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

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

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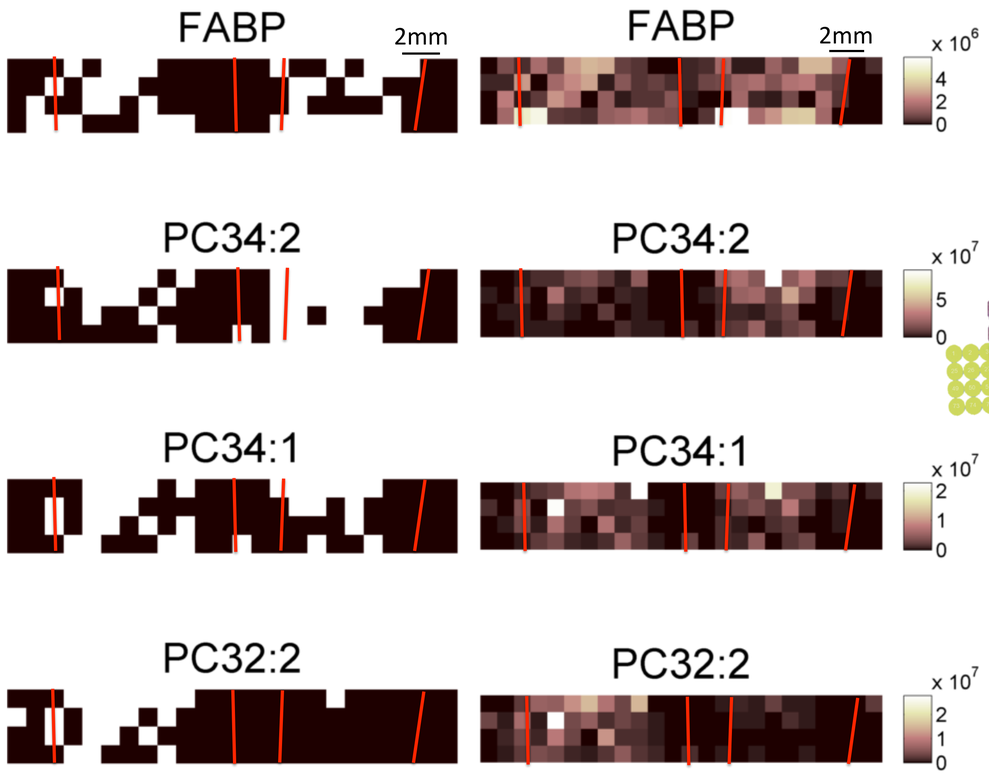
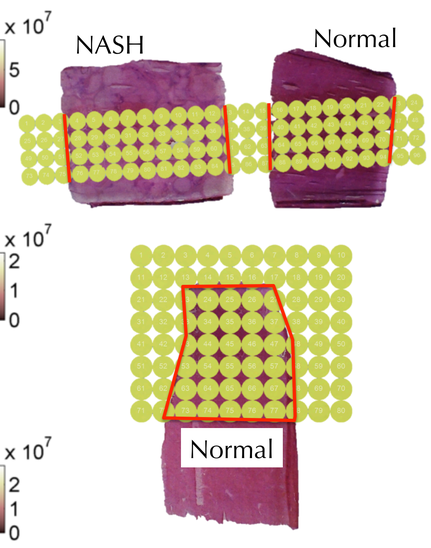
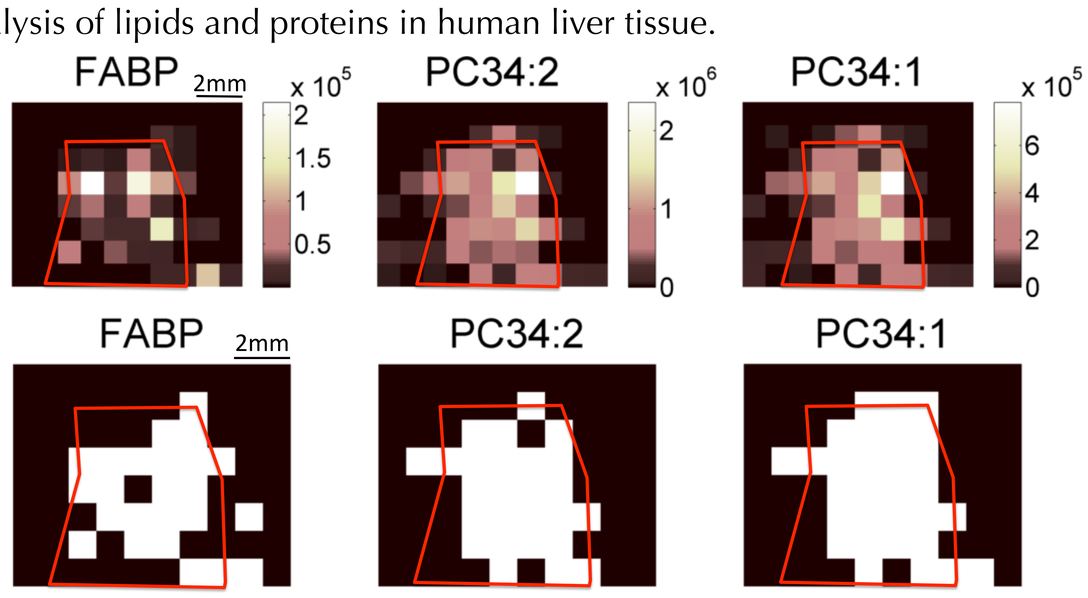


