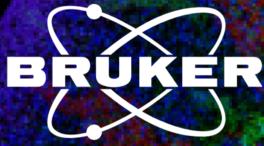

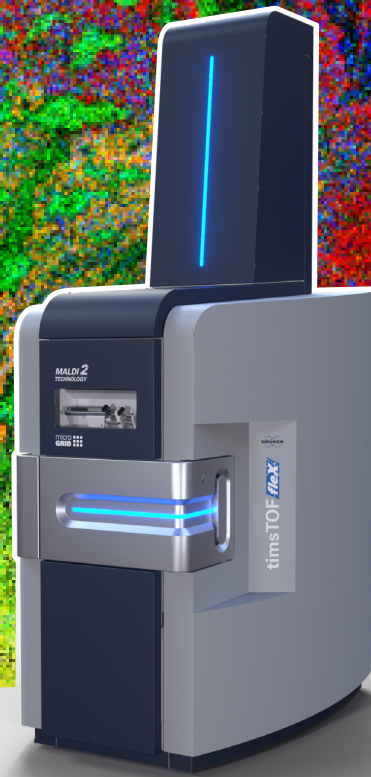

Maximize spatial biology insights with 5 μm MALDI Imaging using microGRID

Targeting proteins specific to relevant pathways and processes using unprecendented spatial resolution for visualizing maximum biological information from tissue.

Abstract

The interdisciplinary field of spatial biology continues to connect omics research areas with the goal of understanding the spatial distribution of biomolecules that influence biological processes and functions. Advanced imaging techniques continue to emerge on the market, but matrix-assisted laser desorption ionization (MALDI) Imaging, a mass spectrometry-based technique, is a widely accepted methodology for determining spatial localization of analytes on tissue and has been around for more than 25 years. Recent improvements in instrumentation regarding stage and laser design enable now MALDI Imaging of tissue on a cellular level.

Keywords:
MALDI Imaging, microGRID, high spatial resolution, HiPLEX, multiomic, IHC, spatial biology

Introduction

Visualizing metabolites and lipids allows for the connection of the immediate cellular metabolism with the proteins that are doing the work of the cell. MALDI Imaging offers the only label-free spatial analysis technique for metabolites and lipids, and additional workflows make released glycans and intact proteins accessible for broad multiomic information. With these workflows and novel photocleavable mass tags applied to fresh frozen or FFPE samples, correlating metabolite, lipid, glycan, and protein information to histology becomes increasingly easy at high spatial resolution for faster and more effective analysis of tissue morphology. This Technical Note will highlight the targeted protein workflow in combination with high spatial resolution enabled by microGRID technology, which enables the highest spatial resolution of 5 μm available by commercial mass spectrometry.

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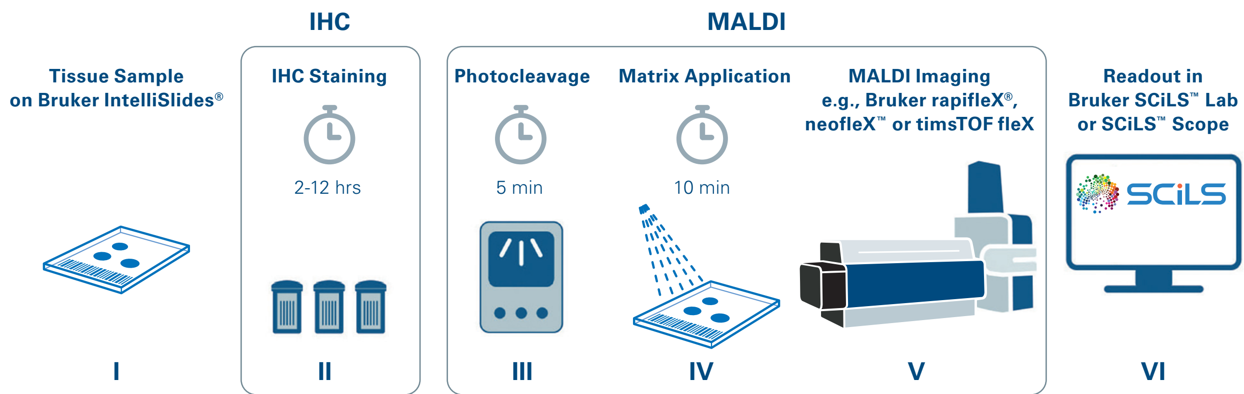

