

*MS-DIAL tutorial:
Universal program for untargeted metabolomics
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1. Prerequisite:

Software installation:

MS-DIAL last version: <http://prime.psc.riken.jp/compms/msdial/main.html>

Abf (Analysis Base File) Converter: <https://www.reifycs.com/AbfConverter/>

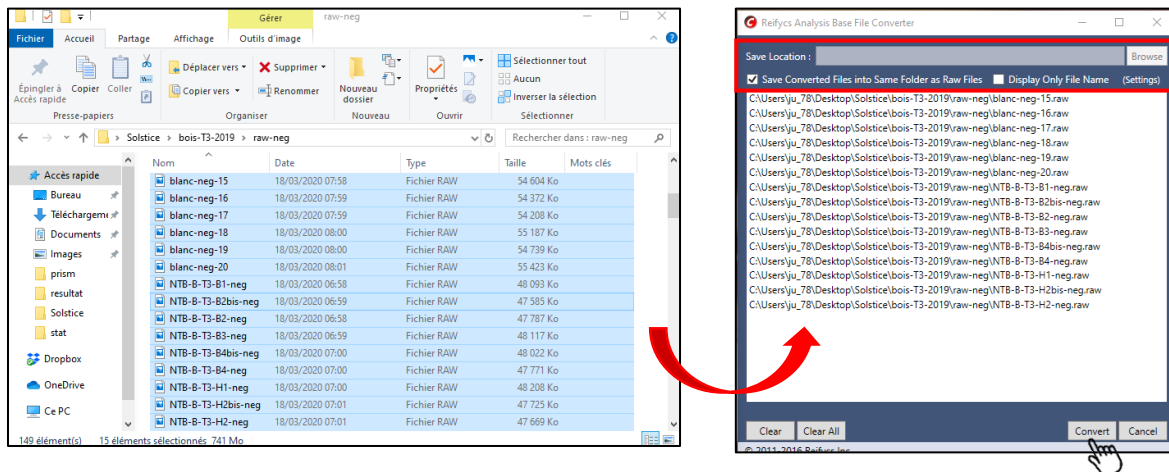
MSFileReader for Thermo data: see the following link which explains how to process <http://fields.scripps.edu/rawconv/>

2. File conversion:

- a. Start the application AnalysisBaseFilConverter
- b. **Drag and drop** .raw files into the application
- c. Choose the folder of arrivals (cf. red rectangle)

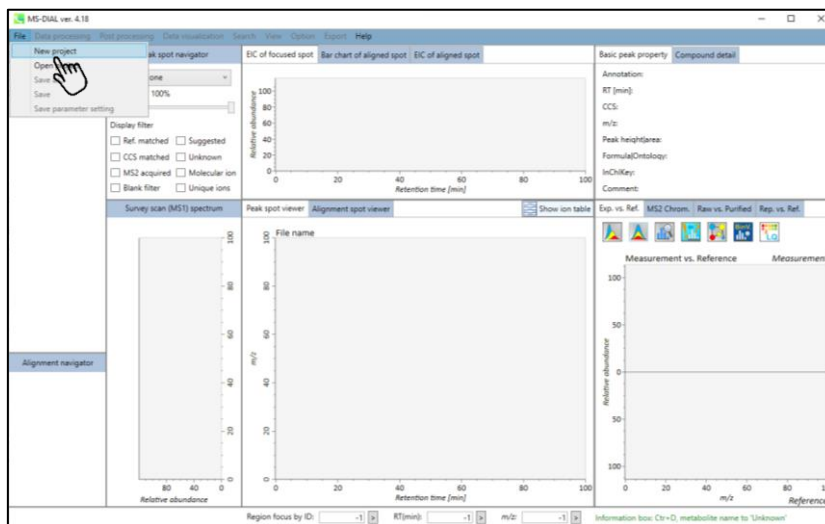
By default, the .abf files will be created in the same folder as raw data since « **Save Converted Files into the Same Folder as Raw Files** » is selected. If you want to generate them into another folder, uncheck this and select the new folder by clicking « **Browse** » on the top right.

