Quick-Start Tutorial

This quick-start tutorial will guide you through all the steps needed to (i) load a dataset (i.e. peak lists with its corresponding intensity values), (ii) visualize 3D spectra, (iii) compare the spectra in the dataset using boxplot and heatmap functions and (iv) carry out different analyses, such as clustering and Principal Component Analysis.

Contents

1. Load dataset

To load a sample dataset, for example, select *Load sample data* followed by SPE dataset (Figure 1a). This dataset contains MALDI-MS peak intensity and its corresponding m/z values observed from a Solid phase extraction (SPE) conditions screening experiment when using six different SPE materials and four different SPE conditions.

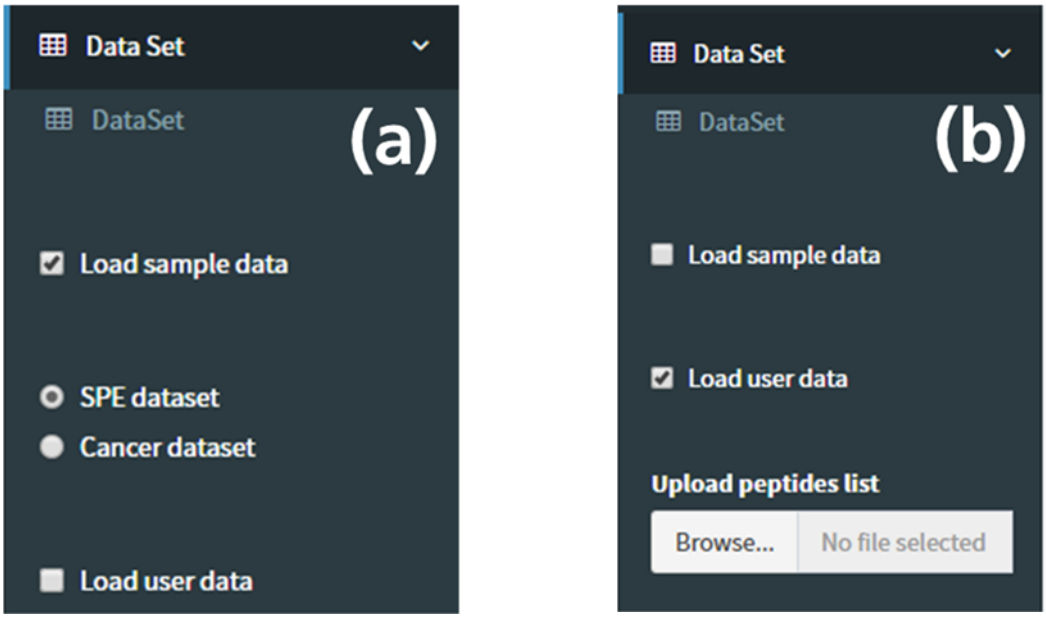


Figure 1. *Load Dataset. 1a. Options need to be selected to load a sample dataset. 1b. Options need to be selected to load a user dataset.*

To load your own dataset, Select the *Load user data* and click *Browse* button (Figure 1b). Then, another dialog box will appear as in Figure 2.

