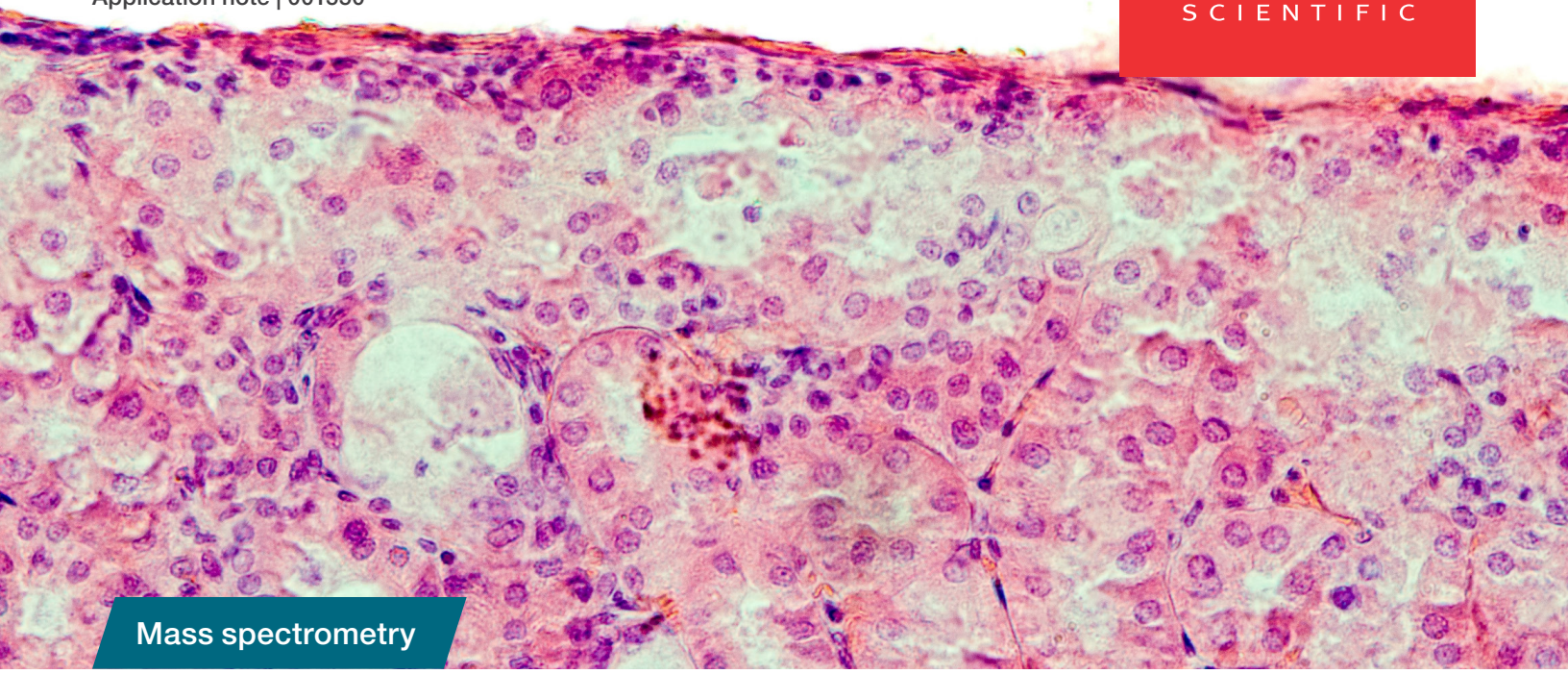
Application note | 001550



Mass spectrometry

Spatial multi-omics at the cellular level by AP-SMALDI MS imaging

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Keywords

Orbitrap technology, high resolution and accurate mass (HRAM), AP-SMALDI technique, high spatial resolution, metabolites, lipids, tryptic peptides, spatial multi-omics, metabolomics, lipidomics, proteomics

Goal

The goal of the experiments described is to characterize small metabolites, lipids and tryptic peptides in complex tissue architectures at the low-micrometer scale by means of spatial resolution when applying AP-SMALDI[5] AF technique coupled to Thermo Scientific™ Orbitrap Exploris™ mass spectrometry.

The results represent a comprehensive spatial multi-omics study—metabolomics, lipidomics, and proteomics—at the cellular level of mouse kidney by means of mass spectrometry imaging.

Introduction

Metabolites are produced and consumed by an extensive network of metabolic pathways involving hundreds of biochemical reactions and corresponding enzymes, which are regulated by various cell- and tissue-dependent factors.[1–3] Organs are composed of tissue architectures that exhibit diverse metabolic functions, chemical uptake and transport processes, thus, resulting in region-specific and heterogenous metabolic activities.[4] Therefore, an analytical platform that provides for comprehensive and spatially-resolved analysis at low-micrometer resolution is essential to investigate functional heterogeneities at the molecular level in tissues and organs. Liquid-chromatography-based (LC)-MS methods provide high specificity and broad molecular coverage but fail to preserve the spatial information about the molecular species of interest. As an alternative,