d. Click on « **Convert** »



3. Create a project on MS-DIAL and Import data

a. Open the application MS-DIAL and on the « **File** » menu select « **New project** »



The following window appears to define the type of experiments you are about to process:

Start up a project

Project file path:

Ionization type
☒ Soft ionization (LC/MS, LC/MS/MS, or precursor-oriented GC/MS/MS)
☐ Hard ionization (GC/MS)

Separation type
☒ Chromatography (GC, LC, CE, or SFC)
☐ Ion mobility (now coupled with liquid chromatography)

MS method type
☒ Conventional LC/MS or data dependent MS/MS
☐ SWATH-MS or conventional All-ions method ☐ All-ions with multiple CEs (cycled like 0V-10V-40V)

Experiment file:

Data type (MS1)
☐ Profile data
☒ Centroid data

Data type (MS/MS)
☐ Profile data
☒ Centroid data

Ion mode
☐ Positive ion mode
☒ Negative ion mode
☒ Advanced: add further meta data

Target omics
☒ Metabolomics
☐ Lipidomics

Select the folder containing the .abf files

Select « Soft ionisation » for ESI experiments
For EI GC/MS select “Hard ionization”

Select the type of chromatography used

Select « Conventional LC/MS » if you want to process LC/MS or LC/MS/MS data in DDA mode
Select SWATCH-MS* if you want to process LC/MS/MS data in DIA mode

Select Profile or Centroid data

Select « Metabolomics » to use your own MSP file database otherwise « Lipidomics » to use the internal lipid database of MS-DIAL

Select the ion mode (you have to process pos and neg separately)

* by selecting SWATCH-MS, you have to indicate a “dictionary file” as tab delimited text file with MS1 scan range and precursor window as follows:

<http://prime.psc.riken.jp/compms/msdial/main.html#Templates>

b. Import your data

The following window appears and click on “**Browse**” to import the .abf files you want to process. During data importation, it is important to note the type (Blank, QC or Sample) and class of every sample in **Class ID column** (blank, sample class, QC). If both ionization modes have been acquired, be careful to have the **same number of samples** between pos and neg modes and in the **same order**.

New project window

Analysis file paths

File path	File name	Type	Class ID	Batch	Analytical order	Inject. volume (µL)	Included
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	blanc-ext-neg1	Sample	blancext	1	1	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	blanc-ext-neg2	Sample	blancext	1	2	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	blanc-neg-15	Blank	Blank	1	3	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	blanc-neg-16	Blank	Blank	1	4	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	blanc-neg-17	Blank	Blank	1	5	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-B1-neg	Sample	NTB-B-B	1	6	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-B2-neg	Sample	NTB-B-B	1	7	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-B3-neg	Sample	NTB-B-B	1	8	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-B4-neg	Sample	NTB-B-B	1	9	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-H1-neg	Sample	NTB-B-H	1	10	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-H2-neg	Sample	NTB-B-H	1	11	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-H3-neg	Sample	NTB-B-H	1	12	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-H4-neg	Sample	NTB-B-H	1	13	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-I1-neg	Sample	NTB-B-I	1	14	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-I2-neg	Sample	NTB-B-I	1	15	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	NTB-B-T3-I4-neg	Sample	NTB-B-I	1	16	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	QC-T3-neg1	QC	QC	1	17	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	QC-T3-neg2	QC	QC	1	18	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	QC-T3-neg3	QC	QC	1	19	1	<input checked="" type="checkbox"/>
C:\Users\ju_78\Desktop\Solstice\bois-T3-20	QC-T3-neg4	QC	QC	1	20	1	<input checked="" type="checkbox"/>

4. Data analysis and parameters setting

a. Data collection parameters

Analysis parameter setting

Data collection

Mass accuracy

MS1 tolerance: Da

MS2 tolerance: Da

☒ Advanced

Data collection parameters

Retention time begin: min

Retention time end: min

MS1 mass range begin: Da

MS1 mass range end: Da

MS/MS mass range begin: Da

MS/MS mass range end: Da

Isotope recognition

Maximum charged number:

Consider Cl and Br elements: ☐

Multithreading

Number of threads:

Execute retention time corrections ☒

☒ Together with Alignment

Indicate the MS1 and MS2 tolerance
Depend of your instrument

Indicate the range of RT

Indicate the range of MS1 mass

Indicate the range of MS2 mass

For small molecules, the maximum charged number can stay at 2 but you can go up to 8 if necessary.

