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MALDI HiPLEX-IHC Imaging of FFPE Human Kidney Tissue at 5 µm utilizing microGRID on timsTOF fleX MALDI-2

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Introduction

Matrix assisted laser desorption ionization (MALDI) imaging is a widely accepted methodology for determining spatial localization of analytes on tissue. Intact protein imaging has always been of interest; however, ionization efficiencies and resolution of the images have been a challenge and trade off when imaging intact proteins. The combination of MALDI HiPLEX-IHC workflow with microGRID overcomes these challenges by allowing for high spatial resolution imaging of intact proteins in a multiplexed fashion. This allows for the imaging of up to 200+ intact proteins simultaneously directly from tissue at up to 5 µm spatial resolution free of artifacts and striping.

Applying this workflow allows for correlation of protein information to cellular features and is accomplished with increasing accessibly for multiplexed markers at high spatial resolutions for faster and more effective analysis of key histological features.

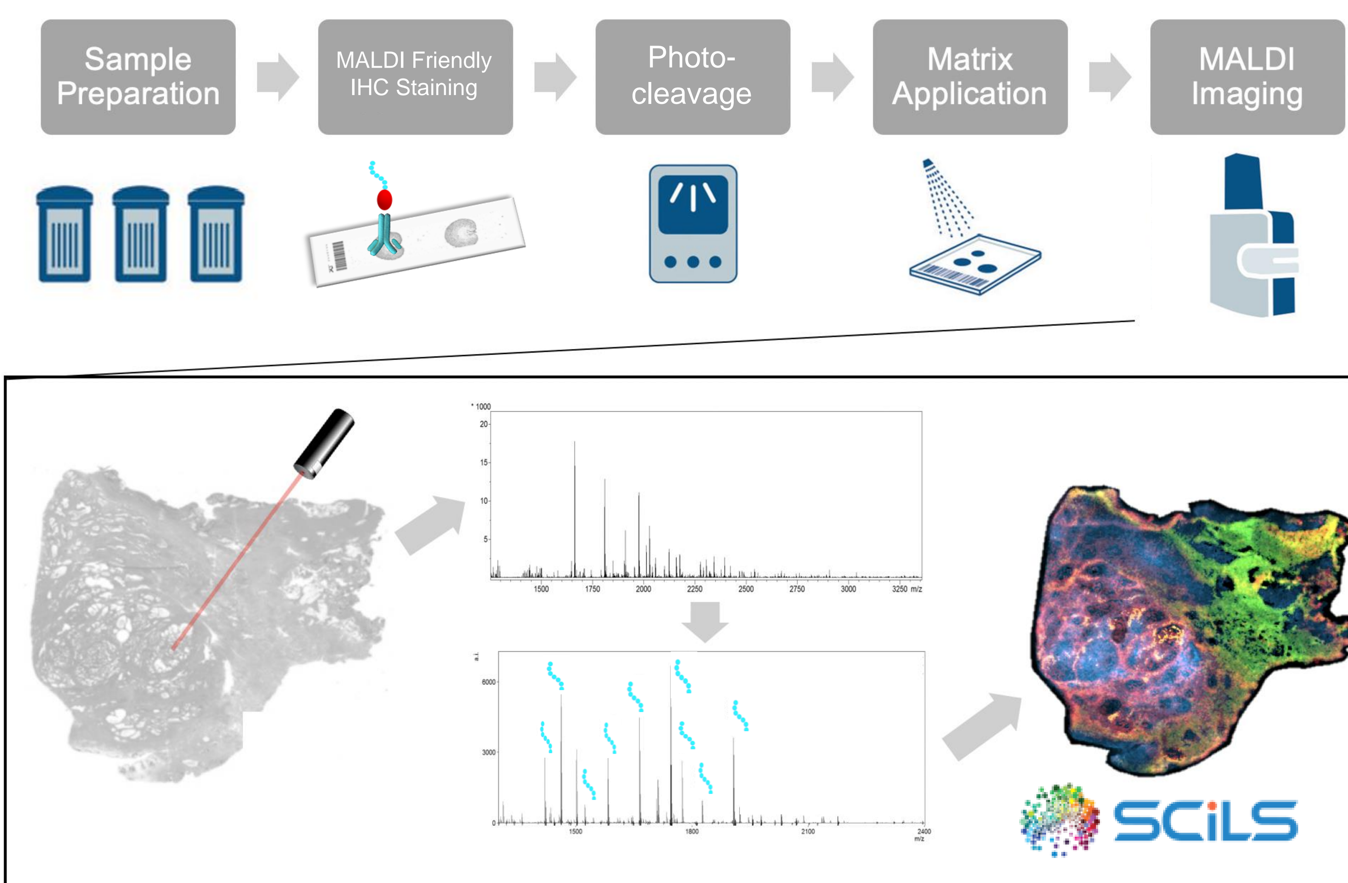

Figure 1. Overview of MALDI HiPLEX-IHC workflow, including sample preparation for FFPE tissue, MALDI-IHC protocols, and MALDI imaging and visualization and data interpretation using SCiLS™ Lab 2023a

