Sequential Imaging and Collagenase Digest Peptide Identification from a Multi-Organ Tissue Microarray

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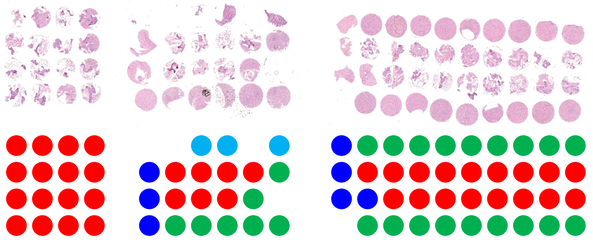
Overview

- Sequential mass spectrometry imaging enables deeper molecular coverage from a single section of tissue
- While there are databases available for the identification of metabolites, lipids, and glycans, standard database tools do not exist for identification of enzymatically produced peptides
- Custom databases can be generated by performing LC-MS/MS on peptides produced using the same sample preparation strategy as used for imaging
- Python scripts were used to convert the library of detected peptides to a database that could be used with SCiLS/MetaboScape to automate the search of the MSI data

Introduction

There is growing interest in spatial multi-omics analysis of clinical samples, however, tissue available for these studies is often limited. When available, it is of utmost importance that as much data as possible be collected from as little tissue as possible. Mass spectrometry imaging allows for the detection of hundreds to thousands of molecules from a single tissue section without the need for target-specific reagents. Additionally, multiple analyte classes can be imaged from the same tissue section with careful experimental planning. However, challenges still exist to identify the molecules detected in the imaging experiment. Here, we present a workflow for sequential imaging and peptide identification from a multi-organ TMA.

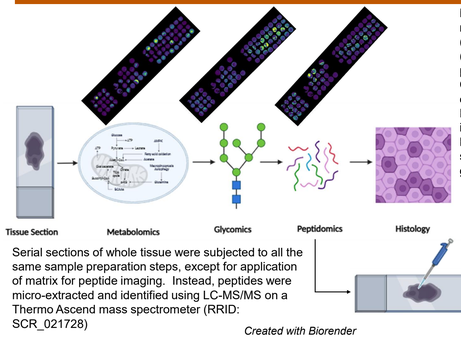
Multi-Organ Tissue Microarray



Multi-organ TMA Top) H&E stained after completion of 3 rounds of mass spectrometry imaging. Bottom) TMA map color coded by organ type

Bone Marrow Liver Skin Spleen

Workflow

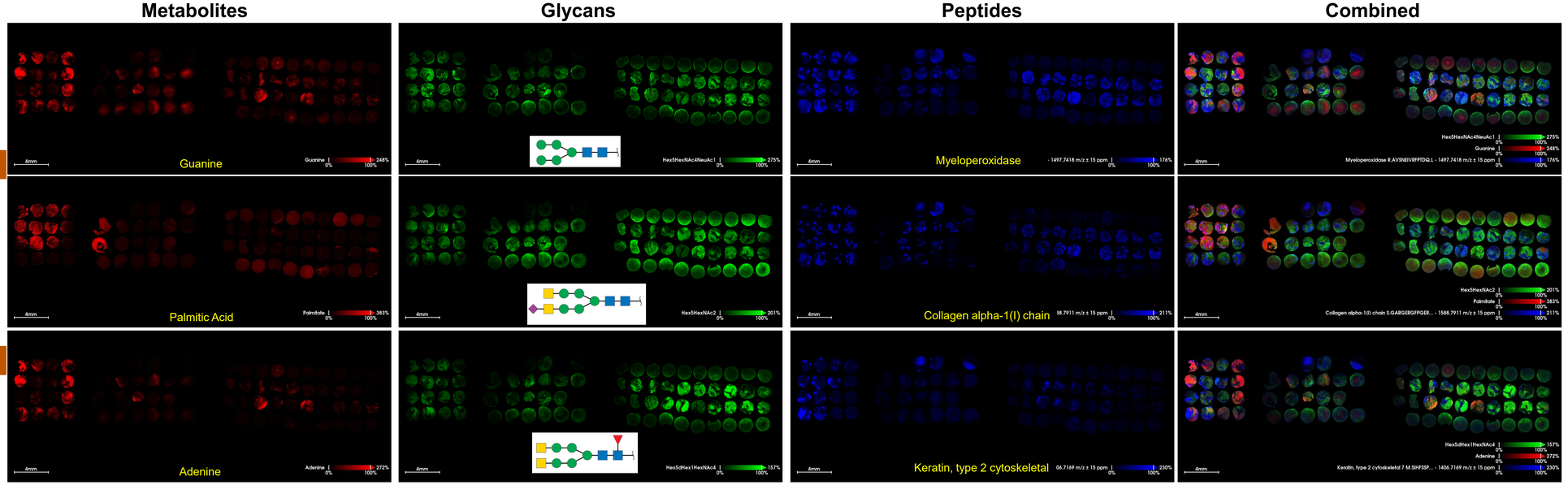


MS images were collected of metabolites in negative ion mode (DAN), N-linked glycans (PNGaseF and CHCA) and peptides (collagenase and CHCA). All matrices and enzymes were applied using an HTX M5 Robotic Sprayer and all imaging data were collected on a Bruker timsTOF flex mass spectrometer. Putative IDs were generated using MetaboScape.