You can fix the count of threads used to process data (see resource monitor in administrative tools (control panel))

b. Peak detection parameters

Fix the peak height threshold*

By default, 0.1 is generally suitable

Linear weighted moving average is set by default with recommended smoothing level 1-3

Fix the peak width threshold (by default 5 is ok)

You can specify some unwanted peaks if necessary

*** Critical value which greatly influence final results: 70% under the observed baseline is a good starting point.**

c. MS2Dec parameters

By default, 0.5 is suitable. Under 0.3, you will recognize noise chromatographic peaks. Up to 0.7, you will reduce peak resolution

Set a cut off value to reduce the MS/MS noise

Recommended for metabolomics and lipidomics

Set at 0.5 by default.

Select this because the algorithm may erase the precursor's isotopic ions.

d. Identification parameters

The screenshot shows the 'Analysis parameter setting' dialog box with the 'Identification' tab selected. The 'MSP file and MS/MS identification setting' section includes a text field for the 'MSP file' (C:\Users\ju_78\Desktop\bois-T3-2019\DB-msdial03\DB-LRSV-neg-0320.msp) and a 'Select' button. Below this are input fields for 'Retention time tolerance' (0.1 min), 'Accurate mass tolerance (MS1)' (0.01 Da), 'Accurate mass tolerance (MS2)' (0.05 Da), and 'Identification score cut off' (80 %). Checkboxes for 'Use retention time for scoring' and 'Use retention time for filtering' are both checked. The 'Advanced' section, indicated by a collapsed arrow, contains a 'Text file and post identification (retention time and accurate mass based) setting' section with a 'Text file' input field and a 'Select' button. Below this are input fields for 'Retention time tolerance' (0.1 min), 'Accurate mass tolerance' (0.01 Da), and 'Identification score cut off' (85 %). The 'Spectrum cut off and report option' section includes a 'Relative abundance cut off' (0 %) and an unchecked 'Only report the top hit' checkbox. At the bottom are 'Load', 'Together with Alignment' (checked), 'Finish', and 'Cancel' buttons.

You can select a msp format database for identification of level 1. It contains information such as accurate mass, retention time, MS/MS peaks and various identifiers.

Set the mass and retention time tolerances.

The identification score cut off should be greater than 70%.

You can select a text format database for retention time and accurate mass search (post identification)

The first two required columns should contain the “metabolite name” and the “accurate mass”. You can add additional information such as the retention time in following columns. If so, set a suitable retention time tolerance, otherwise indicate “100”.

The identification score cut off should be greater than 70%.

Example of msp format database:

Example of text format database:

DB-IRSV-neg-0420 - Bloc-notes

Fichier Edition Format Affichage Aide

NAME: (Metasci) trans-Resveratrol

SCANNUMBER: 174

RETENTIONTIME: 11.77932

PRECURSORMZ: 227.0715

PRECURSORRTYPE: [M-H]⁻

IONMODE: Negative

SPECTRUMTYPE: Centroid

FORMULA: C14H12O3

INCHIKEY: LUKBXSAMLPMWSZ-0W03BTEDSA-N

INCHI:

SMILES: OC1=CC=C(C=C1)C=CC2=CC(O)=CC(O)=C2

AUTHORS:

COLLISIONENERGY: 40

INSTRUMENT:

INSTRUMENTTYPE:

IONIZATION:

LICENSES:

COMMENT: 174

Num Peaks: 16

m/z	Intensity	Theoretical m/z	Mass diff (ppm)	Formula
117.03439	15932	117.034591	1.72	SMILES OC1=CC=C(C=C1)C=CC1, Annotation [
119.05003	14044	119.050242	1.78	SMILES OC1=CC=C(C=C1)C=CC1, Annotation [
138.96481	7961			
141.07118521		141.070425	0	Formula C11H9 ⁺
143.0502	209851	143.04969	0	Formula C10H70 ⁺
144.05347	26046			
145.02922	8625	145.028954	0	Formula C9H502 ⁺
157.06575	28892	157.06534	0	Formula C11H90 ⁺
159.08139	39051	159.08099	0	Formula C11H110 ⁺
181.06555	19544	181.06534	0	Formula C13H90 ⁺
182.07332	24964			
182.95444	9139			
183.08125	55758	183.08099	0	Formula C13H110 ⁺
185.06058	196543	185.060255	0	Formula C12H902 ⁺
186.06398	27923			
227.07161	780266	227.071367	1.07	SMILES OC1=CC=C(C=C1)C=CC2=CC(O)=CC(O)=C2

NAME: (Metasci) cis-Resveratrol

SCANNUMBER: 174

RETENTIONTIME: 12.491

PRECURSORMZ: 227.0715

PRECURSORRTYPE: [M-H]⁻

IONMODE: Negative

SPECTRUMTYPE: Centroid

msdiaDB-txt - Bloc-notes

Fichier Edition Format Affichage Aide

name	MZ	RT
(2-AMINOETHYL)PHOSPHONATE	124.0168	1.146
10-HYDROXYDECANOATE	187.1336	12.42378
1-HYDROXY-2-NAPHTHOATE	187.0399	9.168233
2,3-DIHYDROXYBENZOATE	153.0192	6.998233
2',4'-DIHYDROXYACETOPHENONE	151.04	11.05613
2,4-DIHYDROXYPTERIDINE	163.0259	4.9144
2,5-DIHYDROXYBENZOATE	153.0193	7.91735
2-AMINOISOBUTYRATE	102.0558	1.300117
2-AMINOPHENOL	108.0452	2.778317
2-HYDROXY-4-(METHYLTHIO)BUTANOATE	149.0276	6.95675
2-HYDROXYBUTYRATE	103.0399	3.981767
2-HYDROXYPHENYLACETATE	151.0399	7.821167
2-METHYLGLUTARATE	145.0506	7.2758
3-(2-HYDROXYPHENYL)PROPANOATE	165.0553	10.48115
3,4-DIHYDROXYMANDELATE	183.0296	2.593883
3,4-DIHYDROXYBENZOATE	153.0191	6.994184
3,4-DIHYDROXYPHENYLACETATE	167.0347	3.969783
3,4-DIHYDROXYPHENYLGLYCOL	169.0504	3.22905
3,5-DIIODO-L-THYRONINE	523.8864	11.18588
3,5-DIIODO-L-TYROSINE	431.8596	8.64365
3-AMINO-4-HYDROXYBENZOATE	152.0352	2.763383
3-AMINO-5-HYDROXYBENZOATE	152.0352	3.083767
3-DEHYDROSHIKIMATE	171.0296	2.2125
3-HYDROXYBENZOATE	137.0244	8.760616
3-HYDROXYBUTANOATE	103.0399	8.238883
3-HYDROXYMETHYLGLUTARATE	161.0454	4.483967
3-METHOXY-4-HYDROXYMANDELATE	151.0399	8.7933
3-METHOXYTYROSINE	197.0453	5.3751

e. Adduct ion setting

Analysis parameter setting

Data collection Peak detection MS2Dec Identification Adduct Alignment Mobility Isotope tracking

