

METITREE USER GUIDE

version 0.4
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This User Guide provides an overview of several functional features of the MetiTTree web application. Use the content list to enter detailed information on how to use MetiTTree.

Mass spectrum plot showing relative abundance (y-axis, 0 to 2.0e5) versus m/z (x-axis, 85 to 130). The base peak is at m/z 115 (C₈H₇O₂). Other significant peaks are labeled at m/z 85, 135, 96, 125, 107, 110, 109, 108, 107, 106, 105, 104, 103, 102, 101, 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85. Buttons for 'Zoom', 'Show All', and 'Show Cumulative' are present.

Chemical structures of the base peak (C₈H₇O₂) and its fragments (C₇H₆O₂, C₆H₅O₂, C₆H₄O₂, C₆H₃O₂, C₆H₂O₂, C₆H₁O₂, C₆H₀O₂) are shown along with their fragmentation pathways.

Beta DB

1: Select reference database

database: NMC-database ▾ select

database selected: NMC-database

directory selected: project-ab

file selected: met-A.cml

2: Select directory/file

directory: project-ab ▾ select

file: met-A.cml ▾ select

top similar structures "default"

score	structure	name	file	job
100		ChemSpider	file_234534.cml	2
25		ChemSpider	file_177732.cml	5
17		ChemSpider	file_734577.cml	3
17		ChemSpider	file_900543.cml	1

MetiTTree is a user-friendly, web application dedicated to organize, process, share, visualize, and compare high-resolution multi-stage mass spectrometry (MSⁿ) data. It integrates dedicated features to export and visualize complex MSⁿ data, facilitating the exploration and interpretation of metabolomics experiments.

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Upload MSⁿ data

Upload files with MSⁿ mass spectrometry data and store them into data directories.

MetiTTree accepts the uploading of mzXML files (version 3.1) containing MSⁿ mass spectrometry data. MetiTTree allows the creation of directories for grouping mzXML files, assisting the organization of the data according projects or topics.

Creating a new directory

1. Select “MSⁿ data” (tab), to open the “MSⁿ data” page.
2. First provide a name for the new directory then click 'create' to save your directory.

[create a directory](#)

Use directories to group files. Directories can also be used to process multiple files at once.

name : [create](#)

Add or remove files

1. Select one of the directories by clicking the magnifying glass.

[directories](#)

	name	file	created
	metabolites	19	2012-06-28

2. The page “Files” will be displayed. The files added to the directory will be listed.

files in: [metabolites](#) (change directory / add files)

ID	file	name	sort by: name / date	pubchem	chemspider	owner	delete
19	dilu_c.mzxml	Glutathione				you	
18	dilu_d.mzxml	Glutathione				you	
17	hmdb_a.mzxml	6-hydroxy-m-Anisic acid				you	
16	lab_c.mzxml	5-Hydroxylysine				you	
15	hmdb_f.mzxml	N-Acetylserotonin				you	

- Select “**add files**” to add new files from your computer to this directory. Finally, select “**submit**” to save it.

[add file\(s\)](#)

select a file to add : [Browse...](#) [submit](#)

Note: You can upload a single mzXML file or a zip-file with multiple mzXML files.

An mzXML file is an open data format for storage and exchange of mass spectroscopy data, developed at the SPC/Institute for Systems Biology. All known converters to convert vendor specific files to mzXML files are summarized on this page:

<http://tools.proteomecenter.org/wiki/index.php?title=Formats:mzXML>

[Extended view](#)

To see extended information of a file, click the magnifying glass.

- Once the file is uploaded you eventually can specify the InChI (IUPAC International Chemical Identifier) of the neutral compound. To save the InChI identifier you have to select the “**update**” button. Automatically, the file will be cross-linked to the pubchem and chemspider databases.
- The files can be sorted by name or data created by selecting “**name/date**”.

sort by: name / date				
ID	file	name	pubchem	chemspider
19	dilu_c.mzxml	Glutathione	PubChem	ChemSpider
you x				
2d image		InChI	comments	
		<pre>InChI=1S/C10H17N3O6S /c11-5(10(18)19)1-2-7(14)13-6(4 -20)9(17)12-3-8(15)16 /h5-6,20H,1-4,11H2,(H,12,17) (H,13,14)(H,15,16)(H,18,19) /t5-,6-/m0/s1</pre>	<input type="button" value="update/save"/>	

Process and visualize

MSⁿ data:

MetiTTree integrates the MEF tool (Rojas-Chertó et al., 2011) and uses its functionality to process MSⁿ data. The required input to process MSⁿ data is an mzXML file (<http://en.wikipedia.org/wiki/MzXML>) and the settings of the processing parameters. Processing parameters are grouped into those to extract the mass spectrometry information (m/z, intensity, and scan number) and those to enrich the MS data with chemical information (chemical elements and number of atoms). MetiTTree allows the processing of individual or multiple files at the same time. Furthermore, the same mzXML file can be processed several times with different sets of processing parameters. Results and parameters information are stored to allow posterior revision.

