HUPO2013 POS-01-246

Identification and label-free quantitation of mass spectrometric data via freely available plug-in software, Mass++

oSatoshi Tanaka[1], Yuichiro Fujita[1], Akiyasu C. Yoshizawa[1], Shin-ichi Utsunomiya[1], Shigeki Kajihara[1], Mitsuru Fukuda[2,3], Masayuki Ikawa[2,3], Kentaro Takahashi[2], Tsuyoshi Tabata[2], Ken Aoshima[2], Yoshiya Oda[2], Koichi Tanaka[1]

1 Koichi Tanaka Laboratory of Advanced Science and Technology , Shimadzu Corporation 2 Eisai Co., Ltd. 3 iBio-Tech Co., Ltd.

SHIMADZU

Eisai Co., Ltd.

ms3d FIRST Program

Related Presentation : POS-01-271

1. Introduction

In 2006, we started to develop a visualization and analysis tool for mass spectrometry, Mass++. Recently we have improved identification and quantitation functions of Mass++. In identification, a conventional method begins with extracting peaks, saves them in a text file and then posts it to a search engine; hence it is quite time-consuming. Mass++ can directly post peak lists and parameters to a certain search engine, such as Mascot, X! Tandem or MassBank, which are linked with Mass++. Searched results are stored in Mass++ internal database and can be displayed in the viewer of Mass++. In addition, Mass++ provides quantitation data of peaks and can also manage quantitation results using a “peak matrix”, where its row represents each peak and it column represents each sample. The quantitation results are also stored in the Mass++ internal database and linked to corresponding identification results. Hence, peaks related to target substances can be easily found in original mass spectrometric data.

2. Mass++

Mass++ is a visualization and analysis tool for mass spectrometry and it has some rich functions such as data visualization, smoothing, baseline subtraction, identification, quantitation and so on.

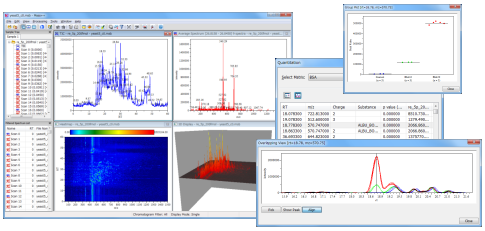


Fig. 1. Mass++

Mass++ has a plug-in structure which allows us to customize software depending on users’ own purposes. In addition, new functions can be developed using C/C++, C#.NET and VB.NET as a new plug-in without editing Mass++ source code.

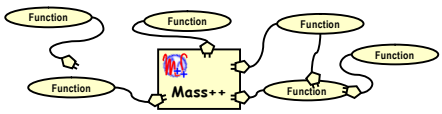


Fig. 2. Plug-in Structure

Some kinds of plug-ins for data reading make Mass++ possible to read various data formats.

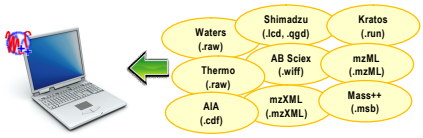


Fig. 3. Supported Data Formats

Mass++ can be freely downloaded from the following website:

http://www.first-ms3d.jp/english/achievement/software/mass2

And users community is open at Google Groups:

https://groups.google.com/group/massplusplus/

References

[1] MassBank: A public repository for sharing mass spectral data for life sciences
H.Horai, M.Arita, S.Kanaya, Y.Nihei, T.Ikeda, K.Suwa, Y.Ojima, K.Tanaka, S.Tanaka, K.Aoshima, Y.Oda, Y.Kakazu, M.Kusano, T.Tohge, F.Matsuda, Y.Sawada, M.Yokota Hirai, H.Nakanishi, K.Ikeda, N.Akimoto, T.Maoka, H.Takahashi, T.Ara, N.Sakurai, H.Suzuki, D.Shibata, S.Neumann, T.Iida, K.Tanaka, K.Funatsu, F.Matsuura, T.Soga, R.Taguchi, K.Saito and T.Nishioka, J.Mass Spectrom., 45, 703-714(2010)

Acknowledgements

This research is granted by the Japan Society for the Promotion of Science (JSPS) through the “Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program),” initiated by the Council for Science and Technology Policy (CSTP).

3. Identification and Label-free Quantitation on Mass++

Identification

Identification function in Mass++ is quite useful. After inputting parameters by users, Mass++ automatically makes peak list, posts it to the search engine with search parameters, gets the result from search engine and registers it to the internal database.