Adduct ion setting

User-defined adduct

Molecular species	Charge	Accurate mass [Da]	Included
[M-H] ⁻	1	-1.00782503207	<input checked="" type="checkbox"/>
[M-H2O-H] ⁻	1	-19.01838971207	<input checked="" type="checkbox"/>
[M+Na-2H] ⁻	1	20.97411921676	<input type="checkbox"/>
[M+Cl] ⁻	1	34.96885268	<input type="checkbox"/>
[M+K-2H] ⁻	1	36.94805661586	<input type="checkbox"/>
[M+FA-H] ⁻	1	44.99765396793	<input checked="" type="checkbox"/>
[M+Hac-H] ⁻	1	59.01330396793	<input type="checkbox"/>
[M+C2H3N+Na-2H] ⁻	1	62.00066831777	<input type="checkbox"/>
[M+Br] ⁻	1	78.9183371	<input type="checkbox"/>
[M+TFA-H] ⁻	1	112.98503896793	<input type="checkbox"/>
[M-C6H10O4-H] ⁻	1	-147.06573383101	<input type="checkbox"/>
[M-C6H10O5-H] ⁻	1	-163.06064845057	<input type="checkbox"/>
[M-C6H8O6-H] ⁻	1	-177.03991300599	<input type="checkbox"/>
[M+CH3COONa-H] ⁻	1	80.99524996793	<input type="checkbox"/>
[2M-H] ⁻	1	-1.00782503207	<input checked="" type="checkbox"/>
[2M+FA-H] ⁻	1	44.99765396793	<input checked="" type="checkbox"/>
[2M+Hac-H] ⁻	1	59.01330396793	<input type="checkbox"/>
[3M-H] ⁻	1	-1.00782503207	<input type="checkbox"/>
[M-2H] ²⁻	2	-2.01565006414	<input type="checkbox"/>
[M-3H] ³⁻	3	-3.02347509621	<input type="checkbox"/>

Load ☒ Together with Alignment Finish Cancel

User-defined adduct setting

Adduct: [M-H2O-H]⁻ Mass: -19.0184 Xmer: 1 Charge: 1 Ion mode: Negative Add

You can select the adduct ions suitable. If necessary, you can add your own adduct ion by clicking “**User-defined adduct**” or load a whole adduct list by clicking “**Load**”.

f. Alignment parameters

Analysis parameter setting

Data collection | Peak detection | MS2Dec | Identification | Adduct | Alignment | Mobility | Isotope tracking

Alignment parameters setting

Result name: alignmentResult_2020_4_14_11_47_44

Reference file: QC-T3-neg3

Retention time tolerance: 0.1 min

MS1 tolerance: 0.015 Da

Advanced

Retention time factor: 0.5 (0-1)

MS1 factor: 0.5 (0-1)

Peak count filter: 0 %

N% detected in at least one group: 0 %

Remove features based on blank information: ☐

Sample max / blank average: 5 fold change

Keep 'reference matched' metabolite features: ☒

Keep 'suggested (w/o MS2)' metabolite features: ☐

Keep removable features and assign the tag: ☒

Gap filling by compulsion: ☐

Load ☒ Together with Alignment Finish Cancel

Specify the QC file. All samples will be aligned on this QC file.

Set the retention time and mass tolerances

To remove peaks with more than N% missing values.

Peaks should be detected in N% of all samples of a class

5. Analysis results

The following window appears after processing:



By clicking on "Ref. matched", you can see the number of matched features based on your imported database. The following example is based on the interrogation of a msp

format database based on retention time, accurate mass and MS/MS spectrum for identification of level 1:



By clicking on **Show ion table**, you will easily see all the identified features and their respective intensities in each defined class:

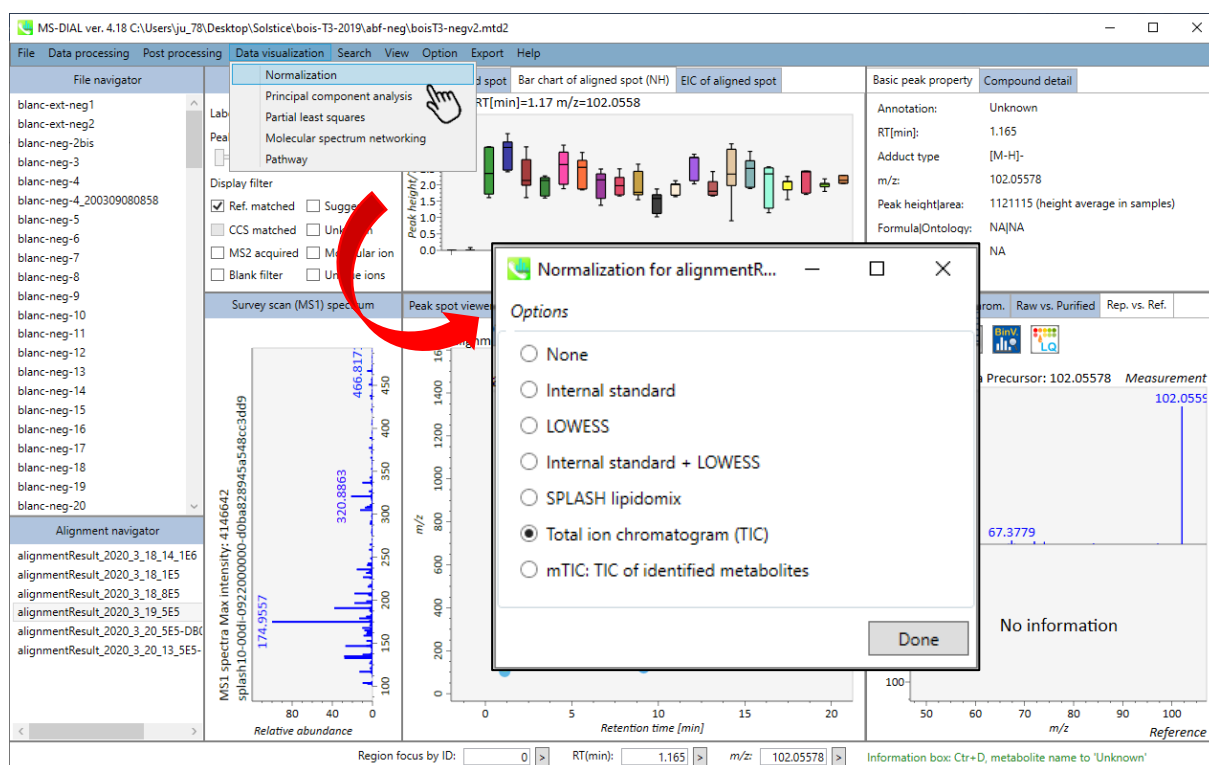
Alignment Table

Num of rows: 27 Metabolite Name Filter Comment Filter

102.06 Mz Range 1472.43 0.6 RT Range 18.4

ID	RT(min)	m/z	Type	Fill %	Metabolite name	Comment	Correlation	S/N	ANOVA P-value	Fold change (Max/Min)	BarChart
1	1.12	104.0351	[M-H] ⁻	0.09	(IROA) SERINE		0.65	131.5	5.38E-05	118.12	
453	1.26	341.1085	[M-H] ⁻	0.84	(IROA) MELIBIOSE		0.51	6289.5	7.17E-03	769.15	
310	8.21	289.0719	[M-H] ⁻	0.84	(Metasci) (+)-Catechin (Hydrate)		0.67	29656.4	9.01E-09	20097.32	
309	8.81	289.0718	[M-H] ⁻	0.84	(Metasci) (-)-Epicatechin		0.67	28564.6	3.13E-09	15026.86	
264	10.62	271.0614	[M-H] ⁻	0.84	(Metasci) (+/-)-Naringenin		0.77	42.9	3.48E-13	1036319.00	
239	1.16	259.0224	[M-H] ⁻	0.84	(IROA) MANNOSE 6-PHOSPHATE		0.80	1069.7	1.28E-15	475.27	
189	12.48	227.0714	[M-H] ⁻	0.83	(Metasci) cis-Resveratrol		0.63	96.2	3.37E-02	5959.37	
185	11.74	227.0713	[M-H] ⁻	0.84	(Metasci) trans-Resveratrol		1.00	9416.3	4.66E-15	20092.79	
146	7.13	203.0825	[M-H] ⁻	0.83	(IROA) TRYPTOPHAN		0.39	1447.9	1.11E-03	3711.93	

