The Non-Instrument Based Preparation of Tissue Samples Destined for Imaging Mass Spectrometry (IMS) Analysis

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Method Article

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Abstract

This Methodology aims to provide a comprehensive set of protocols for the analysis of formalin-fixed paraffin-embedded \(FFPE) tissue via matrix-assisted laser desorption/ionisation imaging mass spectrometry \(MALDI IMS) in a low-cost and highly repeatable and robust way, thereby allowing other research teams to begin their own IMS-centered avenues of research. The descriptions of key steps within allows for easy adoption of the protocol while allowing for desired modifications to be performed with minimal yet intuitive adjustment.

Introduction

This protocol is concerned with the preparation of formalin fixed paraffin embedded \(FFPE) and Fresh frozen \(FF) tissue samples for reproducible analysis via imaging mass spectrometry, that does not require robotic or automated sample preparation instruments. The protocol in current regular use in our laboratory and is based on a method first described in Analytical Chemistry in 2011 by Yang and Caprioli and used sublimation as a method for the application of MALDI matrix. We have expanded well beyond their initial work as we found that the method described therein was not reproducible. We therefore significantly improved their initial work by modifying the equipment used, making the protocol reproducible for every subsequent run. We have also focused on refining the protocol to be highly reproducible as well as characterising the mechanisms behind successful sample preparation. The aim was to provide enough information so that the protocol could be easily modified by other research teams to suit their needs. We also ensured that no individual step required specialised equipment or instrumentation, thereby keeping costs to a minimum.

Reagents

- Xylene - 100% ETOH, - 70% ETOH, - carnoys Fluid \(6:3:1 100%ETOH , Chloroform and glacial Acetic acid), - 20mmol tris HCL Ph8.8, - Procine trypsin, - Acetonitrile, - Sinapinic acid or CHCA Matrix, - 50mmol ammonium bicarbonate,

Equipment

- Sciex 5800 MALDI TOF/ TOF, - Chem Glass Sublimation chamber \(modified), - Vapour chamber \(custom built - instructions in manuscript), - Domestic pressure cooker.

Procedure

1) Prepare nitrocellulose slides: Cut ITO slides as necessary, according to size of sublimator then coat them with a layer of liquid nitrocellulose \(40 mg/ml in 100% acetone) using the same procedure as preparing a blood smear. 2) Section the desired tissue at 12 µm and thaw or float mount onto the surface of the nitrocellulose. 3) Wash the whole slide in order; 75% ethanol, 95% ethanol, Carnoy’s fluid, 95%

ethanol, de-ionised water, 95% ethanol. Each wash is for 30 s except for Carnoy's fluid which is for 2 min. 4) Place slide into a small plastic beaker containing 20 mmol Tris-HCl \(pH 8.8) and place into a pressure cooker \(70 kPa internal pressure at >100 C) and cook for 15 min. 5) Remove slide and allow to dry and cool at room temperature. 6) Pipette 10 µl Trypsin \(1 mg/mL in water) onto the centre of the tissue section. Using the longitudinal side of the pipette tip, Use surface tension to 'drag' the trypsin solution droplet to cover the whole of the section. Be careful not to scratch the tissue section. 7) Place the slide in a fan-forced oven at 25°C with a dust filter placed over the top to protect from keratin contamination and allow to dry. 8) Place sample slide in custom vapour chamber as previously described by O'Rourke et al, and evenly pipette 650 µL of a 50:50 mix of 50 mmol ammonium bicarbonate/100% acetonitrile onto the centre paper tab. 9) Seal the chamber with para-film and leave at 37 C overnight. 10) Disassemble the chamber, remove and weigh slide. 11) Mount slide onto cooling finger of sublimation apparatus, place 300 mg of appropriate matrix evenly onto the bottom petri dish of sublimator and assemble the chamber. 12) Evacuate chamber to ~25 mtorr and pack cooling finger with ice. Allow to settle for 5 mins 13) Place sublimator into oil bath at an appropriate temperature \(this depends on matrix) and allow to sublimate until a coverage of 0.2 mg/cm2 has been achieved \(this will vary with temperature and volatility of matrix e.g. CHCA requires 45 mins at 200-210C). 14) Dissemble chamber quickly and remove slide to prevent condensation forming. Allow to return to room temperature. 15) Mount sample into vapour chamber again and evenly pipette 650 µL of 50:50 100%ACN: 0.1% TFA, onto the paper tab making sure the paper tab and slide align correctly. 16) Leave at 37C for 1 hour. 17) Disassemble the chamber and remove slide. This sample is now ready for analysis in the mass spectrometer.

