Advances in Multi-mode Mass Spectrometry for Tissue Imaging Studies

*by E.Claude, E. Jones, M. Towers and J.I. Langridge**
Waters MS Technologies, Stamford Avenue, Wilmslow, Cheshire, SK9 4AX
**Corresponding author*

Liquid chromatography mass spectrometry (LC-MS) plays an important and increasing role in characterising complex biological mixtures at the protein and metabolite level. However, despite the comprehensive nature of this technique, due to the sample preparation required, the ability to determine the spatial localisation of these compounds in tissues or biological specimens is typically lost. Imaging mass spectrometry (IMS) has been successfully used to overcome this, determining the spatial distribution of molecules within a variety of samples types, without the need of fluorescent or radioactive labelling normally used in histochemistry. In this article we present the combination of two complementary ionisation techniques (MALDI and DESI) that can be used in series on the same tissue section, for imaging mass spectrometry, determining the distribution of multiple classes of biological compounds.

Matrix assisted laser desorption ionisation (MALDI) coupled to MS is a powerful approach for the spatial localisation of a wide range of compounds [1-2]. The molecular distribution of molecules is determined by rastering a laser across a section of tissue, recording a mass spectrum at each position. This provides an X, Y (2D) spatial distribution of the molecules present on the tissue surface. In particular, this approach has been used to study proteins and associated tryptic peptides allowing the protein content of tissues to be explored [3]. It has also been used for other molecules including lipids, DNA and metabolites, however, there are limitations for low mass analysis due to matrix interferences.

More recently the uses of alternative, ambient ionisation techniques, such as desorption electrospray ionisation (DESI) [4] have allowed metabolites and lipids to be studied with high sensitivity and specificity. The advantages of the ambient DESI approach are that minimal sample preparation is required, no matrix is needed, and in addition the technique can also be non-destructive [5-6]. The lack of matrix ions allows a clear picture of metabolites and lipids at relatively low *m/z* to be established, while the non-destructive nature of DESI allows previously analysed tissue sections to be studied by other imaging modalities both MS and non-MS approaches. For example, matrix could be applied and the section could be studied by MALDI MS, or the