microscopy-based imaging methods, such as fluorescent sensors, are limited in visualizing various molecular species simultaneously.[5] In contrast, matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI), introduced in 1994, enables nontargeted, label-free chemical imaging and represents the predominant MSI method in bioanalytical research.[6,7] In particular, atmospheric-pressure scanning microprobe matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-SMALDI MSI) combined with Thermo Scientific™ Orbitrap™ technology emerged as the high end in MALDI MSI instrumentation due to its unmatched performance regarding mass resolution, spatial resolution, sensitivity and experimental flexibility.[8–11] Recent technical developments enable researchers to routinely perform imaging experiments with a spatial resolution of 5 µm, high-speed measurements with up to 18 pixels per second and the possibility for 3D-surface analysis of irregular surfaces and native samples.[12] For instance, metabolite, lipid and drug distributions were visualized with 5 to 10 µm step size for various biological sample systems and native organisms, thereby linking molecular structures to biological functions and origins.[13–17]

Here, applied to mouse kidney as a model system for a complex organ with high metabolic activity, we performed an MSI-based multi-omics approach for the comprehensive and spatially resolved analysis of lipids, metabolites and tryptic peptides in an untargeted fashion with 5 and 10 µm step size. First, we utilized lipid distributions to reveal the complex tissue architecture of mouse kidney. For the subsequent spatial metabolomics experiment, we applied this knowledge to successfully interpret region-specific metabolic activities for over 300 different metabolites, thus allowing analysis of various major metabolic pathways. To demonstrate the potential for identifying and visualizing corresponding enzymes and carrier proteins, we optimized and performed on-tissue tryptic digestion for the unbiased spatial proteomic characterization of over 400 different tryptic peptides.

Experimental

The complete overview of the experimental design is depicted in Figure 1. Frozen mouse kidney was stored intact at –80°C until cryo-sectioning. Thin tissue sections (20 µm) were obtained at –20°C using a cryotome (HM525, Thermo Fisher Scientific, Dreieich, Germany), thaw-mounted on regular glass slides and stored at –80°C until MSI analysis. Before matrix application, tissue sections were brought to room temperature in a desiccator for 15 minutes to avoid condensation of water at the sample surface. Optical images of tissue sections were obtained using a Keyence VHX-5000 digital microscope (Keyence Deutschland GmbH, Neu-Isenburg, Germany). As MALDI matrices, 2,5-dihydroxy-benzoic acid (DHB) was used for lipidomic analysis in positive-ion mode, 9-aminoacridine (9-AA) was used for metabolomic analysis in negative-ion mode and α-cyano-hydroxycinnamic acid (CHCA) was used for proteomic analysis in positive-ion mode. Matrix application was optimized and performed using the ultra-fine pneumatic sprayer system SMALDIPrep (TransMIT GmbH, Giessen, Germany). All measurements were performed using an autofocusing AP-SMALDI[5] AF ion source (TransMIT GmbH, Giessen, Germany) coupled to an Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer (Thermo Fisher Scientific (Bremen) GmbH, Germany). The step size was set to 5 or 10 µm (see Figure captions of Figures 2–6 for details) using the 2D pixel mode. The mass resolution (Full Width at Half Maximum) was set to 240,000 at *m/z* 200 and internal lock mass calibration by EASY-IC (scan-to-scan mode) enabled mass accuracies below 1 ppm for all MSI experiments. Ion images of selected *m/z* values were generated using MIRION (TransMIT GmbH, Giessen, Germany) and no data manipulation steps, such as smoothing, interpolation or cut-off functions, were used. Using RAW2IMZML (TransMIT GmbH, Giessen, Germany), .raw-files were converted to the universal MSI data format .imzML and subsequently uploaded to the METASPACE platform for lipid (database: SwissLipids) and metabolite (database: CoreMetabolome) annotations.

