Waters™

Answering What and Where in complex samples: Advances in Imaging mass spectrometry and the impact of high resolution mass and ion mobility

Roy Martin

Sr Manager Biological Mass Spectrometry, Waters

Outline

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Part 1:

- Imaging and sample selection
- Sample prep and Ionization

Part 2:

- Technology for analysis

Part 3:

- Setup and data flow
- Considerations for quantitation

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SELECT SERIES Cyclic IMS

SELECT SERIES Cyclic IMS

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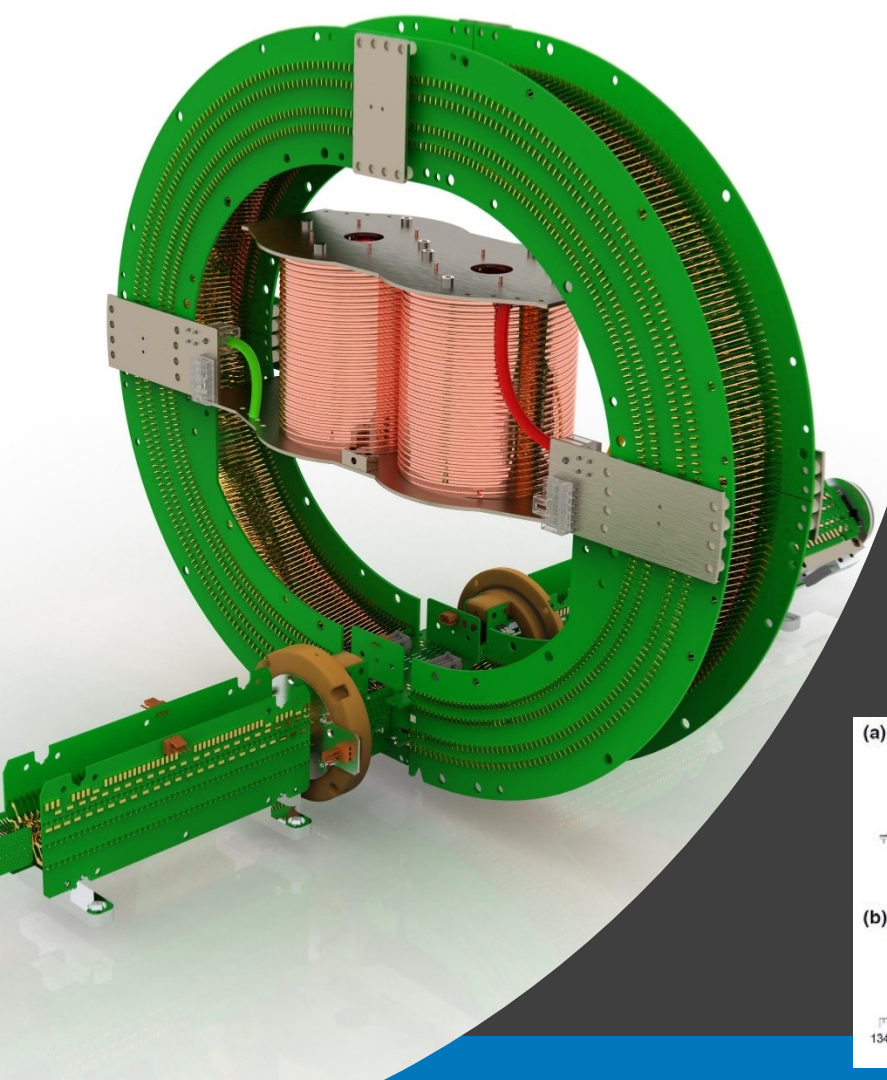
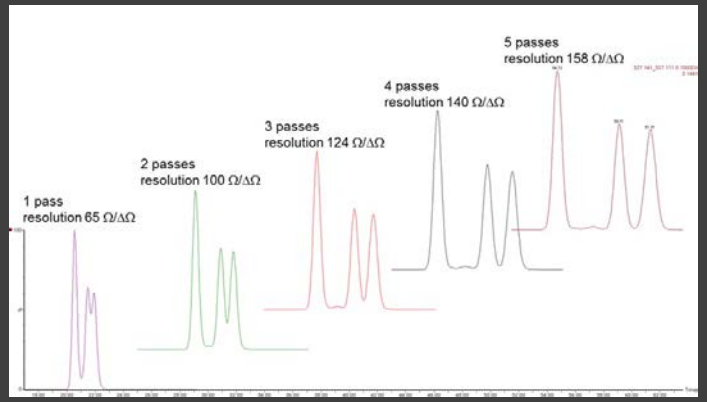
- The SELECT SERIES Cyclic IMS is a unique ion mobility mass spectrometer combining novel cyclic ion mobility with a new high performance oa-TOF

- Key features of Cyclic IMS
  - Ultra high ion mobility resolution
  - Novel multi-stage IMS^n capability
  - Superior Time of Flight performance

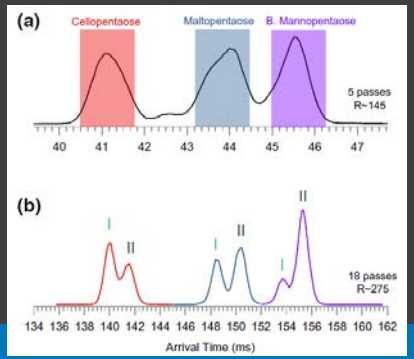


Cyclic IMS Experiments

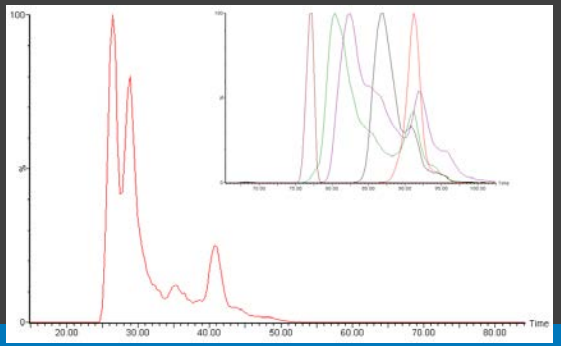
Single Pass and Multi-Pass



IMS^n Selection:



IMS^n with Activation:



DESI imaging
IMS selection and extended separation

Emrys Jones
& Jakub Ujma

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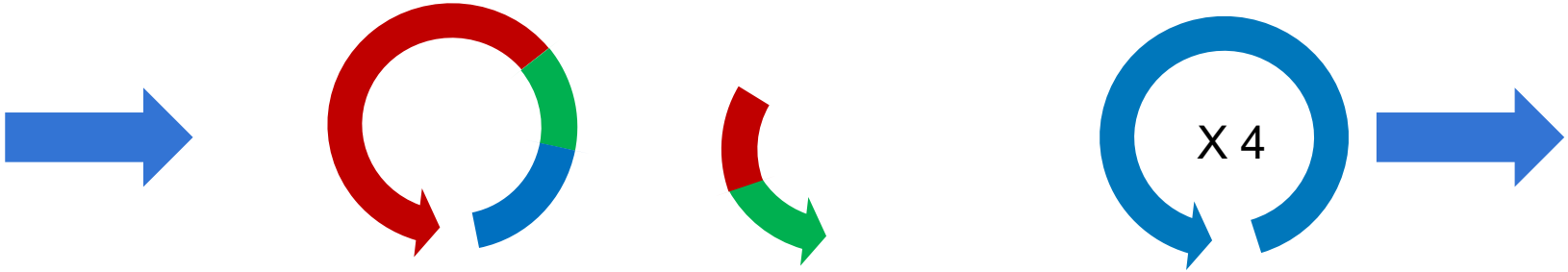


1. Inject into cIMS

2. One pass of cIMS, ejecting everything until the lipid cluster

3. Send lipids onto next cycle, eject everything after it

4. Do four more passes then eject and acquire

This is what the cyclic IMS sequence looks like

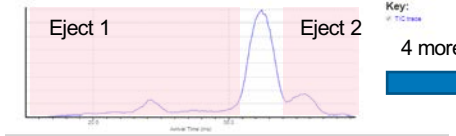
Sequence | Delete | Up | Down

	Time	Time Abs	Pre Array Gradient	Pre Array Bias	Array Entrance	Wave Amp	Array Offset	Array Mode	Array Exit	Post Array Gradient	Post Array Bias	
1 Inject	10.00	10.00	85.0	70.0	10	2	45	Forward	50	35.0	10.0	Reset
2 Separate	5.00	15.00	85.0	70.0	30		70	Sideways	30	35.0	10.0	Reset
3 Eject	13.00	28.00	85.0	70.0	50	25	45	Forward	2	35.0	10.0	Reset
4 Separate	2.00	30.00	85.0	70.0	30		70	Sideways	30	35.0	10.0	Reset
5 Eject	12.00	42.00	85.0	70.0	50	25	45	Forward	2	35.0	10.0	Reset
6 Separate	50.00	92.00	85.0	70.0	30		70	Sideways	30	35.0	10.0	Reset
7 Eject and Acquire	24.40	116.40	85.0	70.0	50	15	45	Forward Eject	2	35.0	10.0	Reset

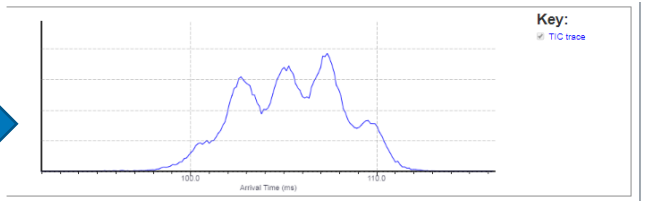
Display of XML | Show XML

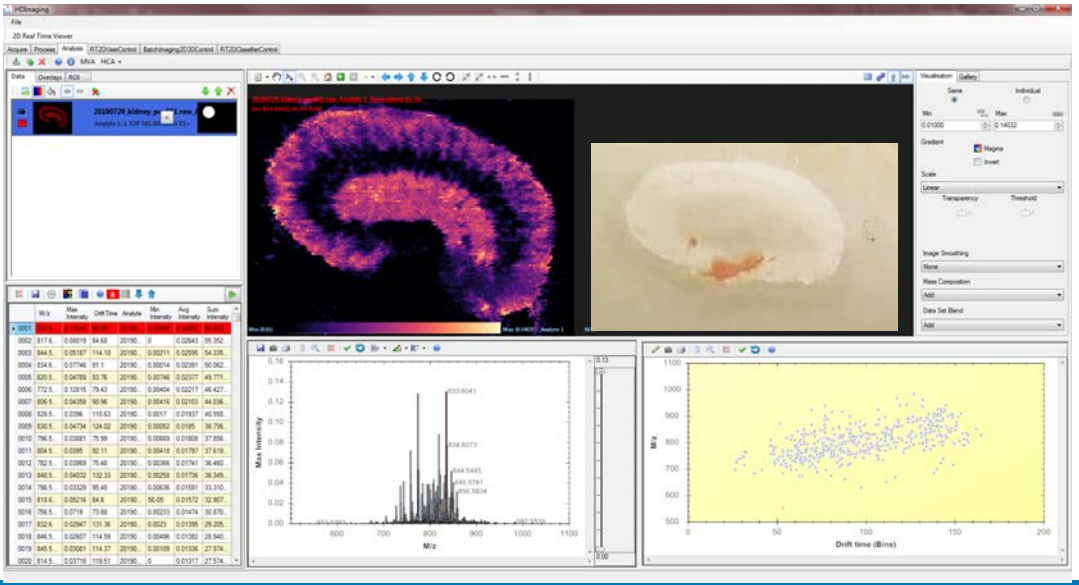
DESI on Cyclic fits into current imaging workflow and software (HDI v 1.5)