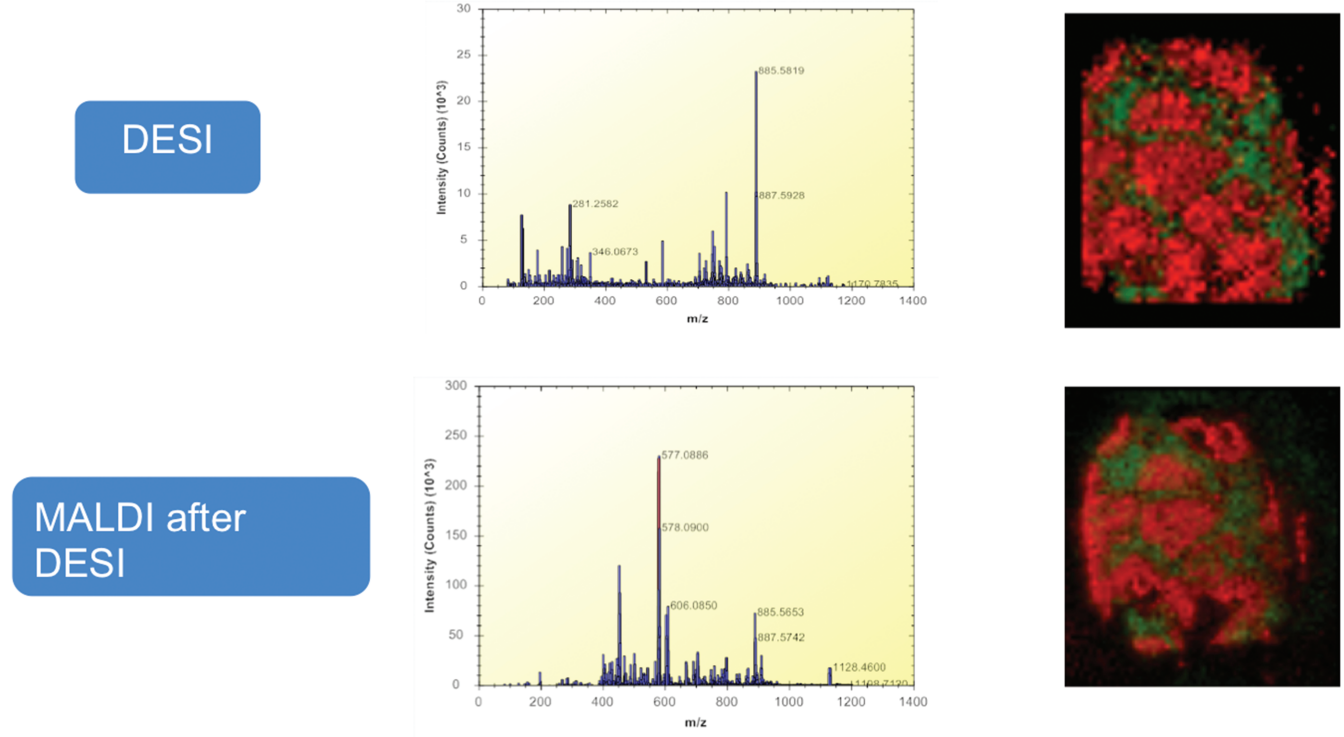


Figure 1: Imaging MS analysis of a human tissue section by a) DESI ionisation and b) MALDI ionisation on the tissue section subsequent to the DESI analysis.

section could easily be subjected to chemical staining, such as Hematoxylin & Eosin (H&E) staining.

Experimental

MALDI MS

Tissue sections were fixed/washed for one minute with 70% cold EtOH followed by 90% cold EtOH to remove part of the salt present on the tissue. A further wash was carried out for 15 seconds in chloroform to remove the highly abundant lipid species present in the tissue. Tissue sections were fully dried in the desiccator prior to tryptic digestion.

Trypsin (Promega) was dissolved in 50 mM ammonium bicarbonate to a concentration of 20 µg/mL with 0.005% of Octyl-α/β-glusocise 10mM solution (Sigma). Ten layers of the trypsin solution were deposited using a nebulising spray device (SunCollect™ (SunChrom, Germany)) with the following flow rate: 5 µL/min first layer, 10 µL/min second layer and 15 µL/min for the remaining layers.

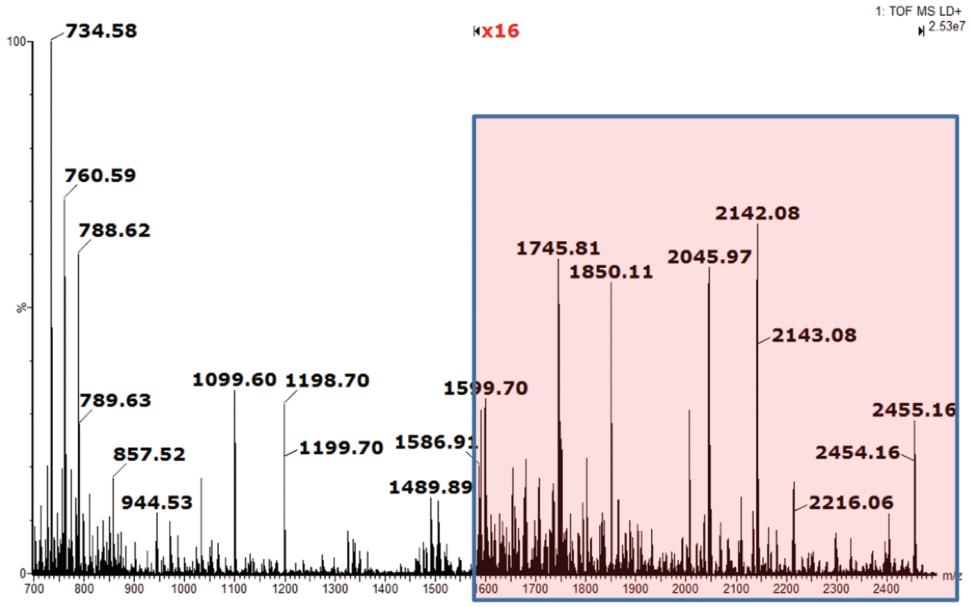


Figure 2: MALDI imaging of an on-tissue tryptic digest from a rodent brain section analysed in positive ion mode. Significant peptide complexity can be observed

The tissue samples were then incubated overnight at 37°C in a homemade incubator.

