

Atmospheric Pressure MALDI Imaging Mass Spectrometry

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OVERVIEW

Purpose: Use AP-MALDI Imaging for direct analysis of biological tissue sections.

Methods: A commercially available AP-MALDI source modified with software and hardware suitable for this type of analysis

Results: AP-MALDI Imaging can be applied for tissue analysis with a resolution of 60 μm wide pixels.

INTRODUCTION

MALDI imaging mass spectrometry is an emerging technique for direct analysis of biological tissue sections. It can profile spatial distribution of drugs, peptides and proteins in tissue sections of plants, animals and humans.¹ In this work, we report development of tissue imaging method by employing atmospheric pressure (AP) MALDI. It offers important benefits as it is a softer ionization technique compared with vacuum MALDI and it can be used for thicker tissue slices without drying. AP-MALDI also enables the use of volatile matrices such as DHA (2,6-dihydroxyacetophenone). AP-MALDI source coupled with ion-trap, QTOF or FT-ICR instruments provides MS/MS possibility for tissue imaging.

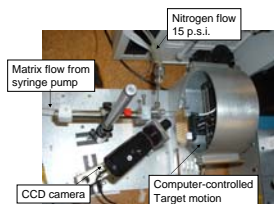
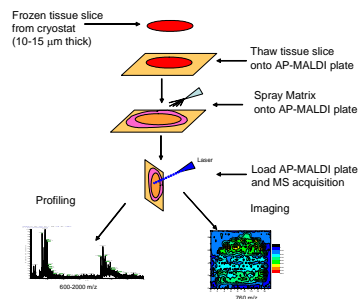
INSTRUMENTATION

Tissue Imaging requirements

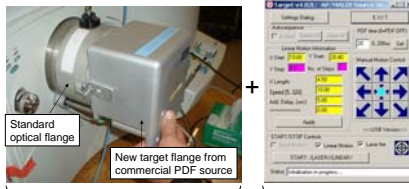
A commercially available AP-MALDI source was adapted for MS imaging. The AP-MALDI source is coupled with an ion trap mass spectrometer. Custom software has been developed to provide scanning of target surface synchronously with the mass spectral data acquisition and to convert the recorded set of mass spectra to 2D-MS image. Laser spot size is a major concern for this application. Also, coating the matrix on the sample needs to be optimized.

AP MALDI Imaging Hardware

The detection sensitivity is the primary concern for MS imaging. For standard peptides, the amount of 10-20 fmole of analyte per image pixel was sufficient to generate a good quality image. To test the spatial resolution of the technique, a mixture of CHCA matrix and peptide was deposited onto the target plate surface by a custom made matrix deposition module employing **pneumatic spray** to achieve uniform matrix coating on the tissue sample. After drying, a cross made of 60 μm thick hair was attached atop of the matrix layer. The recorded MS image proved the resolution of 60 μm for the technique.



Imaging AP UV-MALDI-MS system with 40 μm spatial resolution at 1 kHz repetition rate



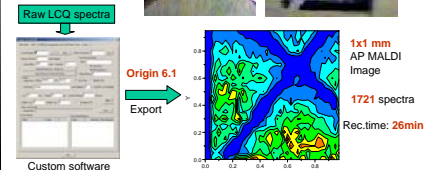
Hardware: Customized AP MALDI source provides smooth linear motion of a target plate

Software: custom program provides zigzag patterned smooth target motion synchronized with computer system timer

Imaging of simple objects

Imaging AP UV-MALDI-MS system with 60 μm spatial resolution at 1 kHz repetition rate

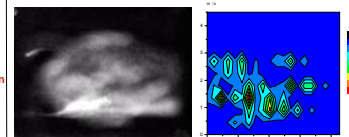
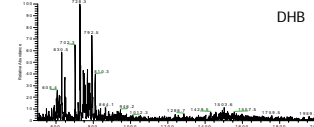
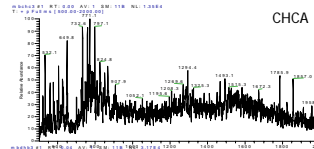
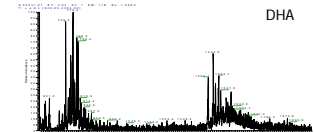
Model object: Hair cross (~70 μm thick) pneumatically-sprayed with matrix/Angiotensin II solution



RESULTS

MS Profiling of tissue sections: Matrix selection

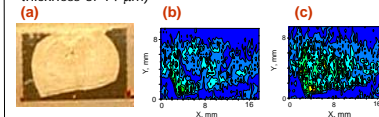
The detection sensitivity is the primary concern for MS. Two issues come into play for this, the ionization efficiency of the analyte and the localization of the matrix crystals. Both depend on the matrix used. Below are MS profiles of similar tissue sections with different matrices



These images show the manual spotted sample of Angiotensin II with CHCA matrix. Visible and MALDI image of the same spot.

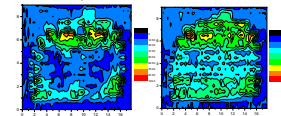
AP-MALDI MS Imaging of tissue sections:

Tissue samples from rat brain have been investigated. Different phospholipids can be mapped on the brain tissue in line with previously published results². Here we show image from the rat brain slice (18x10 mm with thickness of 14 μm)



(a) Sample photograph after the experiment; (b) AP MALDI image of PC 32:0 lipid, [M+H]⁺ 734.6 Da; (c) AP MALDI image of PC 34:1 lipid, [M+H]⁺ 760.6 Da.

Matrix: **dihydroxyacetophenone (DHA)**
Image recording time: **120 minutes**
Number of spectra: **2495**
Laser spot size: **500 μm**
Laser repetition rate: **10 Hz**



Matrix: **dihydroxyacetophenone (DHA)**
Image recording time: **50 minutes**; Number of spectra: **2780**
Laser spot size: **500 μm** ; Laser repetition rate: **200 Hz**

CONCLUSIONS

AP-MALDI Imaging can currently be done on relatively large tissue samples with a 60 μm resolution.

ACKNOWLEDGEMENTS

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REFERENCES

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- (2) Jackson S.N. et al. *J. Am. Soc. Mass Spectrom.*, 2005, 16, 133-138).