

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

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Outline

Part 1:

- Imaging and sample selection
- Sample prep and Ionization

Part 2:

- Technology for analysis

Part 3:

- Setup and data flow
- Considerations for quantitation

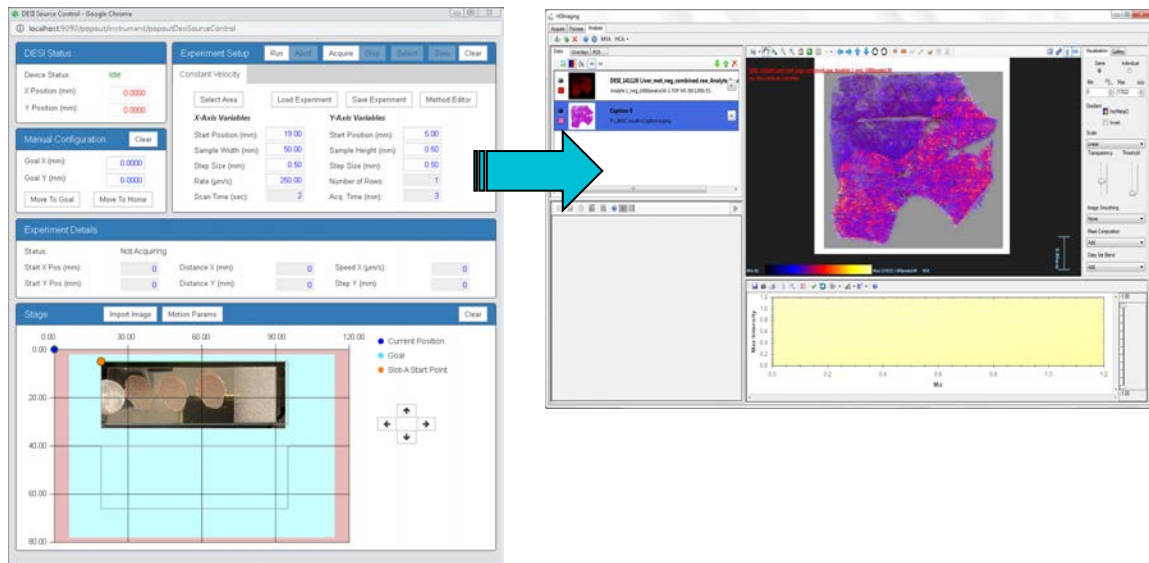


Informatics

SELECT SERIES MRT

Simplified instrument setup, usability and data export

SELECT SERIES MRT Software



Set Up

- User Centric Experimental Design

Acquire

- Compact File Sizes

Export

- Universal file format

Process

- HDI
- METASPACE
- SCiLS Lab
- Lipostar-MSI

Applications

- ☰ Tune
- ☰ Manual Calibration
- ☑ Instrument Setup
- 🏠 Vacuum
- 🏠 Quad Setup
- 🔧 Detector Setup
- 🔧 Resolution Optimisation
- 📏 Scope Mode
- 🏠 Health Status
- 👁 Diagnostic Summary
- 📄 Diagnostic Information

Mass Spectrum
Data View Acquire

Laser Rate (Hz): *100-200 shots per pixel*

ND Filter Position: *depends on laser focus*

Laser Focus (mm): *Change focus to get different spot sizes*

ND 1 Filter:

Positive Negative Single-Push MRT Diamond API Gas
2400

Controls: *2023_2_13_version3 maldi

MALDI StepWave Quad/MS Profile/DRE Ion Guides Transfer Transport ToF ADC RF System +

Sample Plate (V):	<input type="text" value="0"/>	<input type="text" value="142"/>
Extraction (V):	<input type="text" value="10"/>	<input type="text" value="142"/>
Hexapole DC (V):	<input type="text" value="10"/>	<input type="text" value="133"/>
Aperture 0 (V):	<input type="text" value="5"/>	<input type="text" value="122"/>
Maldi Gas:	<input type="text" value="On"/>	
Maldi Gas (mL/min):	<input type="text" value="350"/>	<input type="text" value="350"/>

Camera LED:

Laser Fire:

Laser Rate (Hz):