Figure 1
Overview of the MALDI HiPLEX-IHC workflow.
Tissue samples undergo the following process: (**I**) section on intelliSlides®, (**II**) staining with Miralys™ photocleavable mass-tag antibodies using a MALDI-friendly IHC process, (**III**) cleave photo-cleavable mass tags that are conjugated to the antibody, (**IV**) MALDI matrix application, (**V**) analysis in a Bruker neofleX™, timsTOF fleX or rapifleX®, (**VI**) visualize data in SCiLS™ Lab or SCiLS™ Scope software.

Methods

FFPE human kidney tissues were prepared using the standard MALDI HiPLEX-IHC workflow, using technology from AmberGen [1,2], which is described in Figure 1. Briefly, the slides were heated at 60°C and transferred through a xylene to ethanol/aqueous gradient to remove the wax. The tissue then underwent antigen retrieval in a basic buffer, followed by a tissue blocking step. Next, antibodies with photocleavable peptide tags, available from AmberGen, Inc. (Billerica, MA), (i.e., markers for Vimentin, Histone, ATPase, CD68, Collagen 1A1) were placed on the tissue and allowed to incubate at 4°C overnight. The peptide tags were then released using UV light and MALDI matrix (α-cyano-4-hydroxycinnamic acid) was applied using established protocols on a pneumatic M3+ sprayer (HTX Technologies, Chapel Hill, NC). Finally, recrystallization of the matrix was performed, and the tissue was run on a Bruker timsTOF fleX MALDI-2 instrument at 5 μm lateral resolution using microGRID technology. After MALDI Imaging was performed, matrix was washed off and hematoxylin and eosin (H&E) staining done using standard procedures. Data was analyzed in SCiLS™ Lab software with corresponding H&E staining integrated with pathological annotations, corresponding to protein expression for defining key histological features. The pathologist's tissue annotations were imported to SCiLS™ Lab through the QuPath plug-in.

Results

Two series of experiments were run for proof-of-concept of of 5 μm MALDI HiPLEX-IHC Imaging. Initial experiments were run with three antibodies on human FFPE tissue at both 20 μm and 5 μm, in order to demonstrate the resolution enhancement and identification of molecular markers for key histological features. Additional experiments were done on serial tissue sections with higher complexity of antibodies in order to give a more comprehensive picture of protein evaluation. H&E staining was done post analysis and incorporated with pathologist annotation, showing correlation between protein expression and histological features.

The data shown in Figure 2 represent the first experiment that was done with three different antibodies, Vimentin, Histone H2A, and ATPase-1A1 to preliminarily evaluate the MALDI HiPLEX-IHC workflow on FFPE human kidney tissues. A 20 μm image was captured in one area of the tissue, and a subsequent 5 μm image was obtained in a different area of the tissue. The three peptides associated with the antibodies were at *m/z* 1222.79 for ATPase-1A1, *m/z* 1230.84 for Vimentin, and *m/z* 1226.82 for Histone H2A. For maximum clarity in visualization, mass channels for adducts (protonated peptide and sodium adduct) were combined. Closer

examination of the 5 μm spatial resolution data is shown in Figure 3. Overlay of the three corresponding masses showed significant localization of the peptides to areas predicted to be rich in the protein of interest (glomeruli for Vimentin, histone nuclear marker, and ATPase for proximal convoluted tubules).

