MS Imaging – The SELECT SERIES™ MALDI and MRT

INTRODUCTION

The SELECT SERIES MRT is a state-of-the-art hybrid quadrupole Multi Reflecting Time-of-Flight (Q-MRT) mass spectrometer capable of ultra-high mass resolving power and accuracy independent of acquisition speed. The routine mass resolving power of the MRT analyser of greater than 200,000 (FWHM), and mass accuracy in the parts-per-billion range, is afforded by a ToF flight path of over 47 meters which is delivered in a compact geometry of around $1m^2$. The mass analyzer uses two gridless ion mirrors which reflect ions 46 times without the losses associated with field-demarking grids common to traditional ToFs.

The MRT is available as a mass spectrometry imaging (MSI) system compatible with both the DESI XS ion source and the purpose-built SELECT SERIES MALDI source. The combination of these two source options provides full spectrum molecular imaging - a means to gain wide compound coverage for a range of problems in the field of spatial molecular profiling.

Due to the absence of any upfront separation MSI spectra are highly complex. The high resolving power of the MRT tackles this complexity by decreasing the overlap of analyte signals leading to clearer images and reduced background interference whilst the mass accuracy enables a higher confidence in compound identification.

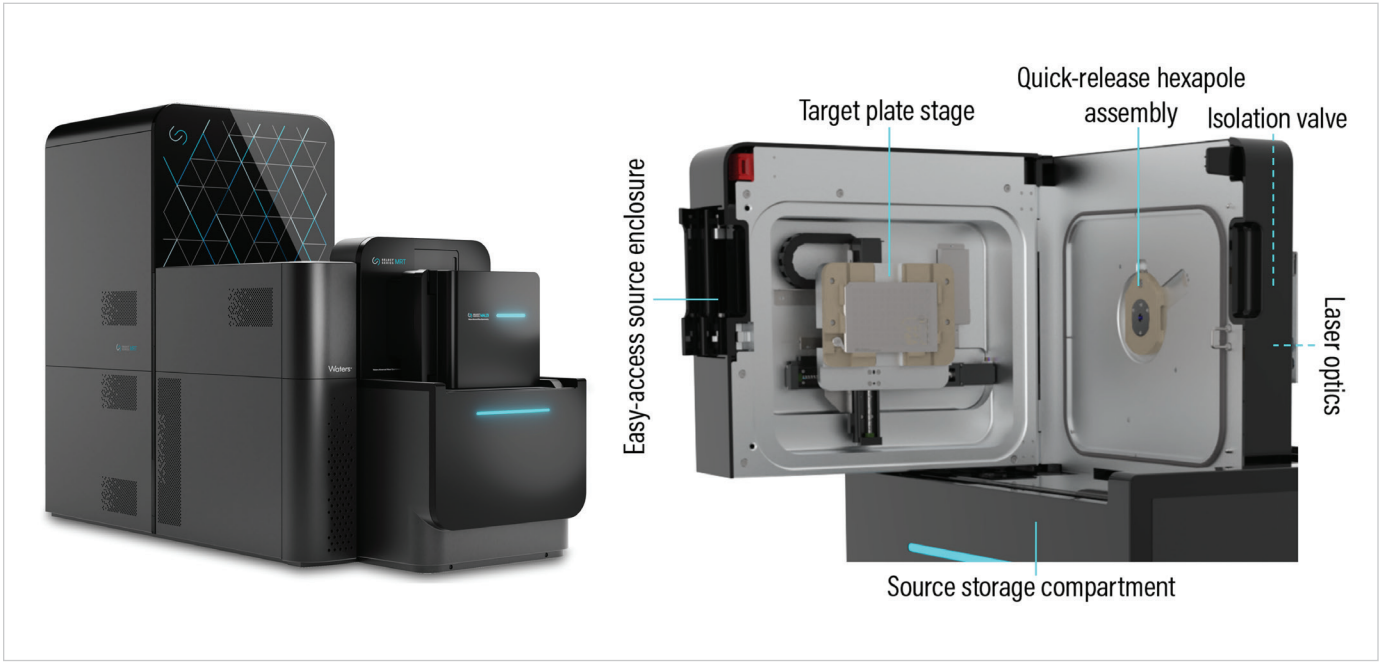

Figure 1. The SELECT SERIES MRT with the SELECT SERIES MALDI source.

