Bruker Daltonics

Application Note # MT-91

High Quality MALDI Imaging of Proteins and Peptides in Small Rodent Organ Tissues

New developments in MALDI instrumentation, laser technology and sample preparation techniques have turned research-style MALDI imaging of proteins and peptides into a routine technique. To evaluate the potential of MALDI imaging with regard to the resolution and information content of the images that can be obtained, biological systems with well-defined structures on both the macroscopic and microscopic level are helpful. Organs of small mammals, such as the brain, kidney, testis, pancreas and spleen of mice or rats have these desired properties and are easily accessible. For these reasons, they are preferred samples for MALDI imaging method development. In this application note we present the current state-of-the-art in MALDI imaging of rodent organs.

Introduction

Over the last few years the popularity of MALDI imaging has increased tremendously. The high potential of MALDI imaging is best recognized in cancer research, where murine organs are often used as model systems. In addition, they represent preferred samples for method development and evaluation of the technique. The main reasons for this, aside from the easy accessibility, ethical implications and sample handling (compared to human tissue), are the size and the clear macroscopic structure of most murine organs. For example, the main features of a mouse kidney are obvious, even to an untrained observer. Coronal brain sections, due to their clear left-right symmetry, are particularly well suited for direct comparison of different experimental parameters.

With the introduction of the Bruker ImagePrep™ system, sample preparations that allow spatial resolution of 50–100 µm or better can be routinely achieved for the first time. Model systems with defined anatomical features of this scale are therefore necessary to evaluate the possibilities of sample preparation. To demonstrate the current possibilities in MALDI imaging, this work shows MALDI images of several rodent organs acquired with the MALDI Molecular Imager™ system.

Experimental

Rat organs were snap frozen in liquid nitrogen and stored at -80 °C before use. Cryosections for MALDI imaging were cut on a Cryo-microtome (Leica) and transferred onto cold Indium-Tin-Oxide (ITO) coated glass slides. To thaw-mount the tissue sections, glass slides were warmed from underneath with the palm of a hand. Sections were then desiccated in vacuum for 5 min, washed twice in 70 % Ethanol and once in 100 % Ethanol for one minute each, then desiccated again. Matrix coating was applied in an

ImagePrep™ station using the pre-installed standard method for sinapinic acid. Spectra were acquired on an autoflex III™ mass spectrometer with smartbeam™ laser in linear mode (200–400 laser shots per pixel). flexImaging™ software (Bruker Daltonics) was used for the visualization and analysis of MALDI images that were acquired at image resolutions from 80-240 µm. For accurate alignment of MALDI images and histological features, H&E staining of the same kidney section was performed after MALDI analysis (post-acquisition staining).

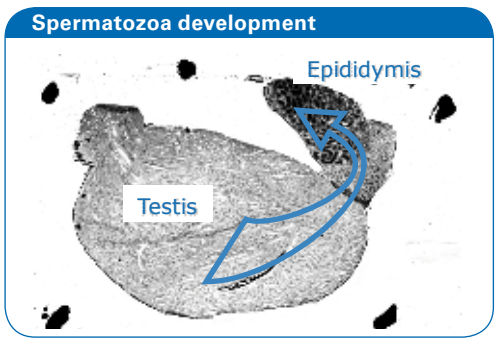

Fig. 1a:
Rat testis:
The maturation occurs during Spermatozoa migration from testis to epididymis.

