TECHNICAL NOTE

#39 Integrating Ultra-High Speed MALDI-TOF and MALDI FTICR IMS For Spatial Proteomics

Application

MALDI imaging mass spectrometry (MALDI IMS) is a powerful technology that allows the detection of analytes from tissue while preserving their spatial distribution.

MALDI IMS of intact proteins is of great relevance for biomedical research, since it provides spatial information of endogenous proteins, as well as their post-translational modifications. Previous work of the Caprioli group has recently shown the use of MALDI Fourier Transform Ion Cyclotron Resonance Imaging Mass Spectrometry (MALDI FTICR IMS) to produce ion images of intact proteins [1]. However, intact protein imaging faces a number of inherent challenges related to throughput, the sensitivity at high spatial resolution, rates of image acquisition, and molecular specificity and identification. Next-generation platforms such as ultra-high speed MALDI-TOF and high mass resolution MALDI FTICR mass spectrometers can override such limitations, improving protein acquisition rates by ten-fold, achieving 10 µm spatial resolution with high sensitivity, and resolving protein isotopes up to ~20 KDa.

Intended Use Of This Technical Note

This technical note describes the power and efficiency of ultra-high speed MALDI-TOF and high mass resolution MALDI FTICR MS for acquisition of spatial proteomics data. The clinical case-studies presented further demonstrate the utility of using high mass accuracy to link IMS and LC-based top-down and bottom-up experiments. HTX, the manufacturers, and the users that have accepted to share their data do not guarantee the performance of the work-flow, and each lab is responsible for applying the corresponding health and safety regulations. For research purpose only.

Background

This technical note addresses two clinically-relevant case studies; cystic fibrosis (CF) and clear cell renal cell carcinoma (ccRCC).

CF is inherited in an autosomal recessive manner, and caused by the presence of mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is involved in homeostasis of sodium channels in the epithelium. Patients with CF present difficulty breathing due to the blockage of airways by thick mucus, as well as bacterial colonization of the lungs [2, 3]. High-speed MALDI-TOF IMS comprises an optimal tool to study the pathogenesis of CF, given that lung tissue from CF patients present high heterogeneity and numerous biologically-relevant substructures.

Cancer diagnosis of the kidney have a ~23% mortality rate, with the vast majority being cases of ccRCC [4]. Visual (by CT and MRI) and histological assessments of tumor margins have proven deficient at the time of surgical removal, leading to disease recurrence [5]. For this reason, determining molecular localization patterns in and around the tumor would aid in the determination of its molecular margins [6].

Here the user was interested in imaging protein expression in 1) human lung tissue of a patient with CF, and 2) human clear cell renal cell carcinoma, using ultra-high speed MALDI-TOF IMS and MALDI FTICR IMS, respectively.

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Imaging Workflow

10 µm cryosections were placed onto conductive ITO coated slides. Tissue was then washed with 70% EtOH for 30 sec, 100% EtOH for 30 sec, Carnoy fluid (6:3:1 EtOH: chloroform: acetic acid) for 2 min, 100% EtOH for 30 sec, H2O with 0.2% TFA for 30 sec, and 100% EtOH for 30 sec, and stored at -80 °C until IMS analysis. Sections were sprayed using an HTX TM-Sprayer (HTX Technologies) as described below:

Flow Rate	0.1 mL/min
Spray Nozzle Velocity	1100 mm/min
Spray Nozzle Temperature	80°C
Track Spacing	2 mm
Number of Passes	4, with offsets, rotations
Nitrogen Pressure	10 psi (0.7 bar)

Images were collected with a single-spot laser setting of ~5 µm and a pixel scan size of 30 µm or 10 µm in both x and y axis, over a mass range of *m/z* 2,000 to 20,000.

Experimental Summary I.

Tissue type	Human lung
Preservation	Cryogenic, in OCT
Tissue cut	10 µm thickness
Matrix deposition	DHA (15 mg/mL) in 9:1 ACN:H_2O
MALDI Laser	Smartbeam II 2KHz Nd:YAG
Acquisition mode	Positive ion

Instrumentation and Supplies

Cryostat	Cryostar™ Thermo NX70
MALDI plate	ITO coated slides
Matrix	Fisher Scientific #114810250
Matrix Sprayer	HTX TM-Sprayer™
MALDI MS	BRUKER raplifleX MALDI Tissuetyper
Imaging software	BRUKER Daltonics flexImaging 4.1

Experimental Summary II.