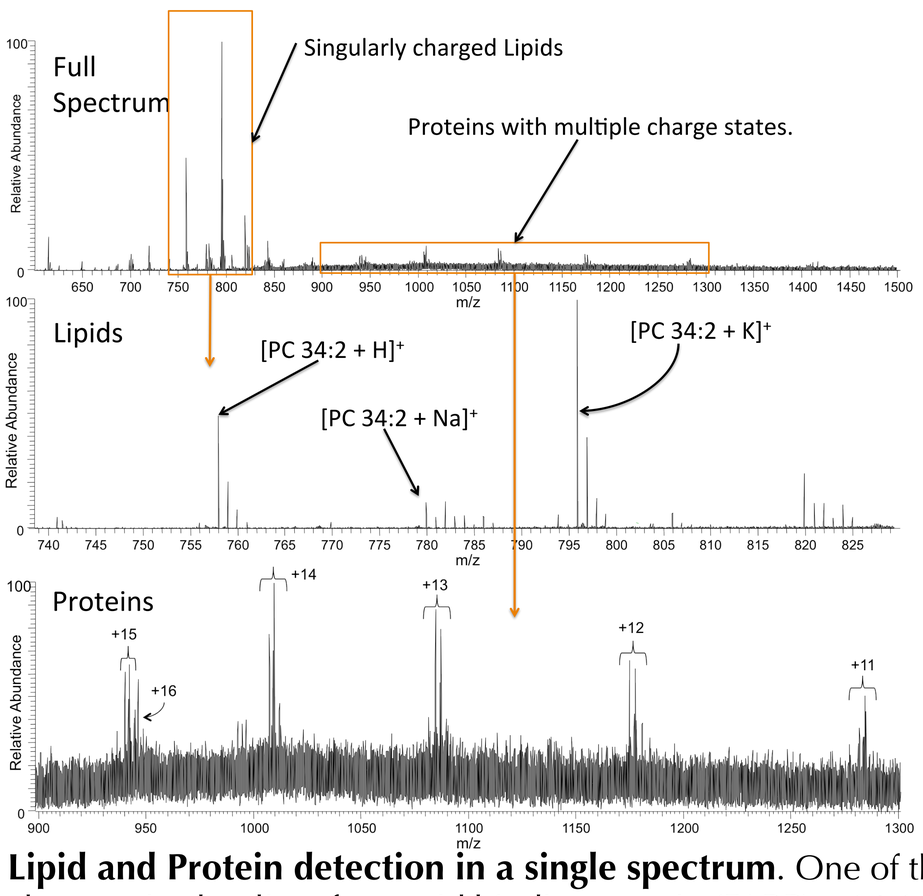


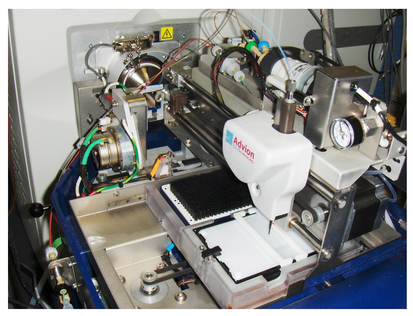


LESA Imaging: Liver Disease is one of the five biggest killers in the UK and is the only one of the big five that is increasing year on year [1]. Liquid Extraction Surface Analysis (LESA) has previously been used in analysis of TLC plates, dried blood spots, whole animal sections and fresh tissue [2-4]. 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.