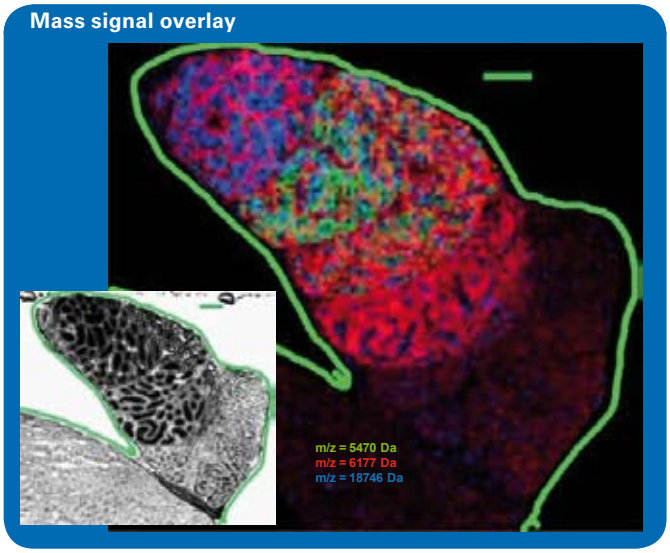

Fig. 1b: The overlay of three specific peptides in different colors shows tubule walls, spermatozoa, and different developmental stages simultaneously. 80 µm image resolution is mandatory to resolve these structures. Scalebar: 1 mm