ND Filter Position:

Laser Focus (mm):

ND 1 Filter:

Maldi Source:

Isolation Valve:

Define imaging Experiment

Select region to image

DESI Status: Moving
X Position (mm): 86.3400
Y Position (mm): 21.0650

Manual Configuration
Goal X (mm): 0.0000
Goal Y (mm): 0.0000

Experiment Setup
Single Region | Multi-Region

Select New Area | Import Area Exp

X-Axis Variables		Y-Axis Variables	
Start Position (mm):	57.53	Start Position (mm):	52.18
Sample Width (mm):	10.96	Sample Height (mm):	6.92
Step Size (μm):	45.00	Step Size (μm):	45.00
Rate (μm/s):	90.00	Number of Rows:	154
Scan Time (sec):	0.5	Acq. Time (min):	333

X-Axis Variables

Start Position (mm):	57.53
Sample Width (mm):	10.96
Step Size (μm):	45.00
Rate (μm/s):	90.00
Scan Time (sec):	0.5

Set scan time (~pixels/sec)

Y-Axis Variables

Start Position (mm):	52.18
Sample Height (mm):	6.92
Step Size (μm):	45.00
Number of Rows:	154
Acq. Time (min):	333

Acquisition time is automatically calculated based on pixel and scan time

Imported scanned image

Green outline of selected region for imaging

RealTime Viewer

Experiment Details

Status: Acquiring (row 153 of 260 rows)

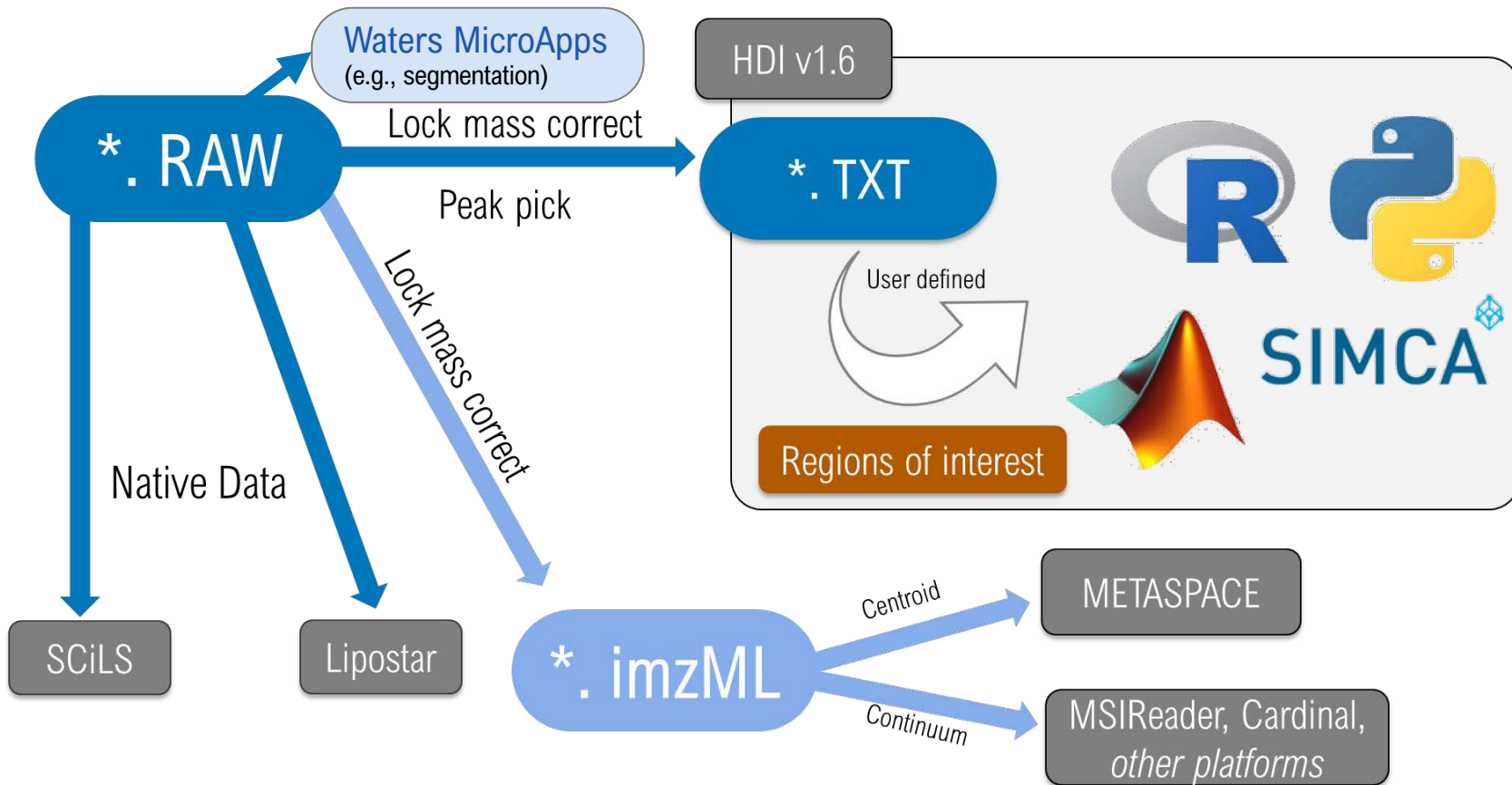
Start X Pos (mm):	57.5256	Start Y Pos (mm):	14.2250
Distance X (mm):	14.0385	Distance Y (mm):	11.6922
Speed X (μm/s):	90	Step Y (mm):	0.04

