Single cell multi-omic mass spectrometry imaging for the human bone marrow microenvironment

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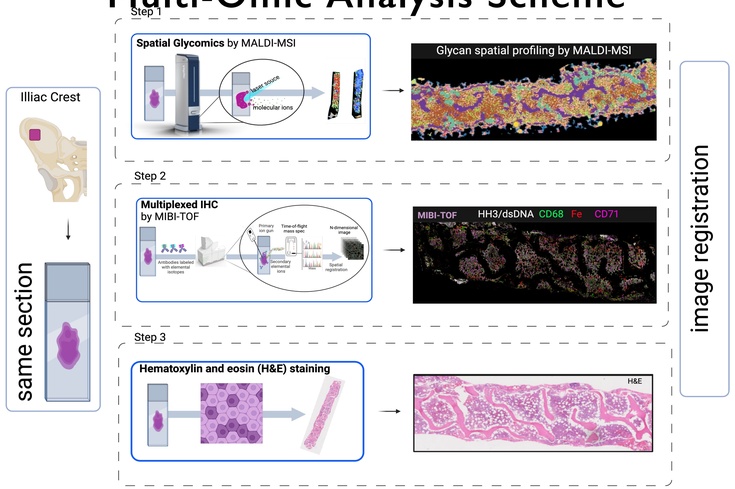
Introduction

- The bone marrow structure reflects evolution-driven mechanisms that have tailored bone to create unique microenvironments catering to the diverse needs of developing blood cells.
- Glycosylation constitutes an essential organizing layer within this system, distinguishing itself from proteomic or transcriptomic properties.
- A scalable workflow has been developed for the multi-platform, sequential mass spectrometry-based imaging of bone marrow tissue sections.
- This workflow incorporates N-Glycan quantification through matrix-assisted laser desorption ionization (MALDI) followed by deep single-cell phenotyping via multiplex ion beam imaging (MIBI).
- The same tissue area is examined using both these methods, along with conventional, high-resolution, light-based Hematoxylin and Eosin (H&E) staining.

Multi-Omic Analysis Scheme

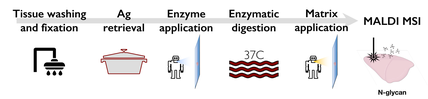


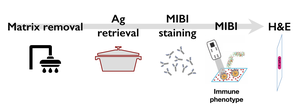
By imaging up to 40 proteins at the same time using **MIBI-TOF**, we can comprehensively identify at a ***single-cell level***, the lineage, function, and spatial location of every cell in a single bone marrow tissue section. **MIBI-TOF** subcellular imaging will be used to capture the composition, functional state, and spatial organization of BM. These data will be complimented by a breakthrough de novo imaging that permits direct identification of glycans using matrix-assisted laser desorption ionization imaging (**MALDI**). The **H&E stain** is the final step, providing additional information about the cellular and tissue organization in the bone marrow.

Disclosure: SCB and MA are founders of Ionpath which commercializes MIBI technology; work funded by NIH U54HL165445, R01AG078702, U24CA224309

Analysis Strategy (see Poster WP377)

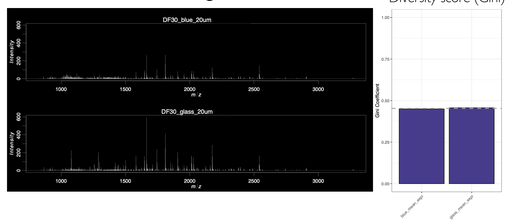
FFPE tissue sections were sectioned onto an organic polymer-coated slide (MIBIblue). The tissue was imaged with a MALDI timsTOFflex using a published protocol for N-glycan imaging (1).



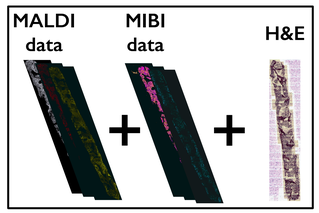


The matrix was washed off and sections were stained with a mix of metal-labelled antibodies targeted to immune phenotypic markers (2). Data was acquired with a MIBIscope and sections were counterstained with H&E.

MIBIblue vs. glass slide



Raw data



Pixel resampling and iterative registration

Image registration methods were tested on simulated data. Bilinear interpolation was used for pixel up-sampling of the MALDI dataset to match the MIBI pixel size. Alignment of the two modalities into a shared coordinate system was achieved by using the H&E as ground truth.

