

● Integrating Ultra-High Speed MALDI-TOF and MALDI MRMS Imaging For Spatial Proteomics

MALDI imaging is a powerful technology that allows the detection of analytes from tissue while preserving their spatial distribution

Introduction

Cystic fibrosis (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. 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]. Lung tissue from CF patients is highly heterogeneous, giving rise to numerous biologically relevant substructures. A greater understanding of these protein

substructures may lead to a greater understanding of the pathogenesis of CF.

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

Keywords:
MALDI-TOF, MRMS,
imaging, rapifleX,
solariX

assessments of tumor margins have proven deficient at the time of surgical removal, leading to disease recurrence [5]. Determining molecular localization patterns in and around the tumor would aid in the determination of its molecular margins [6], aiding in removal of the diseased tissue and prevention of recurrence.

In both of these clinical cases, the ability to locate the presence of diagnostic proteins is of great importance to further understanding of the disease. MALDI imaging 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 Magnetic Resonance Mass Spectrometry (MRMS) imaging to produce ion images of intact proteins [1].

However, intact protein imaging faces a number of inherent challenges related to low throughput, decreased sensitivity at high spatial resolution, and low molecular specificity and identification. Next-generation platforms such as ultra-high speed MALDI-TOF and high mass resolution MALDI MRMS mass spectrometers can overcome these limitations, improving protein acquisition rates by ten-fold, achieving high spatial resolution with high sensitivity, and resolving protein isotopes up to ~20 KDa.

Here we use imaging to examine 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 and MALDI MRMS imaging, respectively.

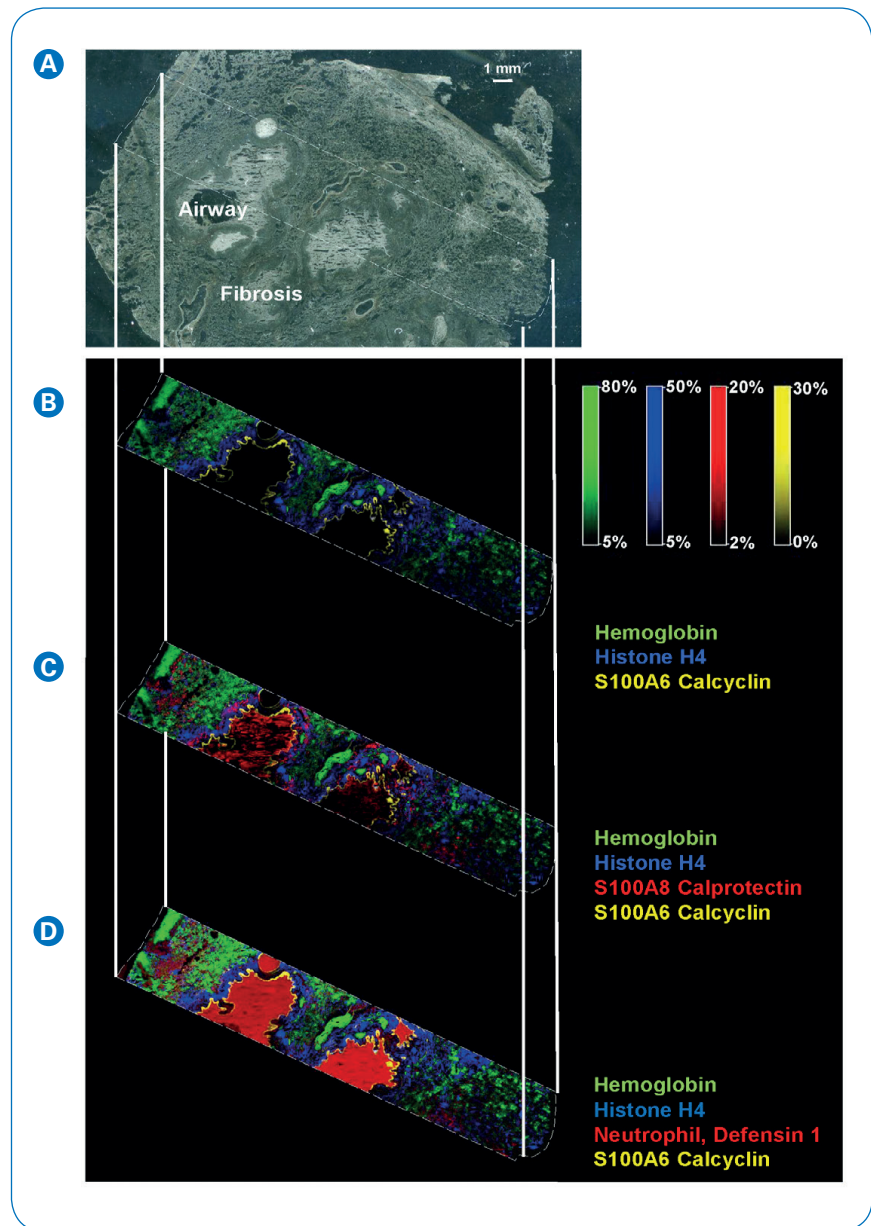


Figure 1. **A** Image of CF human lung with trichome staining prior to IMS acquisition. CF human lung at 30 μ M using the rapifleX MALDI Tissue Imager, on linear positive ion mode. **B** Overlay: m/z 15,125.74 (hemoglobin, green), m/z 11,305.05 (histone H4, blue), m/z 10,095.5 (calcyclin, yellow). **C** 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 (neutrophil Defensin 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 1 mm.