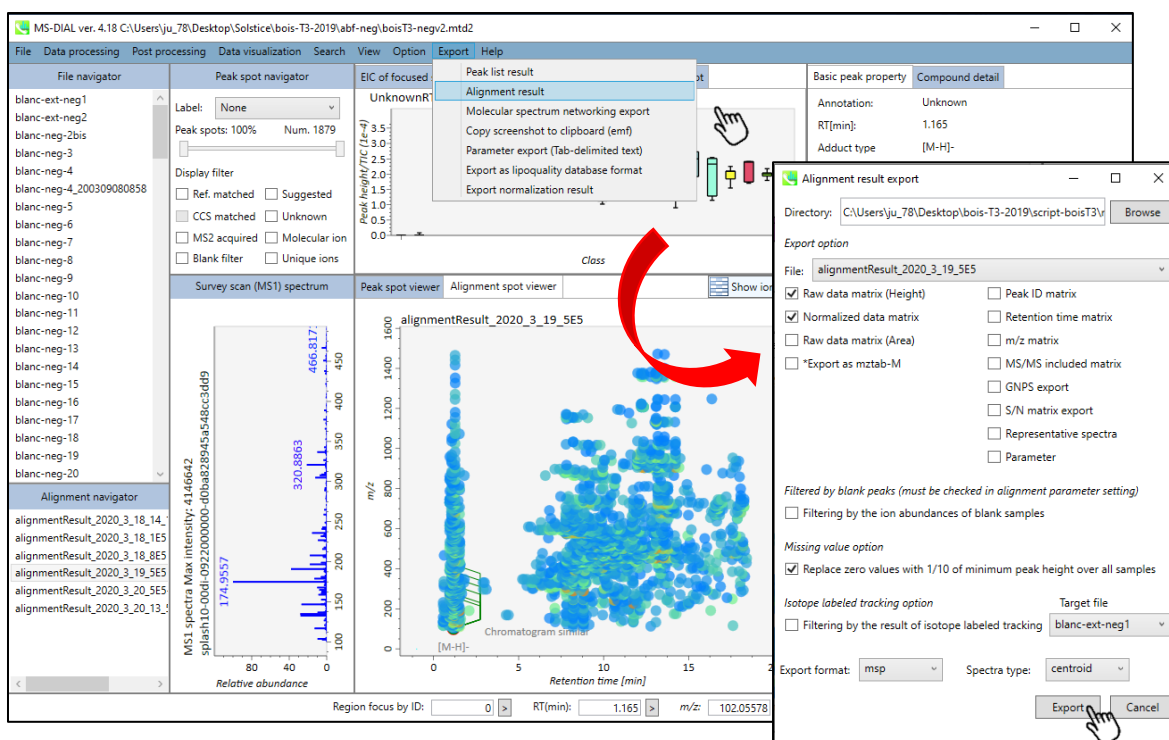
After alignment process, on the **data visualization** menu select **normalization** and normalized data by Total ion chromatogram (TIC) or another method.



On the “**data visualization**”, after normalization, you will be able to do some statistical analysis such as Principal component analysis.

6. Export peak list

On the “**Export**” menu select “**alignment results**” and export both **Raw data matrix (Height)** and **Normalized data matrix**. You can also select the option “**Replace zero values with 1/10 of minimum peak height over all samples**” for further statistical purpose.



7. Export all peaks

By right-clicking on one point, on the “**search formula and structure**” menu select “**Add component to search list**” and export « **all peaks** » for further identification purpose with MS-FINDER (see MS-FINDER tutorial). On the chosen folder, .MAT files will be generated and directly importable into MS-FINDER. At this point, you should be ready to follow the MS-CleanR and MS-FINDER tutorials.