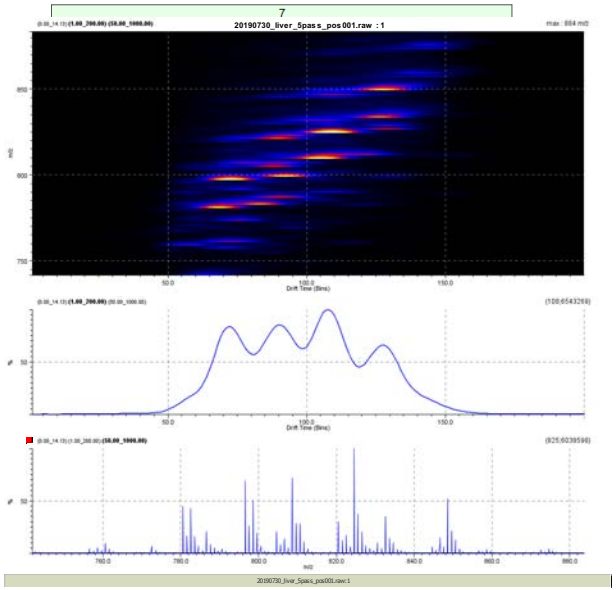
DESI-MS positive ion mode
Mouse kidney
5 pass method with IMS selection
4 pixels per second
50 x 50µm pixel size

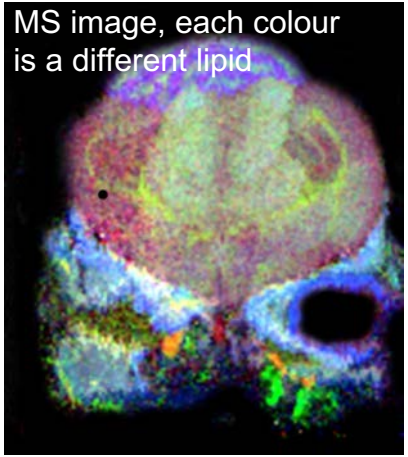


4 more passes of lipids only

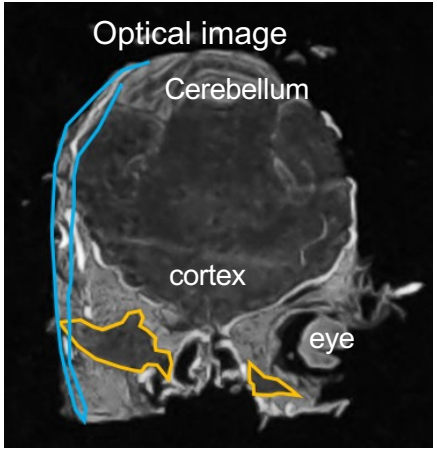








Coronal section of mouse with brain and eye present



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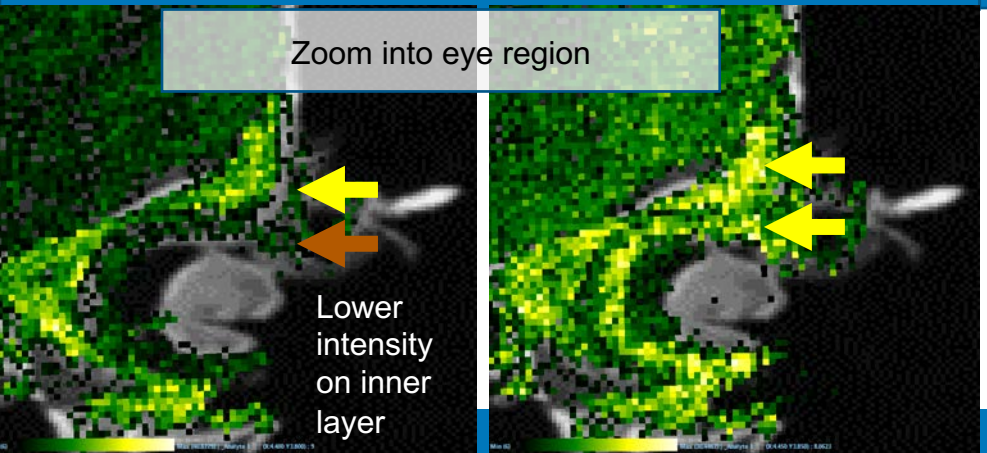
m/z 833.5903

Drift time 74.54

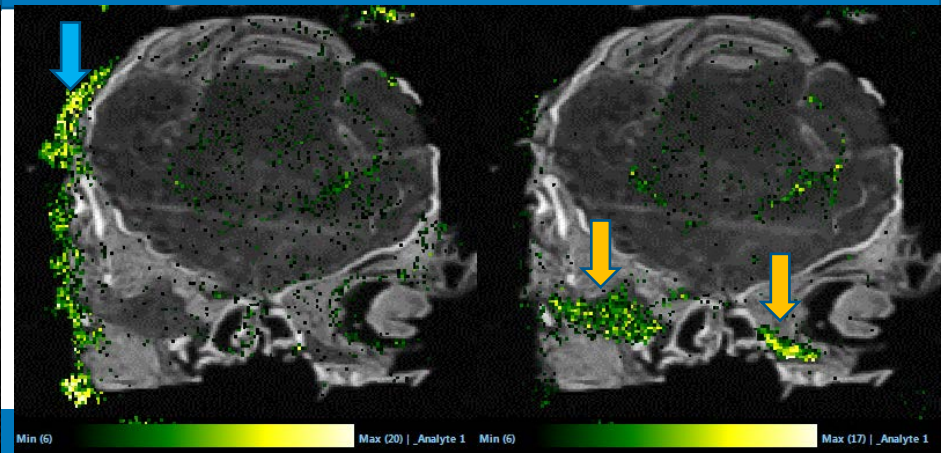
Drift time 112.89



m/z 813.6019 (TG48:8+Na or PG-O 40:4+Na)

Drift time 107.7

Drift time 125.44



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Waters Advanced Mass Spectrometry

DESI Cyclic IMS imaging enhances protein detection and lipid class coverage during the analysis of tuberculosis granulomas

Claire L. Carter, *Ph.D*

Center for Discovery and Innovation

Hackensack Meridian *Health*

Hackensack Meridian *Health* | John Theurer Cancer Center | CDI Center for Discovery & Innovation — Member of Hackensack Meridian *Health*

Isobaric ions with similar spatial distribution separated using Cyclic Ion Mobility

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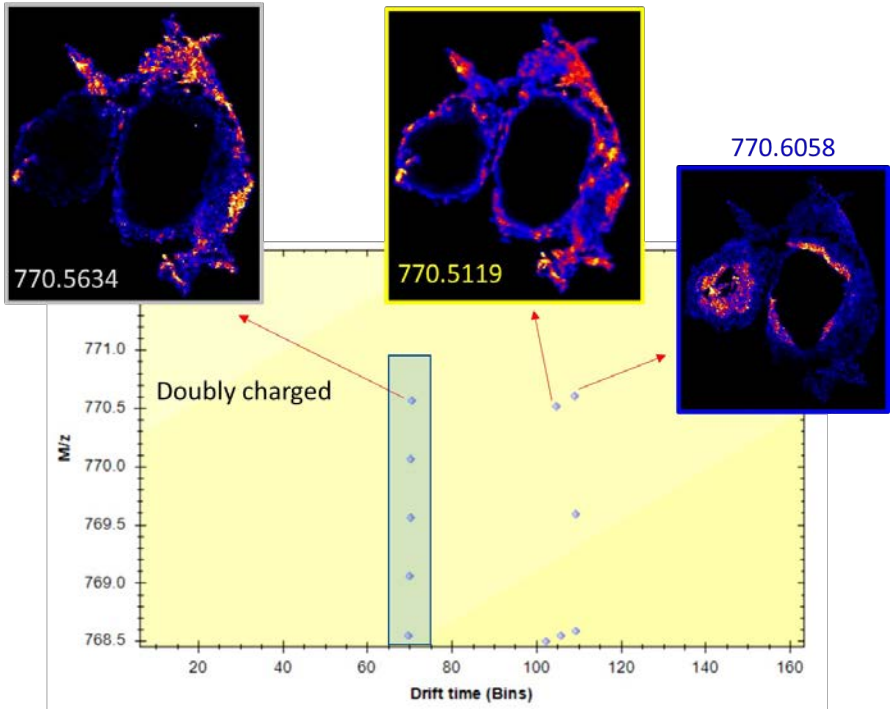
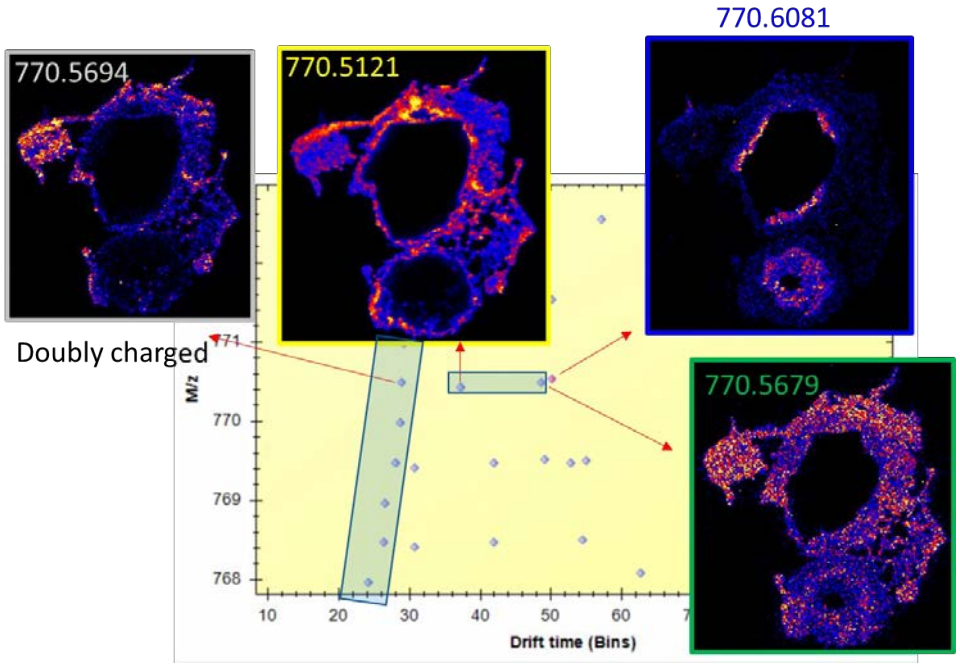
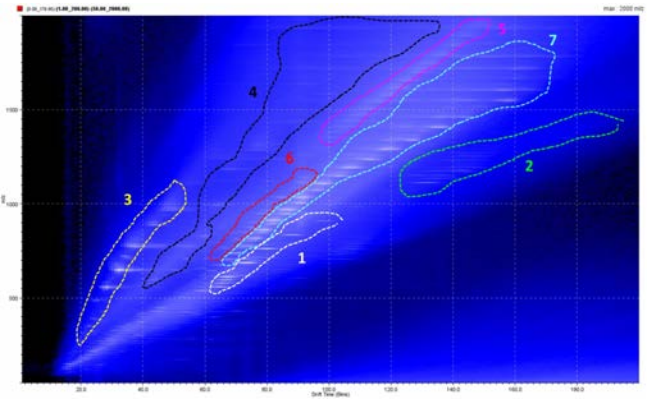
1 Pass



