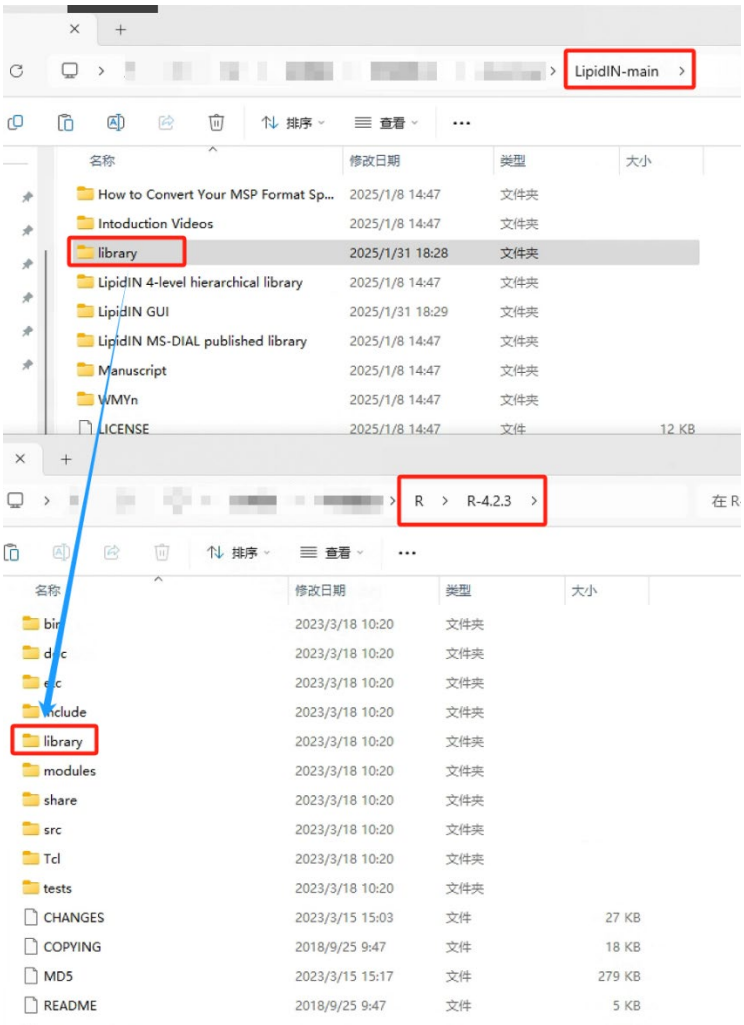
LipidIN Installation and Launch Guide

After downloading the **LipidIN** project from GitHub, navigate to the LipidIN GUI folder. Open the file 'code for launch UI.R' using Rstudio (we recommend using R version 4.1 and above.).

1. **Check Required Packages**

   Lines 1–10 of the script are used to check whether the required R packages are installed. Select these lines, then click **Run** in the top-right corner of your R console. Wait for the necessary packages to be installed. **Besides, we have packaged and uploaded all necessary dependency packages to Zenodo (https://zenodo.org/records/14779264). you can simply download and copy these packages into their R library directory, ensuring consistency in the required R packages.(Recommend methods)**



2. **Modify File Path**

Once all the dependencies are installed, update the file path on **line 15** of the script. Replace the placeholder file path with the path to the downloaded 'LipidIN_2.0.0.1.tar.gz' file. Note that here you need to write the full address of LipidIN_2.0.0.1.tar.gz, the code is only an example, please modify the address according to the actual situation.

3. **Install LipidIN**

   After updating the file path, run **lines 12–19** to install LipidIN. During the installation process, a pop-up window will prompt you to confirm the installation of private packages. Click **Yes** to proceed.

4. **Launch the LipidIN UI**

   Finally, run **lines 22–23** of the script. This will launch the LipidIN user interface.

Parameter input and precautions

1. **Step 1: Input mzML File Path**

   In the first input field of the UI, enter the file path of the mzML file you wish to annotate. If necessary, you can use the **MSConvert** software to convert your files into the mzML format. The default parameters of MSConvert are sufficient for this conversion, and additional operations such as centralization are not required. Please ensure that there are no spaces or special characters in the file path. Please note that the UI does not like long addresses, so please keep your address as accurate, concise, and free of special characters as possible.

2. **Step 2: Input Spectral Library Path**

   In the second input field, provide the file path to the spectral library to be used for annotation. Note that the spectral library must be in **RDA format**. For instructions on converting an MSP-format spectral library to RDA format, refer to the guide: How to Convert Your MSP Format Spectral Library to RDA Format (https://github.com/LinShuhaiLAB/LipidIN/tree/main/How%20to%20Convert%20Your%20MSP%20Format%20Spectral%20Library%20to%20RDA%20Format). Alternatively, you can use the pre-compiled RDA spectral library available on our GitHub repository: LipidIN/LipidIN 4-level hierarchical

library/pos_ALL.rda. As with the mzML file path, ensure that the spectral library path as accurate, concise, and free of special characters as possible.

