# MS Imaging – DESI XS and SELECT SERIES MRT

## INTRODUCTION

The SELECT SERIES MRT (Figure 1) is a novel quadrupole Multi Reflecting Time-of-Flight (MRT) mass spectrometer. Ions are generated in the source and are passed through a second generation StepWave XS ion transfer optics which provides improved sensitivity, especially for more labile compounds. The first mass analyzer is a quadrupole that can be operated in both resolving and non-resolving modes Ions are subsequently transmitted through a series of RF stacked ring ion guides that lead to a segmented quadrupole gas cell that optimizes the spatial focusing of the ion beam. The ions are further collimated and transferred to the focusing region, through ion optics, where a proportion of them are injected into the MRT analyzer via a double orthogonal acceleration approach.

Unlike traditional Time-of-Flight (ToF), the gridless analyzer provides three dimensional focusing through multiple intra-ToF lenses allowing 46 reflections within the ToF and a flight path of over 47 meters with minimal losses. This enables extremely long flight times to be achieved, for example *m/z* 1000 has a flight time of approximately 1.37 ms. Reduced duty cycle is observed with these extended flight times, but this is recovered by employing an encoded pushing approach whereby ions are injected into the ToF prior to ions from previous pushes reaching the detector. The relative injection times are varied to encode the data allowing for accurate de-convolution providing mass spectra with high resolution (>200,000 FWHM) and excellent mass accuracy (<500 ppb).

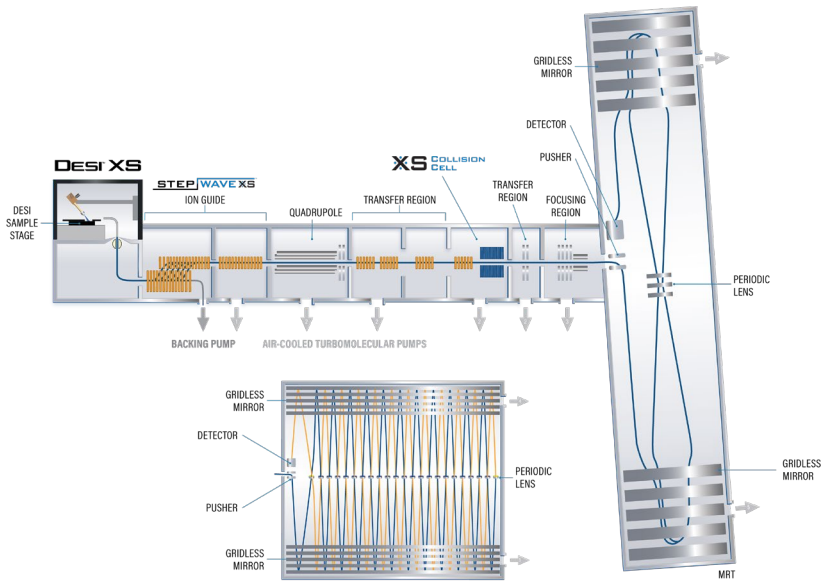


*Figure 1: SELECT SERIES MRT instrument schematic.*

Mass spectrometry imaging (MSI) is a technique used in mass spectrometry to locate and identify endogenous molecules, primarily in biological tissue sections. Typical samples include organ slices, tissue biopsies, and tissue microarrays. Samples require freezing and sectioning but no pre-separation of the analytes, as the retention of spatial information is key. The complexity of the samples requires high resolution mass spectrometry (>70,000 FWHM at *m/z* 200) and sub-ppm mass accuracy such that as many endogenous molecules as possible are resolved, detected, and identified.

Desorption electrospray ionization (DESI) was the first in a range of ambient ionization technologies that have allowed the direct analysis of samples providing a detailed chemical fingerprint of the composition of samples with minimal or no sample preparation. This is performed at atmospheric pressure and temperature and has been applied to a significant number of analytical challenges and applications. The use of DESI for imaging experiments has rapidly accelerated and this approach allows the detailed molecular distribution of a wide range of chemical constituents to be determined in a variety of sample matrices, and has been used for lipid profiling, neuropeptide and neurotransmitter analysis, protein analysis, and xenobiotic drug distribution and metabolism.