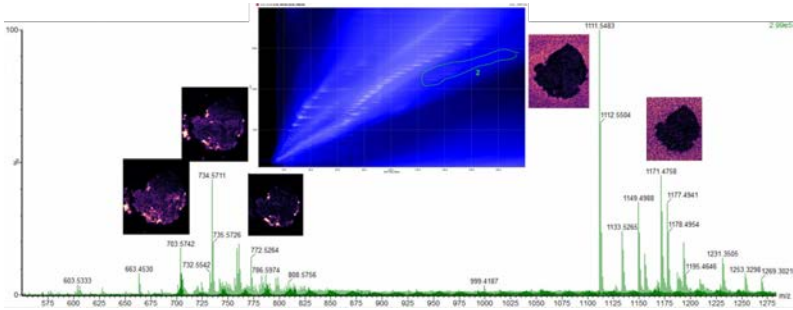
6 Passes



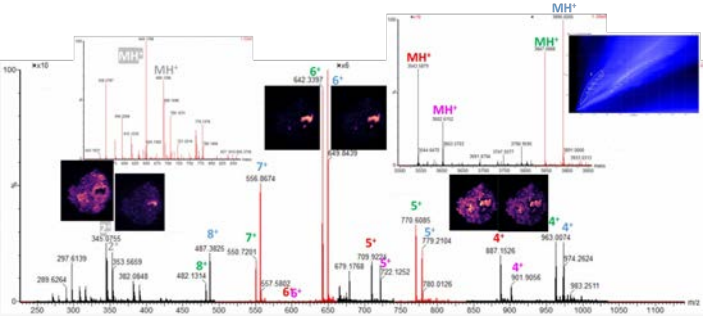
Drift Scope Heat Map from TB granuloma using DESI XS with heated transfer line and High Performance Sprayer



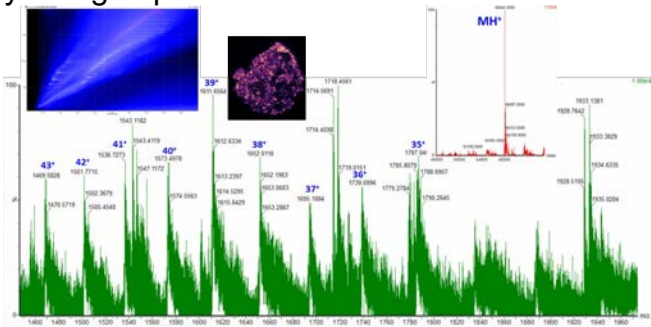
Trend line 2: Singly charged lipids/ Dimers Leu-Enk + adducts



Trend line 3: High mobility multiply charged peptides



Trend line 5: Multiply charged proteins



Overlay of images from the same ion mode and from separate acquisitions of positive and negative ion mode on the same section

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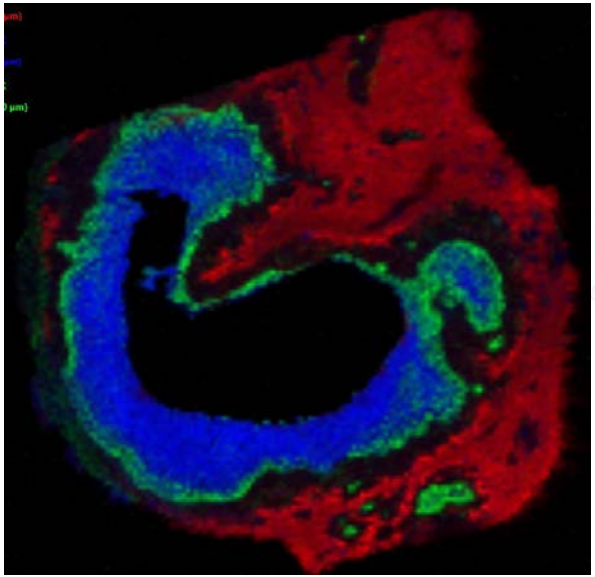
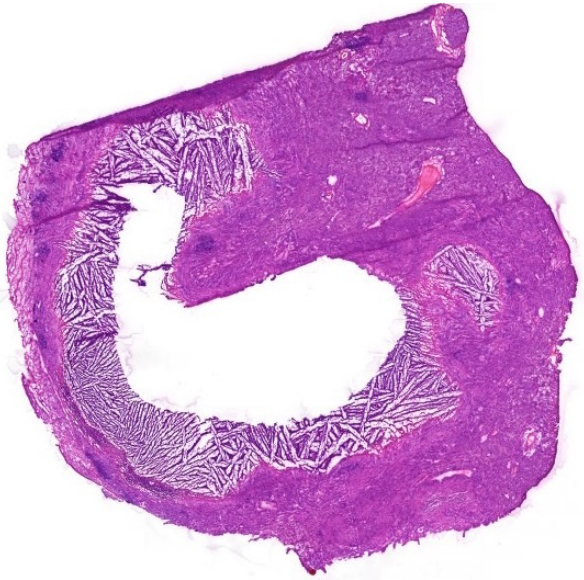
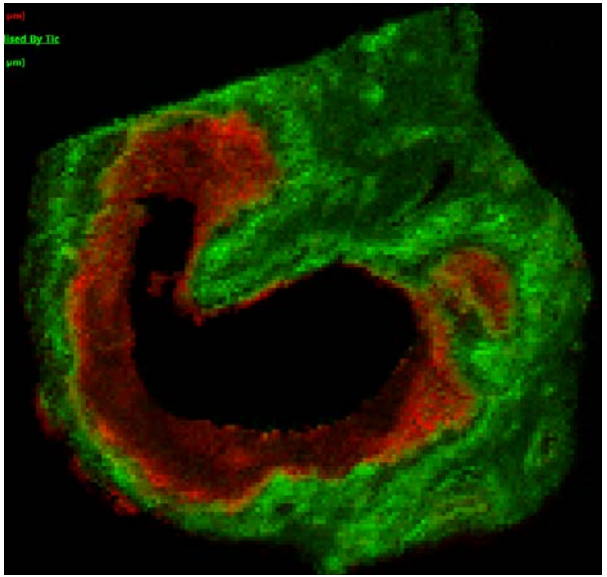
m/z 770.5110 *m/z* 766.5733 *m/z* 926.7655





PC(P-36:5) PI(38:4)



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SELECT SERIES MRT

SELECT SERIES MRT

- The SELECT SERIES MRT is a next generation Q-Tof based upon multi-reflecting time-of-flight technology

- Highlights
  - Routine ppb mass accuracy
  - High mass resolution, irrespective of acquisition rates
  - Highest quality data in both MS and MS/MS
  - Compact data files sizes

Why use Multi Reflecting Time-of-Flight Technology?

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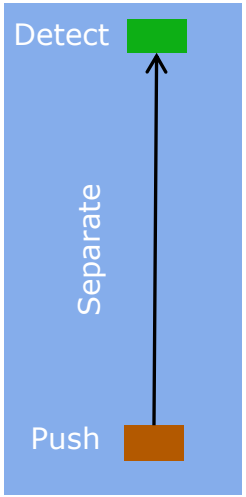
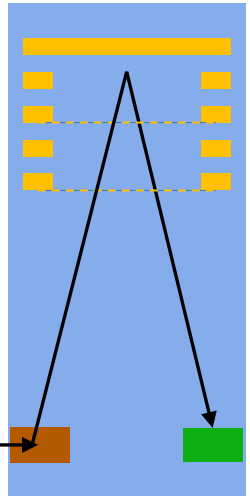
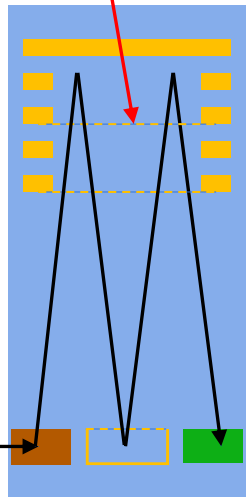




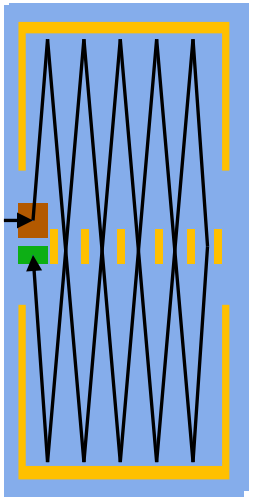
RDa
Xevo G2-XS

Each grid ~92% transmission



SYNAPT
Cyclic IMS

> 90% total transmission (incl. beam focusing)



MRT

MRT enables a longer flight path for the same size mass analyzer

SELECT SERIES MRT

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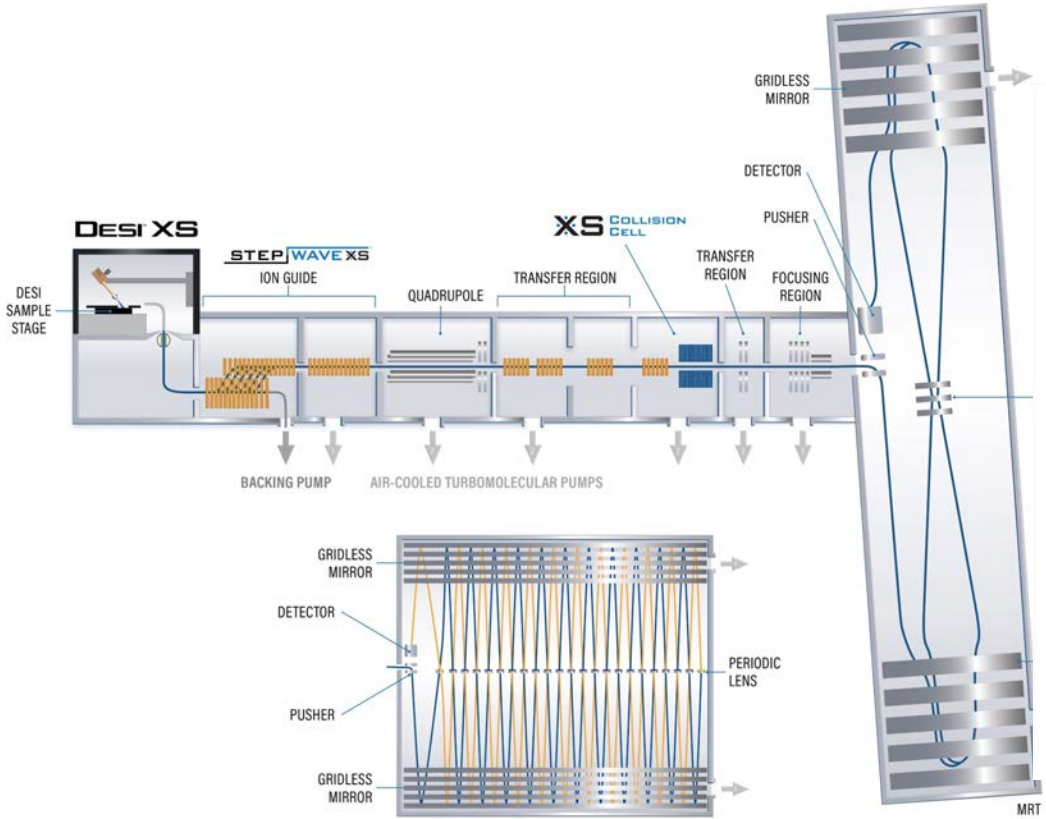