Sample courtesy of Charles Pineau, Rennes, France

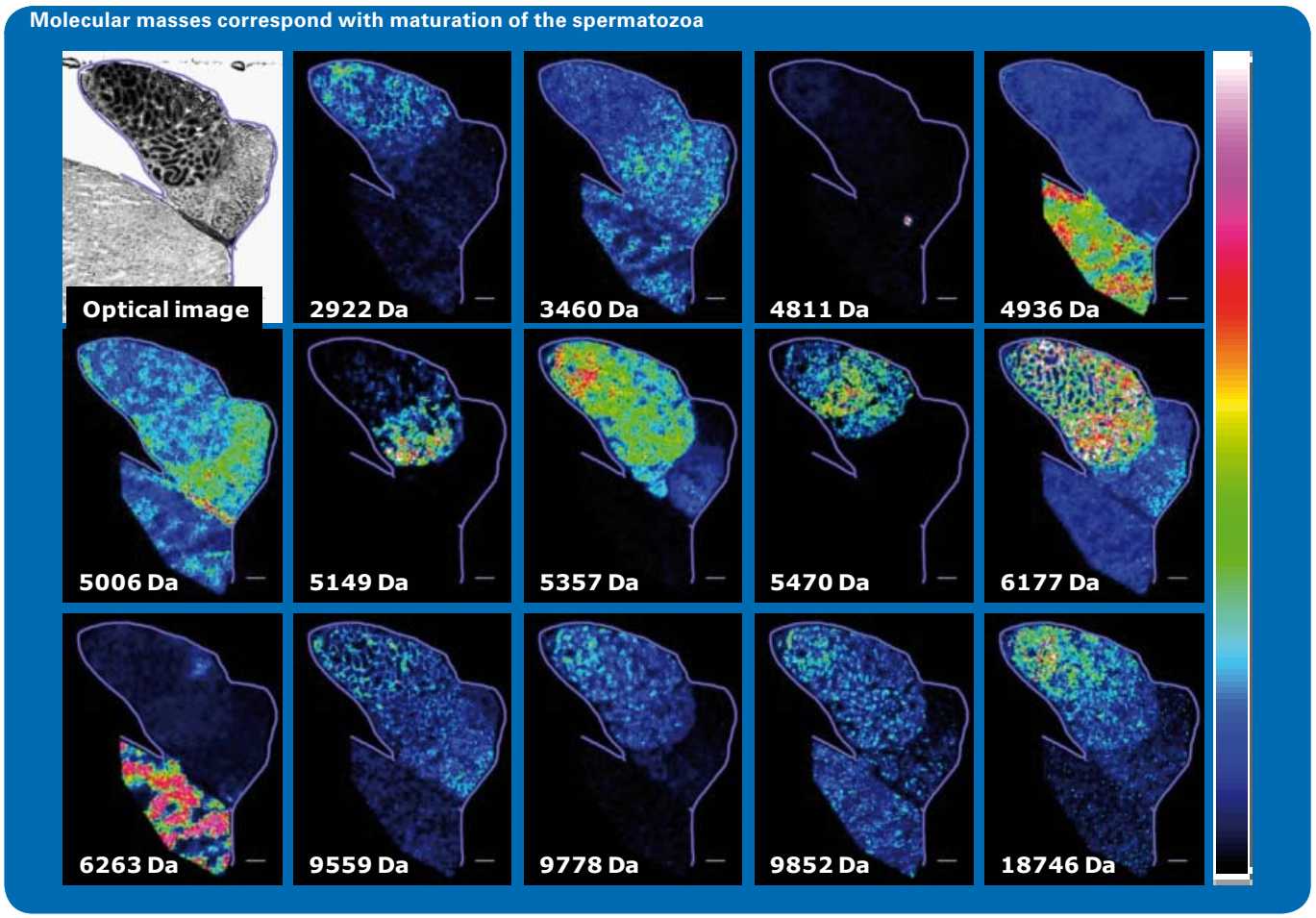

Fig. 1c: Several peptides correspond to the maturation of spermatozoa or tissue morphology. Spermatozoa in the tubuli can be clearly correlated to different peaks. Images taken at 80 µm resolution. Scalebar: 1 mm

Resolution Matters!

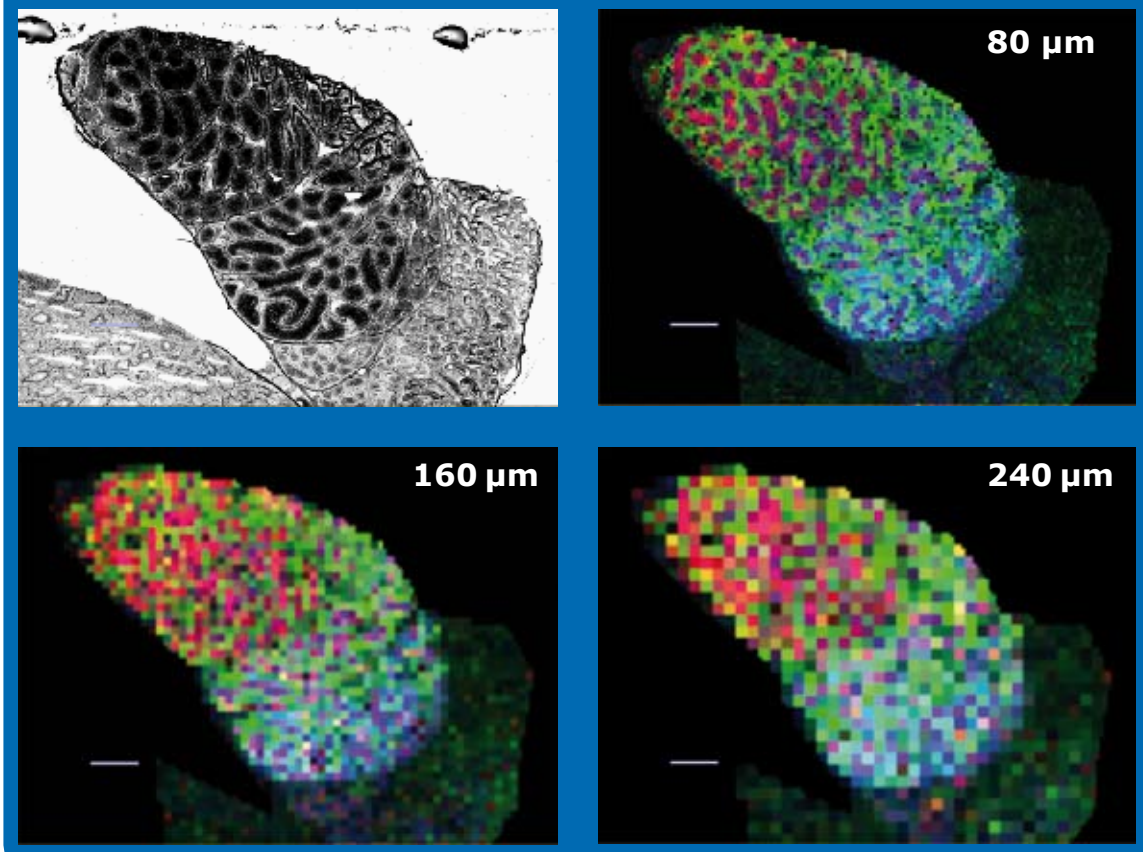

Fig. 1d: Rat epididymis image analysis at varying image resolution (80 µm, 160 µm and 240 µm pixel size). Tubuli (red) are clearly resolved at 80 µm but not at lower image resolution. Scalebar: 1 mm

