Expansion Imaging Mass Spectrometry for High Spatial Resolution Lipid Analysis using a Superabsorbant Hydrogel

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OVERVIEW

- **Purpose:** Improve imaging mass spectrometry (IMS) resolution through tissue expansion.
- **Approach:** Polymerize a swellable hydrogel throughout a mouse brain section allowing for expansion through absorption of water for high resolution lipid imaging.
- **Results and Significance:** Tissue sections have been reproducibly expanded reaching linear expansion factors of ~4.5. Lipid detection was possible in both positive and negative ion mode. While distribution fidelity is maintained across most lipids and regions there remains some future work to ensure identical distributions to unexpanded tissue.

INTRODUCTION

Imaging is an essential tool in biological analyses and IMS uniquely provides a label-free approach with high molecular specificity. It provides a spatial dimension to mass spectrometry which has already been instrumental in proteomic, lipidomic and metabolomic workflows.[1] However, IMS is limited in spatial resolution to ~20 µm on commercial platforms which limits the biological processes and structures that can be studied. IMS resolution is dependent on the diameter to which the matrix-assisted laser/desorption ionization (MALDI) laser can be focused resulting in most super resolution methods proposed being instrument/source modifications[2] Hydrogel based expansion introduces an alternative sample-based method to increase IMS resolution that is applicable to any commercial instrument. Briefly, tissues are incubated in acryloyl-X (AcX) which converts primary amines into acrylamide which allowing for participation in free radical polymerization. An acrylamide/acrylate hydrogel solution is infused throughout the tissue and polymerization is initiated linking the gel network to the tissue. The tissue can then be digested with a protease for mechanical homogenization to ensure isotropic expansion through submersion in deionized (DI) water. Tissue expansion in this manner has been successful with multiple methodologies and applied to a variety of tissues and analytes for fluorescence microscopy (termed expansion microscopy (ExM))[3]. This work seeks to investigate the applicability of this workflow to imaging mass spectrometry of lipids.