Timing

tissue preparation and washing should take ~30 mins depending on speed of sectioning. Tryptic digestion requires >10 hours, overnight is best, sublimation can take up to 2 hours depending on matrix and Recrystallisation takes 1 hour whole time for sample prep is ~8 hours over 2 days.

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Figures

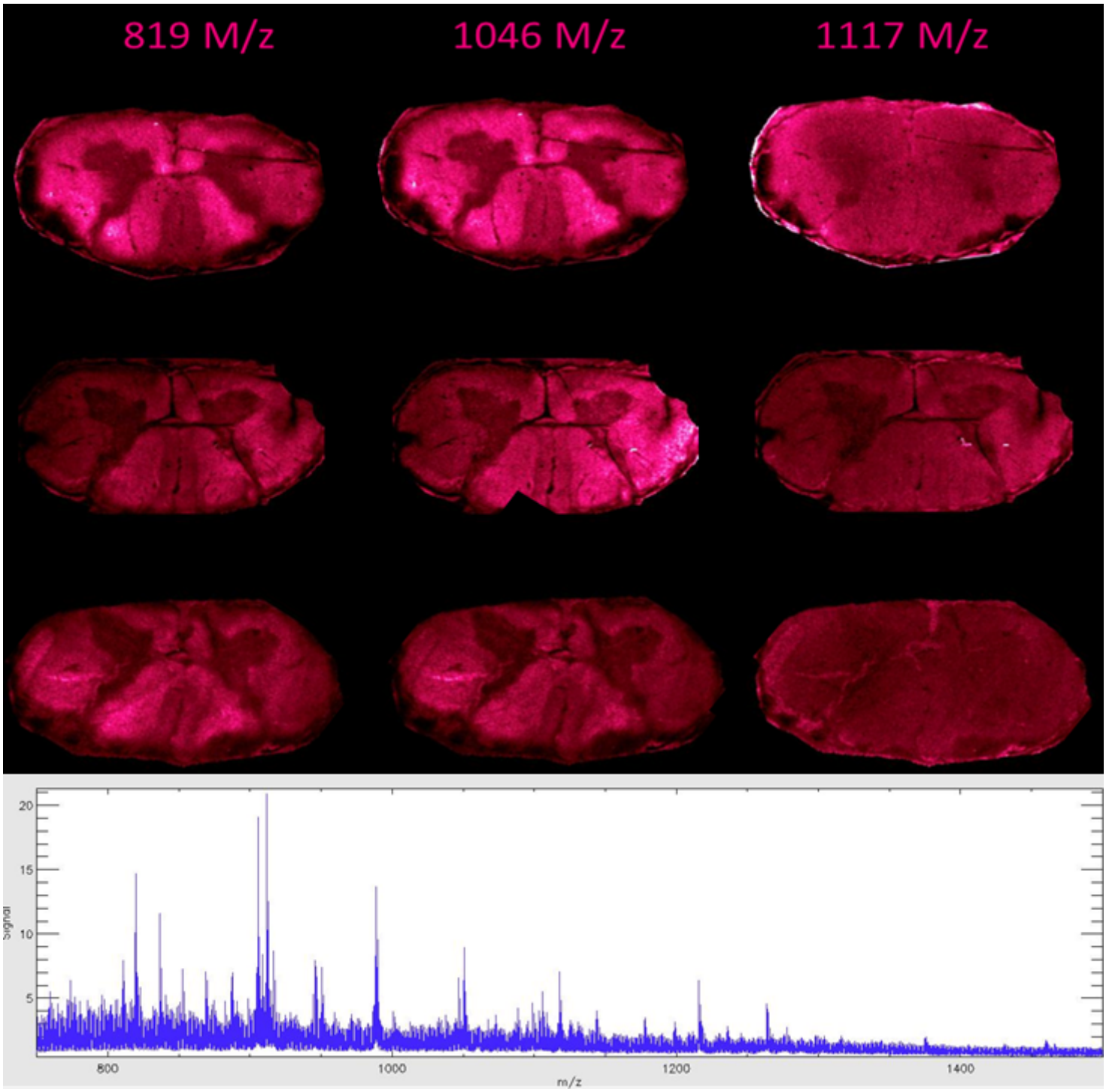

Figure 1

Triplicate data Triplicate serial FFPE Spinal cord sections demonstrating reproducibility by mass. 1,2 and 3 are serial sections taken from the same block of FFPE spinal cord. The m/z values have been selected for display from the attached global spectra. The global spectrum ranges from 750-1500 Da and was created by averaging the relative intensities of all detected masses across every pixel.