Results

Rat testis / epididymis

The testis/epididymis is a good model to evaluate the correlation of MALDI images with conventional histology, since it shows very distinct anatomical features at different scales. The development of spermatozoa begins in the testis and continues along the epididymis (Fig 1a). Changes in protein expression patterns along this path coincide with the maturation of spermatozoa: Several protein signals corresponding with the maturation of the spermatozoa can be detected (Fig. 1c).

On a smaller scale, spermatozoa migrate inside tubuli, which can be seen as dark "islands" in the optical image in Fig. 1b. The good MALDI image resolution obtained using the ImagePrep (down to 25 µm, here: 80 µm pixel size) is particularly noteworthy, as it allows a clear distinction between tubule wall and lumen. In Fig. 1b an overlay of three mass signals shows tubuli walls, spermatozoa and different development stages at the same time. The tubuli of the epididymis represent a structure that can only be resolved at a high image resolution of 80 µm or better (Fig. 1d).

Rat brain

The rat brain is a widely used model for the evaluation of MALDI imaging preparations. The distinct advantage of the coronal brain section as used in this example is the clear left-right symmetry. On top of that, the brain has a wide variety of functionally and anatomically distinct regions, which can be further detailed using molecular marker based histology ("Molecular Histology").
Fig. 2a displays a schematic of major brain regions in the section shown. The different masses selected for molecular imaging in Fig. 2a outline a wide variety of functionally different brain areas. The image resolution in this experiment was 80 µm, sufficient to resolve minute structures such as the granule cell layer of the dentate gyrus (the thin band in the hippocampus visible at mass 6230 Da, arrow in Fig. 2a), which is accurately displayed in the molecular image. Fig 2a shows overlays of different molecular signals simultaneously, underlining the molecular multiplexing analysis capability of MALDI imaging.

