Targeted protein imaging of kidney pathologies using MALDI Imaging

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Introduction

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 20 years.

MALDI Imaging offers the only unlabeled spatial analysis technique for metabolites and lipids, and additional workflows make released glycans and intact proteins accessible for multiomic connections. This work will highlight the targeted protein workflow on human kidney samples, with multimodal comparison to traditional staining. MALDI Imaging has recently garnered attention as a breakthrough method for integration across the spatial omics space.

A description of the MALDI HiPLEX-IHC workflow is presented in Figure 1.

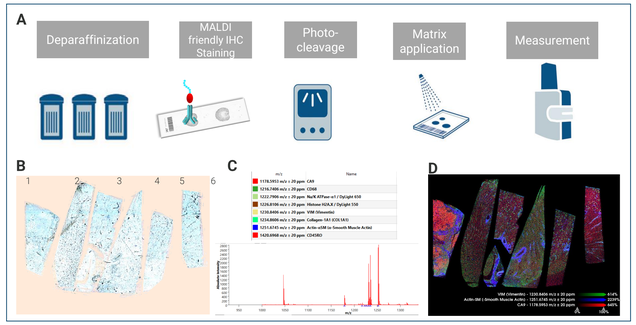

Figure 1. (A) Overview of the MALDI HiPLEX-IHC workflow: Slides were heated at 60°C, rehydrated with a xylene to TBS gradient. Antigen retrieval in basic buffer was followed by a tissue blocking step. Antibodies with photocleavable tags (AmberGen) were placed on the tissue and allowed to incubate at 4°C (8 hr). Peptide tags were released using UV light and CHCA matrix was applied (HTX Technologies) and recrystallized. Tissue was analyzed on a timsTOF fleX MALDI-2 instrument at 50 µm, 20 µm and 5 µm using microGRID technology. (B) Tissues analyzed: 1) clear cell renal cell carcinoma (CCRCC); 2) transplant rejection (TR); 3) thrombotic microangiopathy (TM); 4) vasculitis; 5,6) control (Ctrl). (C) Antibody panel used with an overview mass spectrum, with blue indicators below spectrum to indicate antibody peaks. (D) Overlay of antibodies (Vim, SMA, CA9) visualized in SCiLS™ Lab.

FFPE human kidney tissue samples courtesy of the Hannover Medical School (MHH) (Prof. Dr. Jan Hinrich Bräsen, Dr. Jessica Schmitz)

Results

FFPE human kidney tissues were first prepared using the standard MALDI HiPLEX-IHC workflow (Figure 1). Data visualization within SCiLS Lab incorporated corresponding hematoxylin and eosin (H&E) staining (Figure 2), DAB (3,3'-diaminobenzidine) or AP red staining for single antibodies (Figure 4). In addition, two antibodies used dual-labeled probes (peptide tag and fluorophore); specifically, Dylight 550 (Histone H2A.X) and Dylight 650 (Na/K ATPase alpha) were used (Figure 3). Detailed descriptions and interpretation are in the figure captions.

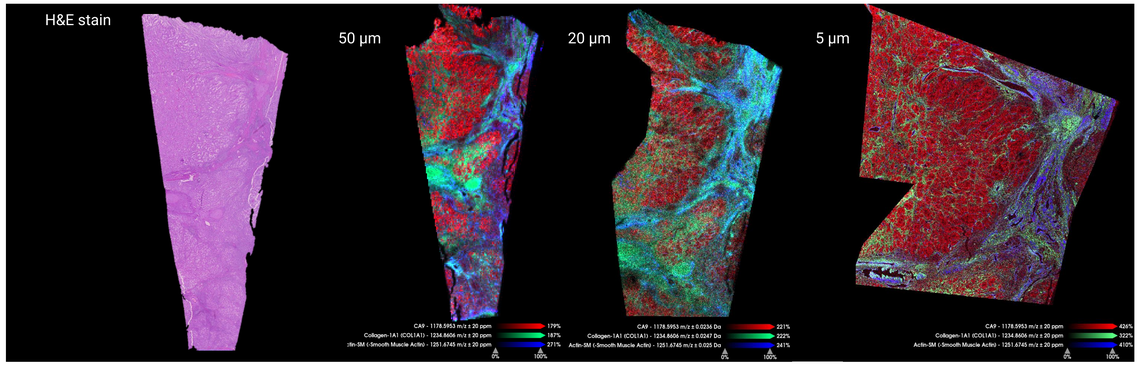

Figure 2. Whole slide field of view comparison between kidney tumor H&E stain, 50 µm imaging run, 20 µm imaging run, and 5 µm imaging run demonstrating the increased resolution and artifact free analysis provided with the microGRID technology. Eight antibodies were included in the stain, with CA9 clearly indicate a clear cell renal cell carcinoma (CCRCC). The smooth muscle actin staining visualizes the tumor border nicely, whereas the collagen 1A1 stain reflects the organization of collagen within the tumor area; all tissue morphology was verified by a pathologist (J.H.Bräsen).