Following desiccation, 15 layers of matrix solution (5 mg/mL of α -Cyano-4-hydroxycinnamic acid (CHCA) in 50/50/0.1 (v/v/v) water/methanol/trifluoroacetic acid + 0.0025% of Aniline) was sprayed with the same device described previously, but with the following flow rates: 10 µL/min first layer, 20 µL/min second layer and 25 µL/min for the remaining layers.

Data were acquired using the MALDI SYNAPT™ G2 HDMS (Waters Corporation, Wilmslow, UK) instrument in the HDMS

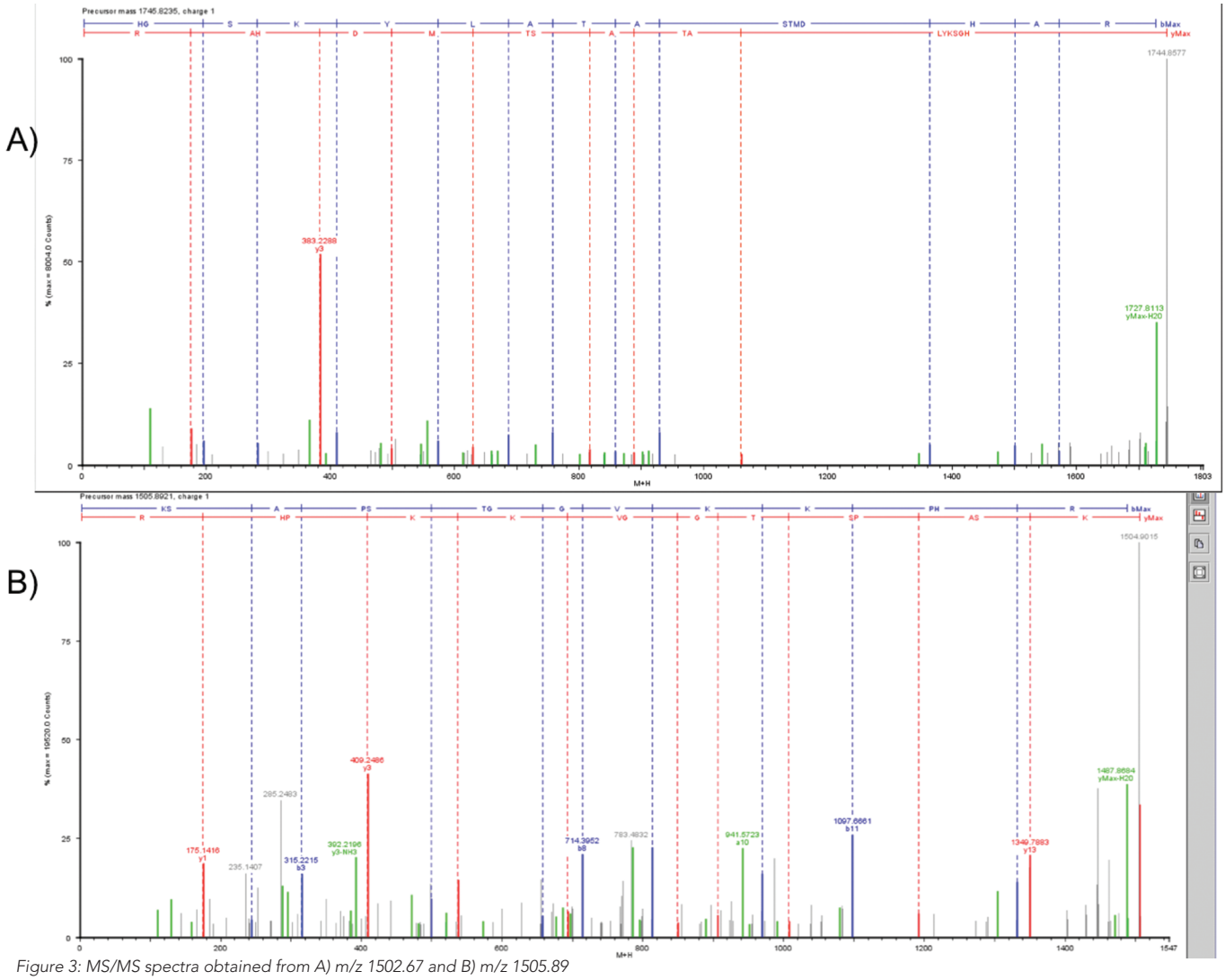


Figure 3: MS/MS spectra obtained from A) m/z 1502.67 and B) m/z 1505.89

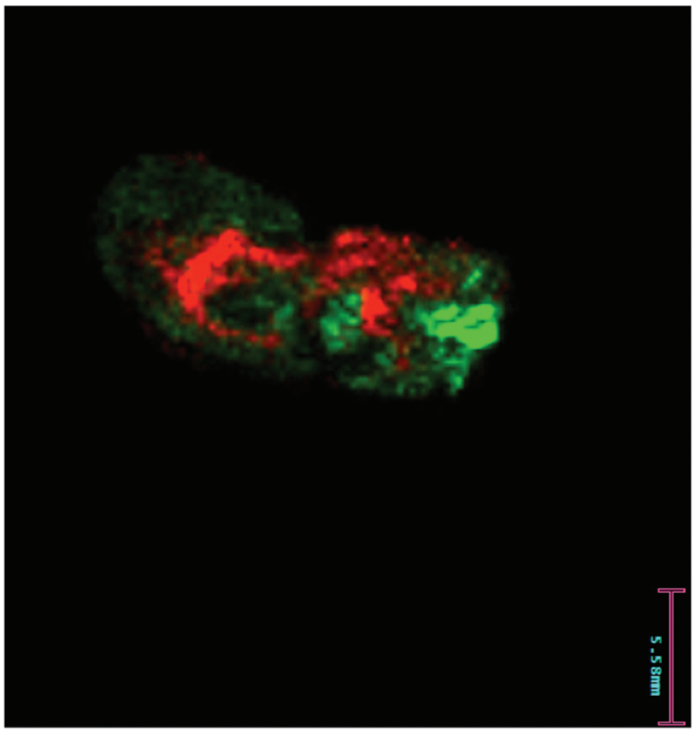


Figure 4: MALDI Imaging MS of tryptic peptides from a sagittal section of rodent brain (250µM spatial resolution)

