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 multiomic 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 were 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.

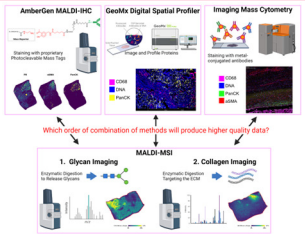


Figure 1. Overview of the purpose of this study with representative images.

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-glycomics and extracellular matrix proteins. Here we evaluate the performance MALDI-MSI of N-glycans and Collagens in serial with the Miralys™ photocleavable 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

Initial Heating & Dewaxing → Antigen Retrieve to break formalin bonds

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 HiPLEX-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

MALDI-MSI of Glycans[4] and Collagen Peptides[5]

PNGase F application → Matrix Application & Glycan Imaging → Antigen Retrieval pH 9 → Collagenase type III application → Matrix Application and ECM imaging

AmberGen MALDI-IHC [6]

Blocking → Staining with photocleavable mass-tag antibodies → Photocleave → Imaging on timsTOF fleX MALDI-2 → Analysis on SCiLS Lab Software

Imaging Mass Cytometry [7]

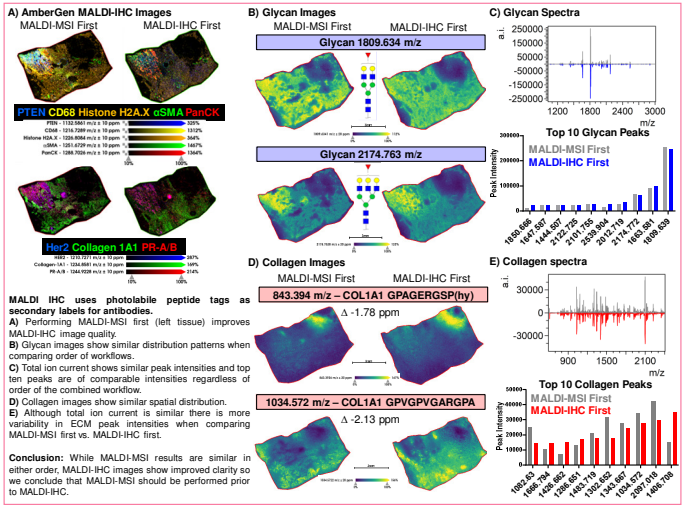
Blocking → Staining with Metal-conjugated antibodies → Washing & DNA Intercalating → Imaging on CyTOF → Analysis on MCD Viewer

GeoMx Digital Spatial Profiler [8]

Blocking → Staining with fluorescent and oligo-conjugated antibodies → Image slide and select regions of interest → Collect oligos from ROIs and count on nCounter → Analysis on GeoMx DSP data center software

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

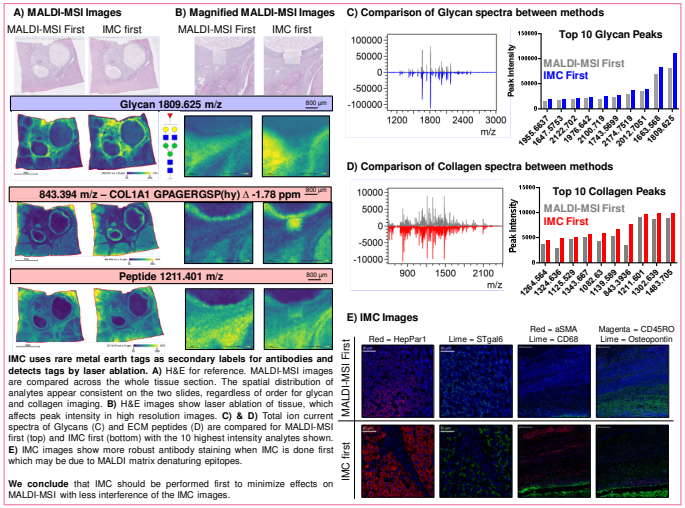
Result 1: MALDI-MSI optimal prior to MALDI-IHC



MALDI IHC uses photolabile peptide tags as secondary labels for antibodies.
A) Performing MALDI-MSI first (left tissue) improves MALDI-IHC image quality.
B) Glycan images show similar distribution patterns when comparing order of workflows.
C) Total ion current shows similar peak intensities and top ten peaks are of comparable intensities regardless of order of the combined workflow.
D) Collagen images show similar spatial distribution.
E) Although total ion current is similar there is more variability in ECM peak intensities when comparing MALDI-MSI first vs. MALDI-IHC first.

