

Improved methods for the analysis of human dried blood spot samples by LESA mass spectrometry

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Introduction

Liquid extraction surface analysis mass spectrometry (LESA-MS) is a useful tool for direct surface sampling of intact proteins from biological substrates. LESA-MS of dried blood spots (DBS) is of interest both from a clinical perspective (disease diagnosis and screening) and from a technical viewpoint.

Here, we demonstrate LESA approaches for native mass spectrometry and differential ion mobility spectrometry (FAIMS) developed for the DBS format. The latter is clinically relevant to the analysis of haemoglobin variants^{1,2,3}. Previous LESA-FAIMS studies have shown improvements in the detection of proteins and separation of molecular classes⁴.

Experimental

DBS were obtained from healthy volunteers on either filter paper (card) or glass. Samples analysed under native conditions (5 % Methanol (+ 0.0125 % acetic acid) and 10 mM ammonium acetate) were analysed using a LESA Nanomate (Advion™) coupled to a Synapt G2S mass spectrometer (Waters, UK).

Samples analysed under denaturing conditions (1:1 Methanol: Water (+ 1% formic acid)) were analysed using a LESA Nanomate (Advion™) coupled to an Ultra-FAIMS device (Owlstone™), which was coupled to an Orbitrap mass spectrometer (Thermo Scientific, UK).

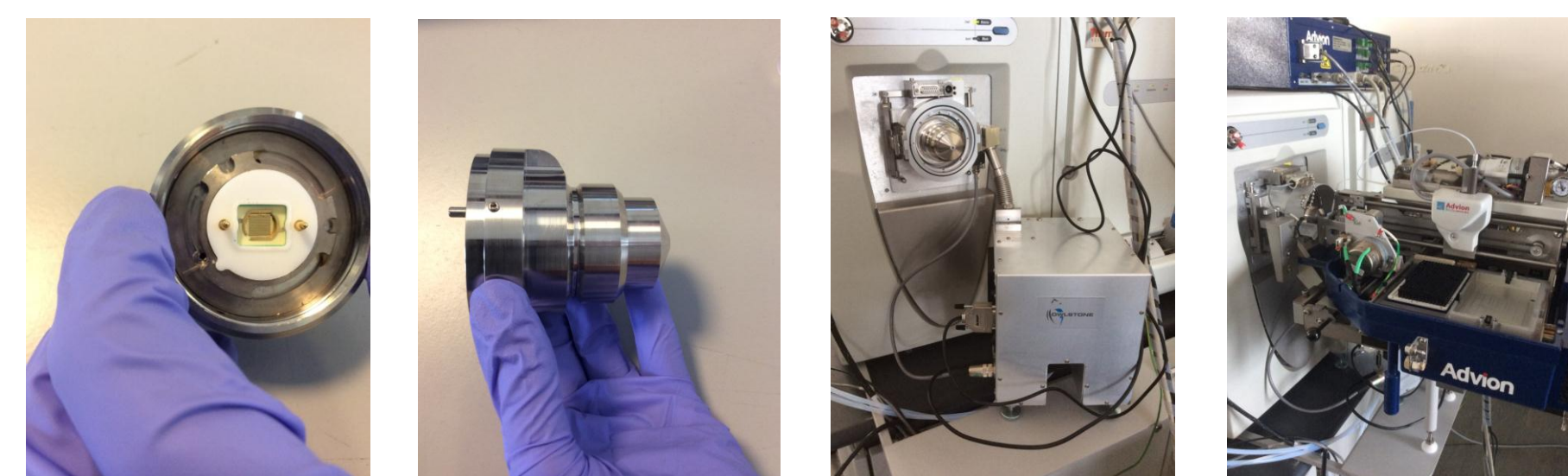


Figure 1 Photographs showing the Owlstone microchip FAIMS device, how this is coupled to the Orbitrap mass spectrometer, and coupling to the LESA Nanomate robot.

