

Combination of Multimodal Single Cell Imaging Methods with Multiplexed Enzyme-based MALDI-Mass Spectrometry Imaging for Translational Studies



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Overview

Objective: We investigated strategies for pairing single cell technology with MALDI-MSI of N-glycans and extracellular matrix proteins. An overall goal is to develop spatial multimodal strategies that allow researchers to investigate both cellular and extracellular features within the tissue microenvironment on a single tissue section.

Methods: Formalin-fixed, paraffin-embedded (FFPE) tissues were subject to optimized protocols for MALDI-IHC, GeoMx Digital Spatial Profiler, or Imaging Mass Cytometry. Each single cell method was followed by MALDI-MSI. The reverse order of the workflows was also performed to determine the best order to combine these spatial imaging modalities using previously optimized protocols.

Results: The data suggests that for each modality combination, there is an optimal order for performing the single cell modality and MALDI-MSI of N-glycans and matrix on the same tissue section.

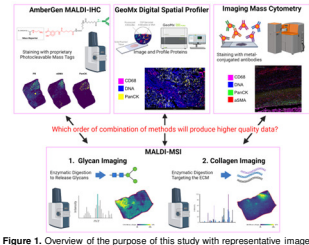


Figure 1. Overview of the purpose of this study with representative images.

Introduction

The tissue microenvironment involves complex, dynamic, dimensional coordination of cell-cell and cell-matrix signaling, chemical flux, and encoded structure which are essential for supporting tissue biology. Multimodal techniques have emerged that combine platforms to simultaneously evaluate very different aspects of the microenvironment, such as single cell expression paired with measurements of the physical properties of the tissue.^{1,3} Single cell modalities work to map single cell expression across tissue sections but have some limitations in reporting other aspects of the tissue microenvironment such as the extracellular matrix. The integration of Matrix-Assisted Laser Desorption/Ionization – Mass Spectrometry Imaging (MALDI-MSI)^{4,5} with single cell spatial omics methods complements single cell spatial information with matrisome imaging targeting N-glycans and extracellular matrix proteins. Here we evaluate the performance MALDI-MSI of N-glycans and Collagens in serial with the Miralys[®] photo-cleavable mass-tags (Ambergen, Inc.)⁶, CyTOF (Standard BioTools Inc.)⁷, or GeoMx[®] (Nanostring, Inc.)⁸

Methods

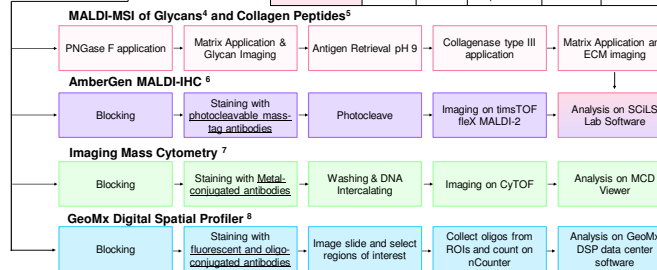
Tissue sources:
AmberGen MALDI-IHC: Breast tissue obtained from Zyagen (San Diego, CA)
Imaging Mass Cytometry: Liver tissue from MUSC tissue biorepository.
GeoMx Digital Spatial Profiler: Breast tissue microarray (TMA; TissueArray.com LLC)
MUSC tissue analyzed with IRB approval exemption 4 status.

Workflows:
 All workflows begin with these two steps



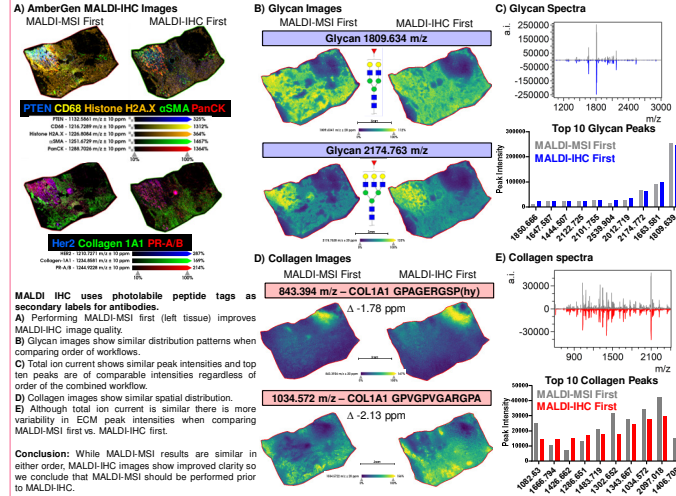
Table 1. Comparison of method parameters for each imaging modality that was investigated.