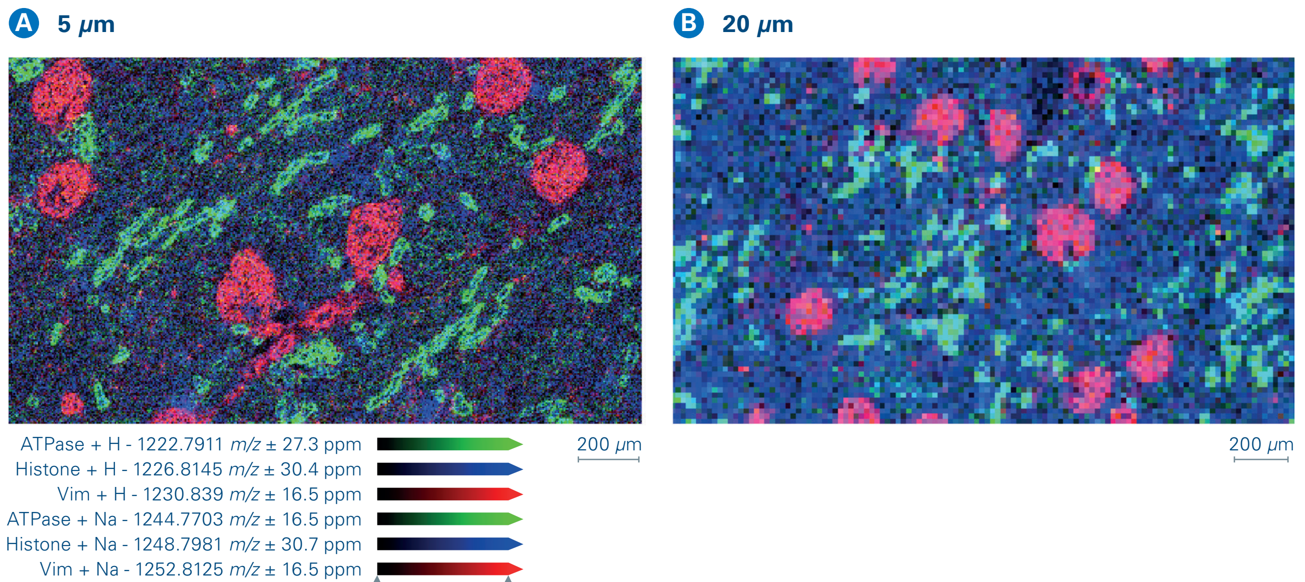

Figure 2

Wide field of view comparison between 5 μm imaging run (A) and 20 μm imaging run (B), demonstrating the increased resolution and artifact-free analysis provided with microGRID technology.