## EXPERIMENTAL CONDITIONS

| | |
|---|---|
| **Sample** | Mouse brain transverse section, 16 µm thickness (stained with hematoxylin and eosin (H&E) post-acquisition) |

**DESI Conditions**

| | |
|---|---|
| Heated Transfer Line temperature: | 330 ºC |
| Spray solvent: | 95:5 methanol: water + 0.1 % formic acid + 250 pg/µL leucine enkephalin (lockmass) |
| Spray solvent flowrate: | 2 µL/min |
| Pixel size: | 50 µm |
| Stage rate: | 100 µm/s |

**MS Conditions**

| | |
|---|---|
| Acquisition: | MS acquisition, in positive ion mode |
| Capillary voltage: | 0.65 kV |
| Nebulising gas: | 7.5 psi |
| Source temperature: | 100 ºC |
| Cone / source offset: | 30 V |
| Mass Range: | 50 – 2000 *m/z* |
| Acquisition rate: | 0.5s/scan |
| Acquisition / Processing Software: | MassLynx v4.2 SCN1024 / HDI v1.6 |

**HDI Processing parameters:**

| | |
|---|---|
| Number of most intense peaks: | 1000 |
| *m/z* window: | 0.05 Da |
| MS resolution: | 200,000 |

## RESULTS

MSI data were acquired and subsequently processed in Waters High Definition Imaging (HDI) software, and a composite image displaying the biolocalization of several phosphocholine (PC) lipids including; PC (38:5), *m/z* 808.59; PC (36:2), *m/z* 788.62; PC (38:6), *m/z* 806.57; PC (40:6), *m/z* 834.60 and heme, *m/z* 616.18 is presented in Figure 2A.

The corresponding H&E stained tissue section is shown in Figure 2B. Selected biolocalized lipids were mass measured and their elemental composition determined. The proposed formulae were searched against the LipidMaps database (Lipidmaps.org) and mass errors were calculated (Table 1).

The observed RMS mass accuracy for these analytes was **392 ppb**. The mass spectrum for a representative lipid, PC (34:1) is shown in Figure 3A, its observed biolocalization is shown in Figure 3B and a zoom of the monoisotopic peak showing the MS resolution is displayed in Figure 3C showing a resolution **>200,000 FWHM**. A benefit of the high MS resolving power is demonstrated in Figure 4, where two lipids within 81 mDa are biolocalized without any interference, these lipids were mass measured and identified as PC (38:4), -473 ppb and PS (40:0), -71 ppb.

