Crommy

User Manual version 1.1.2

System configurations and specifications in this document supersede all previous information received by the purchaser. This document is not part of any sales contract between MS Forge Srls and a purchaser. This document shall in no way govern or modify any Terms and Conditions of Sale, which Terms and Conditions of Sale shall govern all conflicting information between the two documents.

Release history: March 2024 Software version: Crommy 1.x For Research Use Only. Not for use in diagnostic procedures.

Summary

System Requirements

System	Minimum requirements
Hardware	3.4 GHz dual-core processor
	1 6 GB RAM
	1TB hard drive
Software	Windows 10 64-bit operating system or Windows 11 64-bit operating system
System settings	To run processing workflows with online library database, the computer must have unblocked access to the library databases on the Internet.

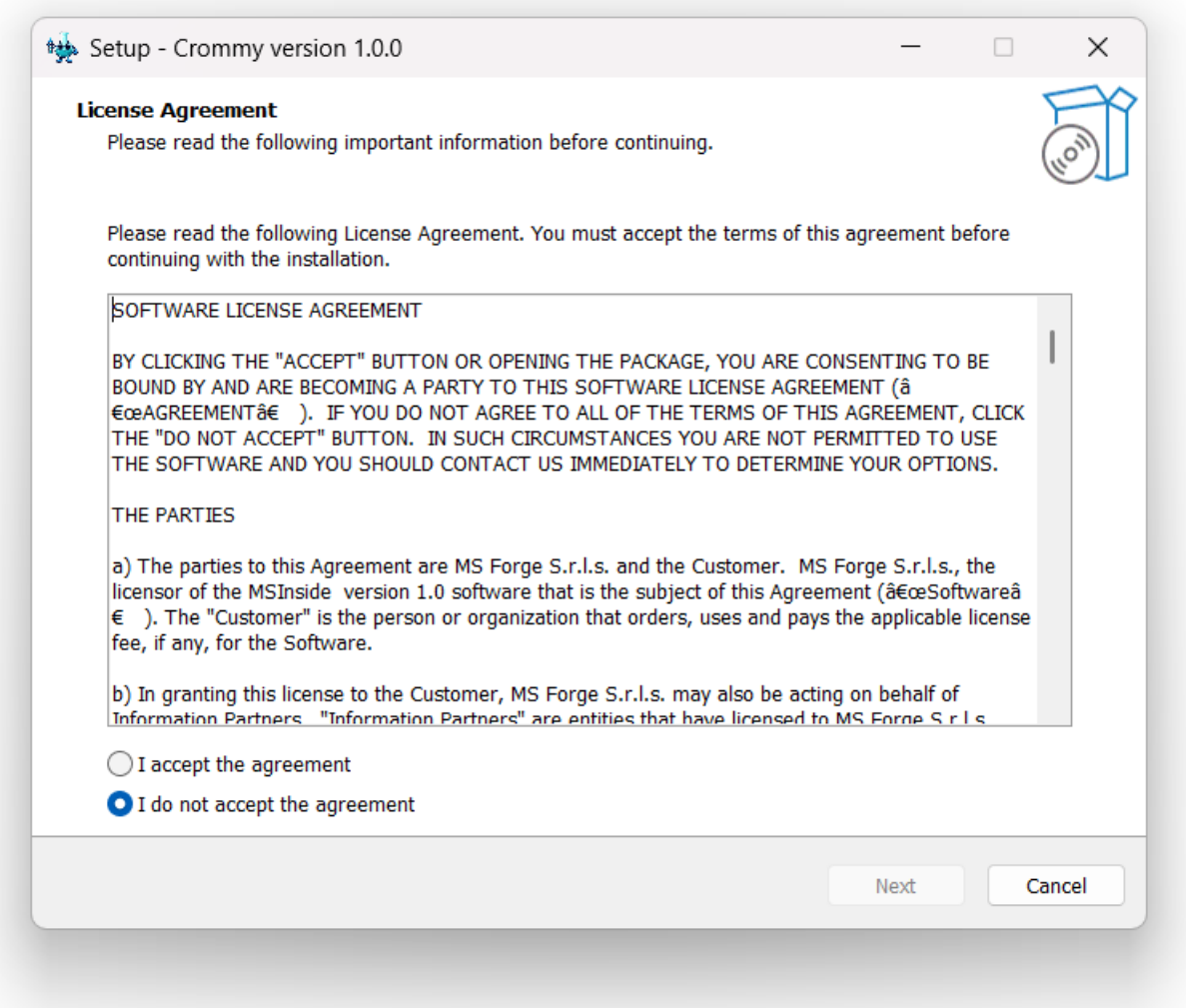
Supported Data Format

Format	Distributon	Built-in
mzML	Open Format	Yes
Raw	Thermofisher Scientific	No*

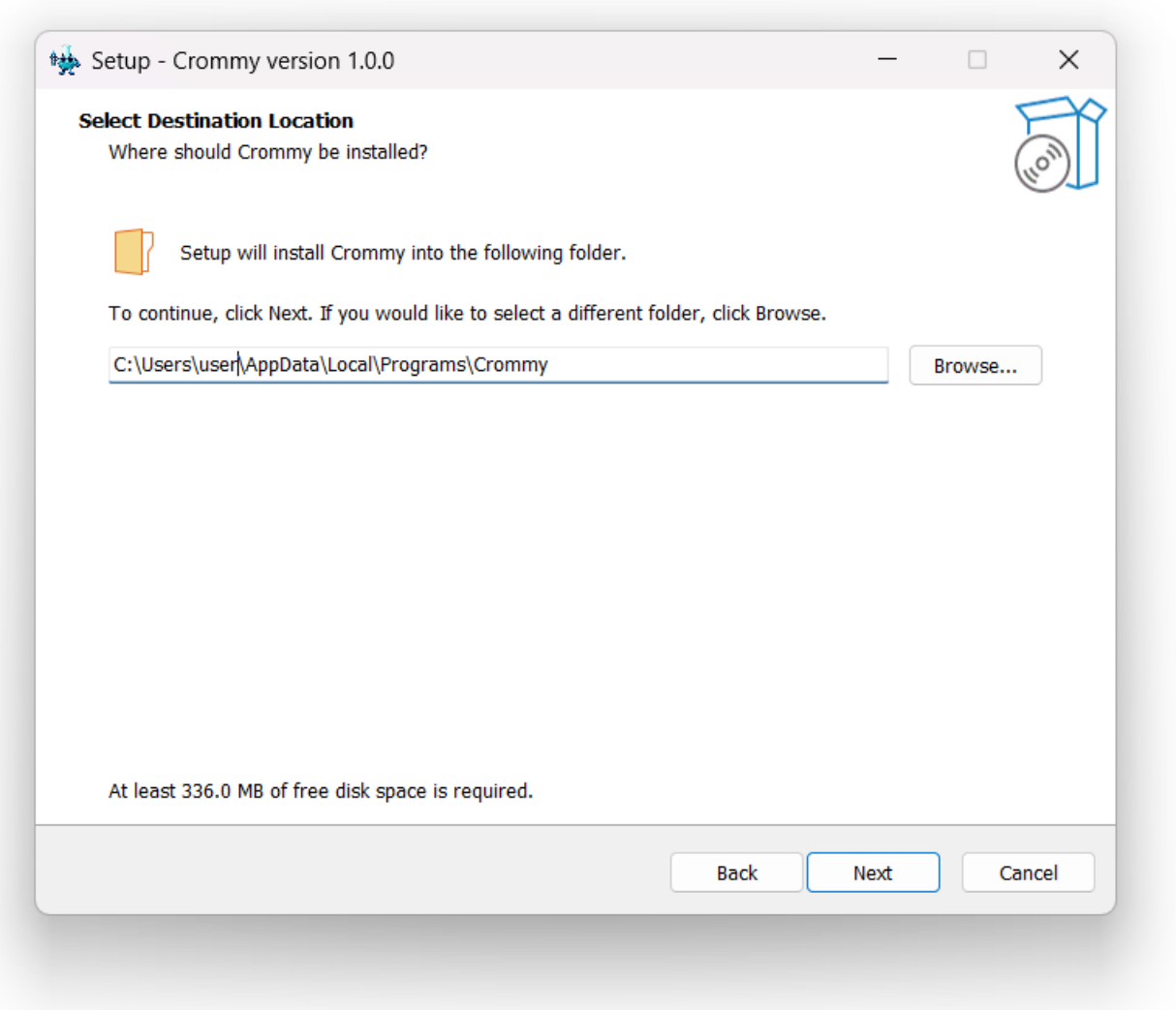
Raw files from Thermofisher Scientific required the download of .dll files which are not included, not redistributed nor provided by the software. When attempting to run analysis directly from raw files, Crommy will ask for providing required dll files.

