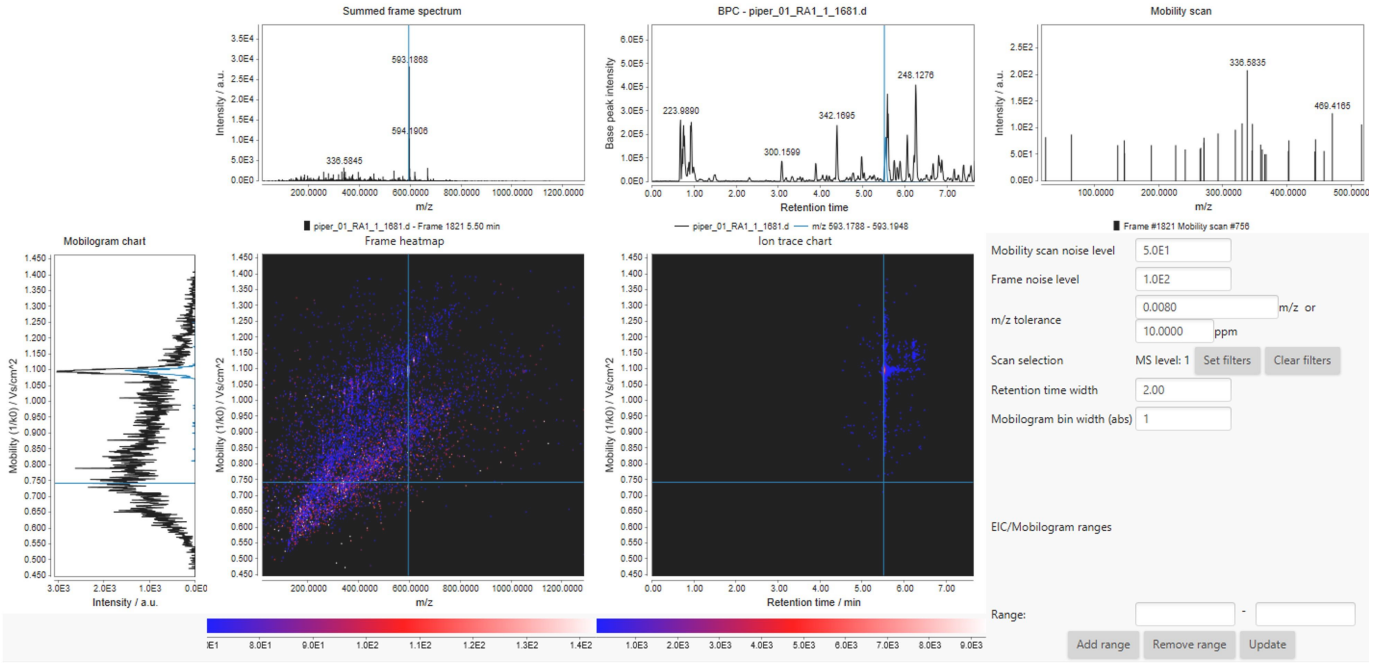
Last update: September 26, 2022 14:55:47

5.3 Ion mobility raw data overview (LC-IMS-MS)

5.3.1 **Description**

Visualization → Ion mobility raw data overview

The "Ion mobility raw data visualization" module allow a comprehensive navigation of the complex LC-IM-MS raw data. The screenshot belows shows an example of LC-IM-MS data acquired with a Bruker timsTOF instrument:



The main window consists of 5 panels and a set of displaying parameters. All the panels are interconnected, which means that moving the cursor in one panel, automatically updates the others. Cursors are displayed as light-blue solid lines in the panels.

5.3.2 Summed frame spectrum panel [1]

The MS spectrum corresponding to each frame is shown in this panel. The displayed MS spectrum is the sum of all the mobility scans acquired over that frame (see Ion mobility spectrometry terminology).

5.3.3 BPC panel [2]

In this panel, the base peak chromatogram is displayed. Each data point corresponds to an individual frame. Moving the cursor frame-by-frame automatically updateds the 'frame heatmap' and 'summed frame spectrum' panels. Moving the cursor frame-by-frame automatically updateds the 'summed frame spectrum' panels as changing data point in regular LC-MS data would display a different MS scan. Since each frame is made of several mobily scans, the 'mobilogram chart' and 'frame heatmap' panels automatically updates too. *Note*. It is currently not possible to display the TIC chromatogram)

5.3.4 Mobility scan [3]

5.3.5 Mobilogram chart [4]

5.3.6 Frame heatmap [5]

5.3.7 Ion trace chart [6]

5.3.8 Displaying parameters [6]

Mobility scan noise level: This parameter controls the signals shown in the XXX panels (panel n°X). For example, a noise level of 5.0E1 will show only the signals above this value (see below)

Frame noise level: This parameter sets a threshold for the signals shown in the "Summed frame spectrum panel" (panel n°X). Signals from MS spectra acquired over the same frame are summed and shown

m/z tolerance

Scan selection

Retention time width

Mobilogram bin width (abs)

EIC/mobilogram ranges

Last update: September 23, 2022 17:08:14

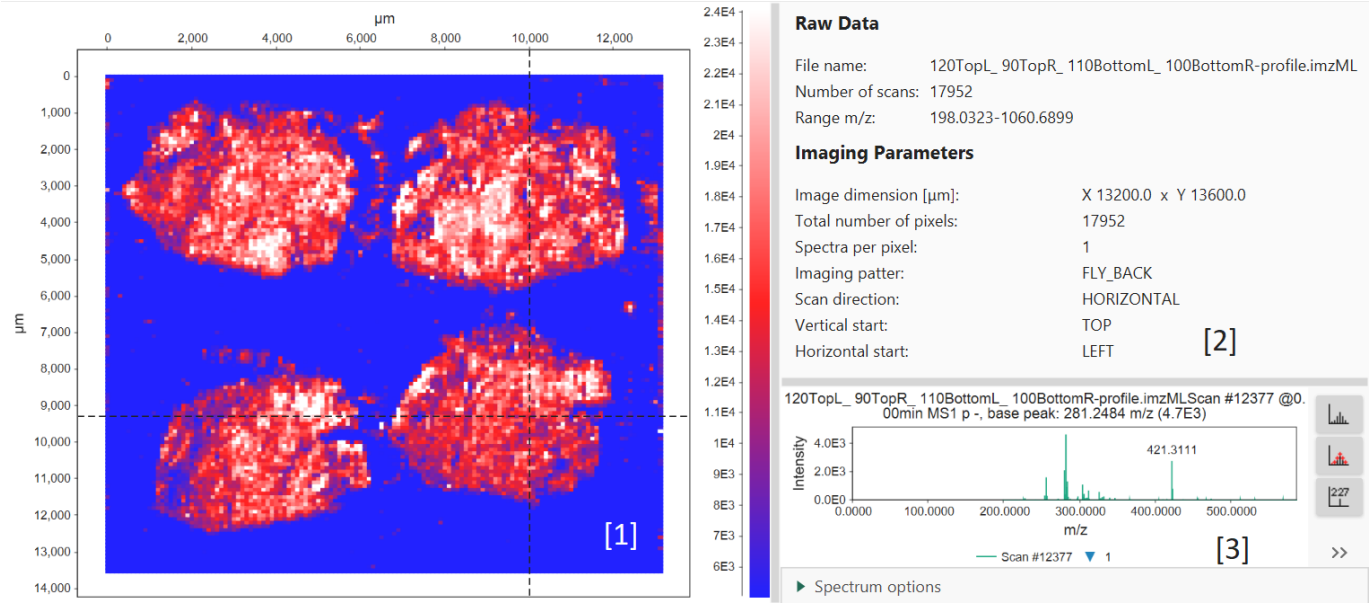
5.4 Image viewer

This module is under active development. Some newer features might not be fully documented.

5.4.1 **Description**

Visualization → Image viewer

This visualization module provides an overview of imaging data.



The interactive imaging plot **[1]** allows to choose any pixel and explore related spectrum. In **[3]** user can choose feature from a feature list to be depicted on a scan (prior feature detection is required).

[2] gives information about file and imaging parameters.

Last update: September 23, 2022 17:08:14

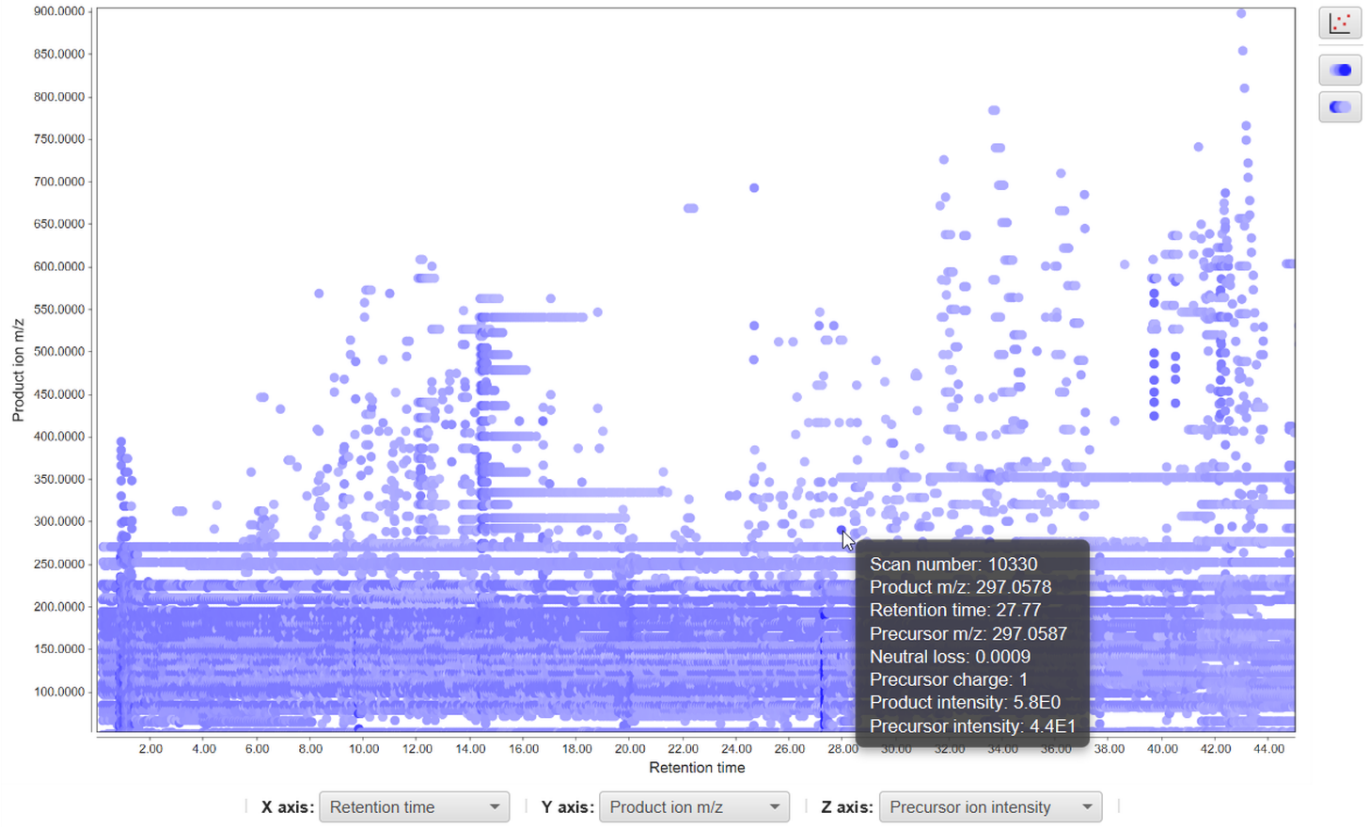
5.5 Processed data visualition

5.5.1 **MS/MS plot**

Description

Visualization → MS/MS scatter plot

This module provides a colored scatter plot of the MS/MS data. There are 4 options for X and Y axes: retention time, precursor ion m/z, product ion m/z, neutral loss and 3 options for Z axis (color): precursor ion intensity, product ion intensity, retention time. The module additionally allows you to filter ions by their intensities and to perform diagnostic fragmentation filtering. In order to focus on the values of interest you can highlight specific data points and sort them by color axis. This tool can be very useful to get an overview of large amounts of MS/MS data by tuning parameters and filters.



Parameters

Raw data file

Selection of the raw data file to visualize. Only one file can be selected.

X axis

Selection of the values for X axis. There are 4 options available: Retention time, Precursor ion m/z, Product ion m/z, Neutral loss.

Y axis

Selection of the values for Y axis. Options are the same as for X axis.

Z axis

Selection of the vales for Z axis. There are 3 options available: Precursor ion intensity, Product ion intensity, Retention time.

MS level

MS level of the scans to be plotted.

Retention time

Retention time range.

m/z range

Range of m/z values for precursor ions in MSn scans.

m/z tolerance

Maximum allowed difference between two m/z values to be considered same.

Intensities filtering

Optional parameter to filter ions by intensity. There are 3 different ways of filtering:

- Number of best fragments - Number of ions with highest intensities from each scan to be visualized.
  For example 5(for each scan 5 ions with highest intensities will be plotted).
- Base peak percent, % - Ions with intensity values lower than the given percent of base peak intensity will be plotted.
  For example 95(ions with intensity values lower than 0.95 multiplied by base peak intensity will not be plotted).
- Intensity threshold - Ions having intensities lower than the given value will not be plotted.
  For example 6.0E6(ions with intensity values lower than 6.0E6 will not be plotted).

Diagnostic fragmentation filtering

Optional parameter for diagnostic fragmentation filtering described below. It has 2 subparameters: diagnostic product ions and diagnostic neutral loss values. Scans not containing any ion satisfying each input criterion will not be considered for the visualization.

Diagnostic fragmentation filtering

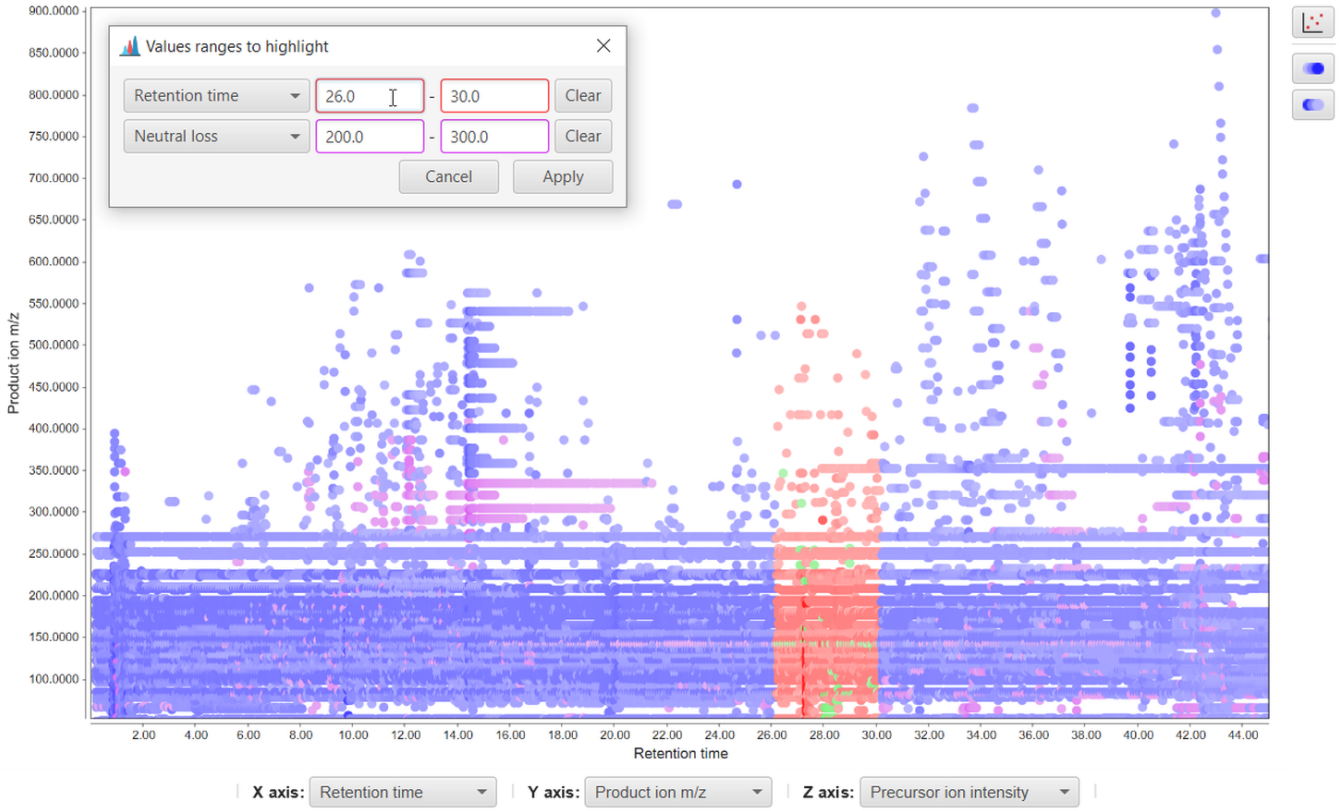
Due to common structural features, compounds within the same class undergo similar MS/MS fragmentation and as a result of many identical product ions and/or neutral losses. Diagnostic fragmentation filter (product ion filter) is a post-acquisition approach to screen LC-MS/MS datasets for entire classes of both known and unknown natural products. This tool searches all MS/MS spectra for product ions and/or neutral losses that has defined as being diagnostic for the entire class of compounds. In other words it screens LC-MS/MS datasets for MS/MS spectra containing production ions and/or neutral losses that are specific to that class of compounds. The user defines the diagnostic product ions and/or the diagnostic neutral loss values (Da) to use in the filtering.

The user can also define the minimum diagnostic ion intensity (% base peak) to use in the filtering. If a recurrent neutral loss occurs, a line pattern in the plot can be observed. If compounds carrying those diagnostic product ions and/or the neutral loss values are detected the resulting plot will show their product ion m/z and precusor ion m/z. Additionally, an output file may be specified that will output the results of the filtering. For a detailed view of diagnostic fragmentation filtering: Walsh, Jacob P., et al. "Diagnostic Fragmentation Filtering for the Discovery of New Chaetoglobosins and Cytochalasins." Rapid Communications in Mass Spectrometry (2018).

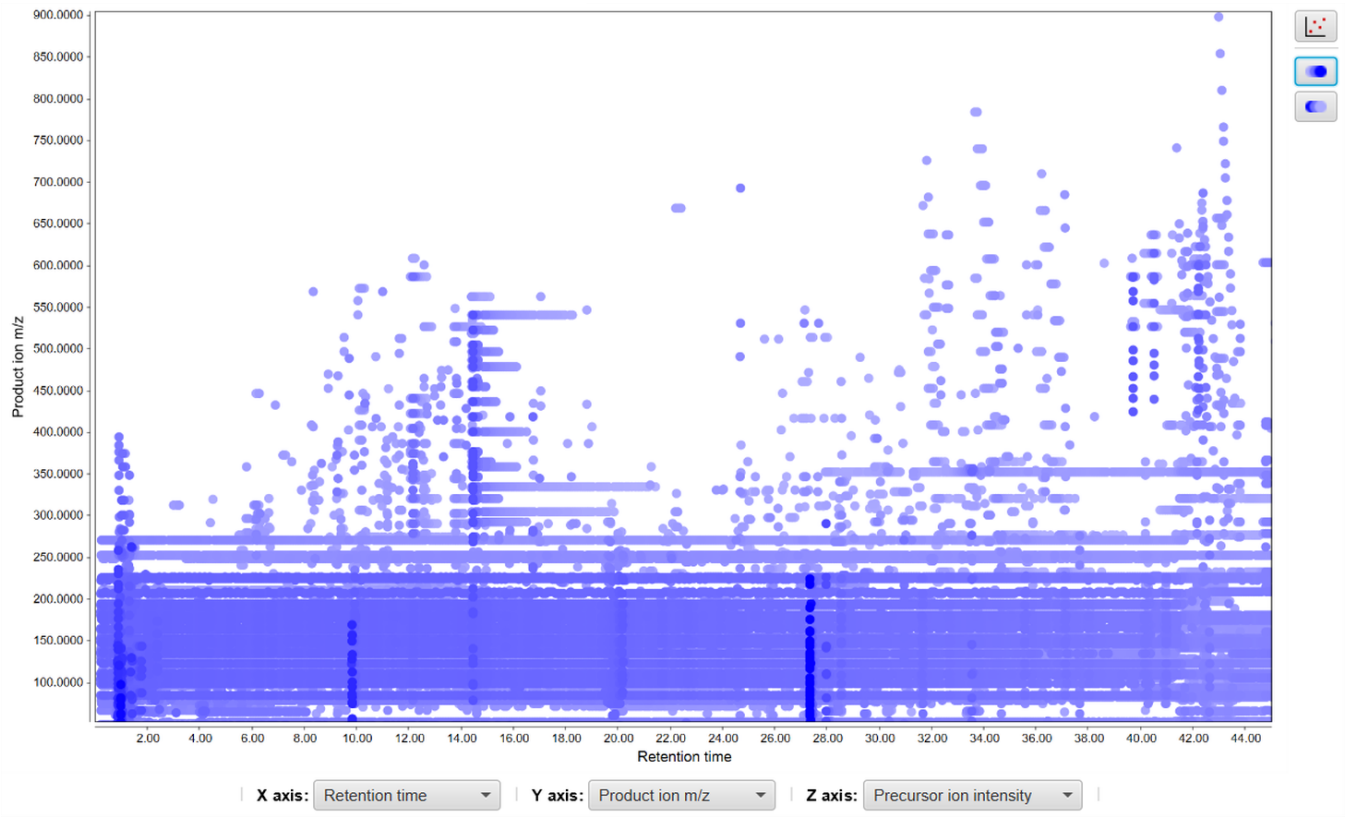
Functionality

This plot is using the third part library JfreeChart for its basic functionality.

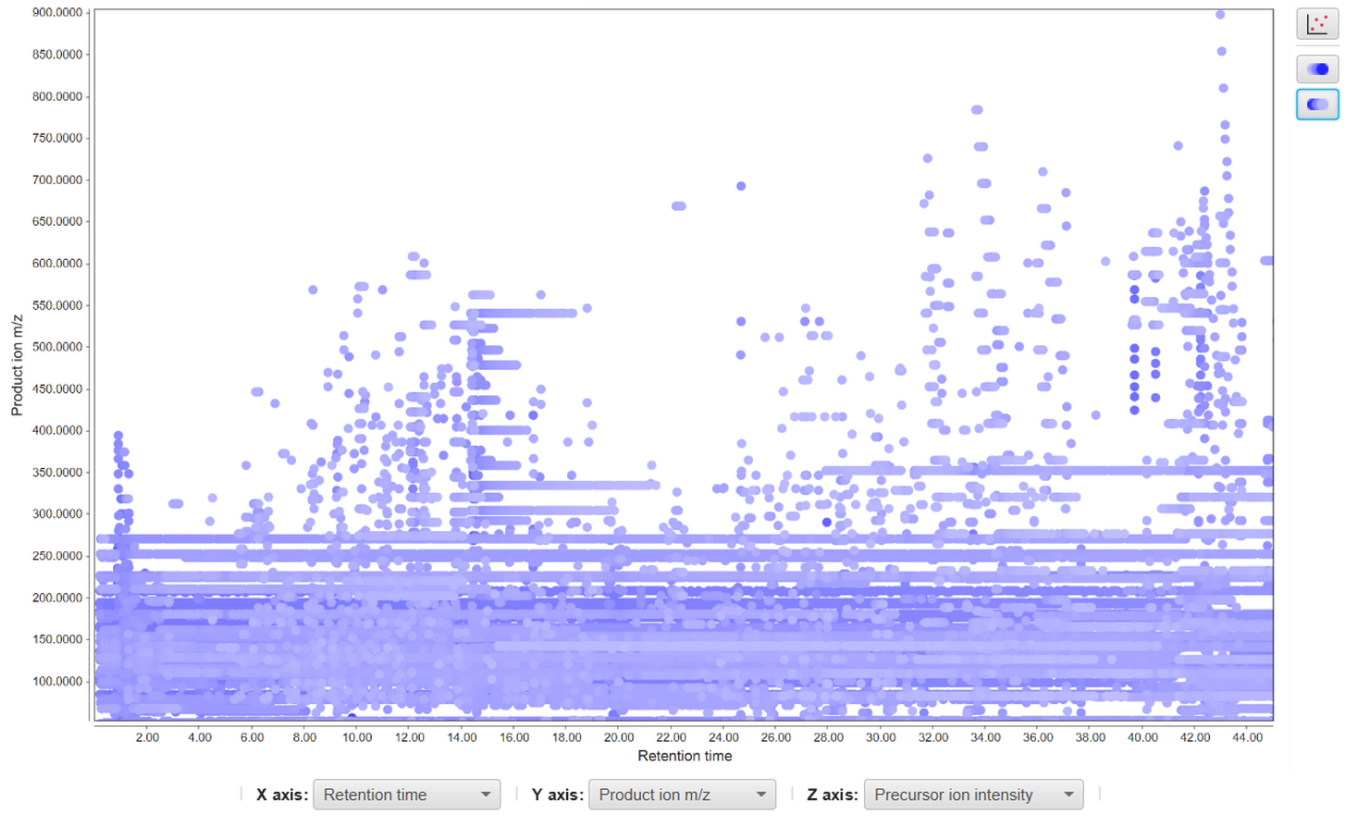
- Drag the mouse from left to right - selecting the area to zoom
- Drag the mouse from right to left - zoom out
- Select combo boxes below - change axes types
- Hold the mouse on data point - show detailed information in a tooltip
- Double click on data point - show spectrum plot
-  - highlight points representing ions with specific values given by input ranges (Note: colors of range input boxes determine the highlighting color, green color denotes ions satisfying both ranges)



-  - show intense points in front



-  - show pale points in front



Last update: September 23, 2022 17:08:14

5.5.2 Interactive ion identity molecular networks

Description

Visualization → Interactive ion identity molecular networks

Molecular networking connects mass spectra of molecules based on the similarity of their fragmentation patterns. However, during ionization, molecules commonly form multiple ion species with different fragmentation behavior. As a result, the fragmentation spectra of these ion species often remain unconnected in tandem mass spectrometry-based molecular networks, leading to redundant and disconnected sub-networks of the same compound classes.