The SELECT SERIES MALDI was designed from the ground up, and is a highly flexible source incorporating a robust 2.5 kHz solid state Nd:YAG laser system delivering spatial resolutions in the range of <15 to >100 µm. As well as generating high quality imaging data the source has been designed with robustness and usability in mind. The source is equipped with an isolation valve, enabling easy access for loading and unloading sample target plates without breaking vacuum. Furthermore, the source hexapole assembly can be easily removed, without the need for tools, and cleaned to maximise instrument up time and system robustness.

In the MALDI configuration the ions generated in the source are first transmitted through the hexapole ion guide into the instrument. The StepWave™ XS ion guide efficiently transfers ions from the hexapole towards the quadrupole which can be operated in resolving (MS/MS) and non-resolving modes. Ions are transferred through a series of ion guides before being introduced into the XS collision cell which can be used to generate product ions by collision-induced dissociation.

Ions are then focused into the double-orthogonal accelerator, which begins the high-resolution mass separation. Gridless ion mirrors and a series of periodic lenses act to confine the ions in a zig-zag-like geometry until they strike the detector. The extended flight length of the MRT analyser leads to long flight times on the order of milliseconds.

The SELECT SERIES MRT employs 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 in the parts-per-billion range.

This document describes the MALDI MRT system as applied to mass spectrometry imaging of murine brain sections. The high mass resolving power and accuracy, at fast scan speeds is demonstrated showing how the system provides excellent quality imaging data.