Conclusion: While MALDI-MSI results are similar in either order, MALDI-IHC images show improved clarity so we conclude that MALDI-MSI should be performed prior to MALDI-IHC.

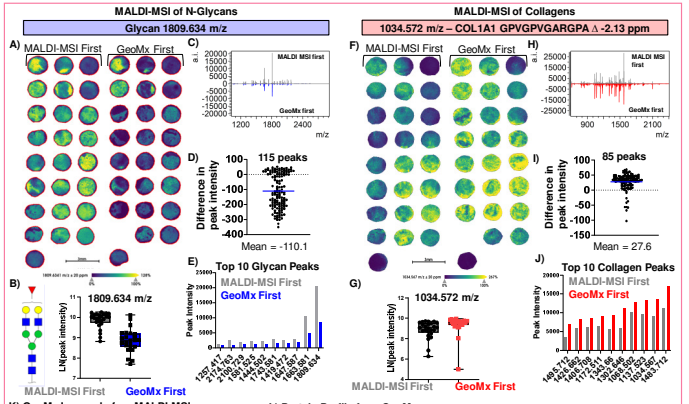
Result 2: Imaging Mass Cytometry optimal prior to MALDI-MSI



IMC uses rare metal earth tags as secondary labels for antibodies and detects tags by laser ablation. A) H&E for reference. MALDI-MSI images are compared across the whole tissue section. The spatial distribution of analytes appear consistent on the two slides, regardless of order for glycan and collagen imaging. **B)** H&E images show laser ablation of tissue, which affects peak intensity in high resolution images. **C) & D)** Total ion current spectra of Glycans (C) and ECM peptides (D) are compared for MALDI-MSI first (top) and IMC first (bottom) with the 10 highest intensity analytes shown. **E)** IMC images show more robust antibody staining when IMC is done first which may be due to MALDI matrix denaturing epitopes.

We conclude that IMC should be performed first to minimize effects on MALDI-MSI with less interference of the IMC images.

Result 3: GeoMx DSP can Spatially Quantify Analytes



GeoMx evaluates fluorescently tagged cell probes to select regions for multiplexed evaluation of UV-cleavable oligotags on protein panels.

A) Example MALDI-MSI of glycan m/z 1809.634 (1809) has significantly lower signal when GeoMx is done first. **B)** Comparison of TMA core intensities for 1809 highlights the decrease in glycan intensity. **C)** Total ion current normalized spectra show major spectral differences when GeoMx is performed first. **D)** Average difference in peak intensity of all peaks is -110% for GeoMx. **E)** The top 10 glycan peak intensities highlight decreases when GeoMx is performed first. **F)** MALDI-MSI of ECM peptides spatial distribution of m/z 1034 (1034) remains consistent regardless of order. **G)** intensities for 1034 are comparable for each core. **H)** Total ion current is similar regardless of workflow order. **I)** For 85 known peptides, show a 27% increase when GeoMx is done first. **J)** top ten known peaks illustrate typical increases per peak. **K)** GeoMx Images prior to MALDI MSI show fluorescent markers that help choose the ROIs for protein profiling. **L)** Protein profile highlighting differences in expression of 35 proteins over 12 ROIs within the TMA.

Conclusion: If GeoMx is performed first, glycan signal intensities decrease. However, using this order for collagen imaging, signal intensity increases. Future work will report on GeoMx signal when MALDI-MSI is performed first.

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 optimally 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

JBD supported by NIGMS T32 5T32GM132055-04; Cellular, Biochemical and Molecular Sciences Training Grant
Supported in part by the Translational Science Lab Shared Resource, Hollings Cancer Center, Medical University of South Carolina (P30 CA138313).
NIH/NCI 1R01 CA253460-01; Collagen Sequence Variants in Racial Disparities of Breast Cancer (PMA)

Conflicts of Interest & References

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

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