

LipidMS app workflow

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LipidMS Overview

LipidMS v2.0.0 is an R-package aimed to confidently identify lipid species in untargeted LC-MS for DIA or DDA data analysis. It combines a set of fragmentation and intensity rules and a parent-to-fragment co-elution score (PFCS, only applied for DIA analysis) calculated in predefined retention time windows. Depending on the MS evidence reached by the annotations, lipids can be identified at three different levels: i) subclasslevel, e.g., PG(34:1); ii) fatty acyl level, e.g., PG(16:0_18:1); and iii) fatty acyl position level, e.g., PG(16:0/18:1). As a general rule, parent ions will be found when no collision energy is applied, while fragment ions will be found when it is. Each lipid class has characteristic ionization and fragmentation properties that allow to filter informative fragments among all fragment ions to reconstruct the parent's structure. Next figure summarizes the basics of LipidMS.

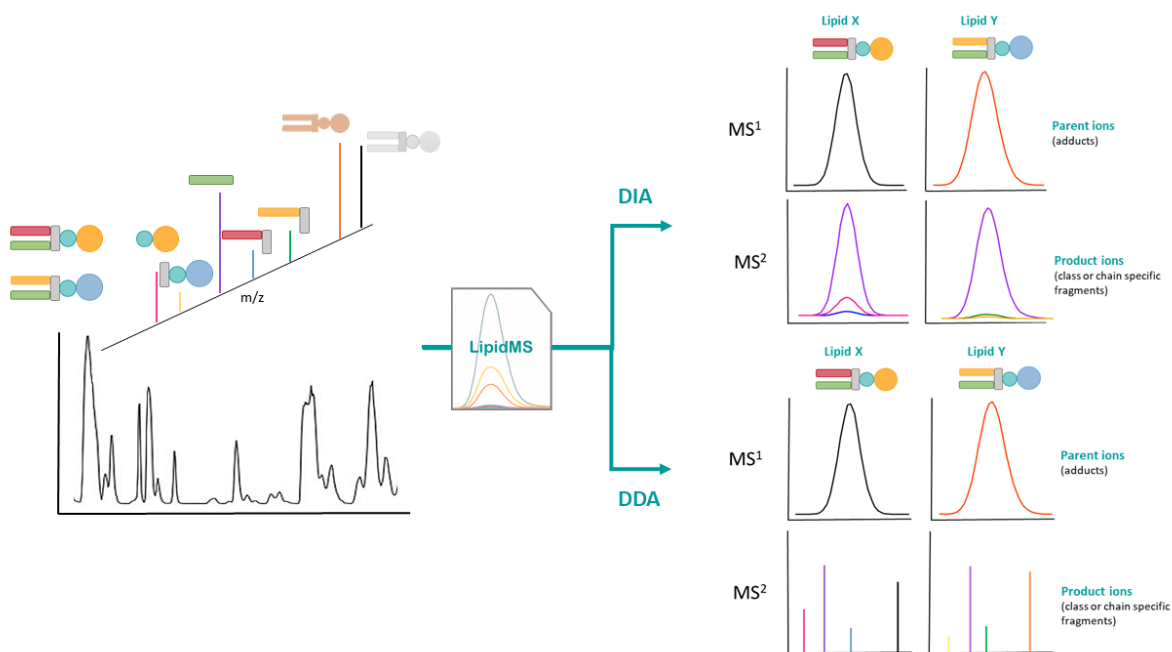


Figure 1: LipidMS abstract

Installation

The LipidMS application can be accessed locally from R or through our website at (<https://www.iislafe.es/es/software/lipidomicstools-description/>). In case you want to run LipidMS locally you need to install the package, otherwise skip this step.

```
# Install LipidMS and LipidMSdata2
install.packages("LipidMS", dependencies = c("Depends", "Imports"))
devtools::install_github("maialba3/LipidMSdata2")
```

Files conversion

To start the MS analysis raw files need to be converted into mzXML format first (you can use any software such as MSConvert from proteowizard) and then, LipidMS can be run. Unlike previous versions, LipidMS v2.0 can read mzXML files directly converted from raw files with no additional steps.

