Step 1. Download PRISM-MS.rar from our Github: https://github.com/CeMOS-Mannheim/PRISM-MS/blob/main/PRISM-MS.rar
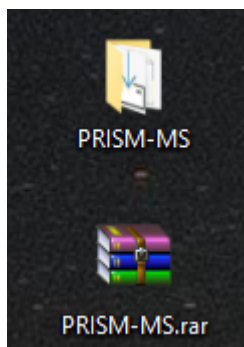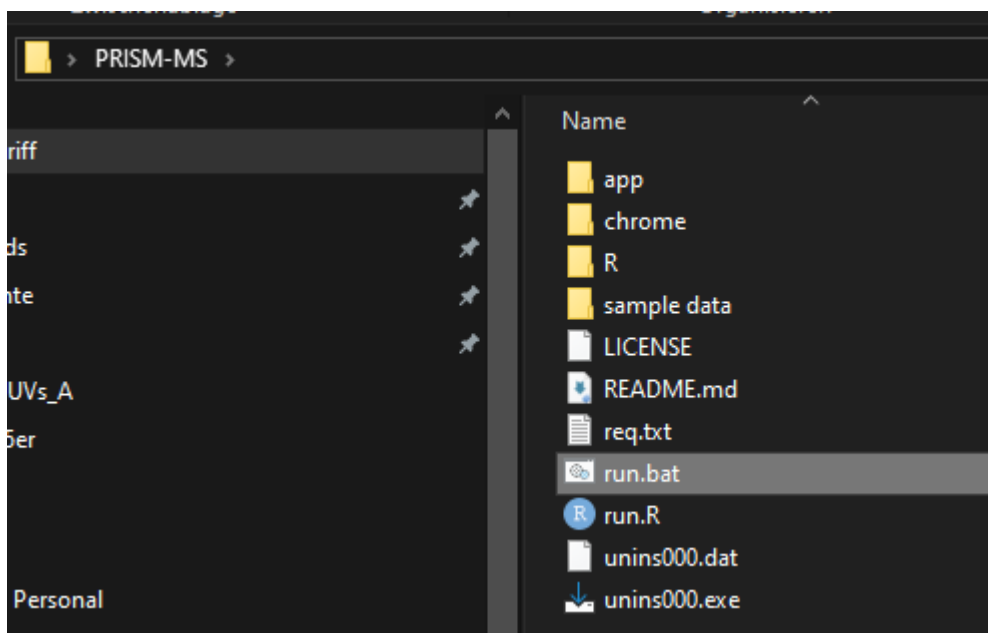
Step 2. Unzip it into any local directory (e.g. Desktop)

Step 3. Open PRISM-MS folder and click on: "run.bat". This will pop open a command prompt starting the shiny application. There is no need to install R or anything else, since it´s all packaged in the .zip folder.

Step 4. Load 1 .imzML and the corresponding .idb file from your measurement (PreScan). Sample data can be found in the unzipped PRISM-MS folder under "..\PRISM-MS\sample data\231027_GUVs_A". This is a small sample dataset to test the application as well as the PreScan selection
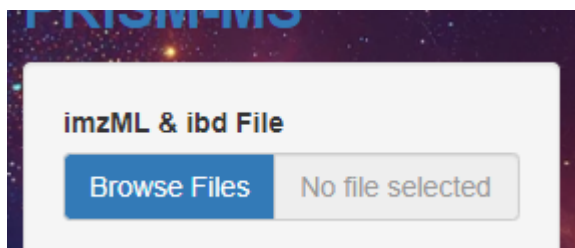
Step 5. Load the corresponding .mis file, a sample file is also included in the same directory as above