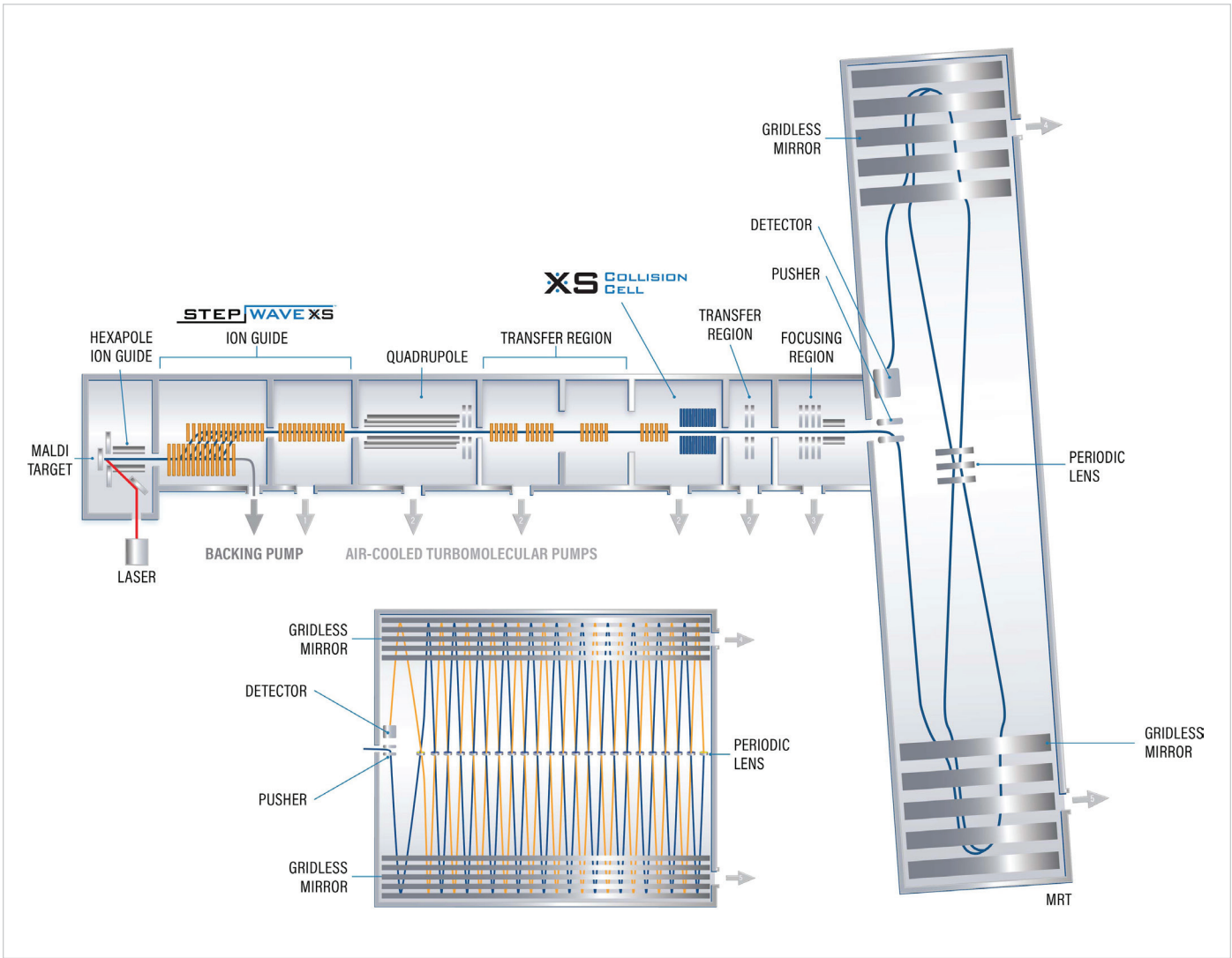

Figure 2: SELECT SERIES MRT with SELECT SERIES MALDI source

EXPERIMENTAL CONDITIONS

Sample	Mouse brain transverse section, 12 µm thickness

MALDI Conditions	
Matrix	2,5-dihydroxybenzoic acid (applied with HTX M5™ automated sprayer)
LASER repetition rate	2 kHz
LASER beam diameter	15/50 µm
MALDI pixel size	15/50 µm

MS Conditions	
Acquisition	MS acquisition (positive ion mode)
Sample plate voltage	20 V
Extractor	0 V
Hexapole DC	10 V
Hexapole RF	350 V
Mass range	50 – 2400 *m/z*
Acquisition rate	100 msec/scan
Acquisition / Processing Software	MassLynx™ v4.2 SCN1024 / HDI v1.6
Continuous lockmass correction	Internal PC (34:1) $[M+K]^+$ at 798.540963 *m/z*

HDI Processing parameters	
Number of most intense peaks	1000
m/z window	0.005 Da
MS resolution	200,000

RESULTS

MALDI-MSI data were acquired on two transverse murine brain sections at 15 and 50 µm pixel resolution at a scan rate of 10 Hz. The time taken to complete each image is related to the number of pixels generated, which for the 15 µm experiment was approximately 410,000 (12 hrs) vs approximately 44,000 (80 minutes) for the 50 µm experiment. The capability to generate these high quality images in such short times at 200,000 FWHM mass resolution is unprecedented for an MSI system. Figure 3A shows a mass spectrum from a single row of the 15 µm image. The main species detected are lipids, with an envelope of signals between 700 and 900 *m/z*. The inset shows the ultra-high resolution of the MS data at 200,000 FWHM. Molecular images were generated from these data by processing using Waters High Definition Imaging (HDI) software. Figure 3B shows a composite image of a number of putatively identified lipids from the 15 µm experiment, namely SM (34:1;O_2) in blue, PS (O-37:1) in green and PS (40:0) in red.