Once the data is processed, it can be displayed using the spectral tree viewer. Here, the user can select a peak or a node, which represents a fragment. For each selection, the corresponding mass spectrum is displayed, together with the reactions that connect the parent ion with the selected fragment. The structures of the fragments are only visible when they have been assigned. The results generated by MetiTTree can be exported to different file formats for further analysis. The CML format allows managing complex chemical content that other software can import to process it even further. The PDF format can be used to visualize the MSⁿ data, which makes it suitable to export images into reports or publications.

1. On the navigation bar select “**Process**” (tab)
2. To process a file you must first select the directory where the file is found. To process all files of a determined directory select “**process directory**”.

select what to process:

please select a directory to process or select files from

or

3. If you want to process specific files from the directory, select “**select file(s) from directory to process**”. A list of files will be displayed. Check those files to process and select “**process files**”. If you like to select all files at once press the box with the sign plus.

files in directory metabolites			
<input type="button" value="Process files"/>			
	file	comments	created
<input type="checkbox"/>	dilu_a.mzxml		2012-06-28
<input type="checkbox"/>	dilu_b.mzxml		2012-06-28
<input type="checkbox"/>	dilu_c.mzxml		2012-06-28
<input type="checkbox"/>	dilu_d.mzxml		2012-06-28

4. The “**Define settings**” page will be displayed introducing the following processing settings:
- MZ gap (bin size) = Minimal distance between adjacent peaks. Peaks with smaller intensity being excluded.
 - Signal to noise threshold = Signal to noise threshold allowed during the peak picking.
 - Elements = It defines upper and lower number of atoms admitted for each element in the elemental composition of the ion (e.g. C1..15, H1..9, O0..4).
 - Rules = It defines the constraint-rules that are applied to verify the elemental compositions generated. It distinguishes between the Nitrogen Rules and the RDB rules.
 - Mass accuracy settings = Mass accuracy error allowed per MS level.

Please change the settings or continue with the defaults

Peak picking settings

MZ gap (bin size as m/z) : m/z (5 e.g 0.5 m/z)
Signal to noise threshold : %

Filter settings

Elements : ('C1..50,H1..100,N0..10,O0..10' or 'C10,N4,H2')
Rules :
 nitrogenR
 RDBER

Mass accuracy settings (ppm)

MS Level 1 :
MS Level 2 :
MS Level 3 :
MS Level 4 :
MS Level 5 :

Process

5. To submit the process select “**Process**”.

6. The “**Process**” page will be displayed containing information about the progress of the different processing jobs.

Status JOB#000000000010 (...finished!)

Output

name	compound	mzxml	cml	export (csv/tab)	visuals	created
hmdb_d.mzxml	3-Aminosalicylic acid					2012-06-28

Settings

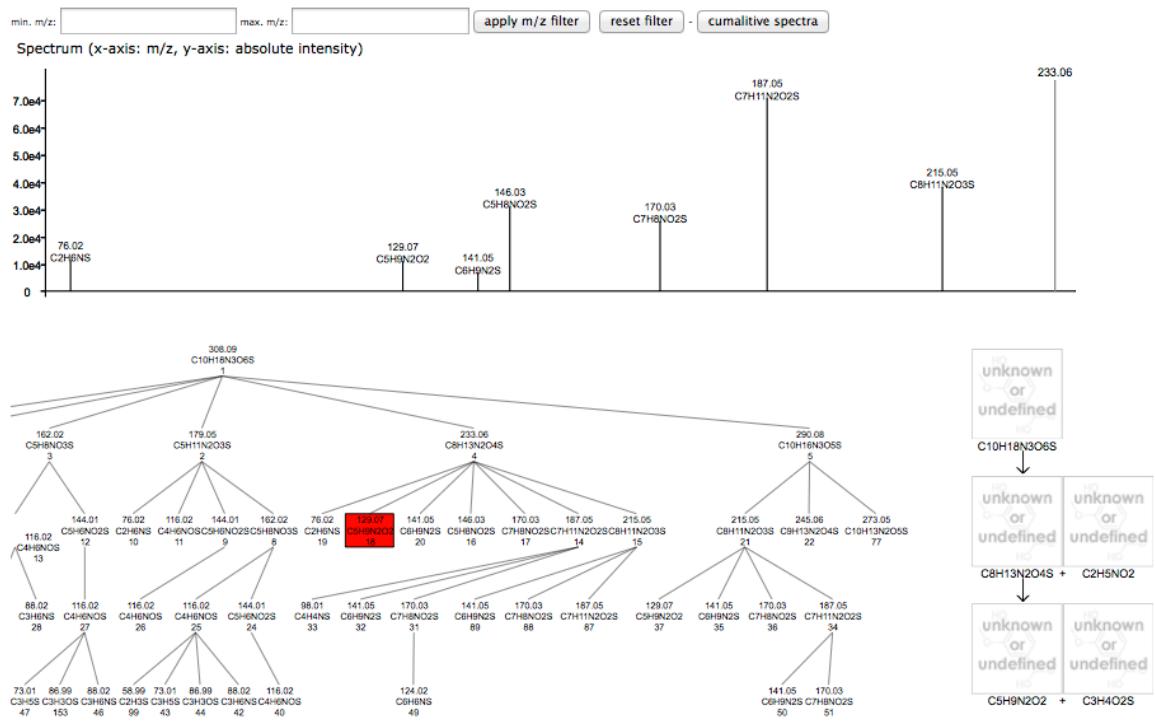
setting	value
accuracy-L1	15
accuracy-L2	15
accuracy-L3	15
accuracy-L4	15
accuracy-L5	15
elements	O3..3,N1..1,H8..8,C7..7
mzgap	0.5
rulenitrogenr	nitrogenR
rulerdber	RDBER
snthresh	1.0