Installation

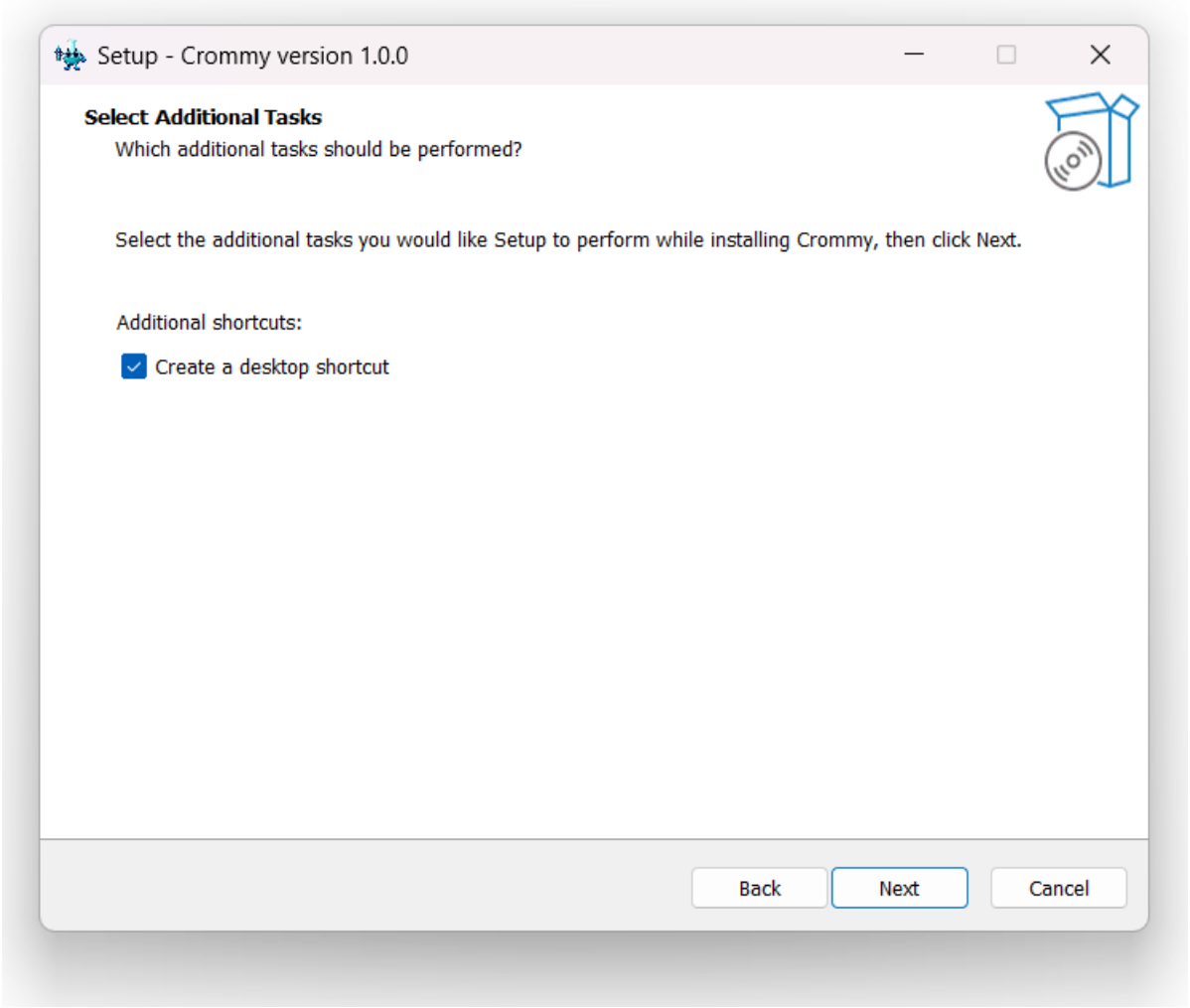
Download the latest Crommy version from www.msforge.it/software and execute for installation.



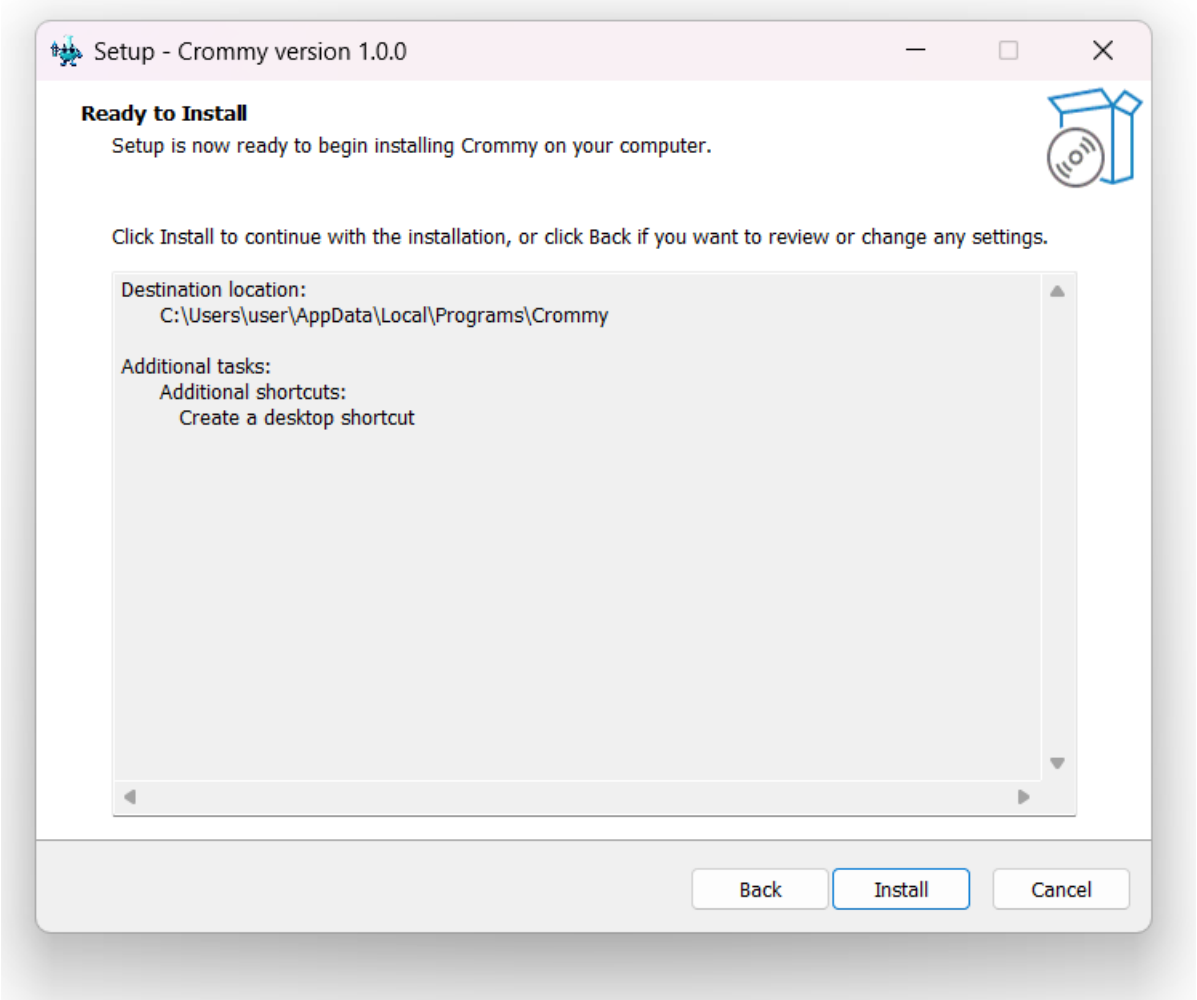
Read the licence agreement and accept by selecting “I accept the agreement” and then click on the Next button for continuing the installation.



You can modify the installation folder (optional) and than click on Next to continue the installation.



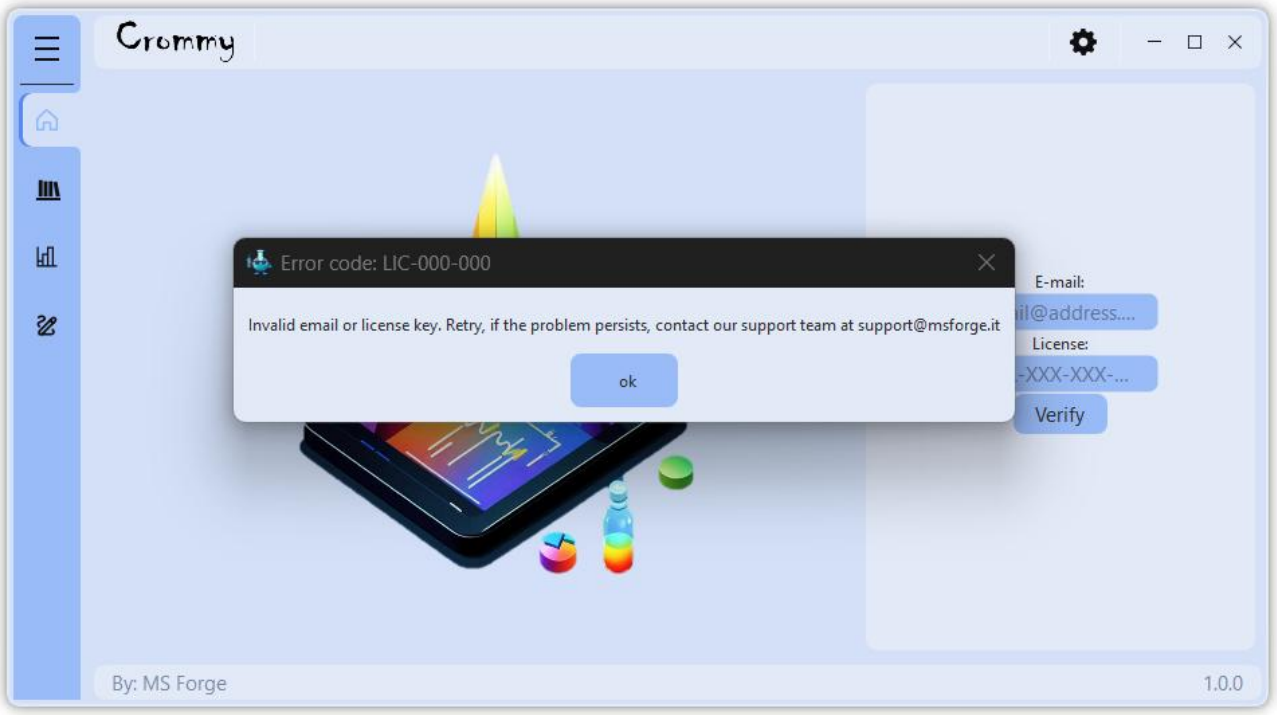
You can now select to create a desktop shortcut. Click Next to move on the summary page.



Here you can click “Install” to complete the installation.

Crommy activation

After starting Crommy, the following screen will appear:

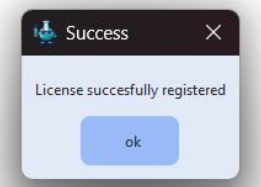


Click «Ok».

