User Manual version 1.0

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

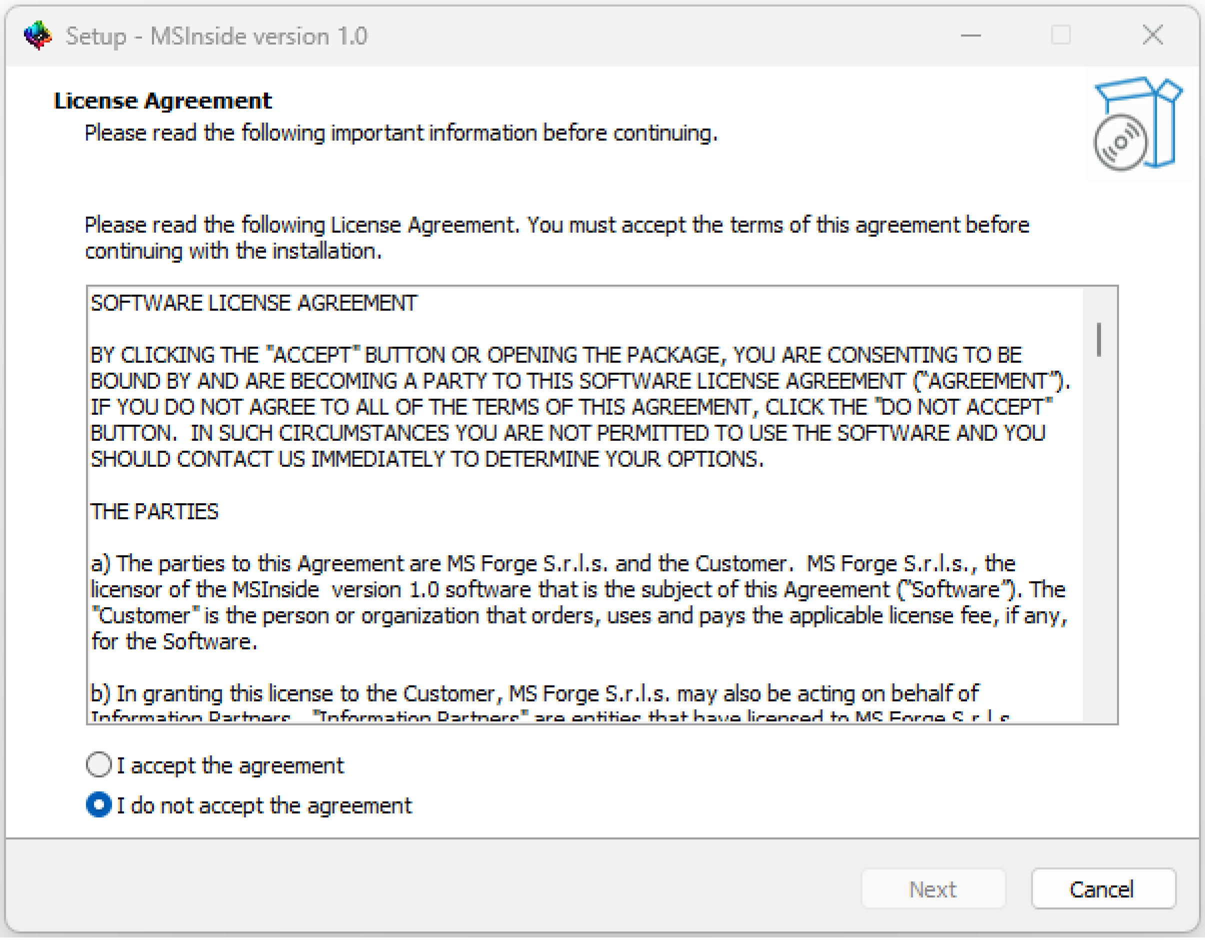
Summary

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.

Installation

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



License Agreement

Please read the following important information before continuing.

Please read the following License Agreement. You must accept the terms of this agreement before continuing with the installation.

SOFTWARE LICENSE AGREEMENT

BY CLICKING THE "ACCEPT" BUTTON OR OPENING THE PACKAGE, YOU ARE CONSENTING TO BE BOUND BY AND ARE BECOMING A PARTY TO THIS SOFTWARE LICENSE AGREEMENT ("AGREEMENT"). IF YOU DO NOT AGREE TO ALL OF THE TERMS OF THIS AGREEMENT, CLICK THE "DO NOT ACCEPT" BUTTON. IN SUCH CIRCUMSTANCES YOU ARE NOT PERMITTED TO USE THE SOFTWARE AND YOU SHOULD CONTACT US IMMEDIATELY TO DETERMINE YOUR OPTIONS.

THE PARTIES

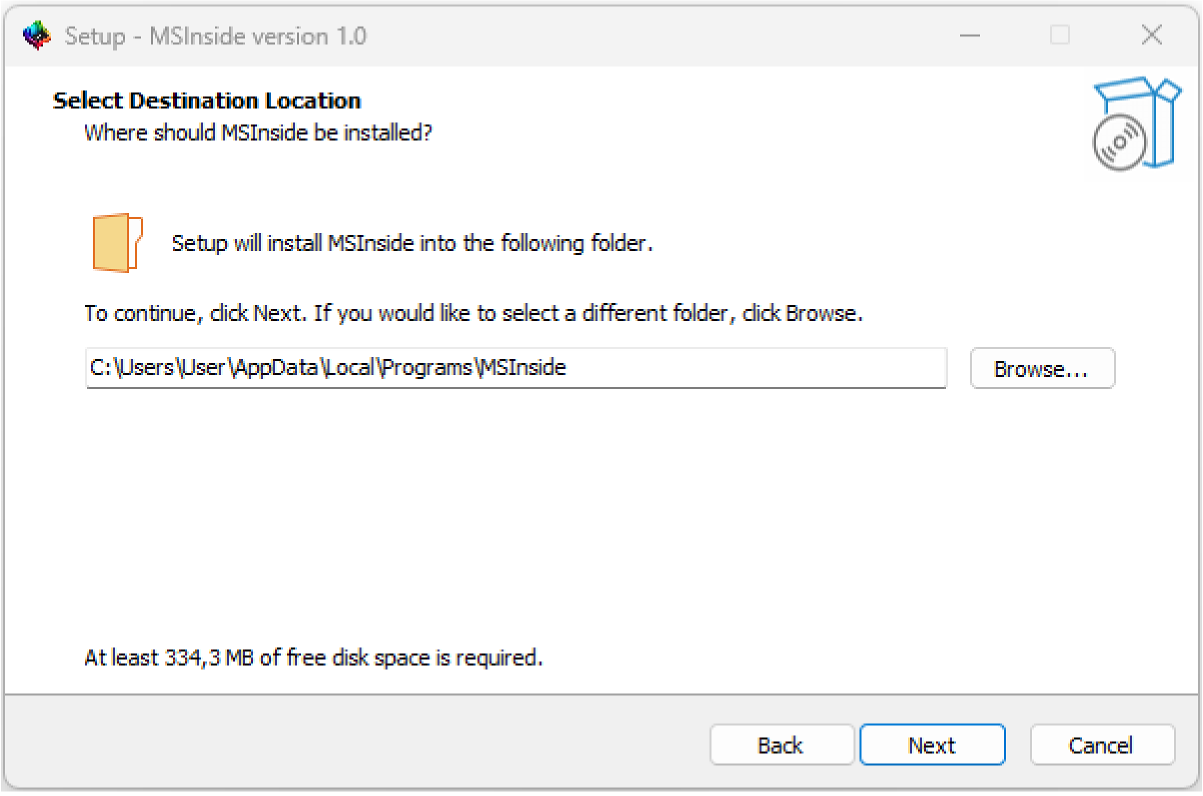
a) The parties to this Agreement are MS Forge S.r.l.s. and the Customer. MS Forge S.r.l.s., the licensor of the MSInside version 1.0 software that is the subject of this Agreement ("Software"). The "Customer" is the person or organization that orders, uses and pays the applicable license fee, if any, for the Software.

b) In granting this license to the Customer, MS Forge S.r.l.s. may also be acting on behalf of

○ I accept the agreement

● I do not accept the agreement

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



Select Destination Location

Where should MSInside be installed?

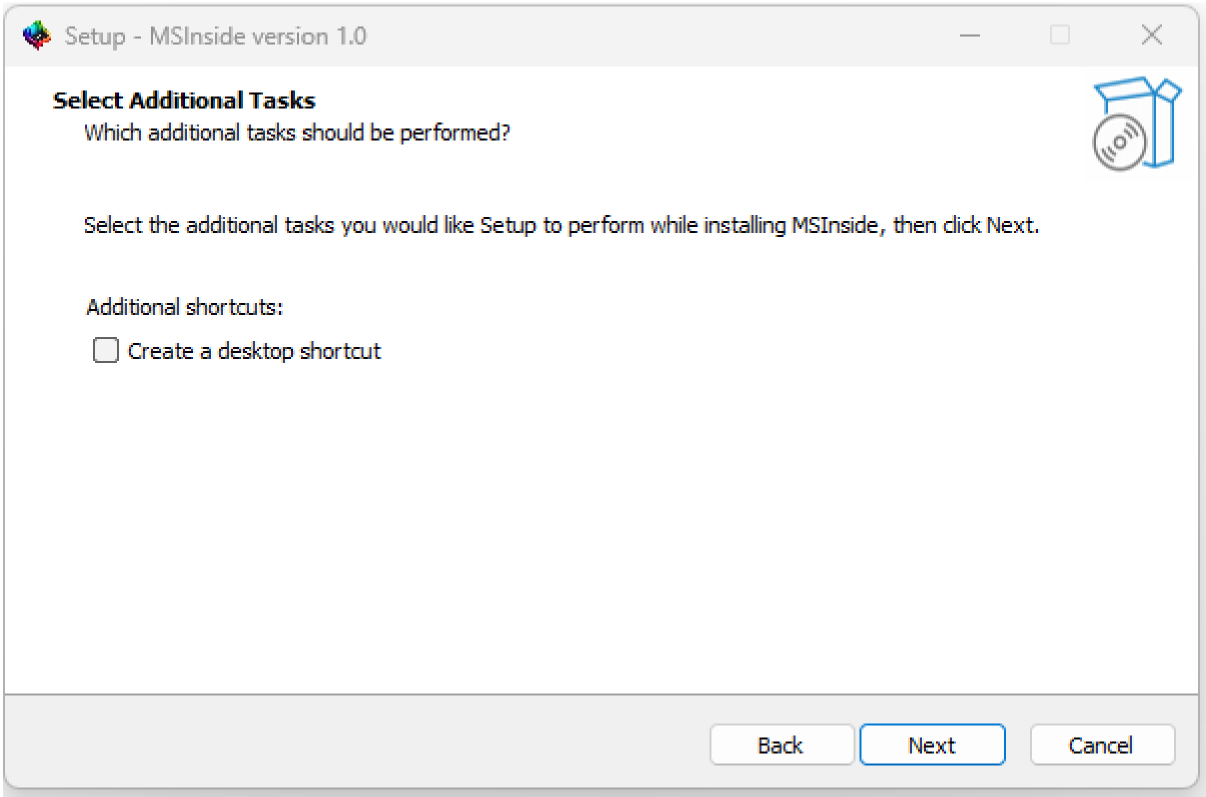
Setup will install MSInside into the following folder.

To continue, click Next. If you would like to select a different folder, click Browse.

C:\Users\User\AppData\Local\Programs\MSInside

At least 334,3 MB of free disk space is required.

