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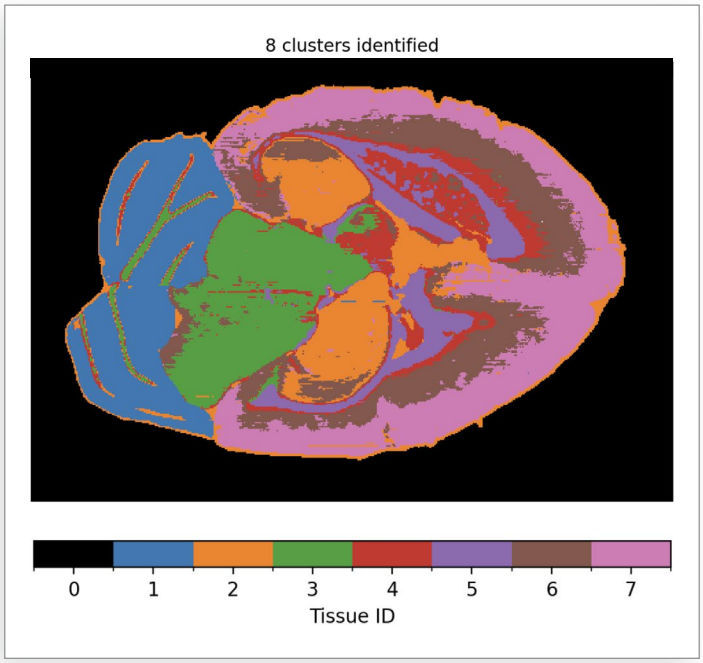
MSI-Segmentation: A Web-based MicroApp for Automated Exploration and Material Segmentation of MS Imaging Data

INTRODUCTION

Mass spectrometry imaging (MSI) is a rapidly developing molecular imaging modality that can map the spatial distribution of thousands of analytes across a sample, enabling exploratory discovery in biological and materials science.

MSI produces large amounts of multi-dimensional data, leading to the need for more powerful statistical techniques and software packages in order to guide and streamline data interpretation.

A new web-based micro-app called MSI-Segmentation is presented which uses unsupervised machine learning to automate the exploration of MSI datasets, including pixel-wise classification of material groups and clustering of colocalized analyte images.



Mass spectrometry imaging (MSI) data are large and complex, as such typical users find understanding their data can be very challenging. In many instances, there are stark chemical differences between regions of the surface being analysed, these are of great interest to the scientist, but there is currently no clear way of identifying them. Even when a *m/z* value of interest is identified, its molecular annotation requires a considerable amount of additional effort to be determined.

To account for this, experts in mass spectrometry imaging often have a plethora of in-house built tools to analyse their data, however new and future users may not have access to these. The MSI segmentation application helps to change the way imaging scientists interact with their data, simply visualizing images colored by chemically differentiated regions without the requirement of multiple tools.

MSI Segmentation accepts input from ASCII text-files containing (x,y) coordinates and *m/z* intensities at each row such as those generated by processing MS imaging data through High Definition Imaging™ (HDI) software (analyte file).

The application performs automated object detection, pixel segmentation using UMAP and HDBSCAN, as well as Ward's hierarchical clustering of ion images. Outputs all results to .csv for downstream processing of raw data.