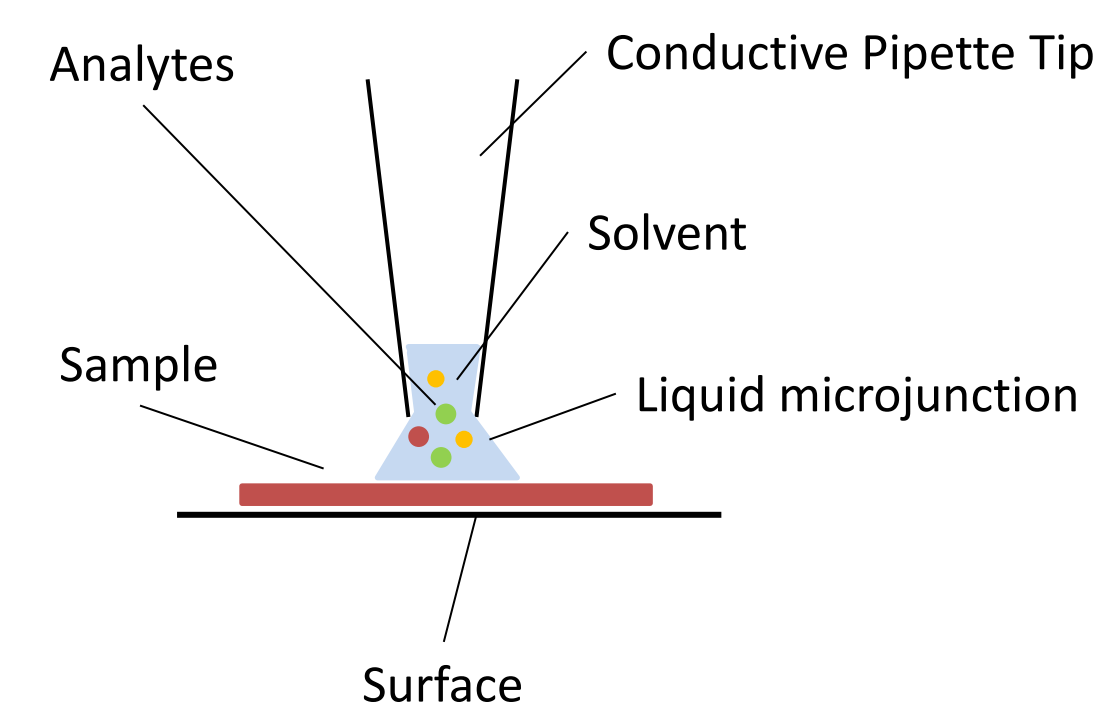
References

- R. L. Edwards, A. J. Creese, M. Baumert, P. Griffiths, J. Bunch and H. J. Cooper, *Anal. Chem.*, **2011**.
- R. Edwards, P. Griffiths, J. Bunch and H. Cooper, *J. Am. Soc. Mass Spectrom.*, **2012**.
- R. L. Edwards, P. Griffiths, J. Bunch and H. J. Cooper, *Proteomics*, **2014**.
- J. Sarsby, R. L. Griffiths, A.M. Race, J. Bunch, E.C. Randall, A. J. Creese and H. J. Cooper, *Anal. Chem. (Under revision)*, **2015**.
- N. J. Martin, R. L. Griffiths, R. L. Edwards and H. J. Cooper, *J. Am. Soc. Mass Spectrom.*, **2015**.
- E. C. Randall, J. Bunch and H. J. Cooper, *Anal. Chem.*, **2014**.

Acknowledgements

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Native LESA-MS



Native ESI mass spectrometry, the study of species in which non-covalent interactions have been maintained enables analysts to further understand the biological condition.

Haemoglobin is present in the body as a non-covalently bound tetramer structure formed of two α - and two β -globin units, each associated with a heme (h) unit. Here we show detection of the intact tetramer via directly sampling the substrate⁵.