Serial sections of whole tissue were subjected to all the same sample preparation steps, except for application of matrix for peptide imaging. Instead, peptides were micro-extracted and identified using LC-MS/MS on a Thermo Ascend mass spectrometer (RRID: SCR_021728)

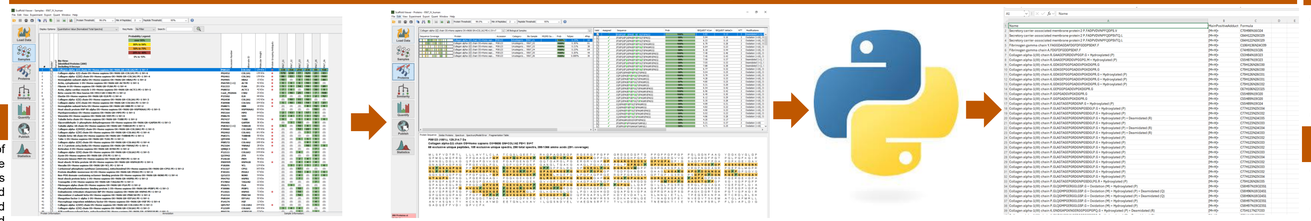
Created with Biorender

Sequential Imaging of a Multi-Organ TMA



Selected metabolite (red), glycan (green), and peptide (blue) ion images showing differences between different tissue types. A total of 671 metabolite, 258 glycan, and 945 peptide peaks were detected in in the imaging datasets. The 3 datasets were co-registered using the SCiLS Ion Image Mapper for simultaneous visualization.

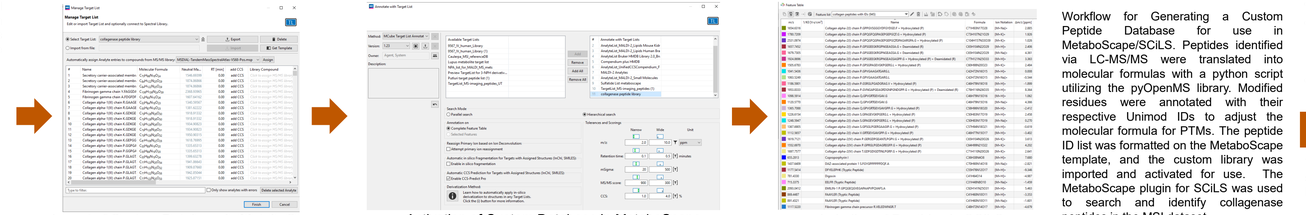
Generation of Custom MetaboScape Peptide Database



Scaffold Sample View

Scaffold Protein View

CSV File of Peptide IDs and Formulas



Import of Custom Database in MetaboScape

Activation of Custom Database in MetaboScape

Labeling of Peptides in SCiLS

Workflow for Generating a Custom Peptide Database for use in MetaboScape/SCiLS. Peptides identified via LC-MS/MS were translated into molecular formulas with a python script utilizing the pyOpenMS library. Modified residues were annotated with their respective Unimod IDs to adjust the molecular formula for PTMs. The peptide ID list was formatted on the MetaboScape template, and the custom library was imported and activated for use. The MetaboScape plugin for SCiLS was used to search and identify collagenase peptides in the MSI dataset.

Collagenase Digest Identification

- LC-MS/MS of peptides produced by on-tissue collagenase digestion resulted in the identification of 4439 peptides which were converted to molecular formulas using pyOpen MS
- After creation of a custom library in MetaboScape, 197 peptides were identified in the MSI data, including 100 peptides from collagen proteins
- 45 unique UniProt IDs were mapped in the dataset

Conclusions

- Sequential MSI revealed differences in metabolite, glycan, and collagenase peptide signals between different tissue types in a multi-organ TMA
- The three MSI datasets were successful co-registered for evaluation of co- and differential localization of molecules between analyte classes
- Python was used to convert peptides sequences and PTMs to molecular formulas
- A custom database of collagenase peptides was generated using on-tissue digestion and LC-MS/MS of extracted peptides for automated searching of the MSI dataset using MetaboScape
- Nearly 200 peptides were identified in the MSI dataset, with over 100 peptides from collagen detected

Acknowledgements/COI

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