MRT – benefits of high mass resolution

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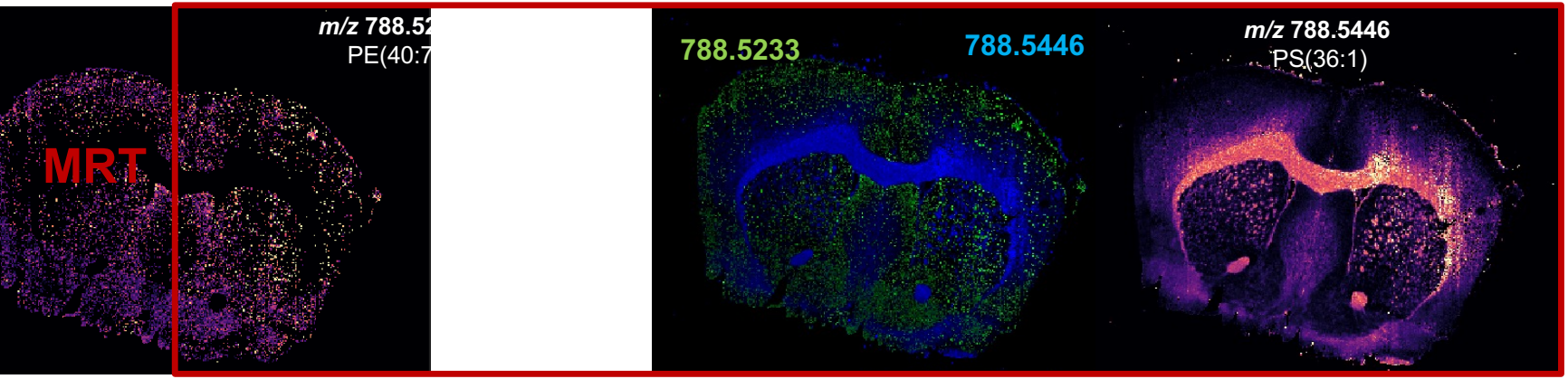
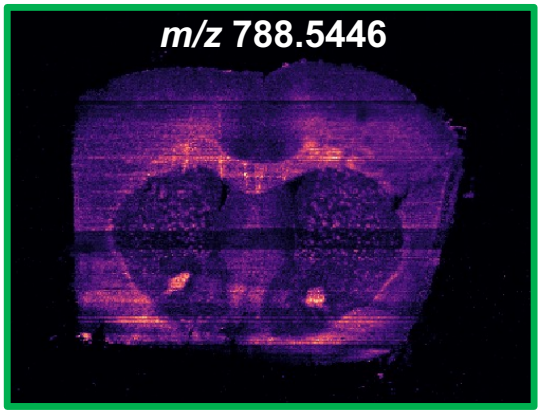
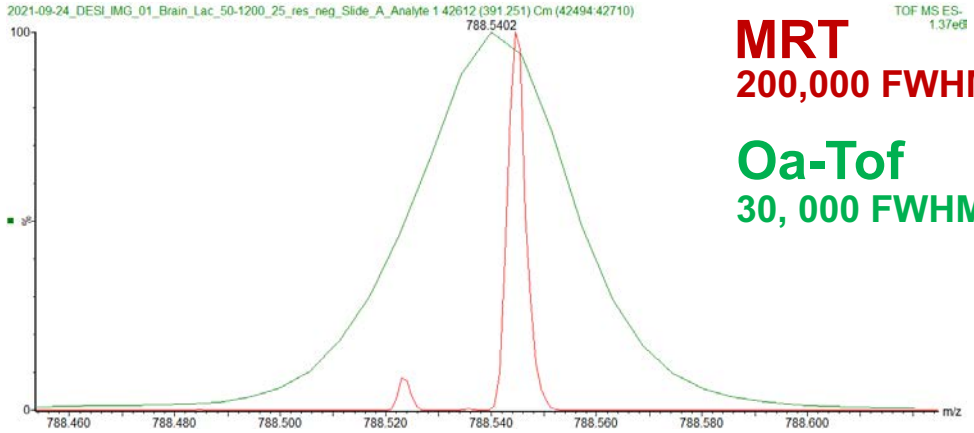


TOF





MRT
200,000 FWHM

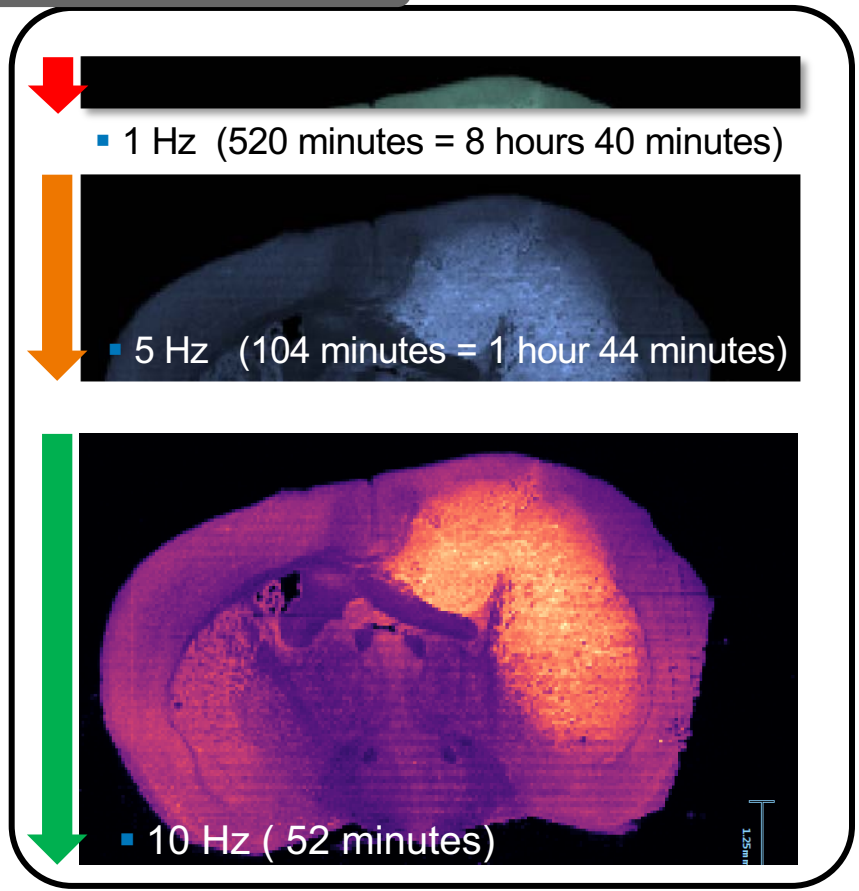
Oa-Tof
30, 000 FWHM

Sampling rate

Progress after 1 hour- total time in brackets

Waters™

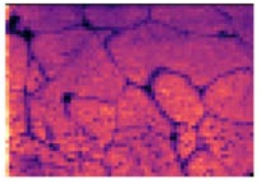
- MS acquisition rates take on a different importance for imaging MS vs traditional LC-MS
- The ability to acquire at up to 10 Hz allow data to be collected at a cellular resolution in a time frame that allows multiple sections to be analysed per day
- Instrument specifications of 200 k mass resolution and 500 ppb mass accuracy are not compromised
- Increase number of samples in a study
- Acquire at smaller spatial resolution in same time frame
- Larger cohorts
- 3D imaging



SELECT SERIES MRT DESI imaging

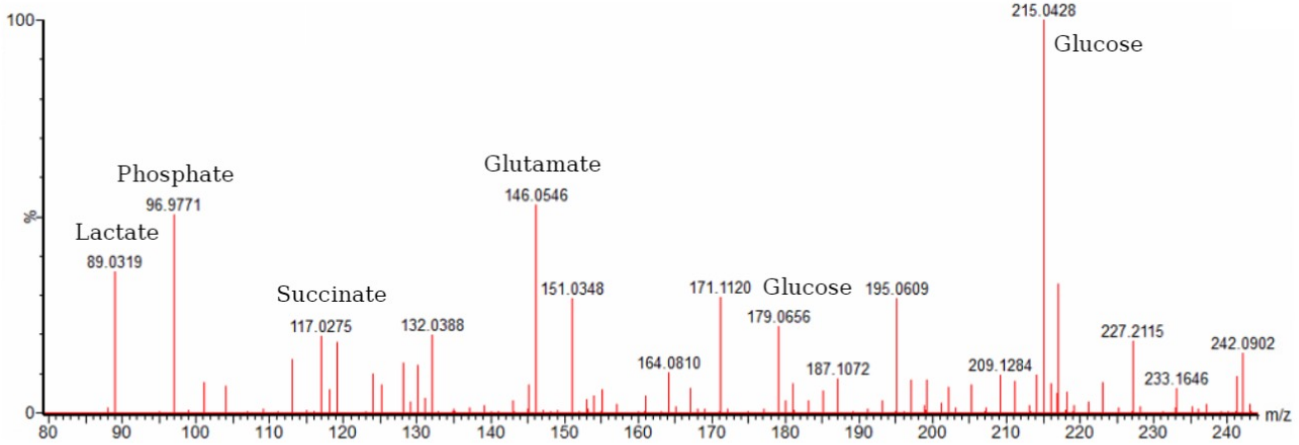
low *m/z* negative ion mode

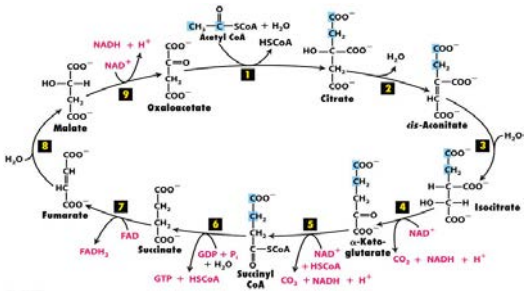
235 ppb mass accuracy



§ Palmitic acid
§ *m/z* 255.2

Analyte	Formula	Expected mass	Observed mass	mDa error	ppb error
Lactate	$C_3H_6O_3$	89.024418	89.0244	-0.018	-202
Phosphoric acid	H_3PO_4	96.969619	96.9696	-0.019	-196
Succinate	$C_4H_6O_4$	117.019332	117.0193	-0.032	-273
Glutamate	$C_5H_9NO_4$	146.045881	146.0459	0.019	130
Hexose	$C_6H_{12}O_6$	179.056112	179.0561	-0.012	-67
Palmitic acid	$C_{16}H_{32}O_2$	255.232954	255.233	0.046	180
Palmitoleic acid	$C_{16}H_{30}O_2$	253.217304	253.2174	0.096	379
Linoleic acid	$C_{18}H_{32}O_2$	279.232954	279.2329	-0.054	-193
Octadecenoic acid	$C_{18}H_{34}O_2$	281.248604	281.2487	0.096	341
Octadecanoic acid	$C_{18}H_{36}O_2$	283.264254	283.2643	0.046	162
Arachidonic acid	$C_{20}H_{32}O_2$	303.232954	303.233	0.046	152
			Mean	0.019	38
			SD	0.051	232
Lockmass corrected with Leu-enkephalin			RMS	0.054	**235**