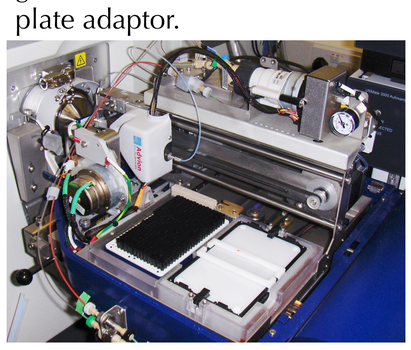
Lipid and protein Images were created using LESA sampling (Advion) coupled to high resolution MS. Images shown as relative intensity and in binary form.
LESA sampling: 0.8 μL of 70% methanol$_{(aq)}$ 0.1% Formic Acid, of which 0.5 μl was dispensed from a height of 0.2mm onto a 10 µm thick tissue section. 0.5 μL was re-aspirated after 10 s delay and electrosprayed into a LTQ Orbitrap velos (Thermo) (tip voltage: 1.75kV, gas pressure: 0.3 psi, capillary temperature: 250°C).
MS Method: Spectra acquired (resolution: 100,000 at *m/z* 400), ~25 scans (3 mins) for normal only and ~15 scans (2 min) for normal and non-alcoholic steatohepatitis (5 co-added microscans per scan). The AGC target was 1 X 10^6 with a max fill time of 2s.

Repeat extractions from a single location revealed changes in species detected. Protocol as for imaging, but with an increased solvent volume of 3.5 μL, of which 3 μl was dispensed and re-aspirated.