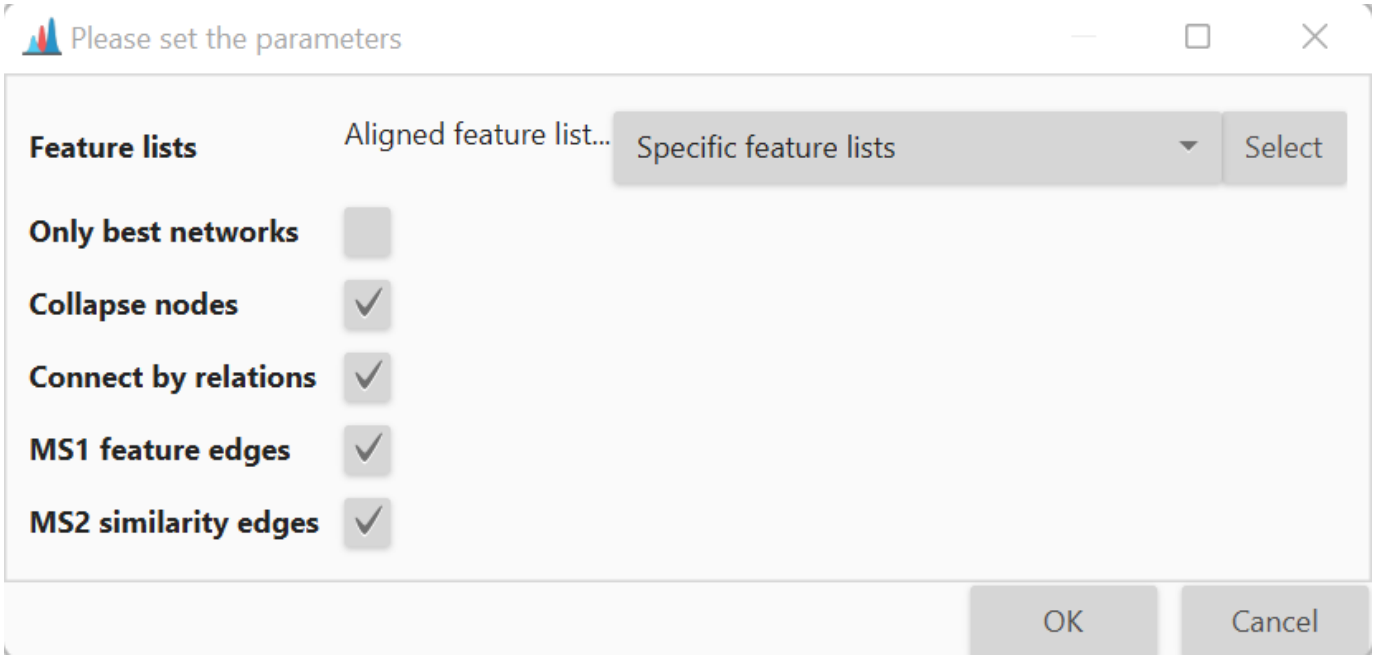
To overcome this bottleneck, MZmine employes **Ion Identity Molecular Networking (IIMN)** module. This module unites chromatographic peak shape correlation analysis with molecular networks, which is able to connect and collapse different ion species of the same molecule. This feature relationships improve network connectivity for structurally related molecules.

This module can be used to reveal unknown ion-ligand complexes, enhance annotation within molecular networks, and facilitate the expansion of spectral reference libraries.

Using IIMN it is possible to query GNPS and other spectral libraries for matches.

More detailed description of theory behind IIMN can be found in the following video:

Parameters



Only best networks

Only the networks that only contain first ranked ion identities for all rows.

Collapse nodes

Collapse all nodes into neutral molecule nodes.

Connect by relations

Connect neutral molecule nodes by network relations.

MS1 feature edges

Include feature correlation edges.

MS2 similarity edges

Show MS2 similarity edges.

Last update: September 23, 2022 17:08:14

5.5.3 Histogram plot

Description

Visualization → Histogram plot

This plot displays a graphic representation of frequencies. Each rectangle represents an interval of frequency. The height is also equal to the frequency density in that interval. The total area of the histogram is equal to the number of data. This tool can use the m/z value, height, area or retention time as frequency value (X axis) and number of peaks in each interval (Y axis).

Parameters

Raw data files

Column of peaks to be plotted.

Plotted data type

Peak's data to be plotted (m/z value, height, area or retention time)

Plotted data range

Range of data to be plotted. This range is automatically loaded with max values from the raw data.

Number of bins

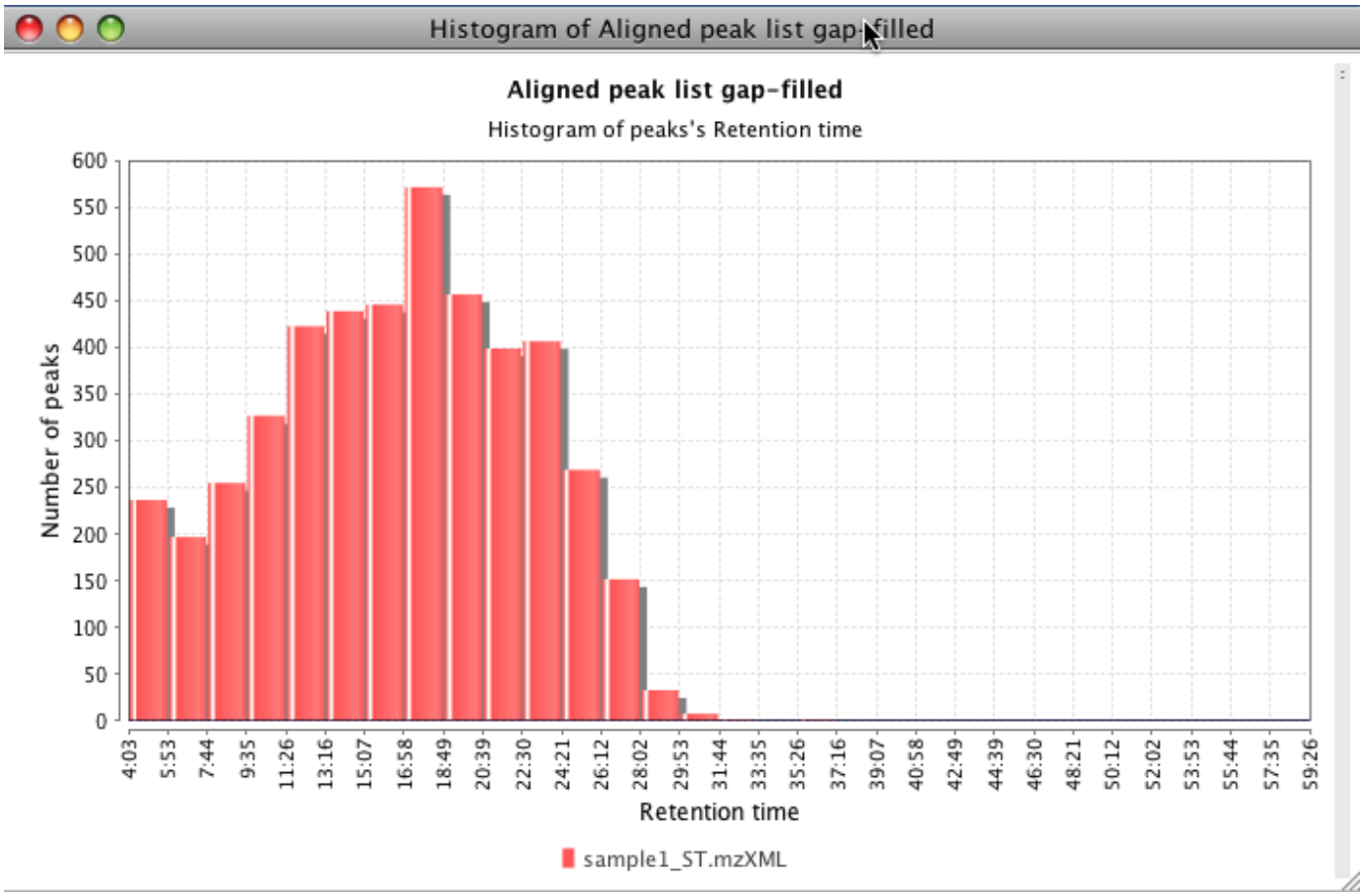
The plot is divides into this number of bins.

Functionality

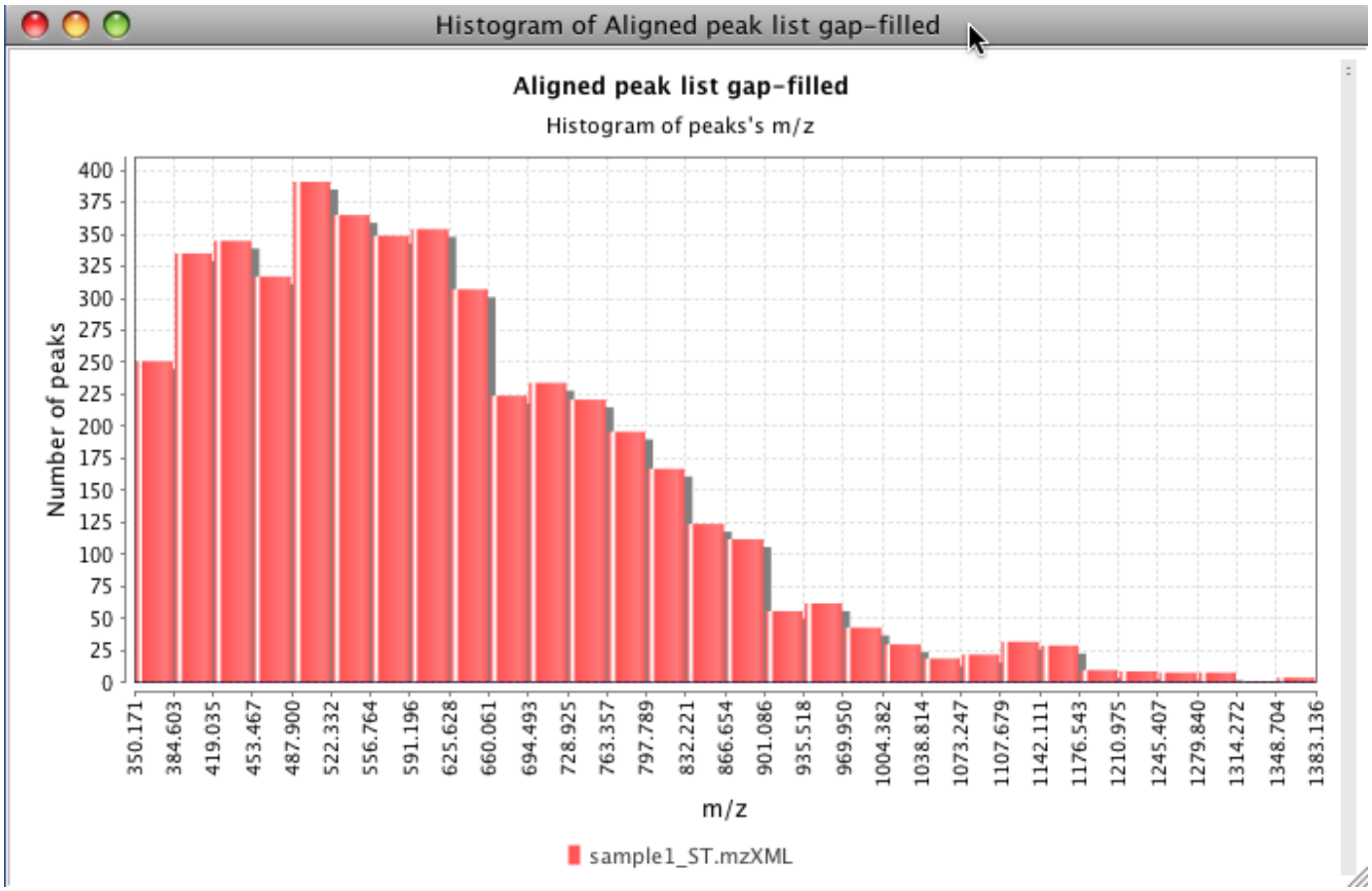
This plot is using the third part library JfreeChart for its basic functionality.

To zoom in, drag the mouse from left to right, selecting the area to zoom. To zoom out drag the zoom from right to left.

The next figure shows a histogram using the retention time value. This data is coming from a raw data with a duration of 60 min. Most of the peaks appears around 17 min.



The next figure shows a histogram using the m/z value. This data is coming from a raw data with a range from 350 to 1400 m/z.



Last update: September 23, 2022 17:08:14

5.5.4 Additional tools

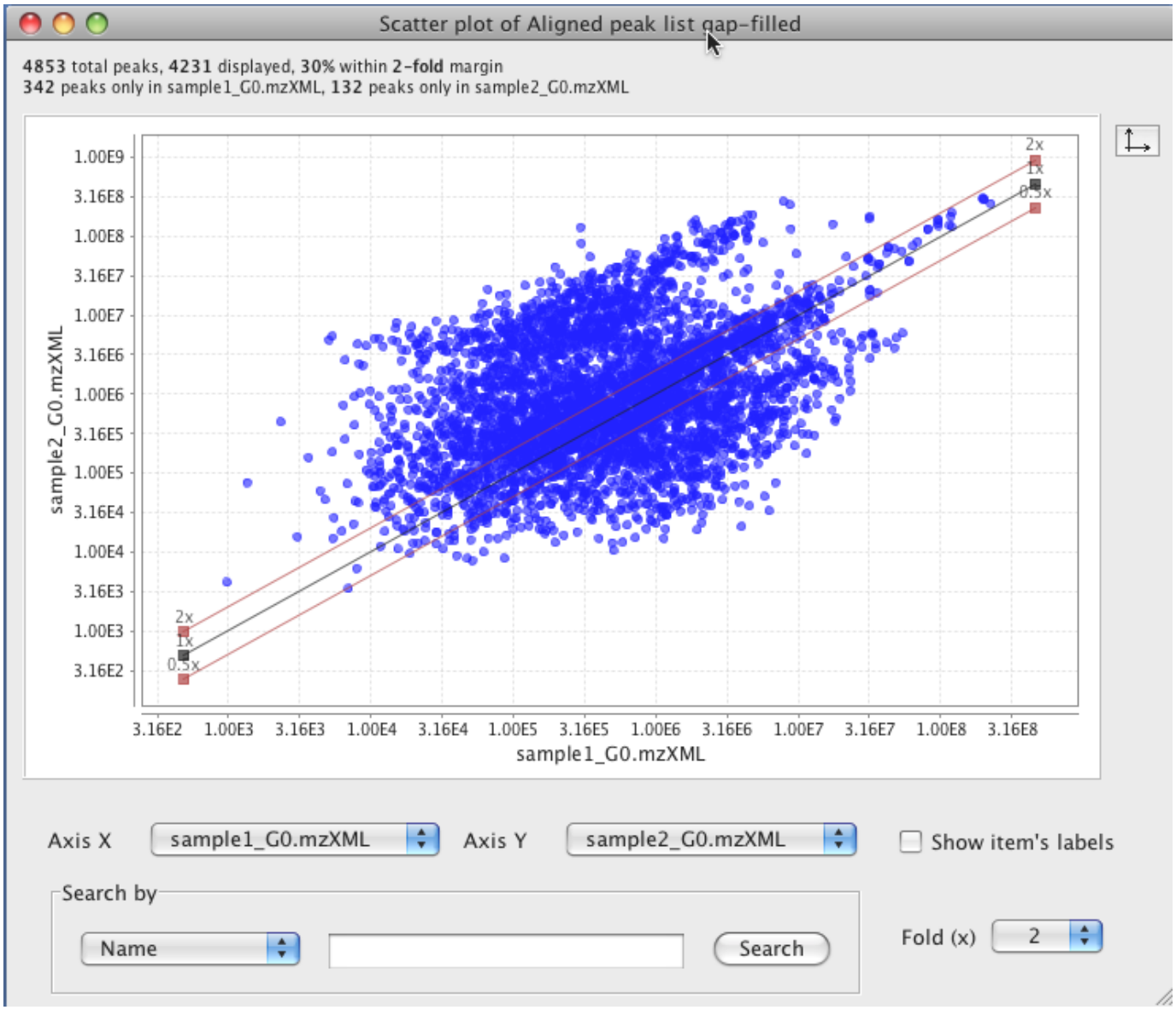
Scatter plot

DESCRIPTION

Visualization → Scatter plot

This tool shows a scatter plot with data from identified peaks in aligned feature list.

A search for a peak can be done using three options (name, retention time and m/z value).



Correlated features \(\Delta\)m/z histogram

DESCRIPTION

Visualization → Correlated features \(\Delta\)m/z histogram

This module plots all m/z deltas between correlated features in a histogram and offers a Gaussian fit.

PARAMETERS

Minimum Pearson correlation

Minimum Pearson correlation of feature shapes.

Limit delta to m/z

Maximum m/z delta is the m/z of the smaller ion (feature list row).

m/z bin width

Binning of m/z values for feature picking

Append to file

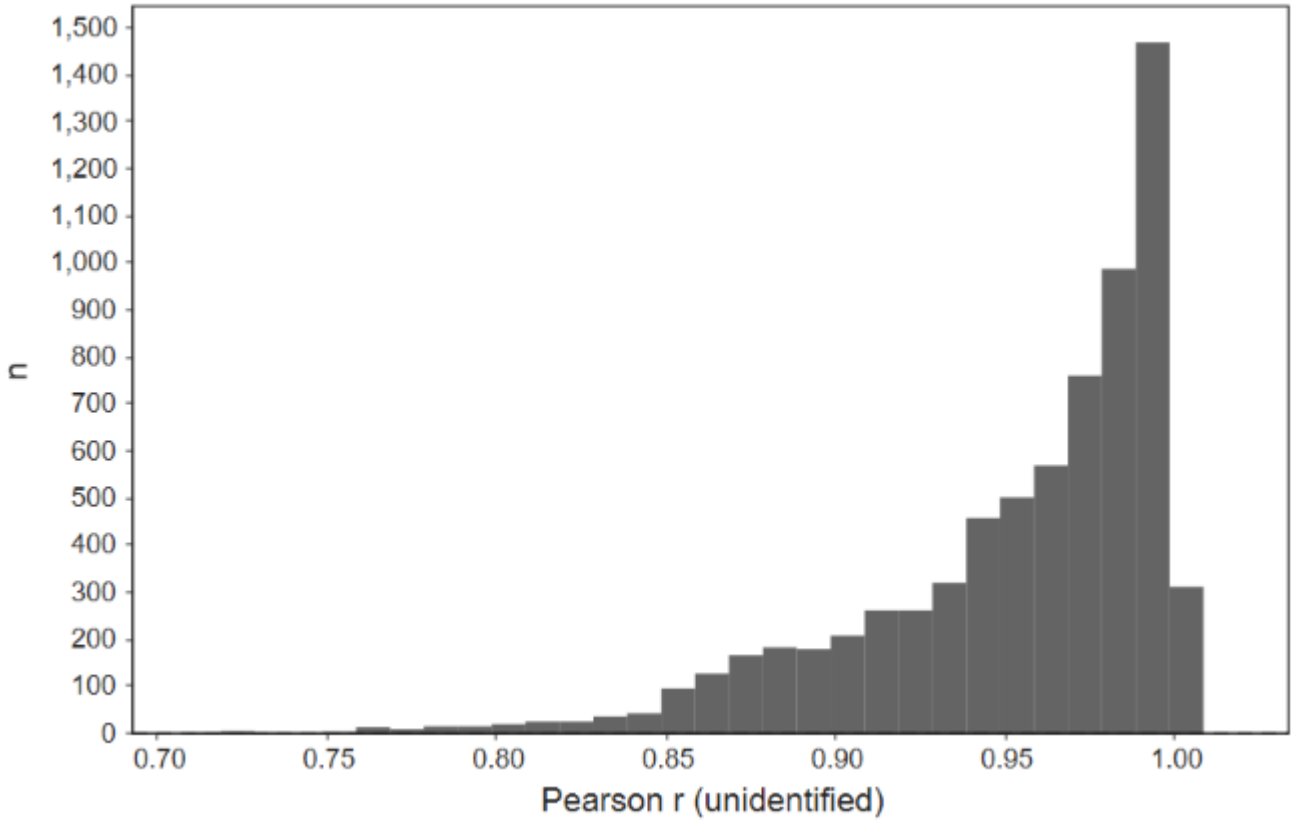
Append the correlated features delta m/z to a csv file.

Correlation coefficient histogram

DESCRIPTION

🚧 This module is being updated. Some newer functionality might not be documented.

This module allows to plot all correlations between feature shapes.



PARAMETERS

m/z bin width

Binning of m/z values for feature picking

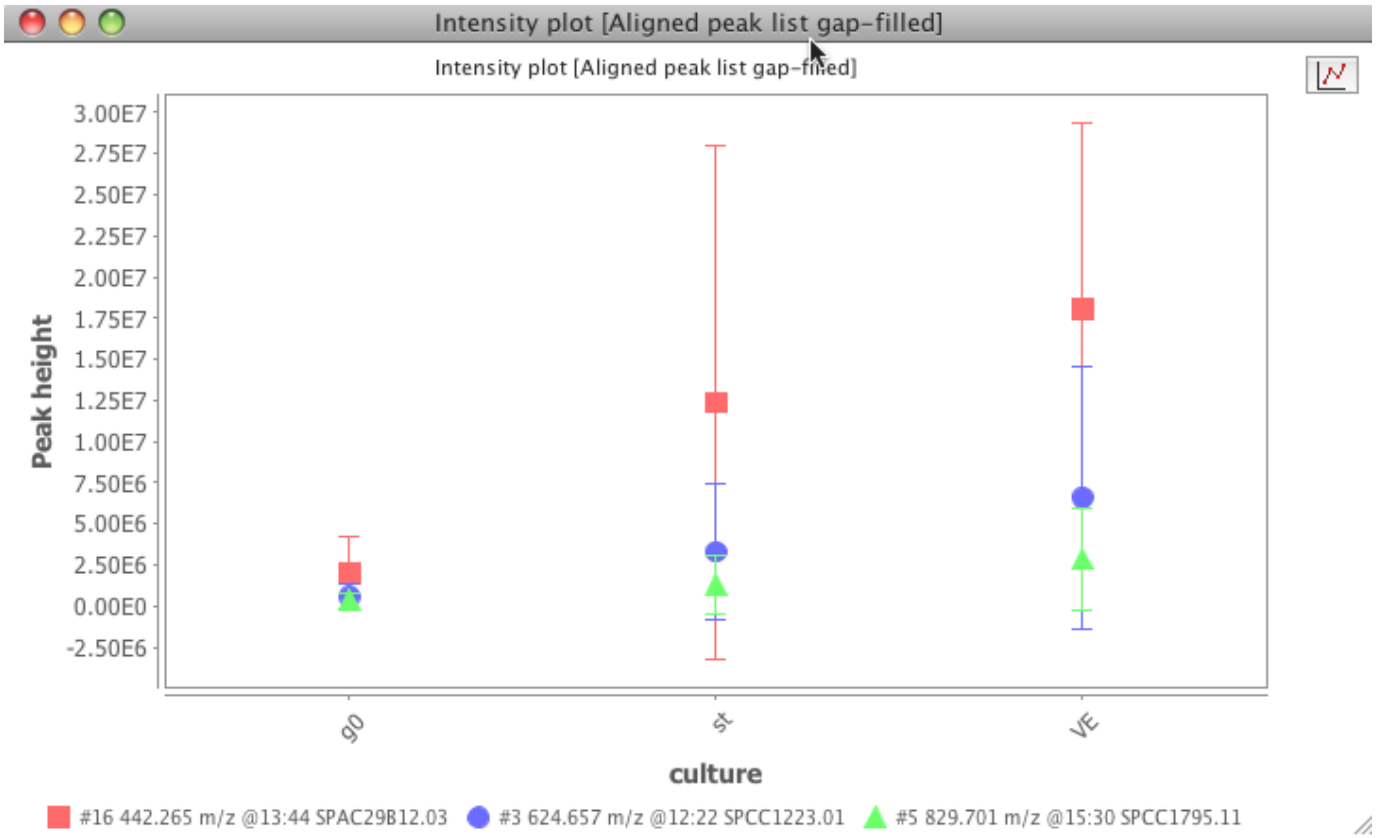
Feature intensity plot

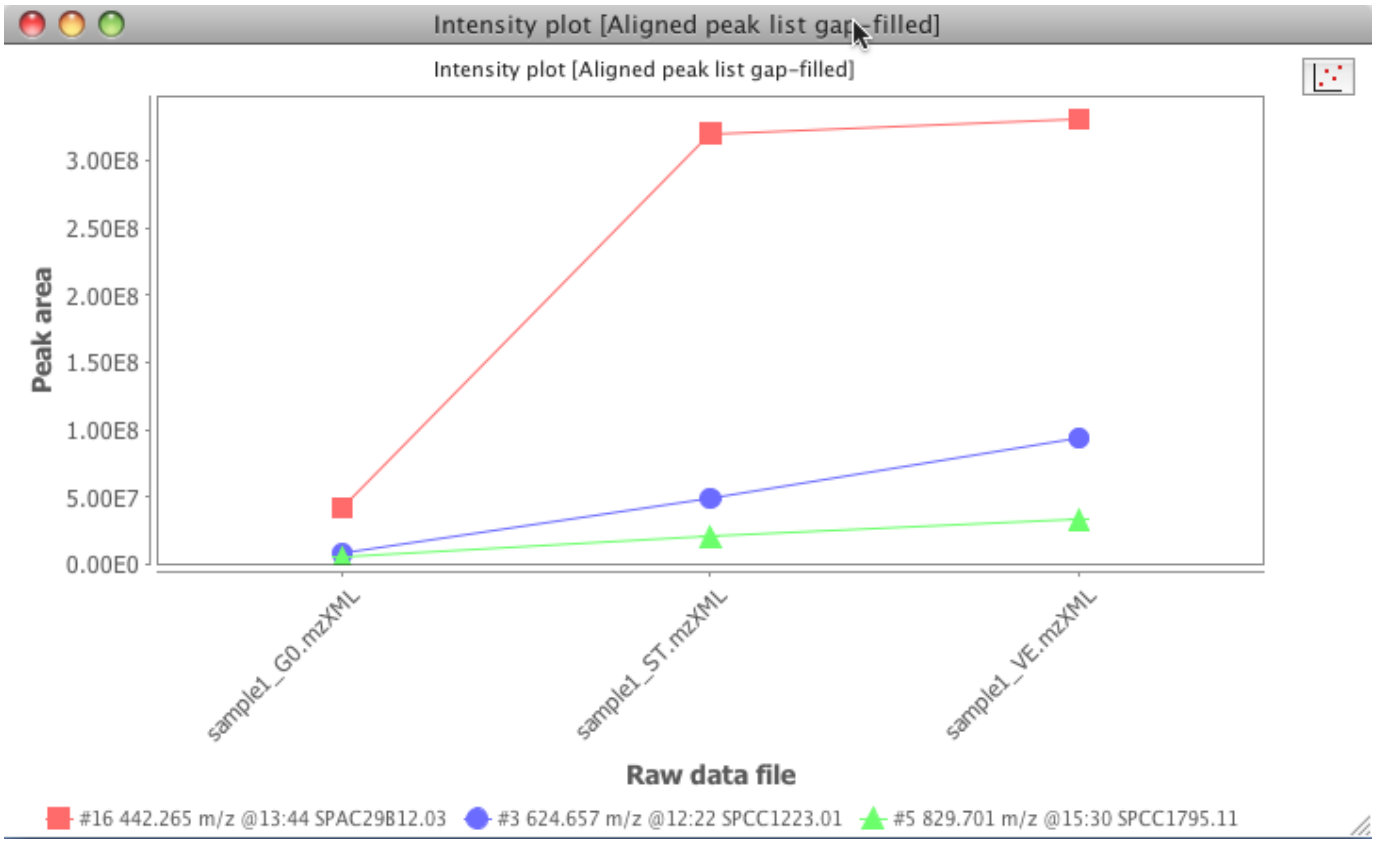
DESCRIPTION

🚧 This module has a known bug and is being updated, which might affect its functionality.