Enter a valid email and a valid Licence Key.

Press «Verify»

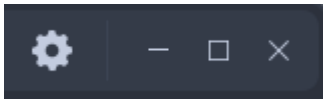
A new popup will appear confirming successful registration.



I you don't own a valid licence, you can send us a licence request on https://www.msforge.it/contact/ asking for private, public or demo license.

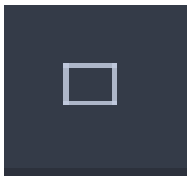
Crommy top bar

Four buttons are present on the top-right of the app

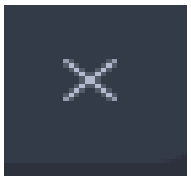


Setting icon display the registration space if the software is not registered yet, otherwise by clicking on setting it will appear a page with the button «User manual» and the box for choosing the number of cores Crommy can used for the analysis.

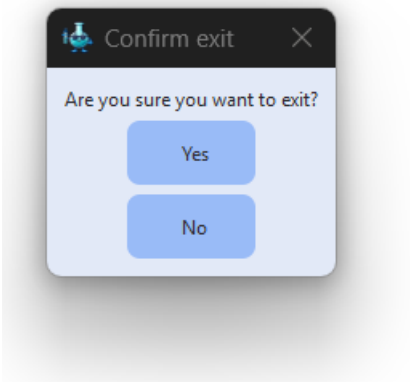
By clicking this button, Crommy will minimize.



By clicking this button, Crommy will maximize in the screen.



By clicking this button, a pop-up will open. By clicking «Yes» the app will close.

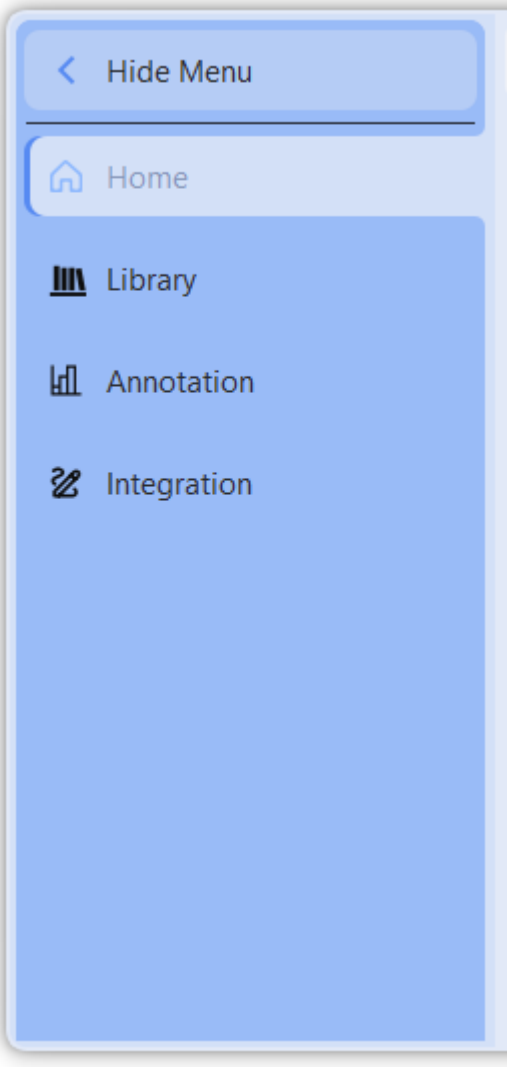


Crommy left bar

Crommy is divided in 5 main pages linked to the relative icon. Moving the mouse of each of the icon will reveal the icon name.

Full icon name can be displayed by clicking on 

After clicking the space next to the icons will expand showing the name of each icon.



Hide the icon names.

Home

Contain the tools for starting a new project or for loading already analyzed project.

Library

Include all common and user spectral libraries and tools for libraries management.

Annotation

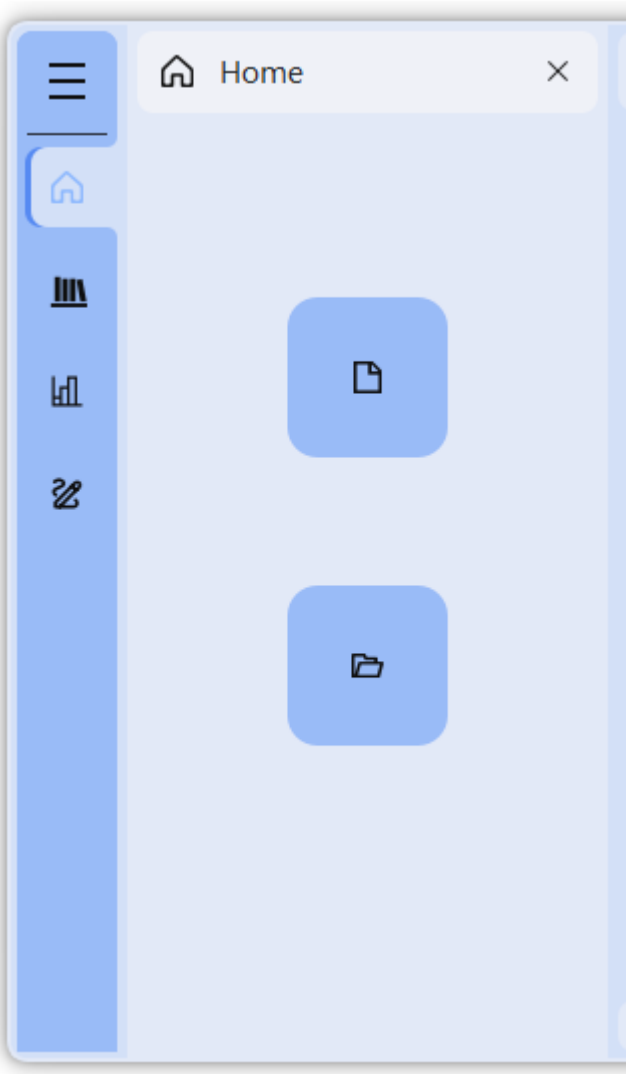
Display compound annotation and relative scores.

Integration

Display the peak area for each detected compound.

Home

Clicking on Home button will open a new window on the left side.

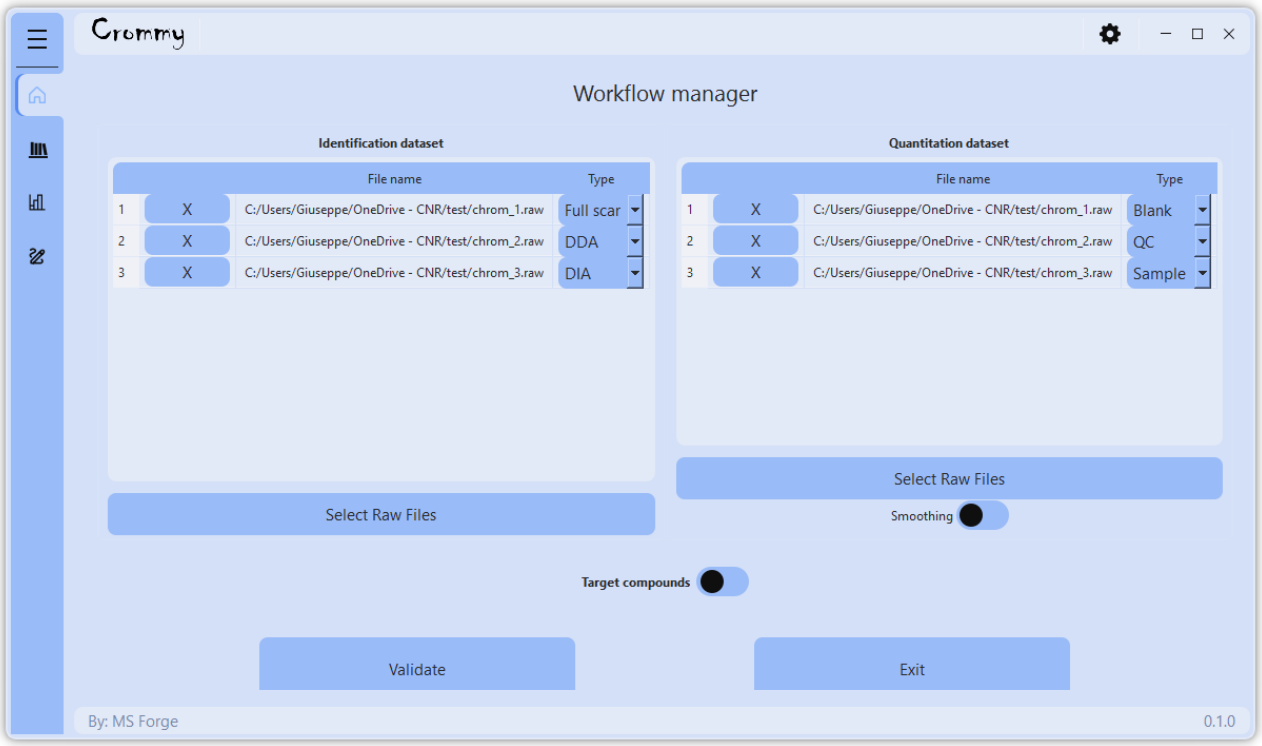


Start a new project.

Open analyzed project

Start new project

After clicking «New Project» the central screen will change by visualizing the Workflow manager.