Tissue type	Human kidney
Preservation	Cryogenic, in OCT
Tissue cut	10 µm thickness
Matrix deposition	DHA (15 mg/mL) in 9:1 ACN:H_2O
MALDI Laser	Smartbeam II 2KHz Nd:YAG
Acquisition mode	Positive ion

Instrumentation and Supplies

Cryostat	Cryostar™ Thermo NX70
MALDI plate	ITO coated slides
Matrix	Fisher Scientific #114810250
Matrix Sprayer	HTX TM-Sprayer™
MALDI MS	BRUKER SolariX 15T MALDI FTICR MS
Imaging software	BRUKER Daltonics flexImaging 4.1

Images were collected with a small laser setting of ~50 µm and a pixel spacing of 100 µm in both x and y axis, over a mass range of *m/z* 1,100 to 25,000. For high *m/z* analysis, special tuning was required: Funnel RF amplitude (250 Vpp), accumulation hexapole (1.4 MHz, 1950 Vpp), transfer optics (1 MHz, 380 Vpp), time of flight delay (2.8 ms) and ICR cell (sweep excitation power 48%).

Results and MALDI MS Images

Fig. 1A shows a representative image from human lung tissue from a CF patient. Selected protein ion images collected with ultra-high speed MALDI-TOF IMS are shown if Fig. 1B-D.

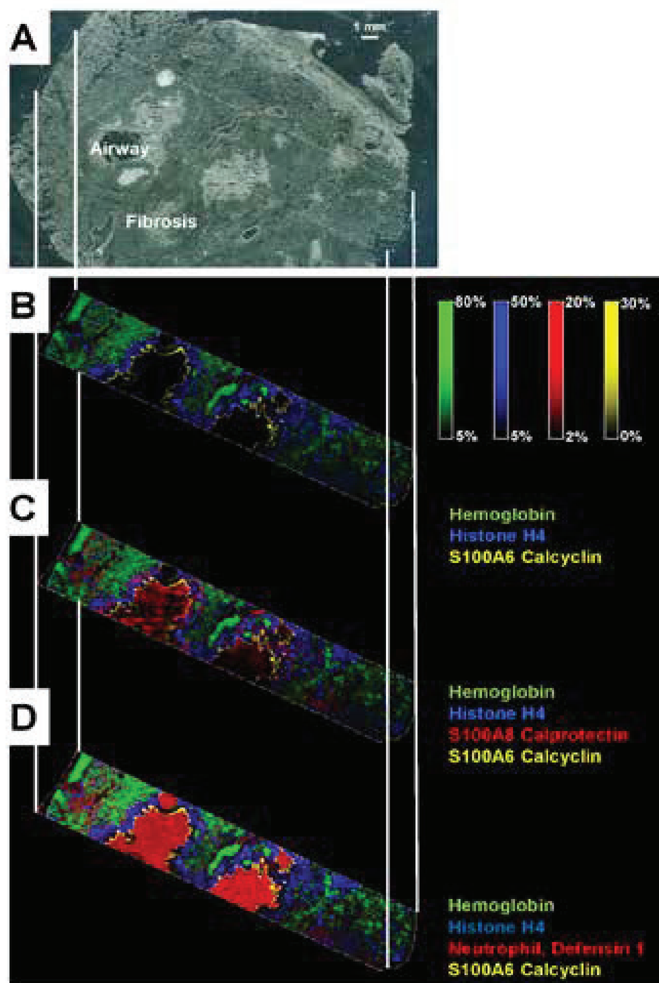

Figure 1. (A) Image of CF human lung with trichome staining prior to IMS acquisition. CF human lung at 30 µM using the RapifleX MALDI Tissuetyper, on linear positive ion mode. (B) Overlay: *m/z* 15,125.74 (hemoglobin, green), *m/z* 11,305.05 (histone H4, blue), *m/z* 10,095.5 (calcyclin, yellow). (C) Overlay: *m/z* 15,125.74 (hemoglobin, green), *m/z* 11,305.05 (histone H4, blue), *m/z* 10834.93 (S100A8, red), *m/z* 10,095.5 (calcyclin, yellow). (D) Overlay: *m/z* 15,125.74 (hemoglobin, green), *m/z* 11,305.05 (histone H4, blue), *m/z* 3450 (neuthrophil defensing 1, red), *m/z* 10,095.5 (calcyclin, yellow). Pixels are beam scanned with a 30 x 30 pixel. 141,000 pixels were acquired in about 1.5 hrs. Scale bar 1mm.

Hemoglobin was detected throughout the tissue while histone H4 and S100A8 (a subunit of calprotectin) were found in abundance in the infected airways. The neutrophil antibacterial peptide defensing 1 co-localized with the former. Calcyclin usually localizes in cells under mechanical strain. The high spatial resolution (10 µm) MALD-TOF IMS data presented in Fig. 2 displays the great spatial heterogeneity of the sample.

