Multimodal investigation of kidney disease states

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Introduction

The MALDI HiPLEX-IHC antibody-based workflow in combination with spatial multiomics data, as obtained on our high-resolution timsTOF fleX platform, has opened new and exciting possibilities to answer disease-related spatial biology questions. The possibility to integrate and align MALDI Imaging data with fluorescence-based images, based on dual-labelled antibodies, enables the alignment of individual cells within a tissue. As a further source of information, the LUMOS II ILIM provides infrared laser imaging information and can stratify cancer and inflammatory areas at high spatial resolution within minutes. Here, a comparative spatial biology analysis of healthy and diseased kidney will be demonstrated using traditional staining, immuno-fluorescence, MALDI Imaging and IR Imaging. The characterization of cellular mechanisms behind solid organ transplant rejection will help to understanding the localization of immune response mechanisms within the transplanted tissue.

Methods

FFPE human kidney samples (provided courtesy of the Hannover Medical School) are explained in detail in Figure 1. Additionally, the following multimodal experiments were performed:

- **Infrared Laser Imaging** (ILIM) was performed on a Bruker LUMOS II ILIM for the IR fingerprint region (1800 to 950 cm^{-1}) at 4.2 µm pixel resolution and analyzed with unsupervised segmentation. The instrument is streamlined for high-throughput and ease-of-use with the fastest chemical imaging at 67 mm^2 per minute based on quantum cascade laser (QCL) technology and a focal plane array (FPA).
- **MALDI HiPLEX-IHC** workflow is described in Figure1. Briefly a standard Immunohistochemistry workflow is performed with the addition of photocleavable antibodies [1]. The cleaved Tag is measured in the timsTOF fleX mass spectrometer and over 100 antibodies can be measured at the same time.
- **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 with the fluorescence scanner (Zeiss).
- **Histochemical staining**, using standard protocols, included DAB (3,3'-diaminobenzidine) or AP red staining for single antibodies known as standard markers in the pathology (Vimentin, CD45RO) on adjacent tissue sections, and H&E on the same section as MALDI Imaging.

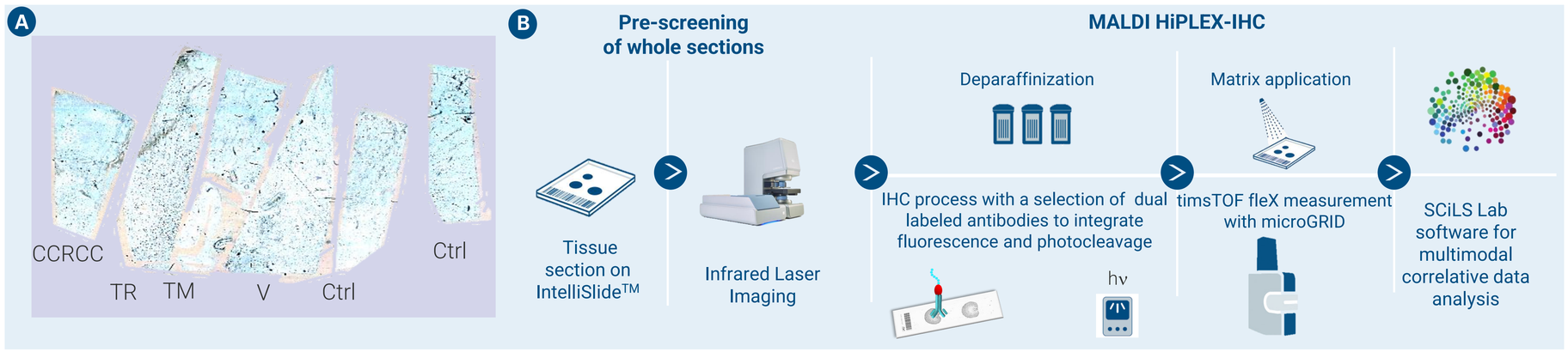


Figure 1. A) FFPE human kidney tissue with indicated pathologies of clear cell renal cell carcinoma (CCRCC), transplant rejection (TR), thrombotic microangiopathy (TM), vasculitis (V) and control. B) Workflow for tissue prescreening using ILIM. Afterwards MALDI HiPLEX-IHC Imaging, using technology from AmberGen Inc. For MALDI Imaging, regions were chosen for high spatial resolution analysis(5 µm) that were discovered as interesting in the previous IR measurement. The antibodies for MALDI HiPLEX- IHC were: CA9, CD68, Na/K ATPase alpha, Histone H2A.X, VIM, Col-1A1, Actin-aSM, and CD45RO.