Visualization → Feature intensity plot

This plot allows to explore specific features against the raw data.





PARAMETERS

Data files

Selects the raw data files from where the peaks were detected

X axis value

X axis display the raw data file name or the parameter defined in the "set sample parameters" window

X axis value

The user can choose from peak's height, area or retention time value to display in this axis.

Peaks

The user can select the peaks to use in this plot.

Kendrick mass plot

DESCRIPTION

Visualization → Kendrick mass plot

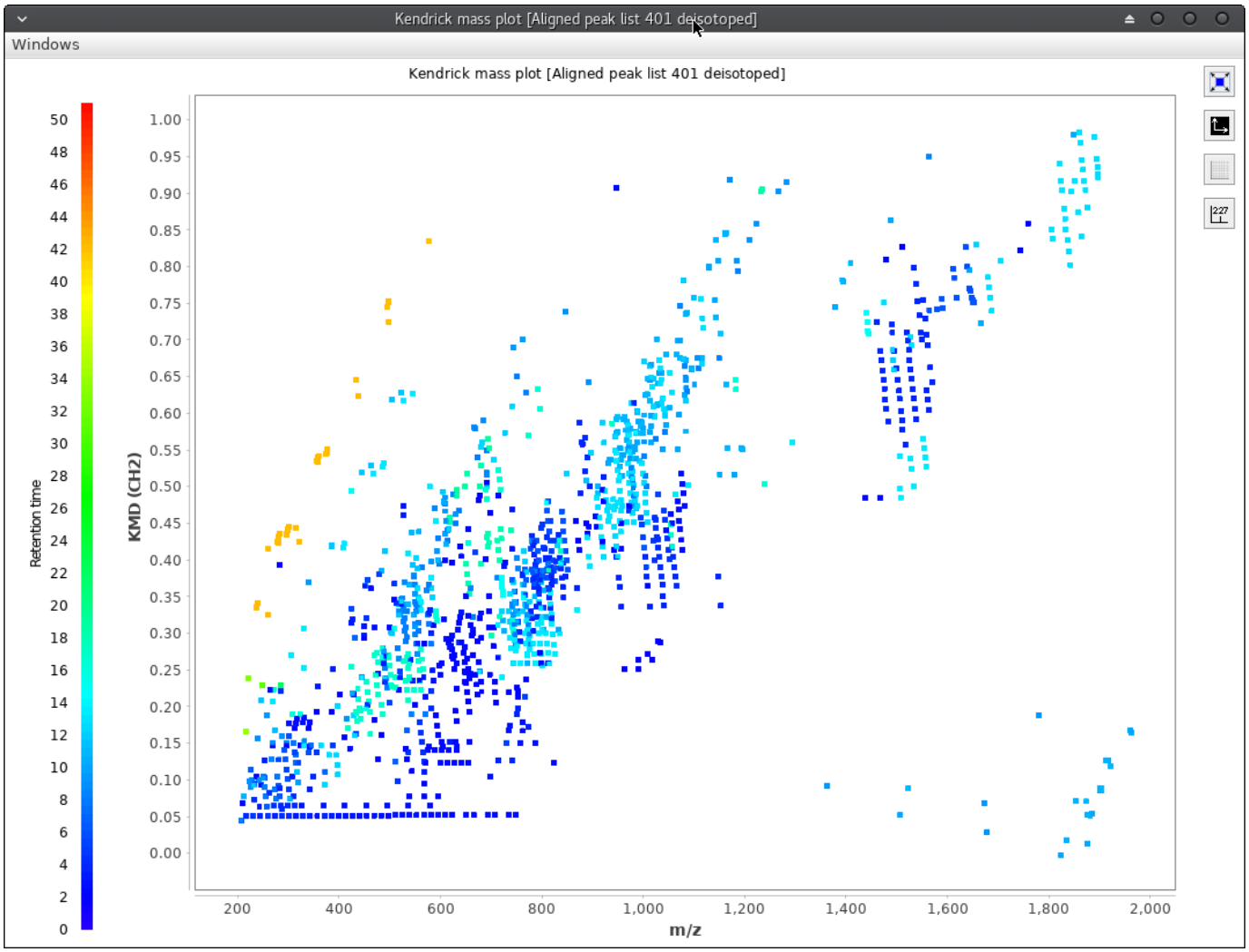
In 1963 Kendrick published his idea of a mass scale, the so-called Kendrick mass scale, which is based on defining the mass of CH2 as 14.0000 u.

The Kendrick mass scale is calculated by multiplying the IUPAC mass scale with the factor 14.0000 u/14.01565 u = 0.9988834. This results in the same mass defect for homologous components, the so-called **Kendrick mass defect (KMD)**. The KMD is defined as the \(\Delta\) of a nominal Kendrick mass and its associated Kendrick mass. Using the Kendrick mass scale has the purpose of data reduction.

\[KM(R)=m/z\cdot\frac{round(R)}{R}\]
\[KMD(R)=round(KM(R))-KM(R)\]

where \(KM\) is Kendrick mass, \(KMD\) - Kendrick mass defect, \(R\) - exact mass of selected base unit

More information here or at https://en.wikipedia.org/wiki/Kendrick_mass.



If you use this module for your analysis or visualization, please cite:

> *Three-dimensional Kendrick mass plots as a tool for graphical lipid identification. A. Korf, C. Vosse, R. Schmid, P. O. Helmer, V. Jeck, H. Hayen, Rapid Communications in Mass Spectrometry 32.12 (2018): 981-991.*

DETAILED FUNCTIONALITY

This module allows to create 2 and 3 dimensional Kendrick mass plots. All possible feature characteristics can be plotted in a third dimension.

Charge dependent Kendrick mass plots

Multiply charged ions can cause splits in Kendrick mass plot. Fouquet et al. have shown considering the charge for the calculation of the KM can help to overcome this problem through clustering of features.

$$KM(R,Z)=Z\cdot KM(R)=Z\cdot m/z\cdot \frac{round(R)}{R}$$

where Z is charge.

> Fouquet, Thierry NJ, et al. "On the Kendrick Mass Defect Plots of Multiply Charged Polymer Ions: Splits, Misalignments, and How to Correct Them." Journal of The American Society for Mass Spectrometry 29.8 (2018): 1611-1626.

Resolution enhanced Kendrick mass defect plots

Fouquet and Sato have shown that a fractional base unit (ivisor) can enhance the resolution of Kendrick mass plots.

$$X>1, KM(R,X)=m/z\cdot \frac{round(R/X)}{R/X}$$

where X is a fractional base unit.

Fouquet, Thierry, and Hiroaki Sato. "Extension of the Kendrick mass defect analysis of homopolymers to low resolution and high mass range mass spectra using fractional base units." Analytical chemistry 89.5 (2017): 2682-2686.

Combining charge and fractional base unit (Divisor)

If both charge and fractional base unit are changed, the following equation is used:

$$KM(R,Z,X)=Z\cdot KM(RX)=Z\cdot m/z\cdot \frac{round(R/X)}{R/X}$$

Fouquet, Thierry NJ, et al. "On the Kendrick Mass Defect Plots of Multiply Charged Polymer Ions: Splits, Misalignments, and How to Correct Them." Journal of The American Society for Mass Spectrometry 29.8 (2018): 1611-1626.

Fouquet, Thierry, Takaya Satoh, and Hiroaki Sato. "First gut instincts are always right: the resolution required for a mass defect analysis of polymer ions can be as low as oligomeric." Analytical chemistry 90.4 (2018): 2404-2408.

Remainders of Kendrick masses (RKM)

Another option to increase the resolution of Kendrick mass plots is the by Fouquet et al. proposed concept of RKM (remainders of Kendrick masses). By clicking the KMD/RKM button in the toolbar on the right side, KMDs are transformed to RKMs.

$$RKM(R)=\Bigg\{\frac{KM(R)}{round(R)}\Bigg\}$$

with $\{ \}$ being the fractional part function defined as $x=x-floor(x)$

PARAMETERS

Peak list

Select the targeted peak list.

Peaks

Add peaks from the peak list.

Kendrick mass base for y-Axis

Enter a sum formula which will be used as Kendrick mass base for the y-Axis.

X-axis value

Select which parameters you want to display on the X-Axis (Kendrick mass (KM) or m/z).

Kendrick mass base for x-Axis

If you want to display a Kendrick mass defect on the x-axis, check the check box and enter a sum formula as Kendrick mass base.

Z-axis value

Select which parameters you want to display in the third dimension. If you select "none", a 2D Kendrick mass plot will be generated.

Kendrick mass base for Z-Axis

If you want to display a Kendrick mass defect on the z-axis in form of a heatmap, check the check box and enter a sum formula as Kendrick mass base.

Z-axis scale value

Choose the bounds for the Z-axis. "Percentile" allows to exclude values of a selected percentile bellow and/or above from the paint scale. Values below will be displayed in black, values above will be displayed in magenta. "Custom" allows to set custom ranges.

Range for z-axis scale

Enter lower bound left and higher bound right. If you have choosen percentile for Z-axis scale the values must be between 0 and 100. If you enter 0 and 100, all values will be included in the paint scale.

Heatmap style

Select the style of your paint scale. You can choose between rainbow and different monochrome color coded paint scales.

Van Krevelen diagram

DESCRIPTION

Van Krevelen diagrams are graphical plots developed by Dirk Willem van Krevelen (chemist and professor of fuel technology at the TU Delft) that are used to assess the origin and maturity of kerogen and petroleum.

The diagram cross-plots the hydrogen:carbon (hydrogen index) as a function of the oxygen:carbon (oxygen index) atomic ratios of carbon compounds.

1. Van Krevelen, D.W. (1950). "Graphical-statistical method for the study of structure and reaction processes of coal", Fuel, 29, 269-84
2. https://en.wikipedia.org/wiki/Van_Krevelen_diagram

PARAMETERS

Peaks

Select peaks from the feature list.

Z-Axis

Select which parameters you want to display in the third dimension. If you select "none", a 2D Van Krevelen diagram will be generated.

Color scale

Select the style of your paint scale. You can choose between rainbow and monochrome color-coded scales.

Last update: September 26, 2022 14:55:47

6. Workflows

6.1 LC-MS Workflow

The workflow proposed herein is intended as a general pipeline for untargeted LC-MS (or LC−MS/MS) data preprocessing. The main goal is essentially to turn the highly-complex LC-MS raw data into a list of features, and corresponding signal intensity, detected across the analysed samples. Such feature lists can then be exported for further downstream analysis (e.g., identification, search against spectral libraries, statistical analysis, etc.). A schematic representation of the workflow is shown below:

6.1.1 Raw data processing

The raw data processing consists of essentially two steps: Data import and Mass detection

Raw data import

Either open (e.g. mzML) and native vendor (e.g. Thermo, Bruker) data formats can be imported in MZmine 3. All the supported formats are listed here (LINK to Doc). For more details see the Data import module.

Mass detection

This step produces a list (referred to as "mass list") of the m/z values found in each MS scan across the LC run that exceed a user-defined threeshold (i.e. noise level). For more details see the Mass detection module.

6.1.2 Feature processing

The goal of the "Feature processing" is to obtain a list of all the detected features (characterized by a RT and m/z value) from the raw LC-MS data.

Chromatogram building

The first step in the "Feature processing" is to build the so-called extracted ion chromatograms (EICs) for each detected mass (see "Mass detection"). There are two modules in MZmine 3 that can fulfil this task: ADAP chromatogram builder (widely used) and **Grid mass** (create docs).

The "detected" features in each file are listed in the so-called "feature lists", which are then further processed and aligned to connect corresponding features across all samples.

Smoothing in retention time dimension (optional)

Depending on the LC peak shape (i.e. data noisiness), the user can perform smoothing in retention time dimension. For more details see the Mass detection and Smoothing modules.

Feature resolving

Feature resolving step enables separation of co-eluting and overlapping chromatography peaks and as such is one of the pivotal steps in data preprocessing. For more detalis on the algorithm used and parameters settings, see the Local minimum resolver module.

^{13}C isotope filter (isotope grouper)

In order to remove redundant features, such as the ones generated due to the presence of isotopologues, isotope filter should be applied. ^{13}C isotope filter (isotope grouper) removes ^{13}C isotope features from the feature list. Use the isotope finder for more sensitive detection of possible isotope signals.

Isotope pattern finder

Isotope pattern finder searches for the isotope signals of selected chemical elements in the mass list of each feature. The isotope pattern detected by the **isotope finder** module has priority over the one detected by the **isotope filter (grouper)** module, if both are available. For more details, see the Isotope pattern finder module.

6.1.3 Feature alignment

Feature alignment enables alignment of corresponding features across all samples.

Join aligner

This module aligns detected peaks in different samples through a match score. The score is calculated based on the mass and retention time of each peak and ranges of tolerance stipulated in the parameter setup dialog. For more information, see the join aligner module.

6.1.4 Gap-filling

Absence of features in some samples can either reflect the truth - the metabolite is absent in the given sample, or it can be due to data preprocessing.To account for this, gap filling is applied as the next step.

Gap-filling (peak finder)

Gap-filling can be performed on the aligned feature lists to cope with missing features that might be artifacts of the feature-detection process. For more details see the Gap-filling (peak finder) module.

6.1.5 Export

Depending on the downstream analyses, there are several export options which are accessible through **Feature list methods → Export feature list**.

For GNPS-Feature based molecular networking, see GNPS-FBMN

6.1.6 References

Karaman, I.; Climaco Pinto, R.; Graça, G. Chapter Eight - Metabolomics Data Preprocessing: From Raw Data to Features for Statistical Analysis. In Comprehensive Analytical Chemistry; Jaumot, J., Bedia, C., Tauler, R., Eds.; Elsevier, 2018; Vol. 82, pp 197–225.

Pluskal, T.; Korf, A.; Smirnov, A.; Schmid, R.; Fallon, T. R.; Du, X.; Weng, J.-K. CHAPTER 7:Metabolomics Data Analysis Using MZmine. In Processing Metabolomics and Proteomics Data with Open Software; 2020; pp 232–254.

Du, X.; Smirnov, A.; Pluskal, T.; Jia, W.; Sumner, S. Metabolomics Data Preprocessing Using ADAP and MZmine 2. In Computational Methods and Data Analysis for Metabolomics; Li, S., Ed.; Springer US: New York, NY, 2020; pp 25–48.

6.1.7 Page Contributors

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6.2 LC-IMS-MS Workflow Overview

Compared to regular LC-MS, LC-IM-MS data is more complex due to the additional separation dimension. Since some terms might not be straightforward for new users, a basic explanation of IM separation principles and the terminology used within this documentation is provided here.

6.2.1 Supported formats

- Vendor formats:
- .tdf (Native Bruker LC-IMS-MS and MALDI-IMS-MSI format)
- .tsf (Native Bruker MALDI-IMS-MS (single shot) format)
- .mzML
- Created via MSConvert from native Bruker data
- Created via MSConvert from native Waters/Agilent data

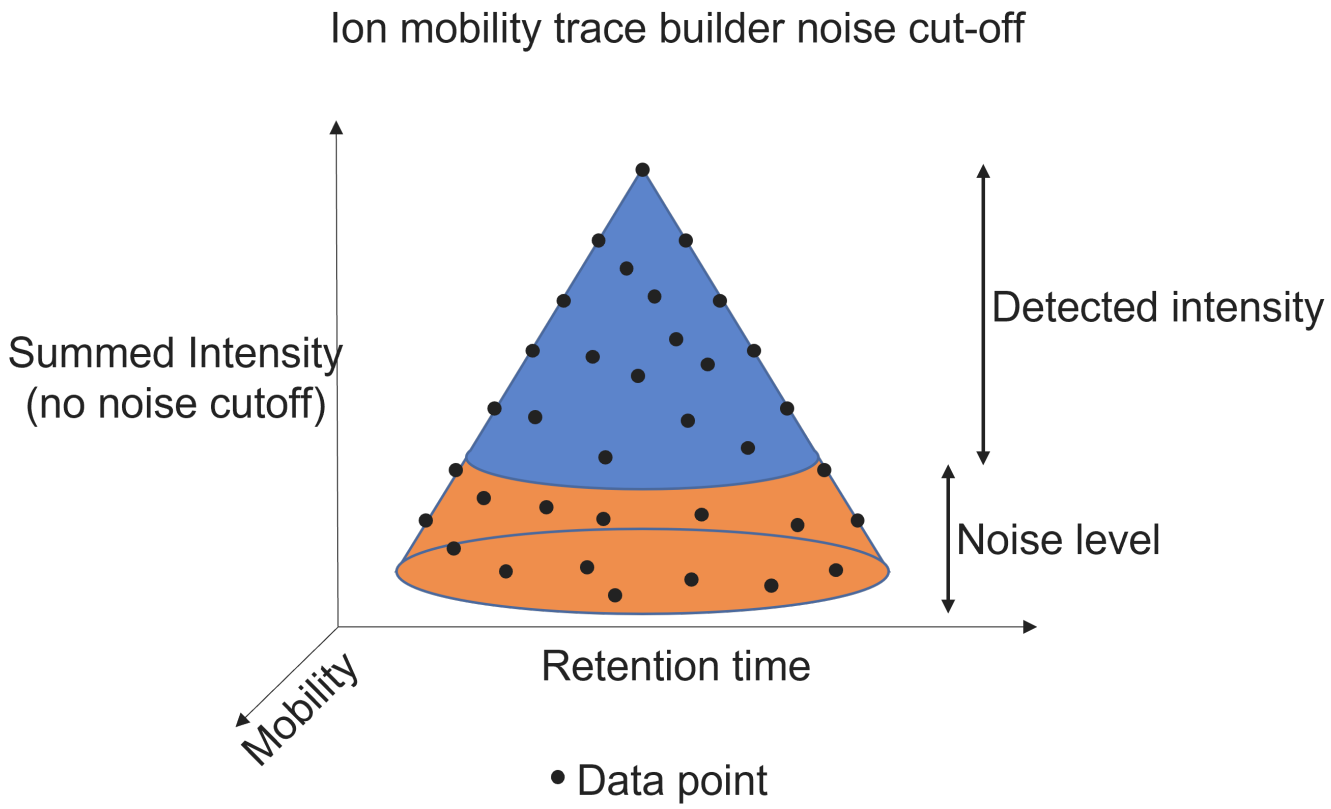
6.2.2 Feature detection workflows

Ion mobility data can be processed in MZmine 3 in two ways. The first few steps are different for the two workflows (see below).

1. LC-IMS-MS workflow via **ADAP Chromatogram builder and IMS expander (recommended)**
2. LC-IMS-MS workflow via **Ion mobility trace builder / Recursive IMS builder**

While these lists might seem fairly similar, there are some differences in the processing approach. The LC-IMS-MS workflow builds ion mobility traces from the data in the mobility scans, whilst the LC-MS workflow builds EICs from the summed frames. For ion mobility data imported from .mzML files, accumulated frame spectra have to be built from the individual mobility scans after mass detection. Since the mass detection impacts the computation of accumulated frame spectra in the same way it would impact the ion mobility trace builder, the differences from this workflow and the ADAP workflow will be negligible.
However, frame spectra for native Bruker .tdf raw data are summed by the vendor library during file import. Here, the frame spectra are generated from the raw data and thus result in higher intensities, since the low abundant data points on the edges of

the mobility and retention time peaks are not cut-off by the mass detection step. (see below)



Therefore, the more low abundant compounds might be detected, if the LC-MS workflow is recommended.

LC-MS workflow (recommended)

LC-IMS-MS data can also be processed via the regular LC-MS modules. If necessary, detected features can be expanded into the mobility dimension.

For this workflow, generation of summed frame spectra via the Mobility scan merging module is a mandatory step, if the data was imported from an .mzML file (automatically generated via native Bruker import).

- Data import
- Mass detection
- Mobility scan merging (mzML data)
- ADAP Chromatogram builder
- Smoothing in retention time dimension (optional)
- Resolving in retention time dimension
- Expanding EICs in mobility dimension
- Smoothing in mobility dimension (optional)
- Resolving in mobility dimension
- Smoothing in rt and mobility dimension (optional)
- Some recognised features might have rather noisy signals (in rt and mobility dimension) after the mobility resolving step. If smoother shapes are required, the smoothing can be reapplied afterwards. In that case, smoothing can be applied to both dimensions at once.

LC-IMS-MS workflow

The LC-IMS-MS workflow will directly build ion mobility traces from the raw data in the mobility scans. This workflow does not necessarily require summed frame spectra. However, if extracted ion chromatograms shall be visualized via the Chromatogram visualizer, the frame intensities are used. In case these are not present, the chromatograms will be blank. Note that feature intensities from the LC-IMS-MS workflow might not exactly match the frame chromatograms due to summing being executed prior to thresholding (for native Bruker data). Furthermore, multiple isomers might hide behind a single chromatographic peak.

- Data import
- Mass detection
- Ion mobility trace builder
- Smoothing in retention time dimension (optional)
- Resolving in retention time dimension
- Smoothing in mobility dimension (optional)
- Resolving in mobility dimension
- Smoothing in rt and mobility dimension (optional)
- Some recognised features might have rather noisy signals (in rt and mobility dimension) after the mobility resolving step. If smoother shapes are required, the smoothing can be reapplied afterwards. In that case, smoothing can be applied to both dimensions at once.

6.2.3 Graphical comparison of LC-MS and LC-IMS-MS data

Data comparison

6.2.4 Page Contributors

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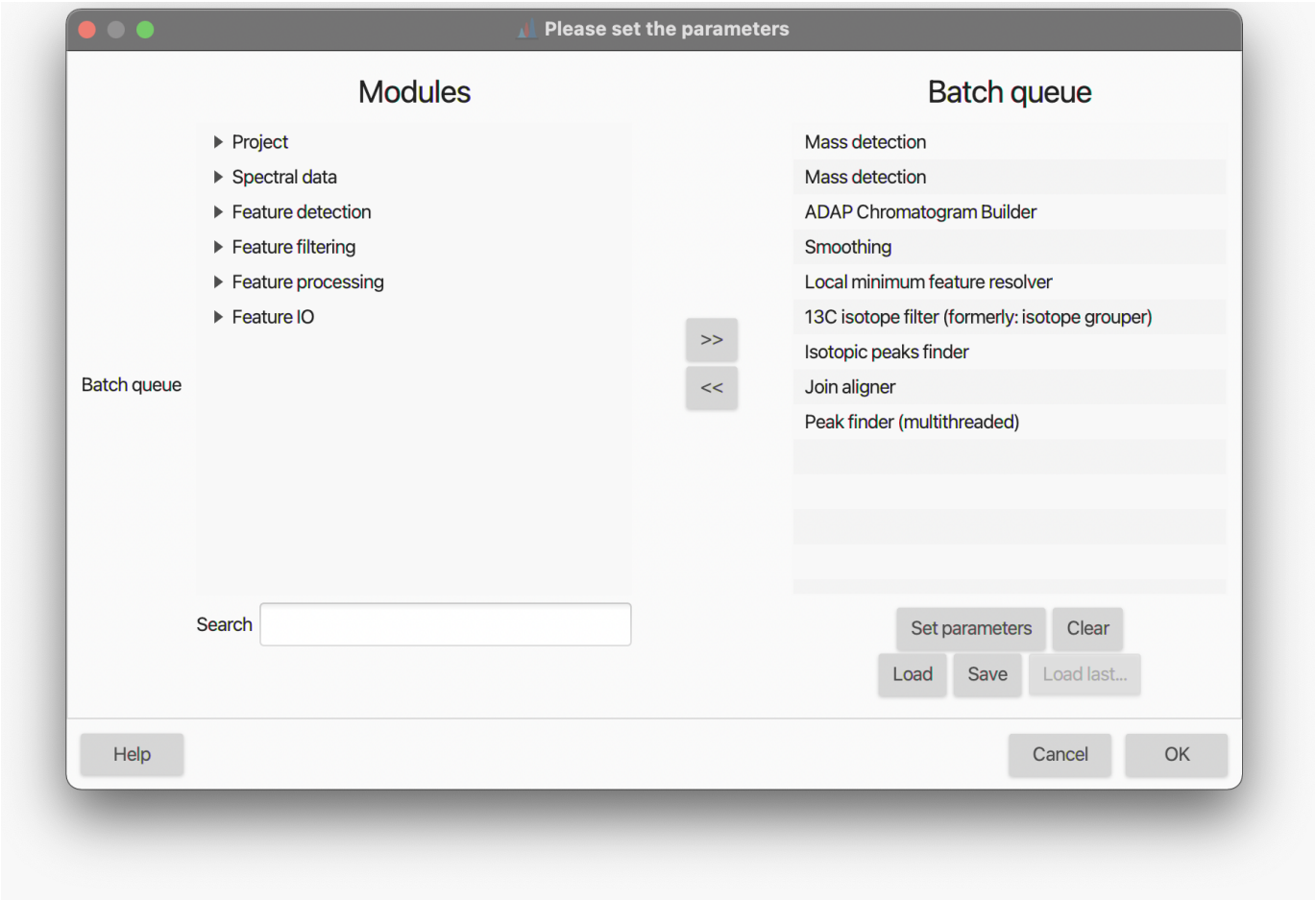
6.3 Batch processing

Besides the interactive GUI, MZmine allows the user to run processing workflows in an automated manner using the "batch mode". Entire processing pipelines (including data import/export) can be run with few clicks, or even through the command-line application. This makes MZmine suitable to be integrated into automated data analysis pipelines (e.g. QC systems).