Job Log

#import job

7. It is possible to visualize the different results.
- mzXML = original mzXML file used to process the spectral data
 - cml = chemical markup language file enriched with chemical information.
 - csv/tab = plain text files with enriched chemical information
 - viewer = The Spectral Tree Viewer (<https://trac.nbic.nl/brsp201017>)
 - pdf = portable document format to visualize the spectral data
8. Select to visualize spectral data with the tree viewer. The Spectral Tree Viewer interconnects three MSⁿ items: the spectrum, which contains mass peaks, the fragmentation tree, which contains fragment nodes/elemental compositions, and the fragmentation reactions, which contain structures.

Glutathione



Storage and manage database(s) with MSⁿ data

Processed MSⁿ data can be stored in one or multiple internal databases. Because the users are organized in groups, they can share files and libraries with other group members. Only the administrator user has the faculty to create a new group and assign users to it.

1. On the navigation bar select the “**Databases**” (tab)
2. The '**Databases**' page will be displayed. From this page, you have access to the functions listed below:
 - Create a new database
 - Modify an existing database
3. To create new database, provide a name for the new database and click 'add' to save your database.

add a new database
name :

4. To modify an existing database, select one of the magnifying glasses in front of the databases.

databases	
name	created
 publicDb	2012-06-28

5. Now modify the files to be contained in the database as required.

publication-database**filter on job**All jobs

Add files to database

Files that can be added to this database.

Files in database			
	File	Job	Created
✗	HMDB_a.mzXML.cml	19	2012-01-15 04:41:43.521
✗	HMDB_b.mzXML.cml	13	2012-01-15 04:39:27.985
✗	HMDB_c.mzXML.cml	15	2012-01-15 04:40:35.075
✗	HMDB_d.mzXML.cml	5	2012-01-15 04:33:15.941
✗	HMDB_e.mzXML.cml	8	2012-01-15 04:35:07.403
✗	HMDB_f.mzXML.cml	17	2012-01-15 04:41:06.914
✗	HMDB_g.mzXML.cml	16	2012-01-15 04:40:55.965
✗	dilu_d.mzXML.cml	21	2012-01-15 04:42:15.268
✗	dilu_e.mzXML.cml	20	2012-01-15 04:42:11.831
✗	lab_a.mzXML.cml	11	2012-01-15 04:37:35.395
✗	lab_b.mzXML.cml	6	2012-01-15 04:33:22.836
✗	lab_c.mzXML.cml	18	2012-01-15 04:41:31.434
✗	lab_d.mzXML.cml	14	2012-01-15 04:39:58.725

The data in this database originates from the files below.

6. On the right side you have the files that actually are in the database. On the left side you have those files that you can upload to the database. Through “**filter on job**” you can filter the files by specific job number.

Querying an existing database(s) for similar MSⁿ data

MetiTTree integrates the functionality to query for similar MSⁿ data stored in your own personal library. The results are presented in a list showing the structures of the compounds with most similar MSⁿ data together with the corresponding similarity value. The results are ranged between 0-100. A value near to 100 indicates that the MSⁿ data is highly similar which would be a complete identification; while a value close to 0 illustrates that it is very different (Rojas-chertó et al., 2012). We expect MetiTTree to contribute two fold in the identification of metabolites. On one hand, we are interested to know if a fragmentation tree of the same compound is present in the library, which would be a partial identification. On the other hand, if similar fragmentation data is found, we can use this knowledge to give hints (e.g. substructure information) which structure the unknown compound could have.