Intensity Preview

Show	Key	Mass (m/z)	Window
<input checked="" type="checkbox"/>	■	554.27	0.20
<input type="checkbox"/>	■	885.54	0.20
<input type="checkbox"/>	■	89.02	0.20
<input checked="" type="checkbox"/>	■	0.00	0.00
<input checked="" type="checkbox"/>	■	0.00	0.00
<input checked="" type="checkbox"/>	■	0.00	0.00

Align X (mm): 0.0000
Align Y (mm): 0.0000

Row Status



Analysis

The screenshot displays the HDImaging software interface with several key components:

- Data Panel (Top Left):** Shows the loaded dataset: MALDI-HDMS-Rat-Brain.raw_MALDI-HDMS-Rat-Brain_Demo_1 TOF MS (200-10000). It includes an 'Overlay' button and a small heatmap preview.
- Table (Bottom Left):** A peak list table with columns for m/z, Max Intensity, Avg Intensity, Drift Time, and Sum Intensity. The peak at m/z 826.604 is highlighted in red.
- Heatmap (Center):** A large heatmap showing the spatial distribution of the selected peak. A red box indicates the Region of Interest (ROI). A 4.50mm scale bar is present at the bottom of the heatmap.
- ROI Panel (Top Right):** Configuration options for the ROI, including 'Same' and 'Individual' modes, 'Min' and 'Max' intensity values (0.00000 and 0.06753), and a 'Gradient' dropdown set to 'jet'.
- Mass Spectrum (Bottom Left Plot):** A plot of Max Intensity vs. m/z, showing a major peak at 379.0886 and other labeled peaks at 825, 826, 86, and 88.
- Interactive Mobility Plot (Bottom Right Plot):** A scatter plot of m/z vs. Drift time (Bins), showing a clear positive linear correlation between the two variables.

Datasets (MALDI, DESI)

Overlay

Peak list
m/z, intensities (max, min, sum), drift time, spatial corr.

ROI (Region of Interest)

Heatmap

Scale bar

Interactive mobility plot

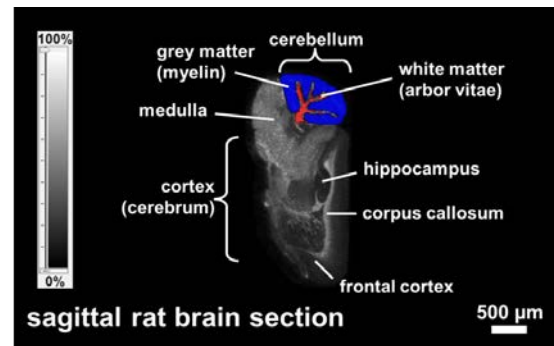
HDI Software – Region of Interests (ROIs)