Imaging Modality	Initial heat time (hours)	Initial heat temp (°C)	Antigen buffer (pH)	Antigen retrieval temp (°C)	Antigen retrieval time (minutes)
MALDI-MSI: Glycans	1	60	Citraconic, pH 3.0	95	25**
MALDI-MSI: Collagen peptides	1	60	Tris, pH 9.0	95	25**
Ambergen MALDI HIPEX-IHC	2	60	Tris-EDTA, pH 9.0	95	30
Imaging Mass Cytometry (IMC)	1	60	Tris-EDTA, pH 9.0	95*	20
GeoMx Digital Spatial Profiler	4	70	Tris-EDTA, pH 9.0	95	15

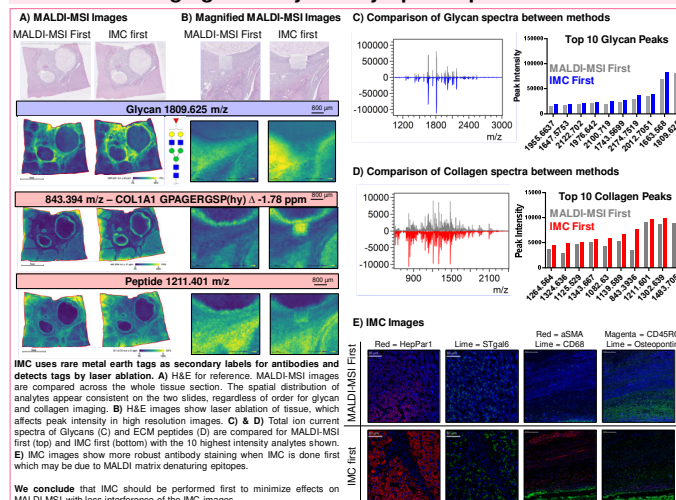


Methods Figure 1: MALDI-MSI was performed before and after single cell type spatial omics workflows on a serial tissue section

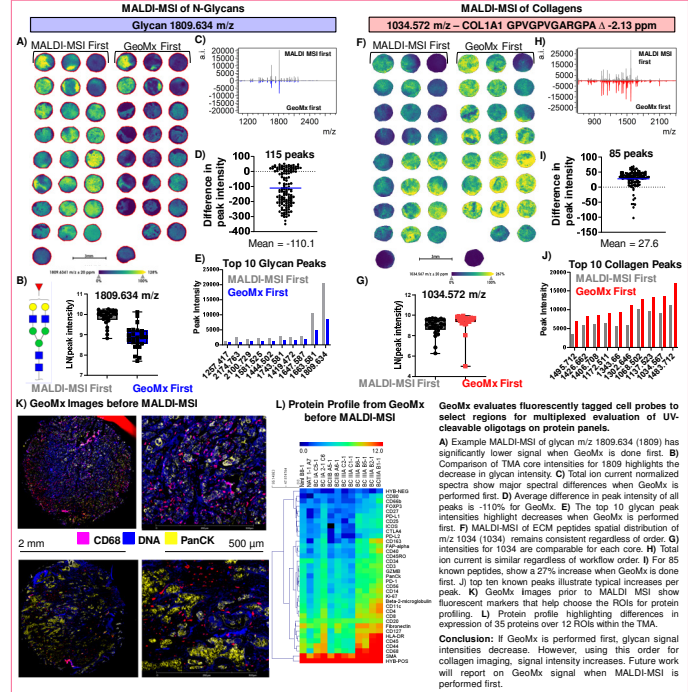
Result 1: MALDI-MSI optimal prior to MALDI-IHC



Result 2: Imaging Mass Cytometry optimal prior to MALDI-MSI



Result 3: GeoMx DSP can Spatially Quantify Analytes



Conclusions

- Using previously optimized workflows for each modality, a novel combination of spatial workflows allows for the joint analysis of cellular & extracellular markers on a single tissue section.
- MALDI-IHC technology (Ambergen, Inc.) allows scanning analytes across a whole tissue or at a single cell level and can be easily integrated with MALDI-MSI because the same workflows are used. Performing MALDI-MSI before MALDI-IHC results in better quality MALDI-IHC imaging.
- IMC (Standard BioTools Inc.) provides high-resolution single cell imaging of small ROIs. Tissue ablation of the ROI due to CyTOF laser caused detectable artifacts in MALDI imaging of N-glycans and collagens. IMC is specifically done prior to MALDI-MSI.
- GeoMx (Nanostring, Inc.) is beneficial for quantifying protein data of target cells in a specific ROI. MALDI-MSI was successfully performed after GeoMx workflows. Ongoing work will address which order of methods will be optimal.

Acknowledgements

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Conflicts of Interest & References

MUSC is a Breaker Center of Excellence for Clinical Glycomics
 RRD and PMA are shareholders and board members of N-Zyme Scientifics

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