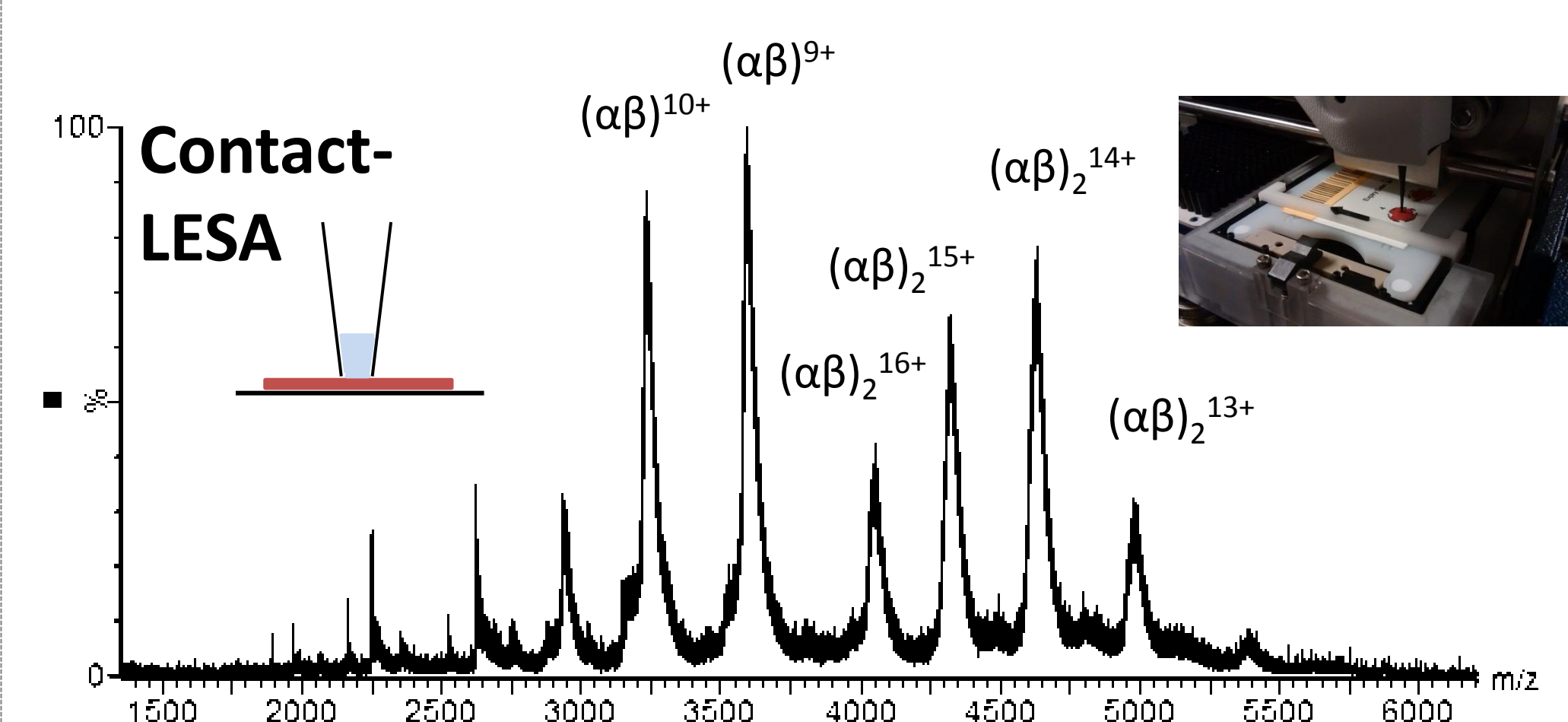
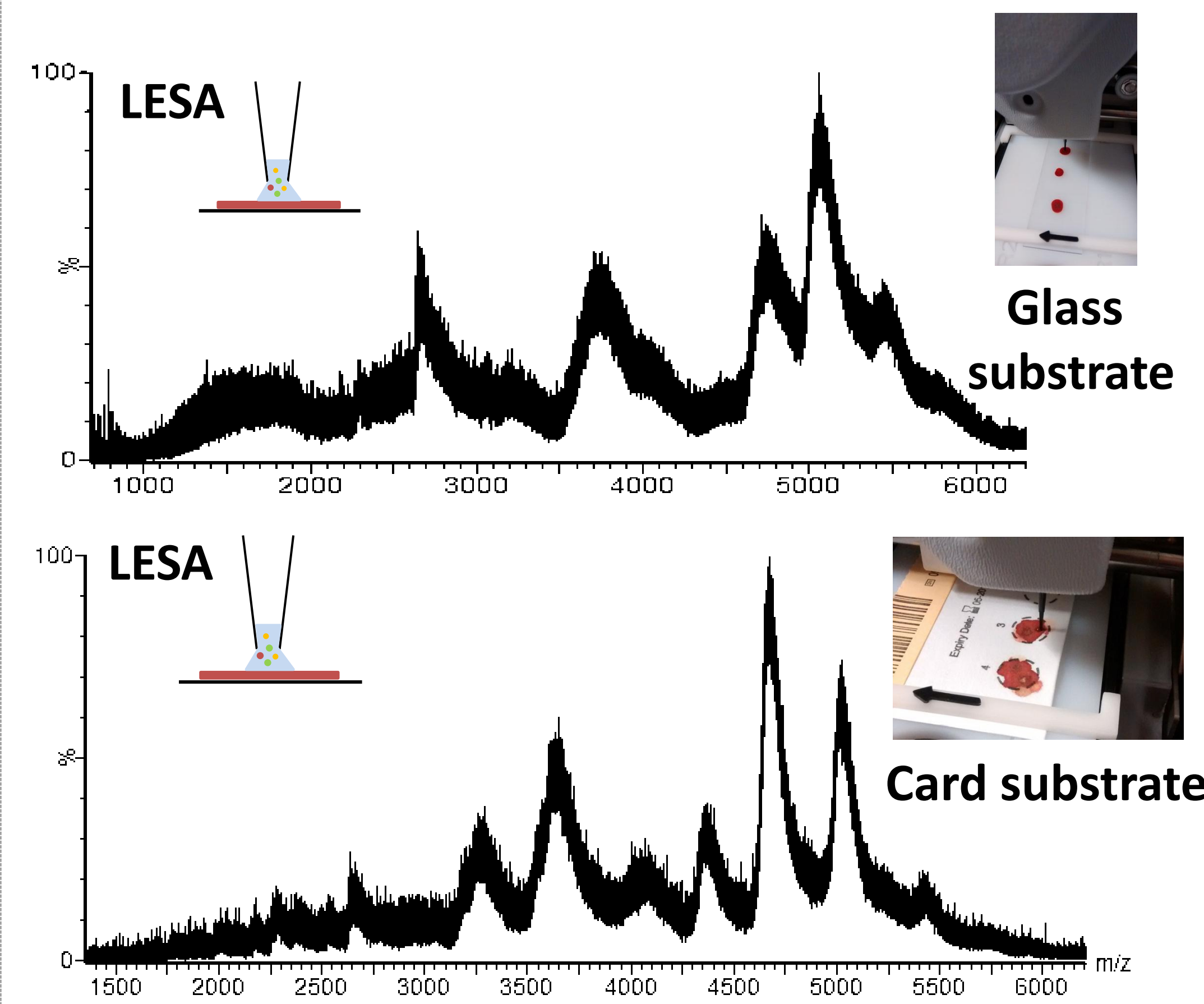