Batch files (XML format) are essentially lists of tasks run by MZmine one after another. Any of the methods available in MZmine 3 can be included in the batch file.

6.3.1 How to run batch processing

Project → Batch mode



When a new step is added to the queue its parameter setup dialog is shown. The "Set paramaters" button allows the user to modify a step's parameter settings. The "Clear" button removes all steps. The "Load" and "Save" buttons make it possible to read and write batch steps to XML files.

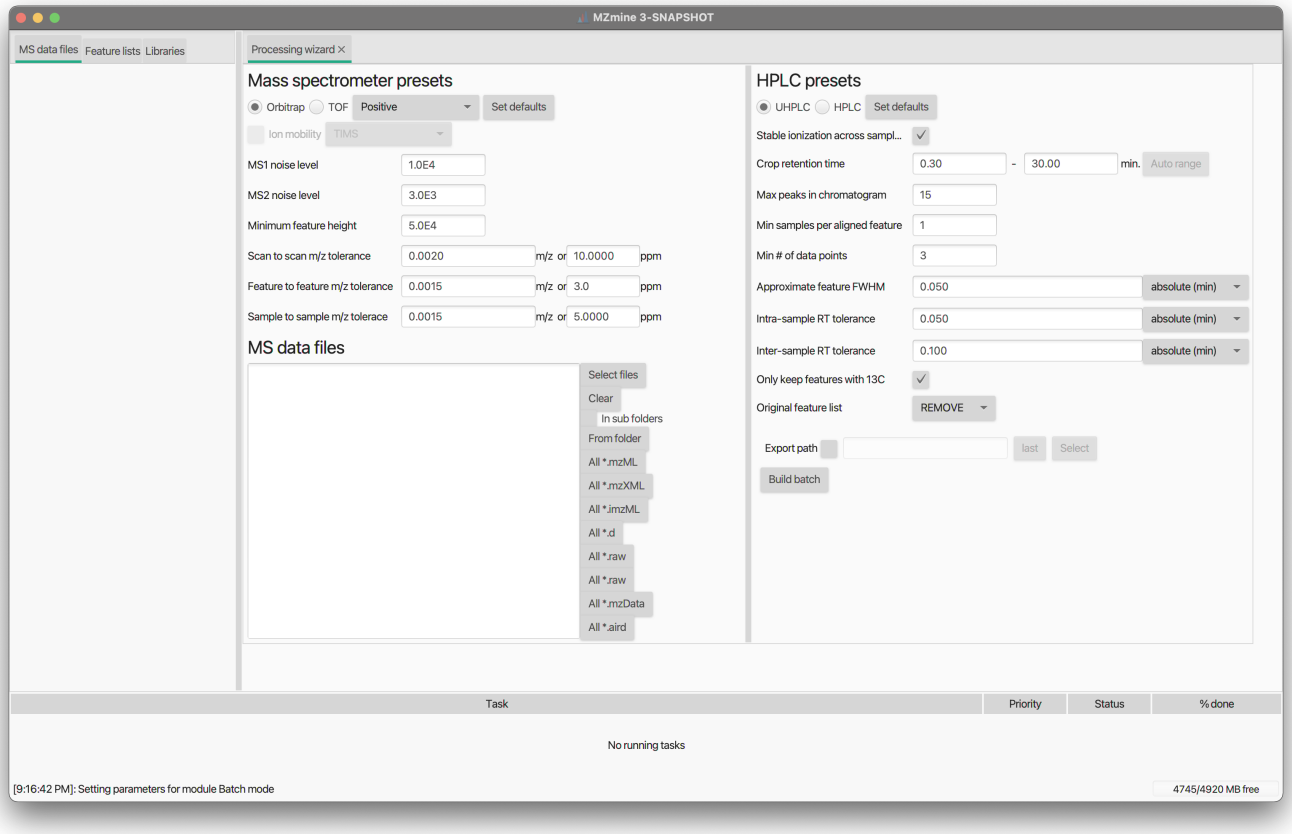
The first step of a batch queue is performed on those raw data files and/or peak lists selected by the user. The remaining steps are performed on the results produced by each preceding step (File/Feature list selection must be set to *Those created by previous batch step*). For example, if the first step of the batch queue is the ADAP chromatogram builder, it will produce peak lists as a result. If the following step were Peak list deconvolution then it will be performed on the peak lists produced by the preceding Chromatogram builder step.

⚠ **Tip** MZmine "remembers" the last settings used.

6.4 Processing wizard

The processing wizard is inteded to quickly set up a general workflow for the processing of untargeted LC-MS and LC-IM-MS data. By clicking the "Set default" button, default settings for mass and feature detection are also provided according to the selected MS type (Orbitrap or TOF) and LC system (UHPLC or HPLC). Once the desired parameters have been set, hit the "Build batch" botton and a pre-populated batch window will open up.

Tools → Processing wizard



Mass spectrometers presets

MS type: When TOF is selected, the "Ion mobility" can be enabled
MS1 and MS2 noise level:
Minimum feature height:
Scan to scan m/z tolerance:
Feature to feature m/z tolerance:

HPLC presets

Stable ionization across samples:
Crop retention time:
Max peaks in chromatogram:
Min samples per aligned feature:
Min # of data points:
Approximate feature FWHM:
Intra-sample RT tolerance:
Inter-sample RT tolerance:
Only keep feature with 13C:

Original feature list:
Export path:

Warning

The default settings were optimized on sample datasets used during the MZmine 3 development. Although probably suitable for many appications, it is strongly recommended not to blindly rely on them. Rather, optimal processing parameters should be chosen based on the LC-(IM)-MS system performance and data acquisition settings.

Last update: August 11, 2022 09:10:52

7. Additional resources

7.1 General terminology

7.1.1 **MS**

Precursor and fragment ions

The **precuror ion** (a.k.a. "parent ion") is the ion that dissociates to a smaller fragment ions in MS/MS experiment.

A **fragment ion** (a.k.a. "daughter ion" or "product ion") is the charged product of an ion dissociation. A fragment ion may be stable or may dissociate further to form other charged fragment ions and neutral species of successively lower mass.

Accurate mass, exact mass and mass accuracy

The **accurate mass** is the experimentally-determined mass of an ion measured with a high-resolution mass spectrometer.

The **exact mass** is the calculated mass of an ion based on its elemental formula, isotopic composition and charge state. While the accurate mass is an experimentally-measured quantity, the exact mass is a theoretically-calculated quantity.

The **mass accuracy** is defined as the difference between the measured value (accurate mass) and the true value (exact mass). It can be expressed either in **absolute (mDa)** or **relative (ppm)** units.

Monoisotopic mass

Exact mass of an ion calculated using the mass of the lightest isotope of each element.

Isotopic pattern

Isotopic (or isotope) pattern describes a set of peaks related to the ions with the same chemical formula but containing different isotopes; e.g. the 16 and 17 mass/charge peaks in a CH4 sample arising from $^{12}CH_4^+$ and $^{13}CH_4^+$ ions.

Mass resolution

Resolution describes an ability of MS method to distinguish two peaks of different mass-to-charge ratios. Can be interchangeably used with mass resolving power

Wikipedia article on MS resolution

Mass resolving power

In a mass spectrum, the observed mass divided by the difference between two masses that can be separated, $m/\Delta m$.

Data acquisition mode

Process of sampling to capture the signals. Different modes have been introduced to better capture signals after LC separation, especially in metabolomics. In MS data can be acquired using three main modes:

- Full scan acquisition mode
- Data-dependent acqusition mode
- Data-independent acquisition mode

Read more: Comparison of data-dependent and data-independent modes

Full scan acquisition mode

In full-scan mode, the mass spectrometer runs on MS1-only mode, and measures m/z values and abundances of all the metabolic features.

Widely used as it allows to capture most of the relevant ions.

Further confirmation of statistically significant features is typically carried out by a separate LC-MS/MS run in a targeted manner.

Data-dependent acqusition mode (DDA)

Mode of the data collection in **tandem mass spectrometry**. In data-dependent acquisition (**DDA**) schemes, the mass spectrometer detects 'suitable' precursor ions in each MS scan and selects them for fragmentation in consecutive MS2 scans.

⚠️ DDA can redundantly identify high-abundance features, while neglecting low-abundance ones.

TopN acquisition scheme

In TopN scheme, the set of N ions is selected for fragmentation by their intensity in the latest MS1 survey scan.

Data-independent acqusition mode (DIA)

DIA can be conducted either by fragmenting all ions that enter the instrument at a given time (called broadband DIA) or by sequentially focusing on a m/z window of precursors and fragmenting all precursors detected within that window.

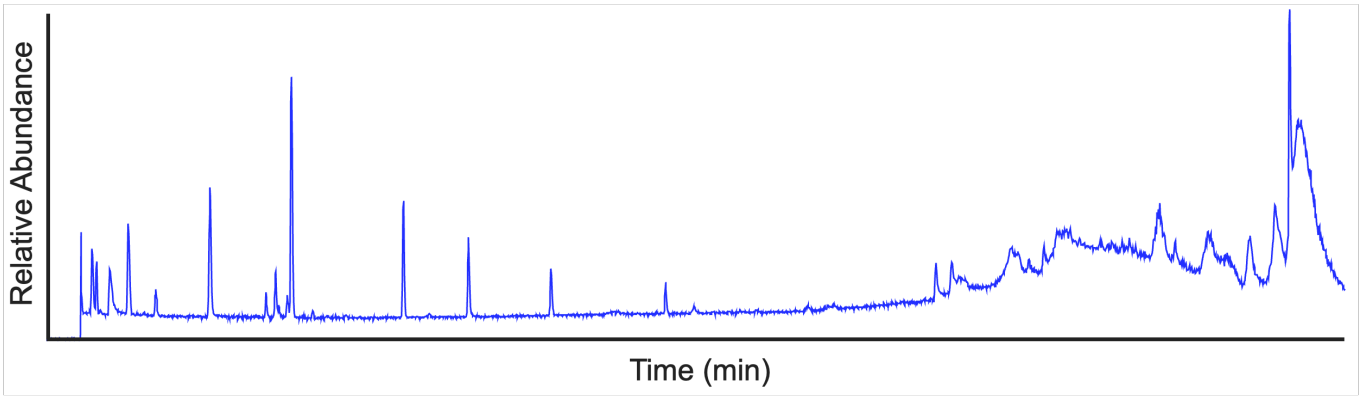
Cycle time acquisition scheme

In cycle time acquistion, a set of precursor ions is selected using m/z values (usually 1.0 to 2.0 m/z range). A full MS/MS fragment ion spectrum is collected for each ion. Cycle time is determined by scan times of all scans in the set.

7.1.2 LC-MS

Total ion current chromatogram

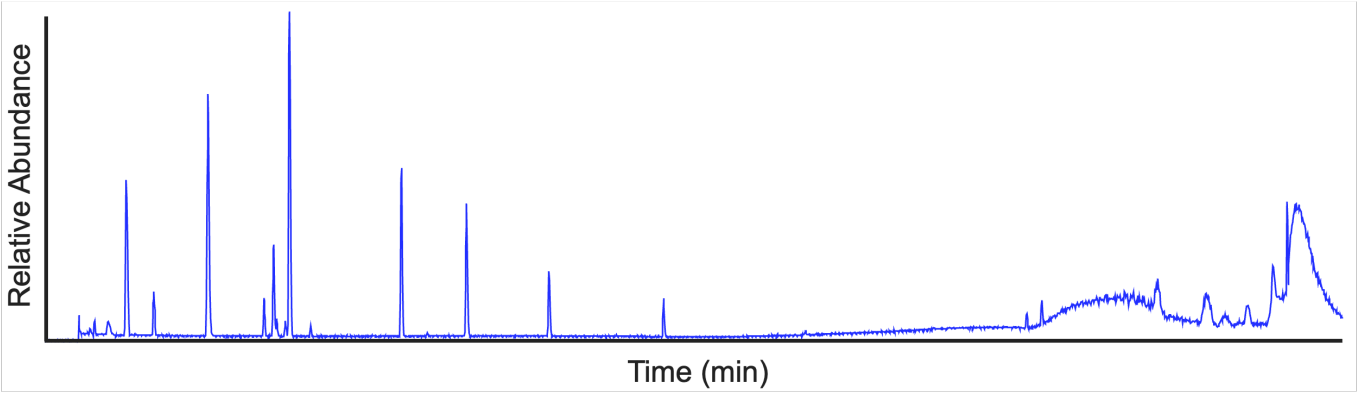
The total ion current (TIC) chromatogram displays the summed signal intensity (y-axis) over the entire m/z range at any one retention time point (x-axis) in the LC-MS run. The following figure shows a TIC chromatogram of a 9-compounds mixture analysed on LC-MS system.



💡 In complex samples, the TIC chromatogram often provides limited information as multiple analytes elute simultaneously, obscuring individual species.

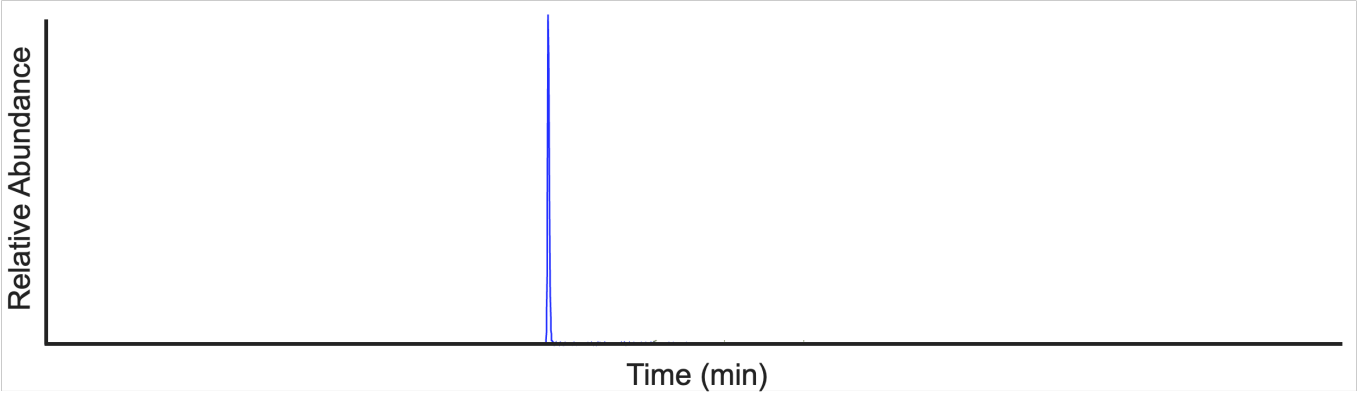
Base peak chromatogram

The base peak chromatogram (BPC) displays the signal intensity of the most intense maass peak in the MS spectra at any one retention time point (x-axis) in the LC-MS run. The following figure shows the same data as above, visualized in BPI mode.



Extracted ion chromatogram

The extracted ion chromatogram (EIC) displays the signal intensity of a specific m/z value, within a defined tolerance (e.g. ± 5 ppm), at any one retention time point in the LC-MS run. The following figure shows the EIC of *m/z* 455.2945 ± 5 ppm (same sample as above).



Chromatographic resolving

Peak overlapping, or co-elution, is a common problem in any chromatographic separation technique. In the case of LC-MS (especially untargeted *omics* analysis), it is virtually impossible to obtain a full baseline separation for the hundreds (or thousands) of analytes eluted through the column. The split of partially-overlapping and shoulder peaks into indivual features is generally referred to as *chromatographic resolving* and is one of the most crucial steps of data processing. TO FINISH.

7.2 MZmine-specific terminology

Masses and Features

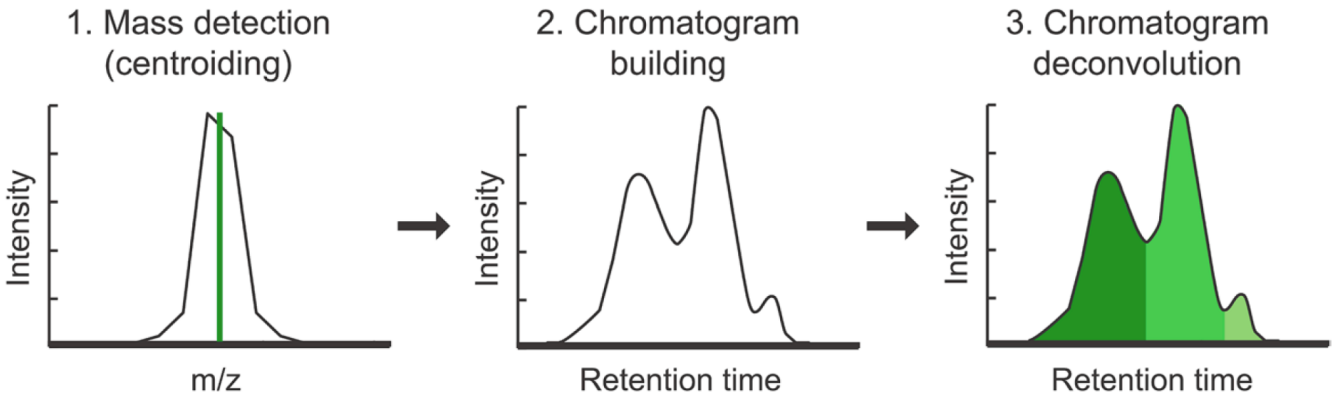
In MS data processing, the term **mass** is normally used to refer to an individual signal in a mass spectrum, which corresponds to an ion detected by the mass spectrometer (see Mass detection).

In LC-MS, a **feature** is defined as a bounded, two-dimensional (*m/z* and RT dimensions) signal characterized by a pair of *m/z* and RT values and associated with the detected signal intensity.

In LC-IM-MS, a **feature** is also characterized by the ion mobility value recorded for the ion (see LC-MS and LC-IMS-MS data comparison.

MZmine 3 provides a selection of different algorithms for LC-(IM)-MS feature detection, depending on the nature of the MS data (*e.g.* mass accuracy and resolution). All the algorithms follow the same logic:

- EICs are constructed starting from each *m/z* value in the mass lists
- Then, EICs are subsequently deconvoluted into individual features (see figure).
- Finally, additional information, such as isotope pattern, adduct type, *etc.* can be assigned to the individual features.



Mass list

In MZmine, we call **mass list** the output of the mass detection module.

A **mass list** is a list of *m/z* values and corresponding signal intensities, found in each mass spectrum (MS or MSn) of each processed raw data file.

Every mass spectrum contained in the raw file is processed individually. The signals exceeding the set noise threshold are included in the mass list. See Mass detection module.

Feature list

In MZmine, **feature lists** are the output of the **feature detection** process (see Masses and features).

The set of detected features in each LC-MS run is stored as a list, hence the name "feature list" (see, for example, ADAP chromatogram builder and Local mimimum resolver for more details). Multiple feature lists can undergo further processing (*e.g.* feature alingment) which results in a table (often referred to as **feature table**) where samples are arranged in columns, features in rows and each entry contains the signal intensity detected for the corresponding feature in the corresponding sample.

Intra and inter-scan tolerances

m/z tolerance is defined as maximum allowed difference between m/z values in order for them to be considered the same. Can be defined as **intra-scan m/z tolerance** for values with one scan (used, *e.g.*, in Mass detection of isotope signals) or **inter-scan m/z tolerance** for values between different scans (in, *e.g.*, ADAP Chromatogram Builder)

Chromatogram resolving

Was referred to as **Deconvolution** in MZmine 2. Process of splitting "imperfect" - overlapping and partially co-eluting - peaks, which are retained as single features, into the separate features.

7.2.1 References

- Pluskal, T., Castillo, S., Villar-Briones, A. & Oresic, M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics* (2010). DOI: 10.1186/1471-2105-11-395
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- Smoluch M., Piechura K. Mass Spectrometry: An Applied Approach, Chapter 3: Basic Definitions (2019). DOI: 10.1002/9781119377368.ch3
- IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. 10.1351/goldbook
- Guo, J., Huan T. Evaluation of significant features discovered from different data acquisition modes in mass spectrometry-based untargeted metabolomics. *Analytica Chimica Acta* (2020). DOI: 10.1016/j.aca.2020.08.065

Last update: September 12, 2022 10:42:38

7.3 Ion mobility spectrometry terminology

7.3.1 **Background**

Ion-mobility mass-spectrometry, here simply referred to as **ion-mobility (IM)**, is an analytical technique where ions are separated through a gas-filled mobility cell prior to the MS acquisition.

In classic **drift tube ion mobility (DTIM)**, ions migrate through an inert buffer gas under the influence of a weak electric field. Ions drift with different **velocity** based on their interaction with the buffer gas, which allows for the separation of different shaped molecules. Modern devices are able to perform IM separation on a millisecond timescale, typically within 10 to 100 ms.

As larger ions have more collisions with the gas, they are more strongly retarded than their smaller counterparts. Thus, smaller ions, having a smaller cross section, arrive earlier at the detector than ions with a larger collisional cross section (CCS).

The **ion mobility** \(K\) is then defined as the ratio of the analyte's steady-state net drift velocity to the applied electric field, and it is convention to calculate the reduced ion mobility \(K_0\) at standard pressure and standard temperature. This value is often reported as the inverse reduced ion mobility \(1/K_0\).

From practical point of view, IM nicely fits in-between LC separation (~seconds timescale) and MS detection of TOF instruments (~microseconds timescale). This allows LC-IM-MS instruments to acquire several MS spectra during each accumulation, without incurring sensitivity loss.

For example, assuming a typical 100 µs MS-acquisition time of TOF analyzers, around 1000 spectra can be recorded within 100 ms of IM separation. Therefore, as opposed to LC-MS, multiple MS (or MS2) spectra are associated to each RT in LC-IM-MS data.

A more detailed explanation of LC-MS and LC-IMS-MS raw data structure is provided here.

Visual explanation of IMS by Waters

Trapped ion mobility spectrometry (TIMS)

Trapped ion mobility spectrometry (TIMS) reverses the concept of traditional drift tube IM. Rather than moving ions through a stationary gas, TIMS holds ions stationary against a **moving gas** and then releases them according to their mobility.

TIMS has the advantage that the physical dimension of the analyzer can be smaller, whereas the analytical column of gas – the column that flows past during the course of an analysis – can be large and user defined. This can lead to increased method sensitivity.

Bruker's TIMS TOF Video

Time-dispersive ion mobility spectrometry (DTIMS and TWIMS)

Time-dispersve IM devices include "traditional" **drift tube (DTIMS)** and **travelling-wave (TWIMS)** devices.

In TWIMS, ions are propelled through a gas-filled stacked ring ion guide with the help of travelling voltage waves.

Higher mobility ions undergo less ‘roll over’ events on the waves than the lower mobility ions. As the waves pass along the device, ions can ‘surf’ on the wave front for a period of time before being overtaken by the wave. Usage of travelling waves makes possible to increase sensitivity, selectivity, and speed of the method.

For more information, see *Fundamentals of Traveling Wave Ion Mobility Spectrometry* DOI: 10.1021/ac8016295

7.3.2 Terminology

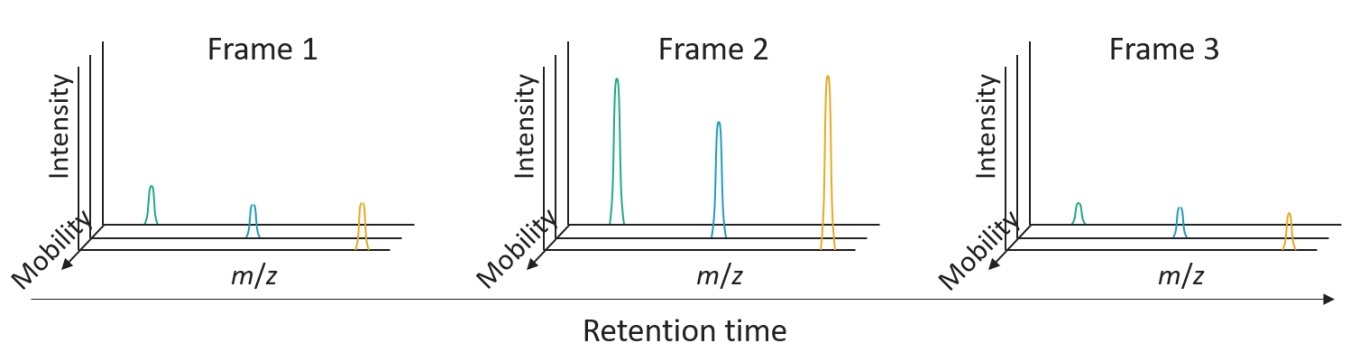
Accumulations, Mobility Scans and Frames

Altough mainly used for TIMS, the term **"accumulation"** refers to the pack of ions gathered at the head of the IM device prior to the release and separation in the IM cell.