Example data files

Some .mzXML example files and extra documentation are available at (<https://www.iislafe.es/es/software/lipidomicstools-about/>).

LipidMS application

How to run it locally

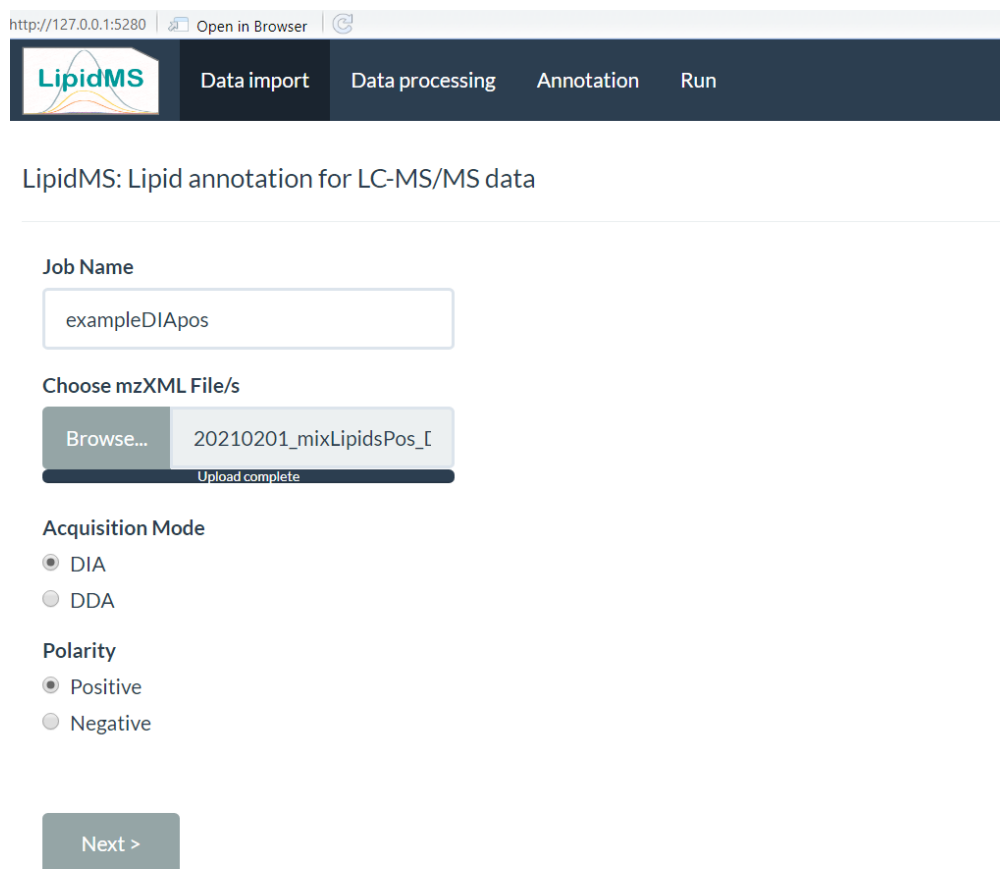
In case you are working locally on your computer execute the following lines and the app will open on a new window:

```
# load LipidMS library
library(LipidMS)

# Lunch the app
LipidMSapp()
```

Data Import

Once all your files have been converted to mzXML and the app is running, upload your files and tune the parameters. At the first tab, data acquisition mode (DIA or DDA) and polarity must be specified:



The screenshot shows the web interface of the LipidMS application. At the top, there is a browser address bar showing 'http://127.0.0.1:5280' and a button to 'Open in Browser'. Below this is a navigation bar with the LipidMS logo and four tabs: 'Data import' (selected), 'Data processing', 'Annotation', and 'Run'. The main content area is titled 'LipidMS: Lipid annotation for LC-MS/MS data'. It contains several input fields and controls: a 'Job Name' text box with the value 'exampleDIApos'; a 'Choose mzXML File/s' section with a 'Browse...' button and a file named '20210201_mixLipidsPos_1' which has an 'Upload complete' status bar; an 'Acquisition Mode' section with radio buttons for 'DIA' (selected) and 'DDA'; a 'Polarity' section with radio buttons for 'Positive' (selected) and 'Negative'; and a 'Next >' button at the bottom.