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



Select Additional Tasks

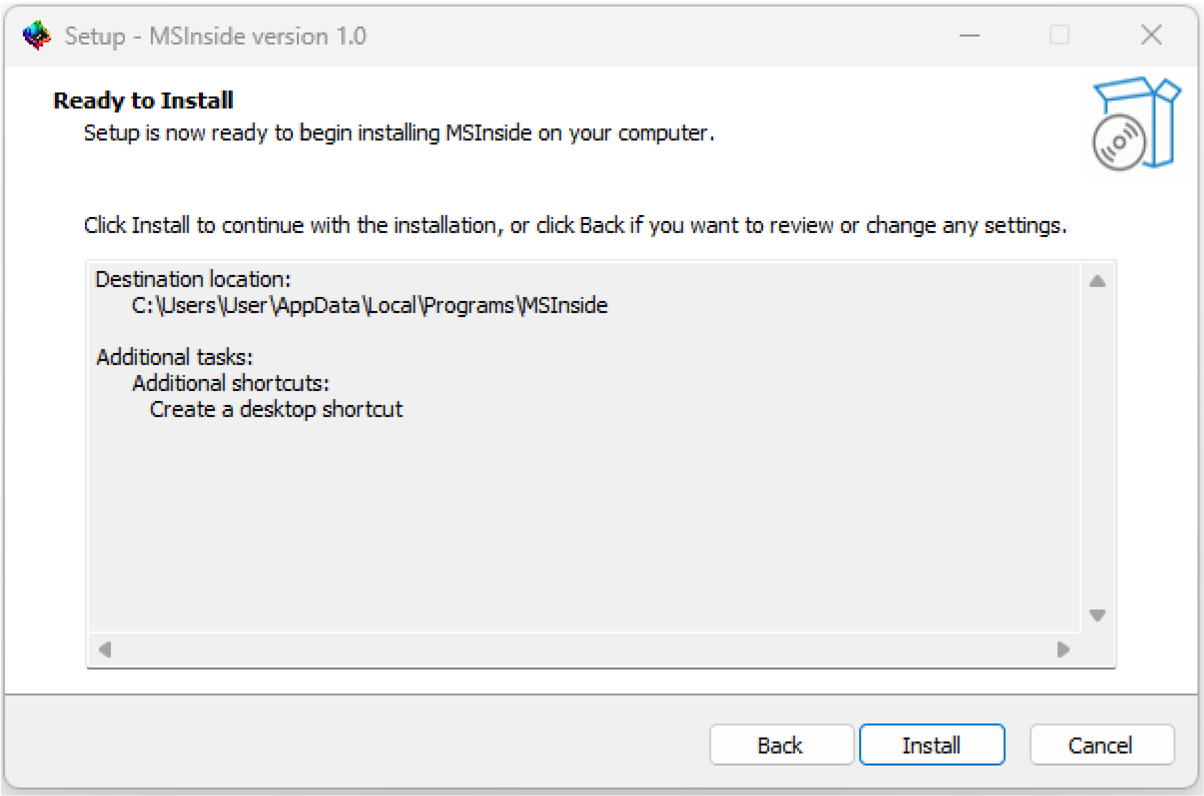
Which additional tasks should be performed?

Select the additional tasks you would like Setup to perform while installing MSInside, then click Next.

Additional shortcuts:

☐ Create a desktop shortcut

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



Ready to Install

Setup is now ready to begin installing MSInside on your computer.

Click Install to continue with the installation, or click Back if you want to review or change any settings.

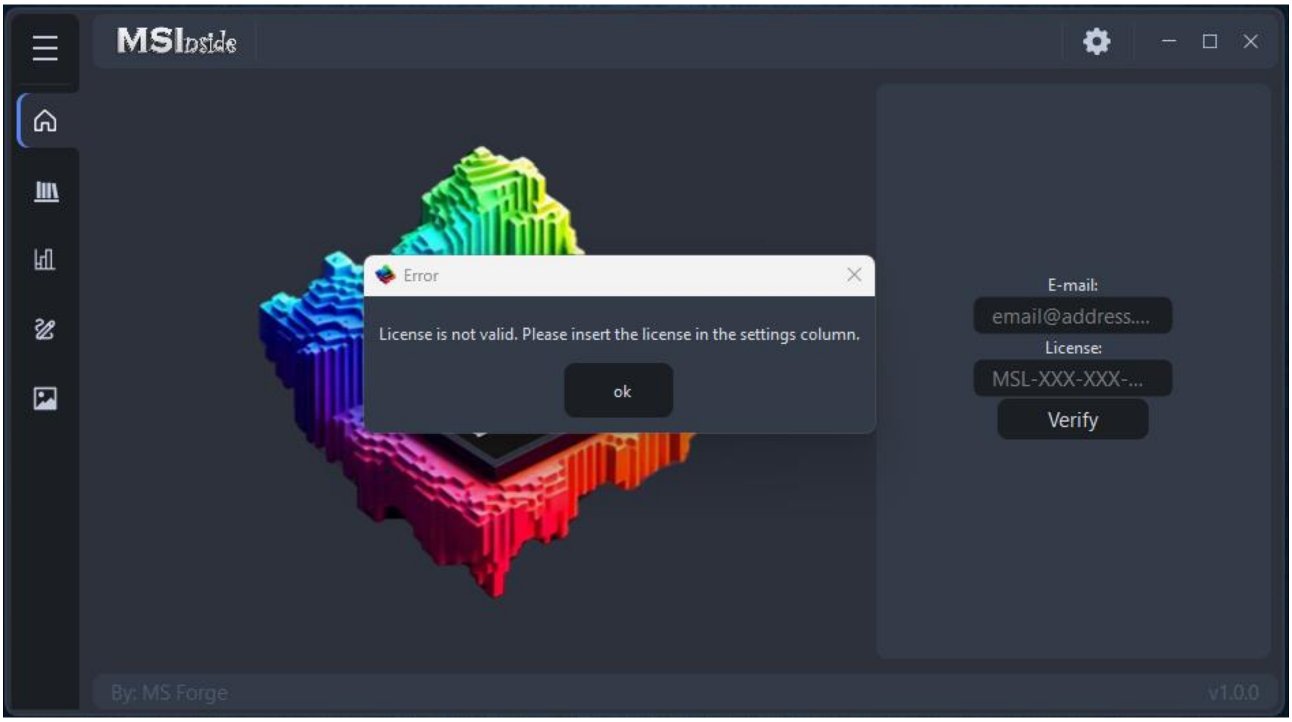
Destination location:
C:\Users\User\AppData\Local\Programs\MSInside

Additional tasks:
Additional shortcuts:
Create a desktop shortcut

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

MSInside activation

After starting MSInside, the following screen will appear:



Error

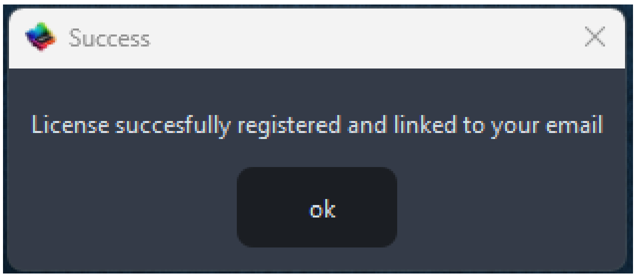
License is not valid. Please insert the license in the settings column.

Click «Ok».

Enter a valid email and a valid Licence Key.

Press «Verify»

A new popup will appear confirming successful registration.



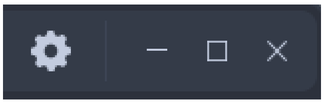
Success

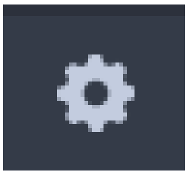
License succesfully registered and linked to your email

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

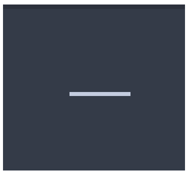
MSInside top bar

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

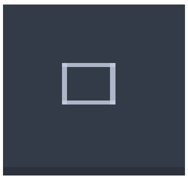




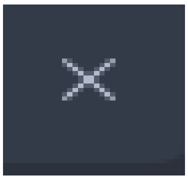
Setting icon display the registration space if the software is not registered yet, otherwise by clicking on setting it will appear a page with the button «User manual». By clicking on the button, this manual will open automatically.



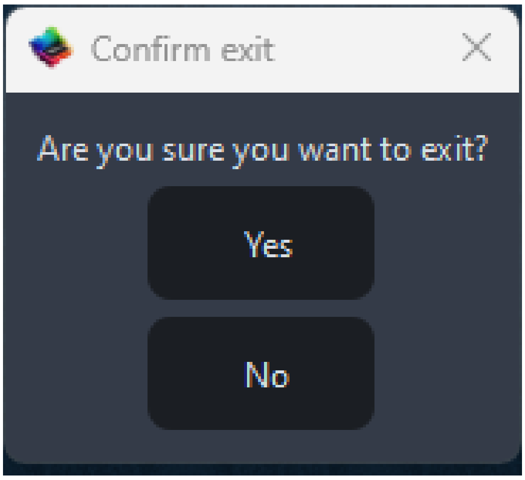
By clicking this button, MSInside will minimize.



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



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



Confirm exit

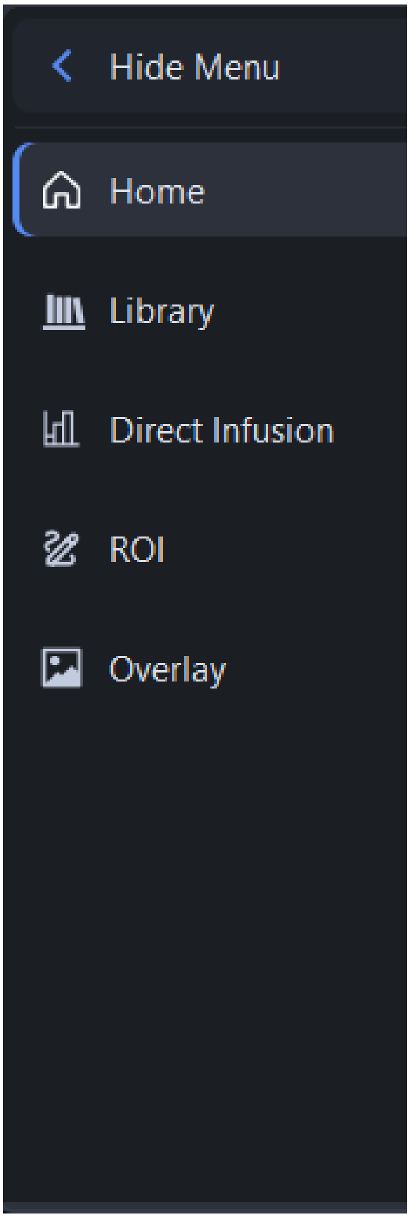
Are you sure you want to exit?

MSInside left bar

MSInside 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.

Direct Infusion

Display compound annotation and relative scores.

ROI

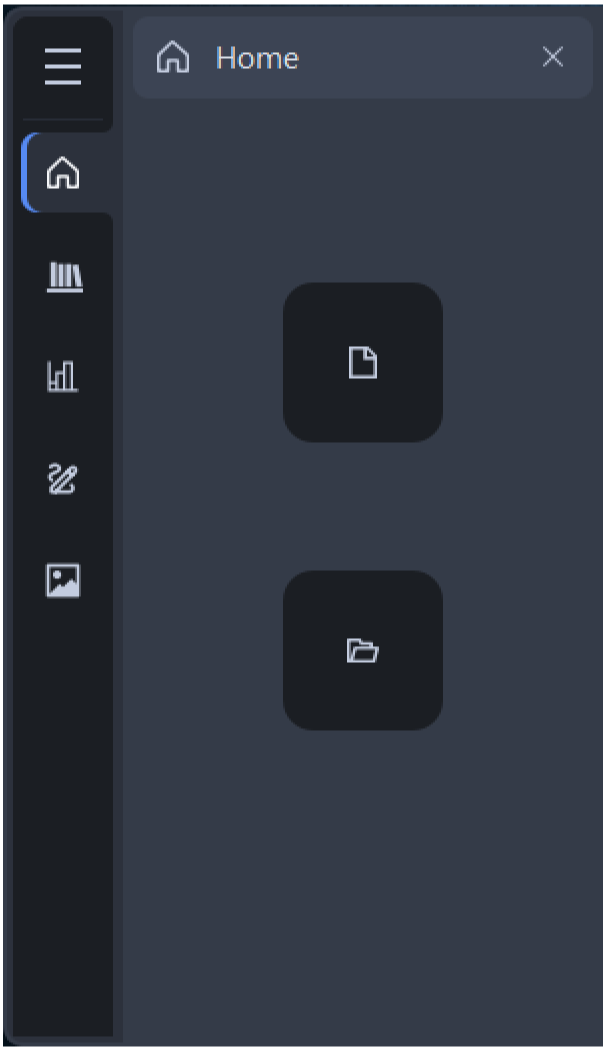
Visual representation of extracted ion maps, ROI selection and ROI export

Overlay

Visual representation of extracted ion maps and multiple color channels for EIMs overlay.