As explained above, since the accumulation-separation cycle typically last ~100 ms, multiple MS spectra (referred to as **"mobility scans"** in MZmine) are acquired during each cycle.

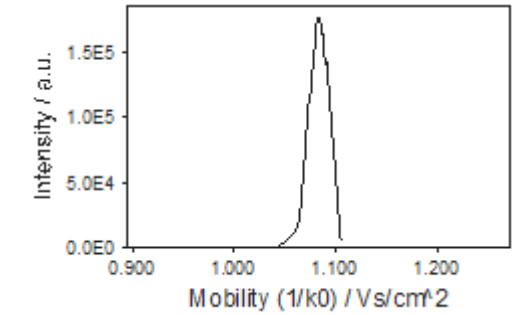
Frame is the set of **mobility scans** collected during each IM separation. A frame can be seen as the IM separation of a single accumulation, along which multiple MS spectra are collected. Several frames are contained within one LC peak. Thus, the **frame number** are a natural unit to measure chromatographic RT.

See here for more details.



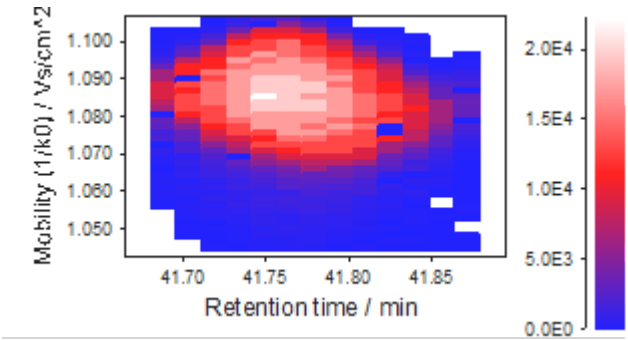
Mobilograms

A "*mobilogram*" represents the intensity of an m/z or m/z range along the mobility axis. A *mobilogram* may be build from multiple frames and summed or built from a single frame.



Ion mobility trace

An "*ion mobility trace*" basically represents a mobility resolved extracted ion chromatogram (EIC).



Collisional Cross Section

Collision cross section (CCS) can be defined as area of interaction between an individual ion and gas molecule. CCS depends on ion's size, shape, and charge. IM-derived CCS values can be used as an additional molecular descriptor to support the compound unknown identification process.

7.3.3 References

- Meier, F., Brunner, A.D., Koch, S., Cox, J., Räther, O., Mann, M. Online Parallel Accumulation–Serial Fragmentation (PASEF) with a Novel Trapped Ion Mobility Mass Spectrometer. *Molecular & Cellular Proteomics* (2018). DOI: 10.1074/mcp.TIR118.000900
- Paglia, G. et al. Ion Mobility Derived Collision Cross Sections to Support Metabolomics Applications. *Anal. Chem.* (2014). DOI: 10.1021/ac500405x

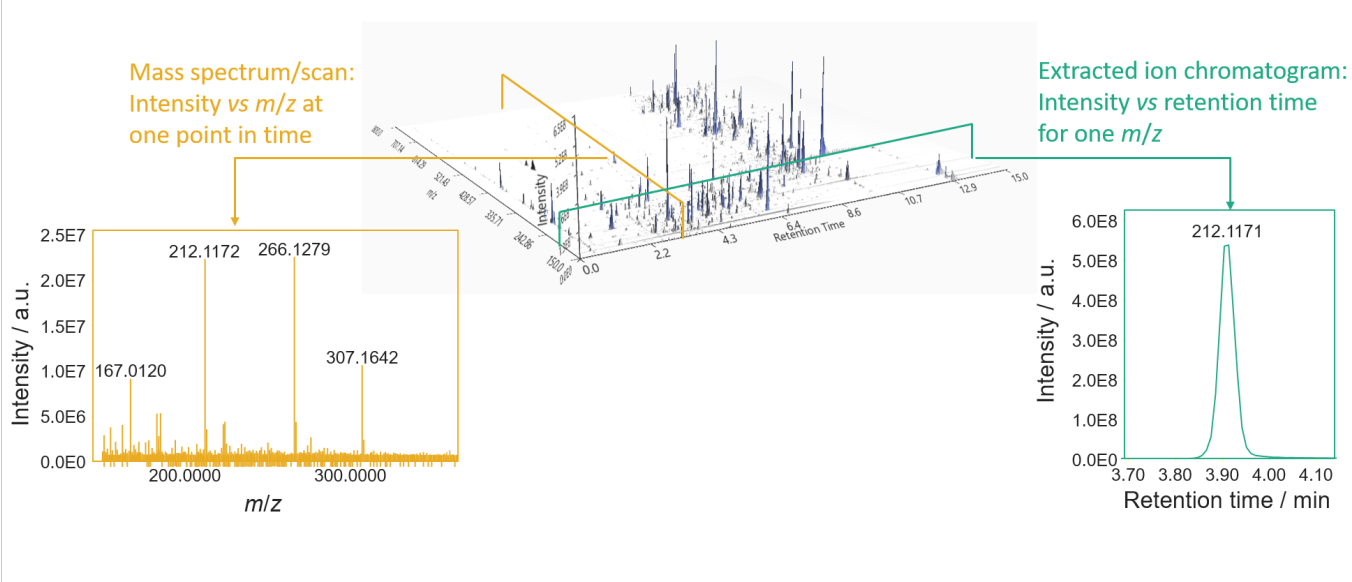
Last update: August 15, 2022 14:16:09

7.4 Graphical comparison of LC-MS and LC-IMS-MS data

Classic LC-MS data consists of three dimensions:

- m/z,
- intensity,
- and retention time.

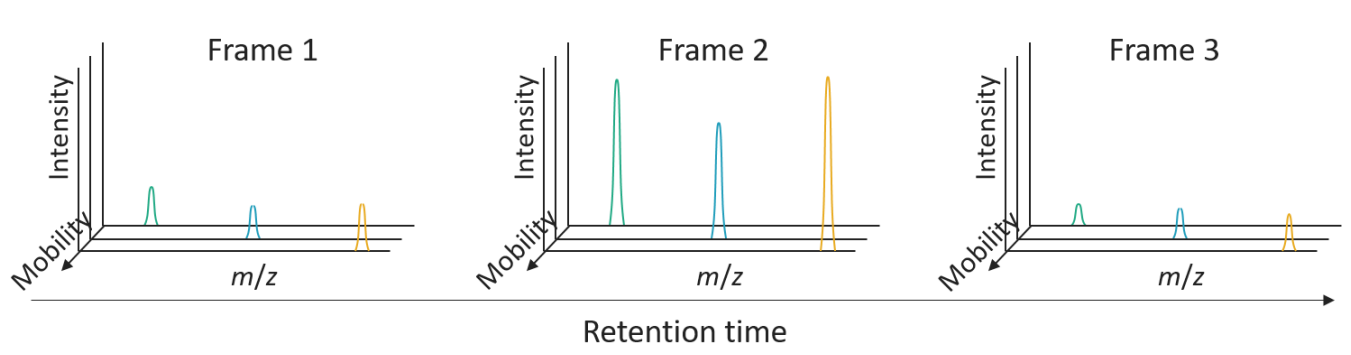
At every retention time, a whole mass spectrum is acquired (yellow). Putting all scans together creates a three-dimensional plane. By slicing the three-dimensional data at a single m/z value (+- a value of tolerance), **EICs** can be created (green).



Ion mobility resolved data, on the other hand, consists of a three-dimensional data plane at each retention time. The three dimensions being:

- m/z,
- intensity,
- and mobility (as drift time (ms) or inverse reduced mobility \(1/K_0 [Vs/(cm^2)]\)).

The 3D projection of regular LC-MS data can be created by summing all mobility scans of a frame to create a frame spectrum. (see Mobility scan merging)



Last update: August 15, 2022 13:17:04

7.5 Kendrick mass defect

Difference between the exact Kendrick mass and the nominal Kendrick mass is called *Kendrick mass defect*.

More information on Kendrick mass can be found here.

7.5.1 Parameters

KENDRICK MASS DEFECT

Set permissible range of Kendrick mass defect per row.

KENDRICK MASS BASE

Enter molecular formula of the repeating unit used as Kendrick mass base (e.g. CH2)

SHIFT

Set shift value

CHARGE

Set charge value

DIVISOR

Set divisor value

USE REMAINDER OF KENDRICK MASS

Select check box to use RKM instead of KMD

Last update: August 22, 2022 16:23:26

7.6 Spectral similarity measures

7.6.1 **Weighted cosine spectral similarity**

The most common spectral similarity measure for library search is the **weighted cosine similarity**. Generally,the cosine similarity is calculated as following:

\[similarity = cos\theta = (u \cdot v) / (\sqrt{(sum(u^2))} * \sqrt{(sum(v^2))})\]

In case of weighted cosine similarity, the previous formula is modified according to the weighting function. In case of weighting function in form \(m/z^a*{Intensity}^b\) (where a, b are weights) the weighted cosine similarity is calculated as follows:

\[weighted\;similarity = (u' \cdot v') / (\sqrt{(sum(u^2))} * \sqrt{(sum(v^2))})\]
\[u' = sum(u_i) = sum(m{u_i}^a*I{u_i}^b)\]
\[v' = sum(v_i) = sum(m_{v_i}^a*I_{v_i}^b)\]

where u and v are the aligned vectors of the two spectra.

It is used to determine the similarity between two spectra (usually library and query spectra). Both spectra are turned into vectors and cosine similarity is calculated by division of vectors dot product over cosine value of the angle between them.

7.6.2 **Composite weighted cosine spectral similarity (identity)**

This similarity measure can be especially useful for very reproducible generation of spectra (GC-EI-MS). This measure is modified by a ratio based on the relative intensities of adjacent m/z signals in the two spectra.

Composite weighted cosine similarity is calculated as follows:

\[composite\;similarity = \frac{N*{cosine\;similarity}+overlap*{ratio\;factor}}{N+overlap}\]
\[ratio\;factor=\frac{min(r_{lib},r_{query})}{max(r_{lib},r_{query})}\]
\[r_{lib}=I_i(lib)/I_{i-1}(lib);\;r_{query}=I_i(query)/I_{i-1}(query)\]

where \(N\) - number of signals in a query spectrum, cosine similarity is calculated as described previously, overlap - number of matching signals in query and library spectra, \(ratio\;factor\) - relative intensities ratio, \(r_{lib}\) - relative ratio of adjacent signals in a library spectrum, \(r_{query}\) - relative ratio of adjacent signals in a query spectrum.

It is used to determine the similarity between two spectra (usually library and query spectra).

7.6.3 **Parameters**

WEIGHTS

For calculating the cosine similarity, different weighting strategies for m/z and signal intensities can be applied.

Several weighting schemes are available:

- None \(m/z^0*I^1\) (weighting only by intensities)
- SQRT \(m/z^0*I^1\) (weighting only by intensities)
- MassBank \(m/z^2*\sqrt{I}\)
- NIST11 (LC) \(m/z^{1.3}*I^{0.53}\)
- NIST (GC) \(m/z^3*I^{0.6}\)

Choice of the similarity measure depends on your data, and intensity-based schemes might work better on homogenous datasets. However, recommended approach for choosing the weighting scheme would be trial-and-error.

MINIMUM COSINE SIMILARITY

This option defines the minimum accepted similarity score that is taken into account for annotation. The similarity score depends on the data handling of unmatched signals.

HANDLE UNMATCHED SIGNALS

Signals that only occur within one scan (query OR library entry) can be handled in different ways to affect the cosine similarity and controlling the quality of matching results.

- KEEP ALL AND MATCH TO ZERO (default)

  This is the standard conservative approach where all unmatched signals weigh negatively on the score. It is used for both GC-EI-MS and MS^2 spectra.

- REMOVE ALL

  The opposite option that discards all unaligned signals, which increases the similarity score artificially.

  This option is only feasible if both the library and query spectrum are considered to be complex mixtures. Therefore, the next two options are more conservative.

- KEEP LIBRARY SIGNALS

  Results in discarding all unaligned signals of the query scan, whereas all unaligned library signals are matched to zero, setting the library spectrum as the ground truth.

  Here, the negative impact of contaminating signals in the query scans are reduced. This might be helpful for mixed spectra of multiple compounds, especially during imaging techniques without any further separation or all ion fragmentation/data independent fragmentation workflows.

- KEEP EXPERIMENTAL SIGNALS

  Results in discarding all unaligned signals of the library scan, whereas all unaligned query signals are matched to zero.

  Has reversed effects compared to the previous option.

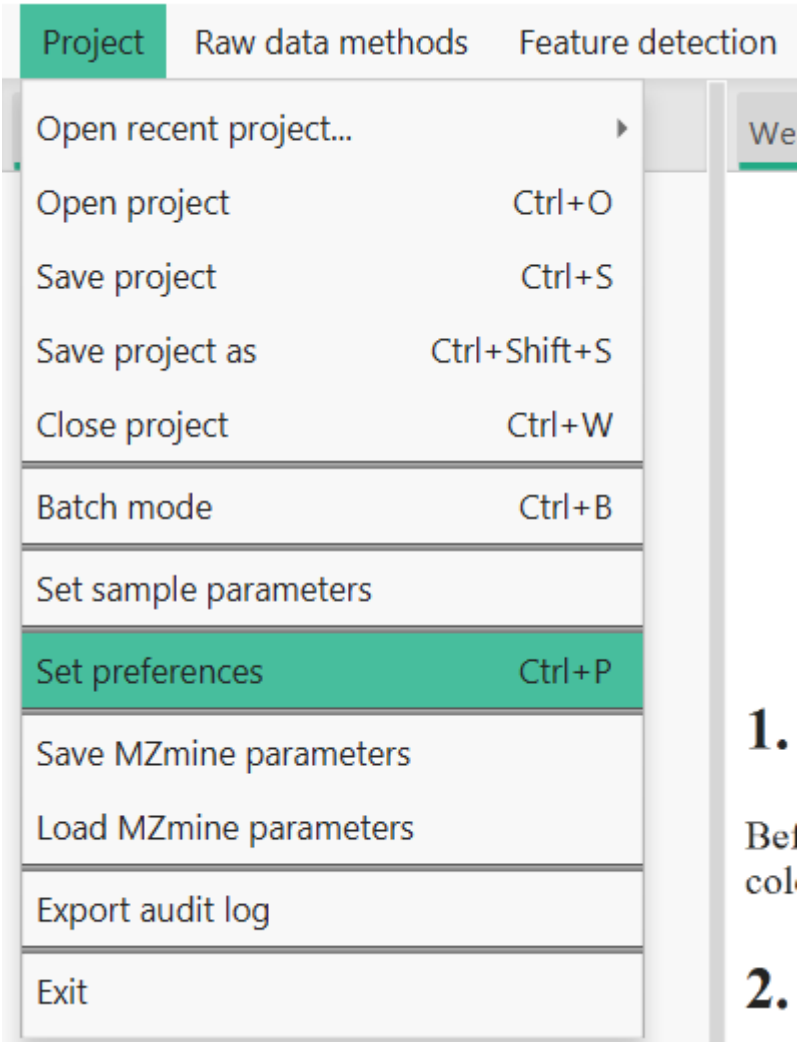
Last update: September 23, 2022 17:08:14

8. Performance options

This section contains information on how to tune MZmine 3 for different systems.

8.1 Preferences

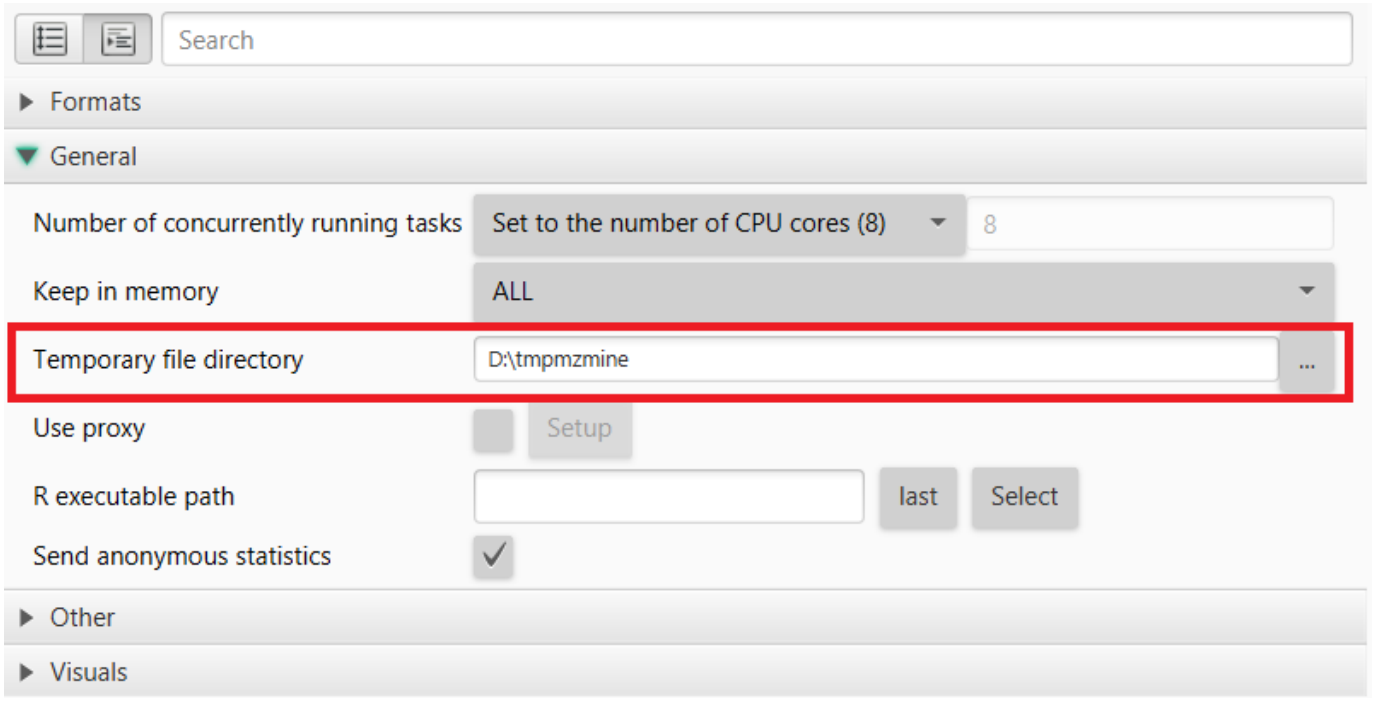
The preferences can be changed in MZmine's graphical user interface by accessing *File/Set preferences* from the menu. The choices will be stored in a (hidden) *.mzmine3.conf* file in the user's home directory (Windows: *C:\Users\USERNAME*) once MZmine is closed.



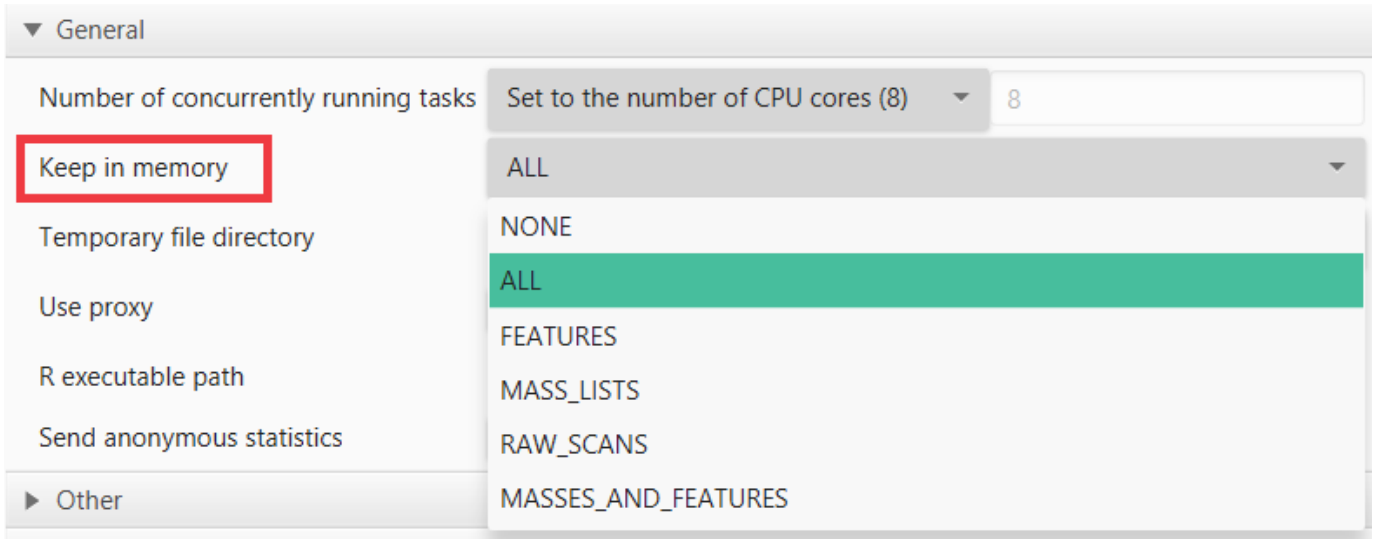
8.1.1 Temporary files

MZmine will create multiple temporary files at various times of the processing stage, e.g., when importing spectral data, running mass detection, or creating feature lists. These files will be stored in a folder that can be specified in the preferences.

We recommend putting this folder on an SSD drive, ideally an M.2 for the best performance. The temporary files will be deleted when MZmine is closed (Mac & Linux) or when a new session is started (Windows).



8.1.2 Memory options



The parameter **Keep in Memory** defines what data is kept in memory (RAM) or otherwise memory mapped to the temp directory.

- Generally this setting should be *none* (**default**).
- If memory is no issue this option might be set to *all* process all spectral and feature data in memory.
- The option *masses_features* keeps centroid mass lists and features in memory while memory mapping raw spectral data.
- The option *mass_lists* will keep only mass lists in RAM, while memory mapping the raw spectral data and features.

8.2 Logs

Currently, the logs are written to an *mzmine_0_0.log* file in the user's home directory. Please submit your log files together with any issues on **GitHub**.

8.3 Maximum memory

The maximum Java heap size (the main part of the RAM available to MZmine) is set to 80%. This is usually a good value, considering that MZmine and its Java Virtual Machine (JVM) will use memory extending over this 80% threshold for specific tasks. There is one way to change the maximum heap size before starting MZmine, however, it requires administrator access.

Find the **MZmine/app/MZmine.cfg** file in the MZmine install directory or portable version. Under Window, this file is write protected, which needs to be changed under **File/Properties/Security/** select Users and click Edit to grant write access. Now change the *MaxRAMPercentage* to grant more RAM.

```
java-options=-XX:InitialRAMPercentage=10
java-options=-XX:MaxRAMPercentage=80
```

Last update: August 14, 2022 10:49:14

9. Command-line arguments

Command-line arguments offer a variety of options that generally override the corresponding parameters in the preferences.

Windows

An easy way to start MZmine with arguments is to create a shortcut to the MZmine.exe, right-click, and define the target with additional arguments. This example runs MZMine in batch mode (headless), imports the specified batch file, overrides the memory management to **none** (which is the default), effectively using memory mapping to store and access spectral, centroid, and feature data from temporary files stored in the defined temp directory. By leaving out the *memory* or *temp* arguments, the values stored in the current *preferences* file will be used, or the default values if no *preferences* file was found.

Start MZmine batch with memory mapping (DEFAULT**)**

```
"C:\Program Files\MZmine\MZmine.exe" -batch "D:\batch\my_batch_file.xml" -memory none -temp "D:\tmpmzmine"
```

Start MZmine batch on machines with enough memory (RAM) with -memory all

```
"C:\Program Files\MZmine\MZmine.exe" -batch "D:\batch\my_batch_file.xml" -memory all -temp "D:\tmpmzmine"
```

9.0.1 Argument table

Argument	Options (default)	Description
-batch	a path, e.g. "D:\batch.xml"	Path to batch file
-memory	**none**, all, features, centroids, raw, masses_features	Defines what data is kept in memory (RAM) or otherwise memory mapped to the temp directory. Generally this setting should be *none*. If memory is no issue this option might be set to *all* process all spectral and feature data in memory. The option *masses_features* keeps centroid mass lists and features in memory while memory mapping raw spectral data.
-temp	a path, e.g., "-temp "D:\tmpmzmine\"	The defined directory should be on a fast drive (usually SSD > HDD > network drive) with enough free space. Local drives are usually preferred. MZmine uses memory mapping to efficiently store and access spectral and feature data. This can lead to a considerable temporary consumption of disk space. Make sure that the selected drive has enough space (maybe 20 GB + 1 GB/10 files; generously over estimated).

