

MALDI imaging of on-tissue digests at high spatial resolution

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Introduction

MALDI imaging resolution of on-tissue enzymatic reaction products can be limited by analyte delocalization induced during enzyme deposition and humidified reaction conditions. Resolution is often affected further when matrix is deposited under conditions conducive to analyte extraction and co-crystallization. Aerosols remain the most reliable method of depositing fine drops of enzyme or matrix onto tissue and the size of the droplets directly affect achievable spatial resolution. Here, is an examination of different preparation conditions that have been carried out to optimize digestion rates with a goal of maintaining laser-limited spatial resolutions.

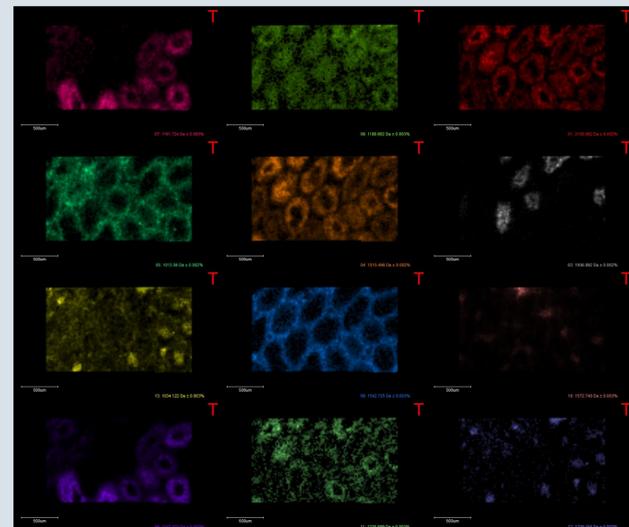


Fig. 1 Series of tryptic ion images from a region of digested rat testis. Image acquired using 7T solariX. 15µm spatial resolution

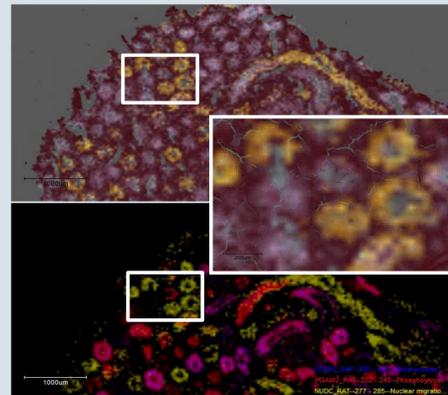


Fig. 2 Ion image overlay from a region of digested rat testis. Image acquired using 7T solariX. 40µm spatial resolution

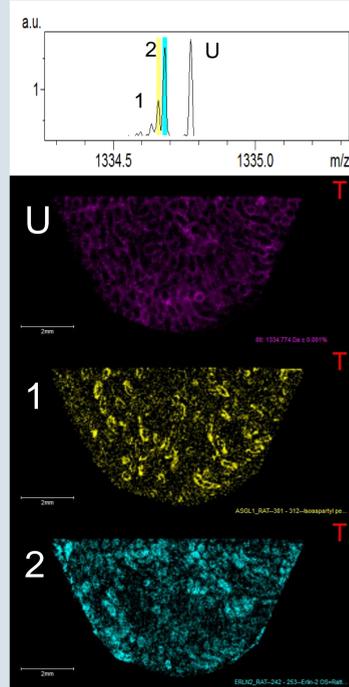


Fig. 3 Isobaric tryptic ion images from a region of digested rat testis. Image acquired using 7T solariX. 15µm spatial resolution

Methods:

The general workflow is illustrated Below. Two or three serial sections were thaw mounted onto ITO coated glass microscope slides (Bruker). Trypsin was proteomics-grade (Sigma). 20µg vials were reconstituted in 20µl of 50mM acetic acid and brought to a final volume of 200µl with 50mM Ambic:ACN 10:1. Trypsin was applied using a TM Sprayer (HTX Imaging) at a rate of 7.5µl/min at 30°C with a stage velocity of 750mm/min and 15psi. using a different number of passes with alternating directions and offset. Slides were incubated at 37°C with 1ml Ambic + 50 µl acetic acid for 2 hrs. Matrix solution (5mg/ml, 1:1 ACN:0.1% Formic acid) was applied with the TM Sprayer (100µl/min, 8 passes, 700mm/min, 15psi, 35°C)