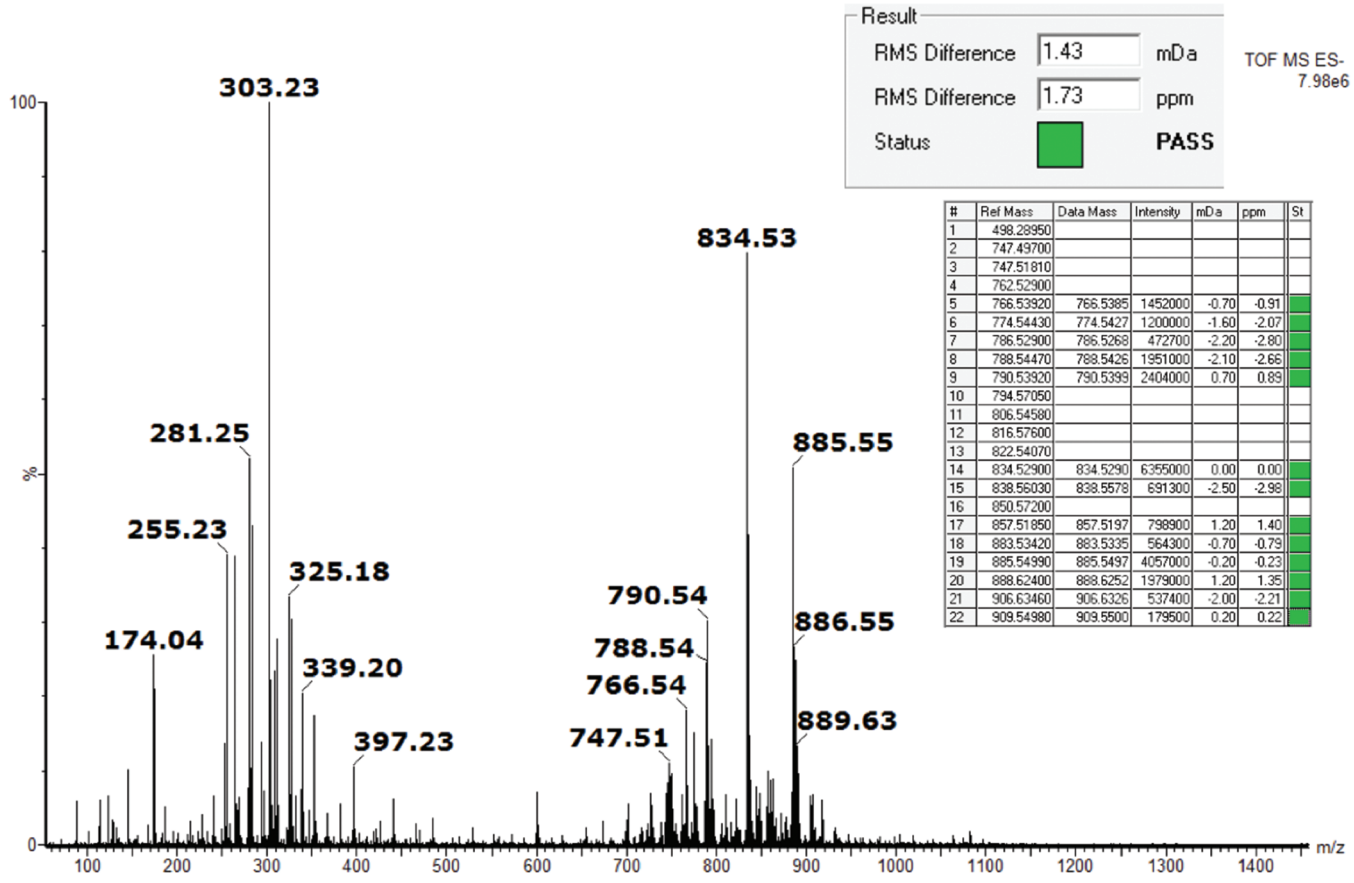
positive ion mode, over the m/z range 700 to 2,500, at an image resolution of 250 x 250 µm and a laser speed of 1 KHz.

DESI MS

Snap frozen tissue was sectioned on a cryo-microtome to 12 µm thickness and thaw mounted onto glass slide. The samples were stored at -80°C. Immediately prior to analysis the samples were brought to room temperature and placed onto the stage, no further sample preparation was required.

The 2D-DESI source was mounted onto a Waters Synapt G2-Si HDMS. DESI spray conditions were set at 3 µl/min, 90:10 MeOH: water, 120psi N_2 gas pressure and a voltage of 5kV for both polarities. Images were acquired at 70 µm spatial resolution with the pixel size determined in the X-direction by the speed of the stage movement (70 µm/s) and acquisition rate of mass spectra (1s). The Y-direction was defined by the distance of 70 µm between two lines of acquisition.

Ion mobility separation occurred in the high-efficiency T-Wave (IMS) cell, filled with nitrogen gas at a pressure of 3mbar. IMS provides an additional dimension of separation based on molecular size, shape and charge.