Orbitrap Exploris 480 mass spectrometer

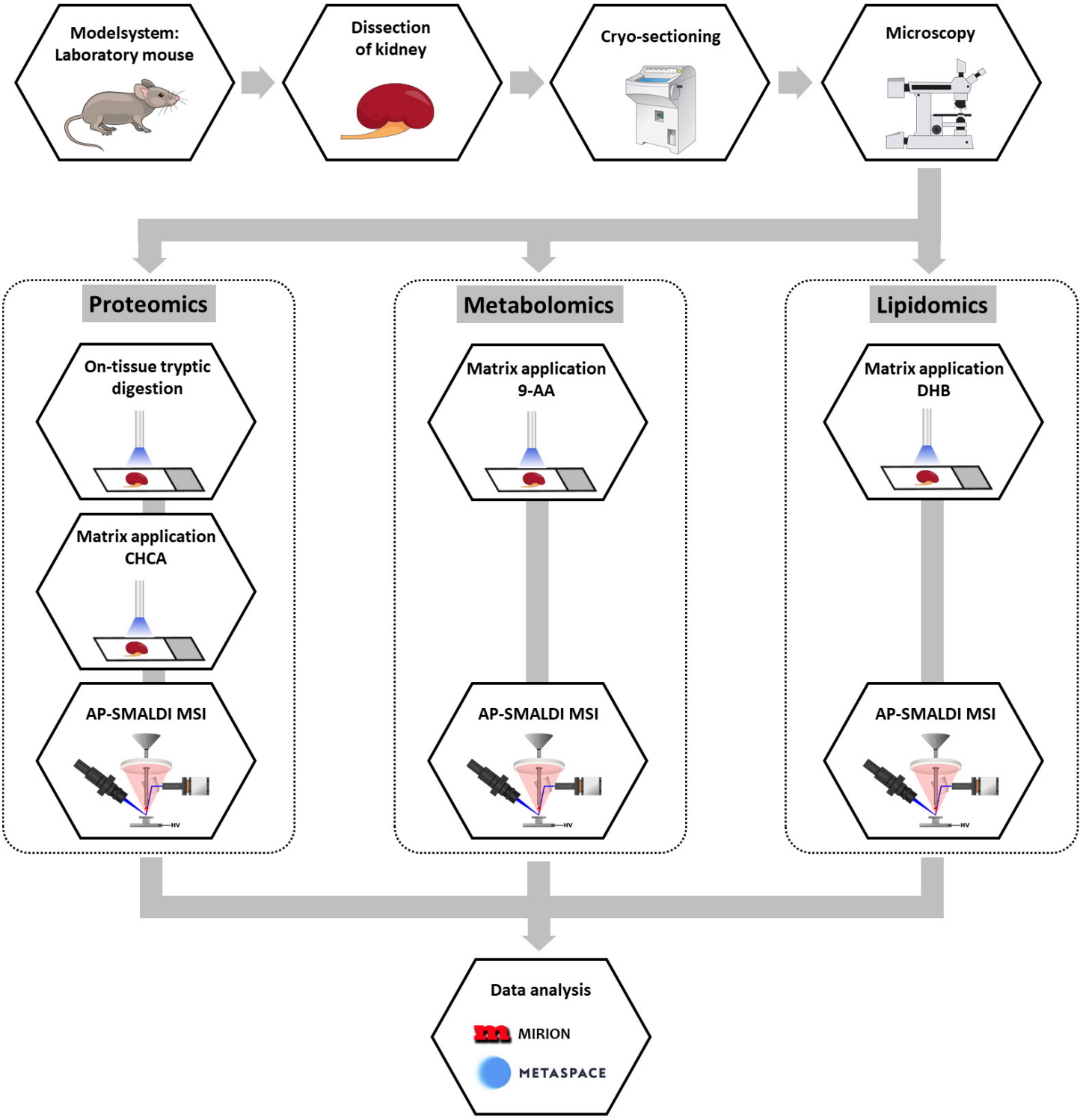

Figure 1. Overview of the experimental design for spatial multi-omics of mouse kidney. Three adjacent mouse kidney tissue sections (thickness: 20 µm) were analyzed with three different sample preparation protocols according to the analytes of interest (i.e., lipids, metabolites and tryptic peptides).

Results

Among organs of vertebrates, the kidney has one of the highest metabolic activities including continuous nutrient uptake, removing waste products and balancing body fluids. In general, filtration and nutrient reabsorption primarily occurs in the outer region (cortex), and the resulting urine is concentrated in the inner region (medulla) of the kidney. The exterior part of the medulla can be divided into the inner and outer stripe (Figure 2a). First, we performed high-resolution AP-SMALDI MSI (10 µm step size) in positive-ion mode using DHB as the MALDI matrix to reveal the complex tissue architectures via various lipid distributions. Figure 2a shows the optical image of the kidney tissue section including the highlighted region of interest. The corresponding red-green-blue (RGB) overlay image shows the spatial distribution for three selected lipid species (Figure 2b). The phosphatidylcholine PC(34:1) at *m/z* 782.5670 ($[M+Na]^+$) is shown in red and was primarily located in the inner- and outer medulla. Depicted in green, the sphingomyelin SM(d34:1) at *m/z* 741.5307 ($[M+K]^+$) was predominantly abundant in the outer medulla (inner and outer stripe). The phosphatidylethanolamine PE(38:0) at *m/z* 814.5721 ($[M+K]^+$) is shown in blue and was found to be distributed in the cortex region of the kidney. Our MSI data demonstrate that by overlaying these spatial distributions we were able to differentiate between the specific regions and highlight fine tissue structures, in particular for the outer medulla (inner and outer stripe) (Figure 2c). In addition, we were also able to visualize the accumulation of sphingomyelin species (e.g., SM(d34:1) in glomeruli, which are the main filtering units of the kidney (Figure 2b and Figure 2c). These networks of small blood vessels filter plasma to produce glomerular filtrate which is subsequently formed to urine.