MALDI analysis
Human Insulin $[M+2H]^{2+}$

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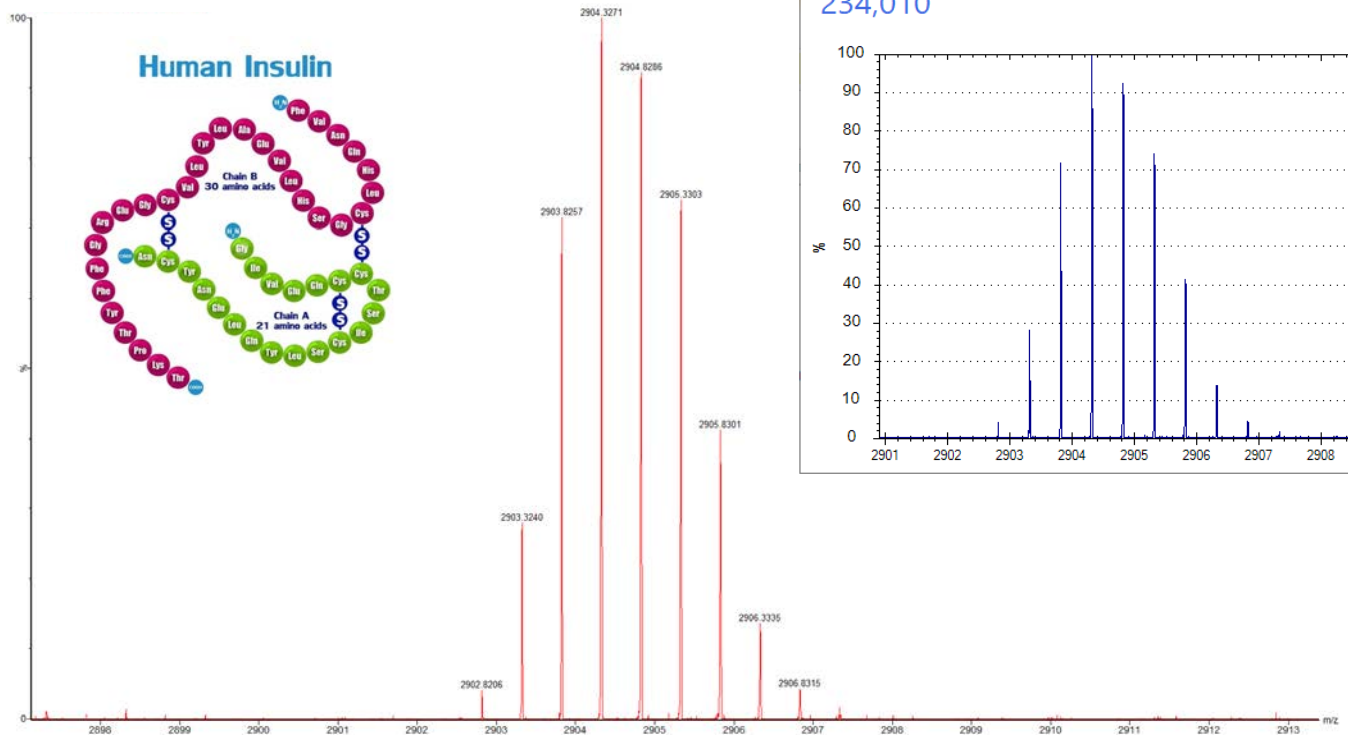

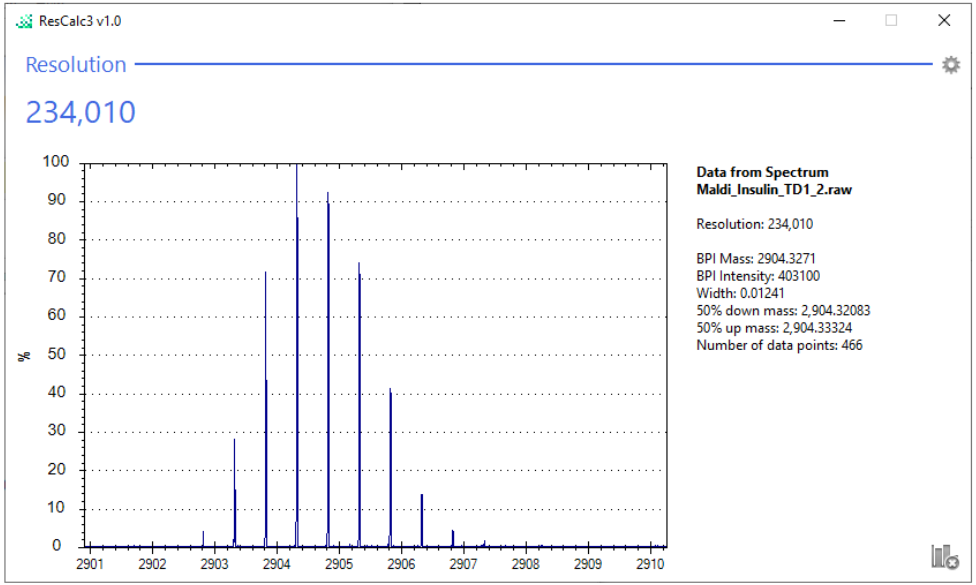

Yeast enolase tryptic digest

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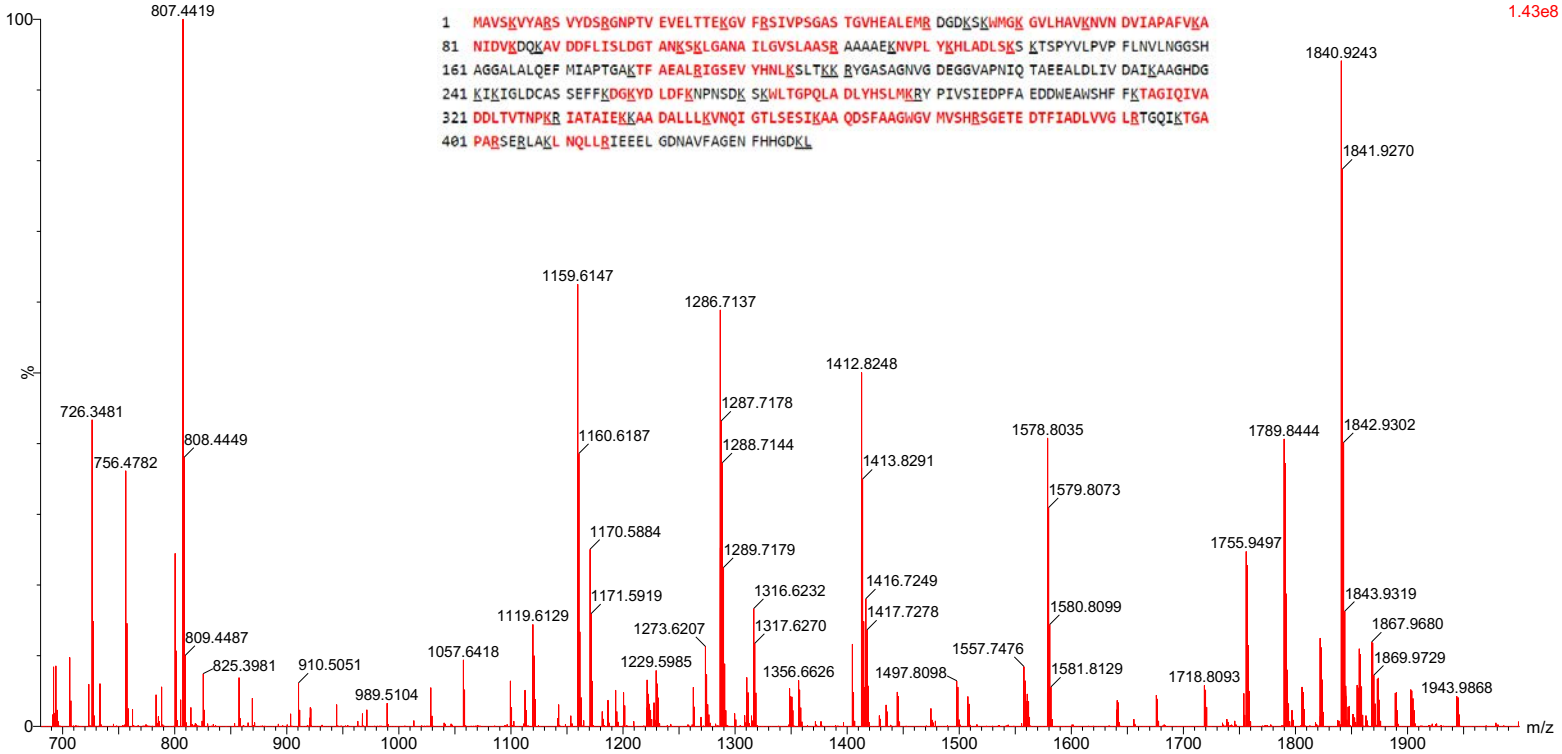
~60% sequence coverage

```
1   MAVSKVYARS VYDSRGNPTV EVELTTEKGV FRSIVPSGAS TGVHEALEMR DGDKSKWMGK GVLHAVKNVN DVIAPAFVKA
81  NIDVKDQKAV DDFLISLDGT ANKSKLGANA ILGVSLAASR AAAAEKNVPL YKHLADLSKS KTSPYVLPVP FLNVLNGGSH
161 AGGALALQEF MIAPTGAKTF AEALRIGSEV YHNLKSLTKK RYGASAGNVG DEGGVAPNIQ TAEEALDLIV DAIKAAGHDG
241 KIKIGLDCAS SEFFKDGKYD LDFKNPNSDK SKWLTGPQLA DLYHSLMKRY PIVSIEDPFA EDDWEAWSHF FKTAGIQIVA
321 DDLTVTNPKR IATAIEKKAA DALLLKVNQI GTLSESIKAA QDSFAAGWGV MVSHRSGETE DTFIADLVVG LRTGQIKTGA
401 PARSERLAKL NQLLRIEEEL GDNAVFAGEN FHHGDKL
```



Tryptic peptide summary

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Tryptic peptide	Sequence	Elemental comp.	Theoretical m/z	Exp m/z	Delta mass (ppb)
T30	YDLDFK	C38H54N7O12	800.38249	800.382507	**19.47**
T20	TFAEAMR	C35H56N10O11S	825.39235	825.392639	**345.99**
T28	IGLDCASSEFFK	C59H89N13O19S	1316.61912	1316.619019	**-77.03**
T15	LGANAILGVSMAAAR	C60H107N19O18S	1414.78350	1414.783325	**-123.28**
T4	GNPTVEVELTTEK	C60H102N15O24	1416.72167	1416.721924	**178.28**
T49	AVYAGENFHHGDK	C64H89N19O20	1444.66041	1444.660645	**164.45**
T42	SGETEDTFIADLVVGLR	C79H129N20O29	1821.92289	1821.922852	**-21.64**
T6	SIVPSGASTGVHEALEMR	C77H129N23O27S	1840.92218	1840.922119	**-33.36**
T30	YDLDFK	C38H54N7O12	800.38249	800.382507	**19.47**