Home

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

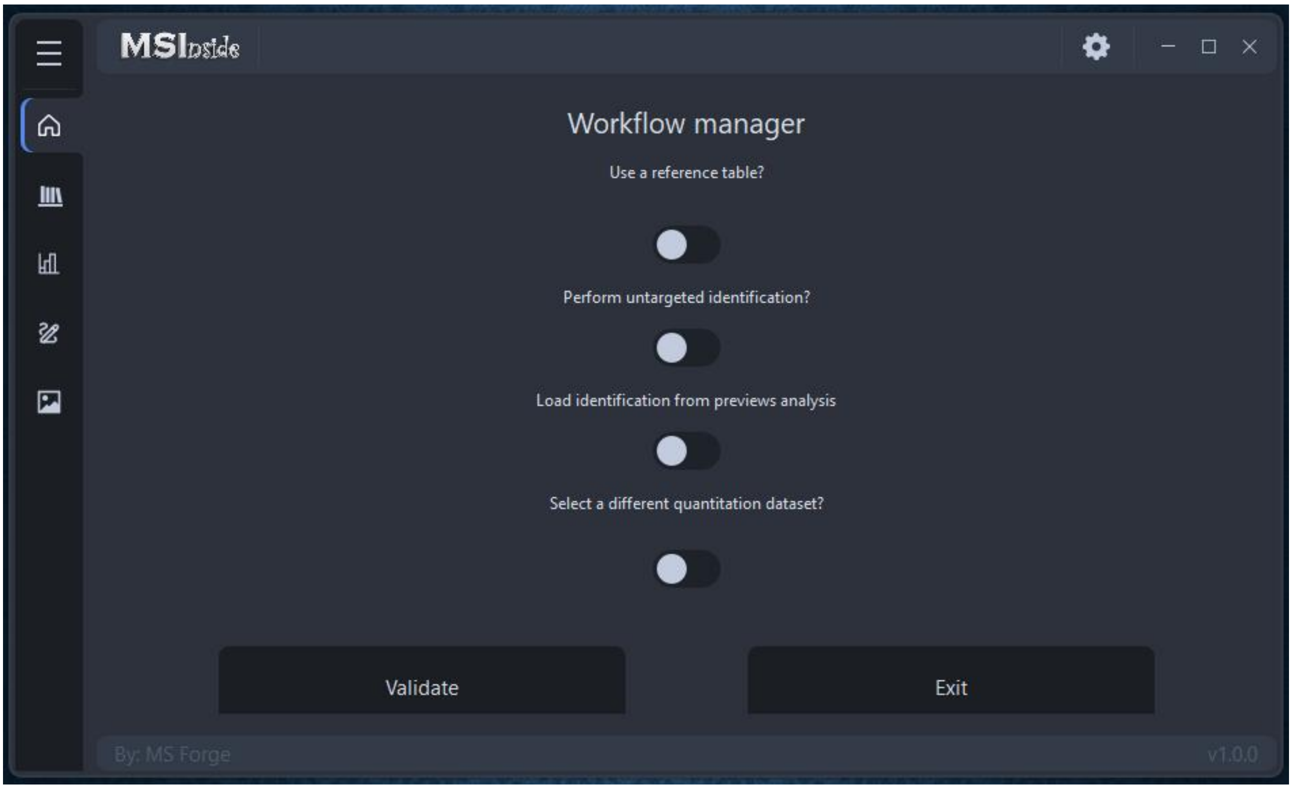


Start a new project.

Open analyzed project

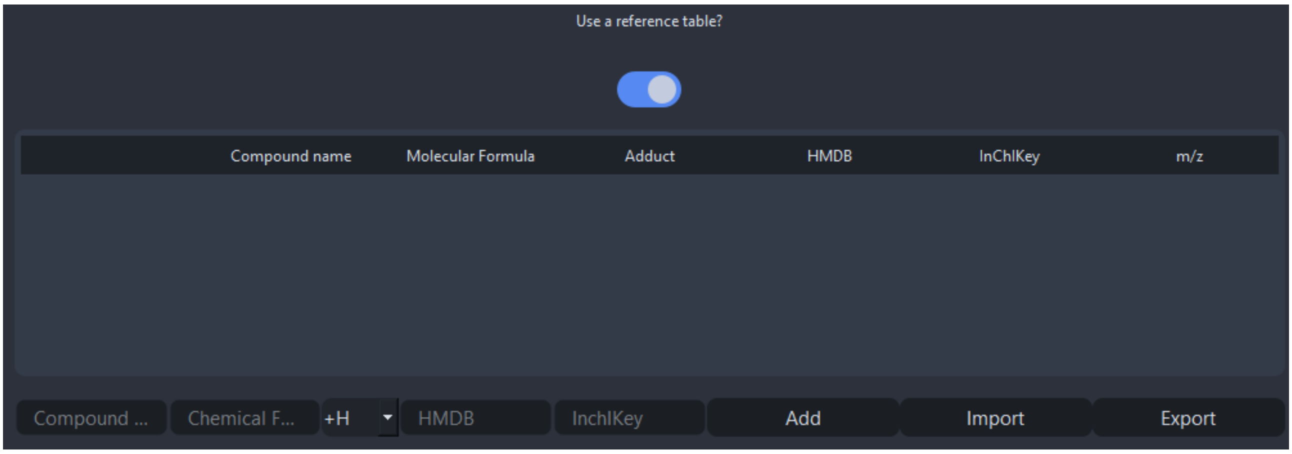
Start new project

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



Different option can be activated depending on the type of analysis.

Use a reference table

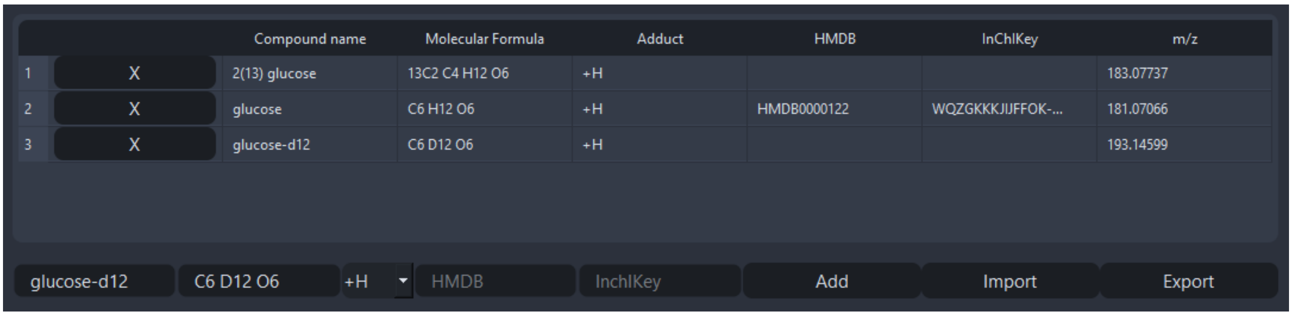


If selected, this session will expand giving the possibility to include compounds as conventionally done in targeted analysis.

Compound name and Chemical formula are mandatory fields.

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

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

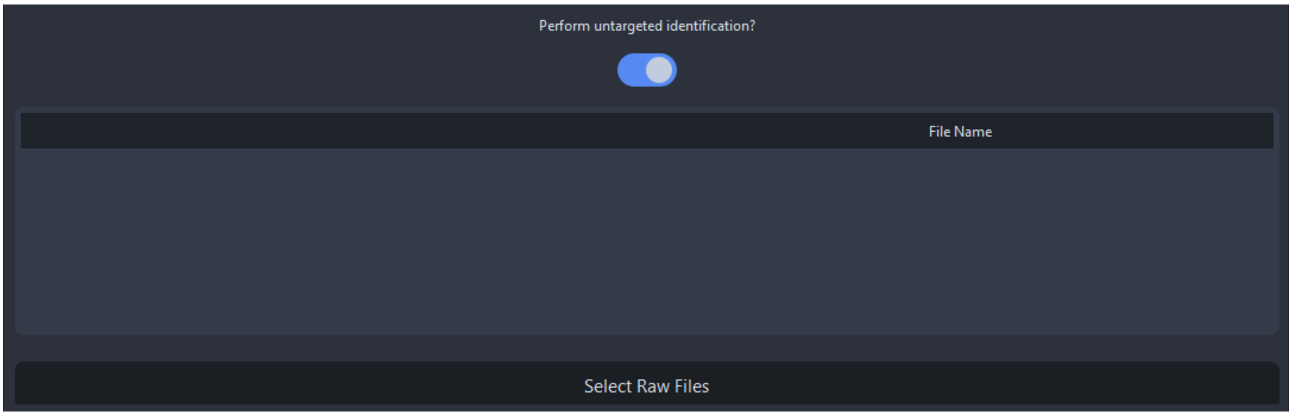


		Compound name	Molecular Formula	Adduct	HMDB	InChIKey	m/z
1	X	2(13) glucose	13C2 C4 H12 O6	+H			183.07737
2	X	glucose	C6 H12 O6	+H	HMDB0000122	WQZGKKKJIJFFOK-...	181.07066
3	X	glucose-d12	C6 D12 O6	+H			193.14599

glucose-d12 | C6 D12 O6 | +H

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

Perform untargeted identification



Selection this option will expand a new table. By clicking «Select Raw Files» you can browse for raw files for identification.

MSInside accept raw files in mzML format only.

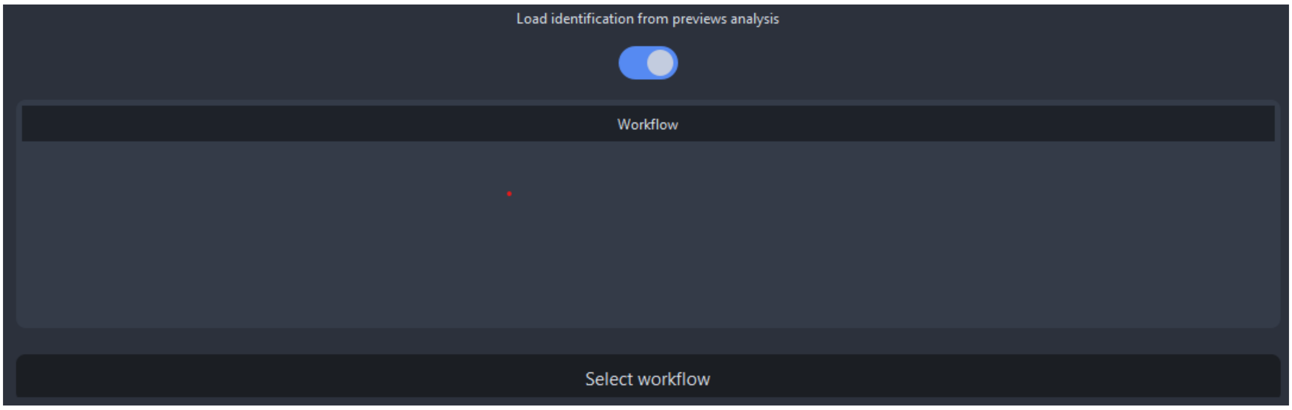
For this step, raw files should be acquired in data-dependent mode. You can upload any kind of DDA file from MALDI-MS experiment, as well as from LC-MS, FIA-MS or DI-MS.

Raw files can be located in different folders. Each time that a list of files is selected by “Select Raw Files”, it will be added to the identification list. In order to remove files from the list, an X button will appear near each uploaded file.

Note: MSInside is design to maximize the coverage and the confidence of annotation for Mass Spectrometry Imaging and therefore the annotation is adapted to this purpose and not suitable for annotation in other approach such as metabolomics experiments with LC-MS.

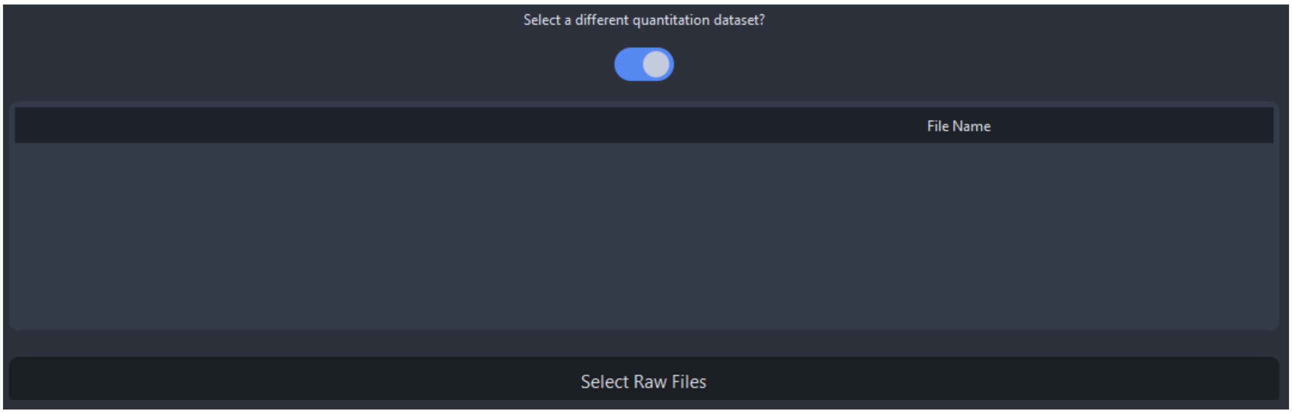
Note: We recommend using DDA acquired or transformed in profile mode for full scan MS and in profile mode for MS/MS spectra as it maximized the quality of annotation. However, we recommend testing empirically the annotation quality by testing different acquisition condition as the used instruments may differ from what already tested by us.