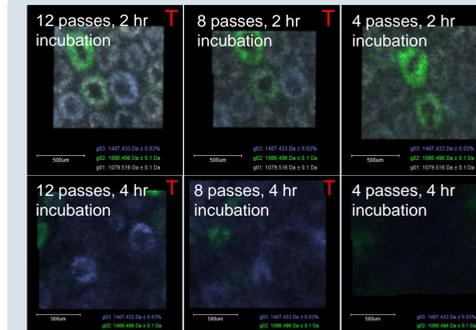
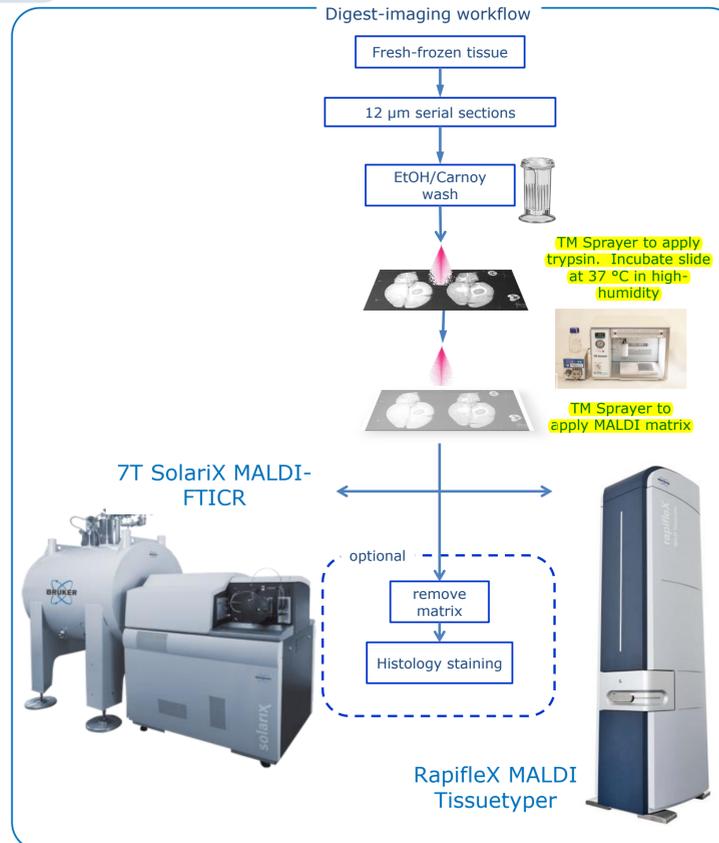


Fig. 4 Effect of different trypsin coverage & incubation times

Fig. 5 Series of tryptic ion images from a region of rat testis. Image acquired using Rapiflex MALDI Tissue typer. 20µm spatial resolution; 81,000 pixels; 114 min measurement time

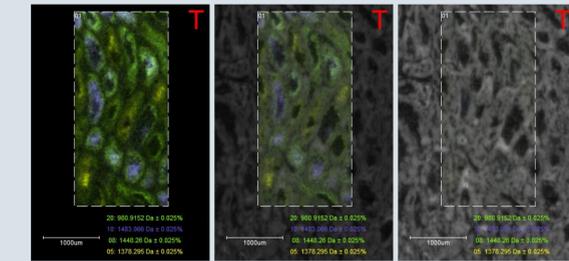
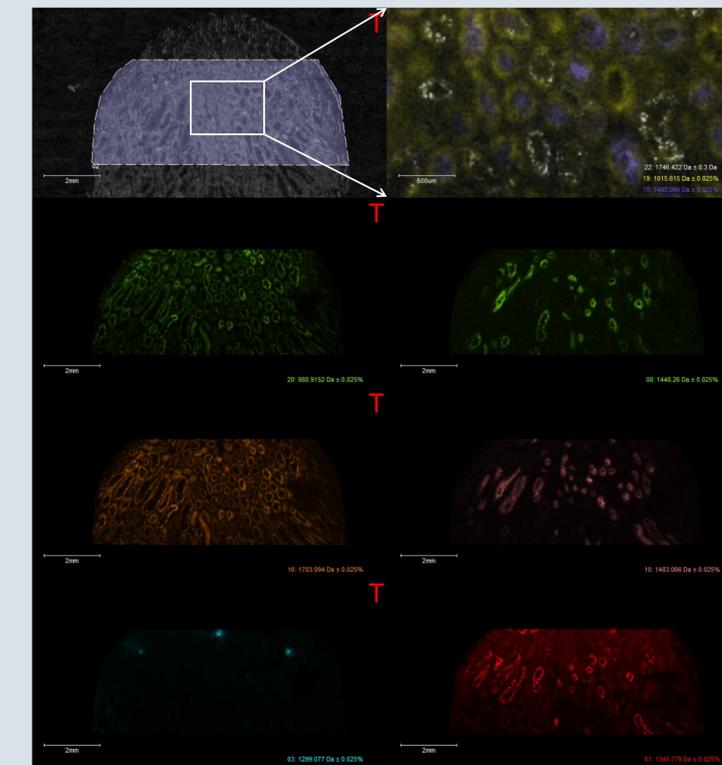


Fig. 6 Overlay of 4 tryptic ion images in a region of rat testis. Image acquired using Rapiflex MALDI Tissue typer.

20µm spatial resolution
14,000 pixels
25 min measurement time

Summary

The microtubule structures of testis provide a good guide for evaluating analyte migration induced during sample preparation. The extra steps of applying enzyme and exposure to the humidified environment of the incubation chamber are expected to promote migration. The current results show that while migration may occur on a cellular scale, the practical scale of MALDI imaging seems to not show significant effect of preparation-induced analyte migration.

Conclusions

- Analyte migration can be minimized during enzymatic treatment of tissue
- The amount of enzyme applied to the sample appears to have some effect on digestion rates.
- Somewhat unexpectedly, current results suggest that longer incubation times may yield lower signal intensity

MALDI imaging