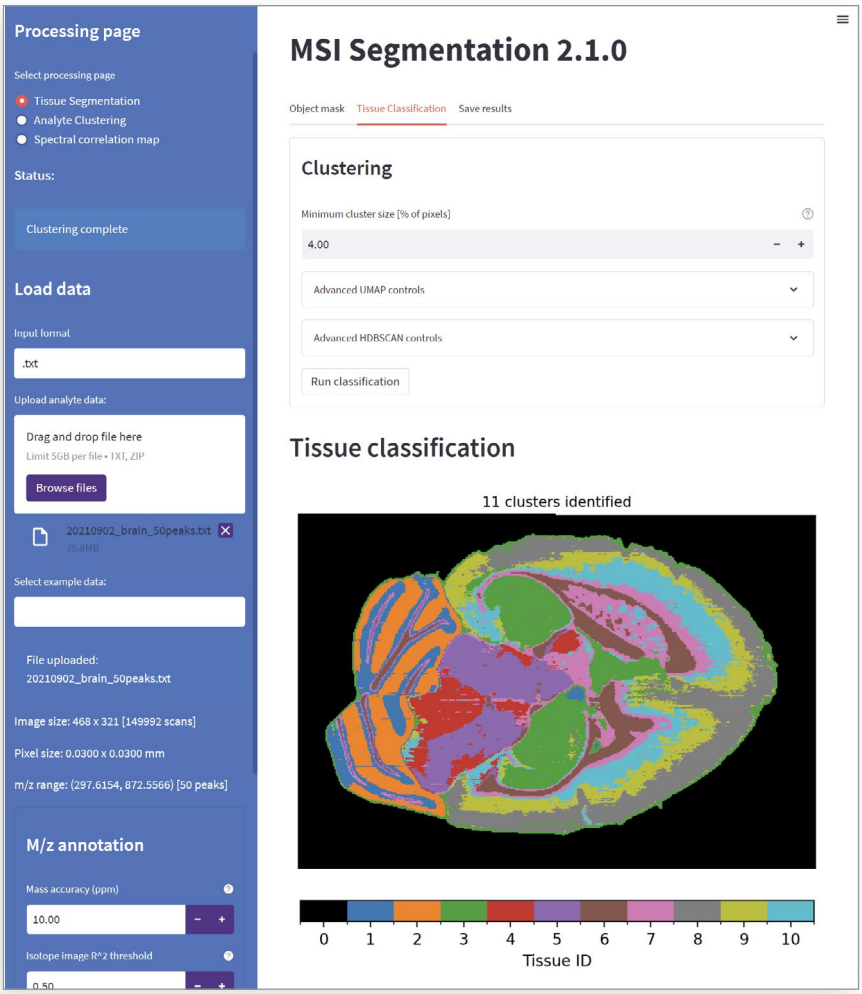


Figure 1. Overview of app layout displaying tissue segmentation of a mouse brain sample.

EXPERIMENTAL CONDITIONS

Sample description

Fresh frozen mouse (Mus Musculus) brain whole organ was frozen at -80°C, the organ was sectioned at -20°C with a section thickness of 18 µm using a cryostat (Leicia). The sections were stored at -80°C until analysis.

Tissue analysis was performed by DESI XS on a SELECT SERIES MRT mass spectrometer, tissue sections were defrosted and dried under ambient conditions for 15 mins prior to analysis.

MS conditions were as follows:

Source type:	DESI
Cone voltage:	40 v
Capillary voltage:	0.8 kV
Nebulizing gas:	Nitrogen
Nebulizing gas pressure:	0.8 bar
Ionization mode:	Negative
Pixel size:	30 µm
Scan rate:	2 pixels per second

RESULTS

The application provides capabilities for automated exploration and unsupervised segmentation of 2D mass spectrometry imaging data. There are three processing pages.

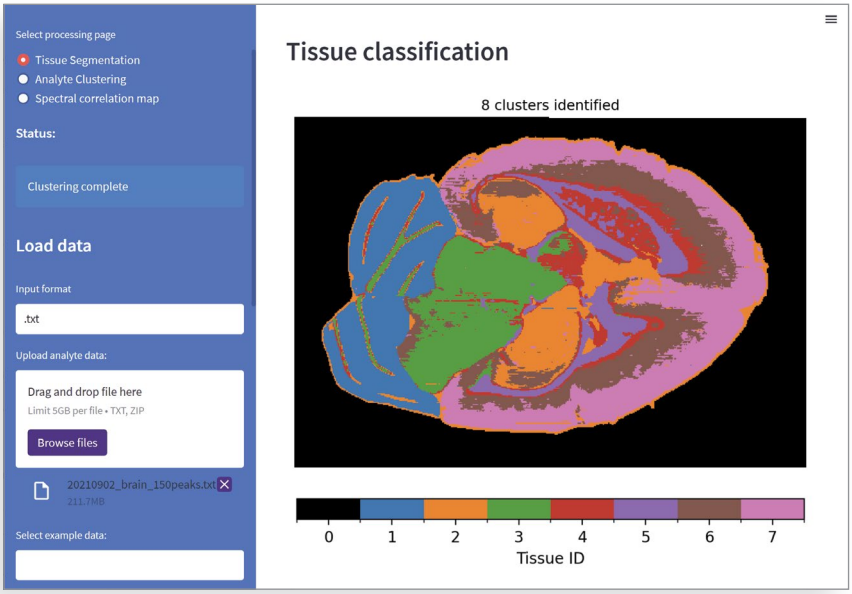


Figure 2. Tissue Segmentation.

"Tissue Segmentation" allows for automated segmentation of pixels into clusters based on similar spectral characteristics. For each cluster the average spectrum of those clusters can be generated and exported as a CSV file. The clusters can also be exported as cluster maps in the form of a binary mask analyte file that can be imported into HDI to overlay with the original imaging data.