Load identification from previous analysis



When this option is selected, a workflow file from a previous analysis can be selected by clicking on “Select Workflow”. MSInside will retrace all the annotation acquired in the previous dataset and use for analysis in the working dataset.

Select dataset for quantification



When this option is activated, a list of raw files can be selected from MALDI-MS experiments. By clicking «Select Raw Files» you can browse for raw files for quantitation. MSInside accept raw files in mzML format only (not imzML).

If this option is not selected, MSInside will use the list of raw files selected for identification. This option is valid only if identification raw files derived from MALDI-MS experiment with DDA.

Warning: MALDI-MS experiment required also XML files. mzML and XML mast have the same name and be located within the same folder.

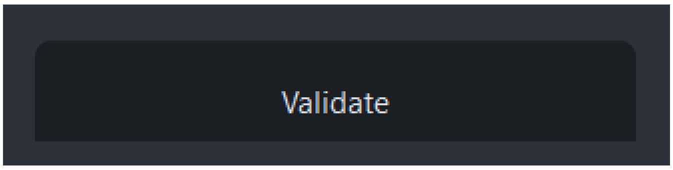
Workflow modularity

The workflow manager will accept the selection of multiple option for the analysis. In the most complete scenario with all option selected, MSInside will evaluate all possible annotation from targeted list, previous acquisition and discovered annotation in untargeted analysis.

Following, the extracted ion maps from the generate cumulative annotation list will be created for each file selected for quantitation.

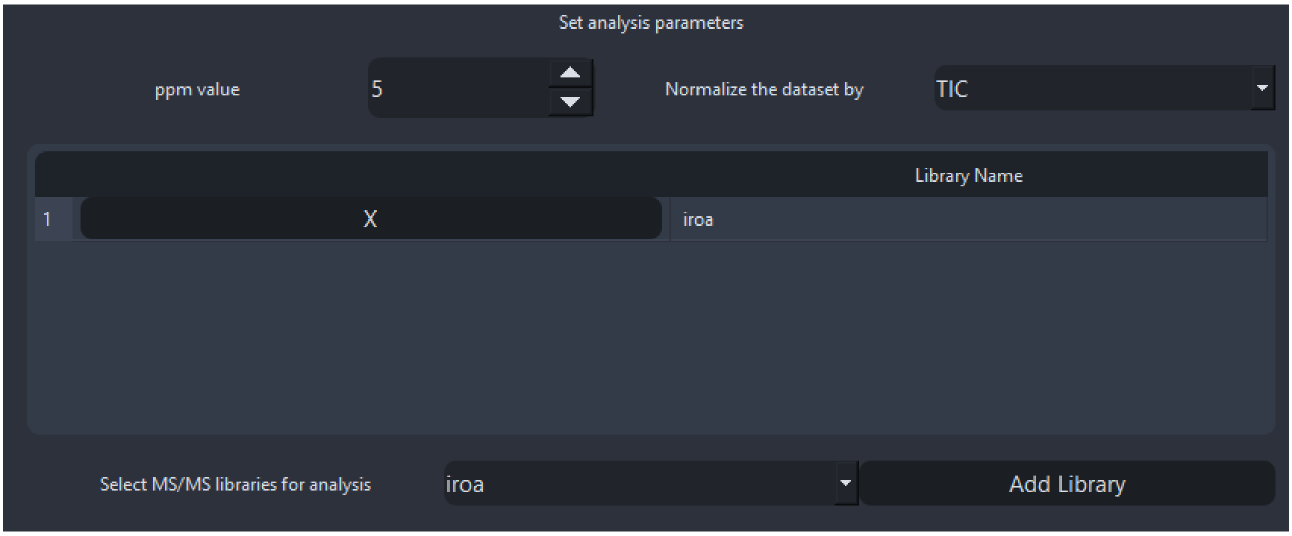
Targeted compounds have higher priority over annotation from untargeted compounds, therefore if the same feature is found both with targeted and untargeted, only the targeted will be returned at the end of the analysis.

After completing the workflow, we can now click on “Validate” button.



If minimum requirements for an analysis are met (e.g. at least the presence of one identification method, and at least one valid file for quantitation), the final workflow panel will appear.

Analysis parameter


Set analysis parameters

ppm value: 5

Normalize the dataset by: TIC

		Library Name
1	X	iroa

Select MS/MS libraries for analysis: iroa

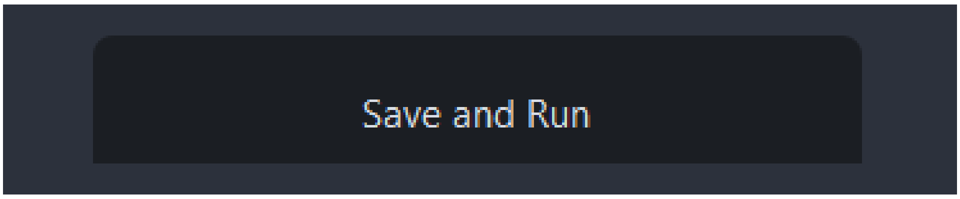
We can now set the analysis parameters.

Part per million value (ppm): this parameter influence the entire analysis. We recommend to select a range in line with the performance of the instrument used for the analysis considering both files for annotation and quantitation.

Normalization can be performed by Total Ion Current (TIC) or not performed by selectin “None”.

On the last table, a list of libraries is available for the analysis and can be added by clicking on Add Library. Those libraries represent available MS/MS library spectra for the analysis (See Library chapter). Multiple libraries can be selected for the analysis. Selection of at least on library is mandatory when untargeted analysis is included in the workflow as MSInside.

After completing the selection of the parameter, we can now click on Save and Run button.



A new window will open, asking for save a workflow file.

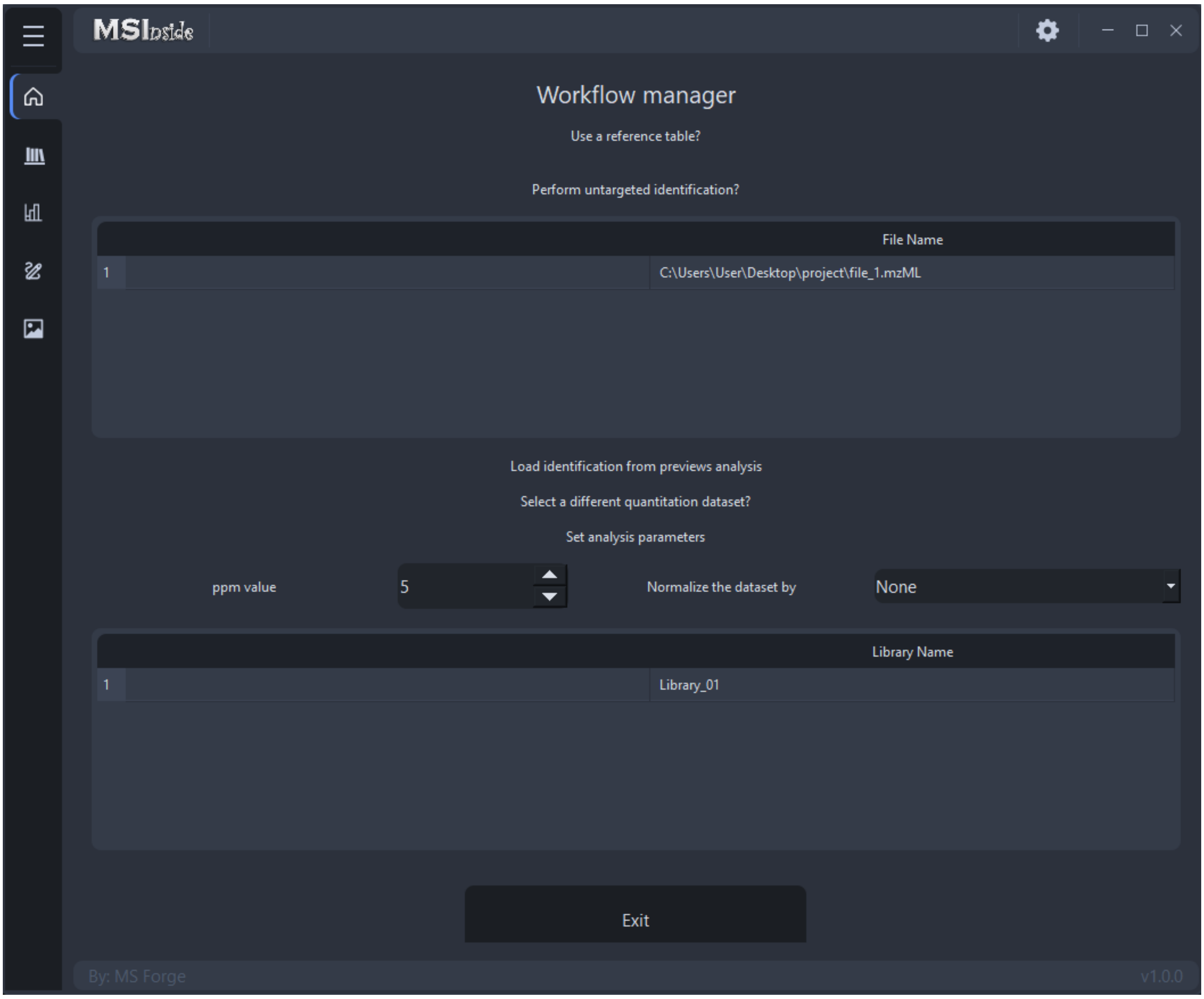
The workflow file can be saved anywhere, and don’t need to be in the same folder with the raw files.

Warning: matafiles are generated inside a folder containing a workflow file. Do not move the workflow file or any of the generated metafiles and folders after analysis.

Warning: do not save the workflow file into a folder containing previous analysis as it may override metafiles from previous analysis.

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.



	File Name
1	C:\Users\User\Desktop\project\file_1.mzML

ppm value: 5

Normalize the dataset by: None

	Library Name
1	Library_01

Direct infusion, ROI, and Overlay are now linked to the project and can be used for exploring the results.

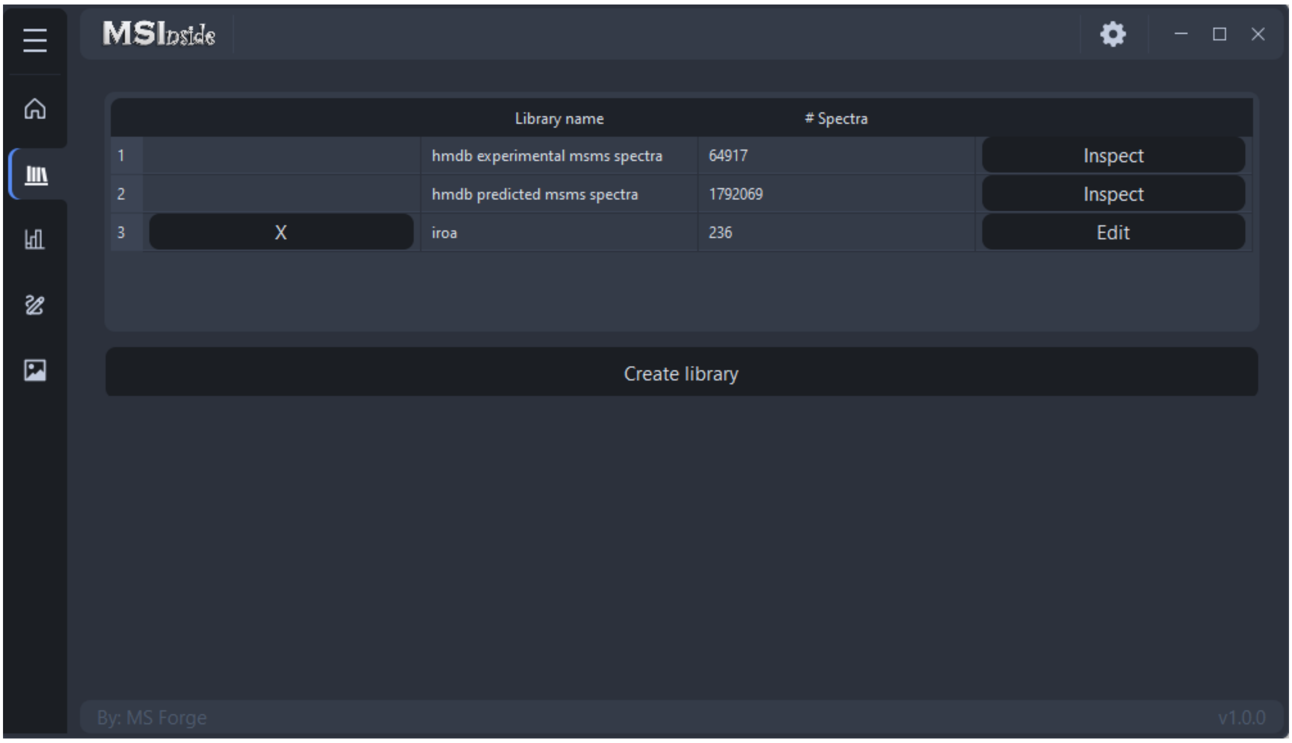
Library

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.