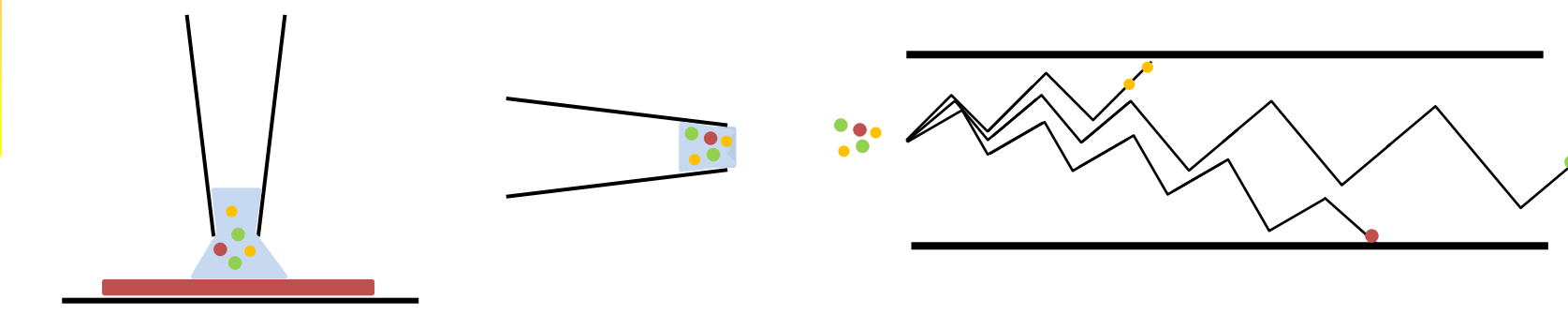
Figure 2 Mass spectra showing ions detected by LESA-MS of DBS samples on a Synapt G2S mass spectrometer. Top: LESA sampling of DBS on glass, Middle: LESA sampling of DBS on card and Bottom: contact-LESA sampling of DBS on card.

Conclusions

Glass is a more convenient substrate as the drying time is much shorter; however, decreased s:n leads to poorer data quality.

Implementation of the contact-LESA method⁶ led to significant improvements in spectral quality for detection of the intact haemoglobin tetramer.

LESA-FAIMS-MS



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 filter or focus ions. By varying both the dispersion field (DF) and the 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.