Last update: April 8, 2022 08:12:41

10. Contribute

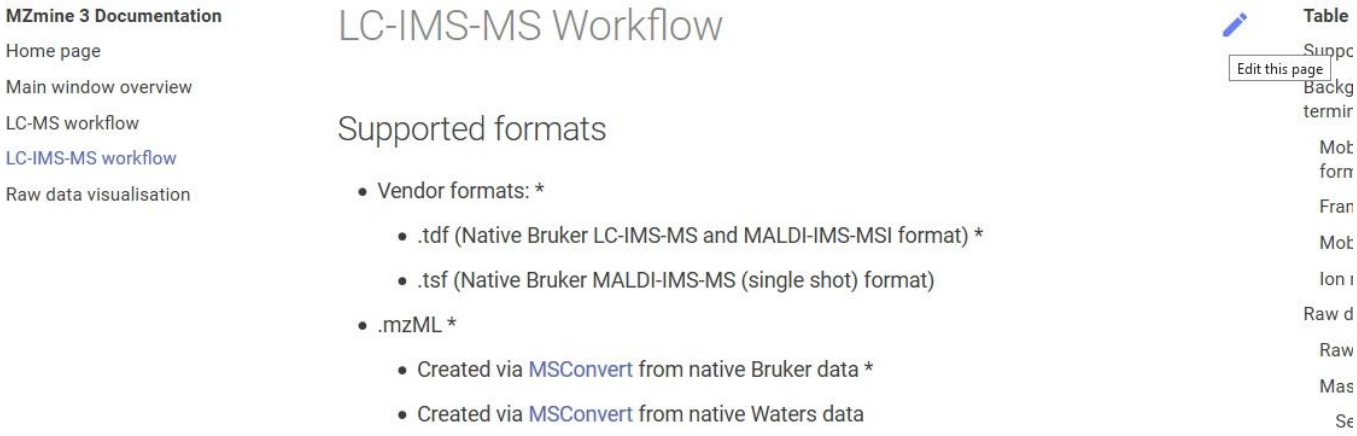
10.1 How to contribute

10.1.1 Contribute to the MZmine documentation

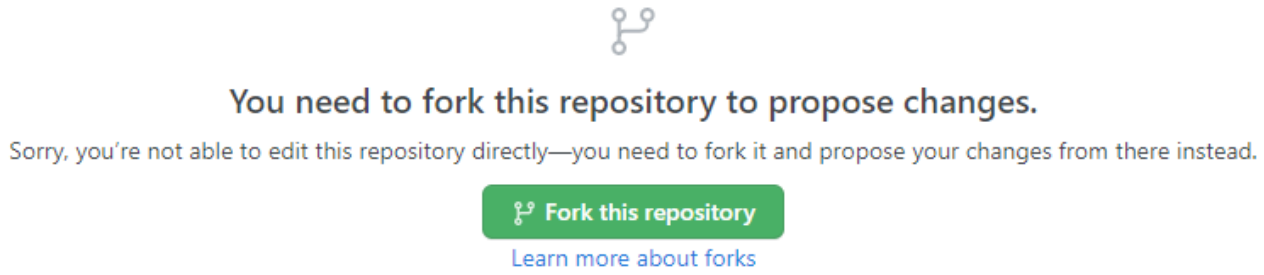
1. Make a GitHub Account

You'll need to make a GitHub Account.

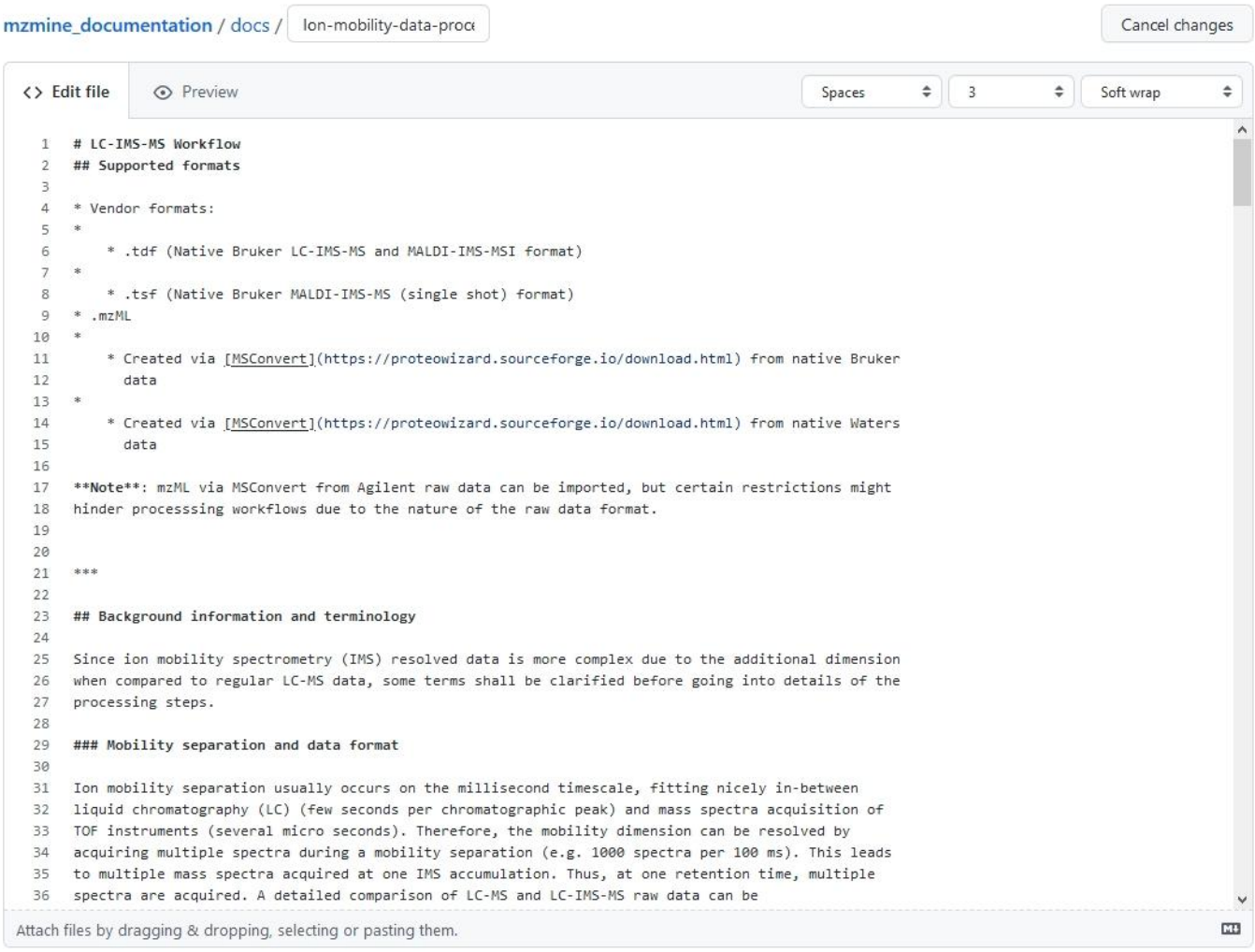
2. Click Edit Button on Page You Want to Edit



3. Fork the Repository When Prompted (only the first time)

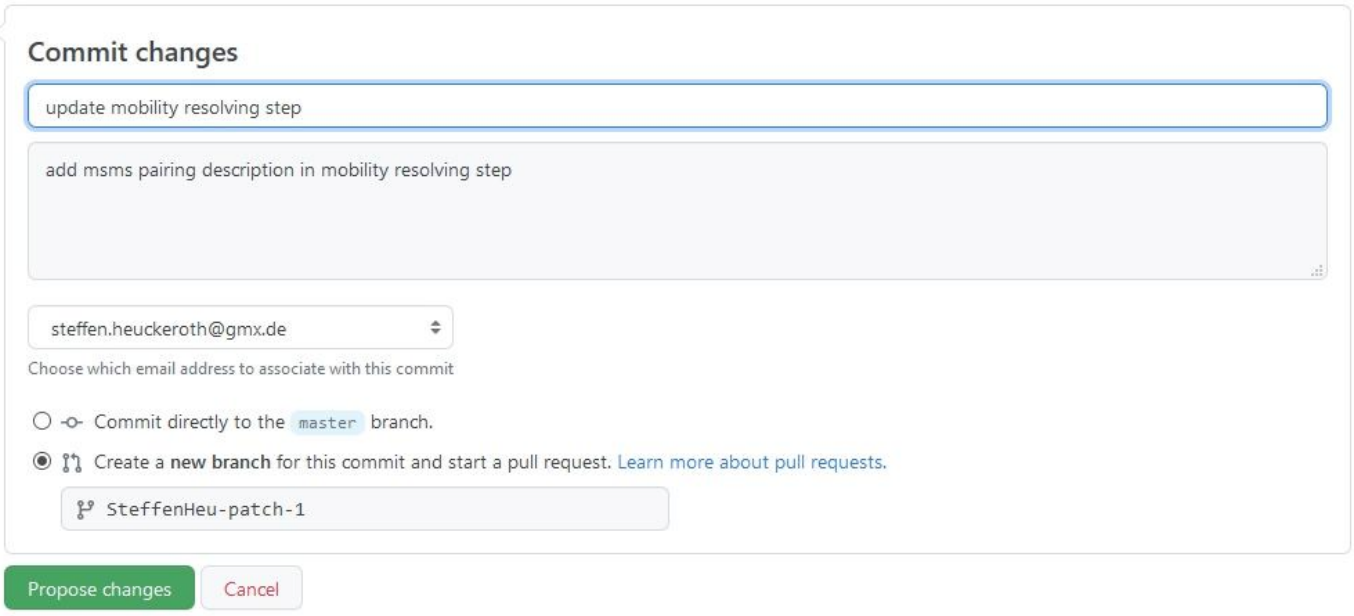


4. Make the Edits in MarkDown

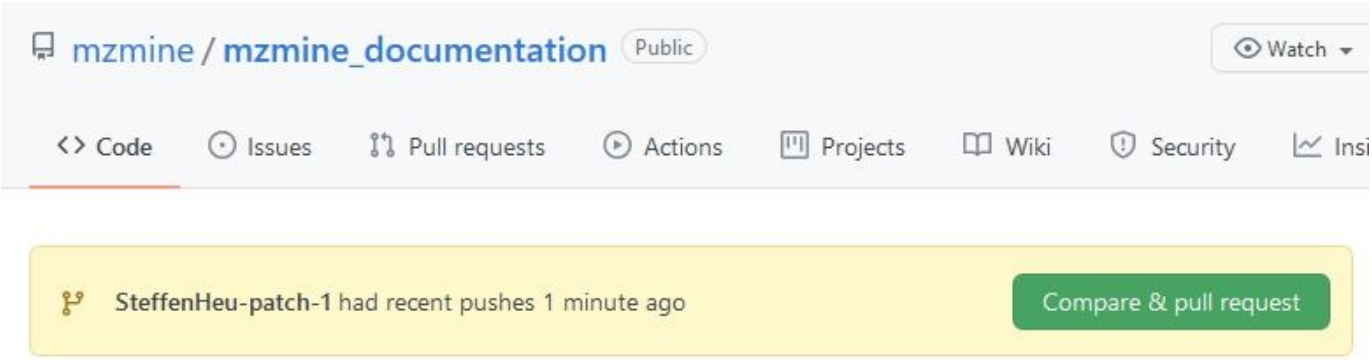


5. Propose Changes

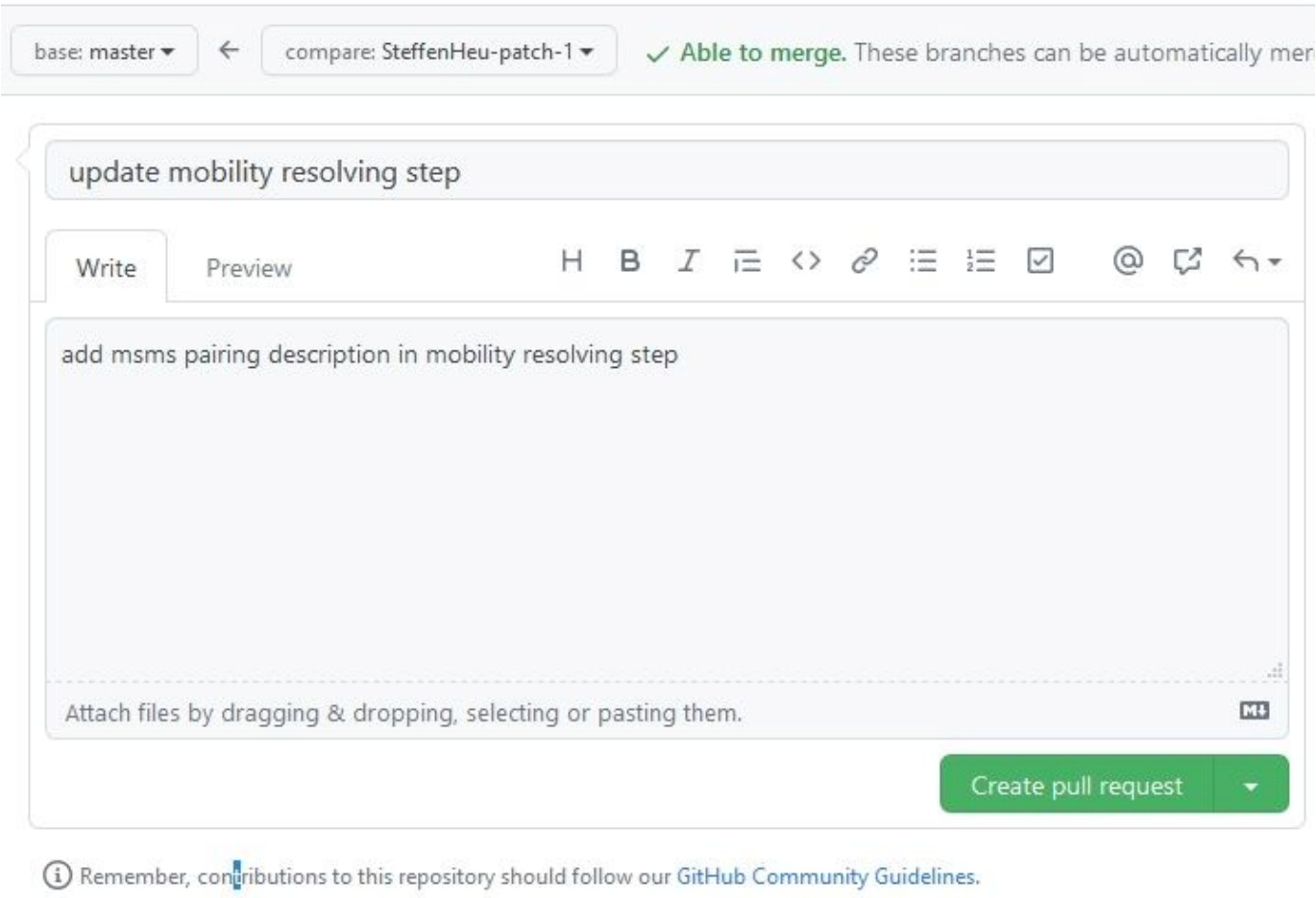
Please describe the change you are making.



6. Create Pull Request



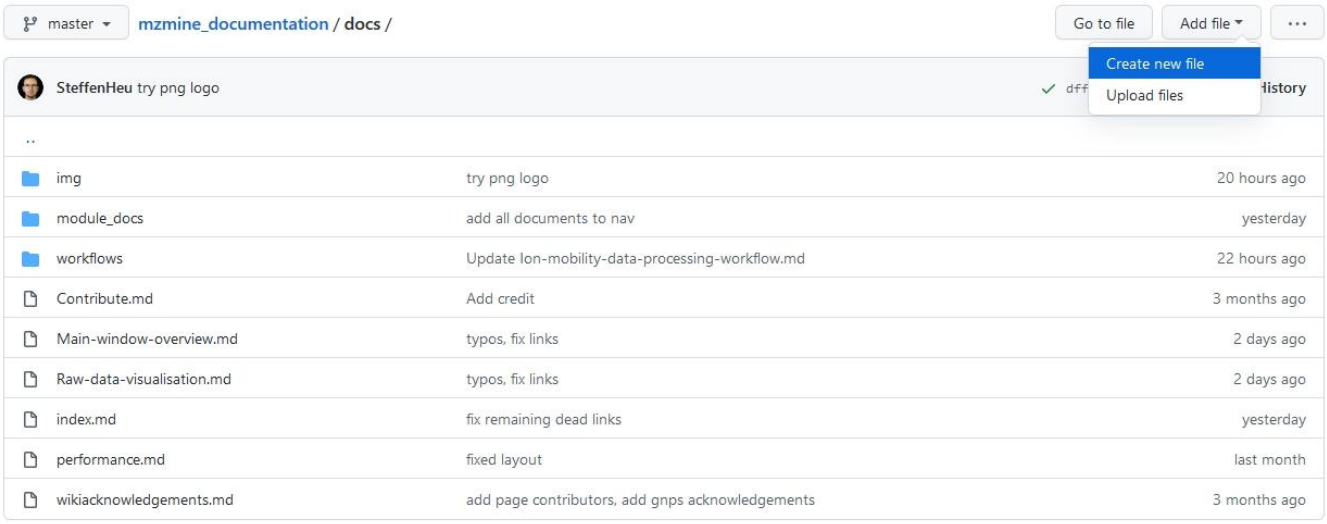
7. Finalize Pull Request with Description



10.1.2 Creating a new page

Follow steps 1 - 3.

Navigate to mzmine_documentation/docs in your fork and create a new file



Follow steps 4 - 7.

10.1.3 Page Contributors

SteffenHeu

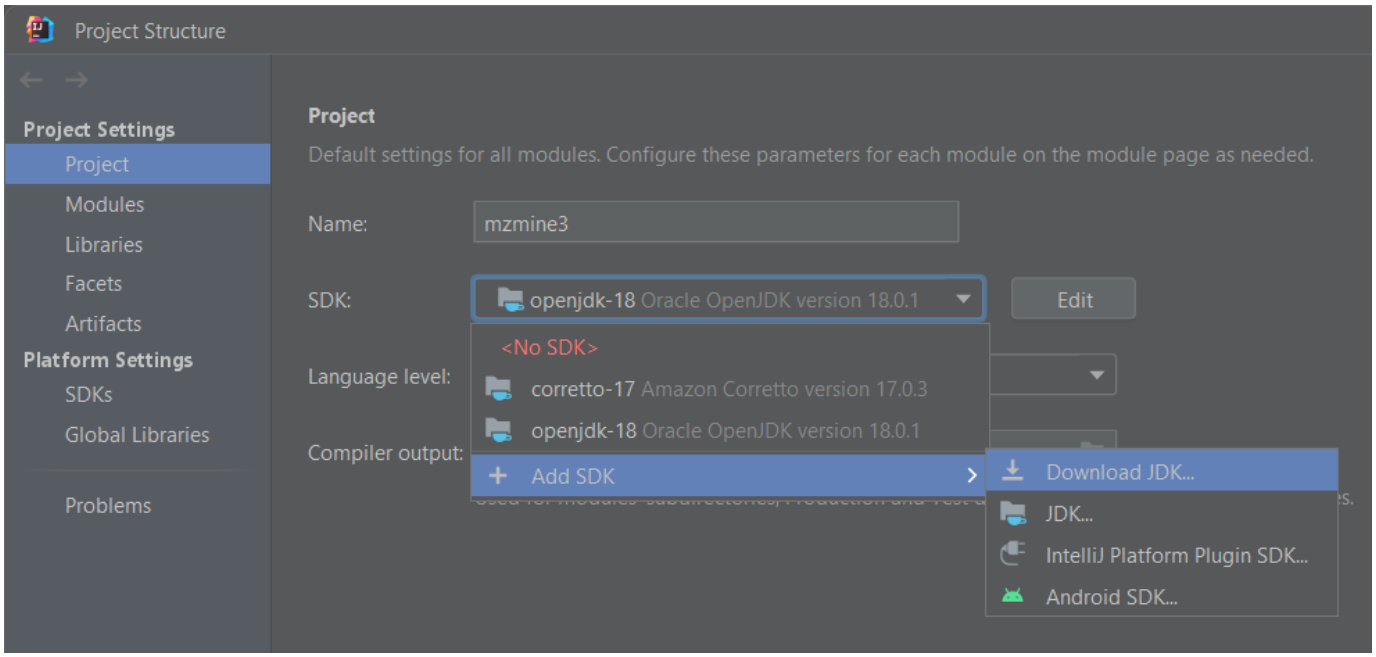
This page was adapted from the GNPS documentation.

Last update: April 5, 2022 13:22:07

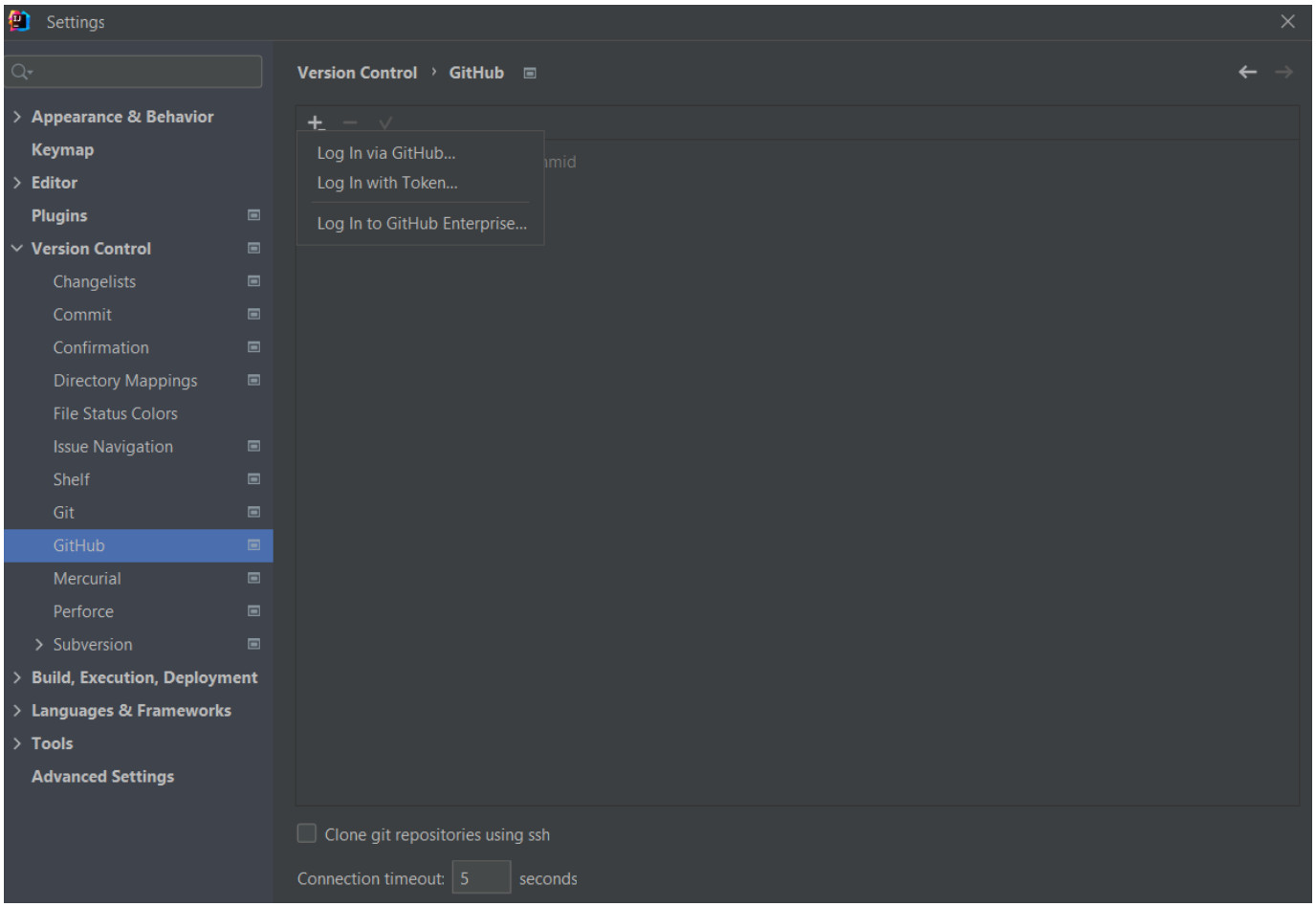
10.2 Development in IntelliJ

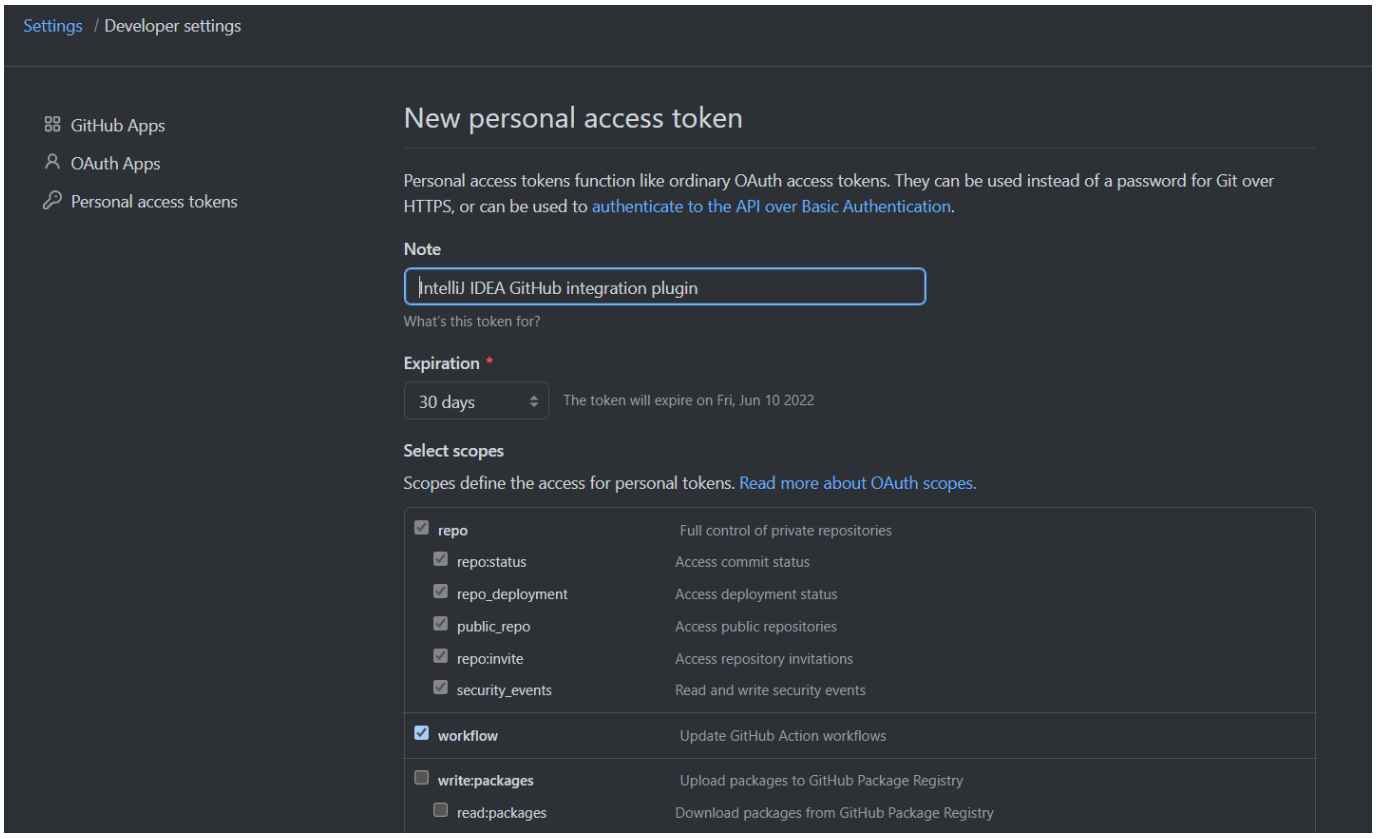
10.2.1 Set up

1. Fork the mzmine (https://github.com/mzmine/mzmine3) GitHub repository (needs free GitHub account) (See https://help.github.com/en/github/getting-started-with-github/fork-a-repo)
2. Download & Install IntelliJ IDEA (there is a free educational license for students and teachers) from https://www.jetbrains.com/idea/download/
3. Download & Install the current JDK. We recommend the OpenJDK. However, you can also use any other distribution, e.g., the Oracle JDK. This can be done from within IntelliJ. Open *File/Project Structure* (CTRL+ALT+SHIFT+S) and select SDKs and add the latest JDK with the +button:

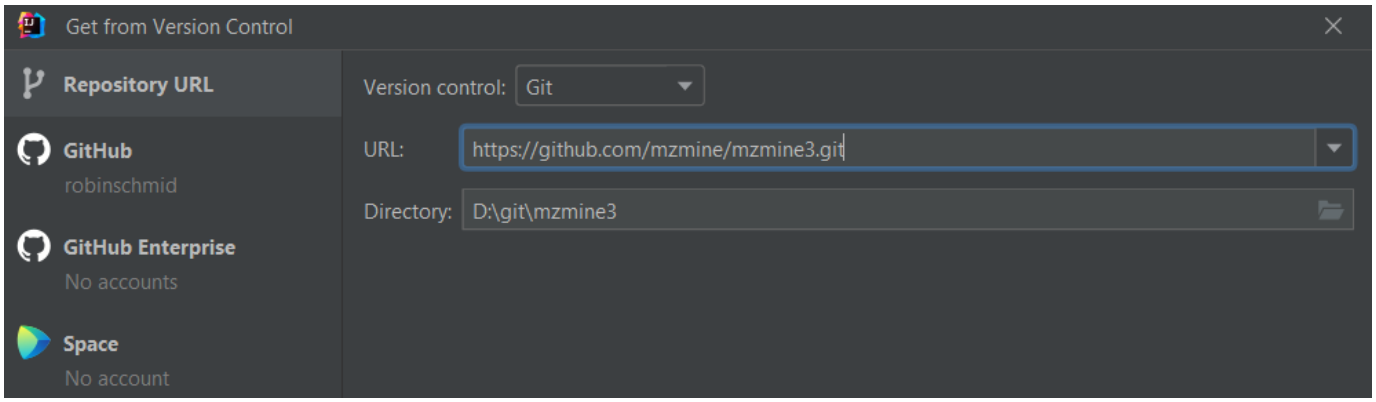


4. Add your GitHub account via **Settings/Version Control/GitHub** +button. Below exemplified with the Log in with Token... option: - Log in with Token... **Generate** - redirects to GitHub - Make sure to select the **Workflow** scope to avoid conflicts that arise from changing GitHub actions

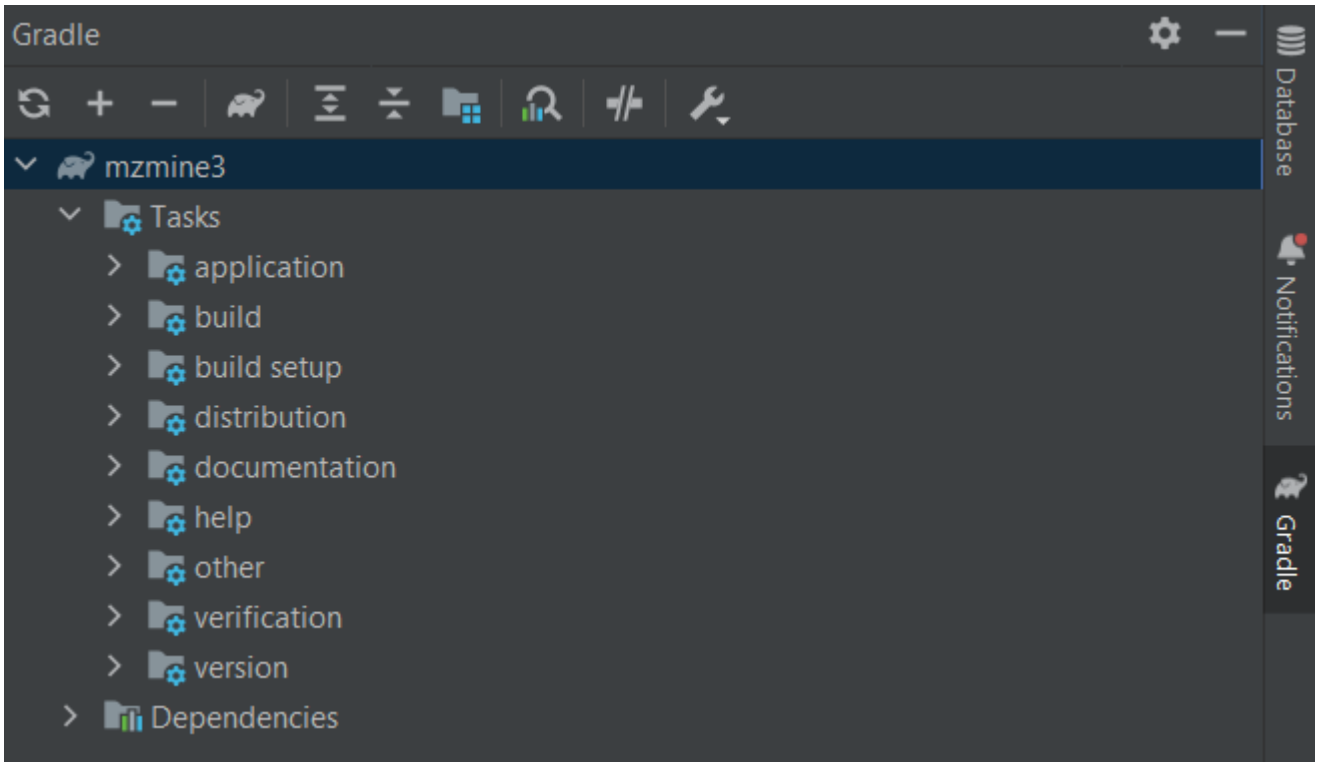


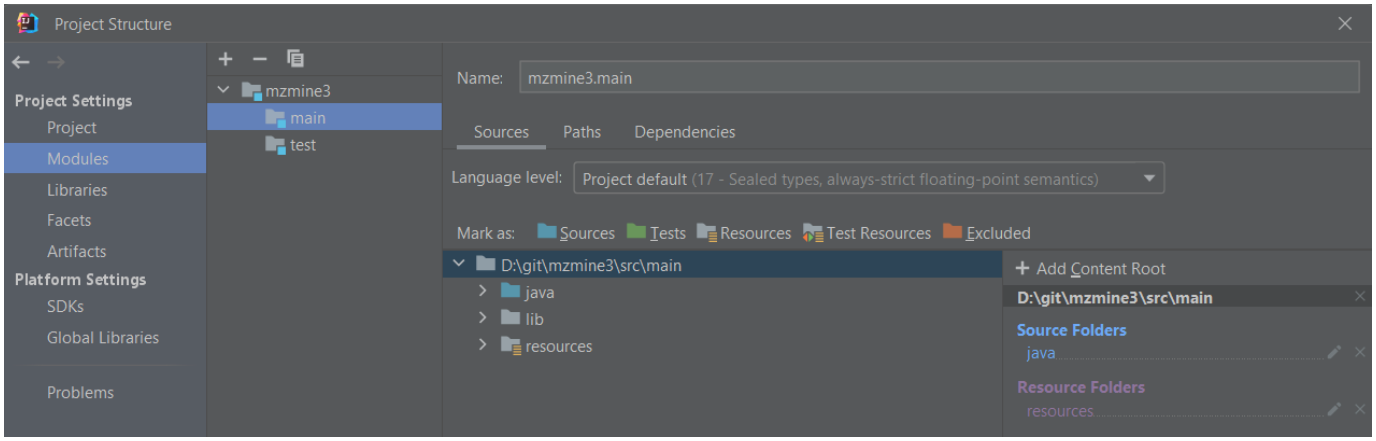


5. Clone GitHub project via version control: **File/New/Project from version control** use your user name to get your fork:
`https://github.com/YOUR USERNAME/mzmine3.git`

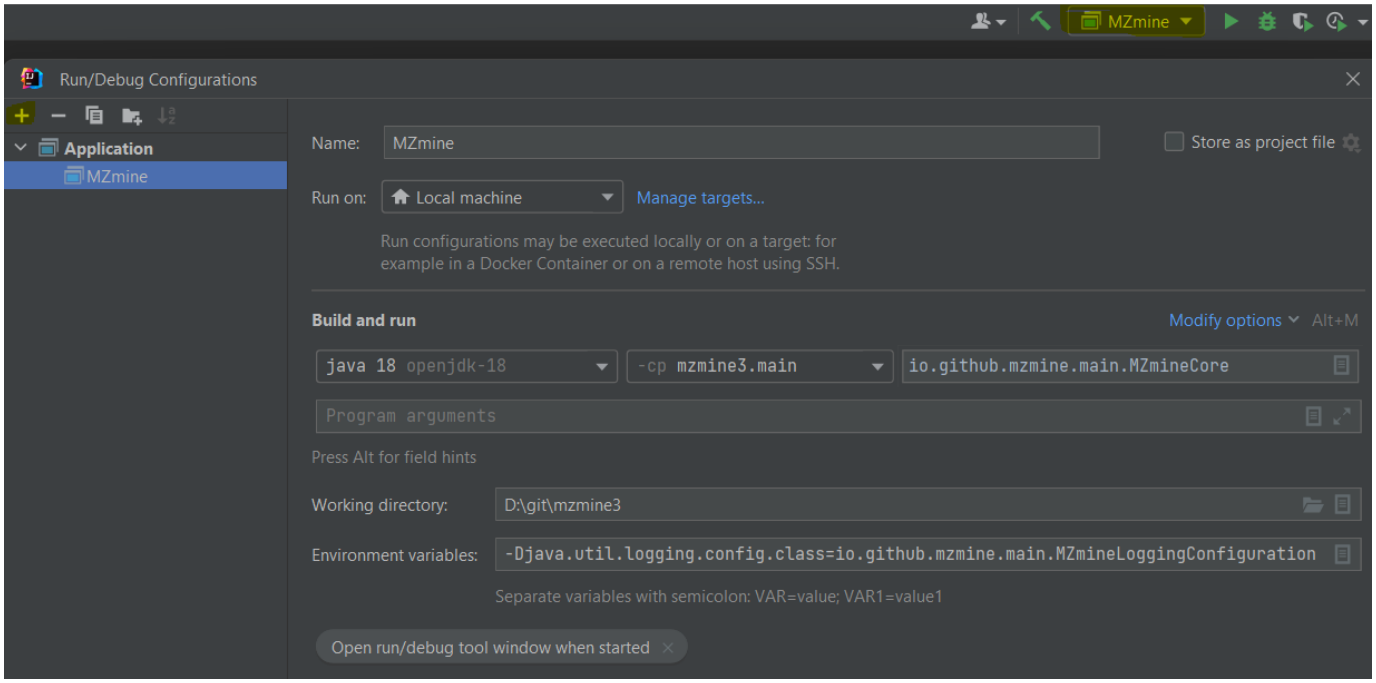


1. Make sure that gradle runs and updates the project. Otherwise, click on **Reload Gradle Project**. Now the project structure (CTRL+SHIFT+ALT+S) should show the source, test, and resource folders which are described in the build.gradle.



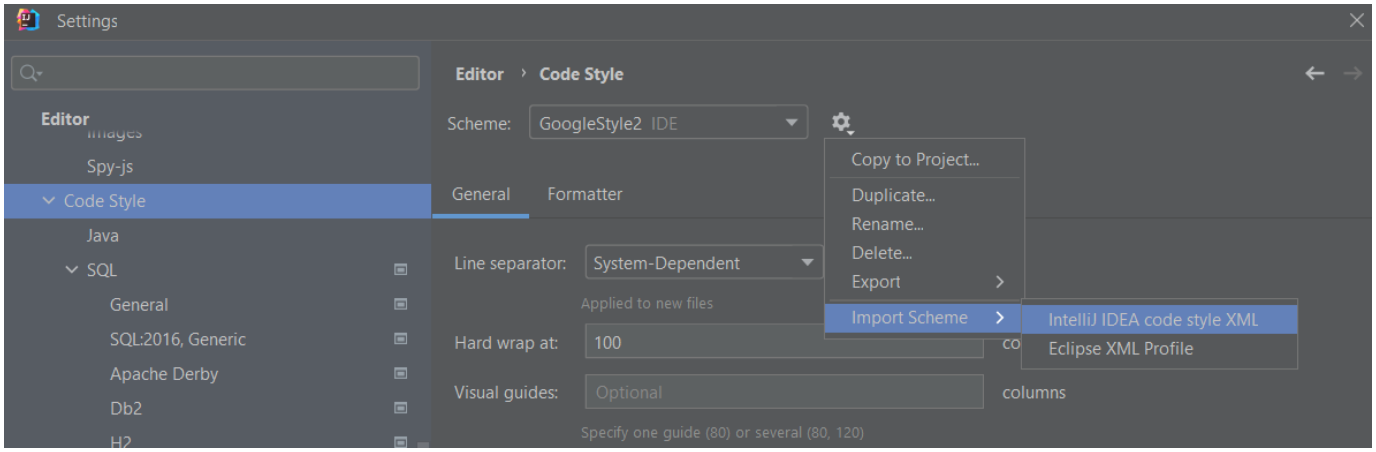


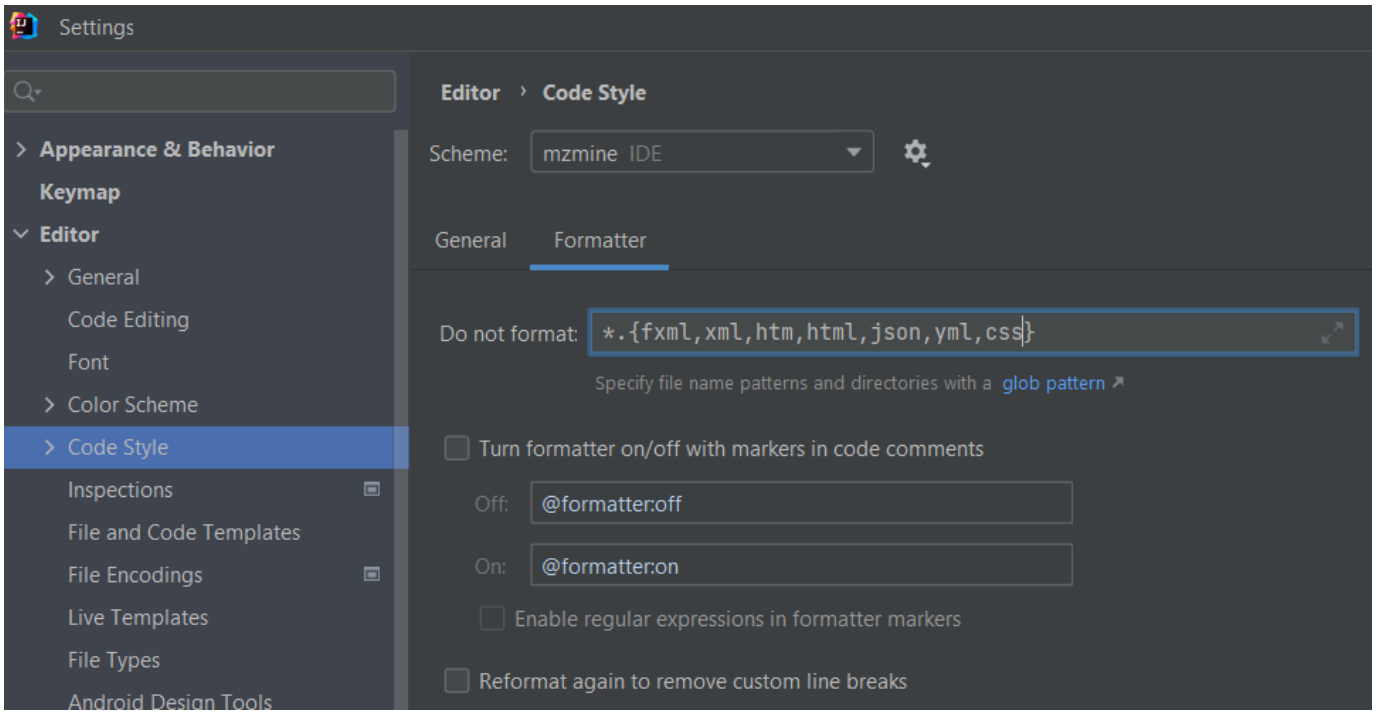
1. Click on Add a Configuration. Select “Application” from the template list). via the + button (don’t just edit the template):
   - Main class: `io.github.mzmine.main.MZmineCore`
   - Environment var: `-Djava.util.logging.config.class=io.github.mzmine.main.MZmineLoggingConfiguration -Xmx12G`
2. Run or debug with this configuration



10.2.2 Code formatter

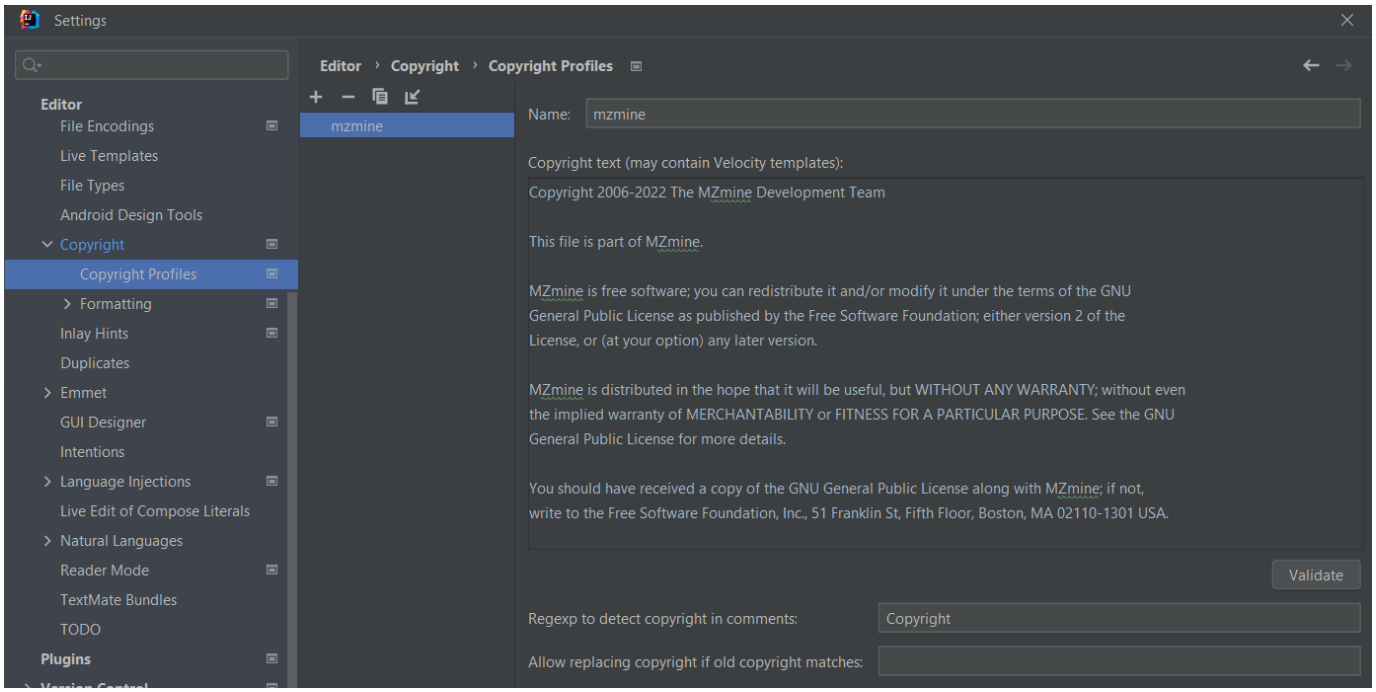
The code formatter is important for contributions to the main version of MZmine and each java file should contain the license header. 1. Import the style format from `mzmine-intellij-code-formater.xml` 2. Apply format to any file with CTRL+ALT+L 3. Can also be applied on each file saved or each commited change (see below) 4. Better exclude specific file formats from being formatted (see below screenshot 2)

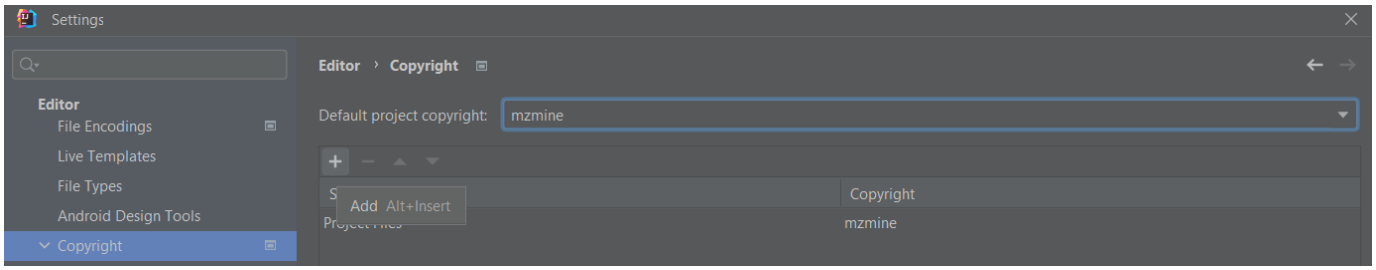




10.2.3 Copyright header

1. Add the license header to each file - the easiest way is to add the copyright profile to intelliJ
2. Import the copyright from `mzmine_intellij_licence_header.xml` or create a new one with the exact text specified in `license_header.txt`
3. Add a new scope for all project files
4. Apply after file save or commit operation or run the **Update copyright...** action