MALDI MRT
Porcine liver sample 15µM x 15µM

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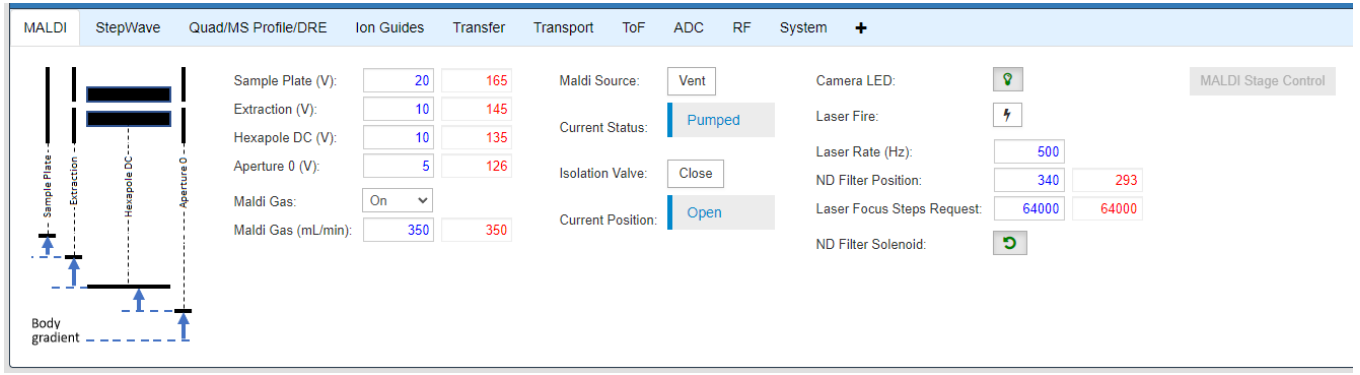
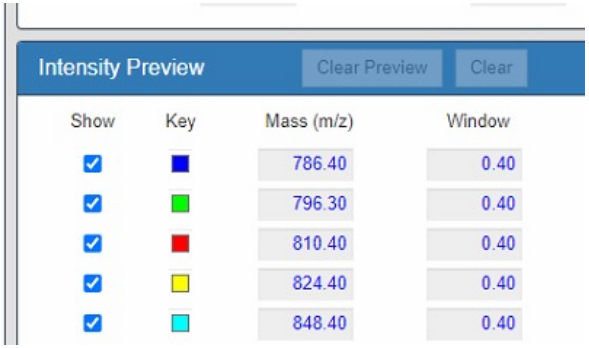




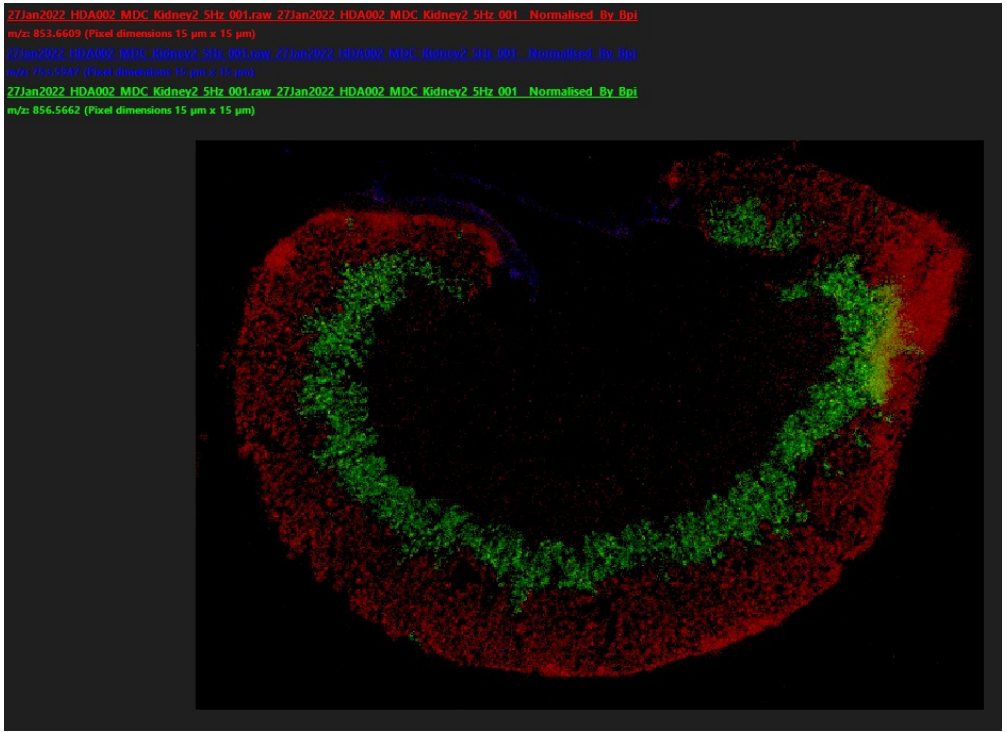
Real time image generation

using up to 5 selected m/z values

MALDI imaging Mouse Kidney

- DHB matrix, 15µM laser spot size
- X, Y step size 15µM
- 5HZ data acquisition
- 267,000 data points (15.5 hours)

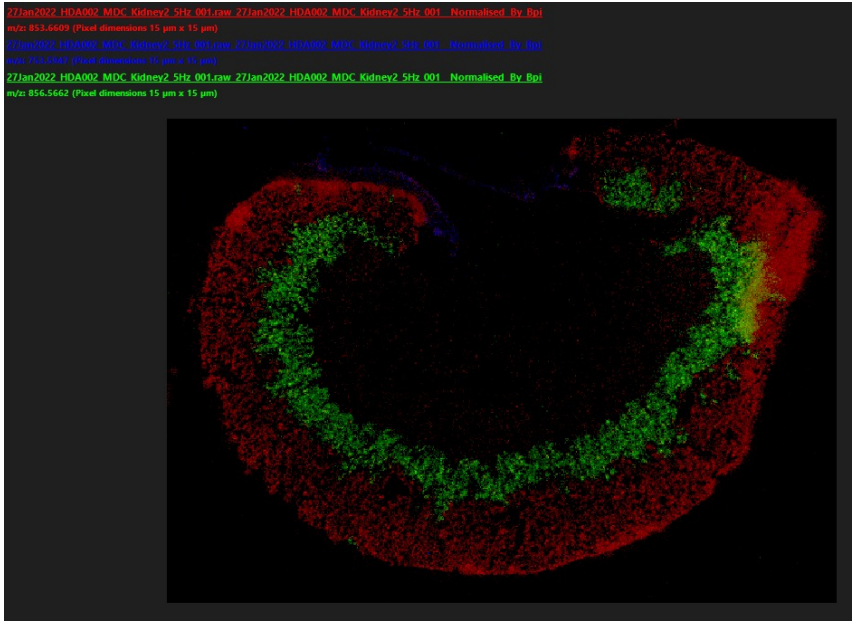
Optical image

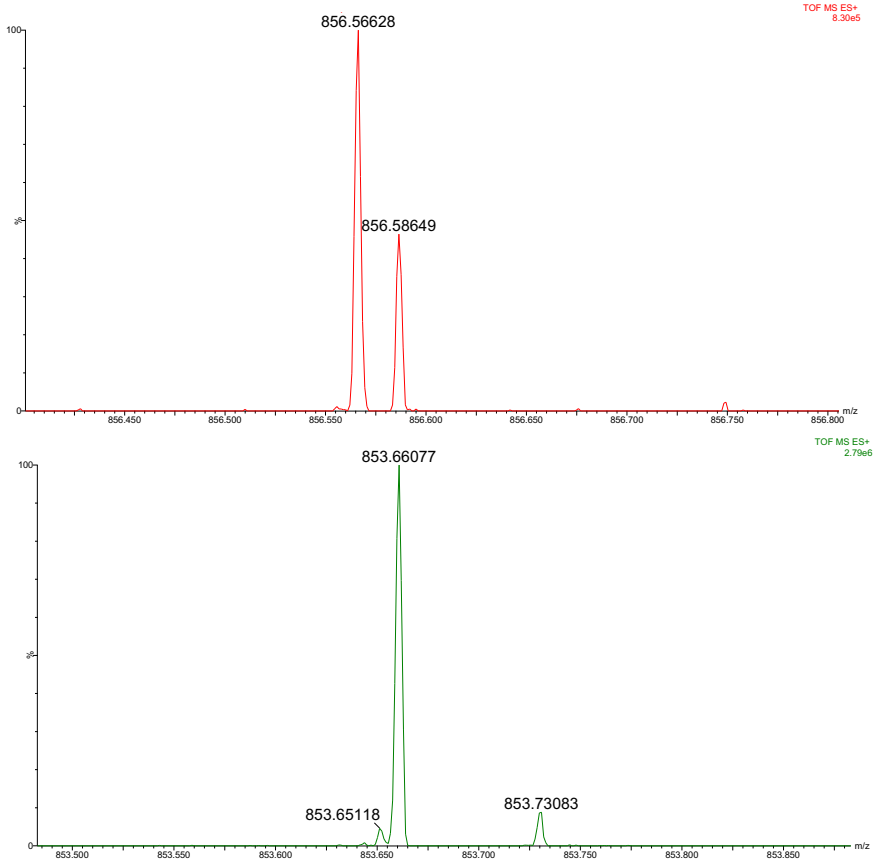


MALDI MSI

MALDI MRT imaging Mouse Kidney

- Phospholipids m/z 853.66 and 856.566
- Locate to the kidney cortex and medulla respectively





Summary

- The SELECT SERIES MRT has state–of–the-art multi reflecting ToF technology that provides benefits in resolution and accuracy at speed
  - The inherent speed of the 200,000 resolution, 500ppb ToF allows it to keep up with the faster acquisition rates without loss of resolution or mass measurement accuracy.