METHODS

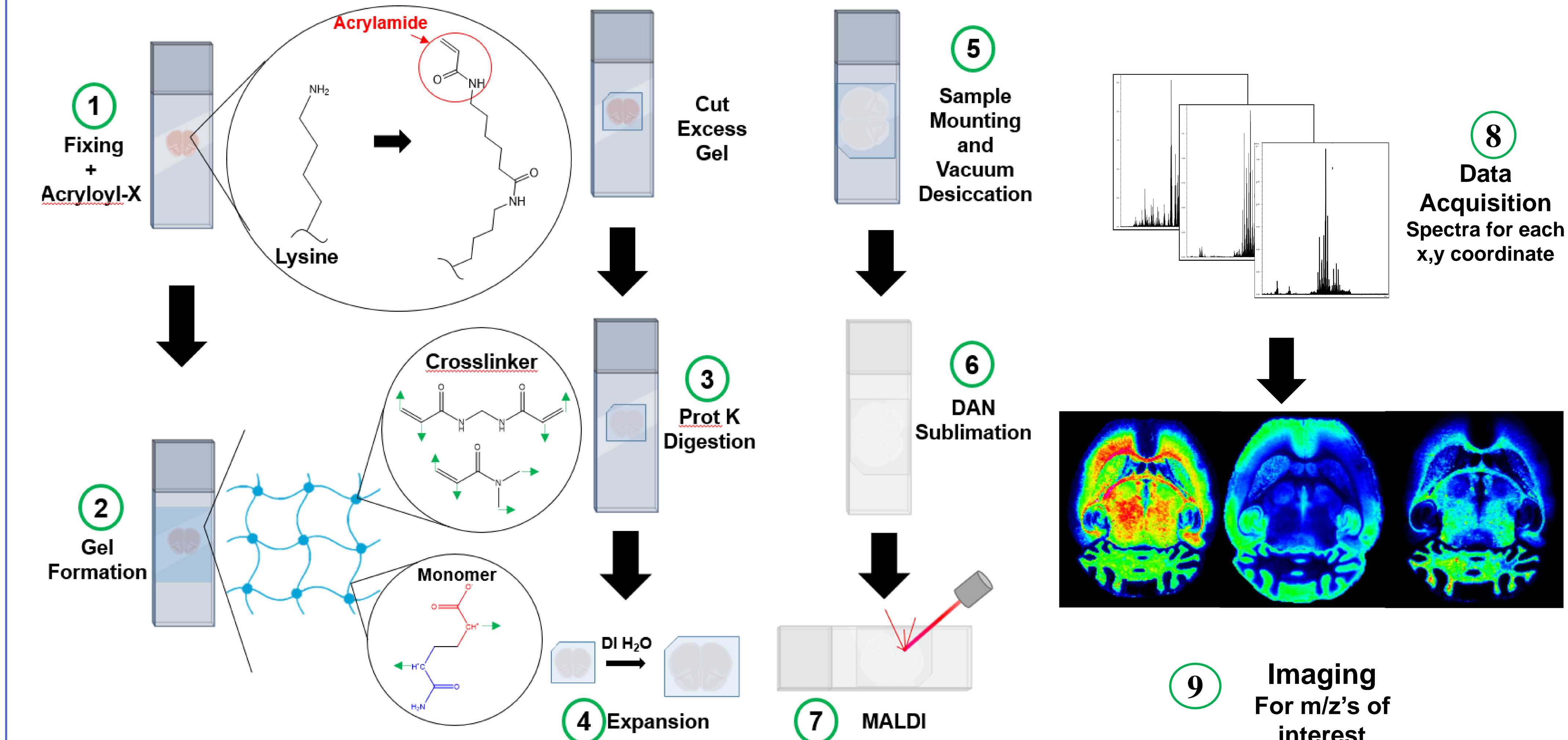

Figure 1 Outline of Lipid Expansion Workflow. The red molecule in the "Monomer" bubble for step 2 is acrylate and the blue is acrylamide. The molecules in the "Crosslinker" bubble are N,N′-Methylenebis(acrylamide) (top) and N,N-Dimethylacrylamide (bottom). Created with Biorender.com

Tissue Sectioning: 30 µm mouse brain sections were thaw mounted to microscope slides using a Leica CM 3050S Cryostat (Leica Biosystems Wetzlar Germany).
Tissue Preparation(1): Tissues were fixed in 4% paraformaldehyde and then incubated in an AcX solution overnight.
Polymer Synthesis(2): Tissue were incubated in polymer solution for 30 minutes at 4ºC and then 3 hours at room temperature.
Digestion(3): Tissues were digested with proteinase K for 12 hours at room temperature.
Expansion(4): Tissues were washed with phosphate buffered saline (PBS) and submerged in excess Deionized (DI) water for expansion.
Desiccation(6): Tissues were mounted onto indium-tin oxide (ITO) slides and dried in a vacuum desiccator under vacuum until uniformly flattened. Samples then sat in a desiccator (vented) overnight.
Matrix application(7): MALDI matrix (e.g. 1,5-diaminonapthalene (DAN)) was applied via HTX M5 TM Sprayer or homebuilt sublimation apparatus
IMS(7-9): Analysis conducted using a 7T solariX Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer in positive ion mode equipped with a Smartbeam II Nd:YAG MALDI laser system. Tentative identifications were based on mass accuracy (< 5 ppm).

