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Spatial Probabilistic Mapping of Metabolite Ensembles in MALDI Mass Spectrometry Imaging

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Introduction

MALDI mass spectrometry imaging (MSI) has shown strong advances over the past years in terms of speed, sensitivity, and spatial resolution. MSI data evaluation, however, still heavily relies on plain reviewing of ion images, which is prone to user interpretation bias and is limiting reproducibility.

In this study, we investigated a systematic, data-driven, and user independent approach to MSI data representation, involving the elements of (A) creating an intensity image for a specific spectral peak, (B) detecting the spatial region where a significantly high relative peak intensity is observed, and (C) combining multiple signals to form images representing molecular ensembles (adducts, classes of ion species, …).

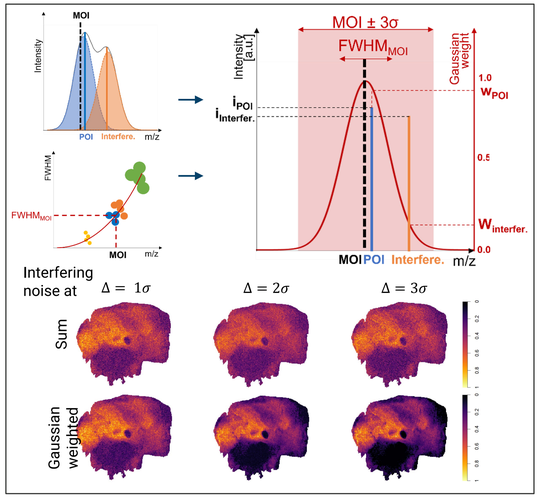

Fig. 1: For a given molecule of interest (MOI), peak width (full width half maximum, FWHM) is estimated, and spot intensities are computed using a Gaussian weighted average. This accounts for small mass variations, while limiting interferences from nearby signals.

Methods

(A) Capturing a peak of interest: The relationship between peak width and m/z is estimated from the data. For any given molecule of interest and its estimated peak width, a Gaussian envelope yields a weighted average of the intensities. This accommodates for small mass variations, while at the same time reducing the impact of nearby interfering peaks (Fig. 1).

(B) Detecting peak hotspots: For a given ion image, Gaussian kernel smoothing is performed, using a kernel size automatically adapted to the spatial scale observed in the sample. Resulting intensity distribution is compared to a complete spatial randomness model as a null hypothesis. This yields upper and lower thresholds outside which a statistically significant relative intensity increase (hotspot) or decrease (coldspot) is observed (Fig. 2).

(C) Going beyond ion images: The statistical framework allows to generate data-integrating probability maps of ensembles of analytes, such as entire lipid classes, groups of adducts of a certain molecule, or any other user-defined set of species.

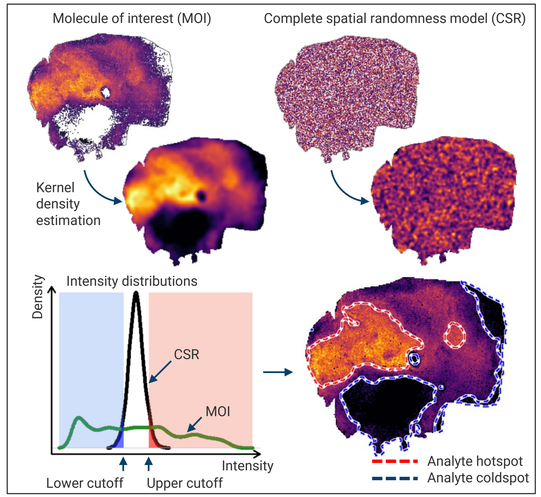

Fig. 2: Kernel density estimation yields a smoothed ion image for the molecule of interest (MOI). Comparing its statistical intensity distribution with the complete spatial randomness (CSR) model results in upper and lower intensity cutoffs and corresponding hot- / coldspot areas.

