A real spatial resolution of any imaging approach is the most important parameter. In biology, we are usually faced with the fact that the samples suffer from beam damage, as in transmission or scanning electron microscopy. This influences the real resolution of final analyzes or images. Mass spectrometry imaging (MSI) with combinations of MALDI ionization offers a broad range of applications in biochemistry and biomedicine, mainly in the studies dealing with distribution mapping of biomolecules, drugs or metabolites in specific tissues. Reported spatial resolution of MSI analyzes reaches the order of micrometers and depends on sample prep setup and laser focus. The limiting factors are laser beam parameters, matrix crystallization and sample ablation during desorption/ionization process. Here we present the results of MALDI MSI experiments on lipid distribution in the kidney tissue sections with following preselected spatial resolution of spectra acquisitions: 50, 20 and 10 µm.

**MALDI sample preparation:** Sectioning of the fresh frozen mouse kidney was performed in coronal plane using Leica CM1950 cryomicrotome. Slice thickness was set to 12 µm; cutting temperature to -18 °C. Slices were cut onto ITO (indium-tin oxide) glass slides using “thaw mounting” method and vacuum-dried in desiccator for approx. 30 min. HCCA matrix (7 mg/ml in 50% acetonitrile/0.1% trifluoracetic acid) was applied by ImagePrep (Bruker Daltonics) deposition device using default method.

**Mass spectrometry:** Data were acquired on Solarix 12T FTICR mass spectrometer (Bruker Daltonics) equipped with a SmartBeam II UV laser. The laser diameter was set to 10 µm and laser intensity of 18% or 30%. Data processing: FlexImaging 3.0 (build 54) software (Bruker Daltonics).

**Scanning electron microscopy (SEM):** The tissue sections were sputter-coated with 20 nm of gold after MALDI MSI analysis. The samples were examined in Tescan Vega LSU scanning electron microscope at 5 kV.

**Results:** Nd:YAG laser beam intensity of 18% did not produce recordable damage of kidney tissue, however it also did not produce sufficient sample ionization. The increase of laser beam intensity of 30% overcome the problem with ionization, but the kidney tissue appeared strongly damaged. With the laser step setting of 50 µm and 20 µm we observed individual laser beam ablation paths in the scanned tissue section and obtained distributional maps of lipids with proper resolution. However, with 10 µm setting, the individual laser ablation tracks overlap resulting in large tissue areas with total ablation. The lipids distributional maps showed insufficient resolution. The SEM analysis of tissue section after MALDI MSI allowed us to properly interpret the distribution maps recorded with different different laser step setting.

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Fig. 1: SEM image of mouse kidney section with ablation tracks in kidney tissue after MALDI MSI analysis. MSI laser step setting: A - 50 µm, B - 20 µm and C - 10 µm. The insert is 2.8 times magnified. Scale bar represent 2 mm.