- The enhanced DESI XS significantly improved compound coverage
  - Higher pixel resolutions are possible (<10µM)
  - Increased sensitivity affords significant reductions in the time per/ pixel reducing total experiment time

- New MRT MALDI source provides high quality data in MALDI mode at high spatial resolution
  - Laser spot size variable <10µM to >100µM
  - Full size MTP format stage (holding 3 microscope slides)
  - FT-MS like performance at up to 10Hz speed

- The performance of the SELECT SERIES MRT enables superior imaging experiments

Two main Mass Spectrometry Imaging (MSI) techniques

Waters™

MALDI

- Matrix assisted laser desorption ionization
- Very High Resolution
- Large Molecules (Peptides & proteins)
- Sample preparation required
- Vacuum environment

DESI

- Desorption electrospray ionization
- High Resolution
- Small Molecules
- Simple sample preparation
- Ambient Analysis
- Rapid analysis

The two different techniques generate complementary information

– ‘Full Spectrum Molecular Imaging’

MALDI and DESI – can now assume similar resolution and sens

Waters™

- DESI
  - Fast, minimal sample prep
  - Surface – labile, easily ionizable
  - Localization based on spray
  - Depth – washing and solvent
  - Tissue is alive – at Room T for analysis
  - Same section available for staining
  - Difficult to get at archival tissue (FFPE)

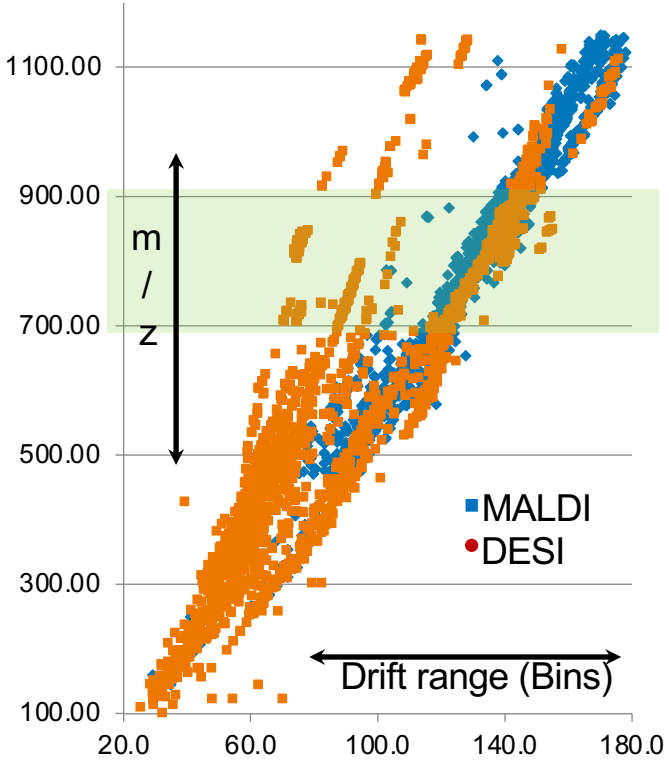
- Core – service model
  - Run fast, under standard conditions
  - Repeat analysis using different conditions
    - Chemistry changes at ionization
    - Automation straightforward

- MALDI
  - Matrix addition – can be complex (or not!)
  - Chemistry targets types of molecules
  - Localization based on crystals/che
  - Depth –washing, extraction into matrix
  - Tissue is “static” preserved in matrix
  - Alternate sections for staining
  - Available extractions for FFPE

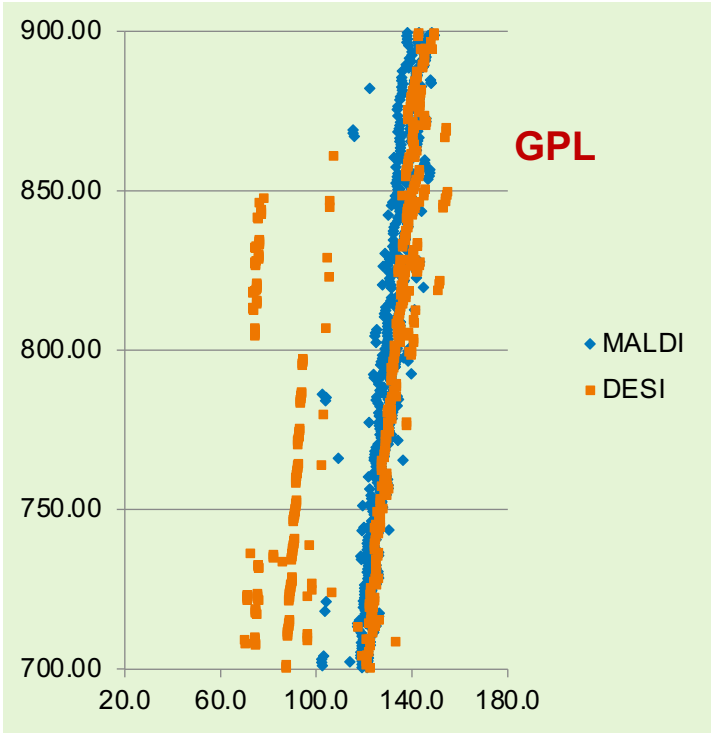
- Core – service model
  - Run standards – target molecules only
  - Additional methods/optimization needed
    - Chemistry changes at sample prep
    - Automation possible

MALDI vs. DESI Ion mobility comparison

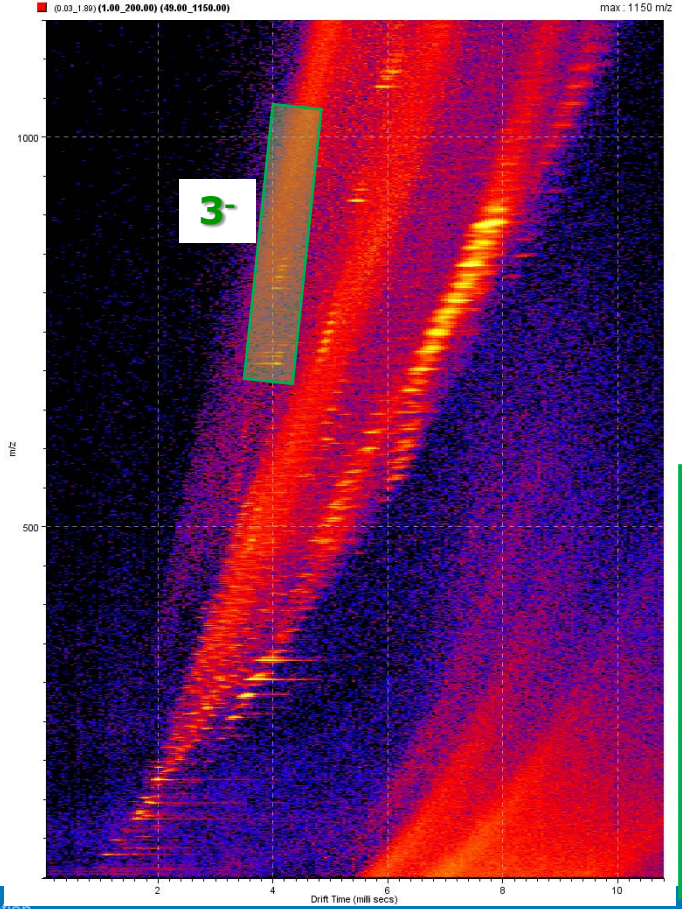
Waters™

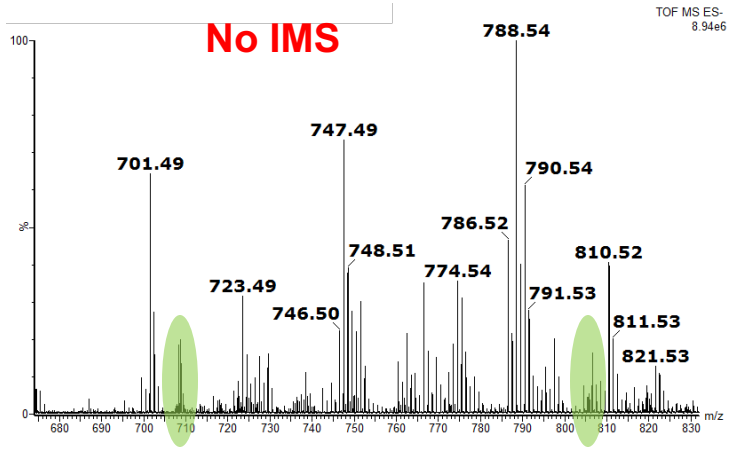


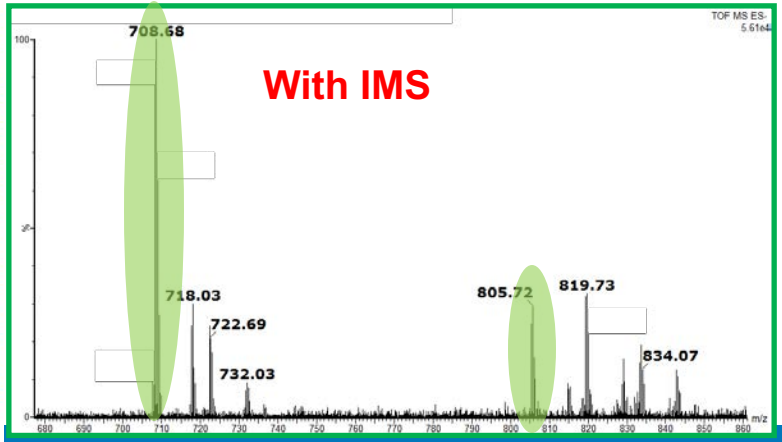
Matrix & background peaks



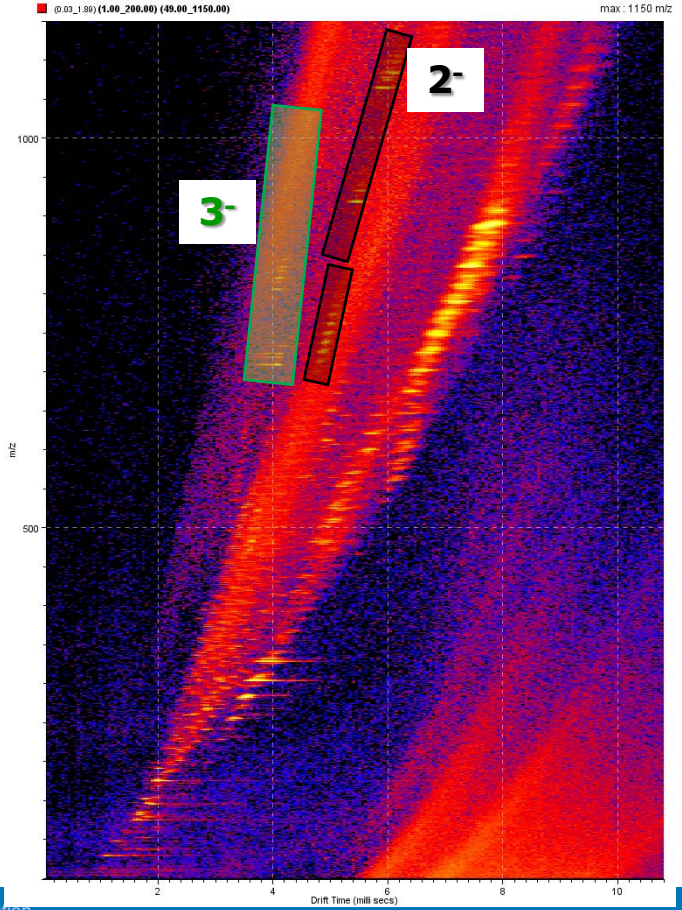
DESI ion mobility investigation, multiply charged species

