Improved methods for the analysis of biological substrates by LESA FAIMS MS

Rian L. Griffiths[1], Joscelyn Sarsby[1,2], Andrew J. Creese [1], Elizabeth C. Randall[1,2], Alex Dexter[1,2], Alan M. Race[1,3], Josephine Bunch[3], and Helen J. Cooper[1]

[1] School of Biosciences, [2]PSIBS Doctoral Training Centre, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

[3]National Physical Laboratory, Hampton Road, Teddington, Middlesex, TW11 0LW

Email: rlg684@bham.ac.uk

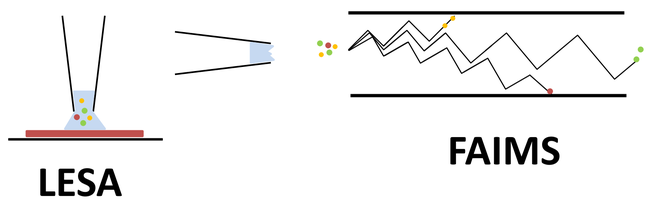
Introduction

Liquid extraction surface analysis mass spectrometry (LESA MS) is a useful tool for direct surface sampling of intact proteins from a range of biological substrates[1-5].

Ions can be separated by movement through an asymmetric electric field as their mobility is dependent on the field strength and gas density. High-field asymmetric ion mobility spectrometry (FAIMS) can be used to either focus or filter certain ions.

By varying the dispersion field (DF) and compensation field (CF), CF only, or by selecting optimal DF and CF settings for the transmission of particular ions, it is possible to perform experiments in the following modes: 2D mode, 1D mode, static mode. Here, we demonstrate LESA FAIMS approaches for improved analysis of biological substrates.

Experimental



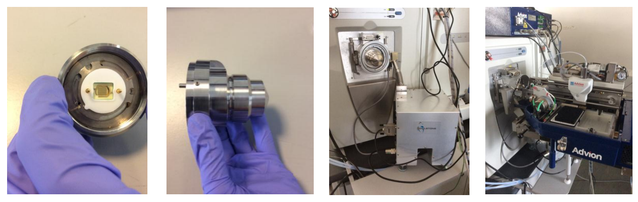


Figure 1 Photographs showing the Owlstone microchip FAIMS device, the device coupled to the Orbitrap mass spectrometer, and coupling to the LESA Nanomate robot.

LESA: Dried blood spots- 5 µl of solvent (50% methanol, 1 % formic acid (FA)) was aspirated before 2 µl was dispensed above the surface. 2.5 µl was re-aspirated after 10 secs. *E. coli*- 3 µl of solvent (40 % acetonitrile, 1 % FA) was aspirated into the robotic arm, 2 µl were dispensed in contact with the surface of the colony. 2.5 µl were re-aspirated after 3 seconds. Mouse brain thin tissue sections- samples were washed in 80 % ethanol for 10 seconds prior to analysis. 6 µl of solvent (40 % acetonitrile, 1 % FA) was aspirated before 3 µl was dispensed above the surface. 3.5 µl was re-aspirated after 10 secs. Mouse liver thin tissue sections- 6 µl of solvent (70 % methanol, 1 % FA) was aspirated before 3 µl was dispensed above the surface. 3.5 µl was re-aspirated after 10 secs.

2D FAIMS: The DF was varied from 130 and 270 Td in steps of 20 Td, the CF was scanned from -1 to +4 Td at each DF.

Mass Spectrometry: LTQ-Orbitrap Velos or Elite.