#	Ref Mass	Data Mass	Intensity	mDa	ppm	St
1	498.28950					
2	747.49700					
3	747.51810					
4	762.52900					
5	766.53920	766.5385	1452000	-0.70	-0.91	
6	774.54430	774.5427	1200000	-1.60	-2.07	
7	786.52900	786.5268	472700	-2.20	-2.80	
8	788.54470	788.5426	1951000	-2.10	-2.66	
9	790.53920	790.5399	2404000	0.70	0.89	
10	794.57050					
11	806.54580					
12	816.57600					
13	822.54070					
14	834.52900	834.5290	6355000	0.00	0.00	
15	838.56030	838.5578	691300	-2.50	-2.98	
16	850.57200					
17	857.51850	857.5197	798900	1.20	1.40	
18	883.53420	883.5335	564300	-0.70	-0.79	
19	885.54990	885.5497	4057000	-0.20	-0.23	
20	888.62400	888.6252	1979000	1.20	1.35	
21	906.63460	906.6326	537400	-2.00	-2.21	
22	909.54980	909.5500	179500	0.20	0.22	

Figure 5: Mass spectrum from the DESI negative mode analysis of rodent brain coronal tissue sections

Processing

Following the MALDI and DESI imaging acquisitions, raw data was processed using the Waters High Definition Imaging (HDI) software, with peak picking of the continuum raw data performed using the Apex 3D algorithm. Each subsequent component is described with a *m/z* and drift time (bin) value.

