

INTRODUCTION

Most of the mass spectrometry imaging (MSI) experiments utilize a targeted strategy, which consist in the visualization and the quantification of the concentration of a certain compound, or a reduced set of compounds along the tissue surface. The untargeted analysis in MSI within and between images is much more challenging due to the difficulty to find the key ions that define the different molecular signatures regions inside an MS image or the ions that are up or downregulated when comparing images in case/control experiments. In this manuscript we will describe an automated workflow for untargeted analysis of metabolomics LDI-MS images, consisting in a first segmentation step, followed by an concentration analysis of ions between clusters independently of the segmentation process. This workflow will be applied to the analysis of a mouse brain tissue sample.

METHODS

Sample Preparation and MSI acquisition

- 10 μm sagittal mouse brain slices has been cutted
- 3 nm gold nanolayer has been sputtered over the sample
- Bruker UltrafeXtreme MALDI TOF lat. resolution of 80 μm .

Data pre-processing

Noise removal, signal normalization, peak alignment and peak calibration has been performed with the in-house rMSIProc R package.

Segmentation

Images has been segmented using k-means clustering algorithm (n = 6 clusters) using an in-house C++ script.

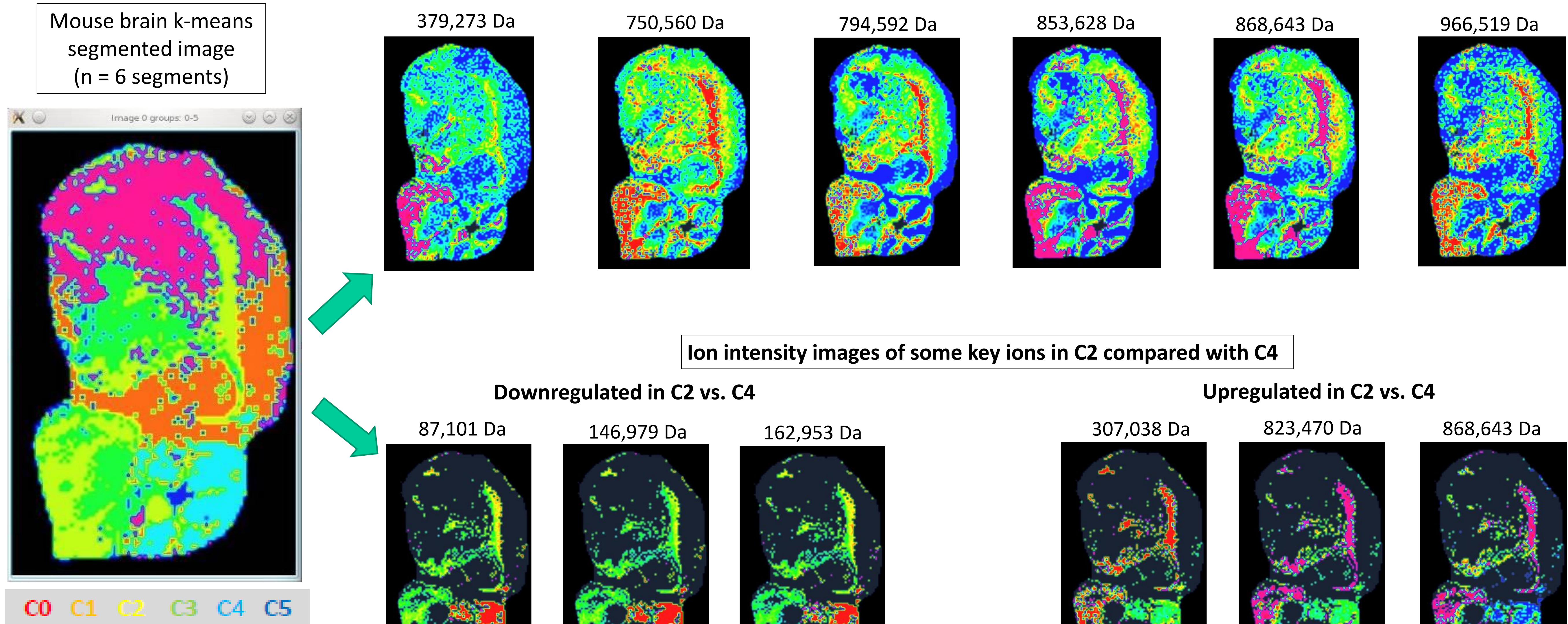
Ion filtering

The purpose is to identify key upregulated/downregulated ions from any combination of clusters, as follow:

- 1) For each pair of clusters, the following parameters are obtained:
 - Z value (quotient between the relative amount of null ion concentration values)
 - P-value (Mann-Witney, similarity of ion concentration values)
 - FC (fold change) value (quotient between ion concentration medians)
- 2) As a result of the application of the above parameters, a key ions list in every cluster pair comparison has been determined.
- 3) The parameters of step 1) has also been used to determine the upregulated/downregulated ions between any cluster compared with the rest of clusters.

RESULTS

Ion intensity images of upregulated ions in cluster 2 vs. the rest of clusters.



CONCLUSIONS

A new automated workflow for untargeted analysis of MS Images has been implemented with an initial step of image segmentation followed by an ion filtering process based on three ion distribution measures. It allows the discovery of the key ions when we analyse one or several MS images.

REFERENCES

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