Figure 2: Data import tab of LipidMS app

If you have samples acquired with different polarities or acquisition modes, analyze them in different batches.

Data Processing

At the second tab we find all parameters required for peak-picking. More detailed information about all these parameters can be found at the documentation page of the dataProcessing function.

http://127.0.0.1:5280 | Open in Browser

 Data import Data processing Annotation Run


LipidMS: Lipid annotation for LC-MS/MS data

dmzaggglom (in ppm) <i>m/z tolerance used for partitioning and clustering. 5 by default.</i>	MS1 <input type="text" value="5"/>	MS2 <input type="text" value="5"/>
drtagglom (in seconds) <i>rt window used for partitioning (in seconds). 25 by default.</i>	MS1 <input type="text" value="25"/>	MS2 <input type="text" value="25"/>
drtclust (in seconds) <i>rt window used for clustering (in seconds). 25 by default.</i>	MS1 <input type="text" value="25"/>	MS2 <input type="text" value="25"/>
minpeak <i>minimum number of measurements required for a peak. By default, 5 for MS1 and 4 for MS2.</i>	MS1 <input type="text" value="5"/>	MS2 <input type="text" value="4"/>
minint <i>minimum intensity of a peak. By default, 1000 for MS1 and 100 for MS2.</i>	MS1 <input type="text" value="1000"/>	MS2 <input type="text" value="100"/>
grtgap (in seconds) <i>maximum rt gap length to be filled. 5 by default.</i>	MS1 <input type="text" value="5"/>	MS2 <input type="text" value="5"/>
drtminpeak (in seconds) <i>minimum rt width of a peak. 15 by default. At least minpeak within the drtminpeak window are required to define a peak.</i>	MS1 <input type="text" value="15"/>	MS2 <input type="text" value="15"/>
drtmaxpeak (in seconds) <i>maximum rt width of a single peak. 100 by default.</i>	MS1 <input type="text" value="100"/>	MS2 <input type="text" value="100"/>
maxeirpeak	MS1	MS2

Figure 3: Data processing tab of LipidMS app

Annotation

The third tab contains parameters related with the annotation step and lipid classes included in the analysis.

Data importData processingAnnotationRun

LipidMS: Lipid annotation for LC-MS/MS data

dmzprecursor
mass tolerance for precursor ions. 5 by default.

5

dmzproducts
mass tolerance for product ions. 10 by default.

10

rttol
total rt window for coelution between precursor and product ions. 5 by default.

5

coelcutoff
coelution score threshold between parent and fragment ions. Only applied if rawData info is supplied. 0.7 by default.

0.7

Lipid classes to annotate for ESI+ :

- ☒ MG
- ☒ LPC
- ☒ LPE
- ☒ PC
- ☒ PE
- ☒ PG
- ☒ Sphingoid bases
- ☒ Sphingoid bases phosphate
- ☒ Cer
- ☒ SM

Lipid classes to annotate for ESI+ :

- ☒ FA
- ☒ FAHFA
- ☒ LPC
- ☒ LPE
- ☒ LPG
- ☒ LPI
- ☒ LPS
- ☒ PC
- ☒ PE
- ☒ PG

Figure 4: Annotation tab of LipidMS app

Run

Finally, run your job. You will obtain two csv files with the results tables (the summary table and the whole peak table with annotations) and a pdf files with plots of the peaks supporting those identifications for each one of your files.

If you are using the web application, an email will be required to send your results back. Otherwise, if you are using LipidMSApp() on your computer, you will find three buttons to download your results. Wait until you can see the results on the main panel to download (you may need to write the extension .zip to save your files properly).