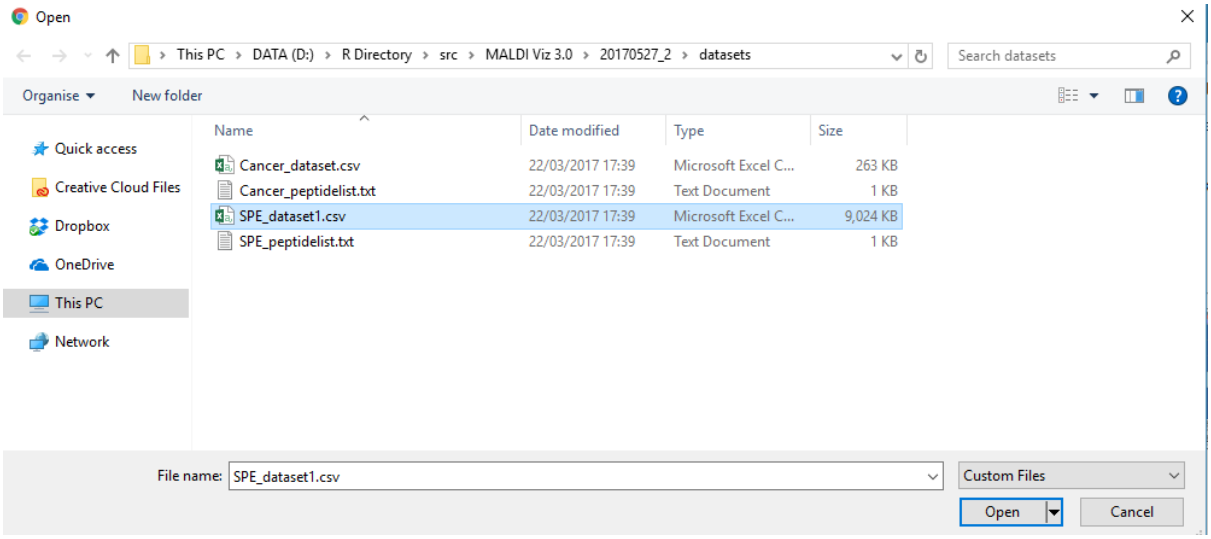


Figure 2. *Dialog box to select and load the user dataset*

You must find the corresponding file from your computer. And click the *Open* button to load the data.

After the data is loaded, you can see it in the DataSet section.

2. Spectra View

If you'd like to compare multiple mass spectra from different samples, simple overlay is not the right way to do it. *3D plot* in *Spectra view* provides much clearer representation of large data sets, where all the differences can be seen immediately. This is very useful for finding features and patterns across the experiment in one quick glance instead of looking at the individual spectra.

The user can inspect the data such as m/z, intensity, and sample details by moving the cursor along the spectrum. Moving, scaling, zooming functions are also made as easy and user friendly as possible. And everything is accessible by mouse.

To view 3D plot, press the Spectra View on the toolbar. Even if 3D plot provides a clear representation to find pattern across the experiment, manual selection of spectra for sub-setting might be essential in some cases.

In case you would like to make the sub-setting select *Edit plot*, there are several options available to select spectra manually (**Figure 3**).

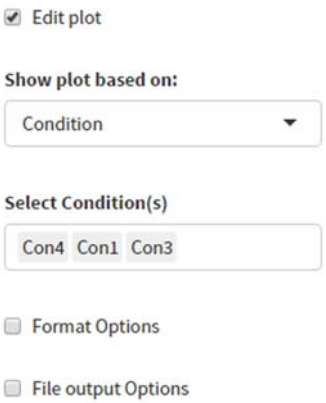


Figure 3. *Options available in the Spectra view data section to edit the 3D plot.*

You must provide the following information:

Show plot based on: In the dropdown box, you can add either select condition or material.

Select condition(s) / material(s): The intra-sample spectra selection. For this, you can select condition or material of your interest. Multiple conditions and materials selection is also possible.

After this operation, you can see the plot section automatically update with the selected spectra. Depending upon the selected spectra, loading time may vary.

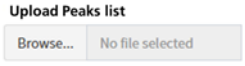
For further help on the 3D plot, please refer to the help.

3. Analysis - Comparison

The *Analysis* section allows you to make comparable a set of spectra with *Boxplot* and *Heatmap* operations. Analyses operations take *Peaks List* of interests as input.

For this quick-start, you can use sample peaks list.

For further help on *Peaks List, Boxplot* and *Heatmap*, please refer to the help.

To upload your own *Peaks List*, press the button 

on the *Boxplot* and *Heatmap 1* data sections. A dialog box will appear as in **Figure 4**:

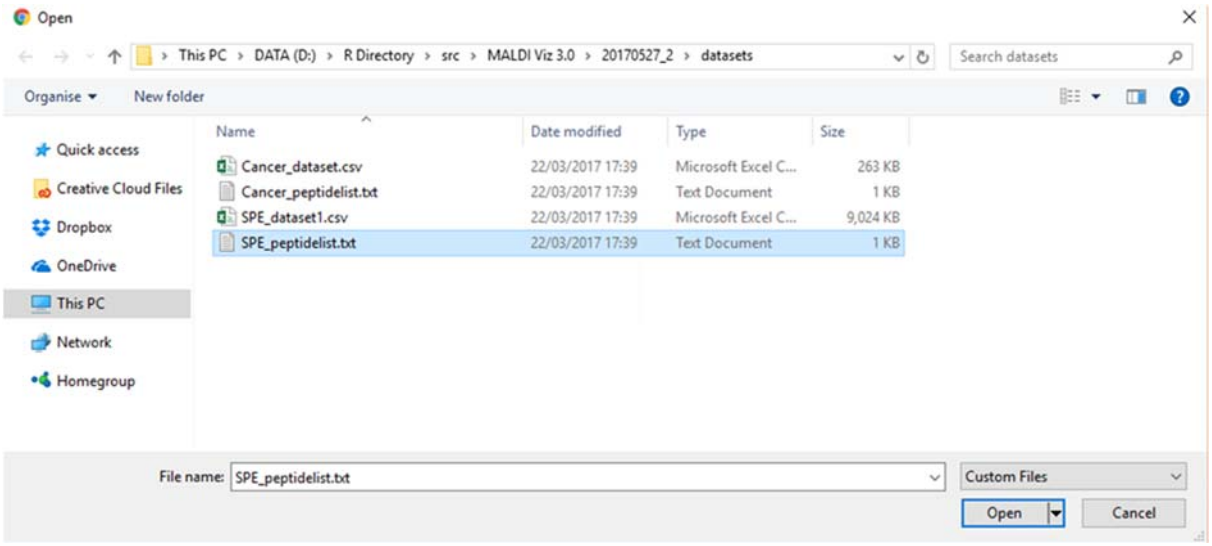


Figure 4. *Dialog box to select and load the user peaks list.*

You must find the corresponding file from your computer. And click the *Open* button to load your *Peaks list*.

3.1. Boxplot

For a *Boxplot* generation, you must provide the following information:

Select Condition(s): in the *Condition(s) to check:* tab you can add the condition of your interest in to compare. Multiple conditions selection is also possible.

After this operation, you can see the updated plot in the *Boxplot* plot section.

3.2. Heatmap 1

For generating a cluster in *Heatmap 1*, you must provide the following information:

Column cluster: in the *Distance - Column:* tab you can select the different distance method of your choice in to compare spectra.

Row cluster: in the *Distance - Row:* tab you can select the different distance method of your choice in to compare spectra.

Clustering: in this tab, you can select the different linkage method of your choice in to compare spectra.

Annotations: Annotations based on the metadata can also be added on top of the heatmap, to get an overview which annotated groups are separated better than others. To know more about metadata and Annotations please refer to the help.

After this operation, you can see the updated heatmap plot with clustering in the H*eatmap* plot section.

For this quick-start, you can use the default distance and clustering linkage method, that is euclidean and ward.D2. For further help on different distance and clustering linkage methods, please refer to the help.

4. Analysis - Classification

The *Analysis - Classification* section allows you to classify a set of spectra with *Heatmap, Cluster* and *PCA* operations.

4.1. Heatmap 2

Heatmap 2 allows finding groups of similar spectra among all the samples being studied. It allows to check if the different conditions present in the input data are separable by means of the m/z intensity values of each sample.

To apply the Heatmap analysis with clustering to the dataset, press the button 

on the toolbar. *Heatmap 2* window will appear.

You must provide the following information:

Column cluster: in the *Distance - Column:* tab you can select the different distance method of your choice in to compare spectra.

Row cluster: in the *Distance - Row:* tab you can select the different distance method of your choice in to compare spectra.

Clustering: in this tab, you can select the different linkage method of your choice in to compare spectra.

Annotations: Annotations based on the metadata can also be added on top of the heatmap, to get an overview which annotated groups are separated better than others. To know more about metadata and Annotations please refer to the help.

For this quick-start, use the default settings and select *Cancer dataset* and **Dataset Type** -> *Expression Profile* under **Dataset** section in the toolbar.

As you can see, the spectra of Cancer dataset can be grouped by their corresponding conditions using Clustering.

4.2. Cluster

Cluster window provides a dendrogram to identify similarity and difference between the groups in the given dataset.

To apply the cluster analysis to the dataset, press the button 

on the toolbar. Heatmap 2 window will appear.

You must provide the following information:

Cluster based on: You can select either column or row based clustering depending upon your dataset in interest

Distance matrix method: In this tab, you can select the different distance method of your choice in to classify the dataset.

Clustering method: In this tab, you can select the different linkage method of your choice in to classify the dataset.

To know more about dendrogram, distance method and clustering method please refer to the help.

4.3. Principal Component Analysis

Principal Component Analysis (PCA) allows the user to visually identify if there is a separation between the conditions (labels) present in the dataset.

To apply the PCA analysis to the dataset, press the button 

on the toolbar. PCA window will appear. For generating a 3D PCA plot, you must provide the following information:

PCA mode: to select PCA plot based on sample or variables.

PCA Normalization: whether input data will be normalized.

After this operation, you can see a 3D PCA plot in the PCA window plot section.

For this quick-start, use the default settings and select *Cancer dataset* and **Dataset Type** -> *Expression Profile* under **Dataset** section in the toolbar.

For further help on *PCA*, please refer to the help.