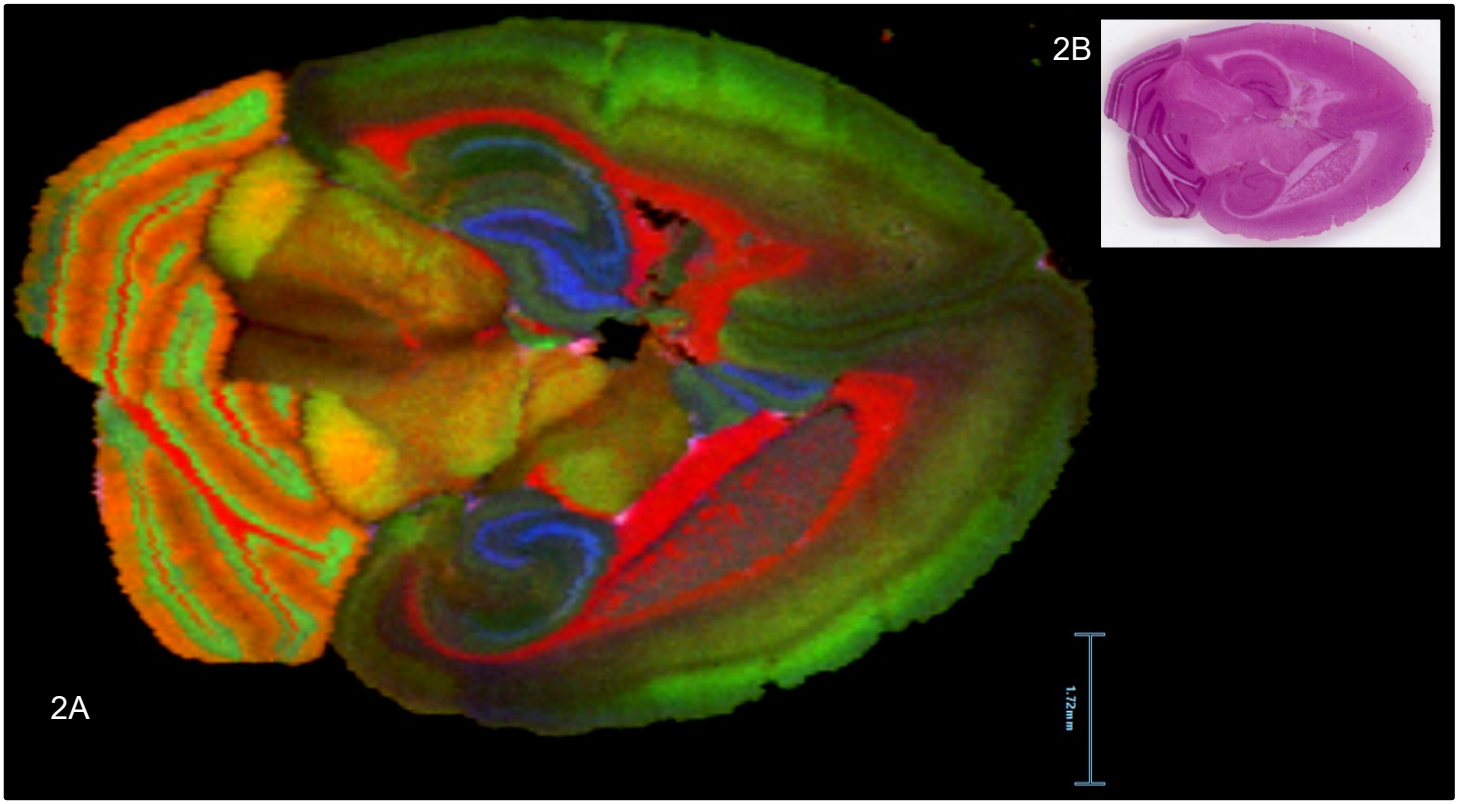


*Figure 2A: DESI MS imaging image of mouse brain (blue m/z 808.59 – PC (38:5); red m/z 788.62 – PC (36:2); green m/z 806.57 – PC (38:6); orange m/z 834.60 – PC (40:6); pink m/z 616.18 – heme). Figure 2B: H&E stained mouse brain section.*

| Putative ID | Formula | Adduct | Expected mass | Observed mass | mDa error | ppm error |
|---|---|---|---|---|---|---|
| PC (32:0) | $C_{40}H_{80}NO_8P$ | H+ | 734.5694315 | 734.569214 | -0.217 | -0.296 |
| PC (34:2) | $C_{42}H_{80}NO_8P$ | H+ | 758.5694315 | 758.569153 | -0.278 | -0.367 |
| PC (34:1) | $C_{42}H_{82}NO_8P$ | H+ | 760.5850815 | 760.584778 | -0.304 | -0.399 |
| PC (32:0) | $C_{40}H_{80}NO_8P$ | K+ | 772.5253131 | 772.524963 | -0.350 | -0.453 |
| PC (34:2) | $C_{42}H_{80}NO_8P$ | Na+ | 780.5513759 | 780.551636 | 0.260 | 0.333 |
| PC (36:2) | $C_{44}H_{84}NO_8P$ | H+ | 786.6007316 | 786.600403 | -0.329 | -0.418 |
| PC (36:1) | $C_{44}H_{86}NO_8P$ | H+ | 788.6163817 | 788.616028 | -0.354 | -0.448 |
| PC (34:2) | $C_{42}H_{80}NO_8P$ | K+ | 796.5253131 | 796.525085 | -0.228 | -0.286 |
| PC (34:1) | $C_{42}H_{82}NO_8P$ | K+ | 798.5409632 | 798.54071 | -0.253 | -0.317 |
| PC (36:4) | $C_{44}H_{80}NO_8P$ | Na+ | 804.5513759 | 804.551453 | 0.077 | 0.096 |
| PC (38:6) | $C_{46}H_{80}NO_8P$ | H+ | 806.5694315 | 806.569092 | -0.339 | -0.421 |
| PC (36:4) | $C_{44}H_{80}NO_8P$ | K+ | 820.5253131 | 820.524963 | -0.350 | -0.427 |
| PC (36:2) | $C_{44}H_{84}NO_8P$ | K+ | 824.5566133 | 824.556274 | -0.339 | -0.411 |
| PC (36:1) | $C_{44}H_{86}NO_8P$ | K+ | 826.5722633 | 826.571899 | -0.364 | -0.441 |
| PC (40:6) | $C_{48}H_{84}NO_8P$ | H+ | 834.6007316 | 834.600342 | -0.390 | -0.467 |
| PC (38:5) | $C_{46}H_{82}NO_8P$ | K+ | 846.5409632 | 846.540649 | -0.314 | -0.371 |
| PC (38:4) | $C_{46}H_{84}NO_8P$ | K+ | 848.5566133 | 848.556213 | -0.400 | -0.472 |
| | | | | Mean | -0.263 | -0.327 |
| | | | | SD | 0.174 | 0.216 |
| | | | | RMS | 0.315 | **0.392** |