Results

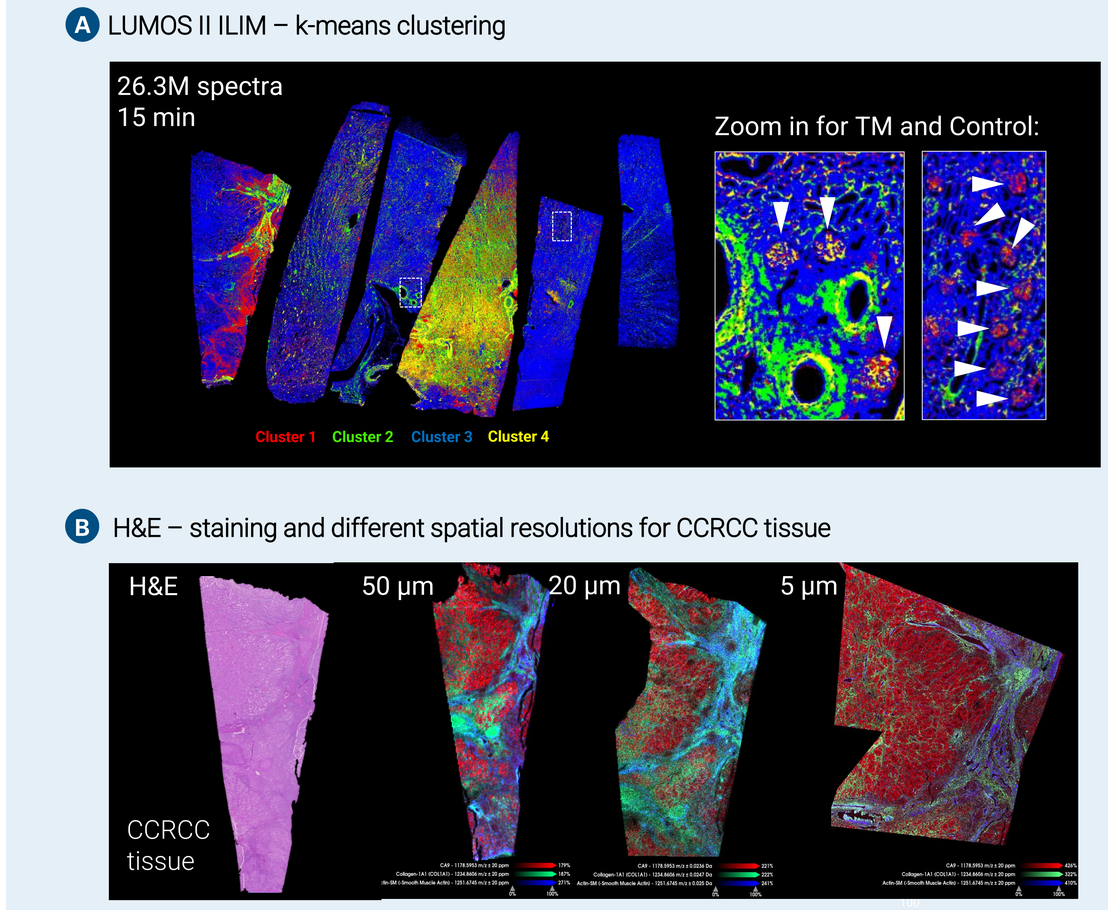
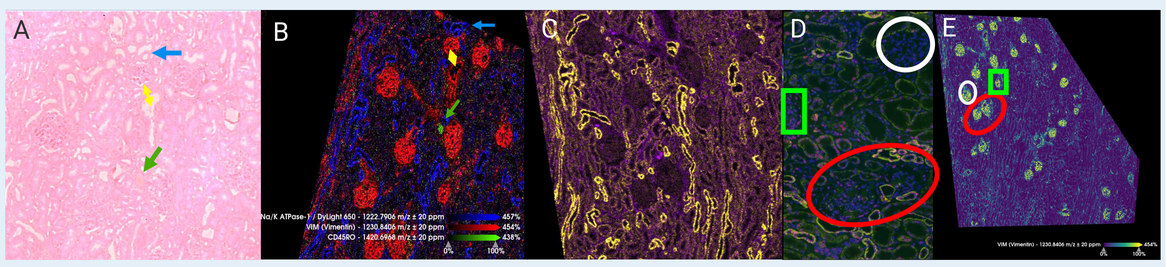


Figure. 2 A) Infrared Laser Imaging (ILIM) of all tissue sections was performed in 15 minutes using the new LUMOS II ILIM microscope and allows to derive molecular and morphological contrast intrinsically from the samples without sample preparation, such as staining or labelling. Different tissue areas with similar IR molecular fingerprints (e.g., lipids, metabolites, proteins, glycans) were clustered together and given a specific pixel color. The obtained segmentation map guided downstream MALDI Imaging to key regions of interest (ROIs), such as glomeruli (as highlighted in the zoom in). B) Cancer tissue as example for different spatial resolution in MALDI Imaging.



