Probabilistic Alignment of Spatial Transcriptomic Experiments for MALDI Imaging Serial Section Alignment

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Introduction

Serial section alignment in is a central theme in MALDI Imaging and allows the understanding of how chemical features distribute throughout a tissue depth. We present a framework using SCiLS™ Lab and the SCiLS REST API for the automated alignment and volumetric reconstruction of tissue serial sections acquired using MALDI Imaging. Three-dimensional volumetric reconstruction and visualization of the aligned serial sections can improve researchers understanding of chemical gradients that are present across different tissue architectures while providing a more accurate interpretation of biological organization versus a slice-to-slice interpretation.

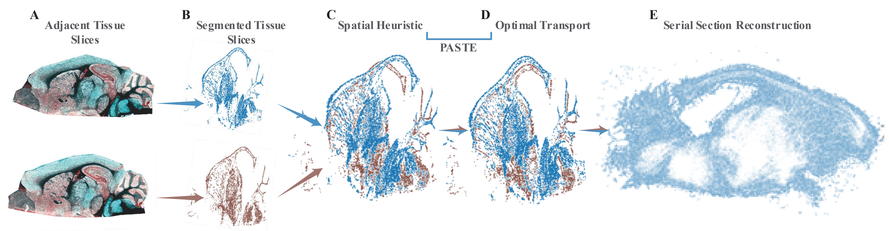


Figure 1. Serial section alignment and volumetric reconstruction workflow diagram. a) MALDI Imaging of serial sections and resulting ion images. b) Segmentation of the tissue allows regions with distinct morphological features to be captured for downstream alignment. c) Spatial heuristic clustering aims to provide a rough alignment of the dataset to stabilize tissue structures across different slices. Spatial heuristic clustering relies only on the xy-coordinates in the dataset. d) After spatial heuristic clustering and optimal transport model can be generated using the metabolite intensity information in the dataset. e) The aligned tissue slices can be then volumetric reconstructed and visualized.

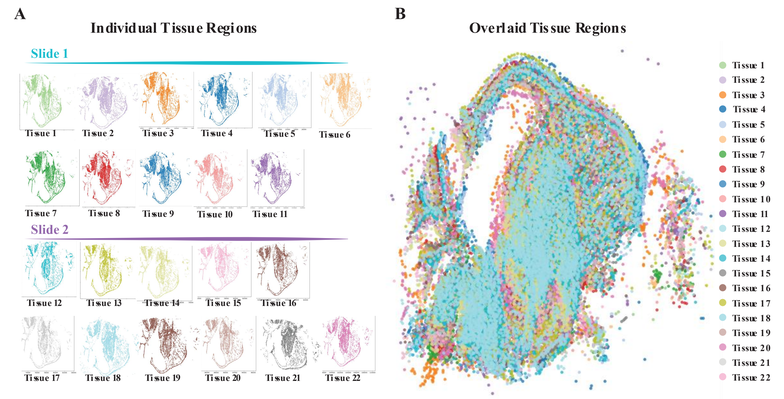
Methods

Mouse brain tissue was sectioned at a 10 µm thickness before thaw-mounting onto IntelliSlides® (Bruker Daltonik GmbH & Co. KG, Bremen, Germany). Data was acquired on a timsTOF fleX at a 20 µm spatial resolution using the *m/z* range 50–1,000. Data visualization and analysis was performed in SCiLS Lab, the SCiLS API and Python. The Probabilistic Alignment of Spatial Transcriptomics Experiments (PASTE) algorithm was developed by the Raphael group (Department of Computer Science, Princeton University, Princeton, NJ, USA).

Developer code here

Results

- 15 pixel/s acquisition on the timsTOF fleX MALDI-2 equipped with microGRID using the smartbeam 3D allowed collection of 22 serial sections.
- Framework for automated serial section alignment using PASTE, SCiLS Lab and SCiLS API.
- No modifications required to MALDI Imaging experiment. Already acquired MALDI Imaging experiments can be used.
- Visualization and interpretation of how multiple analytes distribute throughout a three-dimensional space.



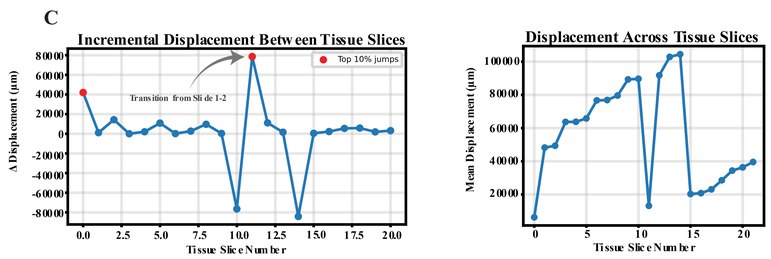


Figure 2. Serial section alignment and visualization a) Individual tissue regions were extracted using segmentation (bisecting k-means) in SCiLS Lab using 24 metabolite and lipid features. The segmented regions and corresponding intensity information was sent to an alternative programing interface via SCiLS Lab API. b) Spatial heuristic clustering on the xy-coordinates was first performed for rough alignment. An optimal transport model is then generated using the rough alignment and metabolite/lipid intensity. The aligned slices are then overlaid for visualization. c) The incremental displacement between and displacement across tissue slices was calculated for assessment of the alignment quality. Both graphs show continuous patterns with incremental displacement from the first slice with large jumps when moving from Slide 1 to Slide 2.

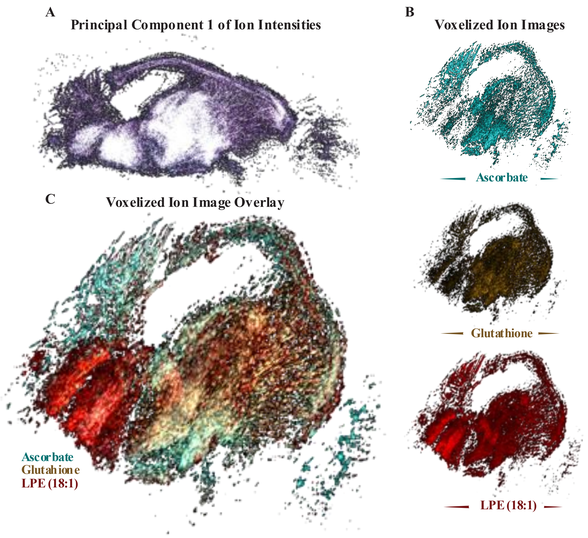


Figure 3. Volumetric reconstruction of aligned tissue slices. a) To visualize the alignment before volumetric reconstruction the principal component 1 of the ion intensities is plotted. b) Voxelized ion images of ascorbate, glutathione and LPE (18:1). c) Voxelized ion image overlay of ascorbate, glutathione and LPE (18:1).

Conclusion

- Full volumetric reconstruction of numerous sequential tissue slices for simultaneous visualization of analytes using PASTE, SCiLS Lab and SCiLS API.
- Not only allows the automated alignment of MALDI Imaging data but it can also be applied for the integration of different instrument modalities for expanded and complementary molecular information.

Imaging MS: Computational Methods, Software, and Analysis

COI Disclosure: N.T., M.E. and D.C. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.