3. **Step 3: Select Filtering Threshold**

   Choose a filtering threshold to exclude peaks with intensities lower than a certain percentage of the maximum intensity in the spectrum. Recommended values for this threshold are **0.01**, **0.05**, or **0.1**.

4. **Step 4: Select Ionization Mode**

   Select the ionization mode for your analysis. The available options are:

   - Positive ionization mode
   - Negative ionization mode ([M+HCOO]-)
   - Negative ionization mode ([M+OAc]-)

5. **Step 5: Set Mass Tolerance for Precursor Ions**

   Specify the mass-to-charge ratio (m/z) tolerance for precursor ions during spectral library matching. Input the desired tolerance value in this step.

6. **Step 6: Set Mass Tolerance for Fragment Ions**

   Specify the mass-to-charge ratio (m/z) tolerance for fragment ions during spectral library matching. Input the desired tolerance value in this step.

7. **Step 7: Select MS2 scan time or MS1 Rt to use for the LCI module.**

   MS1 retention time (RT) provides higher accuracy as it reflects the precise chromatographic peak maxima. However, it requires additional computational time for peak extraction and isotope removal. In contrast, MS2 scan time, while less accurate than MS1 RT, significantly reduces processing steps and improves analysis speed, making it a practical choice for high-throughput lipid annotation workflows.

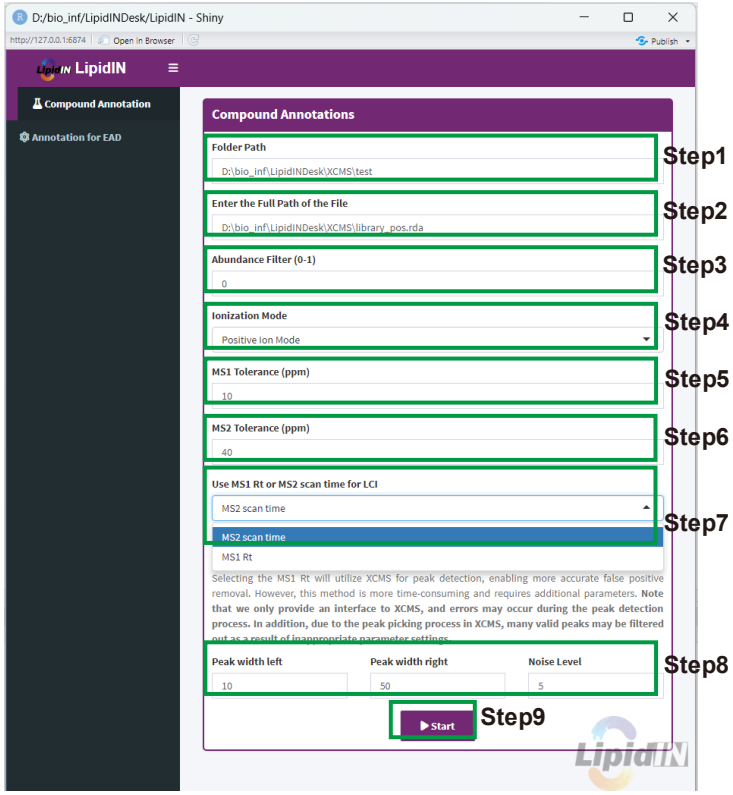
8. **Step 8: Fill in the XCMS peak picking parameters**

   For detailed explanations of the parameters and recommendations on their usage, please refer to the comprehensive documentation available at DOI: 10.18129/B9.bioc.xcms.

9. **Step 9: Start the Annotation Process**

   After verifying the inputs for all the steps above, click the **Start** button to

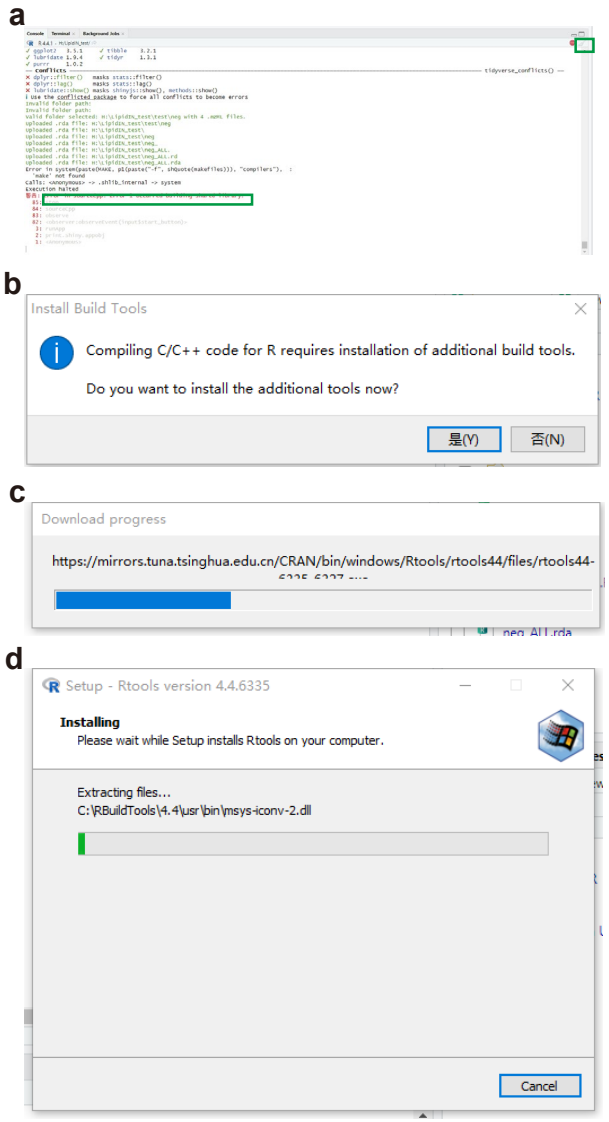
initiate the lipid annotation process.



