LipidMS application workflow

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2021-12-13

LipidMS Overview

LipidMS v3 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 basis of LipidMS annotation:

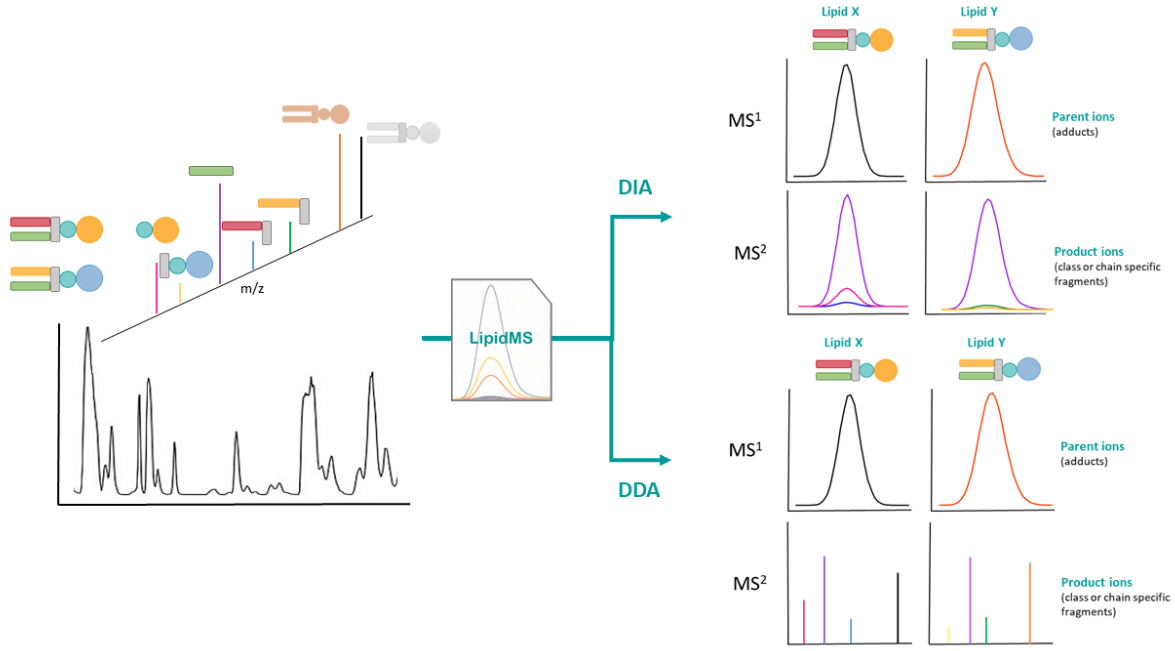

Figure 1: LipidMS abstract

And the following figure shows the overall workflow of LipidMS:

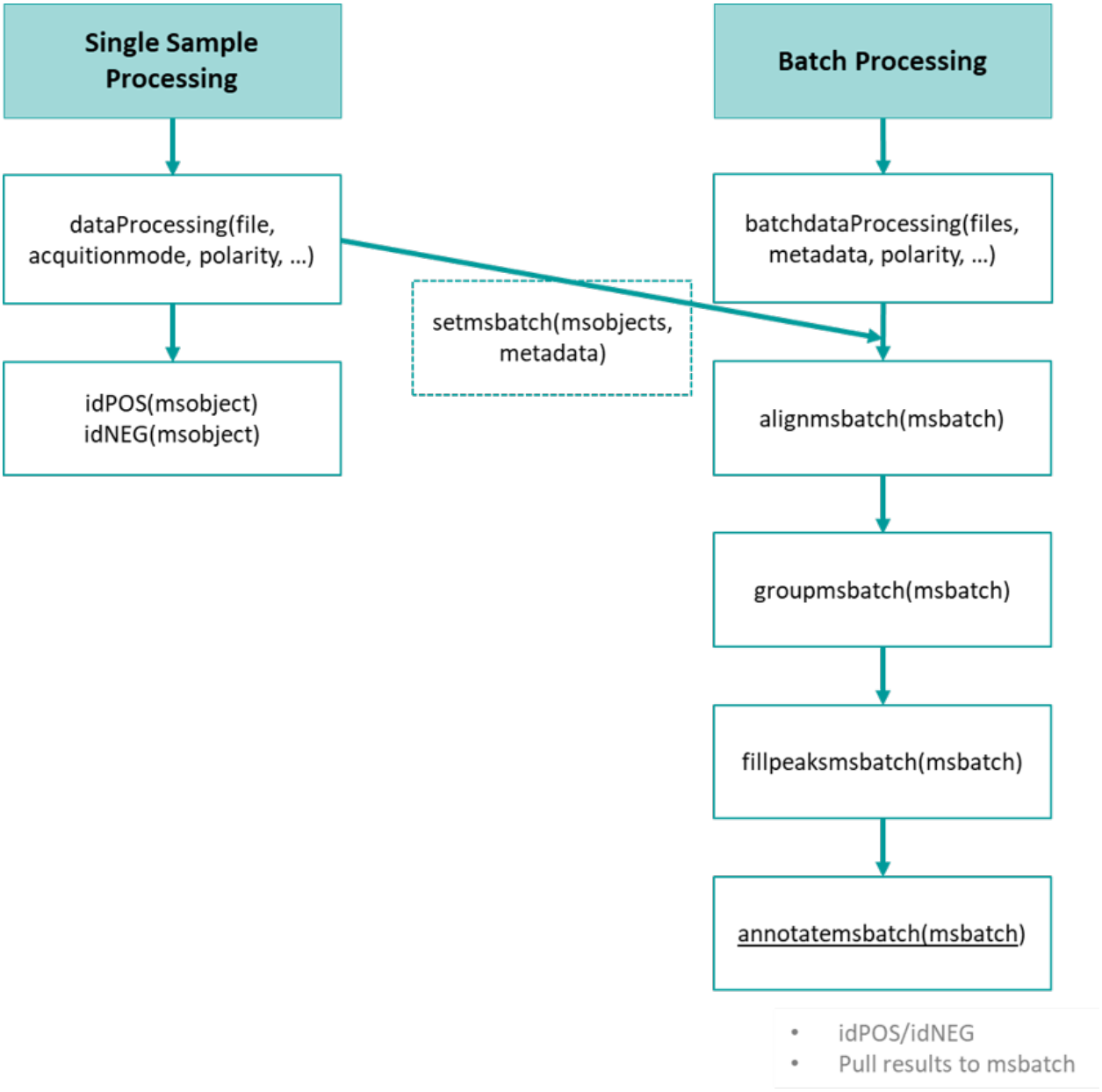

Figure 2: LipidMS workflow

Installation

The LipidMS application can be accessed locally from R or through our website at https://www.lipidms.com. In case you want to run LipidMS locally you need to install the package, otherwise skip this step.

```
# Install LipidMS
install.packages("LipidMS", dependencies = c("Depends", "Imports"))
```

Files conversion

To start the MS analysis raw files need to be converted into mzXML format (you can use any software such as MSConvert from proteowizard) and then, LipidMS can be run.

Example data files

Some example files and scripts can be downloaded (here).

LipidMS application

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)

# Example data files can be downloaded from:
# https://drive.google.com/drive/folders/1hSYrQBkh-rAA-oiaKqGkrNL7uWQraV75?usp=sharing