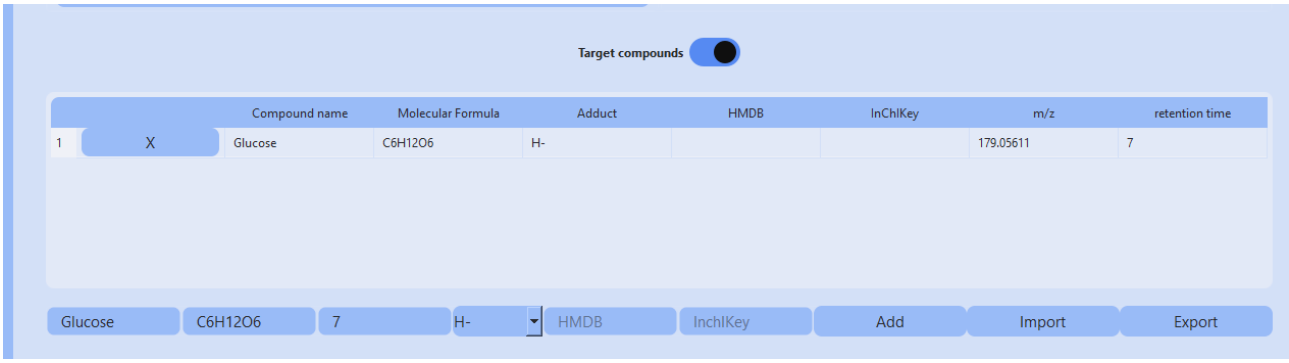


On top, two table are available.

Identification dataset table can be populated by selecting chromatographic files and specify the analysis type (Full scan, DDA or DIA). There are no restrictions to the number of files or the combination of different approaches e.g. DDA and DIA. Based on the uploaded dataset, Crommy will automatically select the workflow optimized for the analysis. Automatic peak detection and annotation will be performed on this selected chromatograms. When running targeted analysis only, this table can be left blank.

Quantitation dataset table can be populated by selecting chromatographic files. This dataset may or may not contain files from identification dataset.

Target Compound table



If selected, this session will expand giving the possibility to include compounds as conventionally done in targeted analysis.

Compound name and Chemical formula are mandatory fields.

Type of adduct can be selected (default is +H). HMDB ID and InChIKey are not mandatory fields.

m/z value will be calculated automatically based on the provided chemical formula and adduct.

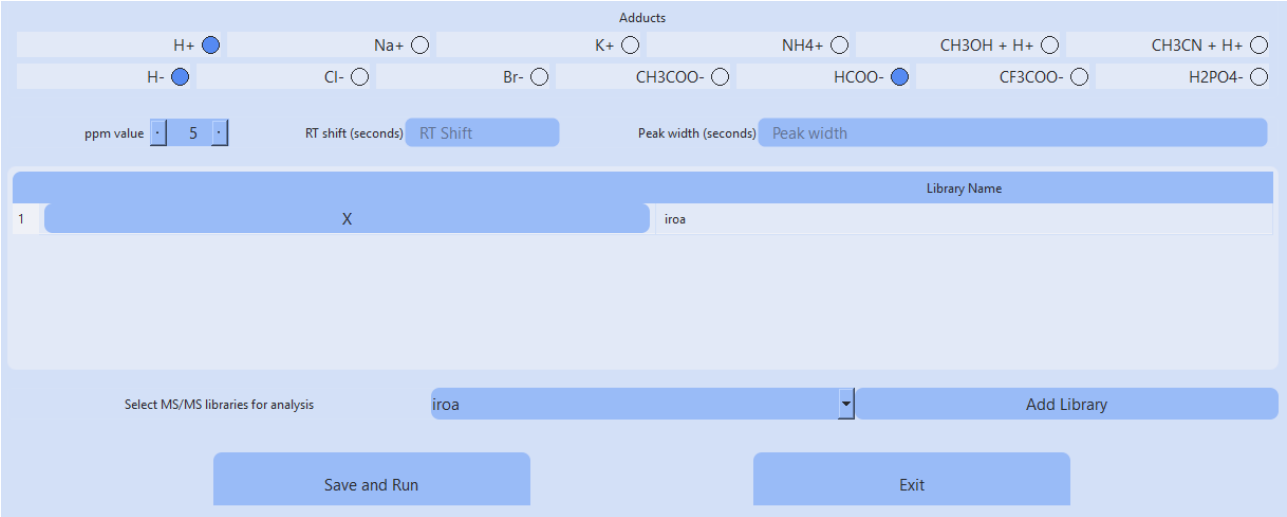
Chemical formulas containing isotopes can be inserted by indicating the isotopologue before the atom letter and by including in square bracket. For example, glucose with 3 heavy carbon (13 Da) can be written as follows:

[13C]3 C3 H12 O6

Generated target list can be exported/imported as .xlsx.

After completing initial information with the list of files for identification, quantification and relative target table we can now press the Validate button.

Complete the workflow



If all mandatory fields are meet, a new part of the workflow will appear. We can now select the adducts we want to search in positive and negative polarities. At least on adduct for each polarity mast be selected. The correct type of adduct will be searched by matching the chromatographic polarity allowing to analysed data acquired in positive, negative or both ionization mode i.e. switching polarity.

Ppm value need to be specified for the analysis. Ppm values are used for matching all possible chemical formulas within the provided error.

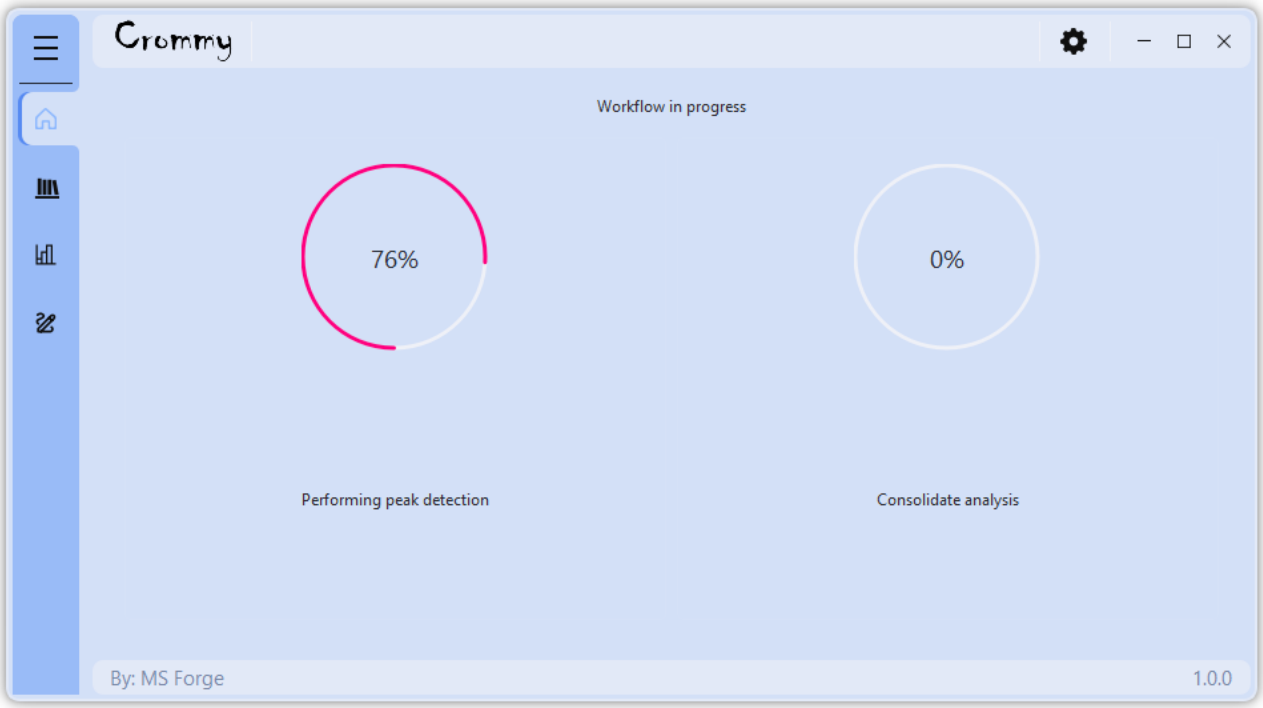
Rt shift need to be specified. This value represent the expected peak shift in retention time within the dataset.

Expected peak width need to be specified only if target compounds are included in the analysis.

Lastly, we can selected a list of fragmentation libraries and add to our analysis. Selected libraries will be used for compound annotation.

We can now save the workflow file and start the run. The process will create metafiles required for the analysis within the folder containing the workflow file. We recommend to save the workflow file in an empty folder and do not override existing workflow files as it may lead to analysis mismatches.

During the analysis, it is possible to navigate in the library, but open existing workflows or starting a new analysis is not allowed.



Open analyzed data

By clicking on the Open button in Home, you can select a workflow file from a previous analysis. The workflow summary will appear.

Annotation and Integration page are now linked to the acquired dataset.

Workflow can not be edited at this stage, but if the workflow was not completed correctly the “Continue” button will appear at the end of the workflow page.

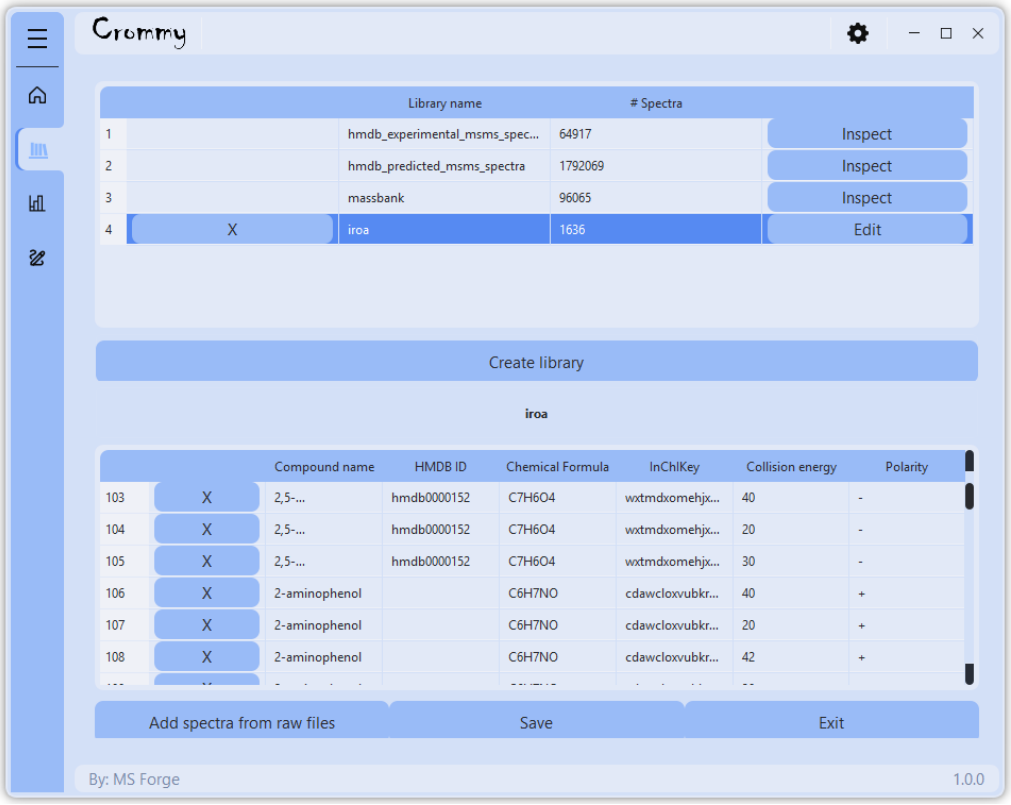
Library

By clicking on the Library Icon on the left menu bar, the library page will open.