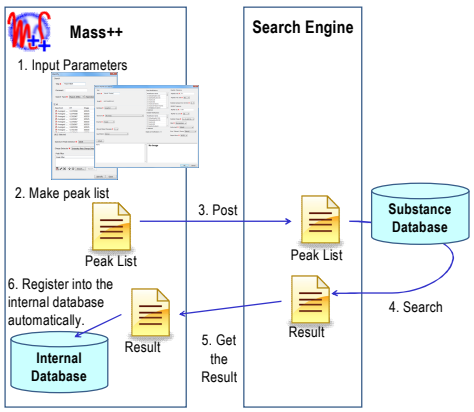


Fig. 4. Identification on Mass++

Anytime users can confirm the search results registered in internal database.

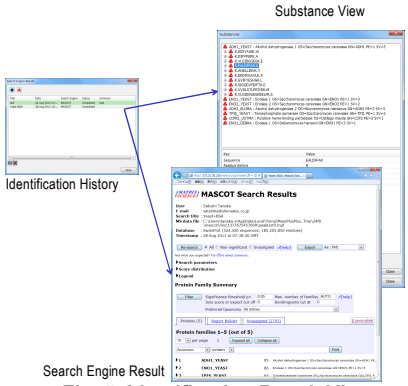


Fig. 5. Identification Result View

Quantitation

During the quantitation process using Mass++, a peak matrix , the differential analysis results of all peaks in a table format, is created.

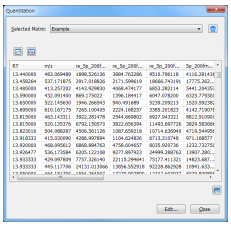


Fig. 6. Peak Matrix

Peak intensity or area can be confirmed by finding the element in the row specified by peak position and the column specified by sample. Peak matrix is created by some steps in the wizard style window.

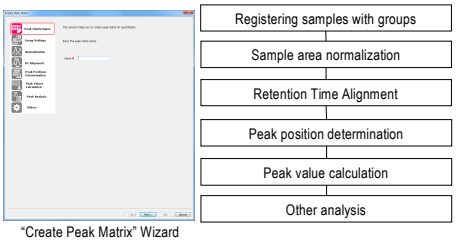


Fig. 7. Steps of Creating Peak Matrix

Users can check the details of each peak by the overlapping view, the group plot and the box plot by double-clicking a row.

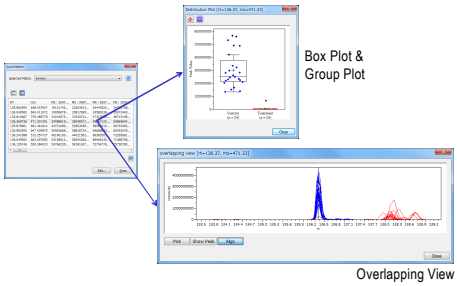
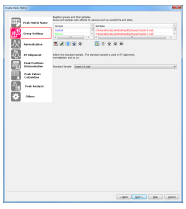


Fig. 8. Overlapping View, Box Plot and Group Plot

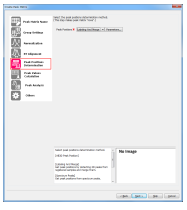
Example

1. Assign each sample to a group



Group Name	Sample	N	Color
Control	Yeast 5 protein 200fmol	3	Blue
BSA 10	Yeast 5 protein 200fmol + BSA 10fmol	3	Green
BSA50	Yeast 5 protein 200fmol + BSA 50fmol	5	Red

2. Peak Position Determination

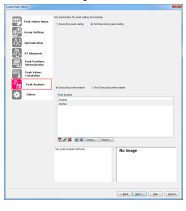


(In this case normalization and RT alignment is not performed.)
Set the “Labeling and Merge” method. This finds peaks by picking from all samples and merge them. Mass++ has peak picking algorithm named AB3D. For more details about AB3D, Check the poster **POS-01-271**.

3. Peak Values Calculation

Set the “Chromatogram Peaks” method. It generates mass chromatogram and calculates peak areas.

4. Peak Analysis

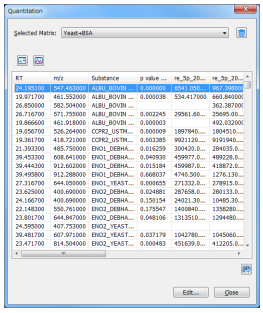


Add the ANOVA (Analysis of Variance) to find the peaks which have difference among groups.

5. Comparative Identification

Perform Mascot search to assign substances to each peaks.
The result of comparative identification is linked to the quantitation result in the Mass++ internal database.

Results



Some peaks are identified as BSA. Their p-value are significantly small. By double-clicking a peak item (row), we can confirm there are differences among groups using the group plot and the overlapping view.

RT=24.20, m/z=547.46