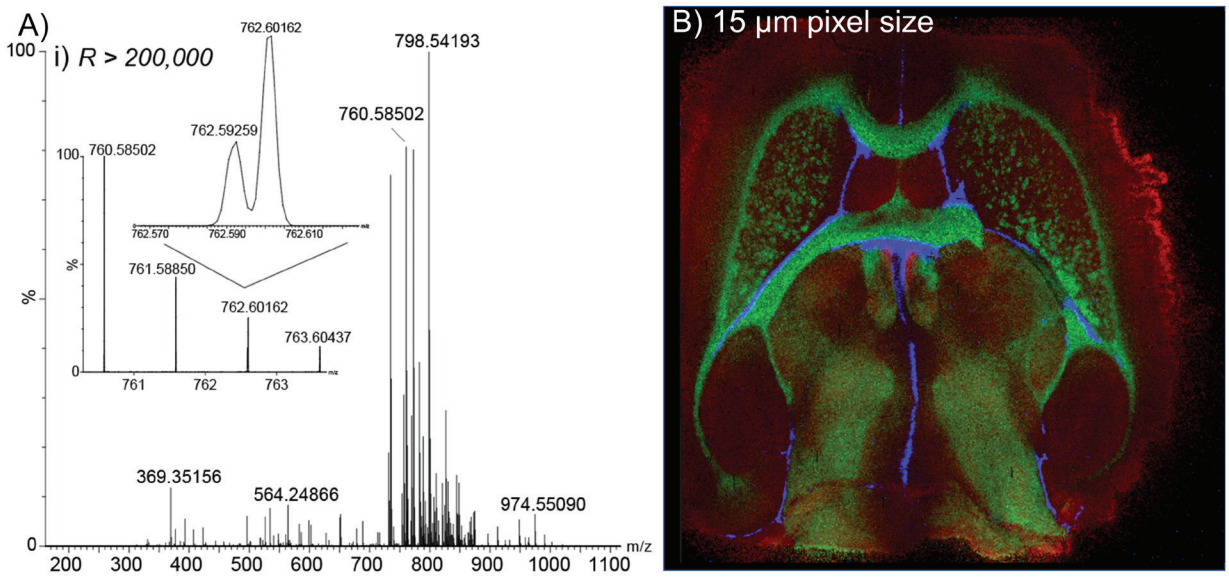

Figure 3: A) Example MALDI mass spectrum showing the ultra-high resolving power of the MRT analyzer from a single row of a transverse brain section. The lipid envelope is visible in the region 700-900 m/z. Inset: zoomed spectrum of PC (34:1) [M+H]+ demonstrating the resolving power of the system. B) 15 µm MALDI MS image of mouse brain (blue m/z 741.53064 – SM (34:1;O_2); red m/z 828.55170 - PS (O-37:1); green m/z 848.63800 – PS (40:0).

To demonstrate the mass measurement accuracy of the system we selected a number of detected lipids and searched their masses against the LipidMaps database (Lipidmaps.org). The putatively-identified lipids along with their chemical formulae and mass errors are presented in Table 1. The observed RMS mass accuracy for these analytes was 348 ppb, demonstrating the high identification confidence achievable on the MRT system.

Table 1 Summary of lipid species mass accuracy

Putative ID	Formula	Adduct	Expected mass	Observed mass	mDa error	ppm error
SM (34:1;O_2)	$C_{39}H_{79}N_2O_6P$	K+	741.53073	741.53064	-0.093	-0.125
PC (34:1)	$C_{42}H_{82}NO_8P$	H+	760.58508	760.58539	0.157	0.207
PC (32:0)	$C_{40}H_{80}NO_8P$	K+	772.52531	772.52551	0.197	0.255
PC (34:1)	$C_{42}H_{80}NO_8P$	NA+	782.56702	782.56750	0.473	0.606
PC (36:2)	$C_{44}H_{84}NO_8P$	H+	786.60073	786.60095	0.218	0.278
PC (36:1)	$C_{44}H_{86}NO_8P$	H+	788.61638	788.61676	0.378	0.480
PC (34:2)	$C_{42}H_{80}NO_8P$	K+	796.52531	796.52539	0.077	0.097
PC (34:1)	$C_{42}H_{82}NO_8P$	K+	798.54096	798.54120	0.237	0.297
PC (36:4)	$C_{44}H_{80}NO_8P$	NA+	804.55138	804.55182	0.444	0.552
PC (38:6)	$C_{46}H_{80}NO_8P$	H+	806.56943	806.56970	0.269	0.330
PC (38:4)	$C_{46}H_{84}NO_8P$	H+	810.60073	810.60077	0.038	0.047
PC (36:4)	$C_{44}H_{80}NO_8P$	K+	820.52531	820.52551	0.175	0.240
PC (36:2)	$C_{44}H_{84}NO_8P$	K+	824.55661	824.55688	0.267	0.323
PC (36:1)	$C_{44}H_{86}NO_8P$	K+	826.57226	826.57251	0.247	0.299
SHEXCER (37:6,O3)	$C_{43}H_{73}NO_{12}S$	H+	828.49262	828.49237	-0.254	-0.307

Putative ID	Formula	Adduct	Expected mass	Observed mass	mDa error	ppm error
PS (36:1)	$C_{42}H_{80}NO_{10}P$	K+	828.51514	828.51563	0.487	0.588
PS (O-37:1)	$C_{43}H_{84}NO_9P$	K+	828.55153	828.55170	0.172	0.208
PC (40:6)	$C_{48}H_{84}NO_8P$	H+	834.60073	834.60095	0.218	0.262
PC (38:6)	$C_{46}H_{80}NO_8P$	K+	844.52531	844.52563	0.317	0.375
PC (38:4)	$C_{46}H_{84}NO_8P$	K+	848.55661	848.55688	0.270	0.318
PS (40:0)	$C_{46}H_{90}NO_{10}P$	K+	848.637511	848.63800	0.489	0.576
				MEAN	0.221	0.275
				SD	0.164	0.201
				RMS	0.276	0.348

Further advantages of performing MSI with the MALDI-MRT are presented in Figure 4. Several isobaric lipid signals are observed within a narrow 86 mDa window and are putatively identified as SHexCer (37:6;O3) (828.49237 *m/z*), PS (36:1) (828.51568 *m/z*), PS (O-37:1) (828.55170 *m/z*) and the A+2 isotope of PC (36:1) (828.57086 and 828.57916 *m/z*). The high resolving power of the MRT analyzer enables each signal to be differentiated and uniquely biolocalized within the murine brain sections, providing high confidence molecular detail. The latter two signals at 828.57086 and 828.57916 *m/z* constitute fine isotope structure observed as a result of the PC (36:1) lipid being detected as a potassium adduct, with the 41K signal being resolved from that of the $^{13}C_2$. Interestingly, this signal pattern might be used to aid in spectrum assignment.