Two types or libraries are available in this page. Common and user libraries.

Common libraries are shared between all users and cannot be edited or deleted.

User libraries, as the name suggest, are builded by each user and can be edited or deleted at any time. The library manager is shared between all the MSforge software. Therefore, if you own a licence for MSInside, our solution for mass spectrometry imaging analysis, you can use the share library between the two software with identical interface. As the library are stored on MSforge server, updates of the library in any MSforge product will sincronized with all the product available with a valid licence.

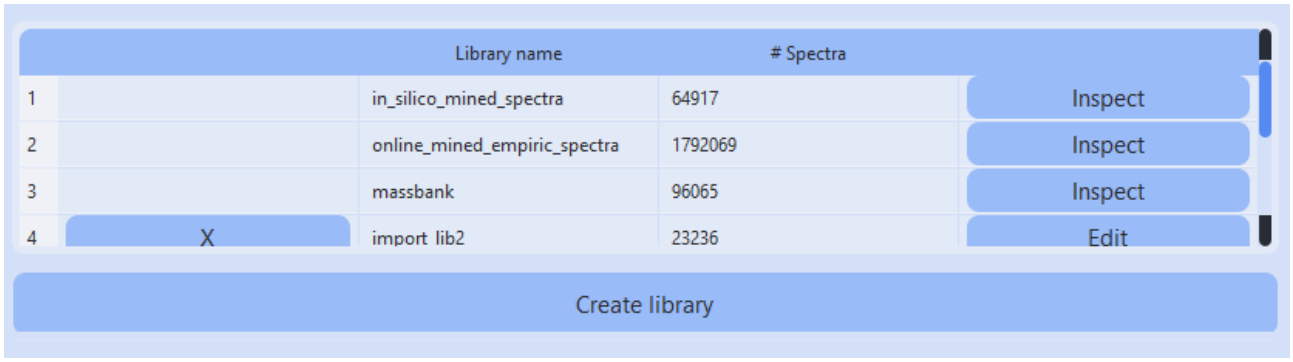


Create a new library

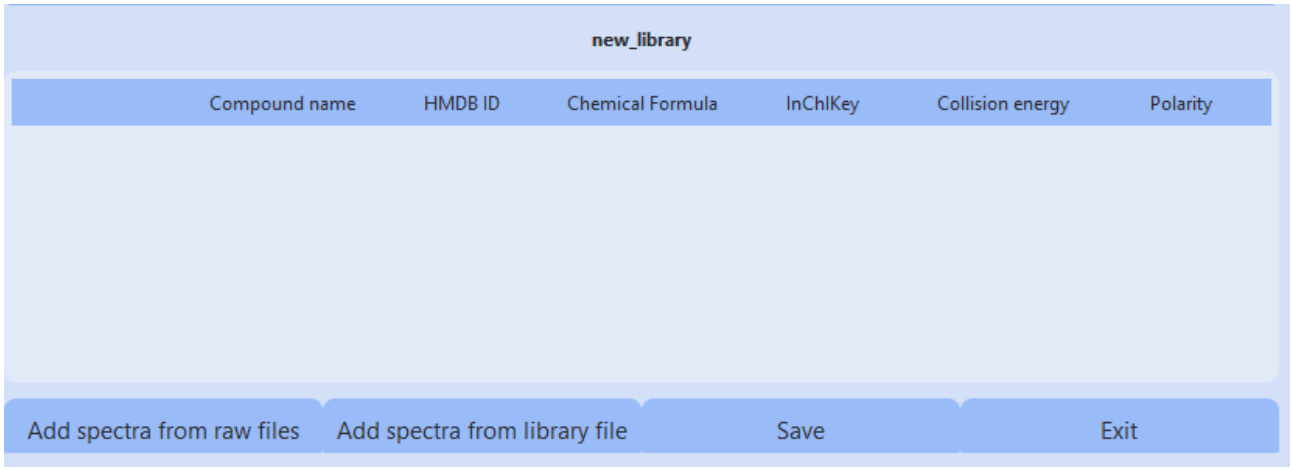
New user library can be created at any time by clicking on «Create Library» button.

You can now type the name of the new library and click Create.

After few seconds the new library will appear as user library with the spectral count equal to 0.



We can now start to populate the new library by clicking the Edit button.

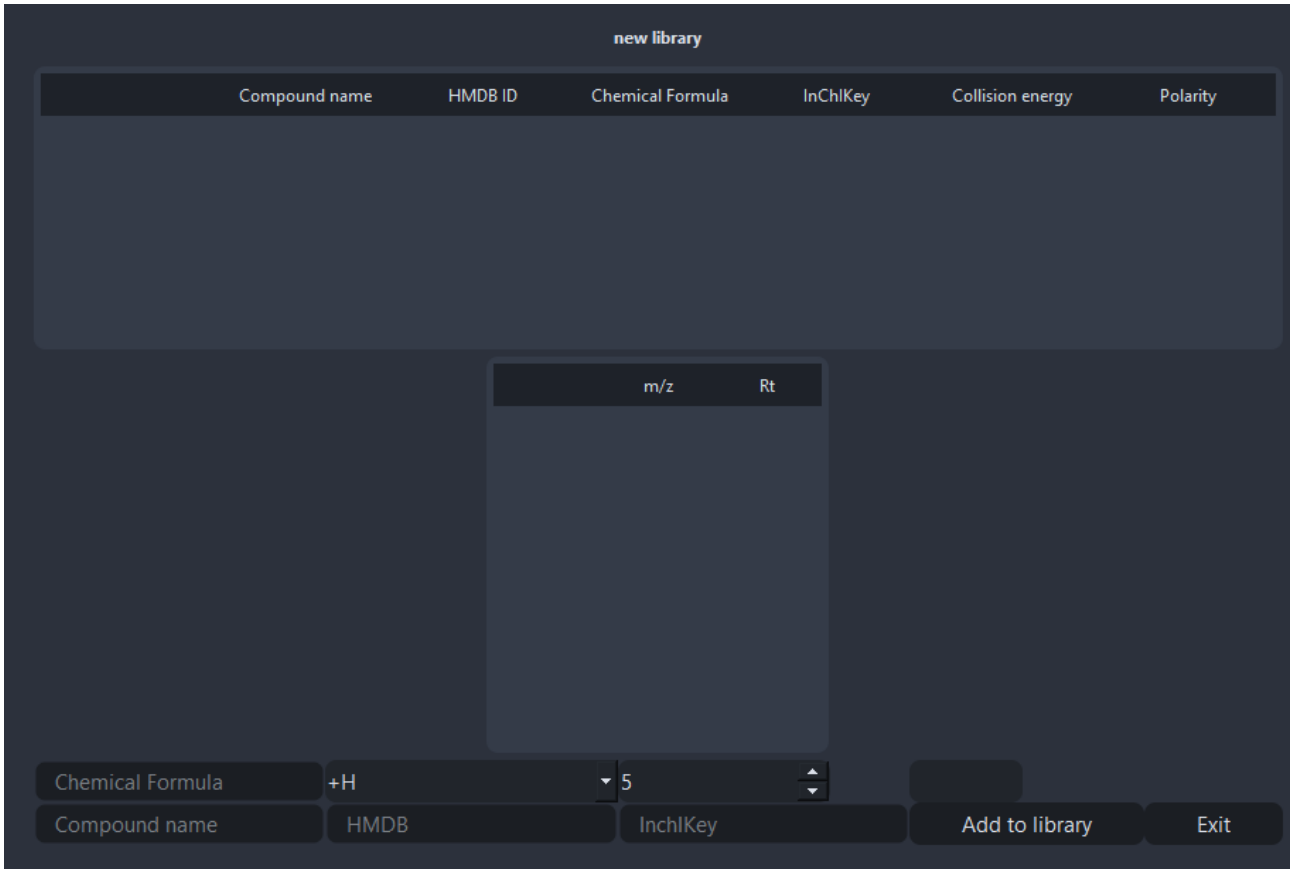


A new area will appear showing all the spectra available in the selected library. External libraries in msp format can be uploaded by using Add spectra from library file button.

Add spectra to library

By clicking «Add spectra from raw files», we can select a raw data in mzML format that contain the fragmentation spectra of our standards.

After loading the file, a new session will appear below.

