IMSC 2022, #940

From Lipids and Drugs to Proteins: Identifying Co-localized Molecules Across Multiple Classes Using MALDI-HiPLEX-IHC

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Objectives and Rationale

- Visualization of proteins through mass spectrometry imaging and immunohistochemistry.
- Demonstrate proof of concept for adapting methods to other experiments.
- Mulitmodal imaging

Experimental

Instrumentation
- timsTOF flex
- HTX M3+

Conditions:
- Positive ion mode
- TIMS off
- MALDI
- 20 µm spatial resolution

- Intact mouse brains dosed with 10 mg/kg of JQ1 (sacrificed at time zero) were provided by St. Jude Children's Hospital. Brains were sliced 10 µm thick and thaw mounted onto Bruker IntelliSlides. Slides were sprayed with DHAP using an in-house method.
- Following the image acquisition with DHAP, slides were stained with Miralys® antibodies. Slides were coated with sublimation using DHB and reimaged.
- All images were acquired on a timsTOF fleX in QTOF mode at 20 µm spatial resolution.
- Imaging data was processed in SCiLS Lab 2022a. Regions were exported to Metaboscape 2022b, using the exact mass for tentative assignments.

Results

Lipid Imaging Results

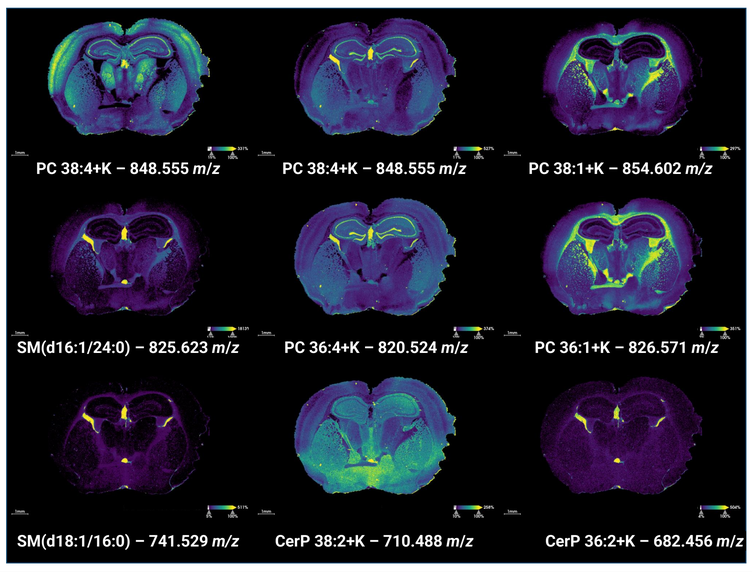

Fig.1: Extracted ion images of selected lipids found in the mouse brain. This is the first MALDI acquisition from this tissue. All images processed with SCiLS Lab 2023a. All annotations performed with Metaboscape 2022b.

MALDI HiPLEX-IHC

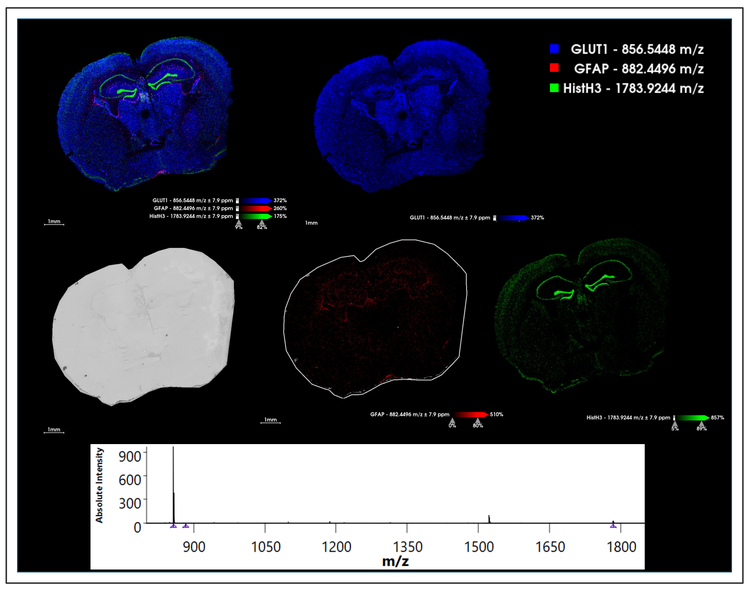

Figure 2. Extracted ion images of mass tags generated through MALDI HiPLEX-IHC. Second MALDI acquisition from tissue. All images processed with SCiLS Lab 2023a. All annotations performed with Metaboscape 2022b.