The individual molecular images for the lipid species can be seen in Figure 4. Firstly, the 50 µm data demonstrate the high speed with which high resolution MS data can be obtained, requiring just 78 minutes for this brain section. Secondly, the 15 µm data show the fine details that can be resolved spatially whilst maintaining high mass accuracy and resolution.

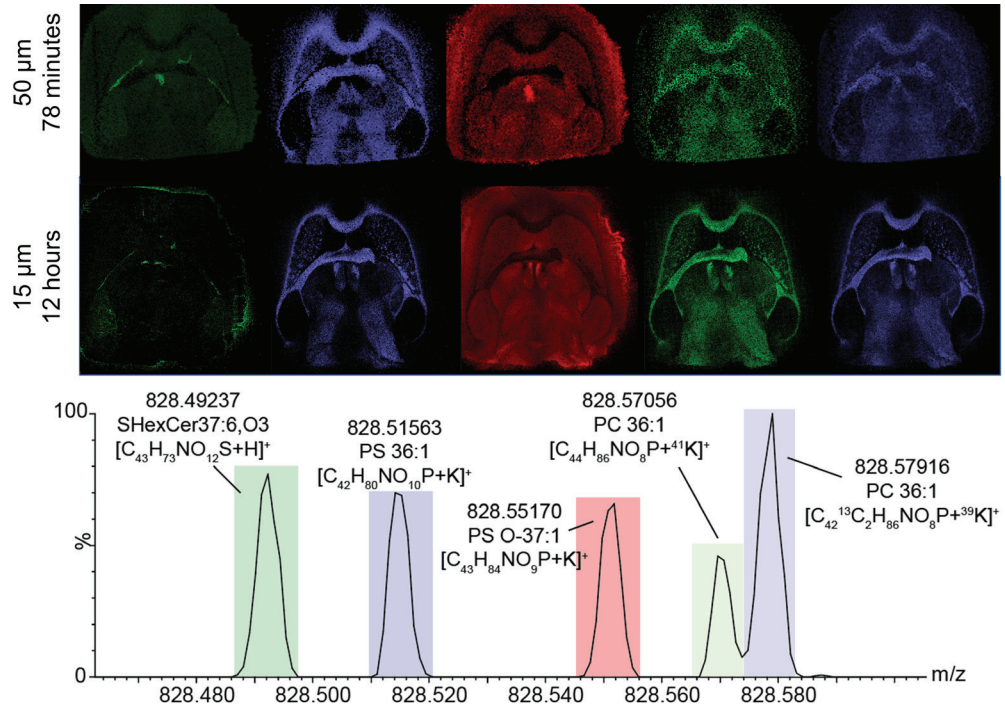

Figure 4: Ultra-high mass resolving power allows interference-free biolocalisation of isobaric lipid signals at high speed and spatial detail. SHexCer (37:6;O3) (828.49237 m/z, 125 ppb), PS (36:1) (828.51568 m/z, 588 ppb), PS (O-37:1) (828.55170 m/z, 208 ppb) and the A+2 isotope of PC (36:1) (828.57086 and 828.57916 m/z, 299 ppb).

SUMMARY

A key analytical challenge in MSI is the ability to confidently identify species from complex samples where prior chromatographic separation is not possible. Here we have demonstrated the addition of unprecedented specificity to the mass spectrometry imaging of biological tissue sections, independent of acquisition speed, with the combination of the SELECT SERIES MALDI source and the ultra-high resolution and mass accuracy SELECT SERIES MRT.

The SELECT SERIES MALDI is a robust, high performance, easy-to-use source which yields excellent spatial resolution resulting in high quality MALDI images of biological tissues. This in combination with the SELECT SERIES MRT enabled the localization of 21 putative lipid species with superb mass accuracy (<400 ppb RMS). The >200,000 FWHM resolving power of the MRT system demonstrated the ability to localize lipids differing by only 19 mDa, but also enabled the separation of fine isotopic structure (less than 9 mDa) at *m/z* 828, revealing previously unseen molecular detail. Equipped with the SELECT SERIES MALDI in addition to the DESI XS source the SELECT SERIES MRT provides a comprehensive full spectrum molecular imaging solution constituting a step-change in the visualization of molecular distributions of biological importance.

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