FFPE human kidney tissue samples courtesy of the Medical University of South Carolina (Drs. Richard Drake, Peggi Angel and Anand Mehta)

Methods

FFPE human kidney tissues were first prepared using the MALDI HiPLEX-IHC workflow. Briefly, slides were heated at 60°C and transferred through a xylene to TBS rehydration gradient to remove the wax. The tissue then underwent antigen retrieval in a basic buffer, followed by a tissue blocking step. Next, antibodies of choice (with photocleavable peptide tags) were placed on the tissue and allowed to incubate at 4°C overnight. The peptide tags were then released using UV light and CHCA matrix was applied. Finally, recrystallization of the matrix was performed, and the tissue was run on a timsTOF fleX MALDI-2 instrument at 5 µm using the new microGRID technology (Figure 1).

Data was visualized in SCiLS™ Lab software with corresponding hematoxylin and eosin staining integrated with pathological annotations, corresponding to protein expression for defining key histological features.

Results

- Initial experiments run with three antibodies on human FFPE tissue at both 20 µm and 5 µm, demonstrating molecular markers for key histological features
- Secondary experiments were done on serial tissue sections with higher complexity of antibodies, demonstrating a higher complexity of protein evaluation
- H&E staining was done post analysis and incorporated with pathologist annotation, showing correlation between protein expression and histological features.