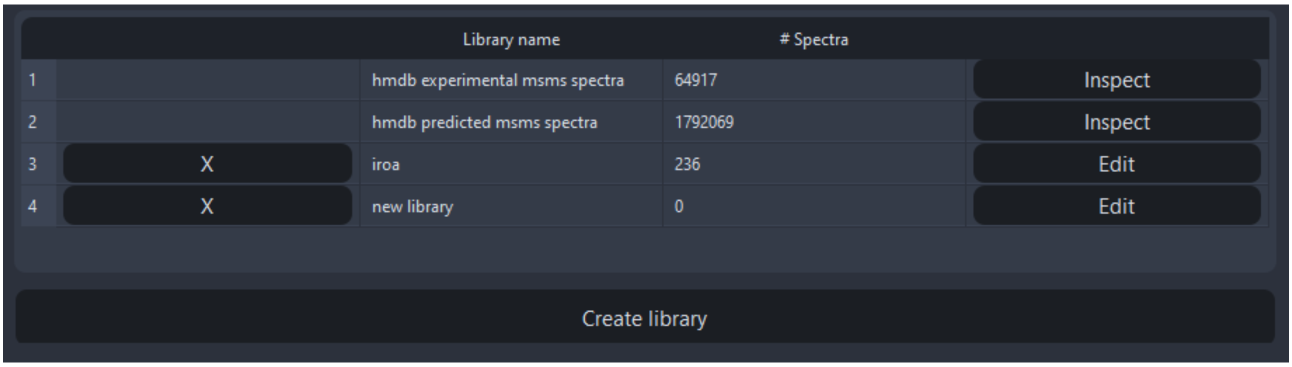
		Library name	# Spectra	
1		hmdb experimental msms spectra	64917	Inspect
2		hmdb predicted msms spectra	1792069	Inspect
3	X	iroa	236	Edit

Create a new library

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

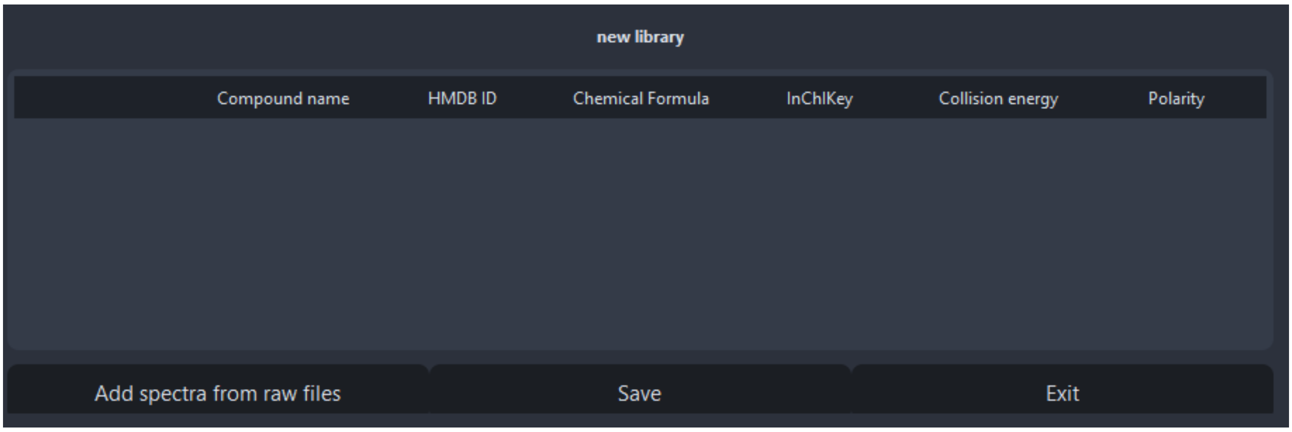
You can now type the name of the new library and click Create.

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



		Library name	# Spectra	
1		hmdb experimental msms spectra	64917	Inspect
2		hmdb predicted msms spectra	1792069	Inspect
3	X	iroa	236	Edit
4	X	new library	0	Edit

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



new library

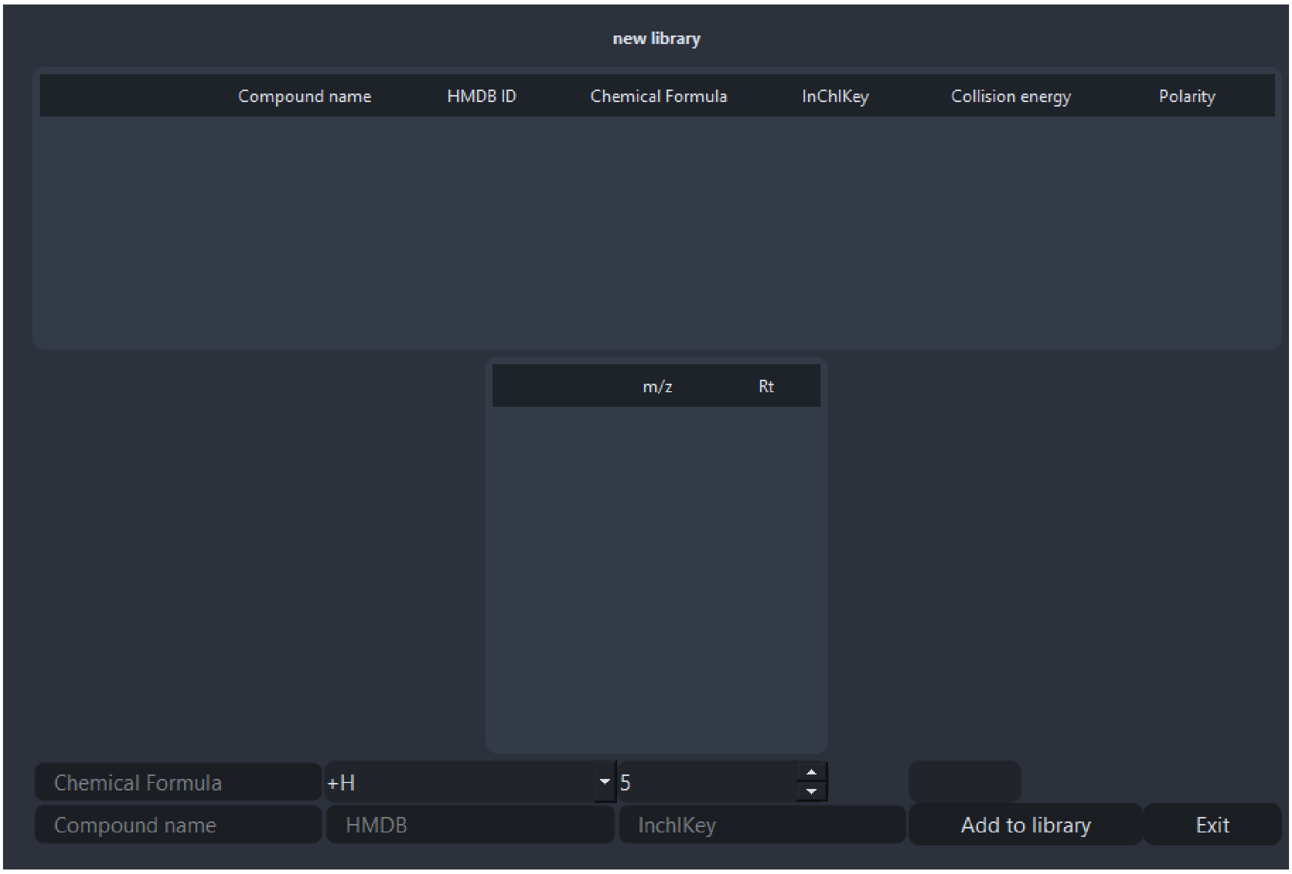
	Compound name	HMDB ID	Chemical Formula	InChIKey	Collision energy	Polarity

A new area will appear showing all the spectra available in the selected library. In this case none as it was just created.

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.

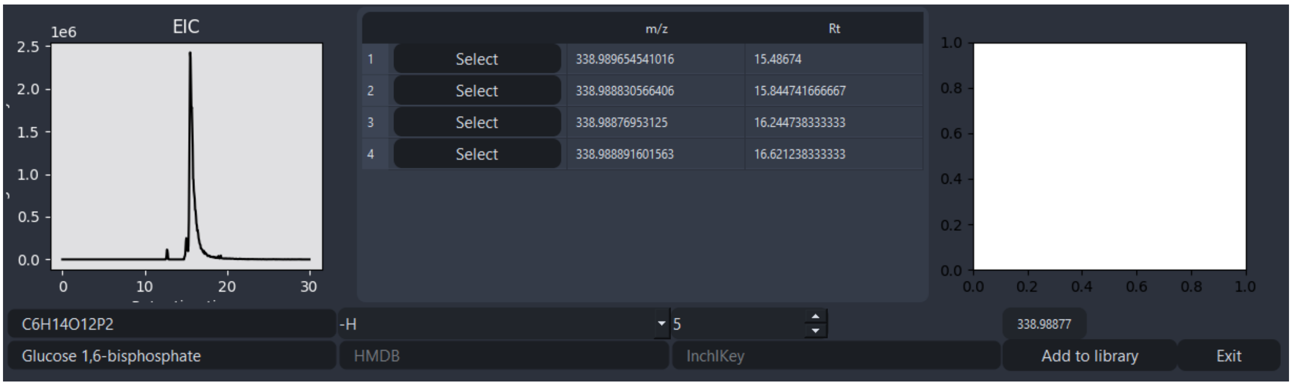


You can now search for your standard by type the chemical formula, adduct, ppm range and name.

HMDB and InchlKey are optional parameters.

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

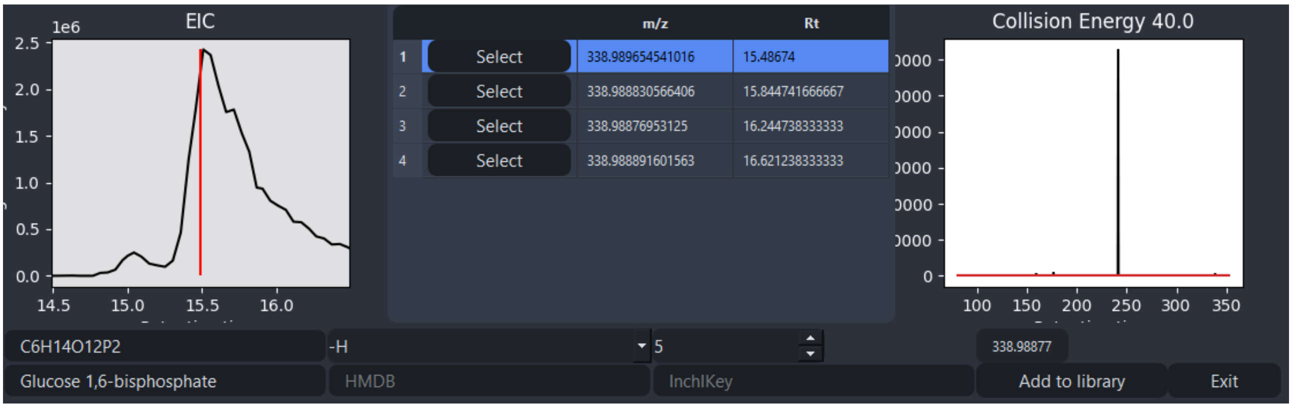
In the central table, all MS/MS scan matching filter criteria will be displayed reporting the precursor found value and retetion time of the MS/MS event.



		m/z	Rt
1	Select	338.989654541016	15.48674
2	Select	338.988830566406	15.844741666667
3	Select	338.98876953125	16.244738333333
4	Select	338.988891601563	16.621238333333

C6H14O12P2
-H
5
338.98877
Glucose 1,6-bisphosphate
HMDB
InchIKey

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.