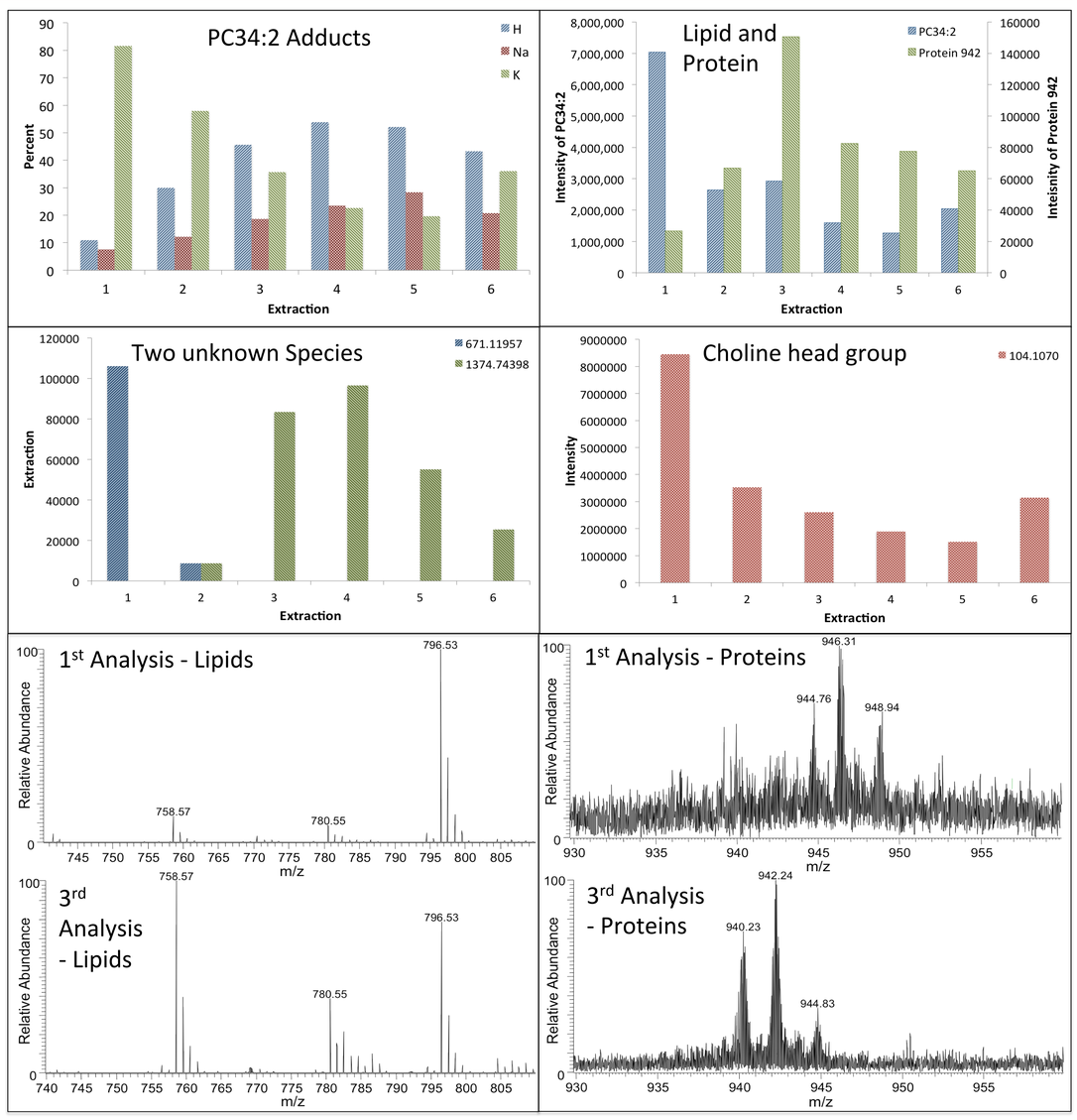




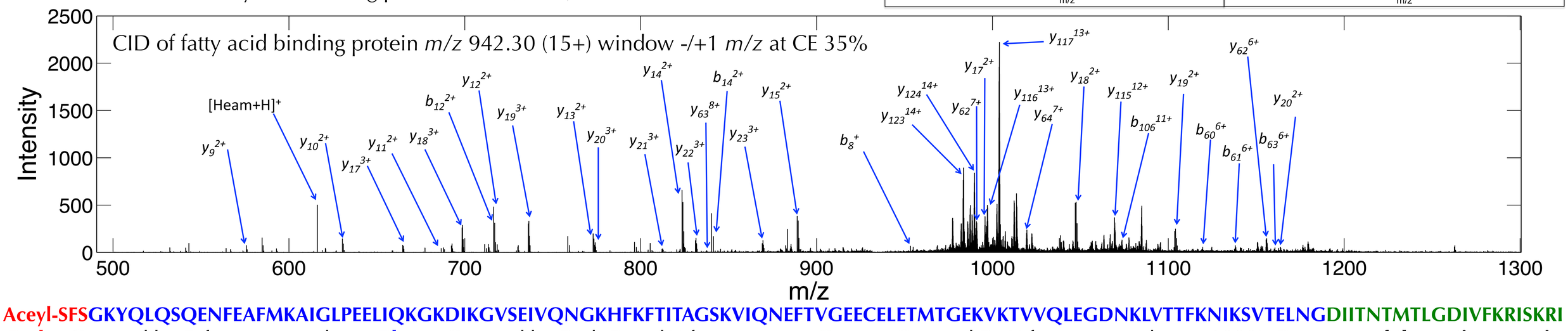
LESA sampling (using a conductive pipette tip) from a glass slide held In a universal plate adaptor.



The solution is injected into the mass spectrometer by chip-based nano-ESI (Advion Nanomate) [5].



Lipid and Protein detection in a single spectrum. One of these proteins has been fully characterised as liver fatty acid binding protein (FABP) – 14,111 Da.



Aceyl-SFSGKYQLQSQENFEAFMKAIGLPEELIQKGKDIKGVSEIVQNGKHFKFTITAGSKVIQNEFTVGEECELETMTGEKVKTVVQLEGDNKLVTTFKNIKSVTELNGDIITNTMTLGDIVFKRISKRI

Red = Covered by B fragments only **Blue** = Covered by Both B and Y fragments **Green**= Covered By Y fragments only **100% Coverage of the entire protein**

Principal 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 numerous constituents directly in tissue.
- Repeat analysis of the same area enables the detection of additional ions following the removal of salts and other abundant molecules.

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

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