Liquid Extraction Surface Analysis Imaging of in-situ Lipids and Proteins from Human Liver Analysed by High Resolution Mass Spectrometry

Joscelyn Sarsby[1,2*], Alan Race[1,2], Patricia Lalor[3], Hamid Dehghani[1,4], Helen Cooper[5], Josephine Bunch[1,2]

[1]Physical Science of Imaging in the Biomedical Sciences, [2]School of Chemistry, [3]Centre for Liver Research Institute of Biomedical Research School of Immunity and Infection College of Medical and Dental Sciences, [5]School of Biosciences, [4]School of Computer Science, University of Birmingham, Edgbaston, Birmingham, B15 2TT UK *author for communication jxs082@bham.ac.uk

PSIBS Physical Sciences of Imaging in the Biomedical Sciences Doctoral Training Centre

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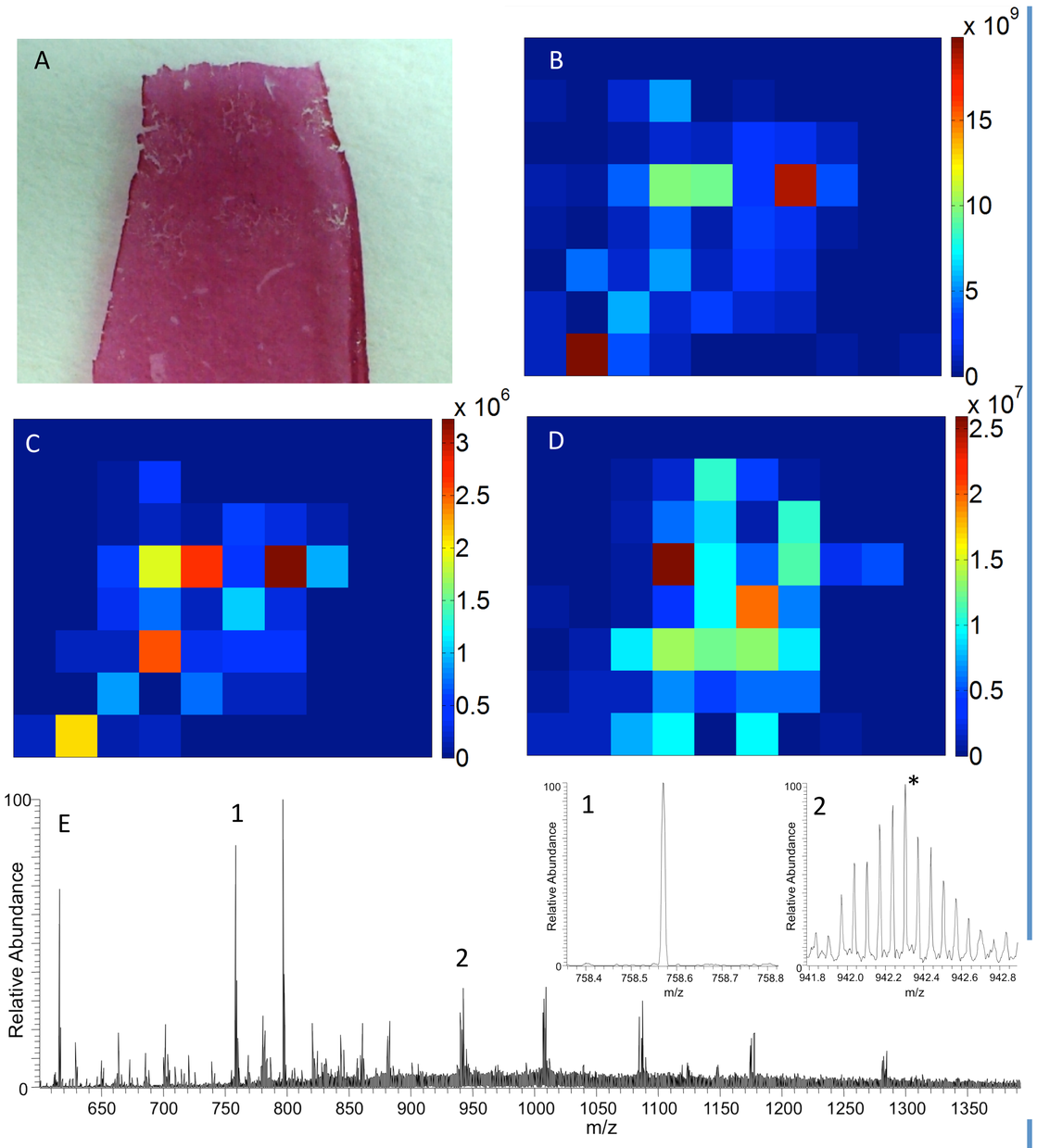


Introduction:

- Liver Disease is one of the five biggest killers in the UK and is the only one of the five that is increasing year on year [1].
- Liquid extraction surface analysis (LESA) is a new sampling technique in which a liquid micro-junction between a sample and a conductive pipette allows extraction of molecules from a surface, the solution is then injected into the mass spectrometer by chip-based nano-ESI (Advion Nanomate) [2,3].
- LESA has previously been used in analysis of thin layer chromatography plates, dried blood spots, whole animal sections and fresh tissue [3-5]
- In this work we explore the use of LESA coupled to high resolution nano ESI-MS and MS/MS for analysis of lipids and proteins in human liver tissue.
- Opportunities for tissue imaging using in-house software, simultaneous detection of lipids and proteins and the effects of repeated analysis are presented.

Methods:

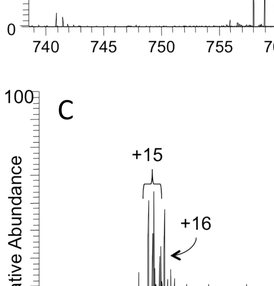
Imaging Lipids and Proteins Liver: LESA sampling - 0.8 µL of 70 % methanol$_{(aq)}$ with 0.1 % Formic Acid was aspirated from a solvent well of which 0.5 µL was dispensed from a height of 0.2 mm onto tissue sectioned at 10 µm thickness followed by a 10 second delay. 0.5 µL was re-aspirated and electrosprayed into a Velos Orbitrap at a tip voltage of 1.75 kV, gas pressure 0.3 psi and a capillary temperature of 250°C. MS Method**:** All spectra were acquired at a resolution of 100,000 at *m/z* 400. Each scan comprised 5 co-added microscans and each spectrum was acquired for ~ 15 scans (2 mins). The AGC target was 1 X 106 with a max fill time of 2 s.

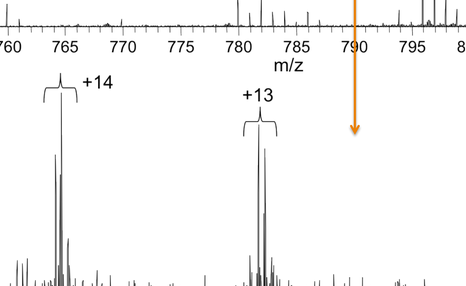
Repeat extractions from a single location were performed under similar condition differing only in the solvent volume, 3.5 µL aspirated of which 3 µl was dispensed and reaspirated. And the data was recorded for 3 min at 5 micro scans.

LESA-MS Images from Normal Liver Tissue. A) H&E stain of the tissue after LESA. Localized regions of damage can be seen where the LMJ was maintained. B) Image showing the total ion current form the data acquired. C) *m/z* 942.3032 (an unidentified protein). D) *m/z* 758.5676 ± 0.005 (identified as the protonated adduct of lipid PC 34:2). E) A spectrum from a single pixel on tissue. 1) and 2) indicate the peaks from which the select ion images were taken. In the case of 2) *m/z* 942.3032, is a single peak (*) from the isotopic distribution from the protein.

Principle Findings:

- Lipids and proteins readily detected in tissue by LESA-MS.
- Direct analysis of tissue via LESA-MS can be used to construct ion images of lipids and proteins in thin tissue sections.
- High mass accuracy and high mass resolving power of the Velos Orbitrap enables identification of some constituents.
- Repeat analysis of the same area enables the detection of additional molecules following the removal of salts and other abundant molecules.