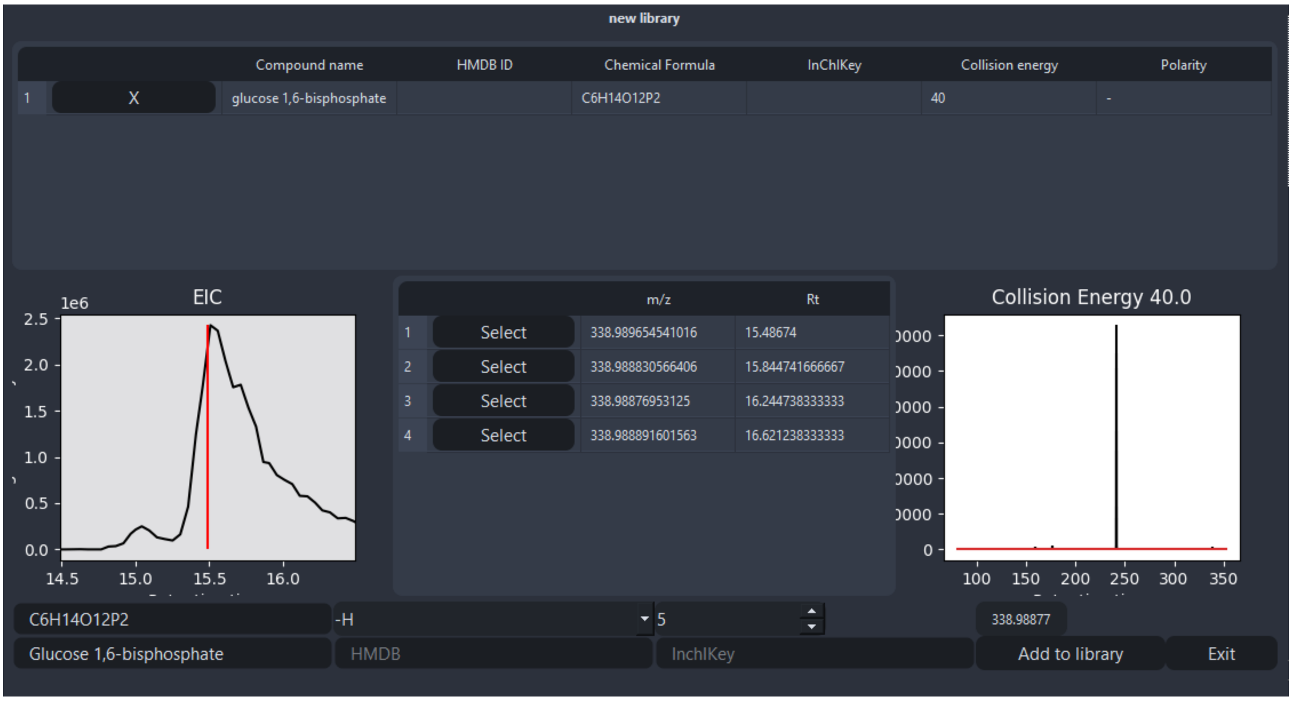


		m/z	Rt
1	Select	338.989654541016	15.48674
2	Select	338.988830566406	15.844741666667
3	Select	338.98876953125	16.244738333333
4	Select	338.988891601563	16.621238333333

C6H14O12P2
-H
5
338.98877
Glucose 1,6-bisphosphate
HMDB
InchIKey

We can now click on «Add to library»,

The compound will now appear on the selected library.

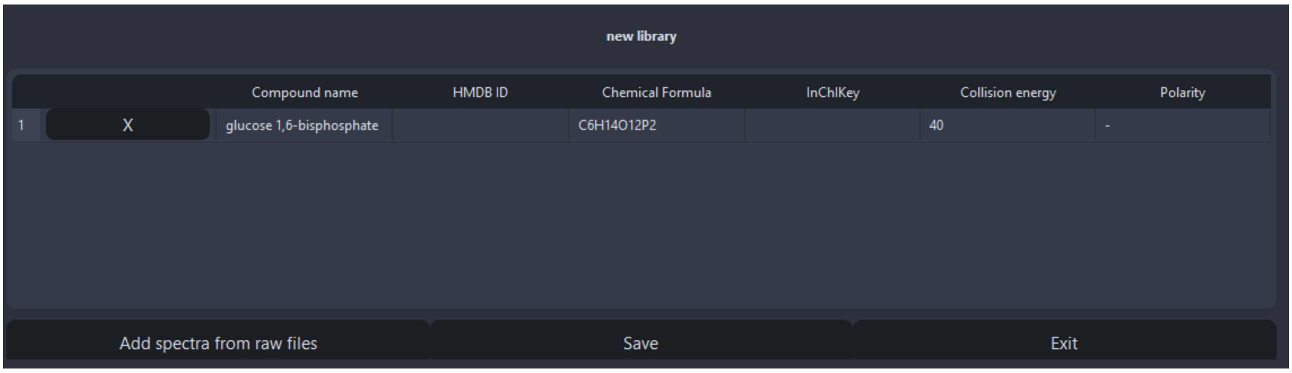


		Compound name	HMDB ID	Chemical Formula	InChIKey	Collision energy	Polarity
1	X	glucose 1,6-bisphosphate		C6H14O12P2		40	-

		m/z	Rt
1	Select	338.989654541016	15.48674
2	Select	338.988830566406	15.844741666667
3	Select	338.98876953125	16.244738333333
4	Select	338.988891601563	16.621238333333

C6H14O12P2
-H
5
338.98877
Glucose 1,6-bisphosphate
HMDB
InchIKey

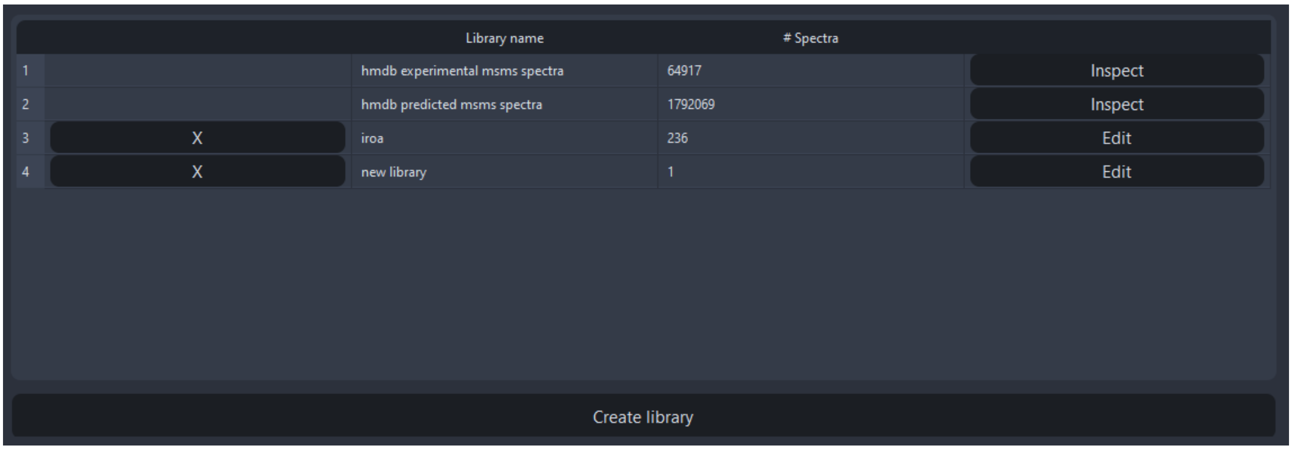
We can continue to include other MS/MS spectra from the selected files or click «Exit» to go back to the library editing screen.



		Compound name	HMDB ID	Chemical Formula	InChIKey	Collision energy	Polarity
1	X	glucose 1,6-bisphosphate		C6H14O12P2		40	-

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.

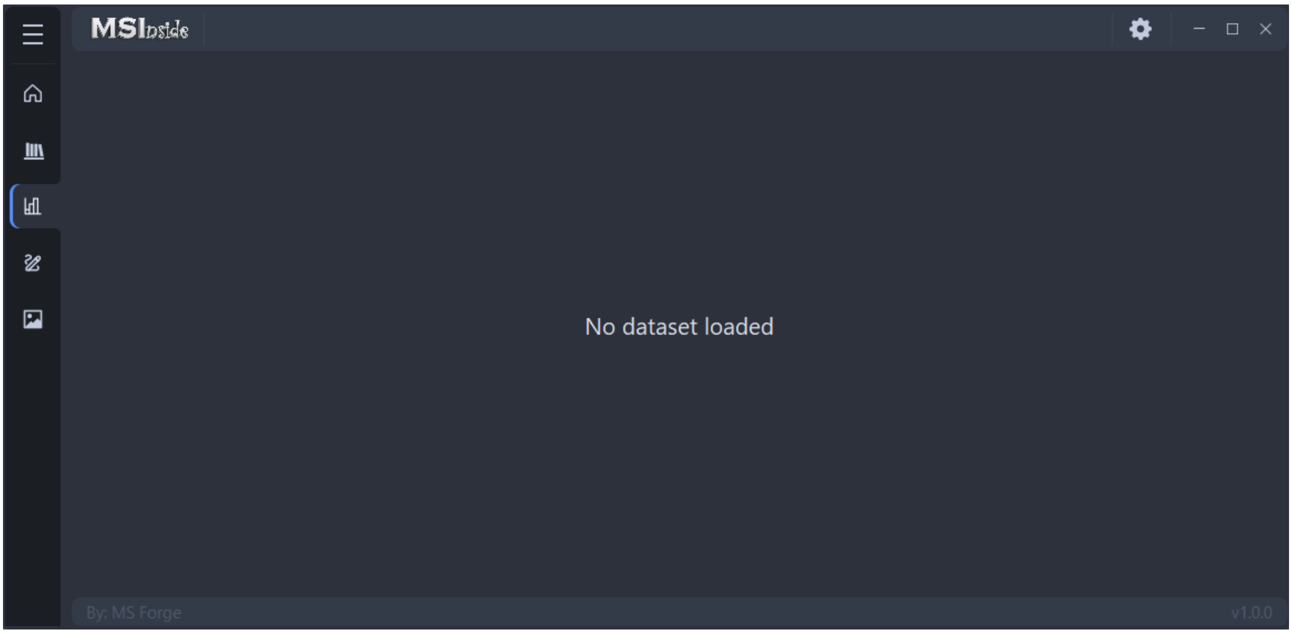


		Library name	# Spectra	
1		hmdb experimental msms spectra	64917	Inspect
2		hmdb predicted msms spectra	1792069	Inspect
3	X	iroa	236	Edit
4	X	new library	1	Edit

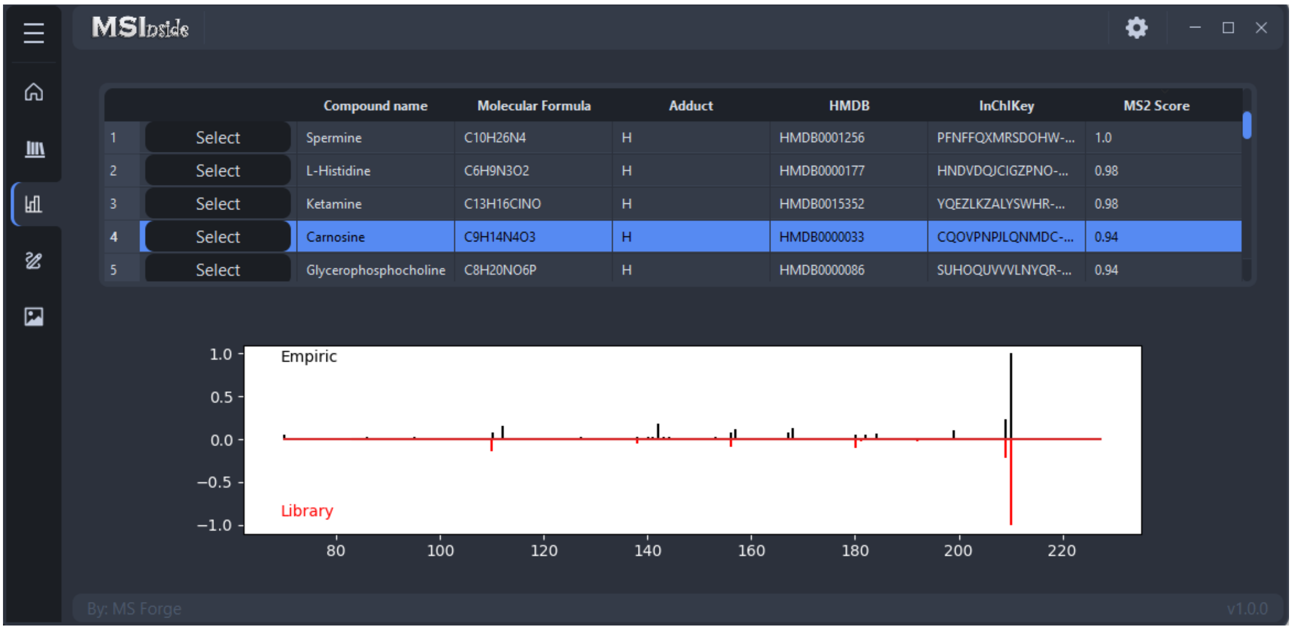
Warning: Common libraries and User libraries are stored on an online server. Poor connection may cause delay in the update process. Connection error (e.g. turning off connection during the process) may cause loss of data. In this case the Library page will change and the logo below will appear indicating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

Direct Infusion

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 found any compound this screen will appear.