MALDI pixel upscaling

Interpolation	Sigma
cubicspline	3.13E-06
bilinear	7.05E-06
lanczos	9.60E-06
cubic	2.21E-05
sum	0.002753259
low_res	0.22075877

Image registration on simulated data

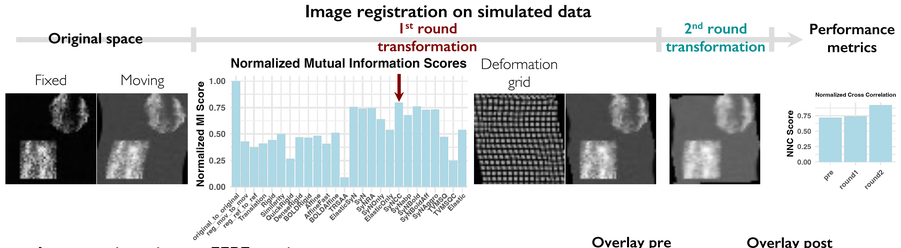
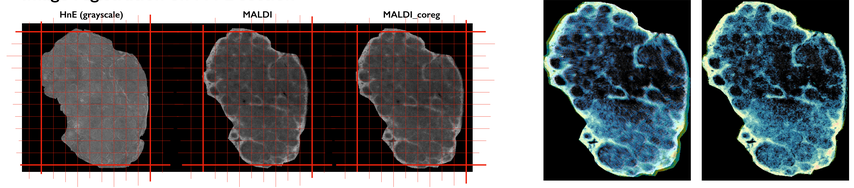


Image registration on FFPE section

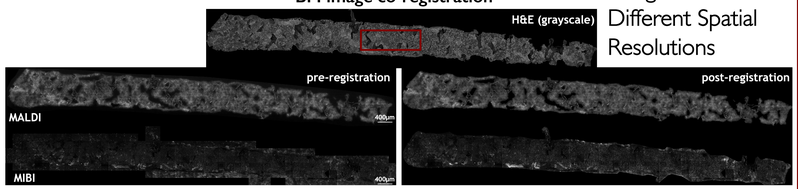


Integration of Cell Features &Glycans

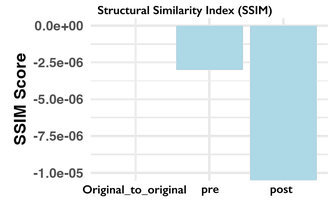
Registered MnM (MALDI & MIBI) imaging data were integrated into a single dataset where each pixel retains bimodal information from N-glycans and MIBI probes. SSIM scores indicates successful structural similarity as result of MALDI pixel up-sampling and landmark image registration to MIBI.

BM image co-registration

Merge 3 Modalities @ Different Spatial Resolutions

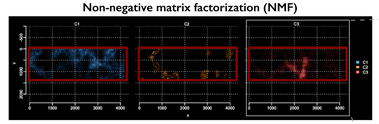


Resolution-based Merging Increases Spatial Similarity



Identify Top Molecular (Glycan) Components with sub micron-scale (Protein) Structures

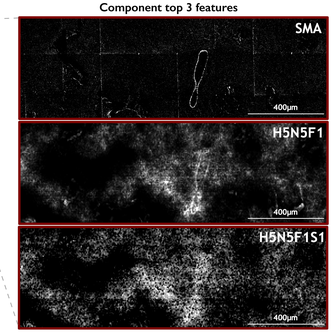
Non-negative matrix factorization (NMF)



Top 10 contributing features to C3

	mz	target	C1	C2	C3
1	2100.7347	SMA	3.0741921	1.1803019	5.859626
2	2012.7187	H5N5F1	3.3423089	0.7450838	5.5850877
3	2303.8141	H5N5F1S1	4.9708876	0	5.3157916
4	2391.8301	H5N4F1S2	2.2648287	3.0191266	4.6623
5	2303.8141	H5N5F1S1	3.5319155	0	4.6398952
6	1938.6819	H4N4F1S1	6.0345997	3.3445849	4.5915748
7	1955.6972	H5N4F2	2.243785	5.6348403	4.2818524
8	1444.5071	H4N3F1	8.2787939	1.1172425	4.1897298
9	1793.6444	Hex4dHex2HexNAc4	7.1782788	3.0018347	4.1337447
10	1622.5548	H6N3	7.1774483	1.0764785	4.1270597

Component top 3 features



Related Presentations

Talk – Ke Leow - Wed Jun 5 – 8:30am (**WOA Room 210ABC**) – Spatial Multi-omics (Single Cell Proteomics, Spatial Glycan, Spatial Trxn) in Human Pregnancy
Poster –Mikaela Ribi – Mon Jun 3 - **MP 291** – MALDI N-Glycomics and High-Grade Glioma
Poster – Davide Franchina – Wed Jun 5 – **WP 377** – Co-Spatial Tissue Imaging (Single cell Proteomics, N-Glycomics, H&E bright field)

References

1. Drake RR ***et al.*** In Situ Imaging of N-Glycans by MALDI Imaging Mass Spectrometry of Fresh or Formalin-Fixed Paraffin-Embedded Tissue. 2018
2. Liu CC ***et al.*** Reproducible, high-dimensional imaging in archival human tissue by multiplexed ion beam imaging by time-of-flight (MIBI-TOF). 2022

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