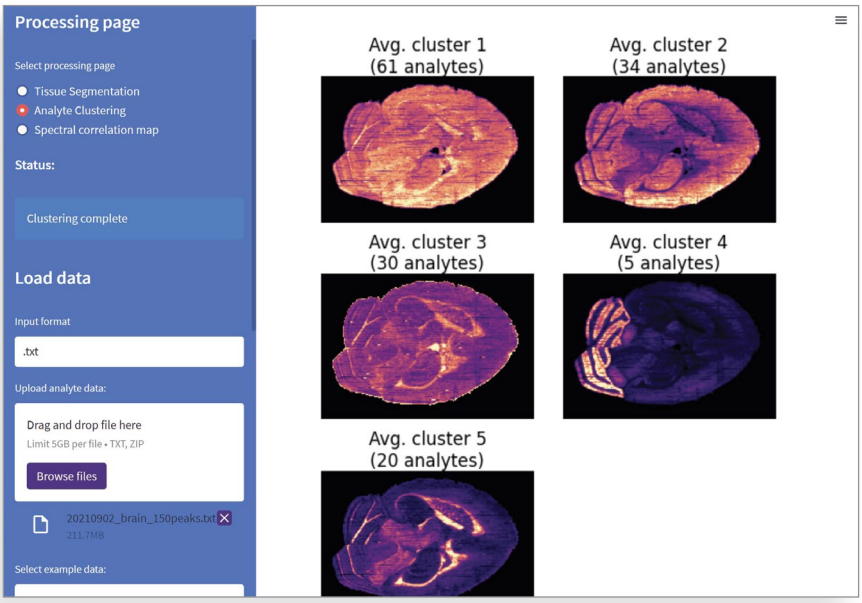


Figure 3. Analyte clustering.

"Analyte clustering" allows for automated clustering of ion images, clustering images with similar distributions then generating an image showing the average of those distributions. The number of clusters the data is split into can be defined as desired. For each cluster a list of peaks present in that cluster can be exported as a csv file. In addition, by applying an intensity threshold to the average cluster images an analyte mask file can be exported. The correlation (R^2) of selected peaks can be calculated for comparison to other peaks within the clusters to aid in cluster assessment.

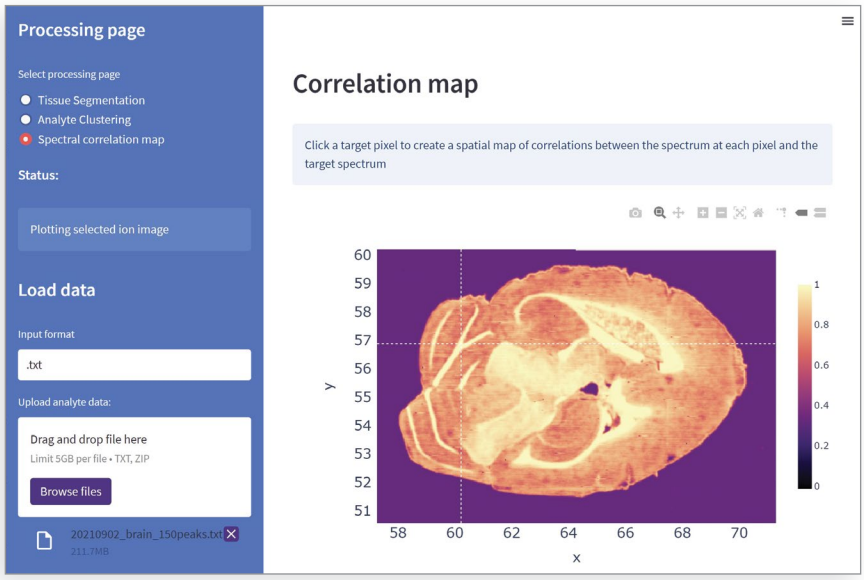


Figure 4. Spectral Correlation map.

Finally, a "spectral correlation map" can be generated which allows for interactive creation of images showing the correlation of all spectra to a given target spectrum (pixel).

Additionally, the app can read .rte files output from the 2D real-time viewer plugin to HDI, or a generic CSV format with columns representing (scan_number, x_pixel_location, y_pixel_location, mz_0, mz_1, ..., mz_N) for N *m/z* bins.

SUMMARY

A new web application was developed that allows for rapid exploration of pre-processed MSI data, using automated machine learning techniques, that allows users of all levels to easily view and identify their data.

The results generated by the MSI Segmentation application can be used in a broader analysis pipeline for targeted analysis of specific spatial regions and ions of interest in raw data.

CONCLUSION

- UMAP automated object detection and pixel segmentation allows for fast, efficient, dimensional reduction of data
- Subsequent segmentation analysis enables the identification of pixels with differing profiles helping to identify regions of interest within highly complex datasets.
- The combination of segmentation analysis, analyte clustering and spectral correlation allows the determination of potential targets that may be important to differentiating specific molecular localizations.

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