Mass Spectra

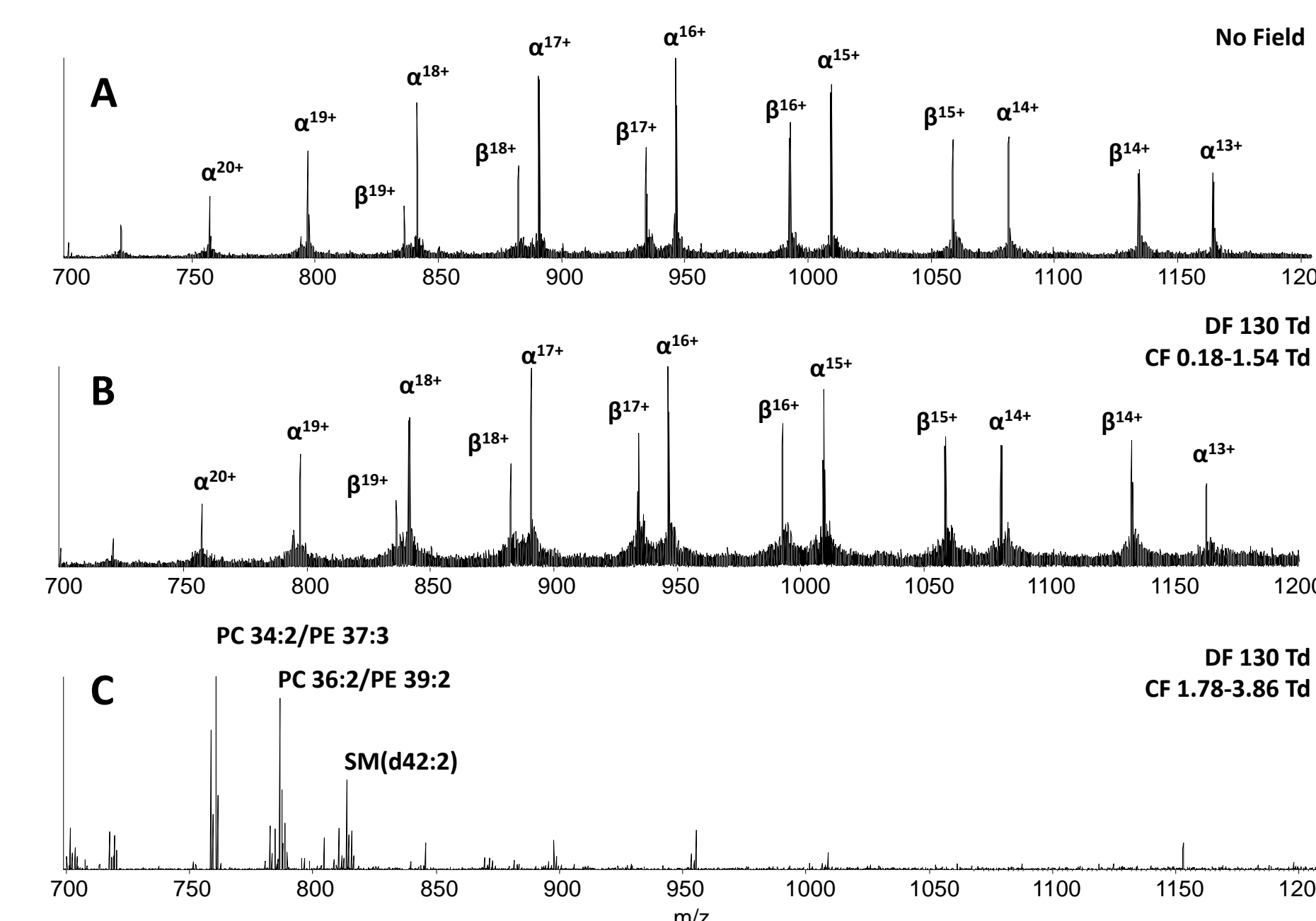


Figure 3 Mass spectra acquired during a 2D mode LESA-FAIMS experiment of a DBS sample on card.