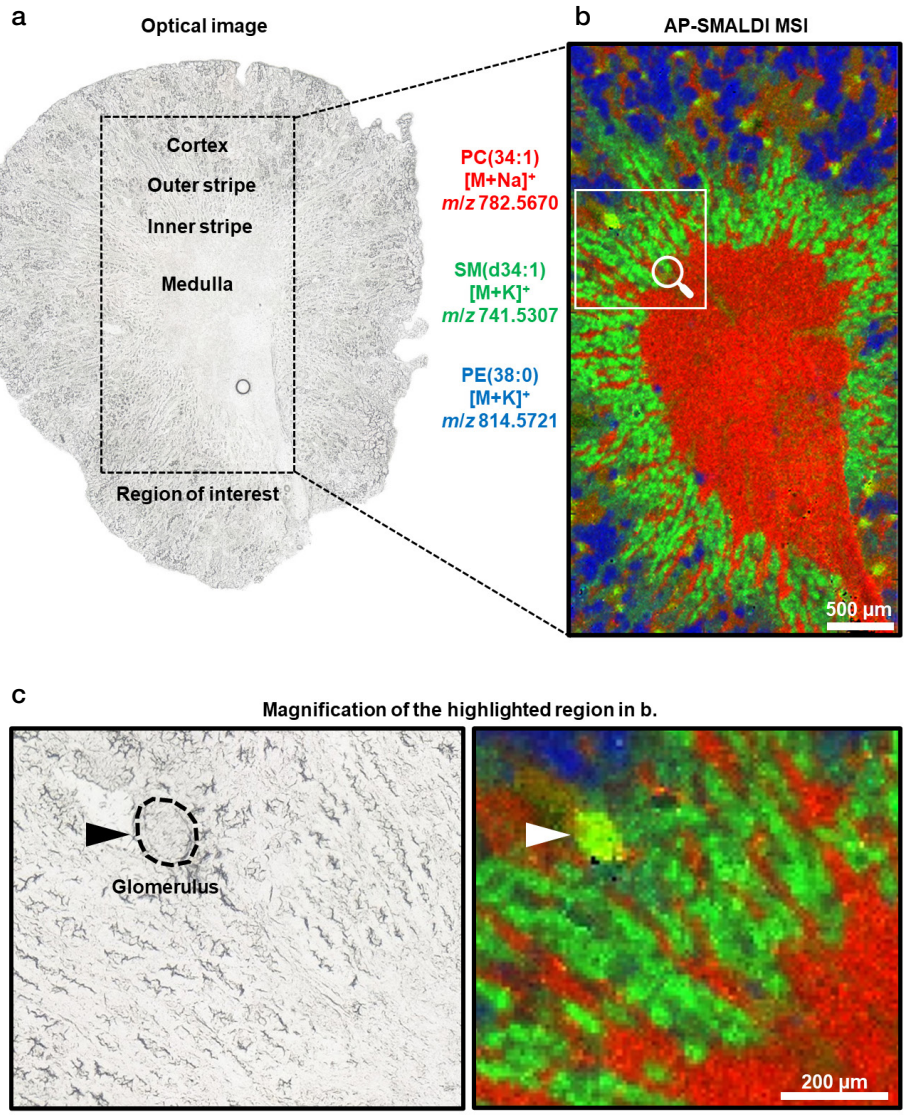

Figure 2. Spatial lipidomics of mouse kidney. (a) Optical image of mouse kidney tissue section, highlighting the analyzed region of interest. (b) AP-SMALDI MSI results (obtained with 10 µm step size) showing the corresponding red-green-blue (RGB) overlay image of PC(34:1) in red, SM(d34:1) in green and PE(38:0) in blue. (c) Magnification of the highlighted region in b, showing fine tissue structures for the inner-/outer stripe region and the accumulation of SM(d34:1) in the glomerulus.

Spatial resolution is the most important factor for discriminating specific compound distributions in biological tissues. Therefore, we analyzed the cortex region using 5 µm step size to reveal previously inaccessible molecular distributions at the cellular level. Figure 3a highlights the analyzed region of interest, and the corresponding RGB overlay (Figure 3b) shows the spatial distribution of three selected phospholipids. The green color channel depicts PC(34:1) at *m/z* 782.5670 ($[M+Na]^+$), which is located in the outer stripe of the medulla. In contrast, the phospholipids PC(P-36:2) at *m/z* 792.5901 ($[M+Na]^+$) in red and PE(P-40:2) at *m/z* 806.6058 in blue show tissue-dependent patterns in the cortex area. Within these characteristic ensembles of similar cells, we were able to reveal that PC(P-36:2) was exclusively abundant at the tissue border (Figure 3c) and PE(P-40:2) in the inner parts of the tissue. Therefore, our spatial lipidomics approach demonstrates that high-resolution AP-SMALDI MSI is a powerful method to spatially resolve the biological organization between different cell-types and their extracellular matrix within tissues.