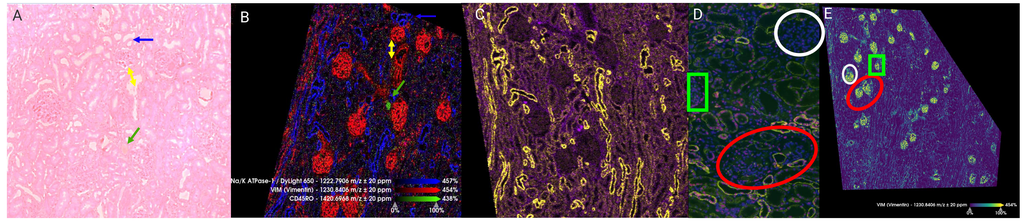

Figure 3. (A) H&E stain after MALDI HiPLEX-IHC analysis with 3 morphologies marked by arrows. (A,B) Protein expression from vimentin, CD45RO and Na/K ATPase using HiPLEX and morphological features from H&E correlate. Areas of interest marked with arrows (yellow, green, blue). (C) Two channel fluorescence data from dual-labeled antibodies on same tissue section as (B), with Histone H2A.X (Dylight 550) in purple and Na/K ATPase alpha (Dylight 650) in yellow. Purple glomeruli outline in (C) correlates to red vimentin in (B), while yellow morphology from (C) aligns with blue in (B). (D) Fluorescence image from dual-labeled probes from (C) shows correlation with histological features. (E) also highlights similar features in (D) using the HiPLEX method for the protein marker vimentin.

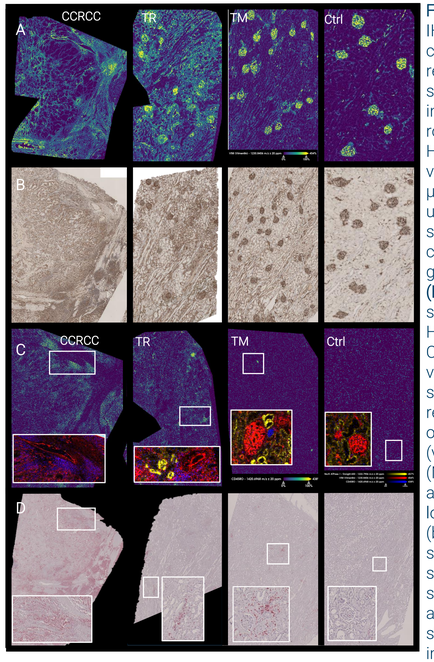

Figure 4. MALDI HiPLEX-IHC ion images in comparison to DAB/AP red single IHC, a standard antibody used in pathology. (A) The first row shows MALDI HiPLEX-IHC images with vimentin displayed at 5 µm spatial resolution, using different disease states from Figure 1. A clear distortion of the glomeruli is visible in TR. (B) Comparison to DAB stained tissue. (C) MALDI HiPLEX-IHC images of CD45RO staining to visualize leukocytes, is shown, with a small region zoomed in with other markers in red (vimentin) and yellow (Na/K ATPase alpha) to allow a clear visual localization of CD45RO (blue). (D) AP red (CD45) stained consecutive sections show similar staining results. Small areas are enlarged to show the localization of immune cells.

To compare the histological quality of MALDI HiPLEX-IHC images with DAB/AP Red single IHC standard antibody stain, we conducted both methods on consecutive slides (Fig.4). Eight different markers were used in the MALDI HiPLEX-IHC experiment whereas vimentin CD45RO were additionally stained with DAB or AP red. A clear distortion of the glomeruli could be visualized with both techniques in transplant rejection (TR) tissue whereas additionally the tubules seem to be affected in this tissue. The CD45RO staining shows in a convincing manner that leucocyte clusters could be visualized by MALDI-HiPLEX IHC which allows a direct overlay with other markers to gain more insight into the different diseases. The Na/K ATPase marker showed a staining of specific tubuli, here close to the inflammation area which needs to get investigated further.

Conclusion

This work demonstrates the highly desirable capabilities of MALDI HiPLEX-IHC coupled with the high spatial 5 µm resolution from microGRID, allowing for correlation of complex intact protein information with key histological features when combined with pathologist annotation.