Results & discussion

The combination of DESI and MALDI ionisation for imaging mass spectrometry studies provides a wealth of complementary information. As previously mentioned DESI can be followed by MALDI as it is a nondestructive technique working at ambient pressures. Figure 1 shows an example of this where a section of human colorectal adenocarcinoma tissue has first been analysed by DESI and subsequently by MALDI. In this case both approaches ionise lipid molecules, as can be seen from the mass spectrum, but differential ionisation of species is observed as the mass spectra look different. Both techniques can determine the tissue morphology as shown in the images from the two techniques.

The real power of MALDI imaging as previously mentioned is in the study of protein and peptide distributions. To enable effective protein identification it is advantageous to analyse tryptic peptides produced from the on-tissue tryptic digestion of proteins [7]. Mass spectral data from the positive ion MALDI analysis of tryptic peptides produced by on-tissue trypsin digestion of sagittal sections from rodent brain is presented in Figure 2. In this case two molecular ions, *m/z* 1502.76 and *m/z* 1505.89 were selected and subjected to MS/MS for identification purposes (Figure 3). These were shown to originate from Myelin basic protein and Histone H3 respectively. From the corresponding ion images (Figure 4) it can be seen that Histone H3 predominantly localises in the cerebellum while Myelin basic protein is more widely distributed throughout the brain section. This analytical approach would also potentially allow for the study of epigenetic modifications on Histone molecules.

Complementary to the analysis of peptides and proteins by MALDI MS, DESI is ideally suited to the analysis of lipids. Figure 5 shows the negative ion DESI analysis of lipids present in coronal sections of rodent brain. Strong lipid related signals with good signal to noise are evident in the data. The vast majority of these correspond to phospholipid derived species. Figure

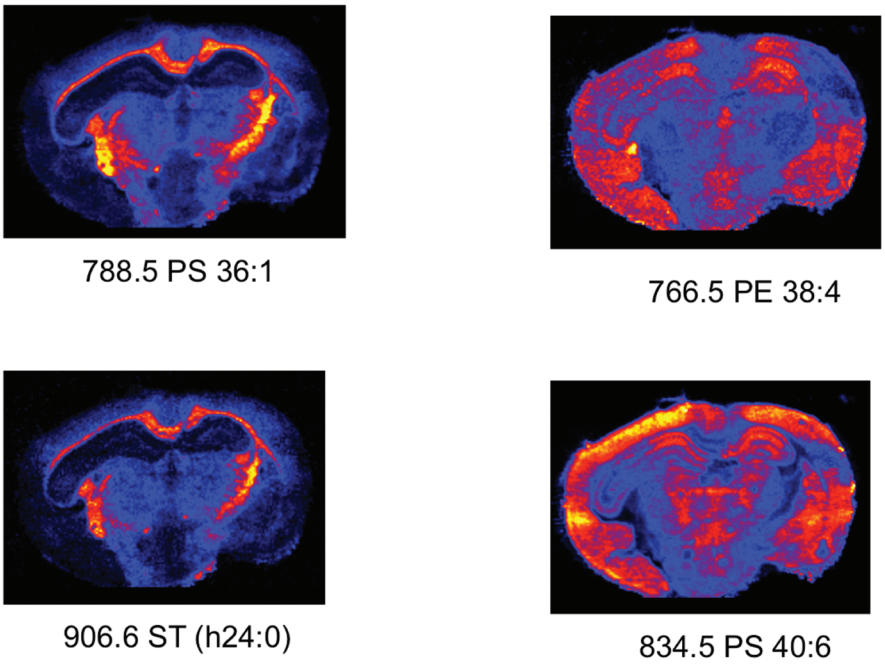


Figure 6: DESI negative mode analysis of rodent brain coronal tissue sections (70µm spatial resolution)

6 shows when investigating the spatial distribution of lipid related ions that an intense phosphatidylserine (PS) related ion *m/z* 834.4 (PS 40:6) clearly locates in the white brain matter. Other significant lipid species, such as phosphatidylethanolamine (PE38:4) *m/z* 766.5 also localise with the white matter. Conversely PS36:1 (*m/z* 788.5) and specific sulphatides (ST h24:0) localise strongly in the grey matter but are absent in the white brain matter. This is consistent with previous DESI studies [8] and these tissue specific lipids are typical of those seen in MS imaging experiments. Further correlation of the data with morphological features can be performed by co-registering the DESI imaging MS data with H&E stained images acquired from the same sections (Figure 7).