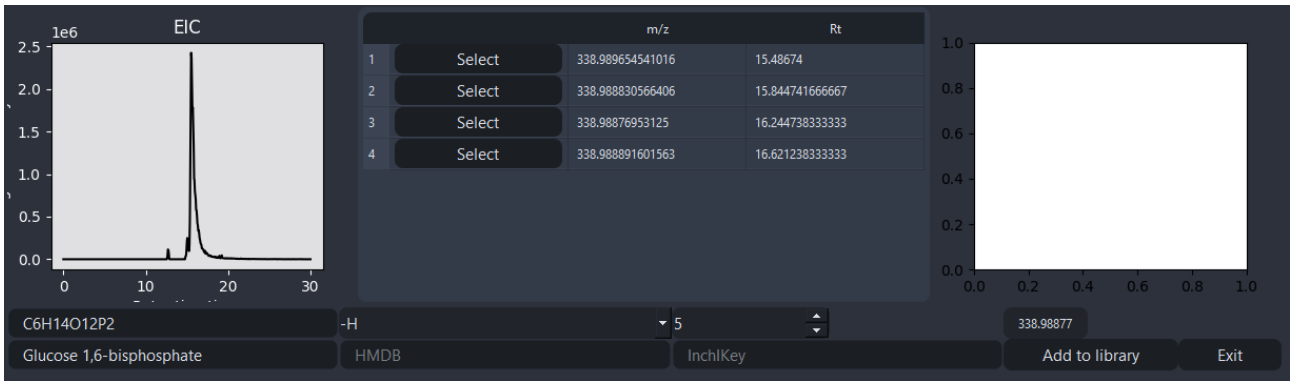


You can now search for your standard by type the chemical formula, adduct, ppm range and name.

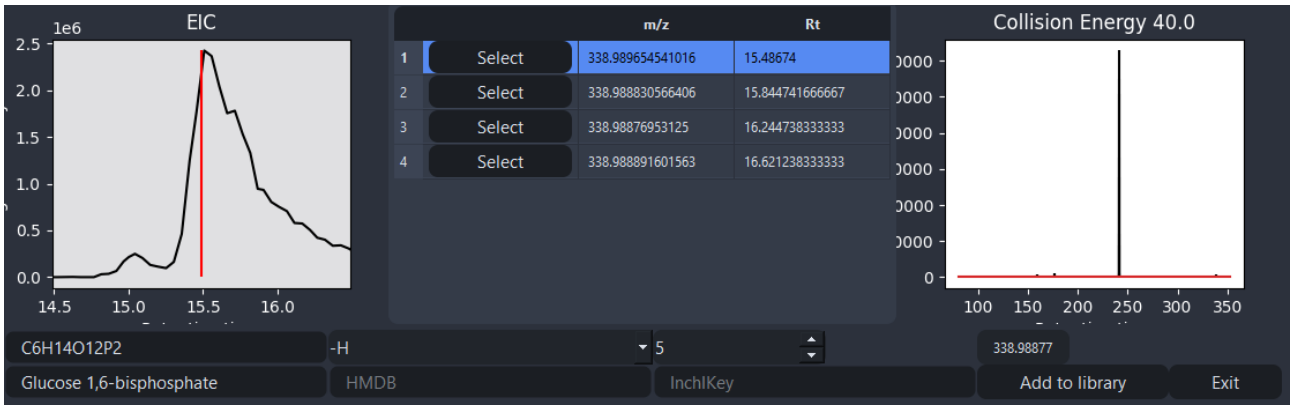
HMDB and InchIKey are optional parameters.

After filling up the required fields, the extracted ion chromatogram will appear on the left side.

In the central table, all MS/MS scan matching filter criteria will be displayed reporting the precursor found value and retetion time of the MS/MS event.

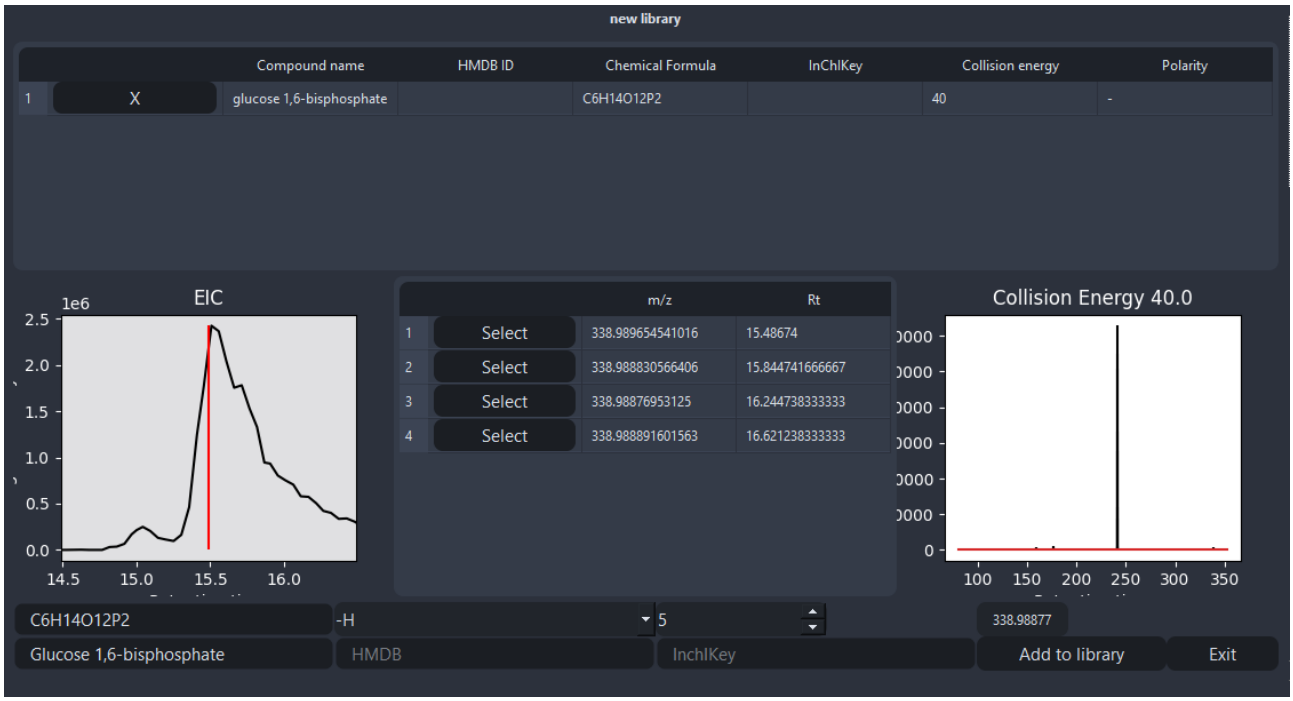


By clicking «Select» to one of the spotted MS/MS spectra, it will update both EIC and MS/MS windows. Showing as a red line the MS/MS event compared with the full-scan event (black line) in the EIC, and the MS/MS spectra with found collision energy on the right side of the screen. Both screen help in visualize the quality of the generate MS/MS scan prior selection.

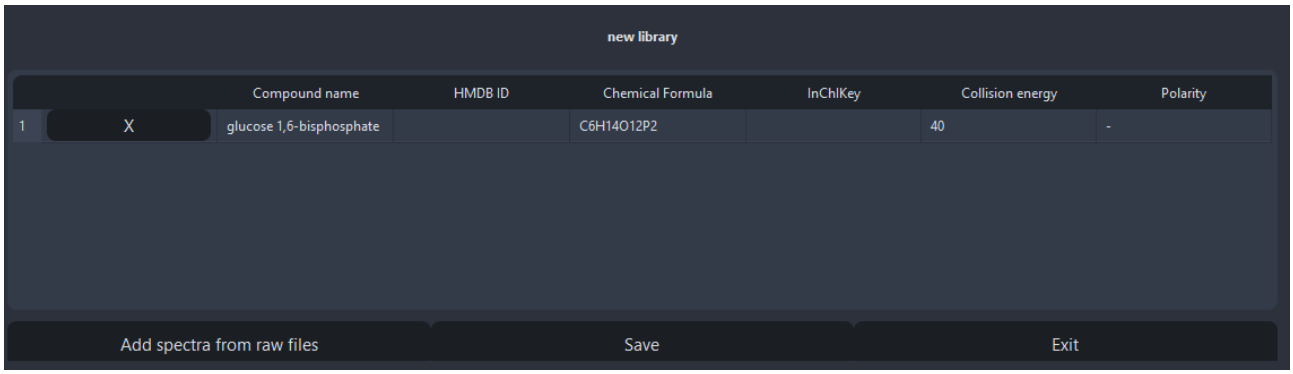


We can now click on «Add to library»,

The compound will now appear on the selected library.

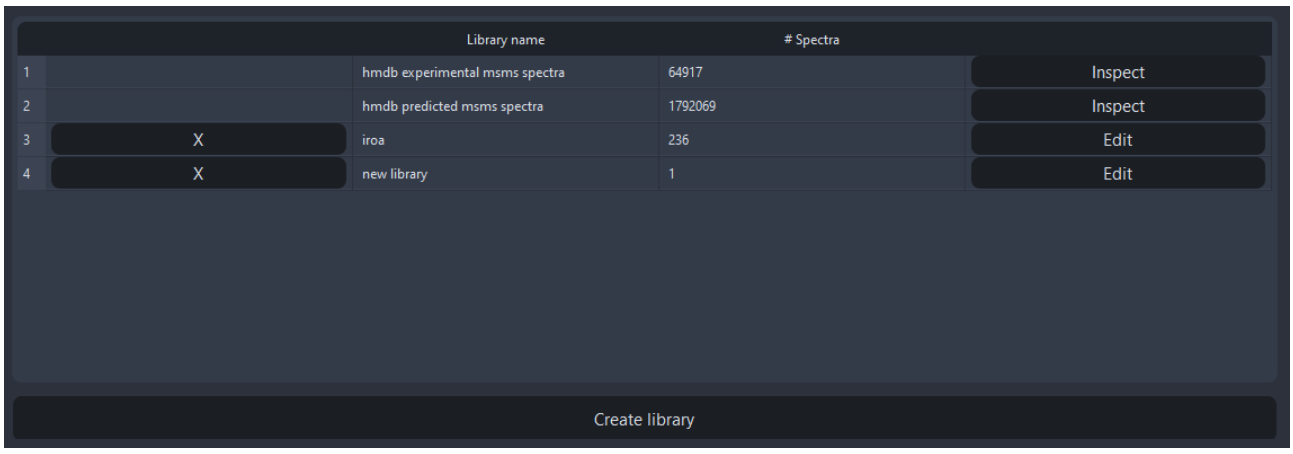


We can continue to include other MS/MS spectra from the selected files or click «Exit» to go back to the library editing screen.