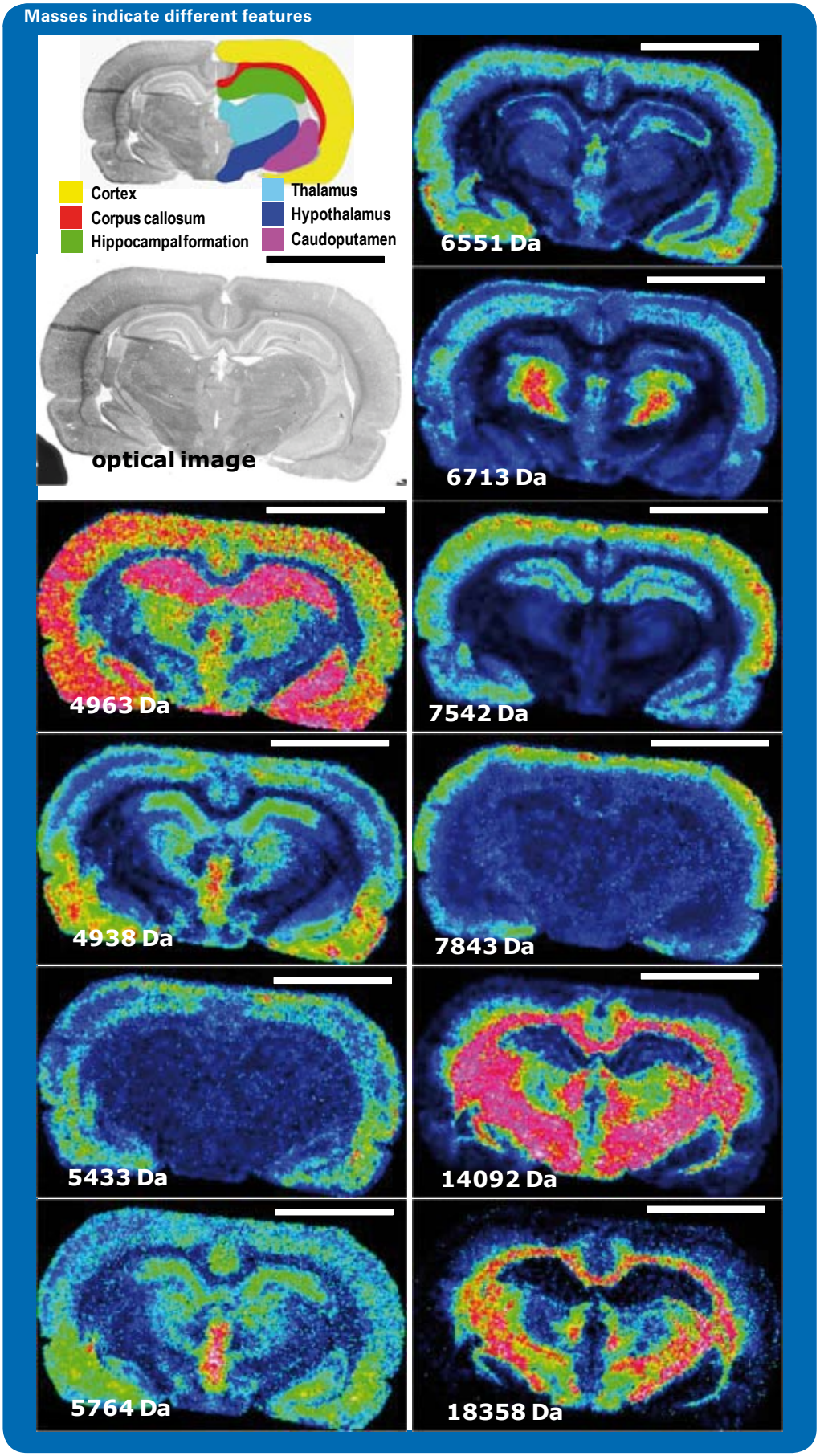

Fig. 2a: Many different brain regions can clearly be associated with specific peptide signals. The image resolution was 80 µm. Scalebar: 5 mm