LipidIN Process Instructions

1. **Package Loading and Installation**

   After clicking "Run," the first message (Message 1) will indicate that the system is checking for missing packages and loading them. If you are running the script for the first time, this process might take longer, as it will automatically install RTools if necessary. Please be patient during this step.



The installation process is as follows: 1. When encountering the error message **"Error in SourceCpp"**, it indicates that **Rtools** needs to be installed. 2. Click the red dot in the upper-right corner of the image to terminate the LipidIN operation. 3. Afterward, an installation prompt for **Rtools** will appear. 4. Click **Yes** to begin the download and installation process.

2. **File Format Conversion**

   Once all required packages are loaded, the second message (Message 2) will notify you that LipidIN is converting mzML files to rda files. If you encounter errors at this step, please ensure the following:

   - Your mzML files contain MS2 spectra.
   - The input file path does not contain spaces or special characters.

3. **Library Loading**

   After file conversion, the system will load the spectral library, which will be indicated by the third message (Message 3). This step can take a significant amount of time, especially if your spectral library contains millions of entries.

4. **Spectral Matching**

   Once the library is loaded, spectral matching will begin, and the fourth message (Message 4) will appear. This process is typically very fast. If it becomes stuck or an error occurs, check the format and content of your spectral library for accuracy.

5. **Lipid Retention Time Analysis**

   If MS2 scan time is selected, the peak extraction step will be bypassed, and the LCI module will proceed directly. Following spectral matching, the system will execute lipid retention time prediction, as indicated by Message 5. This step typically requires approximately 1–2 minutes to complete.

6. **XCMS quantitateion**

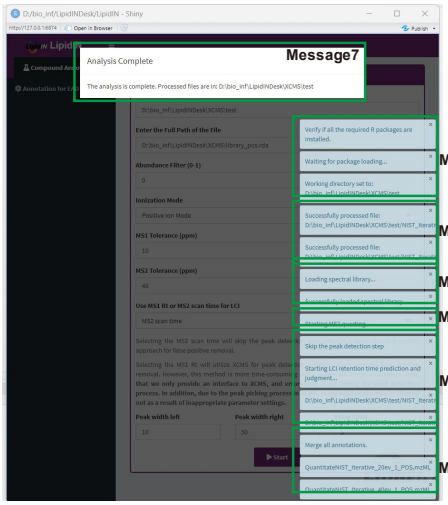
   Upon completion of the LCI step, the XCMS and CAMERA packages will be utilized for quantitative analysis, peak alignment, isotope removal, and gap filling.

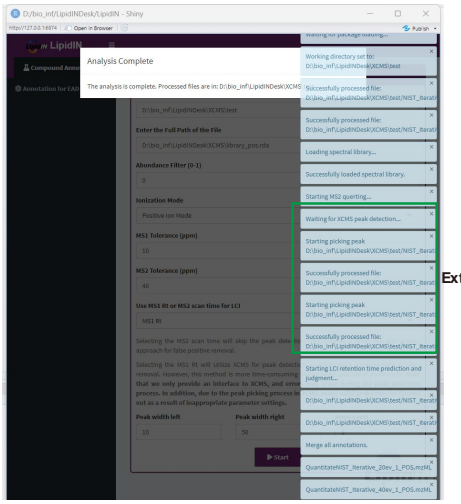
7. **Completion**

   Finally, when all annotation processes are complete, the system will display the sixth message (Message 7).

8. **Extra Message for using MS1 Rt**

   If MS1 retention time (RT) is selected, peak extraction will be performed using XCMS prior to the retention time prediction and evaluation module.


Message7
Analysis Complete
The analysis is complete. Processed files are in: D:\bio_inf\LipidINDesk\XCMS\test
Message1
Message2
Message3
Message4
Message5
Message6


Analysis Complete
Extra Message