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



Mentor: Dr. Peggi Angel

MALDI-MSI of Collagens

-500

2500

angelp@musc.edu

MALDI MSI

GeoMy fir

Jaclyn B. Dunne¹, Jake Griner¹, Martin Romeo², Carsten Krieg³, Mark Lim⁴, Gargey Yagnik⁴ Anand S. Mehta¹, Richard R. Drake¹, Peggi M. Angel¹

¹Department of Cell and Molecular Pharmacology & Experimental Therapeutics, Medical University of South Carolina (MUSC) Charleston, SC: ² Translational Science Laboratory, Hollings Cancer Center, MUSC, Charleston, SC ; 3Department of Pathology and Laboratory Medicine, MUSC, Charleston, SC; 4AmberGen, Inc., Billerica, MA

Medical University of South Carolina

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.



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

AmberCon MALDI-INC Cookiry Disital Spatial Profiler

Staining with proprietary Photocleavable Mass Tags

Imaging Mass Cytometry

CD68 DNA Park

2 Collagen Imagin

+ 5





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 MALDHHC

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

50000

-50000

-100000

5000

-5000

-10000

A) MALDI-MSI Images B) Magnified MALDI-MSI Images C) Comparison of Glycan spectra between methods





IMC uses rare metal earth tags as secondary labels for antibodies and Incluses rare metal earth tags as secondary laures for antonions and detects tags by laser ablation. A) H&E for reference. MALDJ-MSI images are compared across the whole tissue section. The spatial distribution of analytes appear consistent on the two silder, regardless of order for glycan and collagen imaging. B) H&E images show laser ablation of tissue, which directs care, intrading is, bink correlation among C1.8. D). Total ion current and consider imaging. B) that images show laser abalation used, 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 MALDI-MSI with less interference of the IMC image



Top 10 Collagen Peaks MALDI-MSI Firs IMC First 900 1500 2100





100-

0-

-100-

-200-

-300-

-400

E)

MALDI-MSI of N-Glycans

0

1809.634 m/z

Result 3: GeoMx DSP can Spatially Quantify Analytes



MALDI-MSI First GeoMx First

L) Protein Profile from GeoMx before MALDI-MSI

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

clearabile oligotags on protein panels. A E sample MALIONKI of dycam wit 1000.534 (1809) has significantly lower signal when GeoXix is done first. B) Comparison of MAC cere intensities, for 1809 highlights the decrease in glycam intensity, Q) float lon current normalized performed first. Dy Average difference in peak intensity of all peaks is -110% for GeoXix. B) The top 10 glycam peak intensities highlight decreases when GeoXix is performed peaks as ~10%, for Gaokk, B) the for 0 bycken pask in the set of profiling. L) Protein profile highlighting difference expression of 35 proteins over 12 ROIs within the TMA.

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

Using previously optimized workflows for each modality, a novel combination of spatial workflows allows for the joint analysis of cellular &

- Ilular 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

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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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