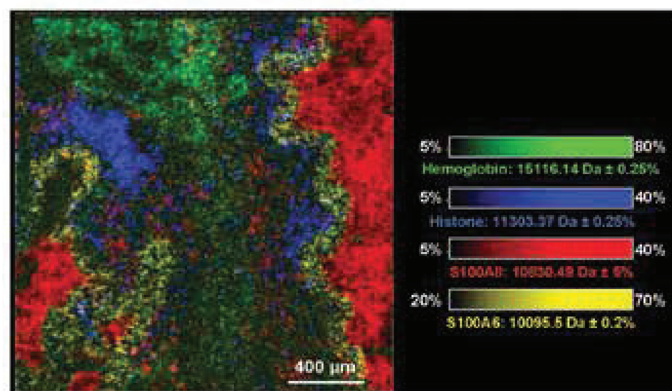

Figure 2. High-spatial resolution (10 µm) MALDI-TOF IMS data of CF human lung, using the RapifleX MALDI Tissuetyper, on linear positive ion mode. Overlay: *m/z* 15,125.74 (hemoglobin, green), *m/z* 11,305.05 (histone H4, blue), *m/z* 10834.93 (S100A8, red), *m/z* 10,095.5 (calcyclin, yellow). Pixels are beam scanned with a 30 x 30 pixel (140,775 pixel size). Scale bar 400 µm.

MALDI FTICR IMS data obtained from human ccRCC tissue is shown in Fig. 3. Note the localization of hemoglobin is the highest close to the tumor regions, a direct reflection of significant carcinogenic angiogenesis taking place. Histone H4 was observed to co-localize with calcyclin (S100A6).

Lastly, a unique feature of MALDI FTICR IMS for protein imaging is the capability to identify the various charge states of highly charged ions. In a recent work from the Caprioli group, the application of this technology allowed for the collection of ion images and isotopic distributions for the [M+H]1+ - 6+ charge states of the subunit of hemoglobin in human ccRCC [7].

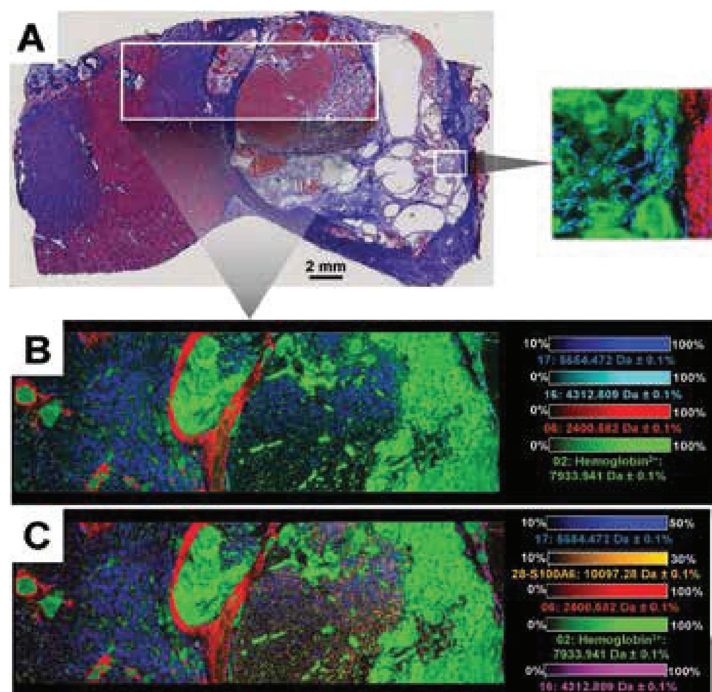

Figure 3. A) Trichrome staining of a clear cell renal cell carcinoma human sample. (B) and (C) show MALDI FTICR protein imaging data collected with a 15T FTICR Bruker SolariX, on positive ion mode, at 100 µm spatial resolution. Overlay for (B): *m/z* 5654.472 (histone H4 with an acetylation and demethylation, blue), *m/z* 4312.809 (turquoise), *m/z* 2400.582 (red), m/z 7933.941 (hemoglobin subunit, green). Overlay for (C): *m/z* 5654.472 (histone H4 with an acetylation and demethylation, blue), *m/z* 10097.28 (orange), *m/z* 2400.582 (red), *m/z* 7933.941 (hemoglobin subunit, green), *m/z* 4312.809 (pink). Acquisition time ~1.5 sec/pixel, total ~6 hrs. Scale bar 2 mm.

Conclusions

Ultra-high speed MALDI-TOF IMS and high-resolution MALDI FTICR IMS are the next-generation technologies for molecular histology. Ultra-high speed MALDI-TOF IMS provides with high spatial resolution, while MALDI FTICR IMS excels at molecular specificity for protein imaging.

References

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