Otherwise you will se a list of identified compounds and their characteristics.



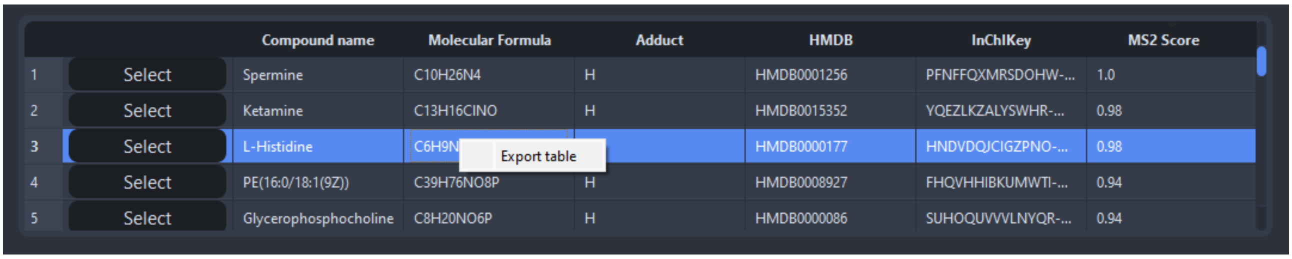
		Compound name	Molecular Formula	Adduct	HMDB	InChIKey	MS2 Score
1	Select	Spermine	C10H26N4	H	HMDB0001256	PFNFFQXMRSDOHW-...	1.0
2	Select	L-Histidine	C6H9N3O2	H	HMDB0000177	HNDVDQJCIGZPNO-...	0.98
3	Select	Ketamine	C13H16ClNO	H	HMDB0015352	YQEZLKZALYSWHR-...	0.98
4	Select	Carnosine	C9H14N4O3	H	HMDB0000033	CQOVPNPJLQNMDC-...	0.94
5	Select	Glycerophosphocholine	C8H20NO6P	H	HMDB0000086	SUHOQUVVVLNYQR-...	0.94

Name, chemical formula, adduct and available identifier from the library will appear for compond found in the annotation dataset. MS

Score represent the similarity between empiric MS/MS spectra and the best matching spectra from the library.

By clicking on the «Select» button next to each compound a plot of the spectra (Empiric and from library) will appear at the bottom of the screen.

For exporting the annotation result, you can Right Click anywhere on annotation table and select «Export Table»,

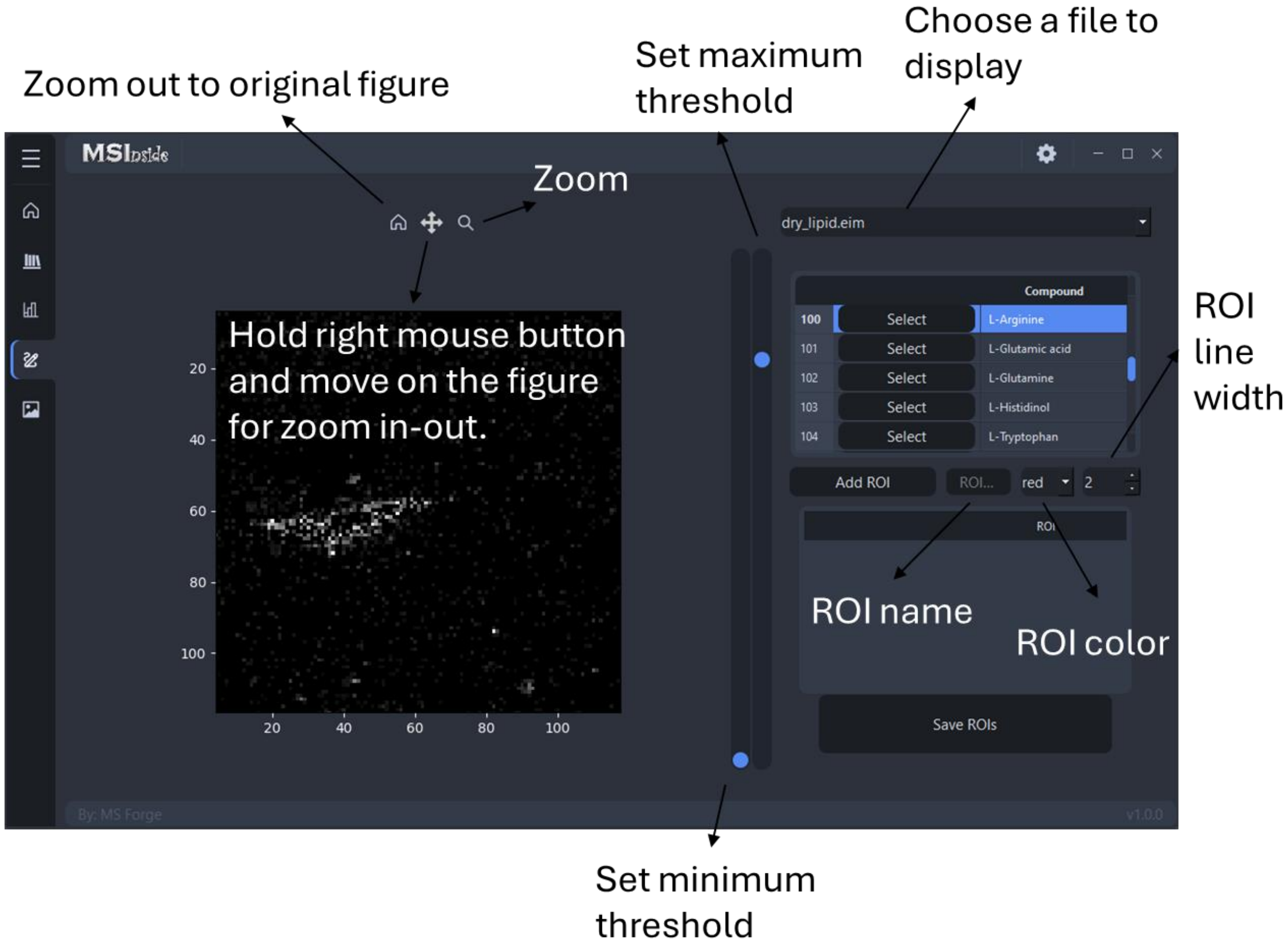


		Compound name	Molecular Formula	Adduct	HMDB	InChIKey	MS2 Score
1	Select	Spermine	C10H26N4	H	HMDB0001256	PFNFFQXMRSDOHW-...	1.0
2	Select	Ketamine	C13H16ClNO	H	HMDB0015352	YQEZLKZALYSWHR-...	0.98
3	Select	L-Histidine	C6H9N		HMDB0000177	HNDVDQJCIGZPNO-...	0.98
4	Select	PE(16:0/18:1(9Z))	C39H76NO8P	H	HMDB0008927	FHQVHHIBKUMWTI-...	0.94
5	Select	Glycerophosphocholine	C8H20NO6P	H	HMDB0000086	SUHOQUVVVLNYQR-...	0.94

Table can be sorted by clicking on the title of the column you want to sort by.

ROI

After loading an acquired dataset (Home – Open), by clicking on ROI icon, the ROI page will appear.



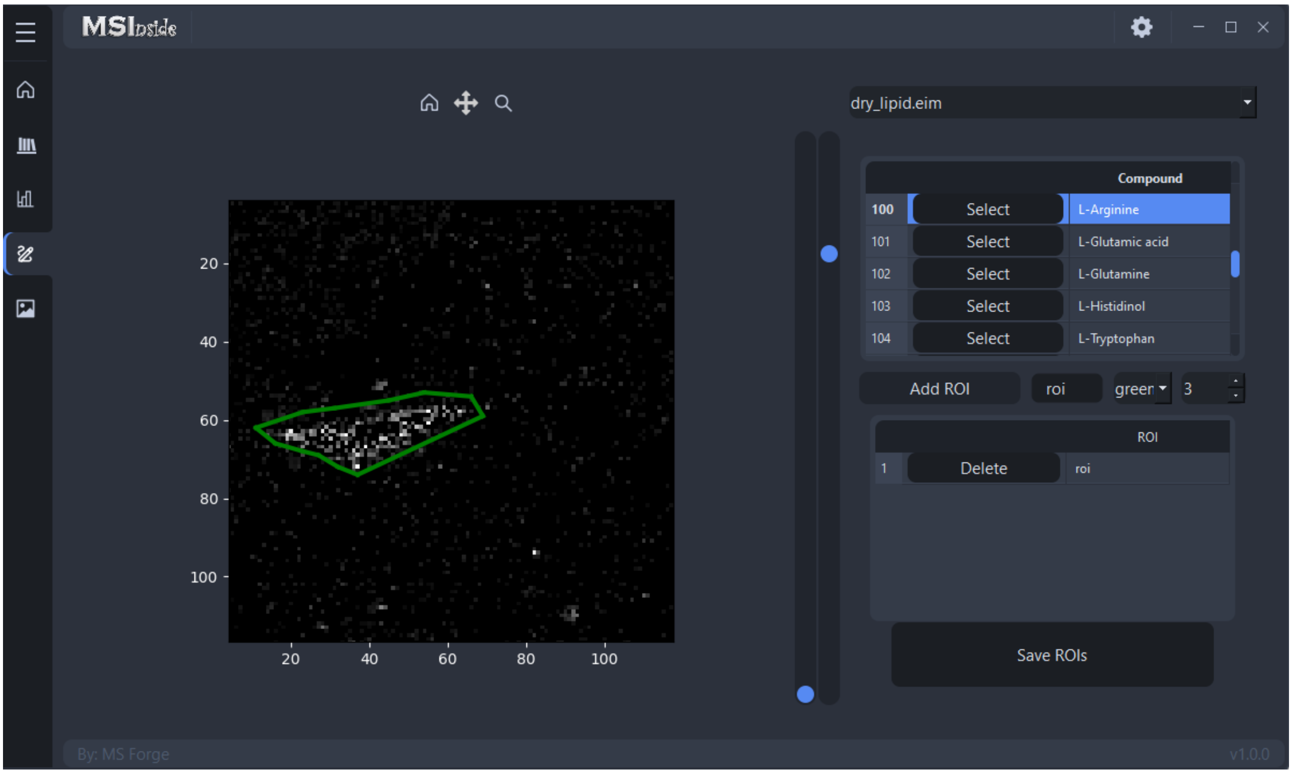
Hold right mouse button and move on the figure for zoom in-out.

		Compound
100	Select	L-Arginine
101	Select	L-Glutamic acid
102	Select	L-Glutamine
103	Select	L-Histidinol
104	Select	L-Tryptophan

By clicking on «Select» next to the compound of interest, the image of the reconstructed EIM will appear on the central screen.

Draw ROIs

We can click on Add ROI and start drawing the region of interest by left-click on the figure.