Sample courtesy of Eckhard Friauf, Kaiserslautern, Germany

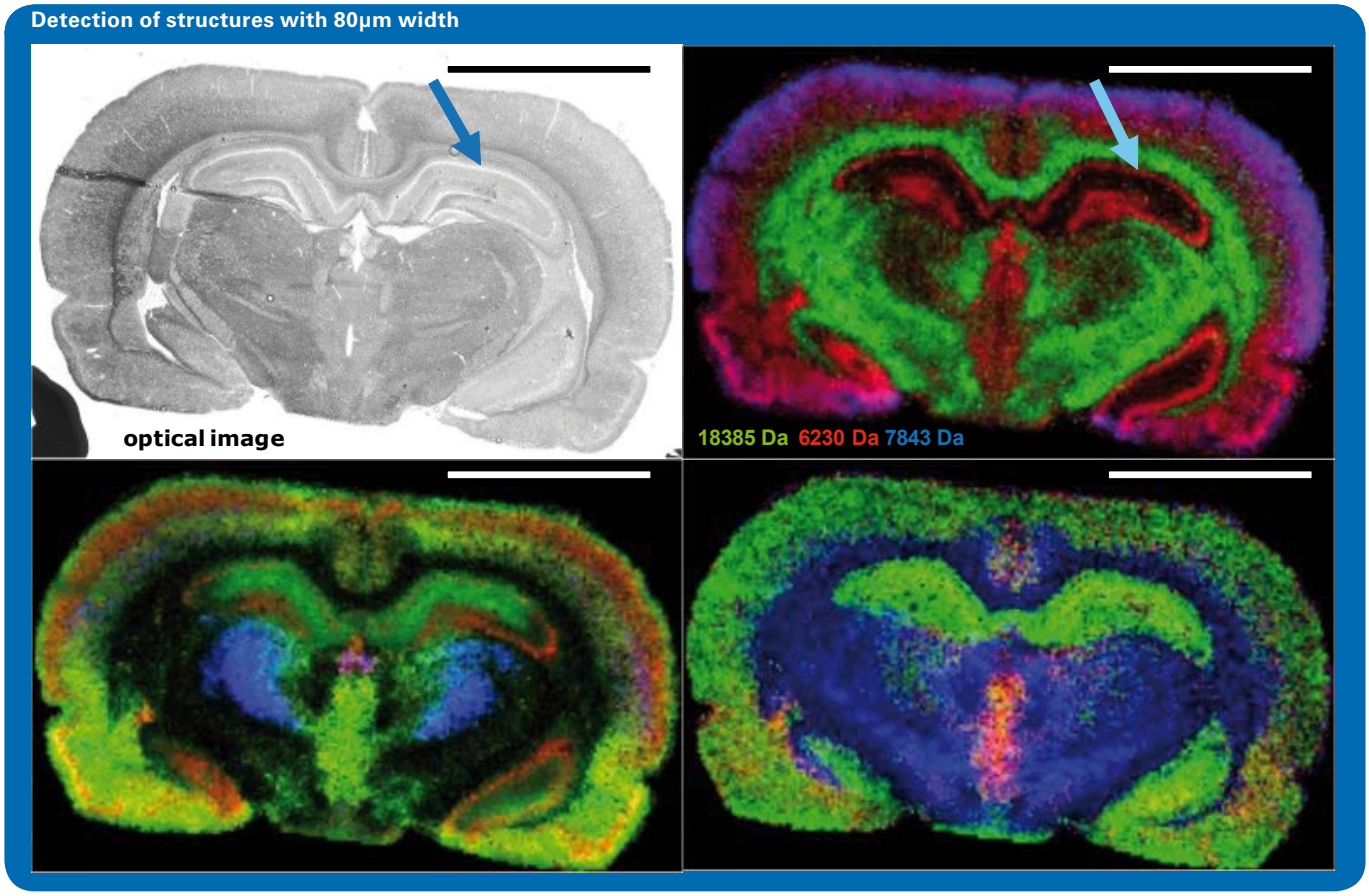

Fig. 2b: The fine structure (~80 µm thickness) in the hippocampus (arrows) is visualized by m/z 6230. This is perfectly reflected by the molecular image (also compare figure 2a). Overlays of different molecular signals are shown. Scalebar: 5 mm