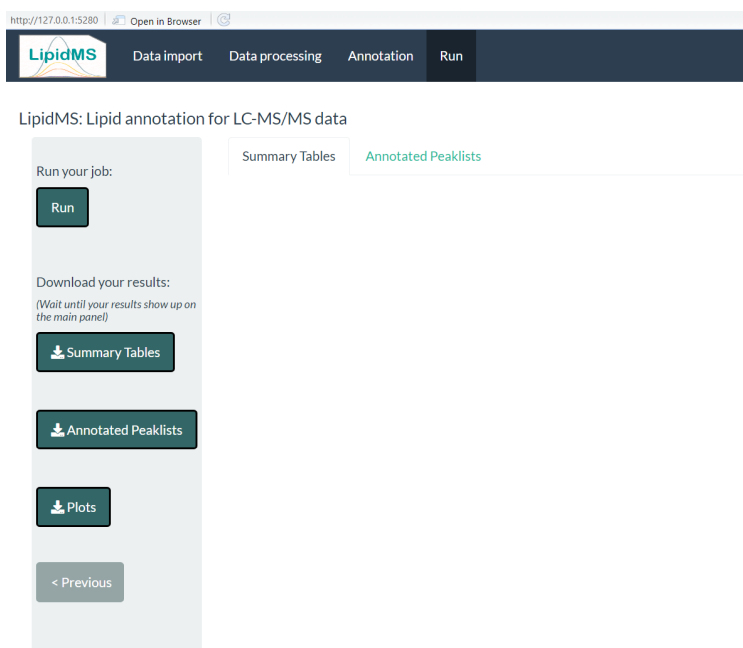


Figure 5: Run tab of LipidMS application

The screenshot shows the main panel of the LipidMS application. The 'Annotated Peaklists' tab is selected, displaying a table of results. The table has columns for ID, Class, CDB, FAcomp, m/z, RT, I, Adducts, ppm, confidenceLevel, and peakID. The results are organized into a table with 11 rows and 11 columns.

ID	Class	CDB	FAcomp	m/z	RT	I	Adducts	ppm	confidenceLevel	peakID
MG(17:0)	MG	17:0	17:0	327.29	917.44	107482700.37	M+H+H2O	3.89	MS-only	MS1_0_67
MG(17:0)	MG	17:0	17:0	327.29	497.37	2028562.13	M+H+H2O	3.06	MS-only	MS1_0_67
MG(20:1)	MG	20:1	20:1	402.36	1510.39	2683129.69	M+NH4	1.88	MS-only	MS1_0_89
LPC(17:0)	LPC	17:0	17:0	510.36	129.93	1241311560.02	M+HM+Na	0.99	FA	MS1_0_12
LPC(17:0)	LPC	17:0	17:0	532.34	129.33	68580483.02	M+NaM+H	0.78	FA	MS1_0_12
PC(17:0/17:0)	PC	34:0	17:0	762.60	627.15	1722179395.55	M+HM+Na	0.64	FA position	MS1_0_26
PC(16:0/18:1)	PC	34:1	16:0	760.58	535.39	5253307834.57	M+HM+Na	1.23	FA position	MS1_0_26
PC(18:2/18:0)	PC	36:2	18:2	786.60	559.53	1478387394.08	M+HM+Na	0.99	FA	MS1_0_29
PC(31:1)	PC	31:1		718.54	559.53	390186546.88	M+HM+Na	1.02	Subclass	MS1_0_22
PC(17:0/17:0)	PC	34:0	17:0	784.58	627.15	118795540.29	M+NaM+H	0.28	FA position	MS1_0_29
PC(16:0/18:1)	PC	34:1	16:0	782.57	534.78	335954991.45	M+NaM+H	0.80	FA	MS1_0_28
PC(18:2/18:0)	PC	36:2	18:2	808.58	559.53	106167538.50	M+NaM+H	0.70	FA	MS1_0_31
PC(31:1)	PC	31:1		740.52	559.53	18113403.04	M+NaM+H	0.44	Subclass	MS1_0_24

Figure 6: Results shown on the main panel (just for the local app)

If you have any further questions, please do not hesitate to contact us at: maribel_alcoriza@iislafes.es or maribel_alcoriza@hotmail.com