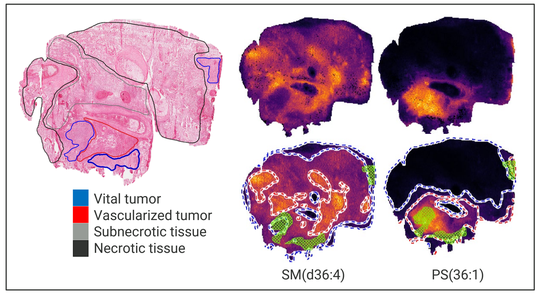

Fig. 3: Hot- and coldspot areas recognized for two exemplary lipids correlate well with histopathologically confirmed anatomical tumor compartments (green areas denote vital tumor regions).

Results

In a tissue section of a human glioblastoma sample, the method reveals hotspot and coldspot regions of two examplary lipids, sphyngomyelin SM(d36:4), and phosphatidylserine PS(36:1). Comparison to a serial H&E stained section shows strong accordance with different tumor compartments, in particular vital tumor areas (Fig. 3).

By generating the complete spatial randomness model from a different reference section, spatially aware cross-tissue comparisons are enabled (Fig. 4).

Generating collective images for whole classes of lipids and their adducts demonstrates the effect of the tissue's alkali ion milieu on ion formation. In particular, the relative contribution of potassium, sodium, and proton adducts to a total of 97 METASPACE-identified ion species of (lyso-) glycerophospholipids (GPL) reveals that potassium-bound GPL are increased, while sodium-bound GPL are reduced in vital tumor areas (Fig. 5).

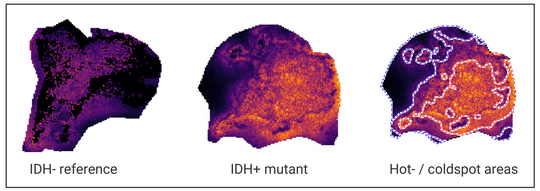

Fig. 4: Cross-tissue comparison. Triptophan signal (m/z 203.0815) in an IDH negative glioblastoma sample is used as a CSR reference model to identify hot- and coldspot areas in an IDH positive mutant.

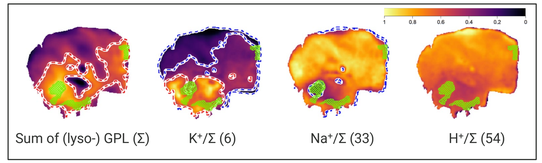

Fig. 5: Collective intensity maps of an ensemble of (lyso-) glycerophospholipids (left) and the relative contribution of K^+, Na^+, and H^+ adducts reveal a correlation between ion formation and tumor vitality.

Summary

Statistical modeling of ion intensities and spatial distributions improves evaluation of MALDI MSI data by reducing operator dependency and user interpretation bias. The method is able to outline analyte hot- and coldspot areas, indicating statistically significant relative presence or absence of ion species.

Application scenarios include correlating spatial molecular profiles with anatomy, cross-tissue comparison and assessment of analyte abundance, as well as collectively visualizing and analyzing whole molecule classes or ensembles of ion species.

Data for this study were acquired from fresh-frozen tissue, using DAN matrix and a 7T FT-ICR mass spectrometer.

Acknowledgements

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References

For full details on method and validation see:

Sammour et.al.: Spatial Probabilistic Mapping of Metabolite Ensembles in Mass Spectrometry Imaging, bioRxiv, DOI: 10.1101/2021.10.27.466114

Conclusion

- Spatial probabilistic mapping enables reproducible and data-driven visualization and interpretation of MALDI MSI intensity images.
- Based on statistical modeling, regions of significant presence or absence of an ion species are obtained without user interaction.
- Cross-tissue comparisons and collective evaluations of molecular ensembles are easily integrated, paving the way to go beyond plain ion images.

Imaging MS: Computational Methods, Software, and Analysis