Lipid Identification

Detected m/z	Calculated m/z	ppm error	Assignment
758.5669	758.5694	-3.3	PC 34:2 or PE 37:3 M+H
760.5826	760.5851	-3.3	PC 34:1 or PE 37:2 M+H
782.5668	782.5694	-3.3	PC 36:4 or PE 39:4 M+H
784.5824	784.5851	-3.4	PC 36:3 or PE 39:3 M+H
786.5981	786.6007	-3.3	PC 36:2 or PE 39:2 M+H
787.6661	787.6687	-3.3	SM(d40:1) M+H
788.6137	788.6164	-3.4	PC 36:1 or PE 39:1 M+H
810.5979	810.6007	-3.5	PC 38:4 or PE 41:4 M+H
811.6664	811.6687	-2.8	SM(d42:3) M+H
813.6815	813.6844	-3.6	SM(d42:2) M+H
815.6971	815.7000	-3.6	SM(d42:1) M+H

Table 1 Lipid species identified in LESA FAIMS mass spectrometry analysis of DBS at DF = 130 Td and CF 1.78-3.86 Td. A number of phospholipid (PC and/or PE) and sphingomyelin (SM) species are identified within 5 ppm mass error by cross referencing with the LIPID MAPS database.

Conclusions

Incorporation of FAIMS in the workflow enables gas-phase separation of lipid and protein molecular classes, enabling analysis of both haemoglobin and a range of lipid (PC or PE, and SM species) from a single extraction sample. The work has implications for multiplexed clinical assays of multiple analytes.

Total Ion Transmission Maps

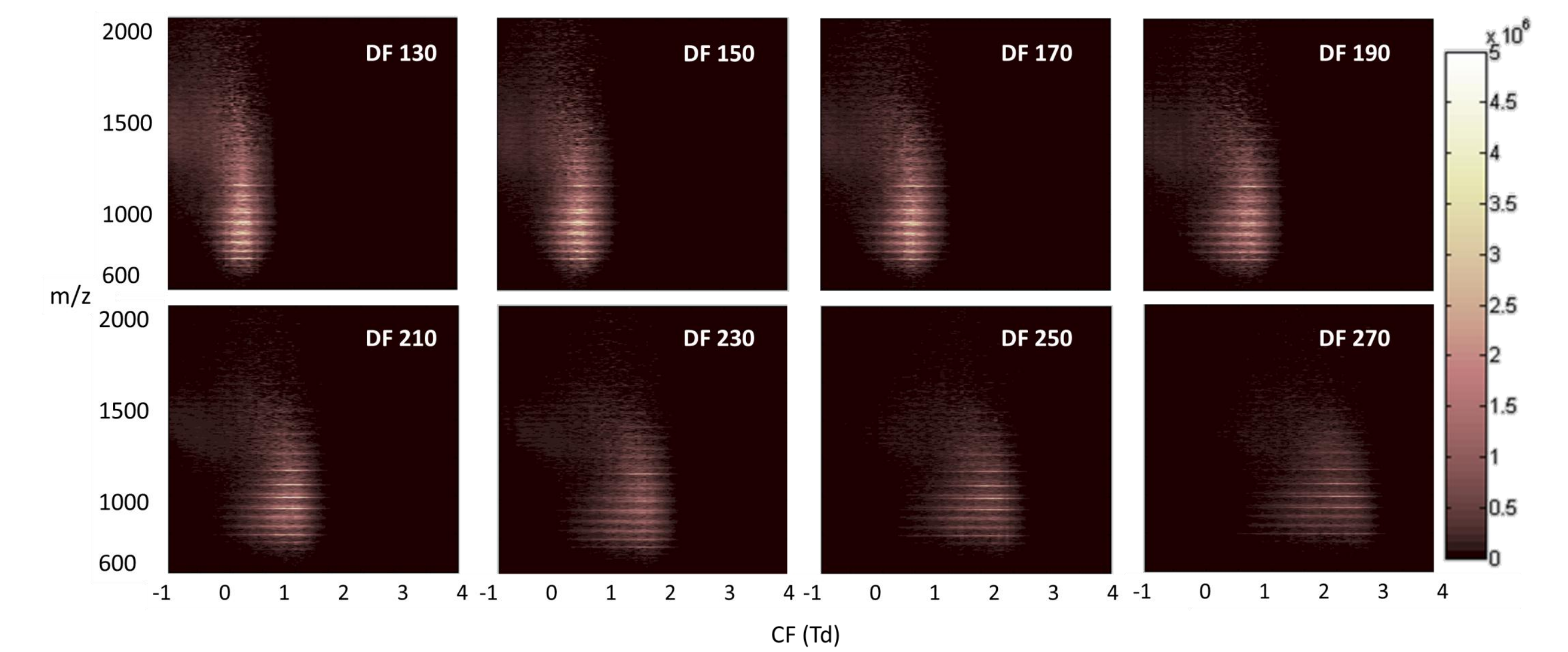


Figure 4 Total ion transmission maps showing ions transmitted over CF ranging from -1 to 4 Td from m/z 600 to 2000. The transmission of ions at DFs from 130 Td to 270 Td in steps of 20 Td are shown.