Data Visualisation

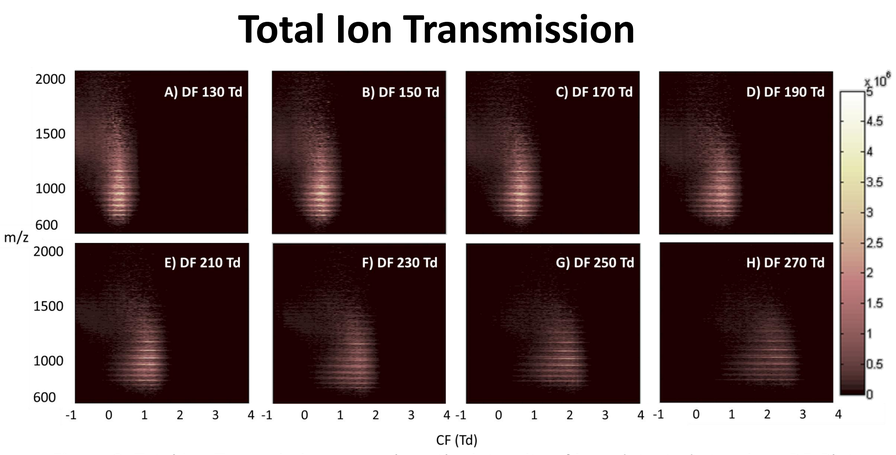


Figure 2: Total Ion Transmission maps show the intensity of ions detected at a given DF. Plots show CF verses m/z. Analytes extracted from dried blood spots are shown in this example

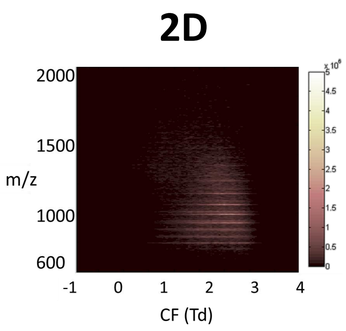


Figure 3: It is also possible to plot the same data in a 3D representation. In this example a mean mass spectrum at the specified DF (270 Td) & the ion chromatogram are shown as projections.

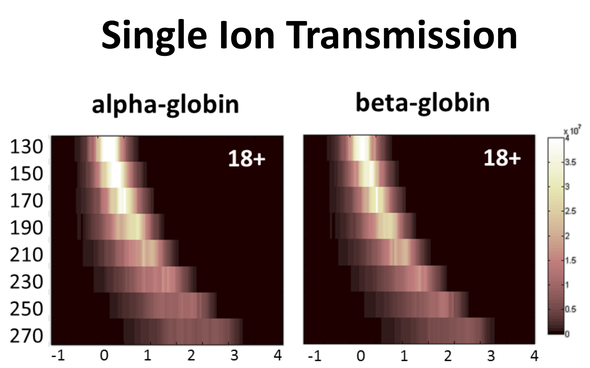


Figure 4: Single Ion Transmission maps showing the intensity of ions relating to the +18 charge state of alpha- and beta-globin proteins extracted from a dried blood spot sample.

Molecular Separation

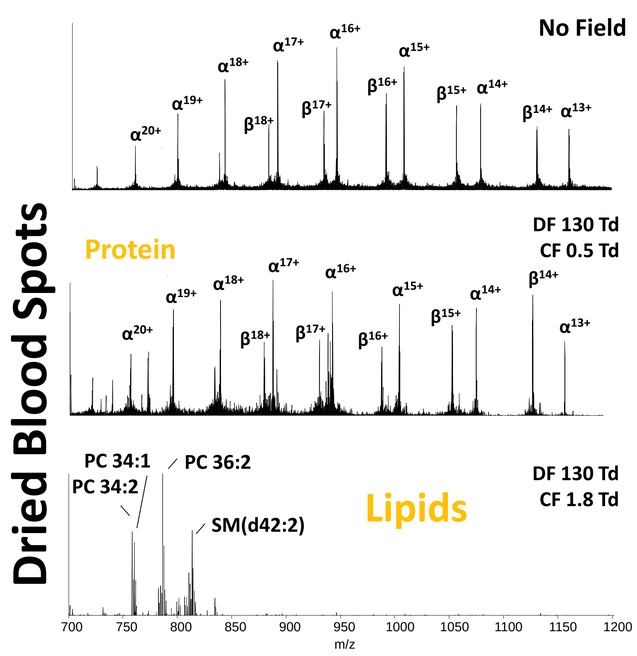


Figure 5: LESA static FAIMS mass spectrometry of dried blood spot sample. Protein species (α-globin and β-globin) are detected at DF 130 Td, CF 0.5 Td whereas lipid species are detected at higher CF (1.8 Td). 1 minute of data has been summed.