Conclusion

The results presented here show that a combination of different MS imaging modalities provides coverage of multiple compound classes. The two ionisation technologies are highly complementary and provide unique information sets that allow a more complete molecular picture of the tissue. The use of DESI imaging allows access to lipids and metabolites with high sensitivity and moderate spatial resolution, with a key attribute being the ability to combine the results with other imaging modalities on the same tissue section. As shown, the combination with MALDI imaging provides access to peptides and proteins, while H&E staining of the same tissue section allows detailed morphological analysis.

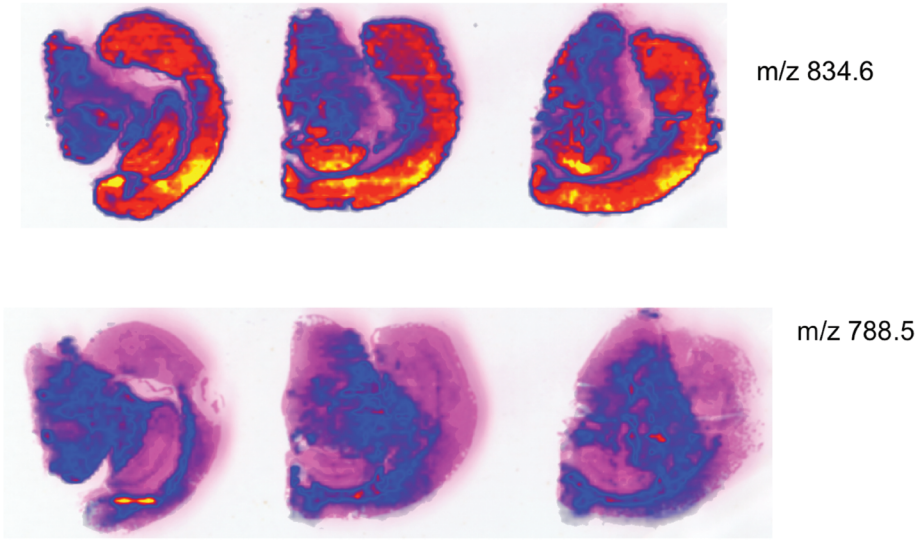


Figure 7. DESI imaging data co-registered with H&E images subsequently obtained from the same tissue section

The ability to define tissue specific molecules at multiple levels (lipids, metabolites and peptides) holds significant promise in multiple, wide-ranging, research disciplines, from environmental to pharmaceutical and clinical research. The approach holds particular promise for the development of an automated tissue classification system, based upon the molecular profiles obtained from Imaging MS experiments.

Acknowledgements

The authors wish to acknowledge Prof. Zoltan Takats (Imperial College, London) and Prof. Ron Heeren (Maastricht University) for access to samples and discussion.

References

[1] R.M. Caprioli, T.B. Farmer, J. Gile Anal Chem. (1997) 69 (23) 4751-4760

[2] M.L Pacholski, N. Winograd, *Chemical Reviews* (1999) 99:2977

[3] P. Chaurand, R.M. Caprioli, *Electrophoresis* (2002) 23 (18), 3125-3135

[4] Z. Takats, J.M. Wiseman, B. Gologan R.G Cooks, *Science* (2004) 306(5695) 471-473

[5] S. Gerbig, et al. Anal Bioanal Chem (2012) 403 (8) 2315-2325

[6] J.C. Vickerman, *Analyst* (2011) 136 (11):2199-2217

[7] B. Cllero-Pator, R.M.A. Heeren, *J. Proteome Res.* (2014) 13: 325-335

[8] L.S. Eberlin, D.R Ifa, C. Wu, R.G. Cooks, *Angew Chem Int Ed Engl.* (2010) 49(5): 873-876