Imaging Workflow

10 μ m cryosections of human lung or kidney tissue 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 coated with 4 passes of DHA (15 mg/mL in 9:1 ACN:H₂O) using an HTX TM-Sprayer (HTX Technologies).

For CF lung tissue, images were collected on the rapifleX in positive ion mode with a single-spot laser setting of $\sim 5\ \mu\text{m}$ and a pixel scan size of $30\ \mu\text{m}$ in both x and y axis, over a mass range of m/z 2,000 to 20,000. For kidney tissue, images were collected on the solariX with a small laser setting of $\sim 50\ \mu\text{m}$ and a pixel spacing of $100\ \mu\text{m}$ in both x and y axis, over a mass range of m/z 1,100 to 25,000.

Results

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

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 Defensin 1 co-localized with the former. Calcyclin usually localizes in cells under mechanical strain. The high

spatial resolution MALDI-TOF imaging data presented in Figure 1 demonstrates the great spatial heterogeneity of the sample.

MALDI MRMS imaging data obtained from human ccRCC tissue is shown in Figure 2. 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).

Acknowledgements

This Technical Note, including figures and illustrations, was produced by Maria J. Torres, East Carolina Diabetes and Obesity Institute, East Carolina University, based on a consultation with Jessica Moore, co-author of the study published in [7]. Tissue images and MS data were provided courtesy of Jessica Moore, and Jeff Spraggins, Mass Spectrometry Research Center, Department of Chemistry, Vanderbilt University School of Medicine, Nashville, TN, USA.

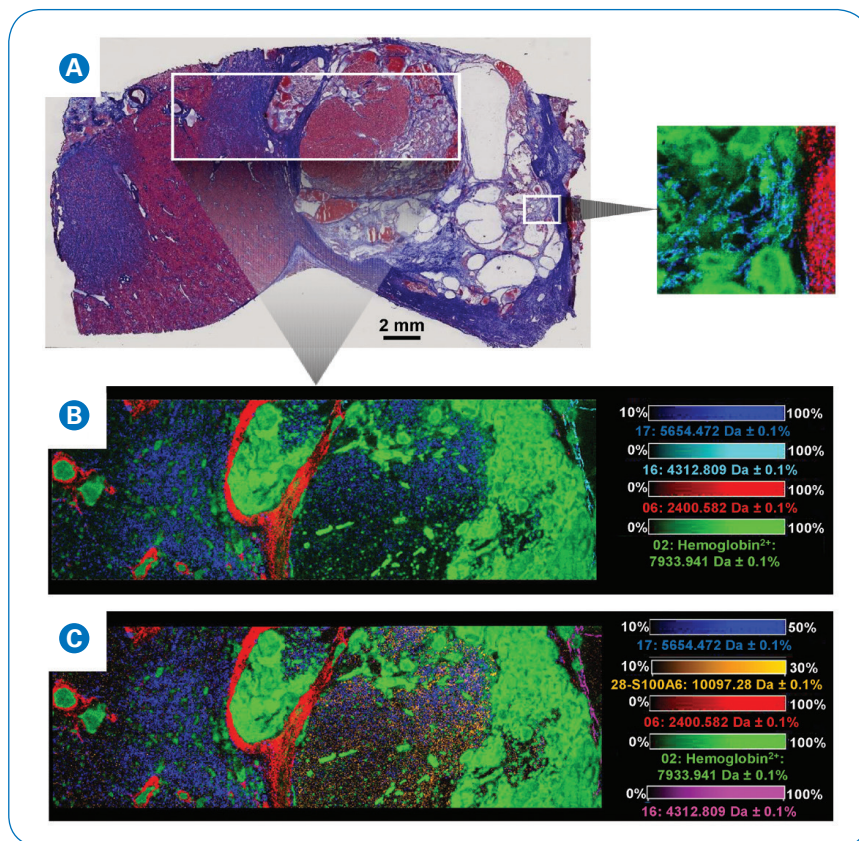


Figure 2. **A** Trichrome staining of a clear cell renal cell carcinoma human sample. **B** and **C** show MALDI MRMS protein imaging data collected with a 15T FTICR Bruker solariX, on positive ion mode, at $100\ \mu\text{m}$ spatial resolution. Overlay for **B**: m/z 5654.472 (histone H4 with an acetylation 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 $\sim 1.5\ \text{sec/pixel}$, total $\sim 6\ \text{hrs}$. Scale bar 2 mm.

Conclusions

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