*Table 1: Summary of lipid species mass accuracy.*

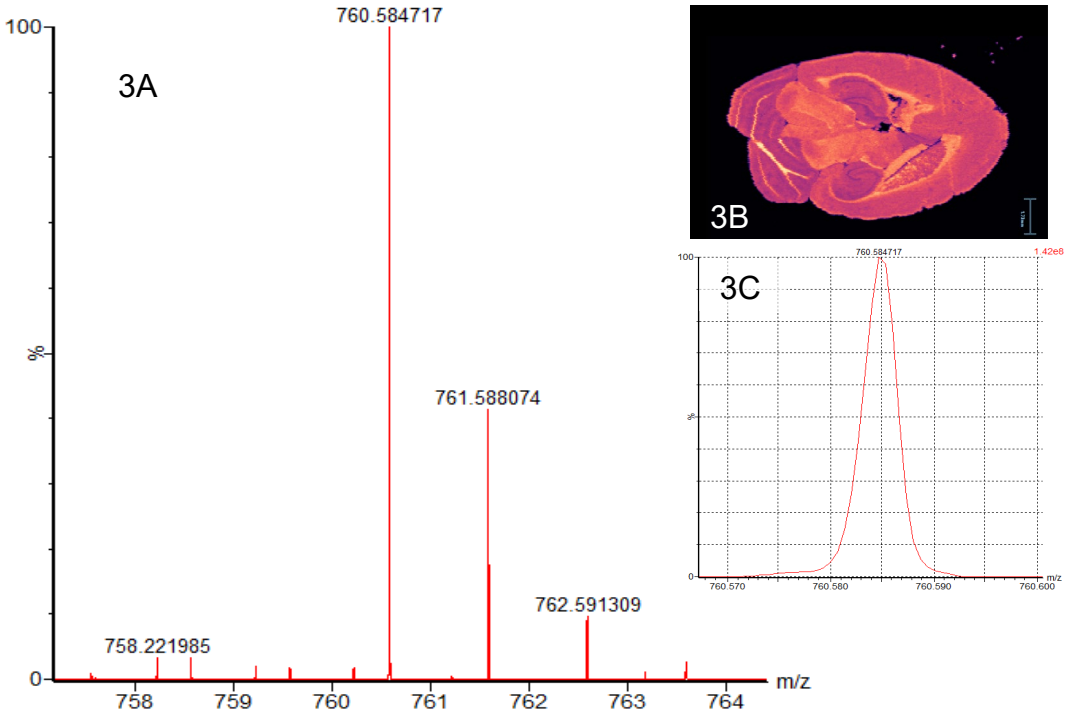


*Figure 3A: DESI MS imaging spectra identified as PC (34:1), 3B: biolocalization of PC (34:1) and 3C: MS resolution, observed half height 0.003784 corresponding to >200,000 FWHM.*

