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Targeted protein imaging of kidney pathologies using MALDI Imaging

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Introduction

Spatial biology is an evolving field that studies the spatial distribution and interaction of molecules in biological processes, connecting multiple areas of omics research. Matrix-assisted laser desorption/ionization (MALDI) Imaging provides a widely accepted method to gain complimentary information for many of the biological processes spatial biology studies.

Methods

FFPE human kidney samples (provided courtesy of the Hannover Medical School), are detailed in Figure 1.

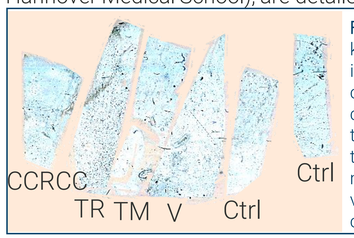
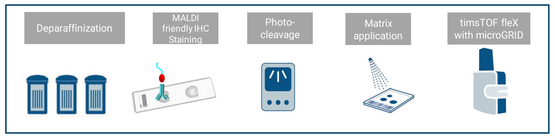


Figure 1. FFPE human kidney tissue with indicated pathologies of clear cell renal cell carcinoma (CCRCC), transplant rejection (TR), thrombotic microangiopathy (TM), vasculitis (V), and control (Ctrl).

- *MALDI HiPLEX-IHC Imaging*, using technology from AmberGen Inc. [1], is shown in the workflow below. The antibodies used were: CA9, CD68, Na/K ATPase alpha, Histone H2A.X, VIM, Col-1A1, Actin-αSM, and CD45RO.



- *Two-channel fluorescence* was performed using dual-labeled antibodies (AmberGen, Inc.) for Histone H2A.X (Dylight 550) and Na/K ATPase alpha (Dylight 650) on the same tissue section as MALDI Imaging.
- *Histochemical staining*, using standard protocols, included DAB (3,3'-diaminobenzidine) or AP red staining for single antibodies on adjacent tissue sections, and H&E on the same section as MALDI Imaging.
- *Infrared Laser Imaging* (ILIM) was performed on a Bruker HYPERION II at 5 µm pixel resolution and analyzed with unsupervised segmentation.

All data analysis and visualization was performed in SCiLS™ Lab 2024b. High-resolution optical image overlay was done using the SCiLS Lab import feature.

Results

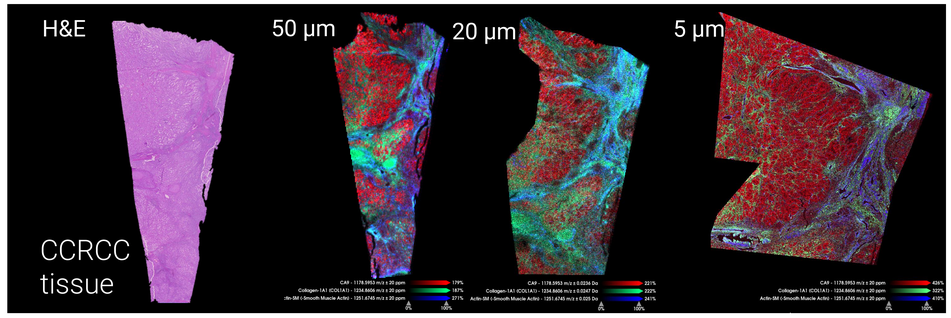
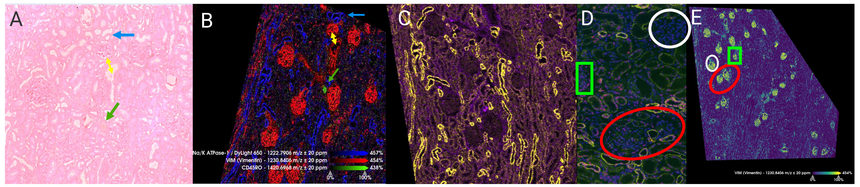


Figure 2. Whole slide field of view comparison of clear cell renal cell carcinoma (CCRCC) with H&E stain, and MALDI HiPLEX-IHC Imaging at 50 µm, 20 µm, and 5 µm spatial resolution, with offset regions. Eight antibodies were included in the stain, with CA9 (red) clearly indicating carcinoma tissue areas. The smooth muscle actin (blue) visualizes the tumor border, whereas Col-1A1 (green) reflects the collagen organization within the tumor area; all tissue morphology was pathologist validated (J.H.Bräsen).

Figure 3. See details below the images. All tissue sections where from the TM pathology.



(A) H&E stain after MALDI HiPLEX-IHC to visualize 3 distinct tissue morphologies indicated by colored arrows.

(B) Corresponding arrows from (A) to view protein expression of vimentin, CD45RO and Na/K ATPase HiPLEX to morphological features.