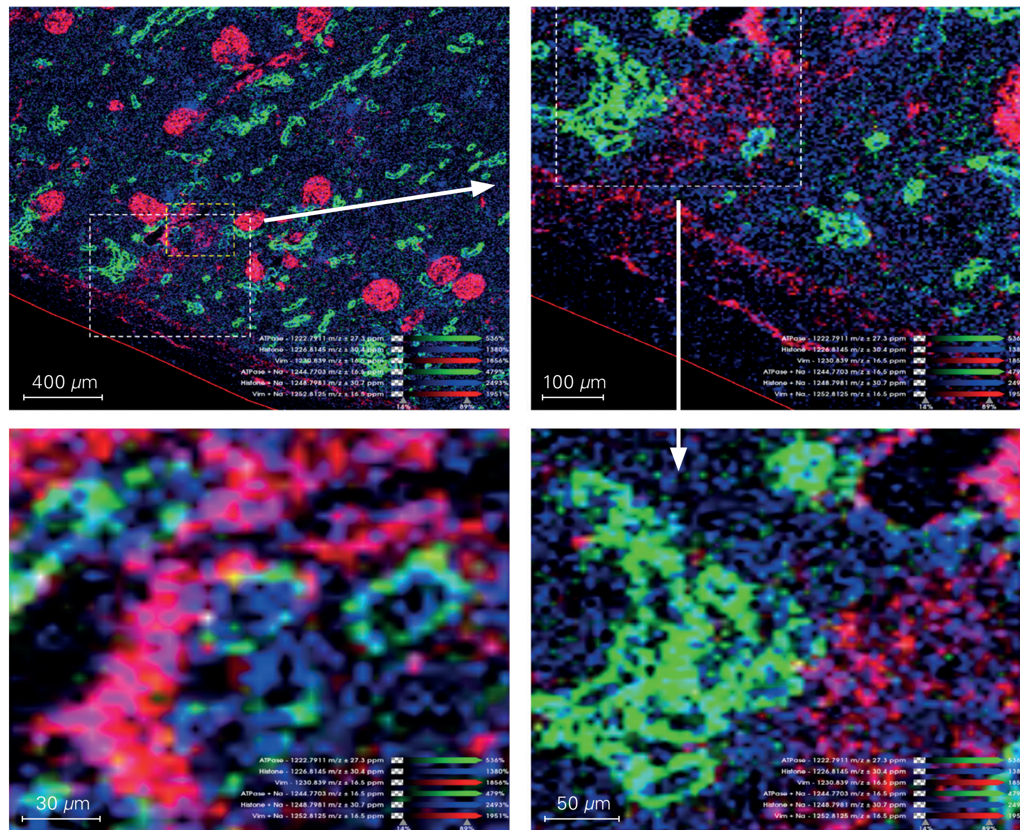

Figure 3

5 μm MALDI Imaging data from human FFPE kidney tissue run with ATPase (green), Vimentin (red) and Histone (blue) markers, zooming in progressively to demonstrate high spatial resolution capabilities with definable tissue features.

A second, higher multiplexed experiment was conducted including additional markers for CD68 (*m/z* 1216.75) and Collagen 1A1 (*m/z* 1234.87) (Figure 4). The experiment was repeated on serial sections of FFPE kidney tissue and run under the same conditions with two adjacent sections at 20 μm and 5 μm. Both imaging runs successfully localized and identified all antibodies used while containing minimal to no artifacts as a result of the 5 μm resolution. The optical image of the H&E-stained kidney section was additionally visualized and annotated for histological features with QuPath, an open source software widely used in digital pathology. The pathologists annotations were then exported to SCiLS Lab via the "Export annotations to SCiLS Lab" extension, which creates a *.sef file. In order to correlate the defined regions to the protein information from the MALDI Imaging result, the .sef file was then imported to the existing SCiLS Lab file containing the MALDI Imaging data for this particular histological section. After co-registration, the annotations from QuPath were directly compared to the MALDI Imaging data. In Figure 4 it is shown that for example the signal for Vimentin nicely correlates to the region that was annotated as glomerulus and CD68 is detected in regions were macrophages are annotated.

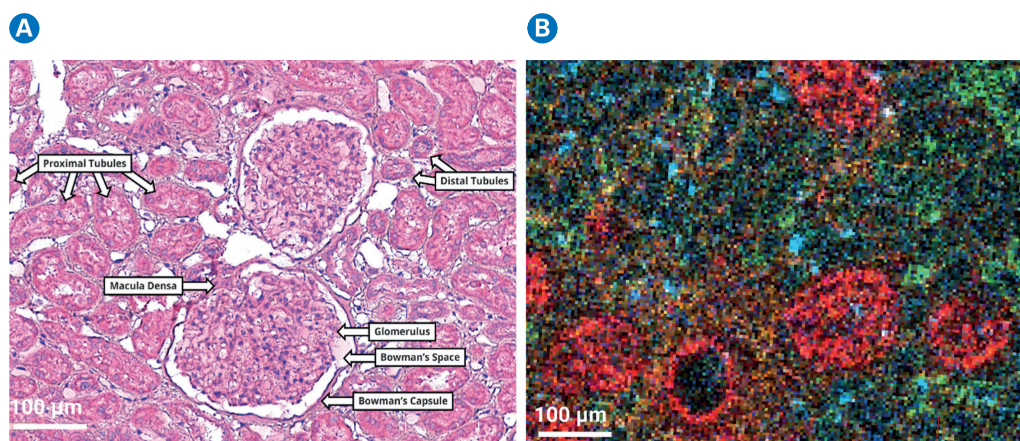

Figure 4
(A) Pathologist annotated H&E-stained tissue and (B) corresponding molecular image via MALDI HiPLEX-IHC, with red being Vimentin, green being ATPase, dark blue being Histone, light blue being CD68 and orange being Collagen 1A1.

Conclusion

This work demonstrates the highly desirable capabilities of MALDI HiPLEX-IHC, using photo-cleavable mass tags from AmberGen, coupled with the high spatial 5 μm resolution from microGRID on the timsTOF fleX. Correlation of complex intact protein information that helps to identify morphological tissue features. In addition, pathologist annotation of histologically stained tissue can be combined within the Bruker SCiLS™ Lab software solution.

Acknowledgements

Human kidney tissue was kindly provided by the Medical University of South Carolina.

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