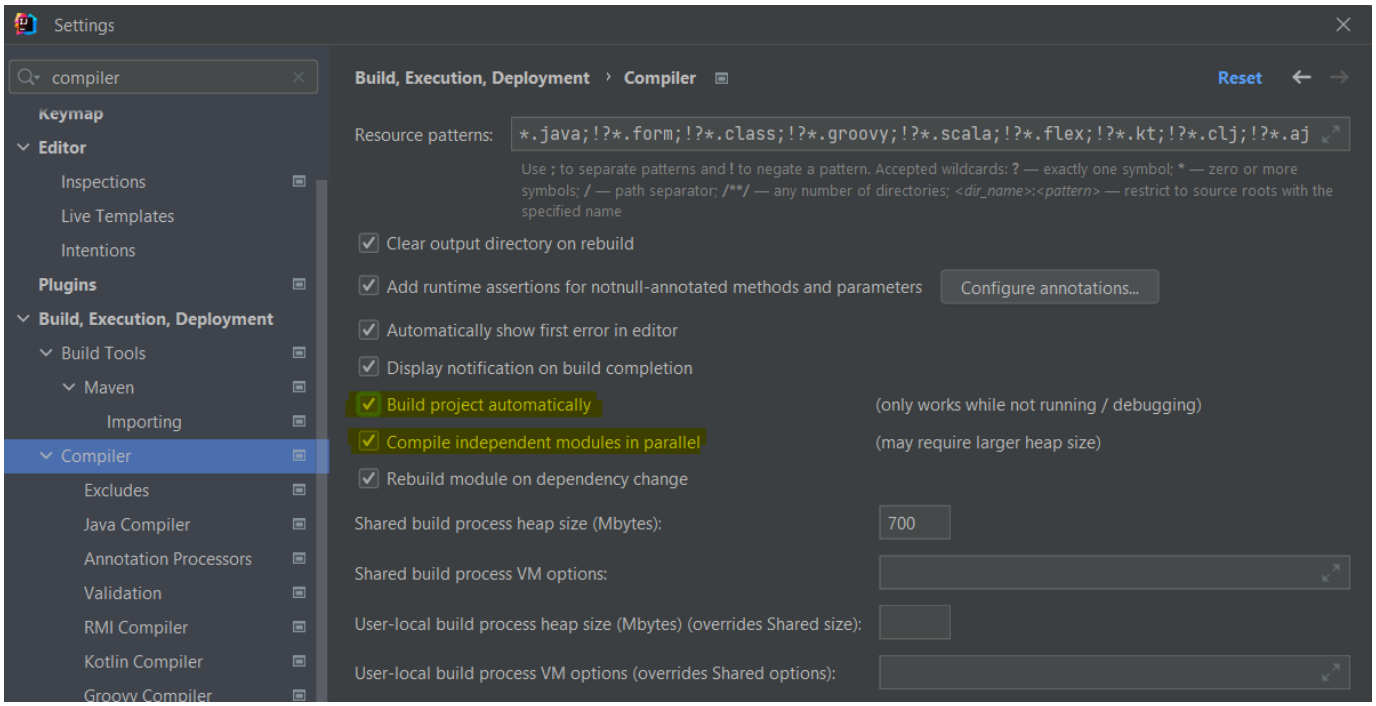


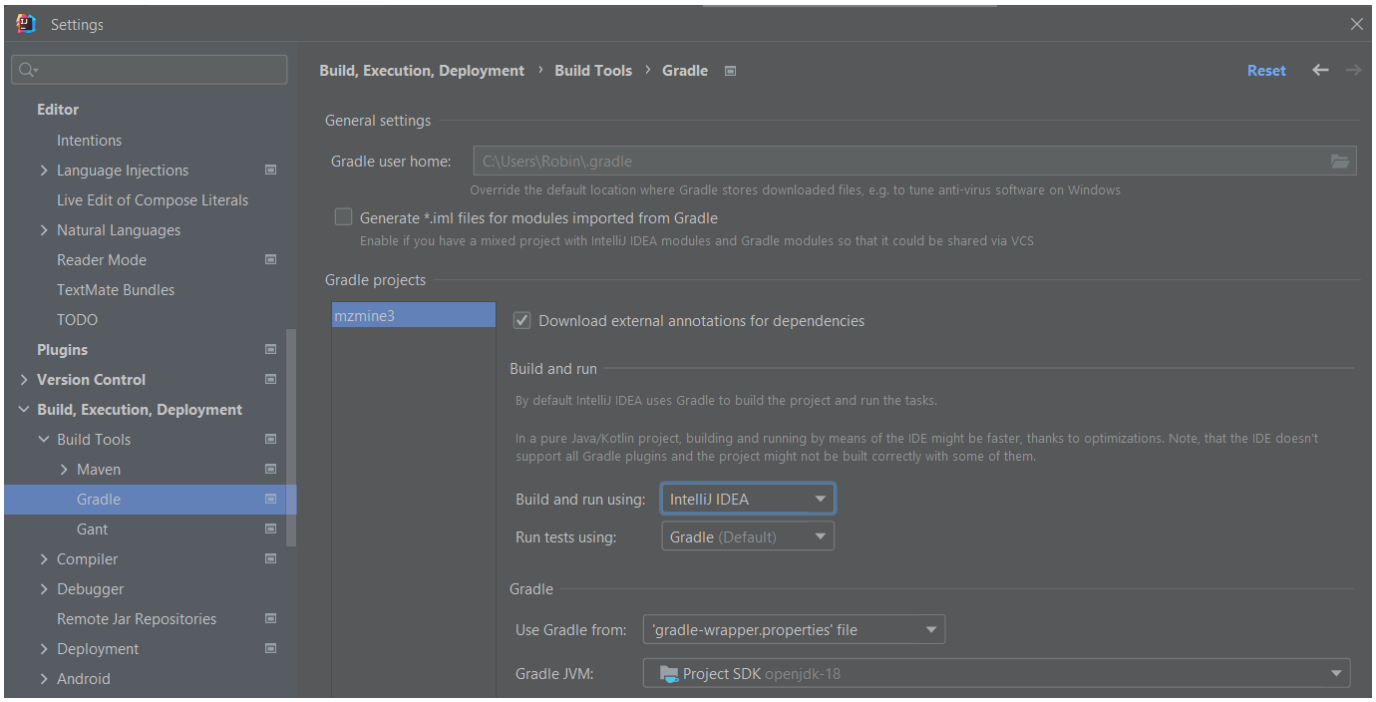


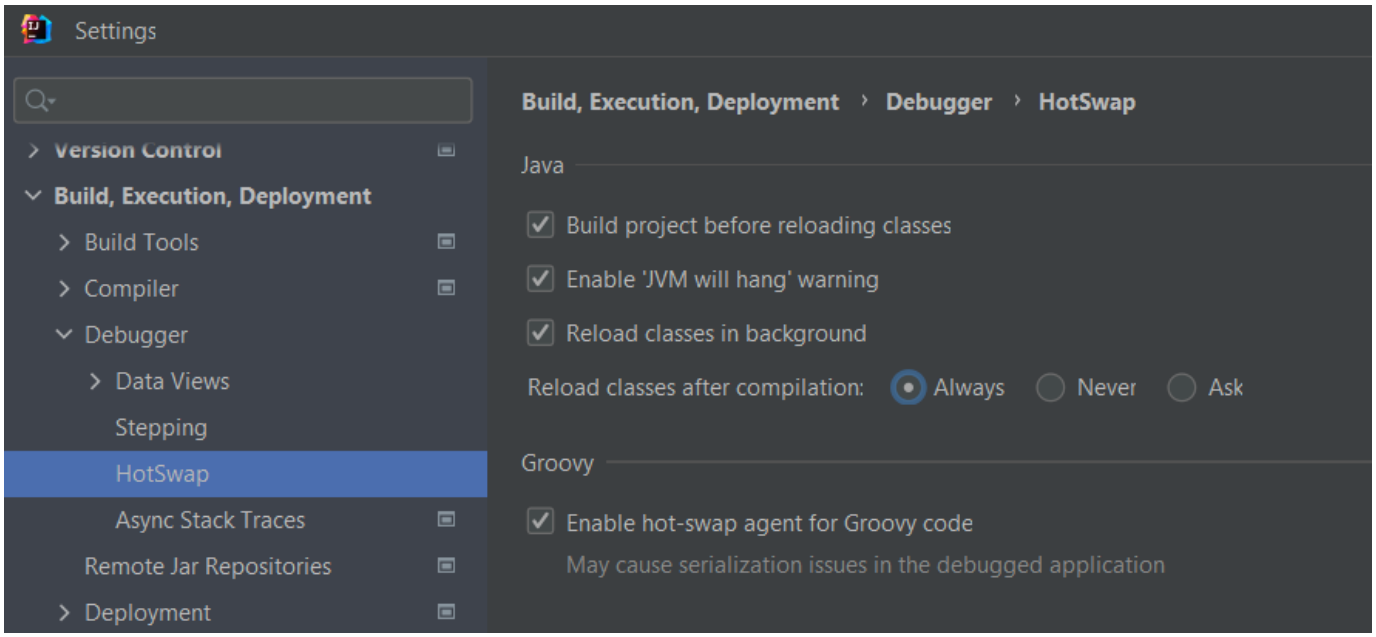
10.2.4 Useful settings

Faster building

1. Activate auto building (consumes more resources)
2. Set **Settings/Gradle/build and run** to IntelliJ
3. Activate HotSwap to automatically load changed classes during debugging

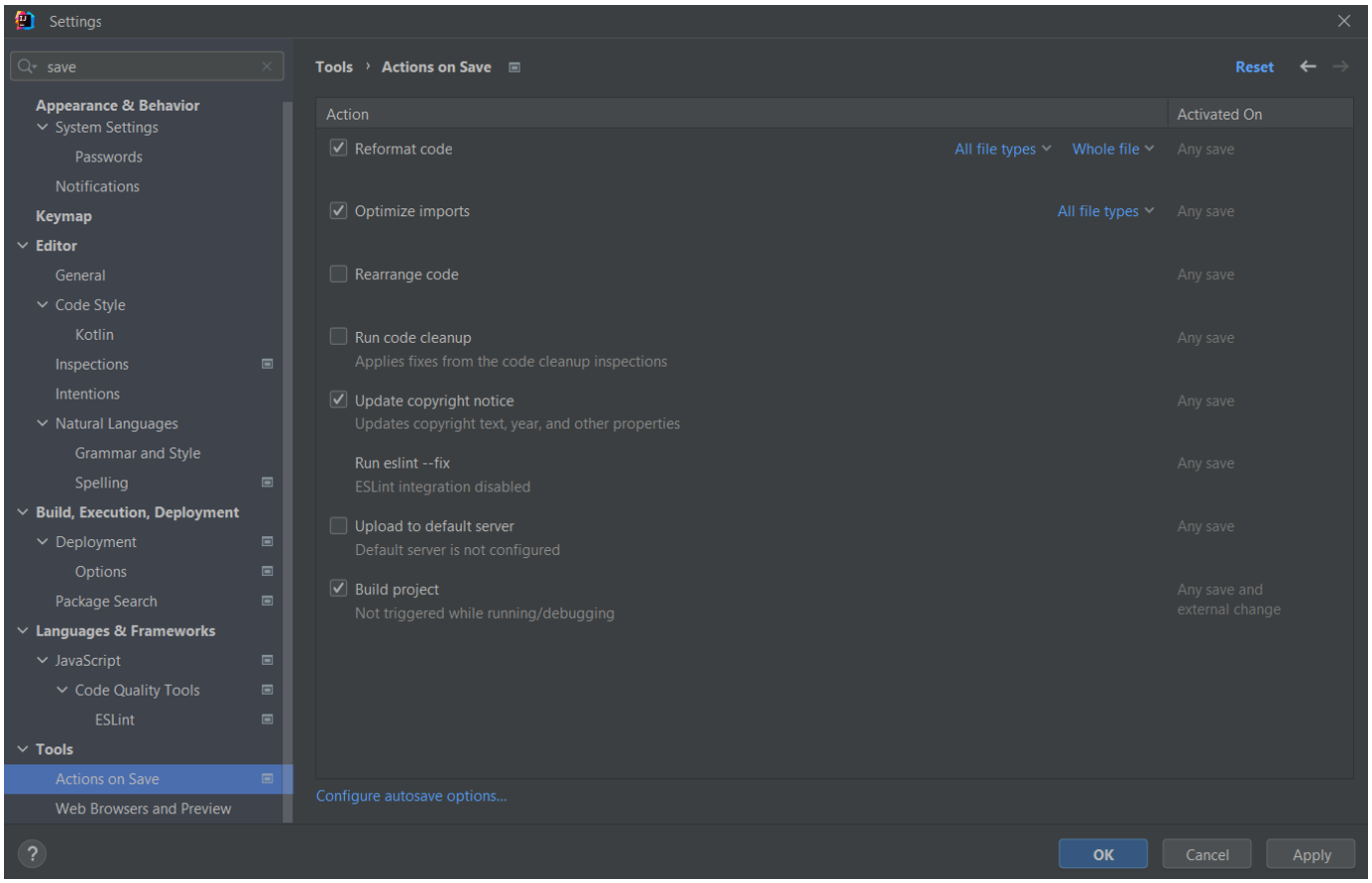


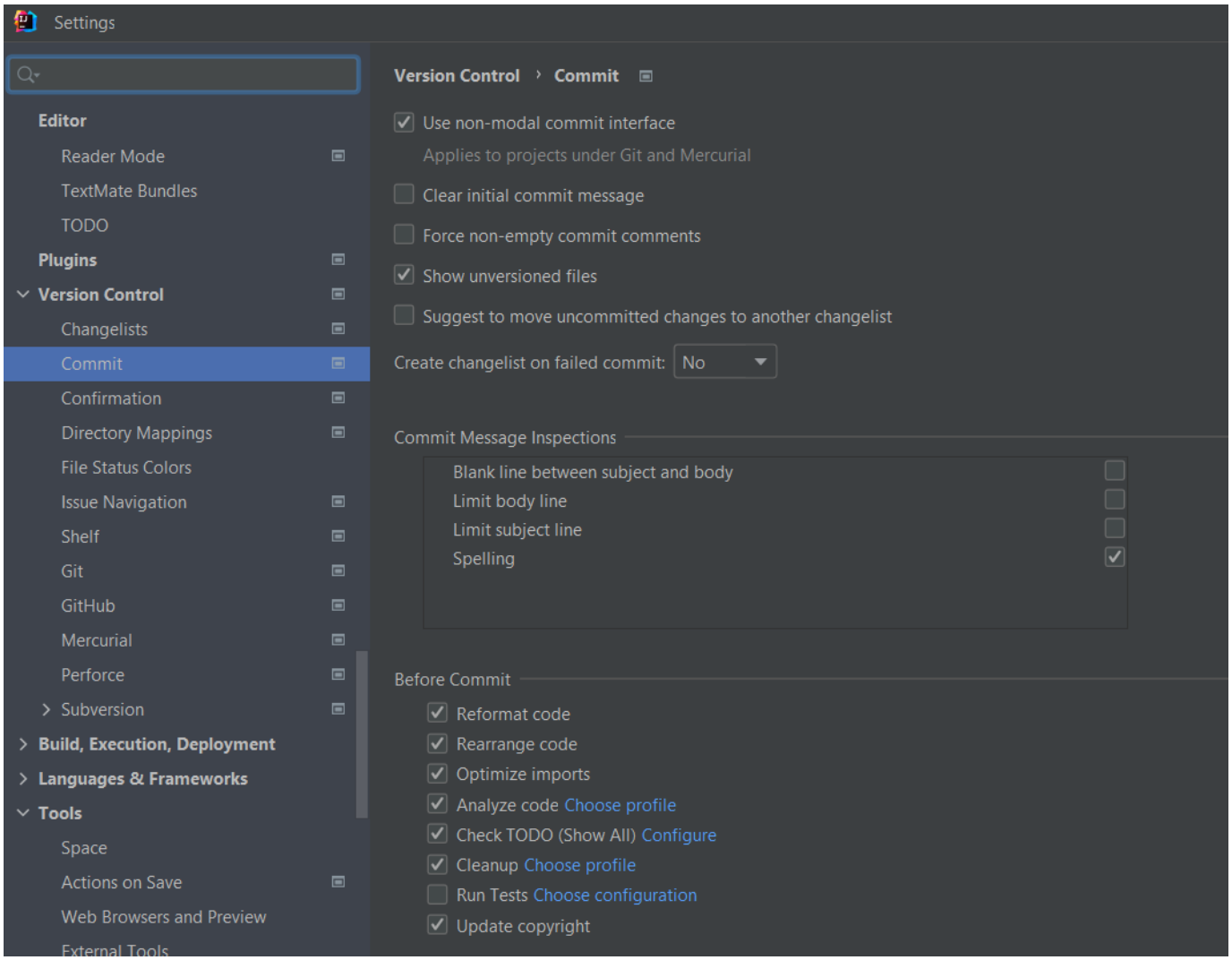




Save and commit actions: Apply copyright, format, etc

Select any actions to perform when a file is saved or committed.





Live templates

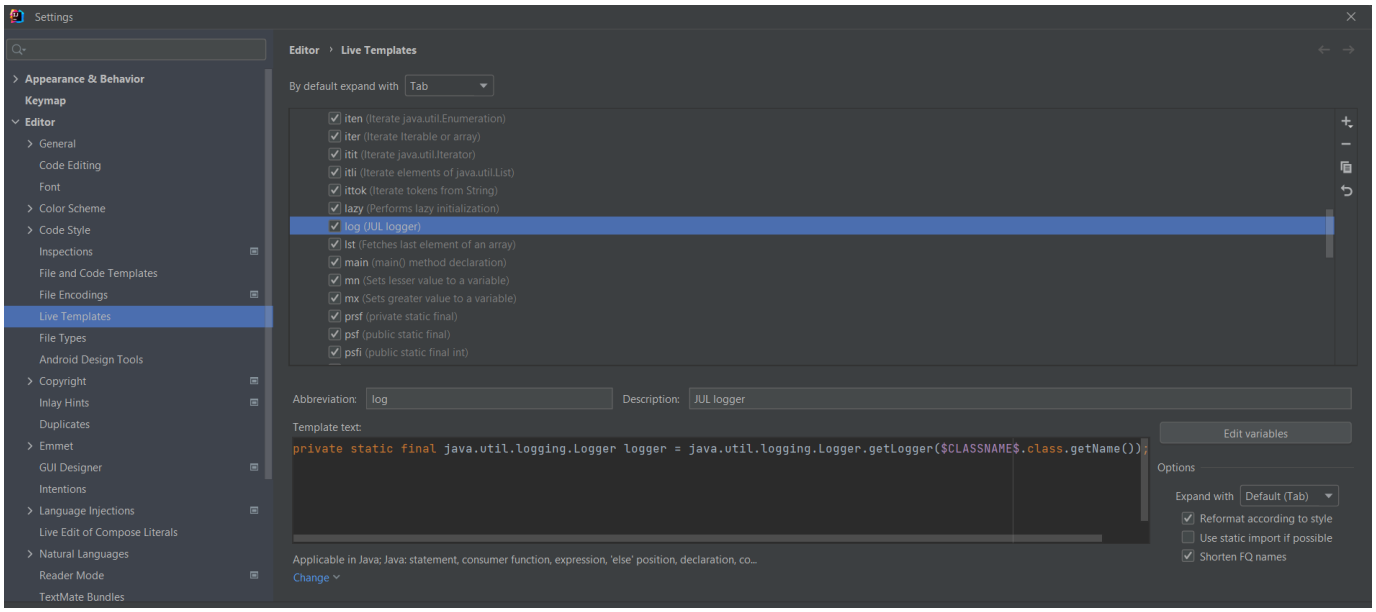
Live templates are used to add custom entries to the code completion, e.g., to quickly create a class-specific logger. Access **Settings/Editor/Live Templates** (CTRL+ALT+S on Windows) and add a new template (+ button). Define the abbreviation to trigger autocompletion at this statement, define the template text, and change the target context ("**Java**") at the bottom. The template below generates a logger after typing log and pressing CTRL+SPACE. The variable **\(CLASS_NAME\)** was set to represent the current className() under **Edit variable**.

The template:

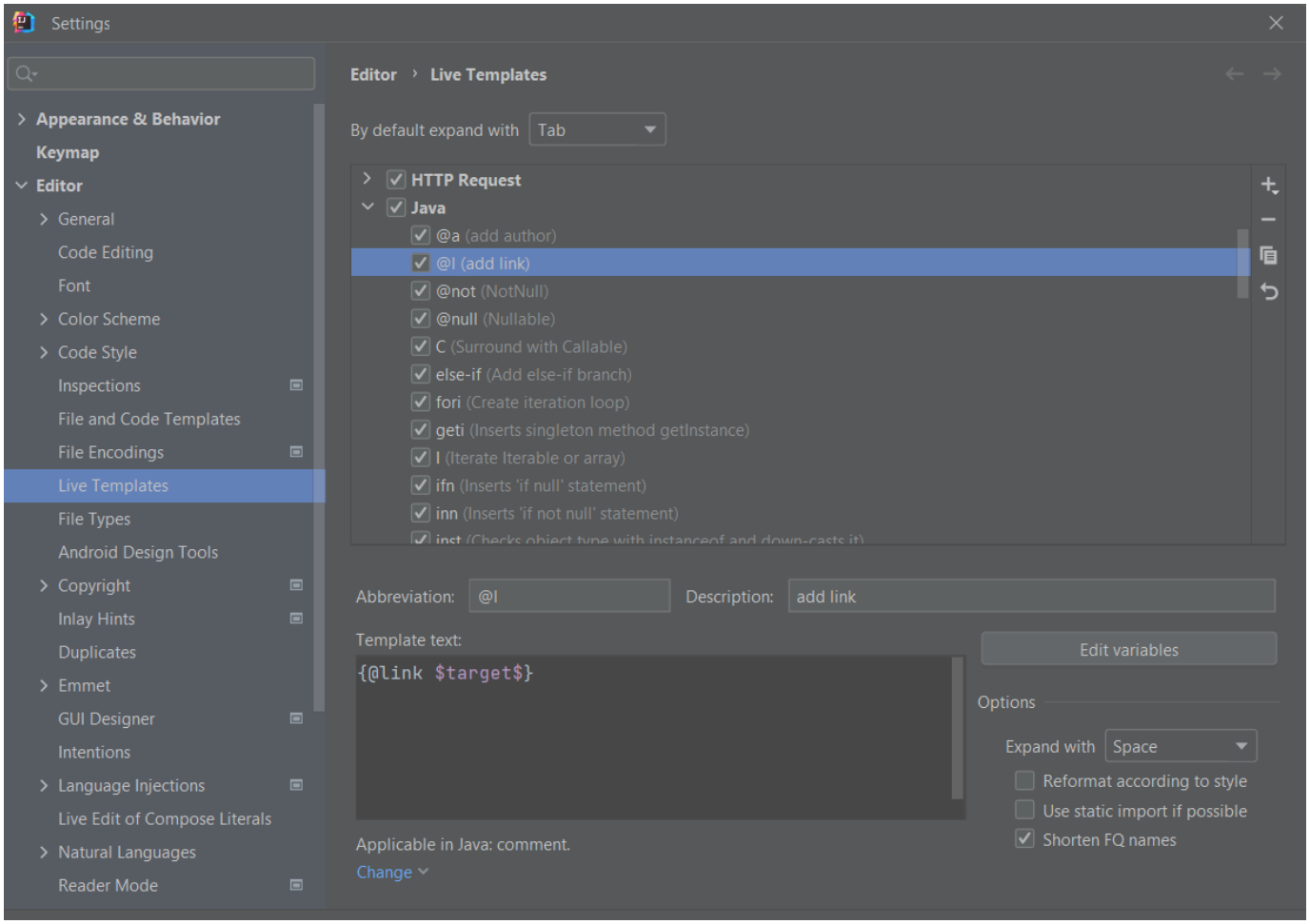
```
private static final java.util.logging.Logger logger = java.util.logging.Logger.getLogger($CLASSNAME$.class.getName());
```

Generates the output in class Scan:

```
private static final Logger logger = Logger.getLogger(Scan.class.getName());
```



Another example to create Javadoc links for @l . The variable (here \(target\)) places the cursor.



10.2.5 Troubleshooting

Correct JDK selection

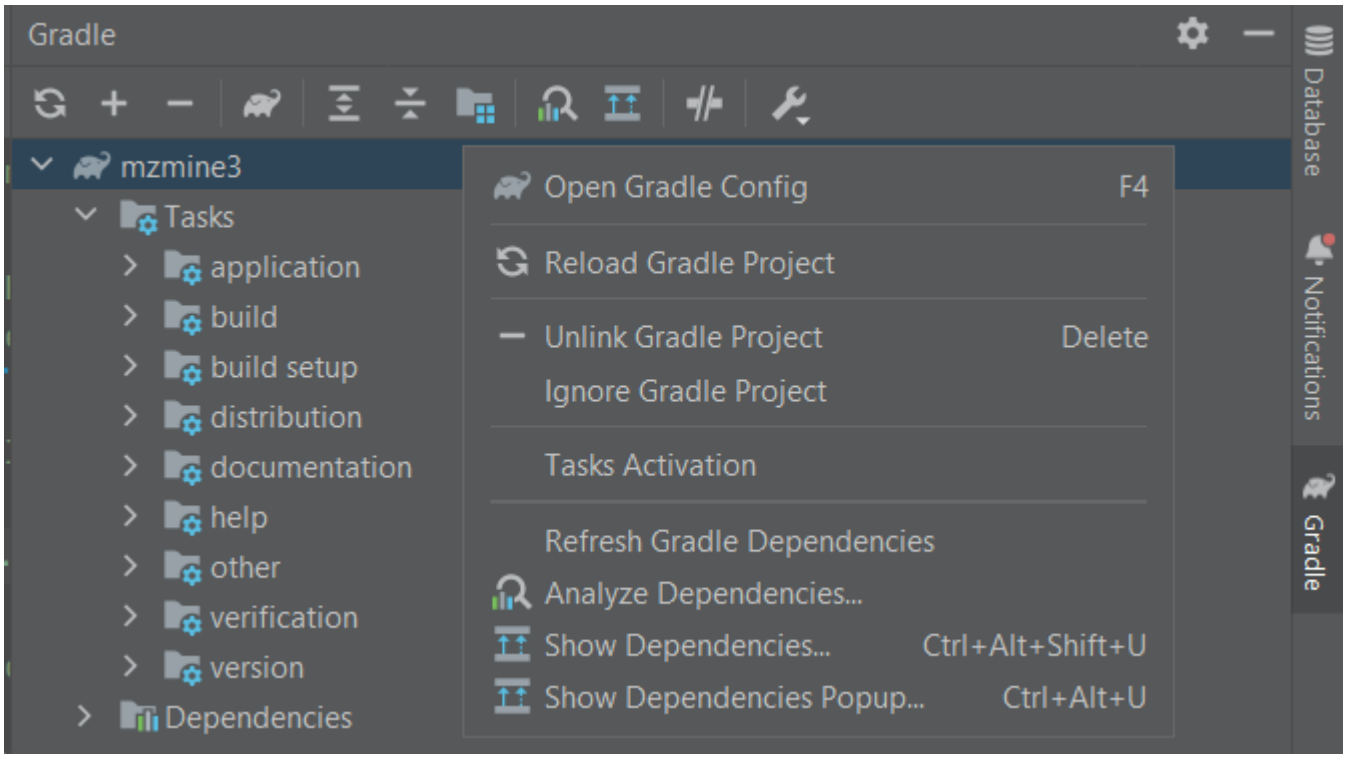
Make sure the correct JDK is set in these places: 1. File/Project Structure/SDKs 2. File/Settings/Build, Execution, Deployment/Build Tools → Gradle → Gradle JVM → “Project SDK” this will update automatically if the project SDK changes. 3. File/Settings/Build, Execution, Deployment/Java Compiler → Project Bytecode version → 17 (for JDK 17) Correct run configuration: Select Default JRE (this will update with the project sdk) or select the correct one manually.

Could not target platform

Error: When building via gradlew: “Could not target platform: 'Java SE 17' using tool chain: 'JDK 13 (13)'” Solution (Windows): Set the JAVA_HOME environment variable to the JDK 17 root directory. See https://docs.oracle.com/cd/E19182-01/821-0917/inst_jdk_javahome_t/index.html

Gradle project not imported

If gradle tool window is not shown: 1. To import the Gradle project navigate to the build.gradle in the project tool window right click → import gradle project. The gradle tool window should now be visible. 2. To update the imports click the update gradle project button in the gradle tool window



Last update: June 1, 2022 08:55:52

11. Acknowledgements

We would like to point out that this wiki was set up in tight collaboration with the GNPS staff. We highly appreciate your help!

11.1 Related projects

- GNPS
- SIRIUS

11.2 Libraries we use in MZmine

- Apache XML Graphics - EPS image export
- Chemistry Development Kit - Isotope pattern and molecular calculations
- Freehep - EMF image export
- Google Guava - Utility classes
- JDK Documentation
- JChemPaint - 2D molecule visualization
- JFreeChart - TIC, Spectra and 2D visualizers
- JMol - 3D molecule visualization
- jmzml - mzML file import
- jmzTab - mzTab file import and export
- NetCDF-Java - NetCDF file import
- VisAD - 3D visualizer
- WEKA - Clustering and other machine learning algorithms
- Bruker TDF SDK - Native tdf/tdf file import (requires VC++ 2017 redist.)
- Thermo raw file parser - Native Thermo raw import

Last update: March 10, 2022 15:35:04