MnM: Co-spatial tissue imaging using MALDI and MIBI

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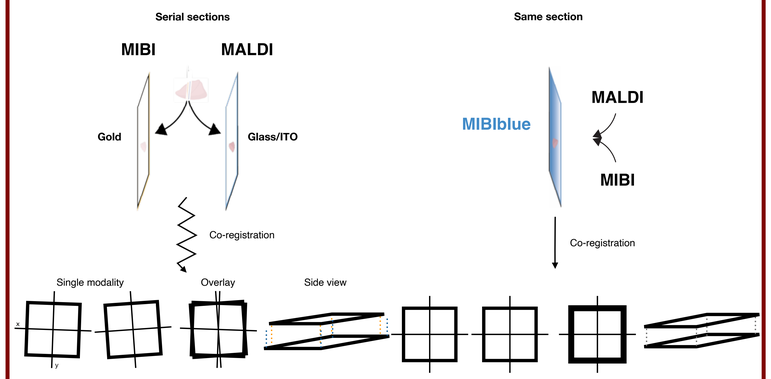
Introduction

Multiple technologies allow measurement of structures, cellular organization, and molecular properties across tissues. These spatial mapping tools offer an approach to study tissue complexity and heterogeneity in (patho-) physiology. However, due to specific platform-to-platform incompatibility (*i.e.* slide type, tissue processing and preparation), protocols that maximize output from a single tissue section are lacking. Also, even serial sections that are immediately adjacent to each other often contain different cells (especially small cells like lymphocytes). Here, we present MnM, a workflow for the sequential imaging of archival material using matrix assisted laser desorption ionization (MALDI) and multiplexed ion beam imaging (MIBI). MnM enables the registration of untargeted molecular composition (MALDI) and targeted single-cell phenotypes (MIBI) from the same tissue section. **We present a bimodal imaging strategy enabling mass spectrometry imaging (MALDI), high-definition spatial proteomics (MIBI), and H&E on the same tissue area, therefore allowing detection of analytes from MALDI and protein expression from MIBI on the same cell.**

On the alignment of multi-modal data

Spatial alignment is non trivial because tissues are subject to processing and "cameras" are not identical across platforms (*i.e.* different pixel resolution).



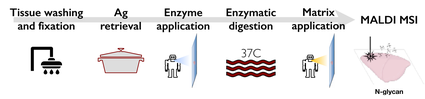
To reduce misalignment:

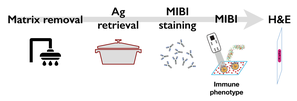
- use the **same tissue section** to retrieve pixels with MALDI and MIBI information.
- eliminate the rotational and warping effects associated with serial sections
- **minimize the spatial deformations** necessary for data integration.

COI disclosure: M.A. is a co-founder and shareholder in Ionpath, manufacturer of MIBI-TOF. S.B. is also a co-founder of Ionpath.

Protocol overview and data generation

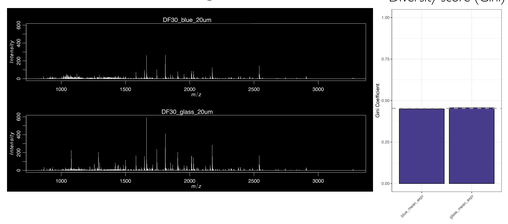
FFPE tissue sections were sectioned onto an organic polymer-coated slide (MIBIblue). The tissue was imaged with a MALDI timsTOF flex using a published protocol for N-glycan imaging (1).



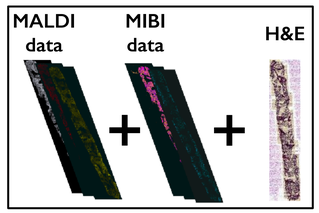


The matrix was washed off and sections were stained with a mix of metal-labelled antibodies targeted to immune phenotypic markers (2). Data was acquired with a MIBIscope and sections were counterstained with H&E.

MIBIblue vs. glass slide



Raw data

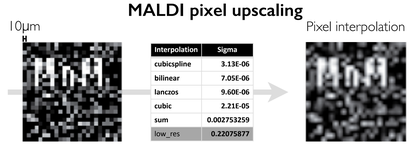


Pixel resampling and iterative registration

Image registration on simulated data:

- **Bilinear interpolation** for pixel up-sampling MALDI→MIBI.
- Alignment into a shared coordinate system 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

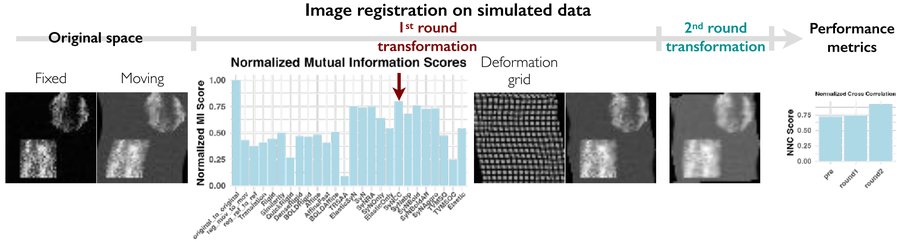
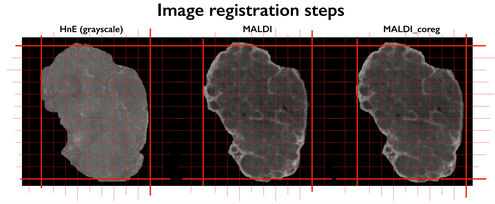
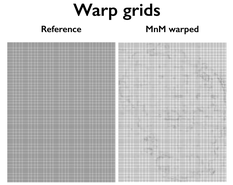


Image registration steps



Warp grids

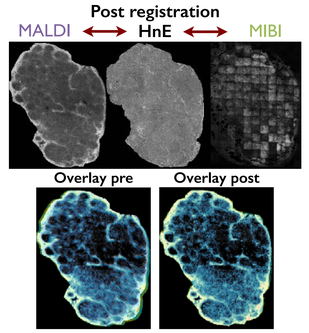


MnM: shared space and a unified spectrum

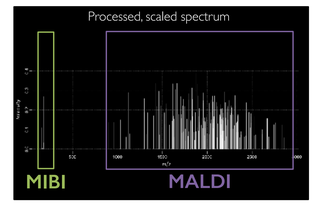
MnM, MALDI and MIBI combined into a single dataset:

- Each **pixel retains bimodal information** from N-glycans and MIBI probes.
- Datasets registration reveals a substantial pixel overlap, indicating satisfactory overall performance of the method.

Post registration



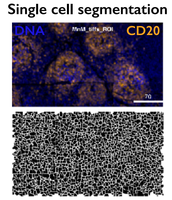
Spectral integration

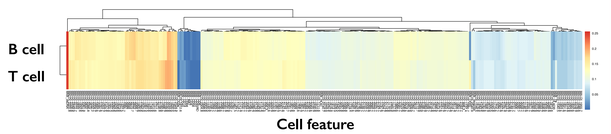


Summary and future work

The conductivity and transparency of MIBIblue slides enable the sequential processing of tissue sections by platforms that were previously incompatible. MnM converts multi-modal-multi-resolution datasets into a shared spatial canvas that can be further analyzed (cell segmentation/analysis, spatial queries etc.). This method upgrades cellular phenotype and activity (antibody-based MIBI) with post-translational and metabolic information (*de novo* MALDI) at the single cell level.

Single cell segmentation





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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