Single Ion Transmission Maps

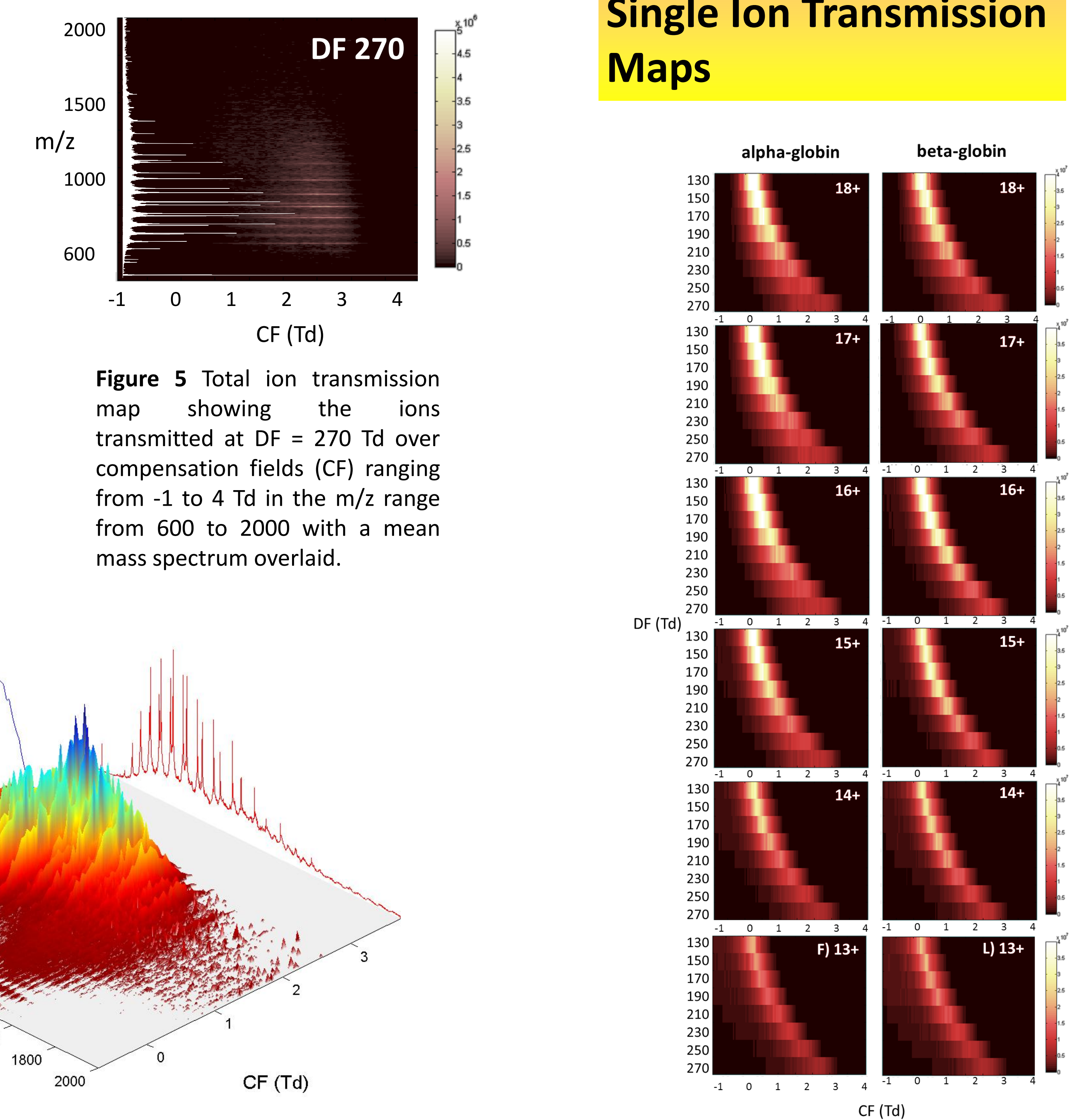


Figure 5 Total ion transmission map showing the ions transmitted at DF = 270 Td over compensation fields (CF) ranging from -1 to 4 Td in the m/z range from 600 to 2000 with a mean mass spectrum overlaid.