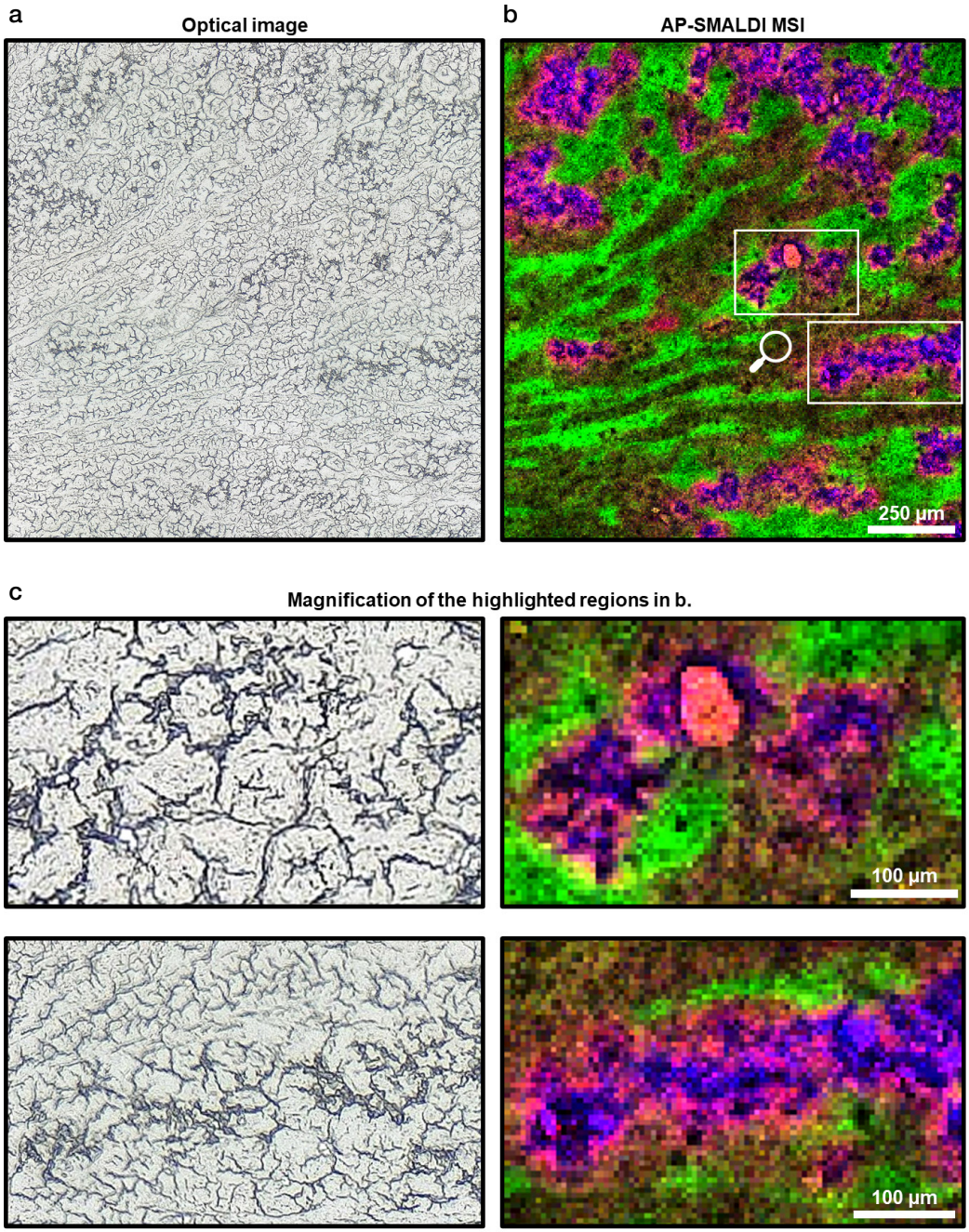

Figure 3. Spatial lipidomics of mouse kidney. (a) Optical image of the analyzed region of mouse kidney tissue section, highlighting the analyzed region of interest. (b) AP-SMALDI MSI results (obtained with 5 µm step size) showing the corresponding red-green-blue (RGB) overlay image of PC(O-38:6) in red, PC(34:1) in green and PE(P-40:2) in blue. (c) Magnification of the highlighted regions in b, showing tissue structures at the cellular level for the cortex area of mouse kidney.

Next, we utilized this knowledge to guide the spatially resolved analysis of small metabolites in mouse kidney. We performed high-resolution AP-SMALDI MSI (10 µm step size) of the adjacent tissue section in negative-ion mode using 9-AA as the MALDI matrix to facilitate the ionization of small molecular species. Figure 4a shows the optical image of the sample surface including the highlighted region of interest. We selected three analytes of interest to demonstrate the spatial organization of metabolic activities in mouse kidney. The red color channel depicts taurine (*m/z* 124.0074, $[M\text{-}H]^-$) and highlights the function of an osmolyte to facilitate urinary excretion in the medulla and to serve as a regulator for the blood flow in the cortex (Figure 4b, c). The green color channel shows the spatial distribution of glucose-phosphate (*m/z* 259.0218, $[M\text{-}H]^-$), which represents a precursor in glycolysis, one of the major metabolic pathways in mammalians. Utilizing the high resolution provided by our instrumental setup, we were able to spatially resolve fine distributions in the medulla including inner and outer stripe, thus highlighting the propensity for glycolysis in these specific tissue areas. We also revealed the accumulation of glucose-phosphate in glomeruli in the cortex region (Figure 4c, d).