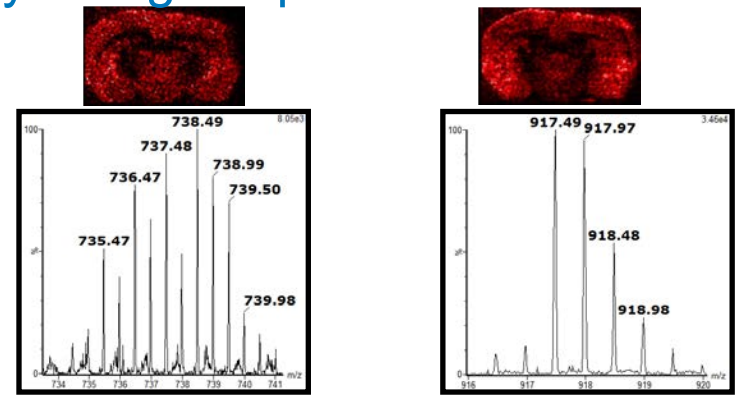




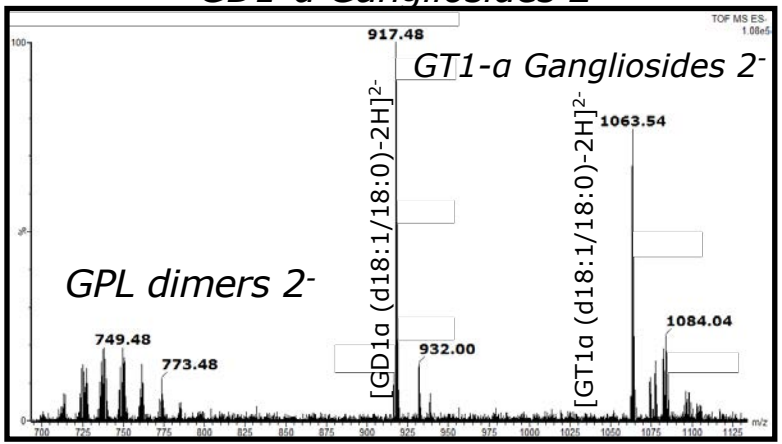


DESI ion mobility investigation multiply charged species

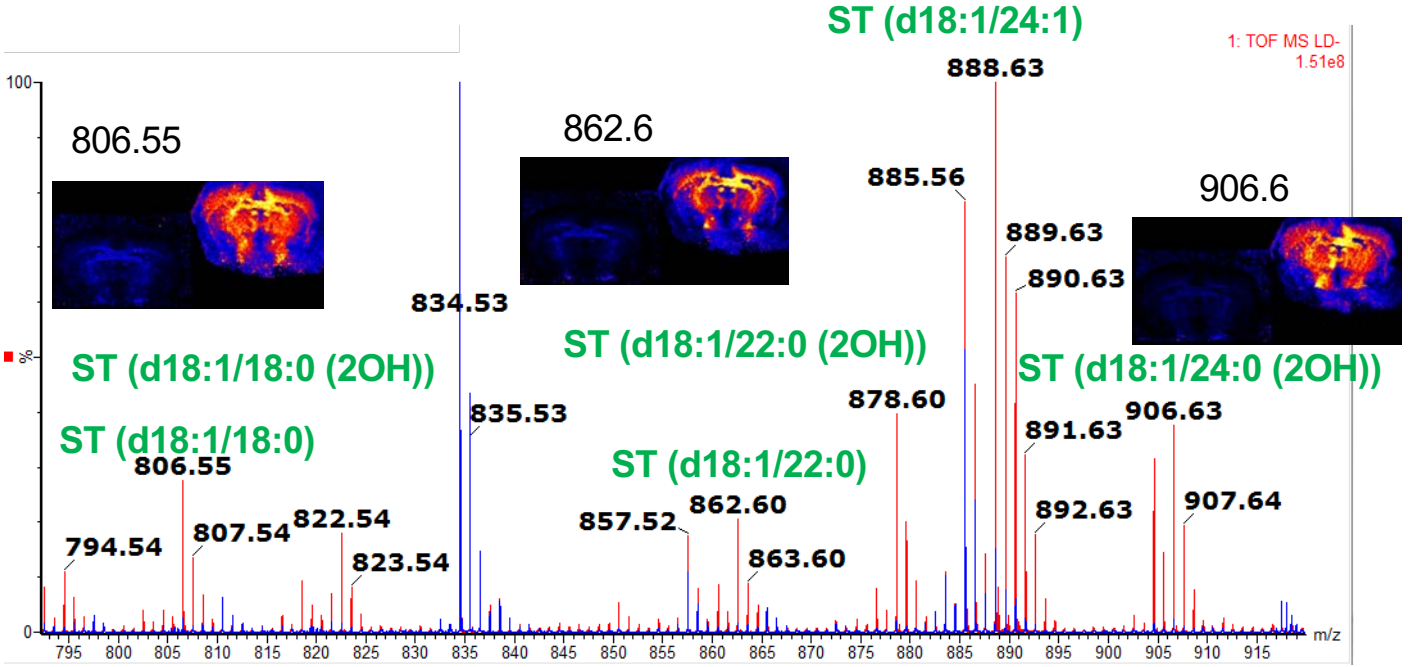




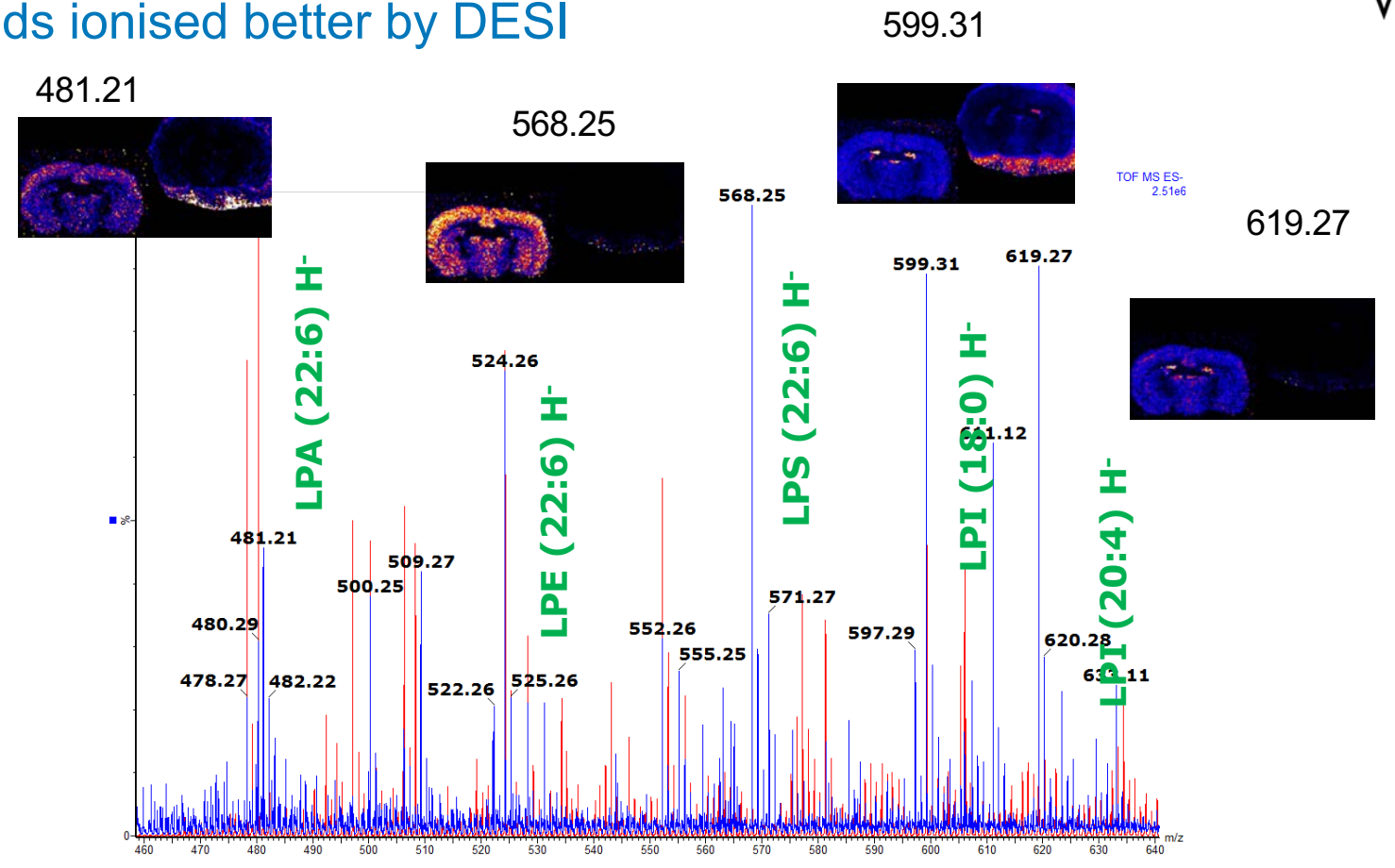
GD1-a Gangliosides 2-



MALDI vs. DESI
Ceramides ionised better in MALDI



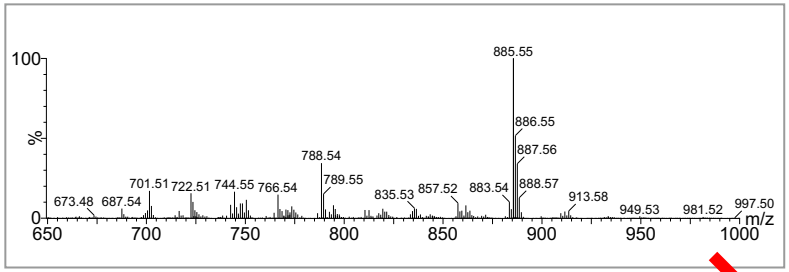
MALDI vs. DESI
Lysolipids ionised better by DESI



DESI followed by MALDI

Waters™

DESI solvent: 90% methanol, 10% water



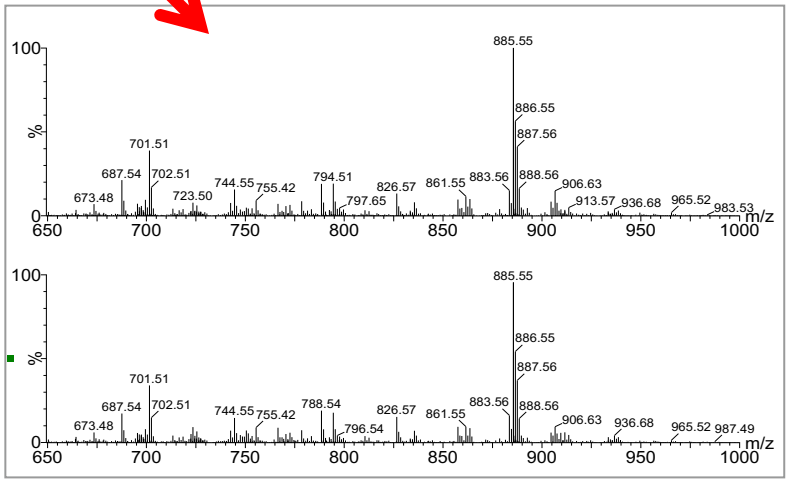
Spectrum of combined tissue area for DESI experiment

No significant drop in signal for tissue previously imaged by DESI (non-destructive)!

Spectra of combined tissue area from MALDI experiments

Top— MALDI imaging data from tissue section previously imaged by DESI.

Bottom— MALDI imaging data from "MALDI only" tissue sections (vertical axis linked).



DESI followed by MALDI

DESI solvent: 90% methanol, 10% water

DESI and MALDI image comparison:

- Left- MALDI images of tissue previously analyzed by DESI (corresponding DESI images in A)
- Right- MALDI images from Pristine Tissue.

Images are displayed on a linked intensity scale.

No significant effect of pre-analysis on the MALDI imaging experiments is observed.