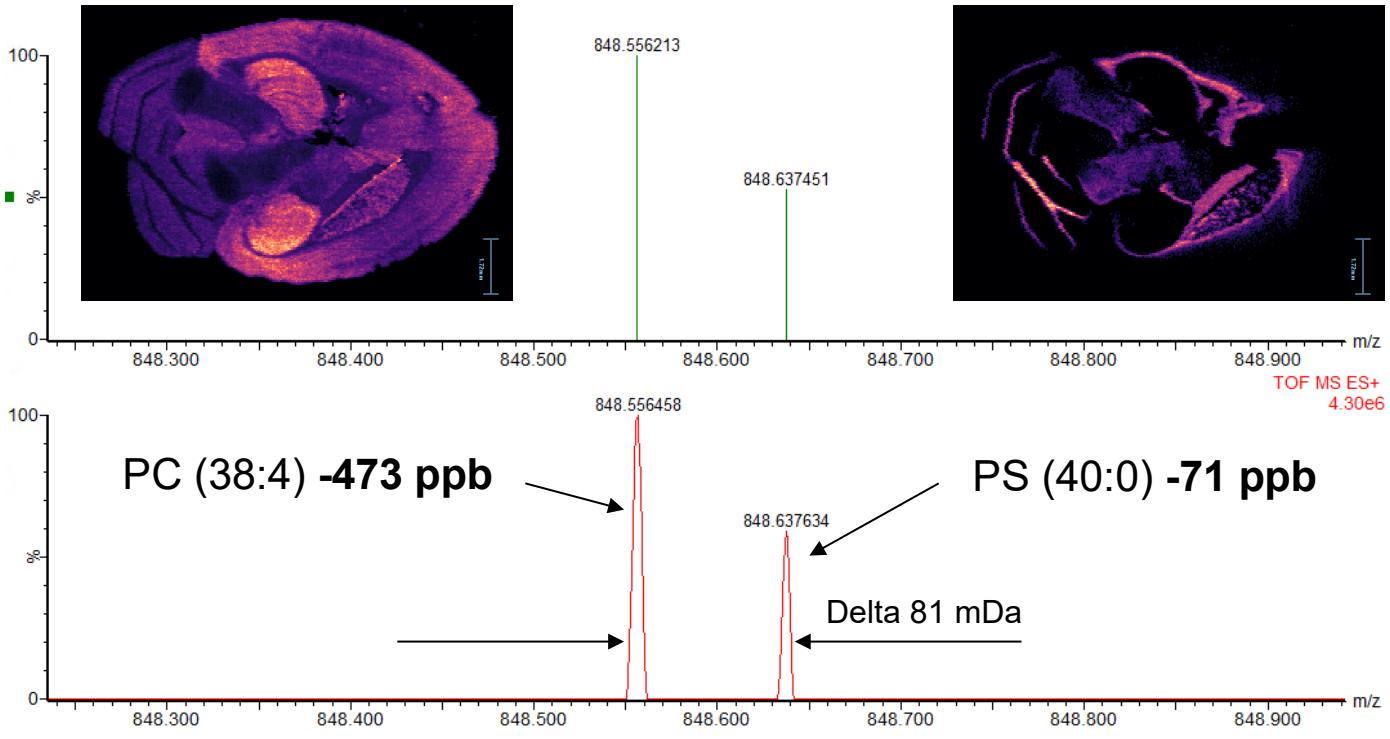


*Figure 4: High mass resolving power allows interference-free biolocalization of two lipids that differ by only 81 mDa. PC (38:4) $+K^+$, expected mass: 848.556614, observed mass: 848.556213, mass error: -0.401 mDa, -473 ppb and PS (40:0) $+H^+$, expected mass: 848.637511, observed mass: 848.637451, mass error: -0.06 mDa, -71 ppb.*

[ PRODUCT SOLUTION ]

SELECT™ SERIES MRT


## SUMMARY

It remains a key analytical challenge in MSI to be able to confidently identify species from complex samples where prior chromatographic separation is not possible. Here we have demonstrated the ability to add unprecedented specificity to the mass spectrometry imaging of biological tissue sections with the combination of DESI XS and the ultra-high resolution and mass accuracy of the SELECT SERIES MRT.

The DESI XS ambient ion source provided excellent spatial resolution resulting in high quality molecular images of mouse brain tissue sections with very limited sample preparation. This in combination with the SELECT SERIES MRT enabled the localization of 17 putative lipid species with superb mass accuracy (< 400 ppb RMS). The >200,000 FWHM resolving power of the SELECT SERIES MRT demonstrated the ability to localize lipids differing by only 81 mDa, revealing previously unseen molecular detail. This high performance imaging system constitutes a significant step forward in visualizing the spatial distributions of confidently identified molecular species.


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