Automated feature finding and evaluation of m/z images based on CCS separation in MALDI TIMS imaging data

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ASMS 2020, ThP 264

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Introduction

Mass spectrometry imaging (MSI) has advanced from single experiments to routine workflows evaluating the spatial distribution of ion images for different mass-to-charge ratios. Ion mobility spectrometry (IMS) for MSI adds another dimension of information and the established workflows are expected to seemingly extend to the mobility dimension.

However, already the amount of data contained in MSI data poses challenging tasks to the analysis. Adding the mobility dimension substantially increases the complexity and remains difficult and tedious to analyze manually. Moreover, manual extraction lacks the impartial evaluation of data, leading to potential selection bias.

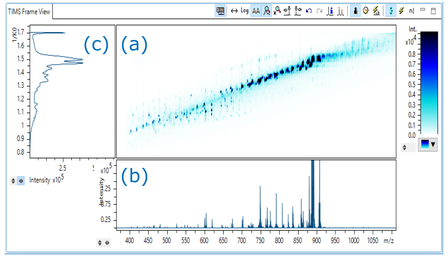

Fig. 1: Average heatmap of TIMS measurement (a), average spectrum (b), and mobilogram (c).

Methods

MSI acquisition: MALDI TIMS imaging data was acquired from fresh-frozen sections of mouse brain in negative mode on a timsTOF fleX instrument (Bruker) with 800 ms ramp time in the 1/K0 range 0.6–1.8 and the mass range for lipids at a spatial resolution of 50 µm.

Feature finding: An average heatmap was generated to locate peaks in the m/z spectrum and features in the mobility data (Fig. 1).

Image extraction: Images were extracted from heatmap features whose spatial correlation measures indicated fixed m/z value, but differing 1/K0 values have anti-correlated spatial distribution.

Analyte list matching: The spatial distribution of highly anti-correlated feature sets were visualized in SCiLS Lab. Automatic annotation of lipids was performed with a preliminary version of MetaboScape.

Tab. 1: Excerpt of mobility image pairs with lowest correlation (177 rows not shown); colored dots denote images in Figure 3.

m/z	1/K0	1/K0	R
916.658	1.51 🟠	1.53 🔵	-0.74
917.660	1.51	1.53	-0.71
726.583	1.34 🟢	1.39 🔴	-0.66
888.559	1.47	1.51	-0.65
894.495	1.48	1.53	-0.64
744.553	1.33	1.36	-0.64
727.587	1.34	1.39	-0.62
906.524	1.50	1.53	-0.62

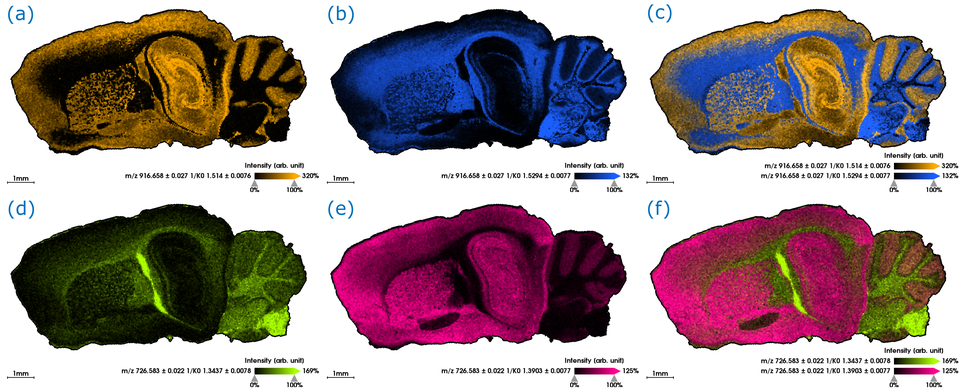

Fig. 3: Visualization of TIMS separated compounds with identical m/z values and their blended joint distribution; (a–c) m/z 916.658 with 1/K0 1.51 (🟠) and 1/K0 1.53 (🔵); (d–f) m/z 726.583 with 1/K0 1.34 (🟢) and 1/K0 1.39 (🔴).

Results

The average heatmap representing the TIMS measurement in a two-dimensional plot of spectral-by-mobility data was used to form the average spectrum. The average spectrum was formed by ignoring the mobility data and comprised in total of 8594 peaks. To search for different spatial distribution based on the mobility dimension, for each of these peaks the mobilogram was generated.

The mobilograms were formed from a slice of the average heatmap by fixing the mass window and preserving the mobility dimension. Each mobilogram was subdivided into 70 subsets of (m/z, 1/K0) windows, only those with at least 0.01% of base peak intensity were considered, totaling into 21,631 images.

Mobility images with identical m/z value were evaluated pairwise using spatial Person correlation measure. Based on the score of correlation, 187 pairs of different mobility images for identical m/z value remained that had a correlation between -0.3 and -1, and thus met our criterion of revealing different spatial distributions (Tab. 1).

The complete TIMS imaging data was imported into SCiLS Lab, disregarding the mobility dimension. With the novel SCiLS Lab API, the 187 pairs of images from (m/z, 1/K0) windows were added to the dataset. For a given image pair with identical m/z value, the two separate mobility images were visualized with individual color gradients and blended to show their joint distribution (Fig. 3).

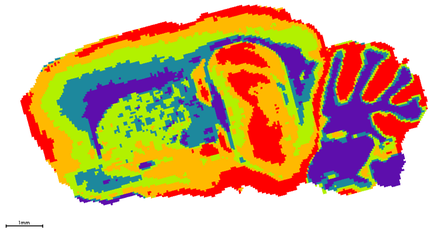

Fig. 4: Segmentation map based on mobility images leading to anatomical features.

Through unsupervised clustering in SCiLS Lab, a segmentation map was produced from the mobility images. The resulting segmentation map correlates with the major anatomical regions of the mouse brain (Fig. 4).

TIMS data from the anatomical regions were loaded into the SpatialOMx workflow of MetaboScape, to perform automatic annotation against a lipid database. For the image pairs with lowest correlation, this potential candidates are HexCer 41:11 and SHexCer 44:2 for m/z 916.658 and CerP 42:2 and HexCer 36:1 for m/z 726.583.

Summary

We presented a novel bioinformatics pipeline to automatically extract anti-correlated mobility images with different spatial intensity distributions from TIMS imaging data. The TIMS imaging-specific regions of interest were matched against lipid annotations.

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