We can now give a final inspection on the selected library and than click on «Save» to save current changes.

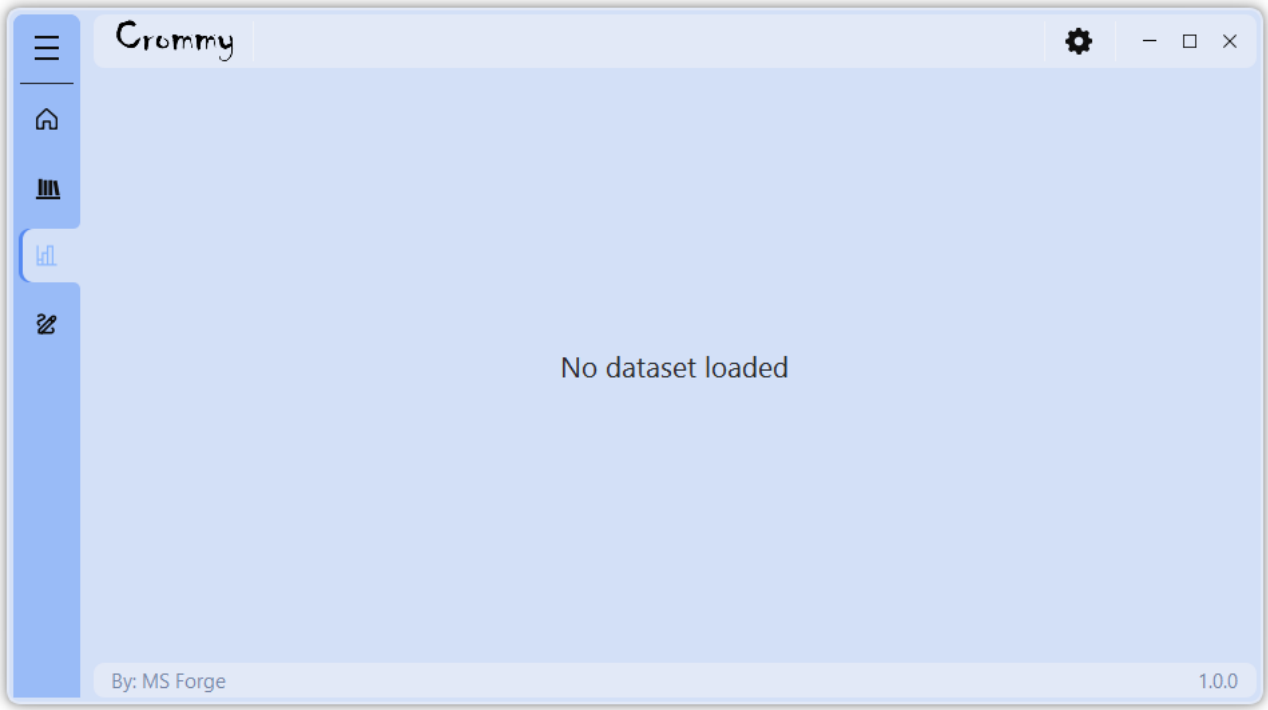
After saving, the number of spectra on the user library will update.



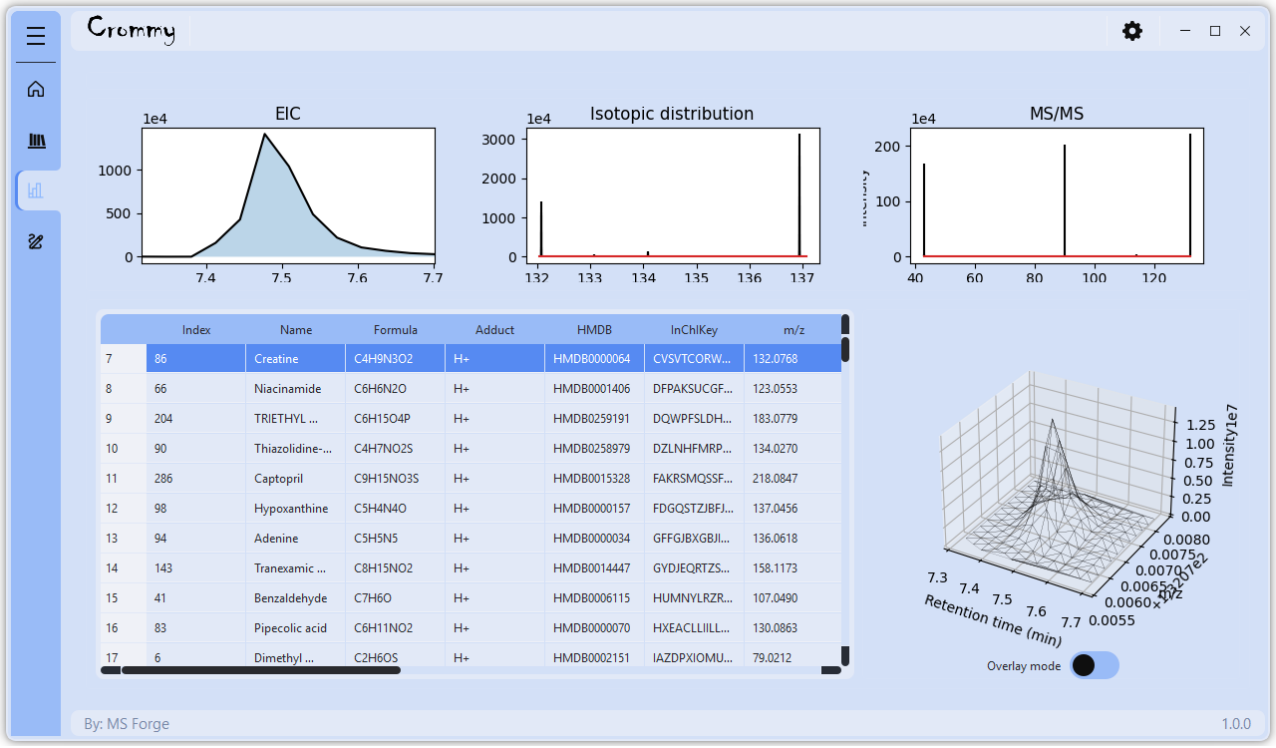
Warning: Common libraries and User libraries are stored on an online server. Poor connection may cause delay in the update process. Connection error (e.g. turning off connection during the process) may cause loss of data. In this case the Library page will change and the logo below will appear indicating missing communication between the machine and the server.

Annotation

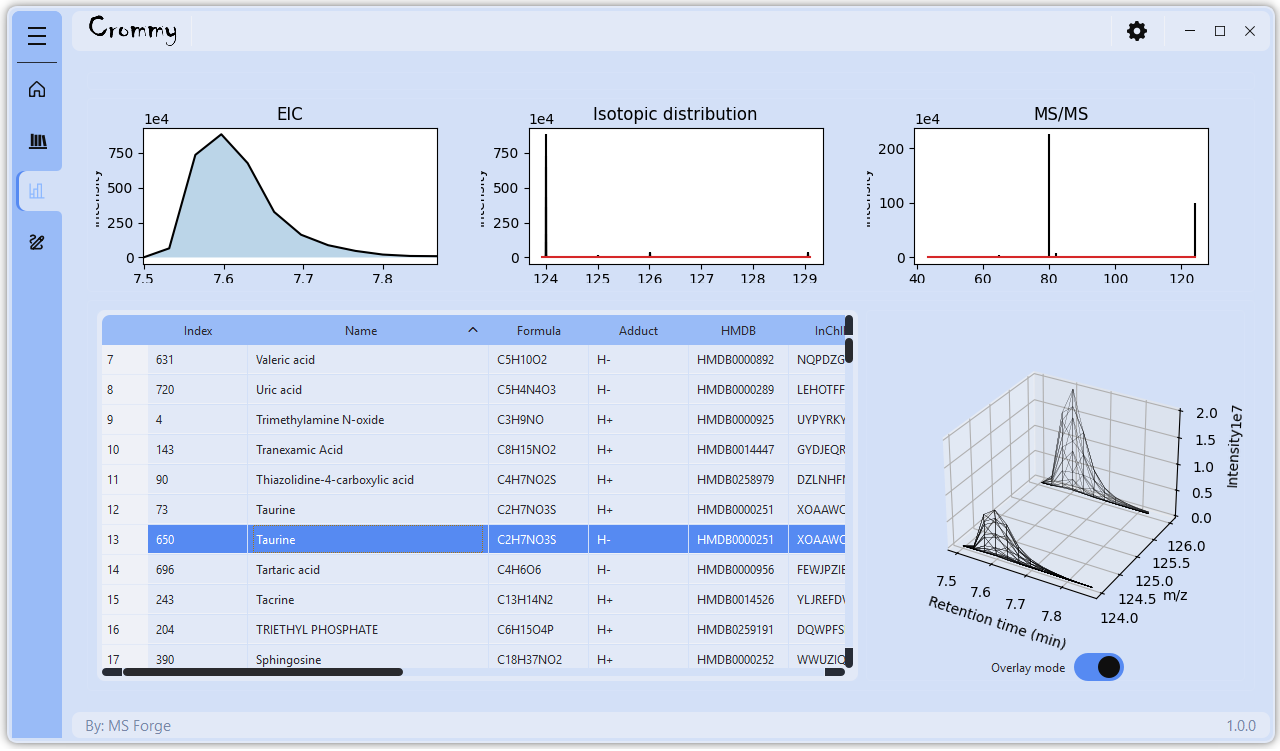
Direct infusion page allows to evaluate all the annotated compounds in the dataset. If no dataset is open (Home -> Open), or the analysis fail to find any compound this screen will appear.