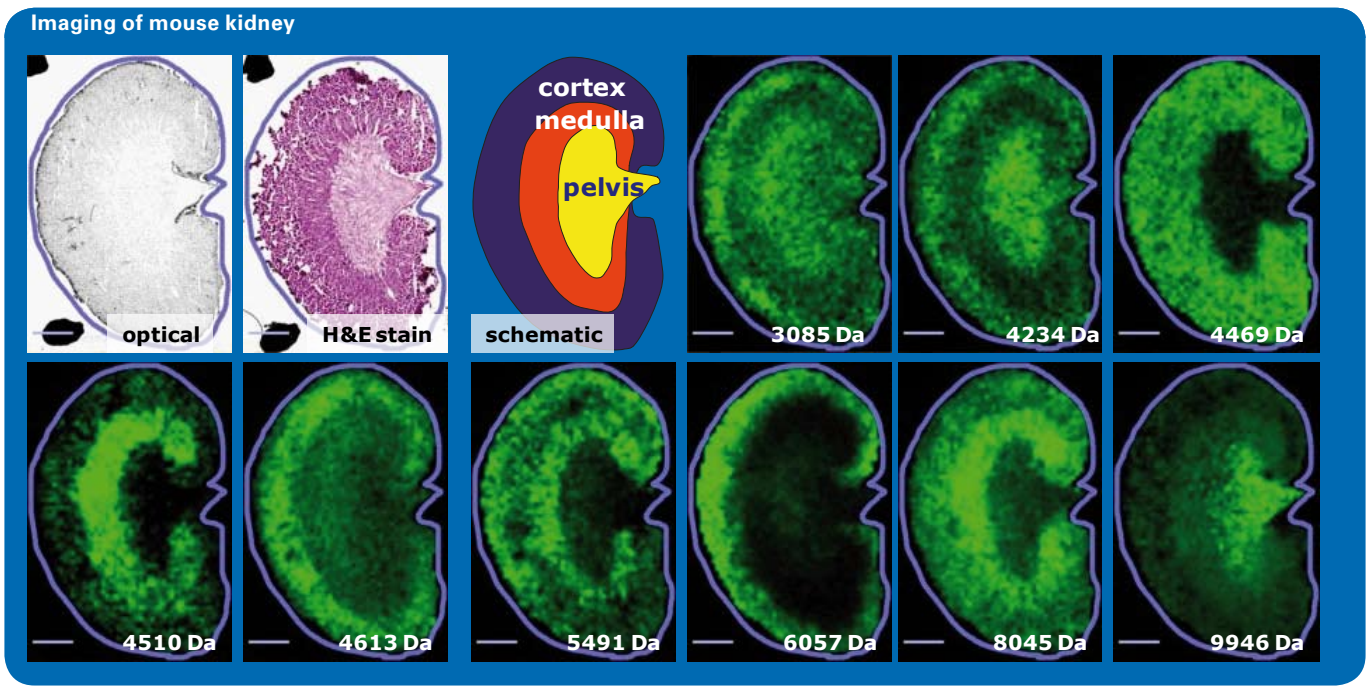

Fig. 3a: The main, functionally different regions (see schematic) are clearly separated by the molecular signals at 200 µm resolution. Scalebar: 2 mm

Mouse kidney

The kidney can be subdivided into three major, functionally different regions: The renal cortex, the renal medulla and the renal pelvis, as seen in the schematic (Fig 3a). In the example shown, a mouse kidney was analyzed with 200 µm image resolution, thus only the main features are resolved. Cortex, medulla and pelvis are clearly distinguishable by molecular markers, and a layered structure becomes apparent inside the cortex. Fig. 3b shows selected masses as multi-colored overlays.

Smaller scale features are present in the kidney, such as the glomeruli, but in the mouse these are below 30 µm in diameter and are therefore not resolved in the MALDI image shown.

Conclusion

The presented data provide insights into state-of-the-art MALDI imaging data quality. Recent crucial improvements in MALDI technology and sample preparation techniques, namely the smartbeam™ laser technology [1] and the ImagePrep™ station [2], currently allow the routine generation of high quality MALDI imaging data. An image resolution of 80 µm, as shown here for the epididymis and brain sample, provides good imaging results at reasonable acquisition time in a robust regime, but certainly does not touch the current resolution limits. Protocols and instrumentation enabling image resolution of 30–50 µm are available and even 10 µm seems in reach – methods are currently under development at Bruker Daltonics.

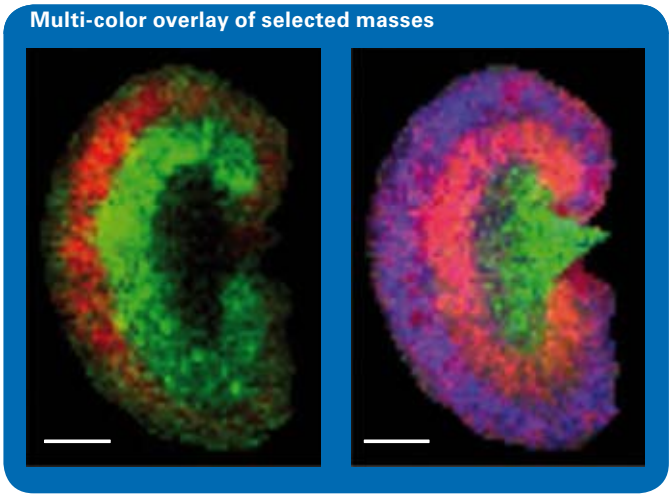

Fig. 3b: Cortex, medulla and pelvis are clearly separated at 200 µm image resolution by the molecular signals shown. Scalebar: 2 mm

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[1] Holle, A., Haase, A., Kayser, M. & Hoehndorf, J. Optimizing UV laser focus profiles for improved MALDI performance. J. Mass Spectrom. 41, 705–716 (2006).
[2] Bruker Daltonics Technical Note-18 „ImagePrep“

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