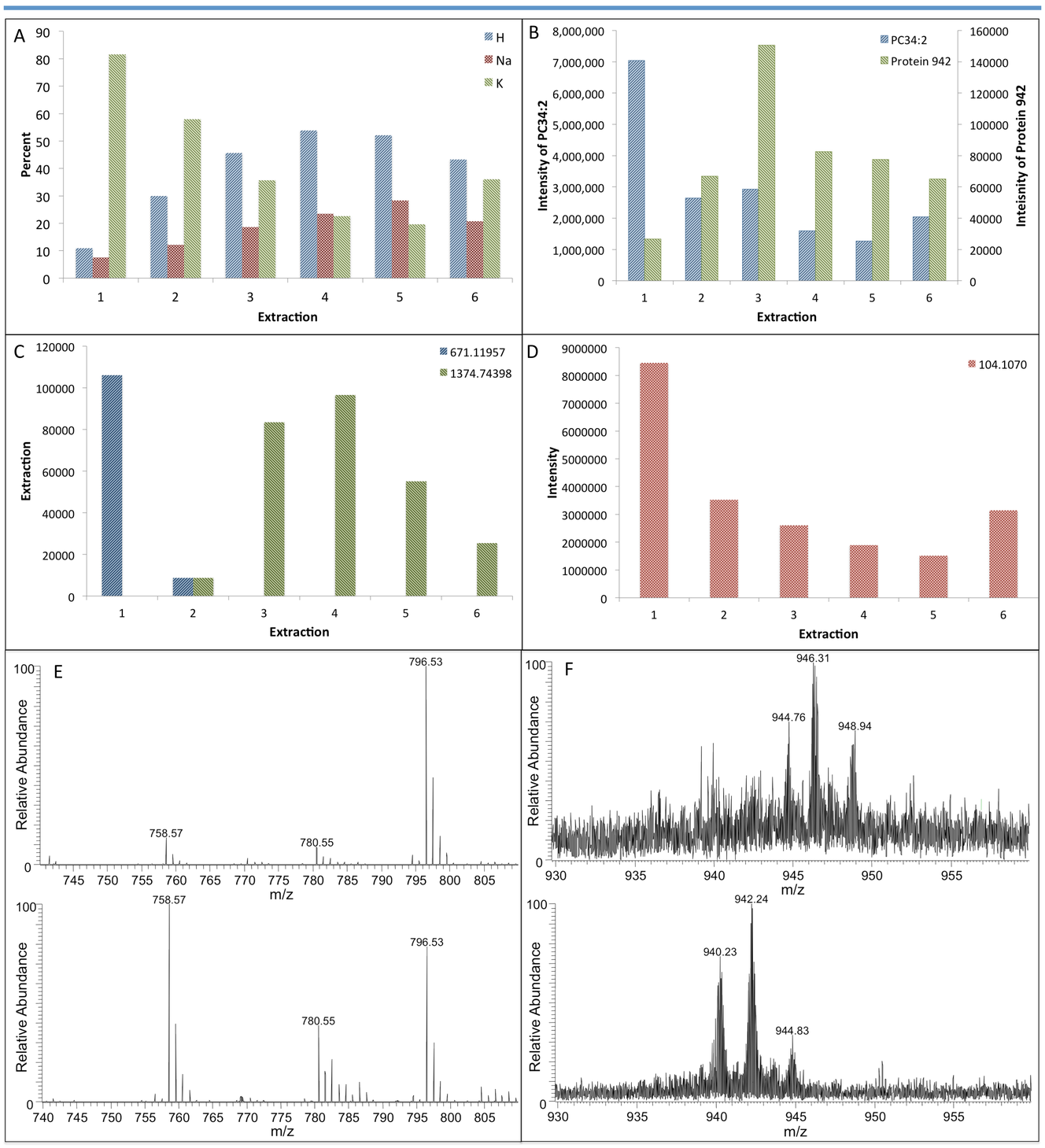


Advion nanomate LESA.

A) Liquid micro-junction maintained between a conductive tip and a surface.

B) Conductive tip is used to introduce the sample for chip based nano-electrospray.

Simultaneous analysis of Lipids and Proteins. A) Typical mass spectrum from liver tissue (*m/z* 600 – 1500) B) singularly charged lipids detected between *m/z* 740 and *m/z* 825. C) Proteins detected as multiple charged species between *m/z* 900 *and* m/z 1300.



Repeat Sampling of Tissue via LESA. A) The relative abundance of the lipid PC 34:2 adducts showing the decrease of abundant salts such as potassium and the relative increase of the protonated adduct. B) The absolute intensity of the potassium adduct of PC 34:2 and an unidentified protein *m/z* 942.3032. C) The 'appearance' and 'disappearance' of two unknown species identified by mass only. D) The depletion of *m/z* 104.1070 identified as the choline head group from PC lipids. E) Top: first injection, Bottom: third injection. The predominant salt adduct detected changes from [PC 34:2 + K]$^+$ to [PC 34:2 + H]$^+$. F)Top: first injection, Bottom: third injection. A protein detected at *m/z* 946.3134 is no longer detected in third injection but a previously undetected protein is observed at *m/z* 942.2350.

References: [1] British Liver Trust www.britishlivertrust.org.uk/home/about-us/media-centre/facts-about-liver-disease.aspx accessed 10/04/12. [2] Kertesz, V.; Van Berkel, G. J. *Journal of Mass Spectrometry* **2010**, *45*, 252. [3]Edwards, R. L.; Creese, A. J.; Baumert, M.; Griffiths, P.; Bunch, J.; Cooper, H. J. *Analytical Chemistry* **2011**, *83*, 2265. [4] Eikel, D.; Vavrek, M.; Smith, S.; Bason, C.; Yeh, S.; Korfmacher, W. A.; Henion, J. D. *Rapid Communications in Mass Spectrometry* **2011**, *25*, 3587. [5] Walworth, M. J.; Stankovich, J. J.; Van Berkel, G. J.; Schulz, M.; Minarik, S. *Rapid Communications in Mass Spectrometry* **2012**, *26*, 37.

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