# Lunch the app
LipidMSapp()
```

In case you would prefer to use the web application, it can be accessed from https://www.lipidms.com.

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, a metadata csv file is required. It must have 3 columns: sample (mzXML file names), acquisitionmode (MS, DIA or DDA) and sampletype (QC, group1, group2, etc.)

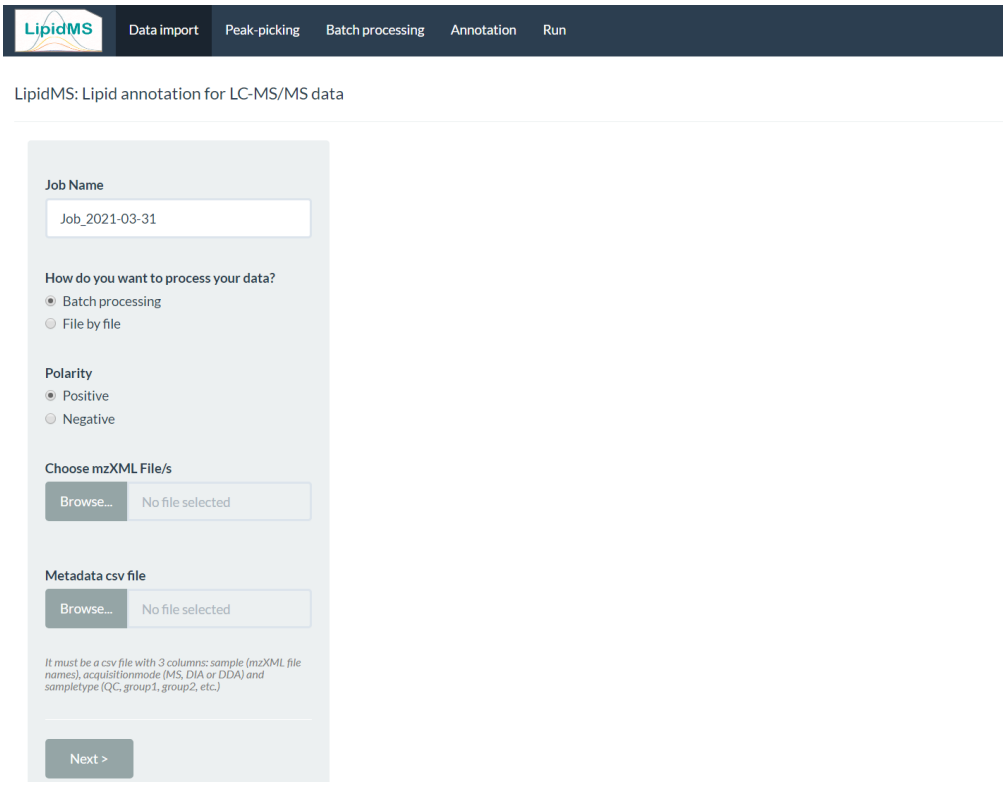

Figure 3: Data import tab of LipidMS app

If you have samples acquired with different polarities they must be analyzed in different batches.

Peak-picking

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.