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

(B) Corresponding arrows from (A) to show 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 3. See details below the images. All images where from the TM pathology tissue.

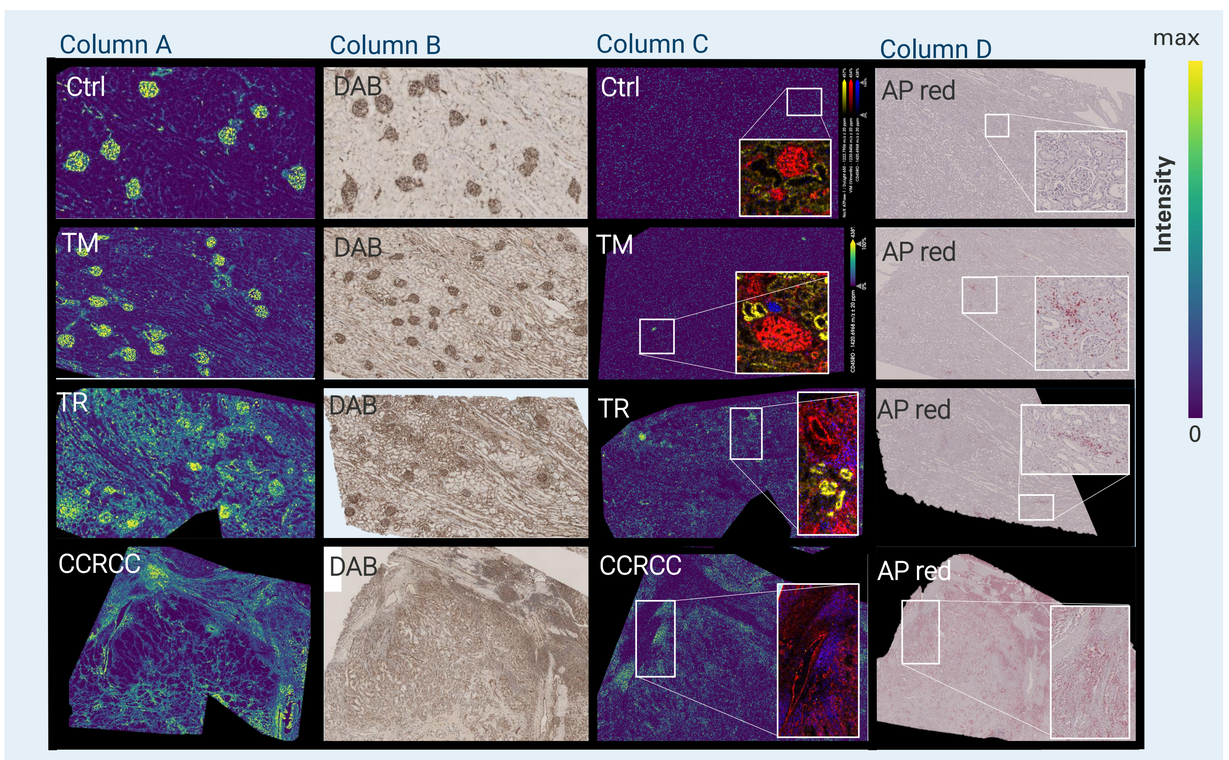


Figure 4. HiPLEX images for tissue from Figure 1, at 5 µm spatial resolution for comparison to single antibody DAB/AP red on consecutive slides. **(Column A)** HiPLEX images of VIM. **(Column B)** DAB stain (VIM) comparison to visualize glomeruli. **(Column C)** HiPLEX images of CD45RO to visualize leukocytes. 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.

Discussion

Complementary multimodal information from single tissue sections allowed to visualize tissue morphology and to understand and explore occurring cellular disease-based processes as shown in our examples:

- 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.

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.

Multimodal MALDI Imaging

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