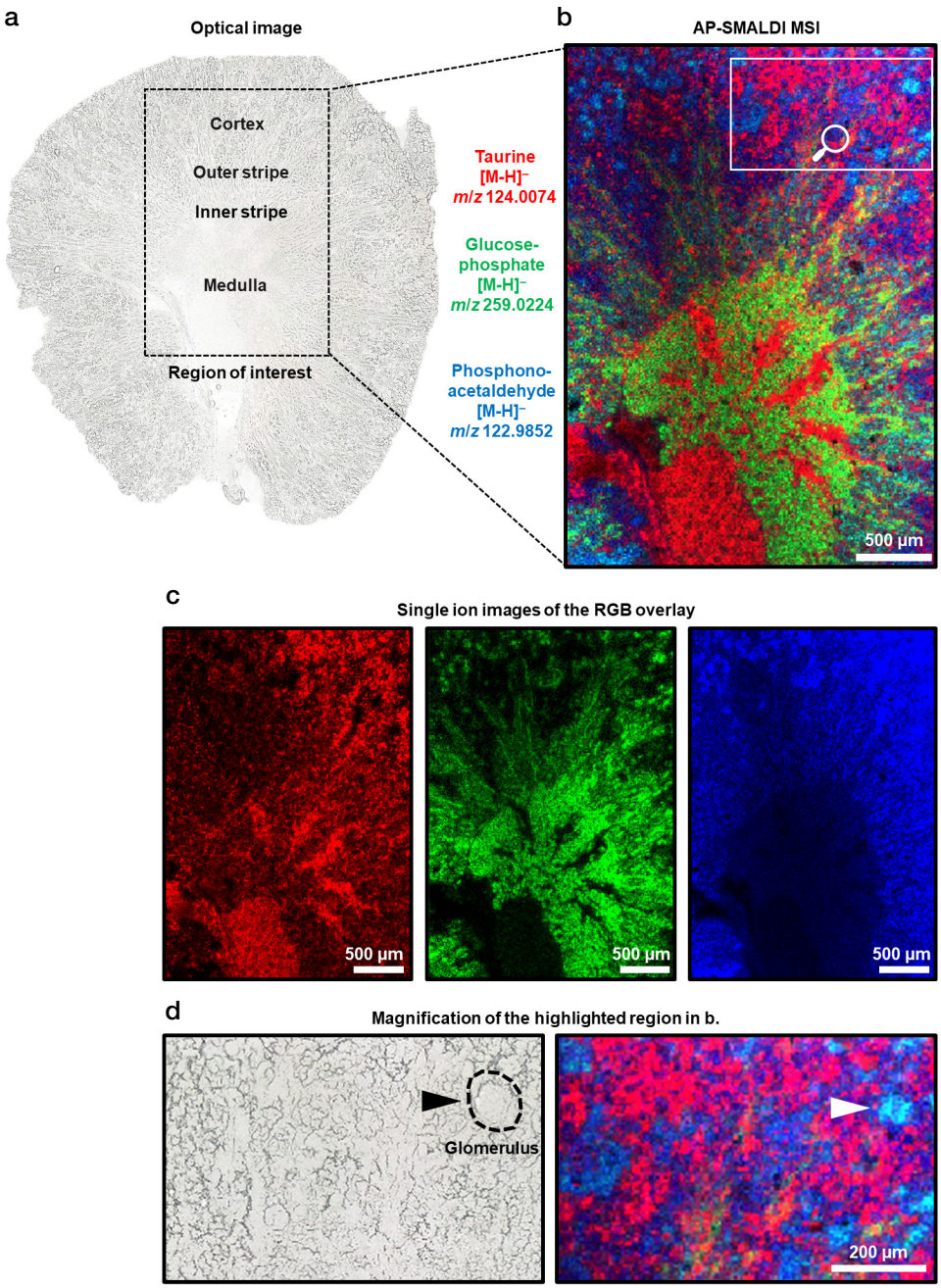

Figure 4. Spatial metabolomics of mouse kidney. (a) Optical image of mouse kidney tissue section, highlighting the analyzed region of interest. (b) AP-SMALDI MSI results (obtained with 10 µm step size) showing the corresponding red-green-blue (RGB) overlay image of taurine in red, glucose-phosphate in green and phosphonoacetaldehyde in blue. (c) Single ion images for the three selected metabolites shown in the RGB overlay. (d) Magnification of the highlighted region in b, showing the spatial distribution of taurine in the cortex and the accumulation of glucose-phosphate in the glomerulus, demonstrated by cyan-colored pixels (overlay of glucose-phosphate and phosphonoacetaldehyde).

The high mass resolution and high mass accuracy of the Orbitrap mass analyzer allowed to annotate metabolites based on accurate mass measurements. For instance, the root-mean-square error for taurine at *m/z* 124.0074 calculated from 92136 spectra (pixels) was 0.8 ppm. In total, we were able to annotate 323 metabolites via METASPACE (database: CoreMetabolome), thus, covering several major metabolic pathways (Figure 5). These metabolic pathways include hundreds of enzymes and carrier proteins, which are spatially segregated within the kidney. However, proteins are generally too large to be detected via MALDI MSI. Therefore, we performed on-tissue tryptic digestion (OTPD) for the spatially-resolved analysis of tryptic peptides, which can be subsequently correlated to the original protein. Due to additional sample preparation steps, such as trypsin application and incubation with increased humidity, OTPD methods can be limited in quality due to analyte diffusion and are typically performed at 30 to 100 µm spatial resolution. Therefore, we optimized the sample preparation protocol and utilized the ultrafine pneumatic sprayer system SMALDIPrep device (TransMIT GmbH, Giessen, Germany) to enable spatial proteomic characterization with 10 µm step size. Figure 6a shows the optical image of the sample surface including the highlighted region of interest. The peptide Ala-Cys-Cys-Thr at *m/z* 397.1210 ($[M+H]^+$) is shown in green and correlates to a protein, which is predominantly abundant in glomeruli and blood vessels in the cortex region of the kidney (Figure 6b, c). In contrast, the blue color channel shows the spatial distribution of Lys-Lys at *m/z* 275.2078 ($[M+H]^+$), which is distributed homogenously in the cortex and outer medulla. In total, we were able to detect and visualize the spatial distribution of over 400 tryptic peptides, thereby demonstrating the potential of integrating MSI-based spatial proteomic information for the unbiased identification and visualization of enzymes and carrier proteins of specific metabolic pathways.