(C) Fluorescence from tissue in (B) using dual-labels. Histone H2A.X (Dylight 550) in purple and Na/K ATPase alpha (Dylight 650) in yellow.

(D) Fluorescence data from dual-labeled probes indicating histological features, correlated in (E).

(E) Similar features to (D) using HiPLEX for the protein marker vimentin.

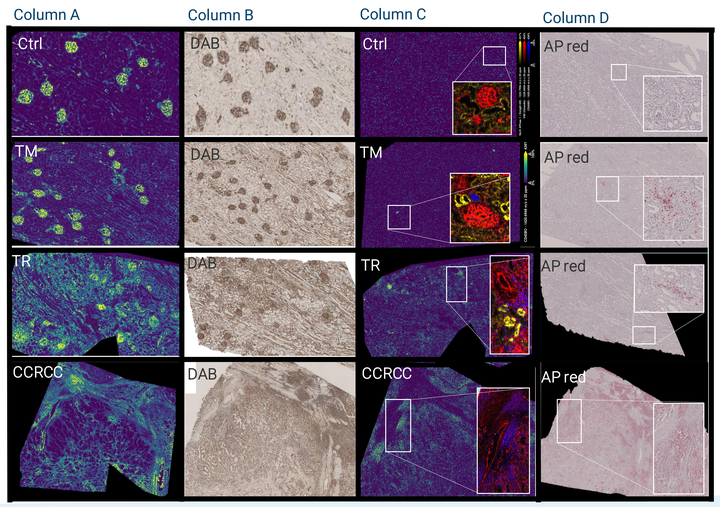


Figure 4. HiPLEX images for tissue from Figure 1, at 5 µm spatial resolution for comparison to single antibody DAB/AP red on consecutive slides. (Column A) HiPLEX images of VIM. (Column B) DAB stain (VIM) comparison to visualize glomeruli. (Column C) HiPLEX images of CD45RO to visualize leukocytes. Zoomed in region includes other markers in red (VIM) and yellow (Na/K ATPase alpha) for better view of CD45RO. (Column D) AP red (CD45RO) stain. Small areas are enlarged to better view immune cells.

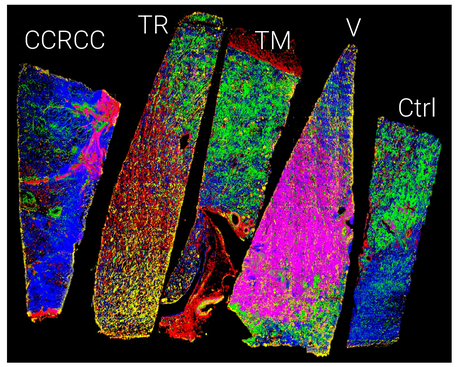


Figure 5. Infrared Laser Imaging (ILIM) of all tissue sections was performed in 27 minutes using the HYPERION II ILIM microscope and is a fast method for pre-screening tissue to determine key ROIs for further measurement. The instrument is based on quantum cascade laser (QCL) technology and a focal plane array (FPA). This combination enables the simultaneous acquisition of 250 x 250 MIR fingerprint spectra (950 to 1800 cm^{-1}, 8 cm^{-1} spectral resolution) at a pixel resolution of 5 µm. The ILIM data was analyzed by unsupervised segmentation using k-means clustering. Different tissue areas with similar IR molecular fingerprints (e.g., lipids, metabolites, proteins, glycans) were clustered together and given a specific pixel color.

Discussion

Visualizing tissue morphology is critical to understand occurring cellular processes and to explore disease-based research courses of action. Transplant rejection, thrombotic microangiopathy, and vasculitis involve damage to kidney microstructures, in particular the glomeruli, which affects kidney function. Here, in carcinoma samples, the spatial location of proteins helps to understand margins, infiltration, and key tumor markers.

- CCRCC carcinoma showed carcinoma, tumor border, and collagen differentiation.
- Figure 3 showed unique protein expression that corresponds to distinct tissue morphologies. Fluorescence images and MALDI HiPLEX-IHC images of the same protein marker correlated to each other with good visualization of histological features.
- Single antibody staining methods that are routinely used in clinical situations, including DAB and AP red.
  - Analysis of consecutive slides showed good visual overlap of vimentin, specific here to the glomeruli. Some distortion could be visualized using both techniques in the TR tissue. Additionally, some tubules appear to be affected.
  - CD45RO staining shows in a convincing manner that leukocyte 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 alpha marker showed staining of specific tubuli, which were near areas of inflammation, which requires further investigation.

Reference

[1] Yagnik *et al., J. Am. Soc. Mass Spectrom.* **2021**, *32*, 4.

COI Disclosure

C.H. and K.S. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study.

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.

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