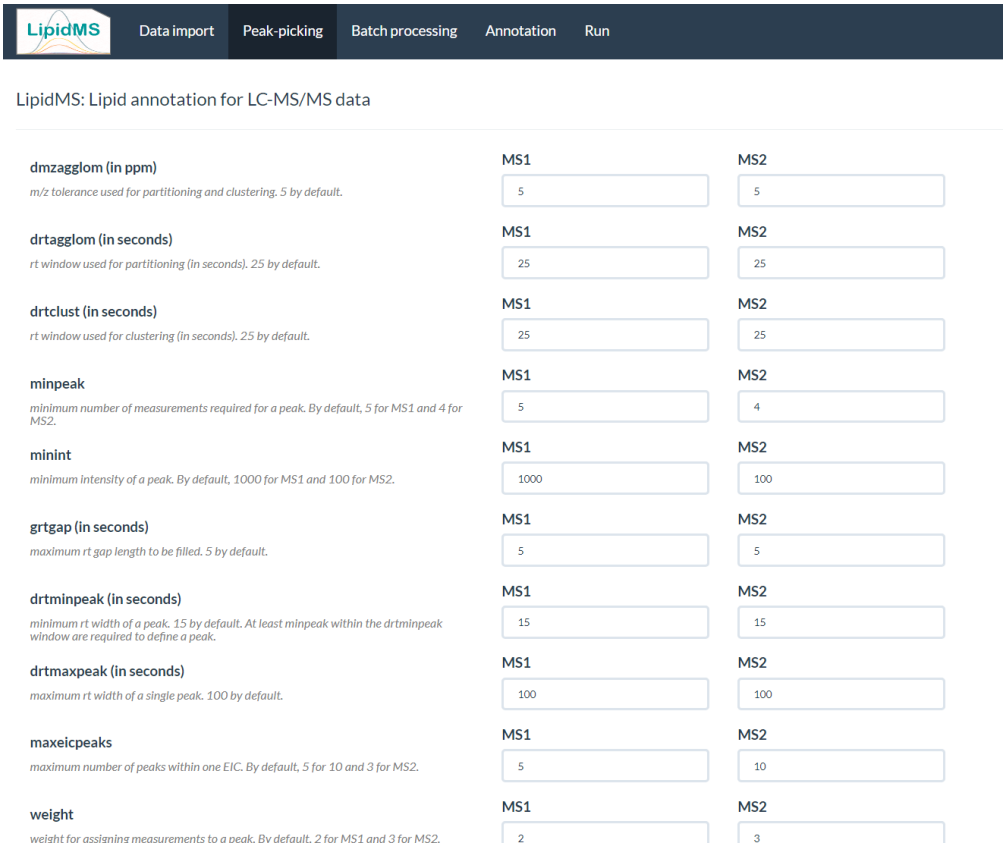

Figure 4: Peak-picking tab of LipidMS app

Batch processing

The third tab contains parameters required for alignment, grouping and filling missing peaks.

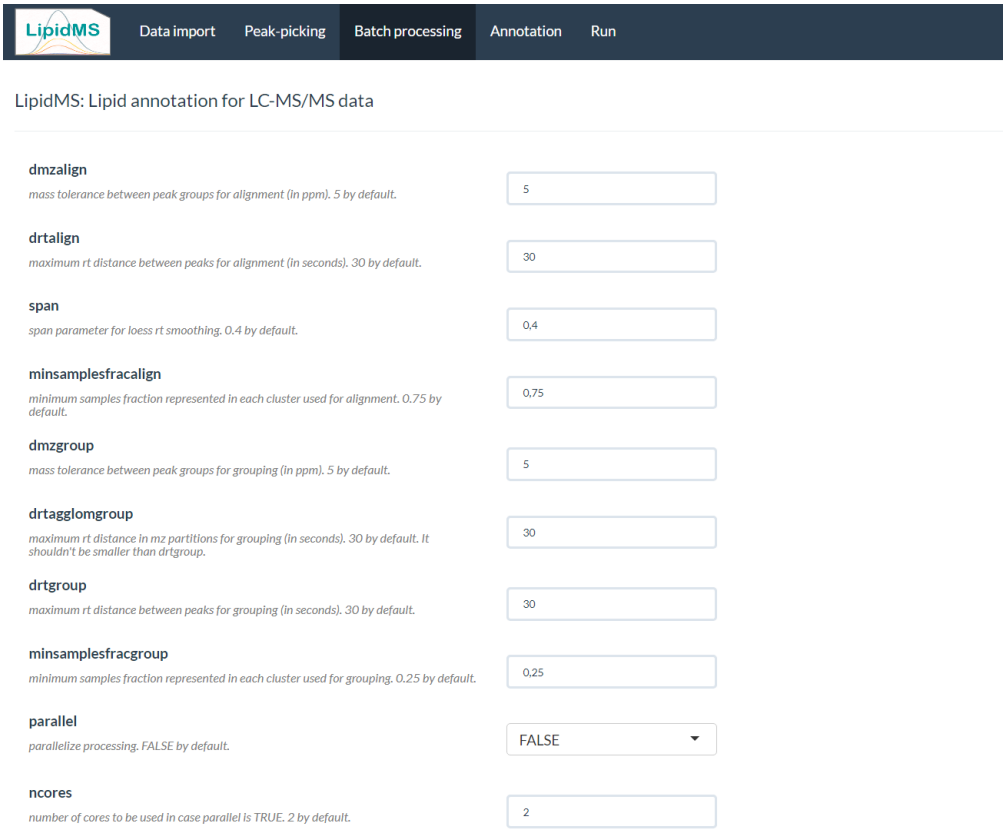

Figure 5: Batch processing tab of LipidMS app

Annotation

This tab contains parameters related with the annotation step and which lipid classes will be included in the analysis.

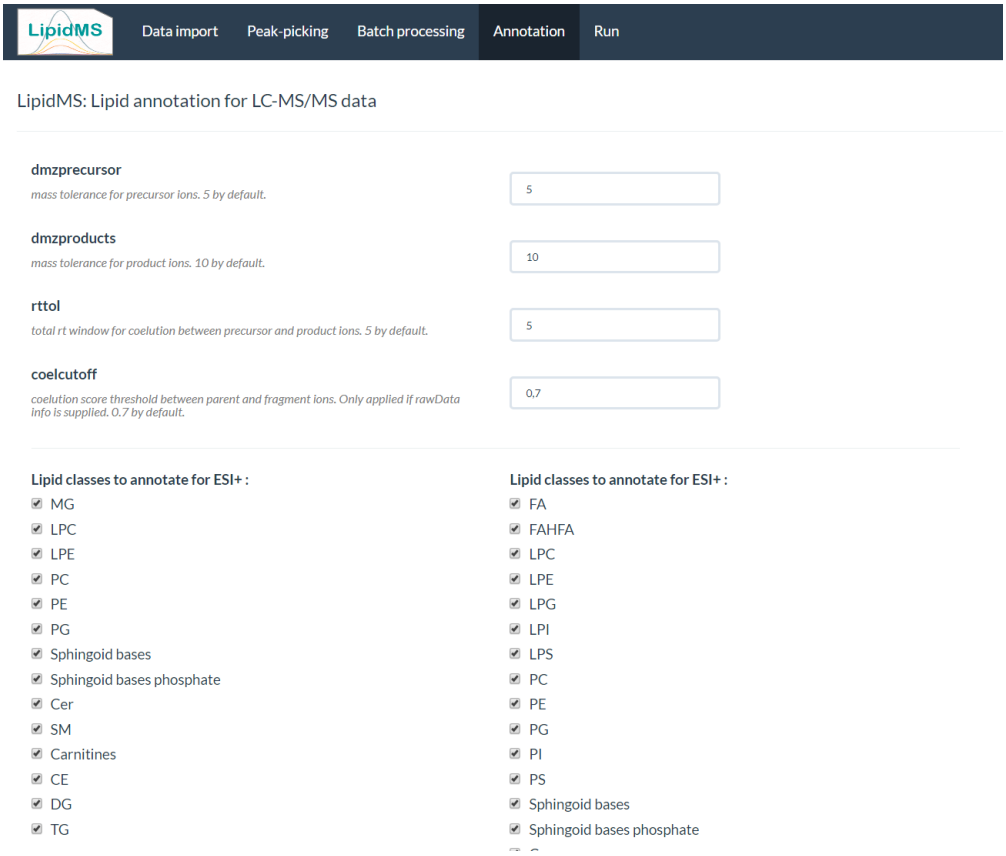

Figure 6: Annotation tab of LipidMS app

Run