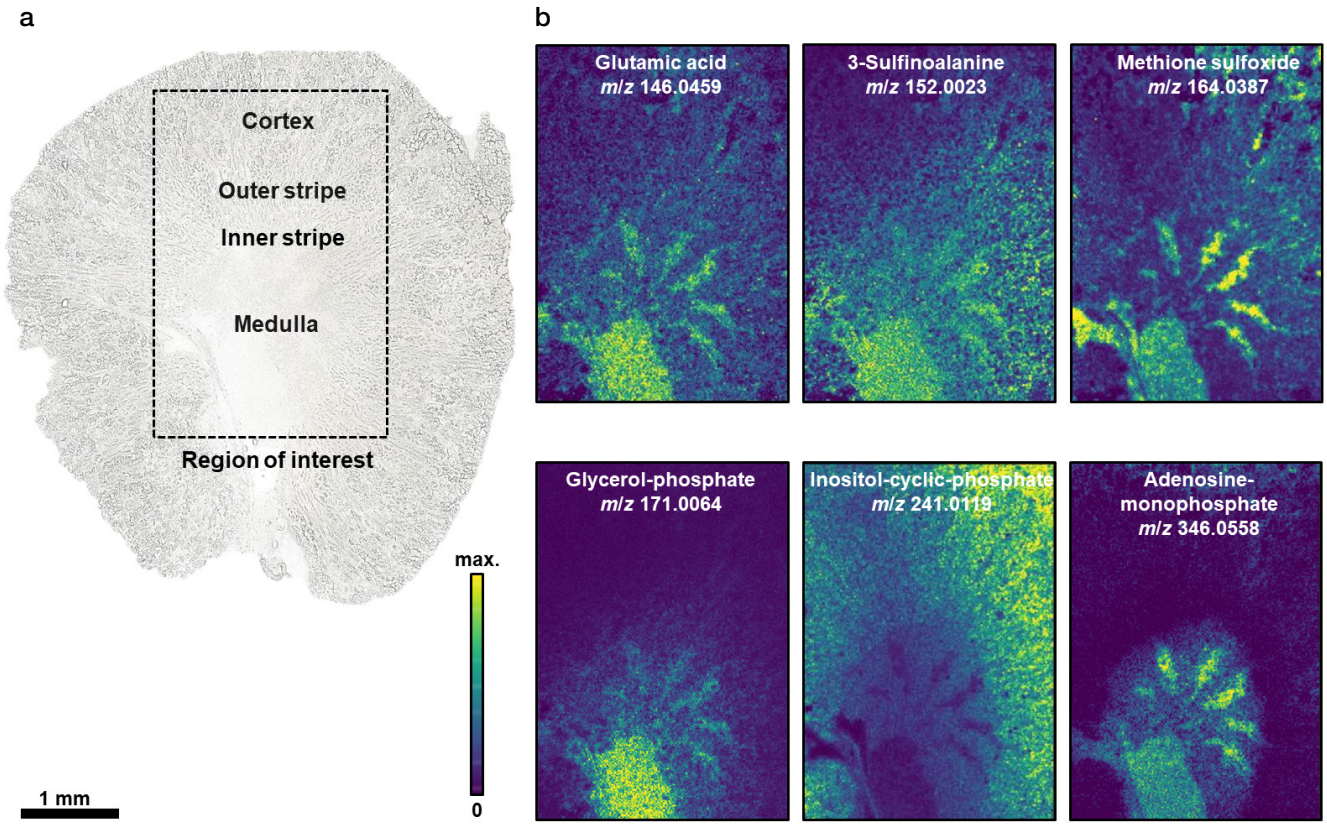

Figure 5. Spatial metabolomics of mouse kidney. (a) Optical image of mouse kidney tissue section, highlighting the analyzed region of interest. (b) AP-SMALDI MSI results (obtained with 10 µm step size) showing single ion images for six additional metabolites. In total, 323 metabolites were annotated via METASPACE (CoreMetabolome database).