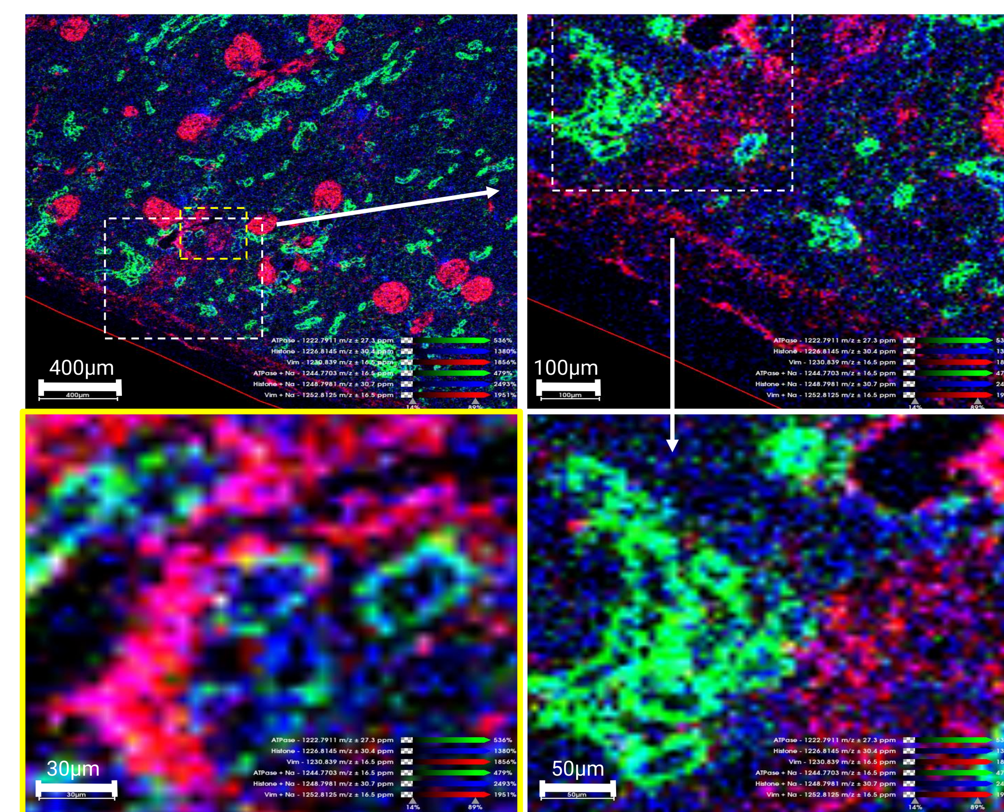

Figure 3. MALDI Imaging 5 µm data from initial FFPE kidney tissue run with ATPase, Vimentin and Histone markers, zooming in progressively to demonstrate high resolution capabilities with definable tissue features

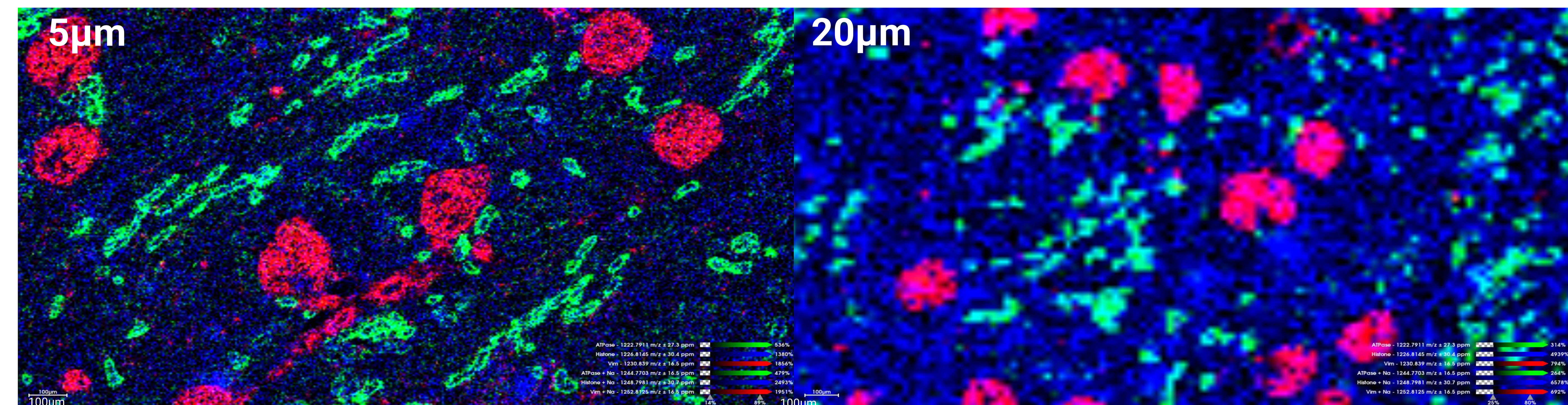

Figure 2. Wide field of view comparison between 5 µm imaging run (left) and 20µm imaging run (right), demonstrating the increased resolution and artifact free imaging provided with the microGRID technology

Initial experiments were done with 3 different antibodies: Vimentin, Histone H2A, and ATPase-1A1 to preliminarily evaluate the on the human 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 (Figure 2). Here, the three targeted proteins were Vimentin, Histone H2A and ATPase-1A1. Overlay of the three corresponding peptide masses showed significant localization of the peptides to areas predicted to be rich in the protein of interest (Table 1, Figure 3)

Following initial optimization, a second experiment was conducted which included more antibodies for a higher multiplexing of protein markers. The original three antibodies were included, along with additions such as markers for CD68 and Collagen 1A1. The experiment was then repeated on a serial section and run under the same conditions with two adjacent sections at 20 µm and 5 µm, ensuring a direct comparison of pixel density for raster size. Both imaging runs successfully localized and identified all antibodies used while containing minimal to no artifacts as a result of the 5µm resolution. These protein images were then directly compared to pathologist annotation of the histological features of the human kidney tissue (Figure 4).