Choose MIS File

Browse... No file selected

Step 6. Select Normalization method, usually "none" is sufficient

Normalization

none ▼

Step 7. Set your mass range and tolerance, for the sample data the preset values are fine
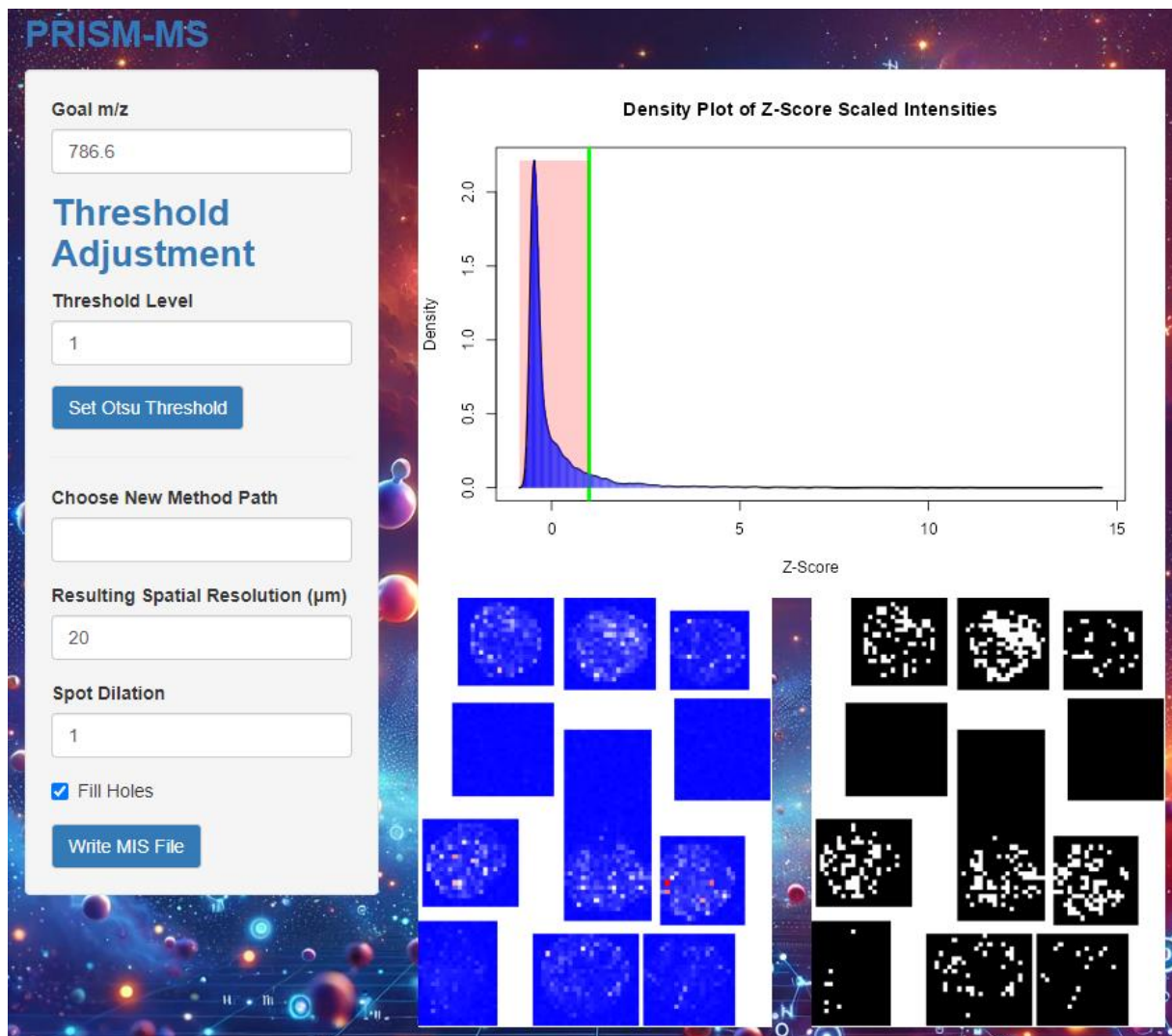
Mass Range

650 — 800   5,000

1 501 1,001 2,001 3,001 4,001 5,000

Tolerance (ppm)

10

Step 8. If all data was loaded correctly a Load button should appear, go and press this

Load

Step 9. Set your Goal/Target m/z e.g. 786.6 or 678.5 for this GUV dataset

Step 10. Adjust threshold levels manually, get the otsu threshold until you find the selected mask you prefer. Enter the path for the method of the second acquisition and adjust spatial resolution of the DeepScan as wished. Also spot dilation can be adjusted at this point. The thresholded image (bottom right) will be converted into a new .mis file as soon as you press "Write MIS File".

Step 11. Store this mis file in a folder and start your measurement.

Step 12. Example result of a PreScan is also in the sample data folder called: "231027_GUVs_A_5_1.mis" which is the guidance to a DeepScan with 5 micron spatial resolution and a spot dilation factor of 1