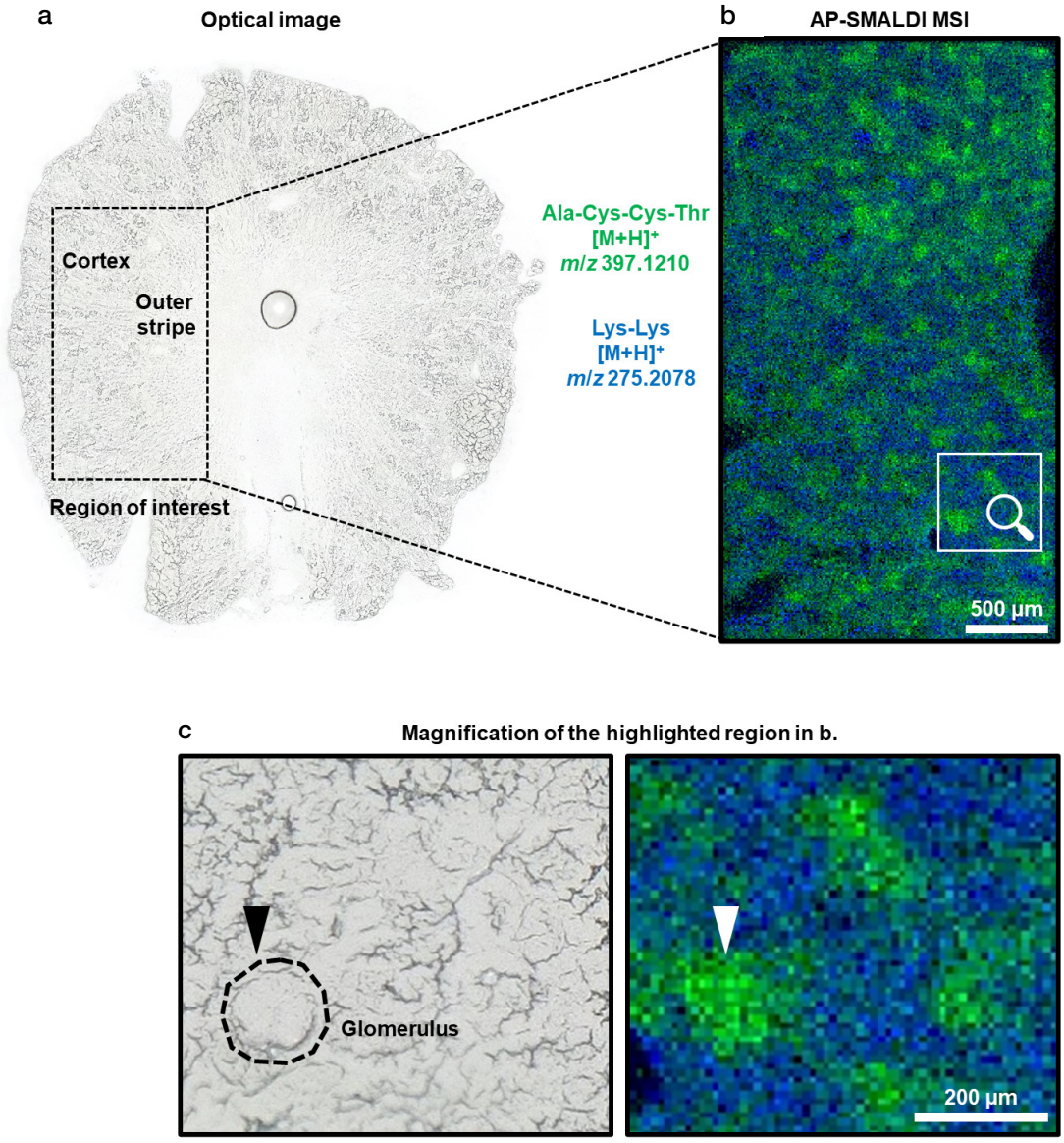

Figure 6. Spatial proteomics of mouse kidney. (a) Optical image of mouse kidney tissue section, highlighting the analyzed region of interest. (b) AP-SMALDI MSI results (obtained with 10 µm step size) showing the corresponding green-blue overlay image of Ala-Cys-Cys-Thr in green and Lys-Lys in blue. (c) Magnification of the highlighted region in b, showing the accumulation of Ala-Cys-Cys-Thr in the glomerulus.

Conclusions

- A spatial multi-omics workflow was developed by taking advantage of the capabilities of the AP-SMALDI-Orbitrap platform, including unmatched resolution in mass and space, high mass accuracy and excellent sensitivity.
- AP-SMALDI[5] AF ion source coupled to Orbitrap Exploris technology enables the collective spatial characterization of bioactive molecules for metabolomics, lipidomics and proteomics in architecturally challenging sample systems, such as kidney tissue.
- Region-specific metabolism including potential enzymes were successfully revealed for complex tissue architectures and cellular features with unprecedented detail.

Why choose AP-SMALDI-Orbitrap MS?

- AP-SMALDI[5] AF technique provides highest spatial resolution (5 µm) among the commercially available MALDI MSI platforms.
- High resolution accurate mass (HRAM) is provided by the Orbitrap technology.
- The high number of 323 metabolite annotations (METASPACE: CoreMetabolome database; see Figures 4 and 5) demonstrates exceptional sensitivity for various compound classes including small molecule metabolites. This high sensitivity is provided by the unique co-axial source design of the AP-SMALDI[5] AF ion source.
- Unmatched experimental flexibility and technical reproducibility, from high-speed to accurate 3D-surface MS imaging analysis[12], are supported without the need for additional hardware features.

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