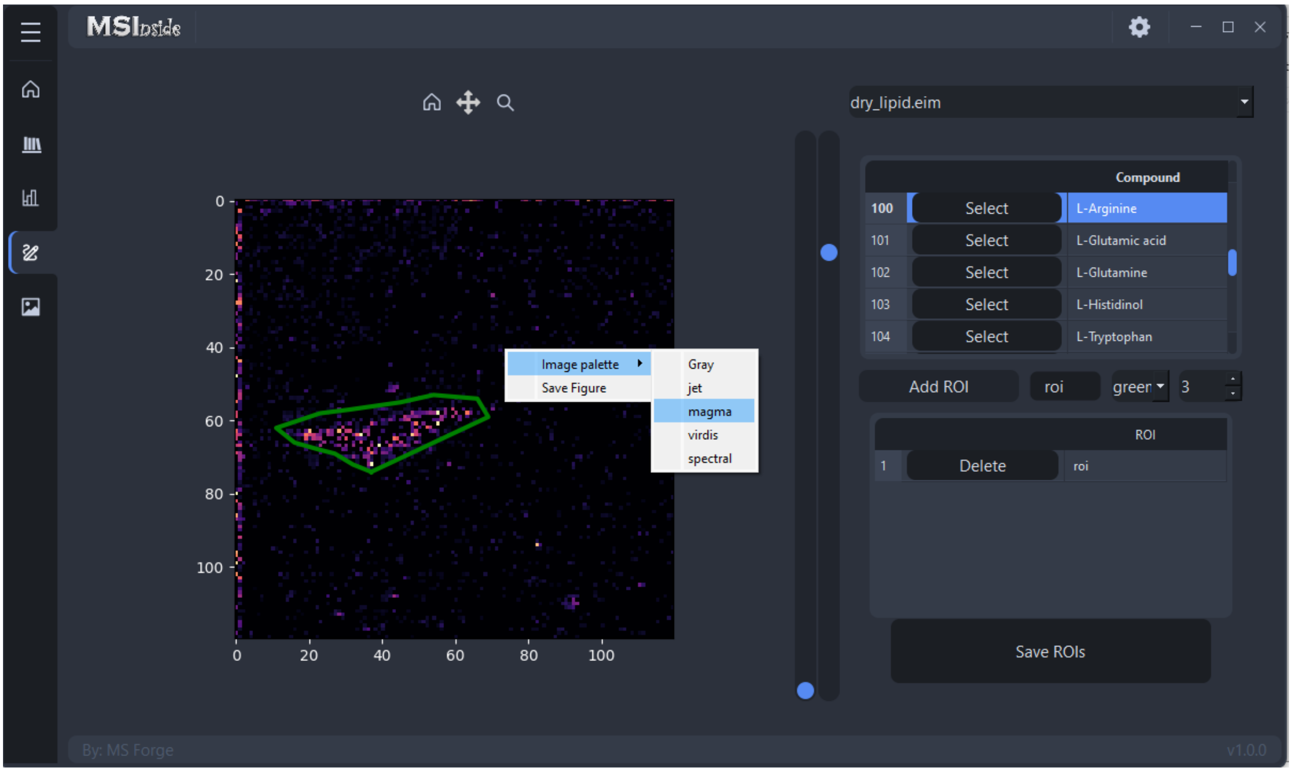
		Compound
100	Select	L-Arginine
101	Select	L-Glutamic acid
102	Select	L-Glutamine
103	Select	L-Histidinol
104	Select	L-Tryptophan

		ROI
1	Delete	roi

When the ROI is closed (by clicking on the starting point) it will appear on the ROI table.

Multiple ROIs can be drawed.

Right click on the figure to change the color map or for save the figure (including the ROIs).



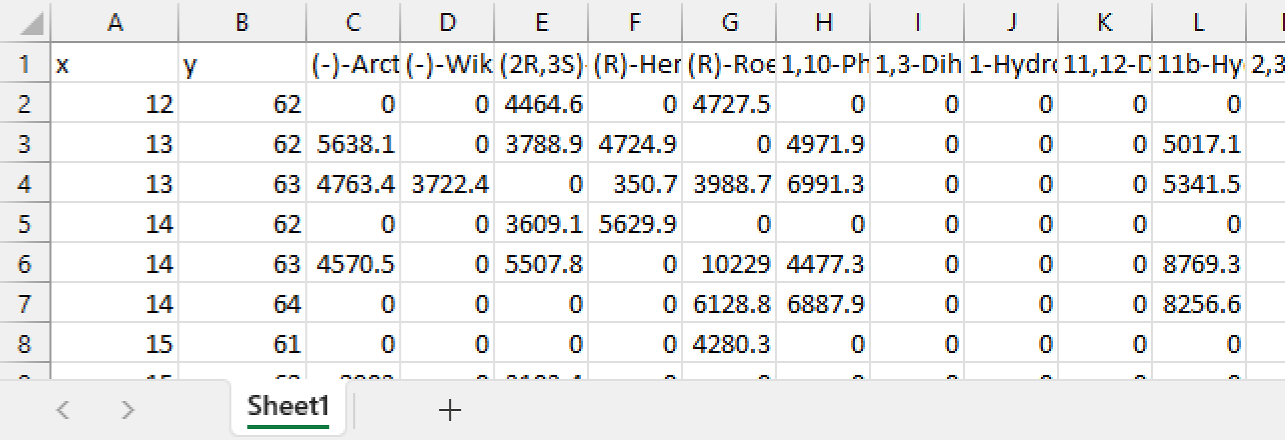
After selection all the ROIs, we can now export the complete pixels content by clicking on «Save ROIs».

The selected ROIs will be saved as Excel file. A folder named «ROIs» will be created inside the folder containing the workflow file. Each drawed ROI is saved as a separated Excel. The name of the file is automatically composed by the file name plus the relative ROI name.

Example of extracted ROI

In this example the file will name “dry_lipid_roi.xlsx”.

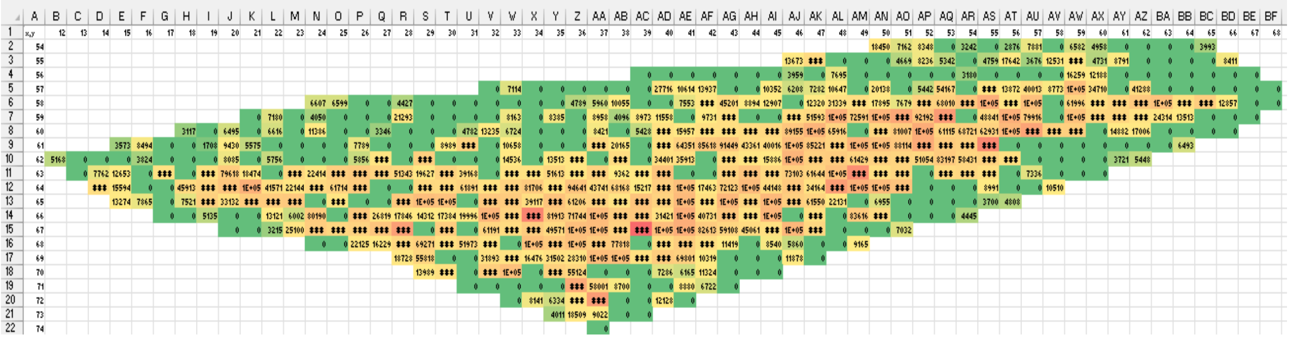
The Excel file will have the following structure.



	A	B	C	D	E	F	G	H	I	J	K	L
1	x	y	(-)-Arct	(-)-Wik	(2R,3S)	(R)-Her	(R)-Roe	1,10-Ph	1,3-Dih	1-Hydr	11,12-D	11b-Hy
2	12	62	0	0	4464.6	0	4727.5	0	0	0	0	0
3	13	62	5638.1	0	3788.9	4724.9	0	4971.9	0	0	0	5017.1
4	13	63	4763.4	3722.4	0	350.7	3988.7	6991.3	0	0	0	5341.5
5	14	62	0	0	3609.1	5629.9	0	0	0	0	0	0
6	14	63	4570.5	0	5507.8	0	10229	4477.3	0	0	0	8769.3
7	14	64	0	0	0	0	6128.8	6887.9	0	0	0	8256.6
8	15	61	0	0	0	0	4280.3	0	0	0	0	0

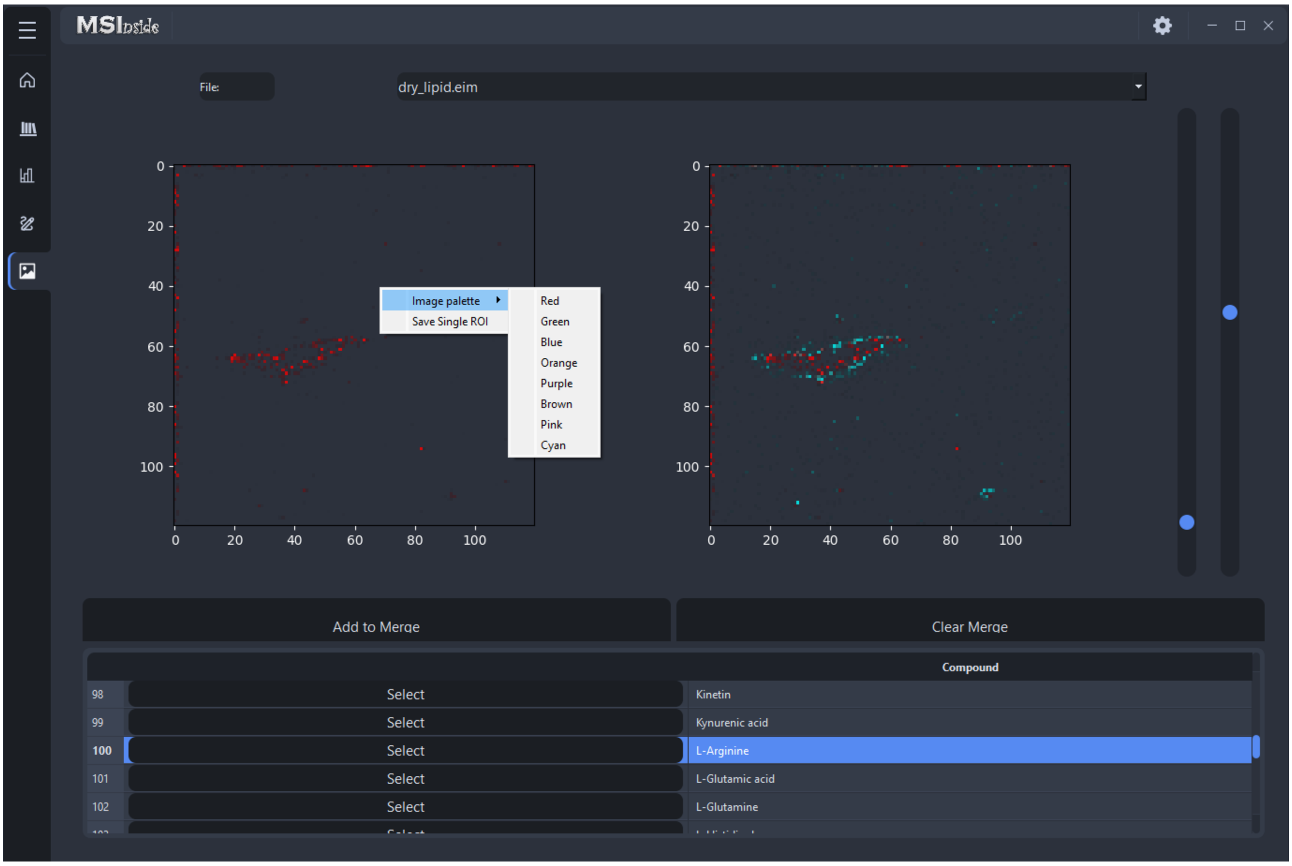
The x and y column are respectively the coordinates of each pixel inside the ROI. Each compound is stored in a separated column reporting the found signal intensity for each pixel.

For explanation purposes, if we rearrange the column of a single compound based on the x,y location, we will reconstruct the original figure inside the ROI as it is showed in the example below for L-Arginine.



Overlay

Overlay page is design for generate graphical representation of multiple compounds within a single picture.



File: dry_lipid.eim

		Compound
98	Select	Kinetin
99	Select	Kynurenic acid
100	Select	L-Arginine
101	Select	L-Glutamic acid
102	Select	L-Glutamine

By clicking «Select», we can choose the compound of interest. With a right click, we can select the color and adjust minimum and maximum values on the slide bars and finally clicking to «Add to Merge». The selected image will be added on the right screen.

Save the EIM with a single compound or with the merge figure can be done by right click on the picture of interest and click on the Save button of the right menu.

MSInside 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 MSInside 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/quote_msinside/

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 MSInside updates, unless the custom request is expressly requested to be kept private.

Otherwise, if it is doable but not suitable for an MSInside 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).