Segmentation Region Extraction

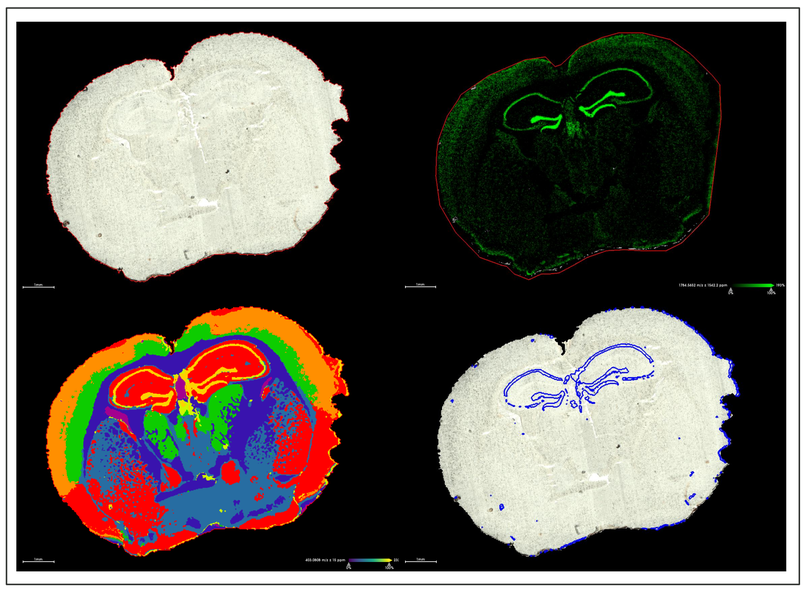

Figure 3. Optical scan (*top left*), result of segmentation analysis (clustering) (*bottom left*), extracted ion image for HistH3 (*top right*), plot of extracted region from segmentation over original tissue matching HistH3. All images processed with SCiLS Lab 2023a. All annotations performed with Metaboscape 2022b.

JQ1 Co-localization

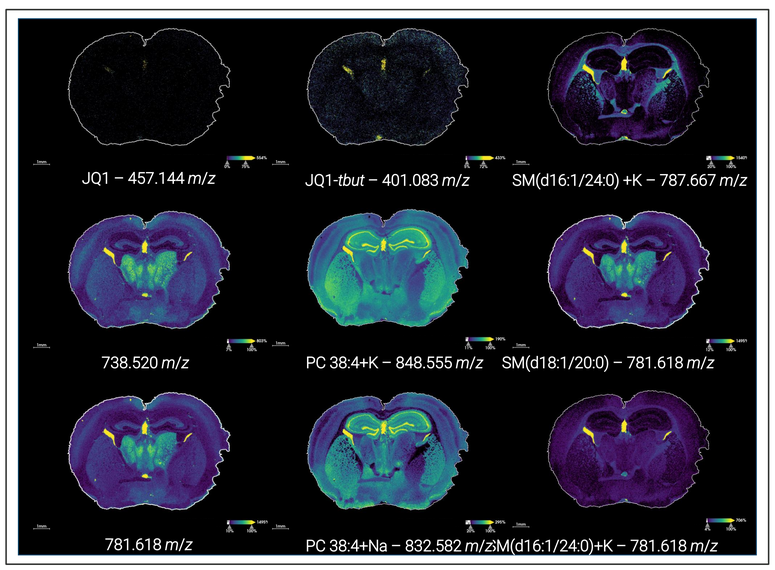

Figure 4. Ion images of features co-localized to JQ1-*tbut*. JQ1 image provided for reference. If annotations were unavailable, only the *m/z* is provided. All images processed with SCiLS Lab 2023a. All annotations performed with Metaboscape 2022b

MALDI HiPLEX-IHC Neural Suite

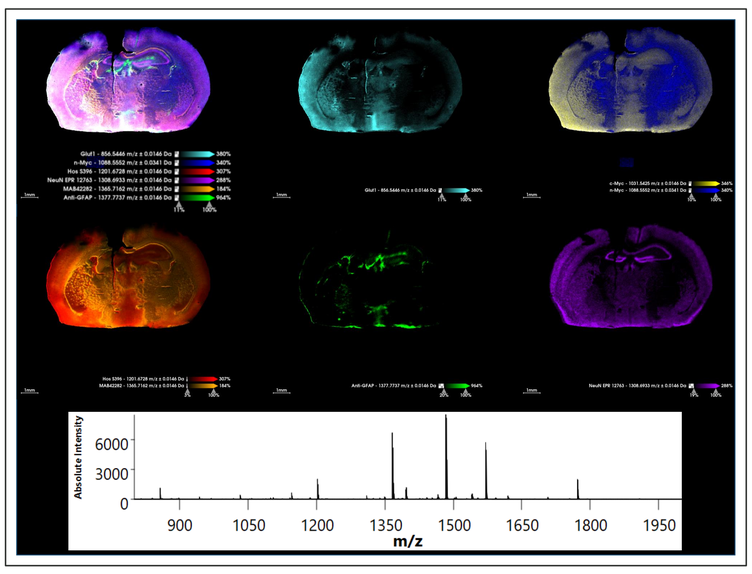

Figure 5. A new section of brain subjected to MALDI-HiPLEX-IHC with a more complete neural suite. All images processed with SCiLS Lab 2023a. All annotations performed with Metaboscape 2022b

- Abundant lipid signal was observed, as well as HiPLEX tags from the same tissue.
- Local regions extracted through clustering used to cross reference acquisition from same tissues.
- JQ-1 detected in low abundance, but enough to generate images from co-localized lipids.
- Advancement in MALDI HiPLEX-IHC furthers future possibilities.

References
- Yagnik, G. *et al*, *J. Am. Chem. Soc*., 2021, *32*, 977-988.

Acknowledgements
- Ethan Yang, Kate Stumpo, Cristina Silvescu, and Shannon Cornett for significant disscusions and assistance.

- MALDI HiPLEX-IHC was applied on a dosed mouse brain for true multimodal imaging.
- Alignment in features across histological regions between acquisitions provided confidence in overall workflow
- Combining all methods for downstream statistical analysis, e.g., co-localization, leads to significant insight into the molecular distribution of the tissue.

Innovation