Otherwise, if the annotation was correctly finished, the annotated compound will be displayed.



In this table, each compound represents a unique identified compound within the dataset. Multiple annotations are already resolved by Crommy e.g. if two peaks may have identical annotations based on mass and fragmentation spectra, the annotation is assigned only to the peak with the best MS2 score and lower MS2 FDR. The assigned compound is removed from the library and the second peak is evaluated against the new library without the already identified compound. Identical annotations may still be present in the dataset if multiple polarities are present.



In this example, we found Taurine twice with index 73 and 650 because this dataset contain data acquired in negative and positive mode and the two peaks represent the same compound at different polarities (H+ and H- adduct) as visible from the overlay of the two peaks in the 3D plot.

In this page, by double click with the left button on the 2d plots is possible to zoom out, while for zoom in we need to click and hold the left button on the x axis of the plot of interest and release the button to zoom in.

For each compound the following columns are present:

Index – This identifier is shared between the dataset, and it is particulary useful for matching peaks with unknown annotation.

Name – Compound name (annotated only otherwise is blank)

Formula – Chemical Formula (annotated only otherwise is blank)

Adduct – Chemical adduct (annotated only otherwise is blank)

HMDB – Human Metabolome ID (annotated only otherwise is blank)

InchIKey – International Chemical Identifier

m/z – mass on charge value

rt – retention time expressed in minutes

charge – evaluated charge

height – maximum peak intensity

area – representative peak area

MS2 type – report the acquisition mode i.e. DDA, DIA or if it is a target compoudn

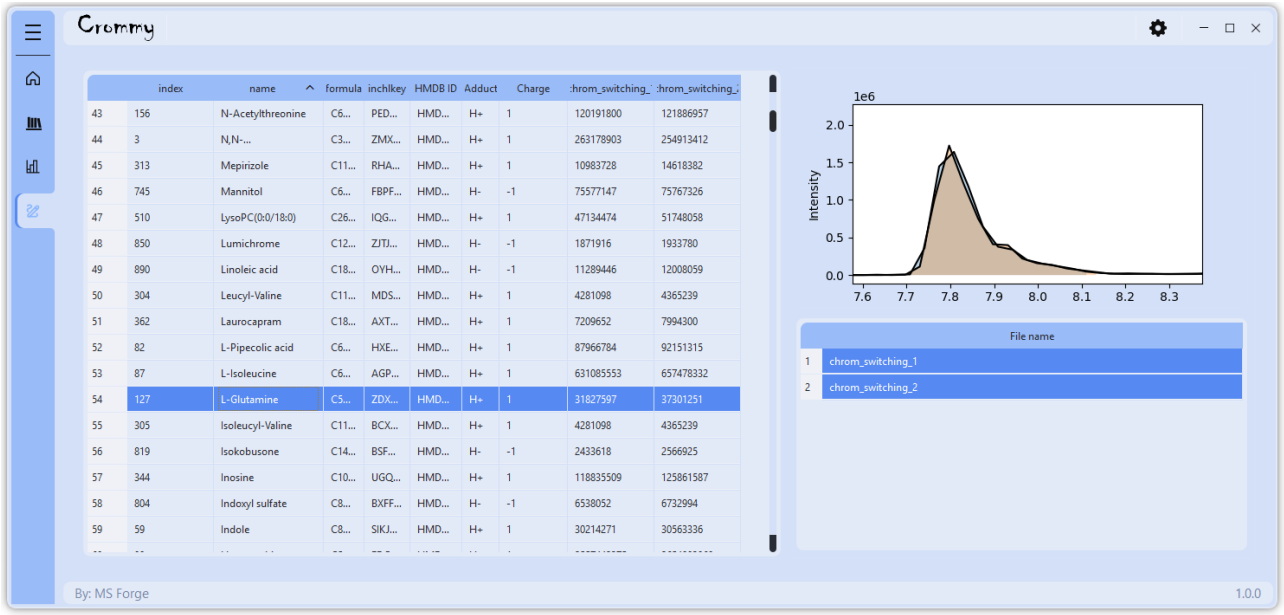
Ppm – part per million error between the m/z and the chemical formula (annotated only otherwise is blank)

MS2 score – best annotation score against the library relative to annotation

MS2 FDR – false discovery rate estimation based on permutation of the best matching fragmentation spectra in the library

Integration

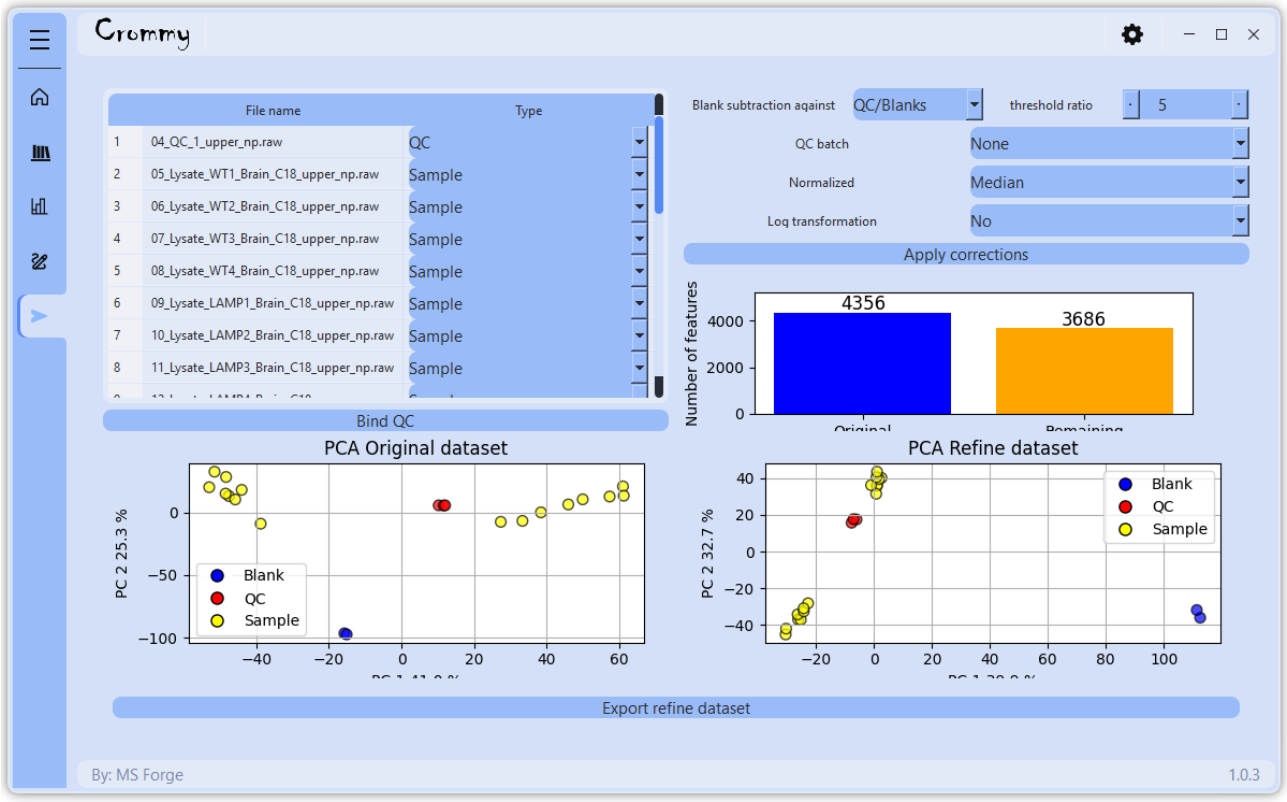
After loading an acquired dataset (Home – Open), by clicking on Integration Icon we can now display the results or our analysis.



Together with the summary information for each compound, in the main table we can read the peak area in each sample. To visualize the integrated peak areas, we can select one compound from the main table and the list of chromatograms from the File name table. If we select more than one chromatograms, the peaks are overlayed on the same image space. Double click with left button will zoom out in the chromatogram, while holding the left button and selecting a region on x axis will zoom in. In similar way, by holding the right button and selecting a region on x axis we can reintegrate all the peaks that are displayed. Peak area of the reintegrated peaks will be updated automatically.

By pressing the right button on the central table, we can export the results in Excel.

Data Pre-Processing



We can now pre-process the results of our experiment using Crommy. In this pannel, all samples related to the dataset are included in the left table. We can now modify sample types in order to correct errors, if any, in the sample labels. We can also change the type to “exclude” in order to remove samples from the analysis.

We can now applied different refinements including features subtraction by comparing blanks and QC or blanks and Samples, QC batch correction by binding each sample to its QC, normalization and log scaling of the dataset and click on Apply Correction button. We can now display samples distribution of original data (bottom left) and refine dataset (bottom right) using the first 2 components from a PCA analysis. Both PCA can be exported by right click on the PCA of interest.

Crommy custom tools

The mission of MS Forge is to create software that lightens the workload and increases production in analytical chemistry labs. We are aware that each user may have different needs and requests for their analysis, but may not have the time, financial support, or expertise to develop what it lacks.

For this reason, you can request custom modifications of Crommy to better adapt to your personal needs. From having a different color palette for the GUI to adding a brand-new page with missing functionalities and AI integration, just ask us!

info@msforge.it

or

https://www.msforge.it/

by selecting “Request custom content” on Reason field.

Our team will carefully evaluate your request in terms of feasibility, interest to the general audience, and compliance with general and internal rules.

If we consider the custom modification to be feasible and of general interest, WE WILL DEVELOP IT FOR FREE and include it in the release of the Crommy updates, unless the custom request is expressly requested to be kept private.

Otherwise, if it is doable but not suitable for an Crommy update, we will contact you with an offer regarding the custom request.

Note: Custom requests are accepted only from users owning a regular license (Demo not included).