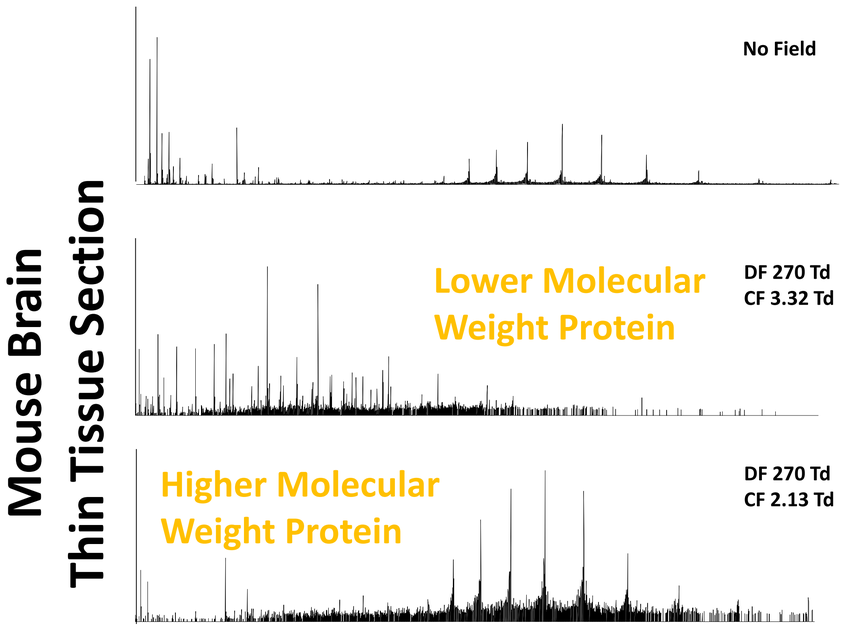


Figure 6: LESA 2D-FAIMS mass spectrometry of mouse brain tissue. Different protein species were detected at DF 270 Td with changing CF. Protein species at CF 2.13 Td has a molecular mass of ~ 22 kDa whereas smaller protein species, 17 species ~ 1-9 kDa, were detected at higher CF (3.32 Td)

Static FAIMS for MS/MS

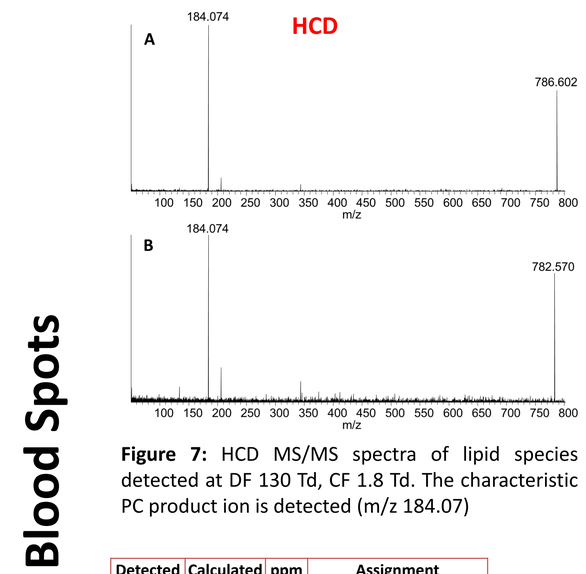


Figure 7: HCD MS/MS spectra of lipid species detected at DF 130 Td, CF 1.8 Td. The characteristic PC product ion is detected (m/z 184.07)

Detected m/z	Calculated m/z	ppm error	Assignment
758.5673	758.5694	2.8	PC 34:2 M+H
760.5833	760.5851	2.4	PC 34:1 M+H
782.5668	782.5694	3.3	PC 36:4 M+H
784.5828	784.5851	2.9	PC 36:3 or PE 39:3 M+H
786.5984	786.6007	2.9	PC 36:2 M+H
787.6670	787.6687	2.2	SM(d40:1) M+H
788.6132	788.6164	4.1	PC 36:1 or PE 39:1 M+H
810.5987	810.6007	2.5	PC 38:4 or PE 41:4 M+H
811.6661	811.6687	3.2	SM(d42:3) M+H
813.6824	813.6844	2.5	SM(d42:2) M+H
815.6990	815.7000	1.2	SM(d42:1) M+H

Table 1: Lipid species identified by a combination of HCD and accurate mass measurement.