3D Plot

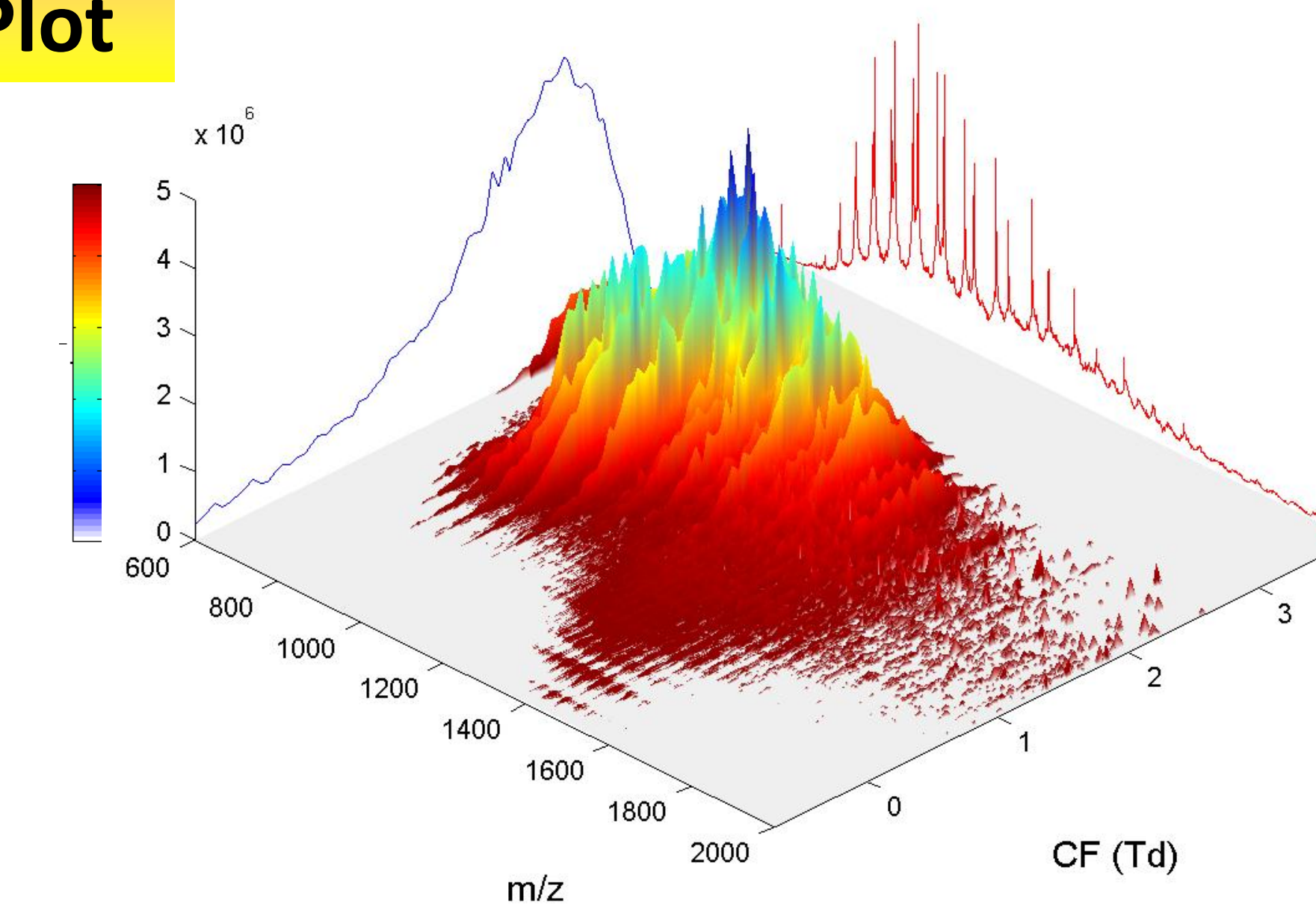


Figure 6 Total ion transmission map showing the ions transmitted at DF = 130 Td in the m/z range 600 to 2000 (x axis) and compensation fields (CF) range -1 to 4 Td (y axis). The relative peak intensity is indicated (between 0 and 1) in the z axis. The mean mass spectrum of detected ions is also projected.

Figure 7 Single ion transmission maps showing the transmission of α - and β -globin protein ions between DF 130 Td to 270 Td (step size of 20 Td) and with varying CF between -1 to 4 Td. A variety of charge states, from +13 to +18, are shown.