1. Select the “search/compare” (tab), to open the “database search” page.
2. Choose a database to query “database”, and the spectral tree file to be compared in “directory” > “file”. To see the database search result push “search/refresh” button.
3. The 'database search' page will display the results into a list of compounds ranked from more similar to a less similar. On the left side it will shows the similarity value when the fragmentation trees are compared to each other.

1: select reference database:

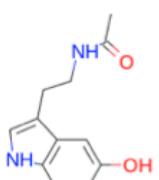
database : publicDb

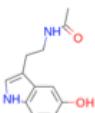
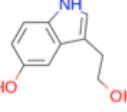
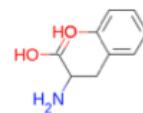
2: select query directory/file:

directory : metabolites
file : hmdb_f.cml - JOB#000C

search / refresh

database selected : publicDb
directory selected : metabolites
file selected : hmdb_f.cml
compound : N-Acetylserotonin
InChIkey : MVAWJSIDNICKHF-UHFFFAOYNA-N
InChI : InChI=1/C12H14N2O2
/c1-8(15)13-5-4-9-7-14-12-3-2-10(16)6-11(9)12
/h2-3,6-7,14,16H,4-5H2,1H3,(H,13,15)



score	structure	name	file	job
100.0		N-Acetylserotonin PubChem ChemSpider	hmdb_f.cml	13
16.66		3-(2-Hydroxyethyl)-1H-indol-5-ol PubChem ChemSpider	hmdb_g.cml	4
7.17		2-Hydroxyphenylalanine PubChem ChemSpider	hmdb_e.cml	1

Visualize MSⁿ data with chemical structure of the fragments

The spectral tree viewer is capable to show the chemical structure of the fragments in the MSⁿ data. At the moment it is only possible if you supply it with a JSON file describing the MSⁿ data.

1. On the bottom of any page, select the “**MsnViewer Demo**”.



The Netherlands Metabolomics Centre in collaboration with The Netherlands Bioinformatics Centre (NBIC). A collaborative institute of the bioinformatics groups in the Netherlands.

Wiki - Roadmap - MsnViewer Demo
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2. You can upload an example by selecting “**Load example**” > “**view**”. You can modify the json message and add your own values.

Paste a JSON formatted Tree Structure to view with the MSnViewer.js application (load example)

```
{  
    "1" : {  
        "id" : "1",  
        "parent" : "",  
        "mass" : "164.01",  
        "intensity" : "2601726.5555",  
        "inchi" : "InChI=1S/C5H9N03S/c1-3(7)6-4(2-10)5(8)9/h4,10H,2H2,1H3,(H,6,7)  
(H,8,9)/p+1, InChI=1S/C3H7N02S/c4-2(1-7)3(5)6/h2,7H,1,4H2,(H,5,6)/p+1",  
        "inchiloss" : ""  
    },  
    "2" : {  
        "id" : "2",  
        "parent" : "1",  
        "mass" : "122.01",  
        "intensity" : "378006.0052",  
        "inchi" : "InChI=1S/C3H7N02S/c4-2(1-7)3(5)6/h2,7H,1,4H2,(H,5,6)/p+1",  
        "inchiloss" : "InChI=1S/C2H20/c1-2-3/h1H2"  
    },  
    "3" : {  
        "id" : "3",  
        "parent" : "1",  
    }  
}
```

3. After the selected view run, the MSⁿ viewer screen will be shown.

FAQs

Where can I address for more help?

For any questions that could not be answered through this guide, you can send us an email directly to info@metitree.nl. We will answer you as soon as possible.

How many files might I upload to process?

At the moment there is not a limit during the uploading process. If the number is big may be you can collapse the server.

Biography:

Rojas-Chertó, M., Kasper, P. T., Willighagen, E. L., Vreeken, R., Hankemeier, T., & Reijmers, T. (2011). Elemental Composition determination based on MSn. *Bioinformatics*, 27(17), 2376–2383. Oxford Univ Press.
doi:10.1093/bioinformatics/btr409

Rojas-chertó, M., Peironcely, J. E., Kasper, P. T., Van, J. J. J., Hooft, D., Vos, R. C. H. D., Vreeken, R., Hankemeier, T., & Reijmers, T. (2012). Metabolite identification using automated comparison of high resolution MSn spectral trees. *Anal. Chem.*.
doi:10.1021/ac2034216