Table 1: Protein markers, mass tags, and histological significance

Protein Marker	Mass Tag (*m/z*)	Histological Significance
Vimentin	1230.84	Glomeruli
Histone H2A	1226.82	Nuclear marker
ATPase-1A1	1222.79	Proximal tubules
CD68	1216.75	Macrophages
Collagen 1A1	1234.87	Fibrillar Collagen

The multiplexing capabilities illuminate spatial interactions between cell types, such as macrophages with the CD68 marker, the extracellular matrix with the fibrillar collagen marker (COLA1A) and other key histological features like the glomeruli and proximal tubules.

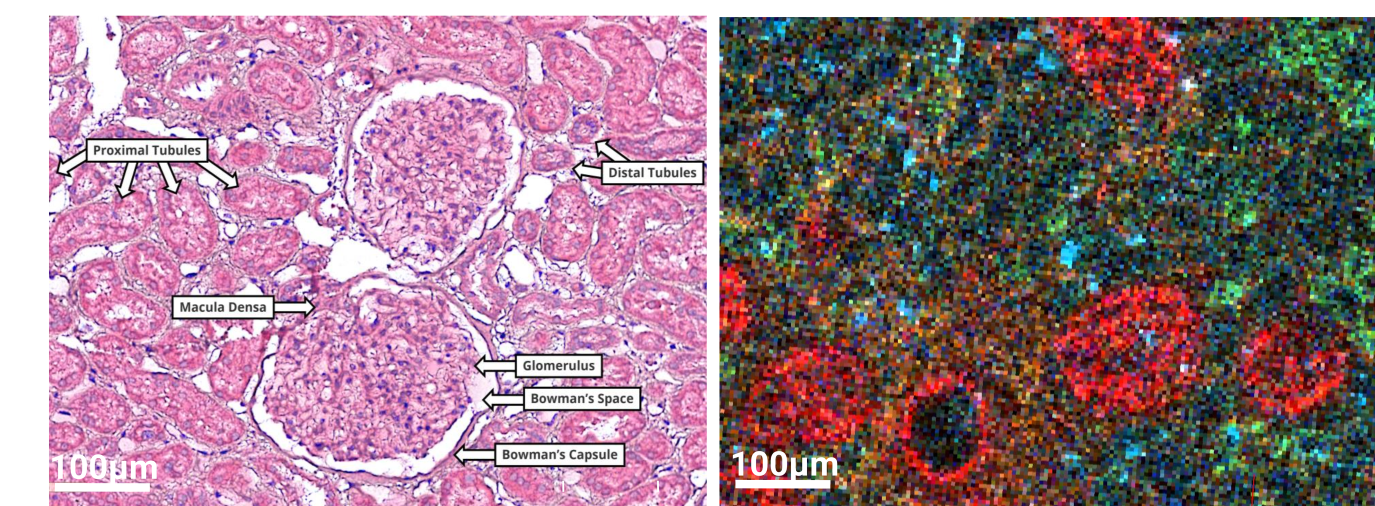

Figure 4. Pathologist annotated hematoxylin and eosin-stained tissue and corresponding molecular image via MALDI HiPLEX-IHC, with red being Vimentin, Green being ATPase, dark blue being Histone, light blue being CD68 and orange being Collagen 1A1.

Conclusion

- This work demonstrates the highly desirable capabilities of the MALDI HiPLEX-IHC workflow coupled with the high spatial 5 µm resolution from microGRID.
- This can address key biological questions and elucidate spatial interactions between different cell types in the tissue microenvironment.

Imaging MS: Instrumentation

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