RESULTS

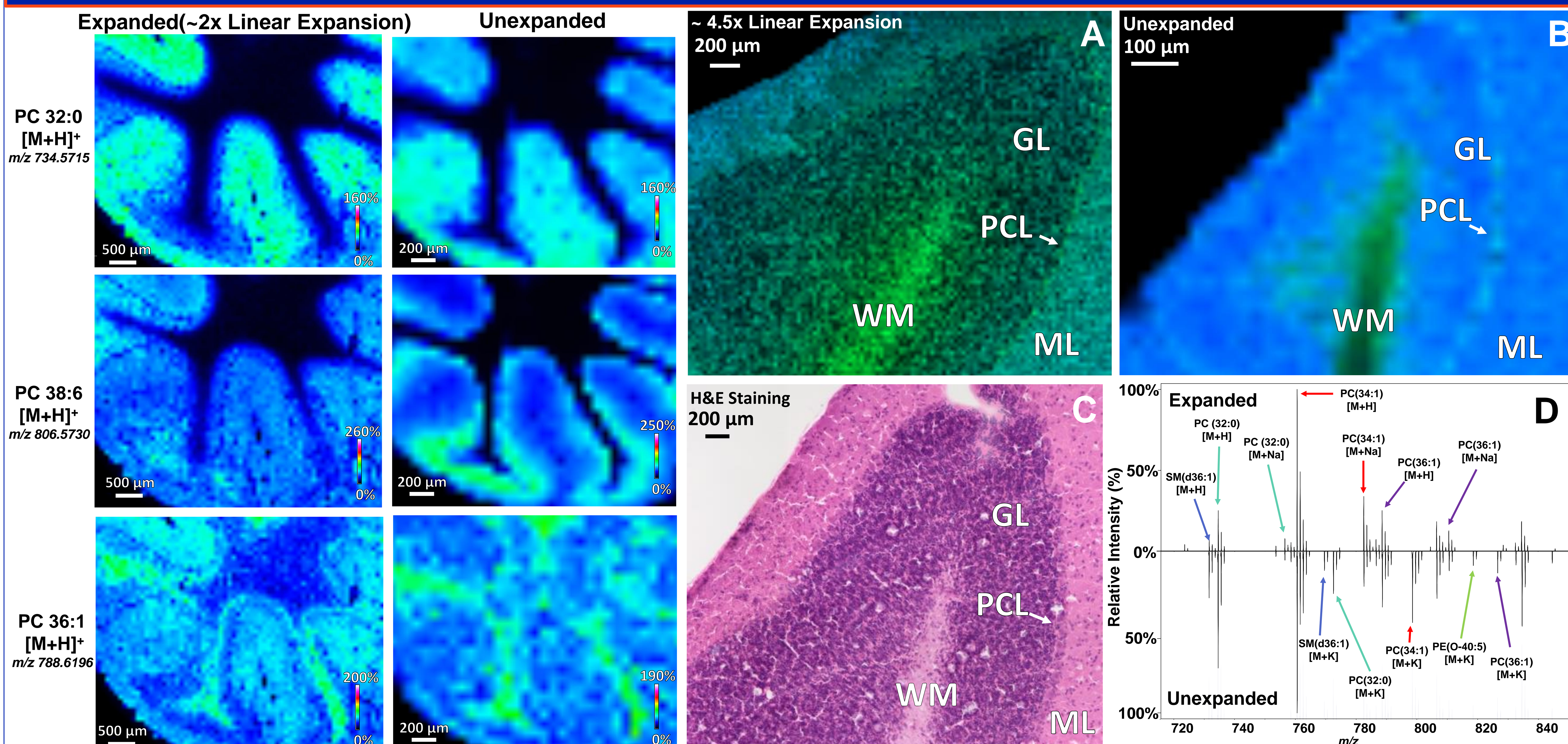

Figure 2 Comparison of Lipid Distributions in Expanded vs Unexpanded Tissue. IMS images of expanded and unexpanded mouse cerebellum taken at an 80 µm raster width for lipids with different distributions. The image for PC 36:1 unexpanded is TIC normalized. Distributions generally match but there are discrepancies in lipids concentrated in the granular layer as seen in the images for PC 38:6

Figure 3 Comparison of Resolution in Expanded vs Unexpanded Mouse Brain Cerebellum. Images show a close up of mouse cerebellum with layers labeled (WM = white matter, GL = granular layer, PCL = Purkinje cell layer and ML = molecular layer). IMS images were taken with a 30 µm raster width and display PC 36:1 [M+H]+ against PC 32:0 [M+H]+. D shows mass spectra from expanded (top) and unexpanded (bottom) tissue. Peaks only seen in the unexpanded spectra have been labeled and tentatively identified as potassiated adducts. The protonated or sodiated adduct of the corresponding lipid is labeled in the expanded spectra with the same color arrow.

CONCLUSIONS

- IMS lipid analysis has been performed on expanded mouse brain tissue with a ~4.5 gain in imaging resolution
- Expansion visibly increased resolution in mouse cerebellum imaging allowing for much clearer distinction of cell layers.
- Expansion analysis was performed both in positive and negative ion mode on mouse brain with minimal loss of detected lipid species.
- Lipid distributions in the cerebellum are generally maintained post expansion but not currently for lipids concentrated in the granular layer.

FUTURE WORK

- Investigate and implement practices to minimize lipid delocalization
- Implement computational workflows to more accurately quantify expansion factor and distortion

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