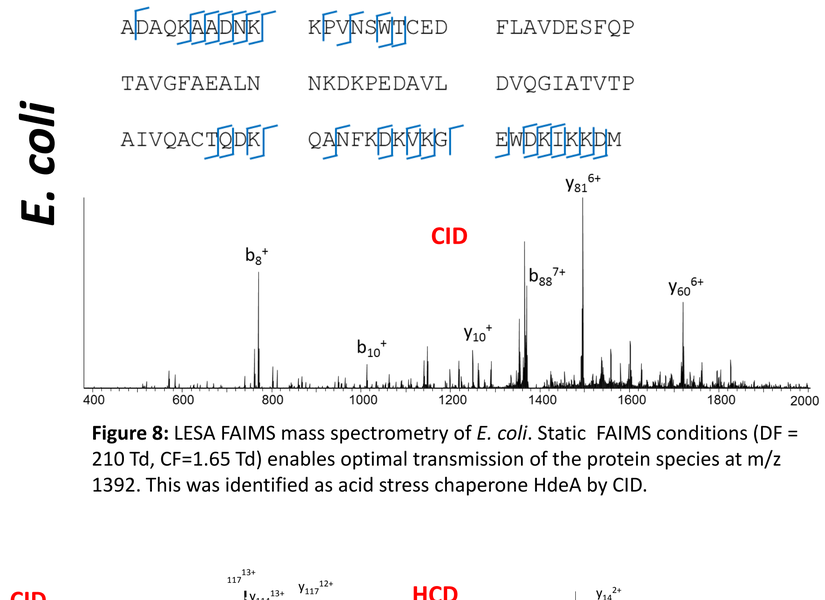


Figure 8: LESA FAIMS mass spectrometry of *E. coli*. Static FAIMS conditions (DF = 210 Td, CF=1.65 Td) enables optimal transmission of the protein species at m/z 1392. This was identified as acid stress chaperone HdeA by CID.

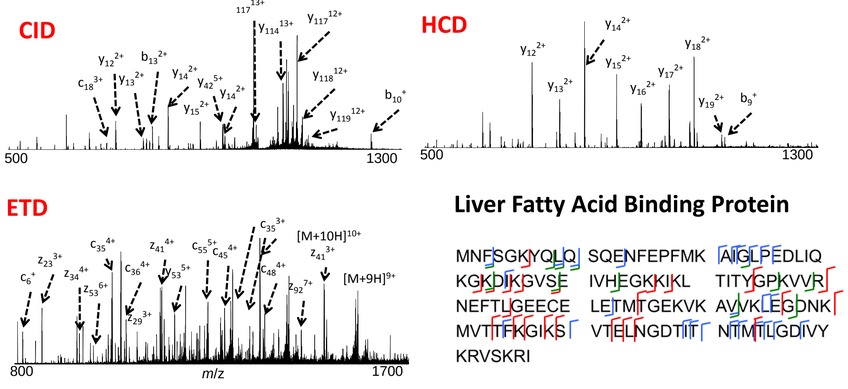


Figure 9: LESA FAIMS mass spectrometry of mouse liver. Static FAIMS conditions (DF 130 Td, CF 0.3 Td) enables optimal transmission of the protein species at m/z 1021. Via a combination of CID, HCD and ETD this species was identified as liver fatty acid binding protein.

Conclusions

The following benefits are highlighted via coupling of FAIMS to LESA MS:

- Molecular separation of, for example, lipids and proteins.
- Improved detection of a range of protein species from biological substrates.
- MS/MS identification of proteins and lipids, via optimal transmission of particular species at specific DF and CF conditions.

References

[1]R. L. Edwards, A. J. Creese, M. Baumert, P. Griffiths, J. Bunch and H. J. Cooper, *Anal. Chem.*, **2011**. [2]R. Edwards, P. Griffiths, J. Bunch and H. Cooper, *J. Am. Soc. Mass Spectrom.*, **2012**. [3]R. L. Edwards, P. Griffiths, J. Bunch and H. J. Cooper, *Proteomics*, **2014**. [4]N. J. Martin, R. L. Griffiths, R. L. Edwards and H. J. Cooper, *J. Am. Soc. Mass Spectrom.*, **2015**. [5]E. C. Randall, J. Bunch and H. J. Cooper, *Anal. Chem*, **2014.**

For further details refer to: J. Sarsby, R. L. Griffiths, A.M. Race, J. Bunch, E.C. Randall, A. J.Creese and H. J. Cooper, *Anal. Chem.* **2015**.
R. L. Griffiths, A. Dexter, A. J.Creese and H. J. Cooper, *Analyst.* **2015**.

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