Finally, run your job. You will obtain two or three types of csv files with the results tables (feature matrix if batch processing has been performed, summary tables and the whole peak tables with annotations) and pdf files with plots of the peaks supporting lipid identifications for each one of your files.

If you are using the web application, an email will be required to send back your results. Otherwise, if you are using LipidMSapp() on your computer, you will find four 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).

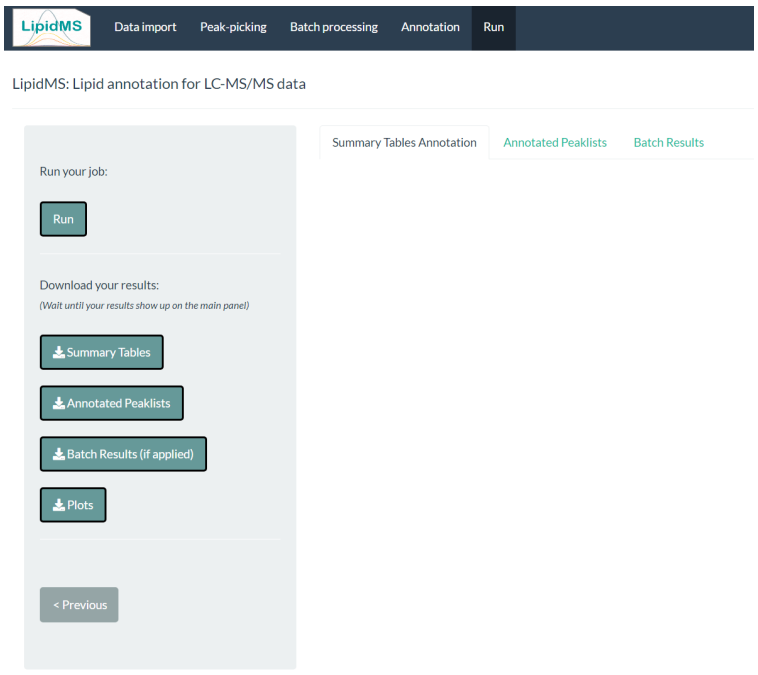

Figure 7: Run tab of LipidMS application

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