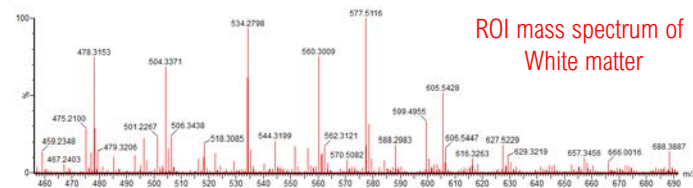
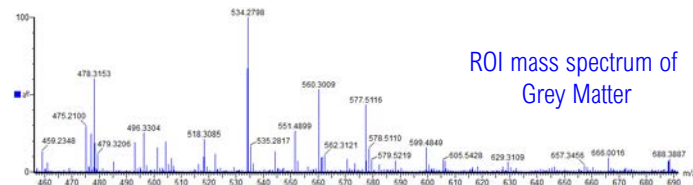
Waters™



Draw ROI shapes	Draws circular, rectangular, freehand or polygonal ROI on the image.
Draw ROI pixel	Sets the brush size to 1 square pixel.
Draw ROI paint brush	Changes the shape and pixel size of the brush.
Erase ROI Brush	Removes pixels from an ROI.
Clone ROI	Clones an ROI into an image brush that paints an exact copy of the ROI onto other areas.



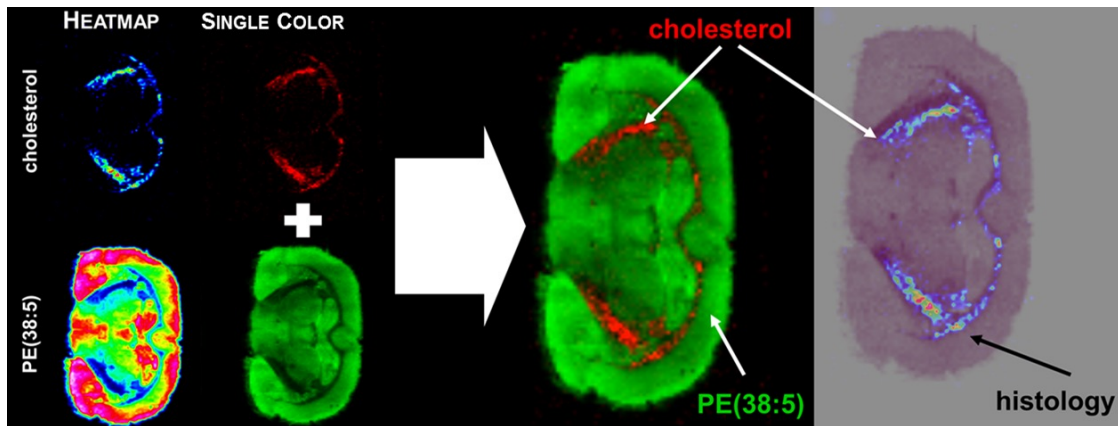
- ❑ Region of interest (ROI) can be created by drawing shapes using paint toolbar
- ❑ ROI facilitates ion intensities or mass spectrum of a specific area for further analysis (e.g., multivariate statistics)



Overlay of Multiple Ions and Histological (Digital Image) Overlay



Waters™

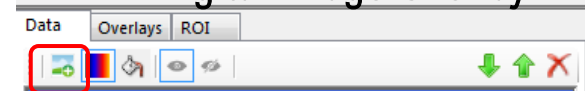


- ❑ Multiple ion image overlay shows single-color images of each ion, each pixel undergoes data set blend (add, max, min, intensity correlation)
- ❑ No limit on colors or number for a overlay, intensities of each ion can be defined
- ❑ Overlay molecular ion image over a histological image (or any digital image)
- ❑ Transparency and threshold of overlay can be defined

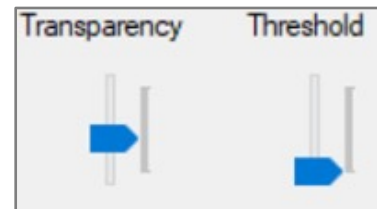
RGB Overlay



Digital Image Overlay



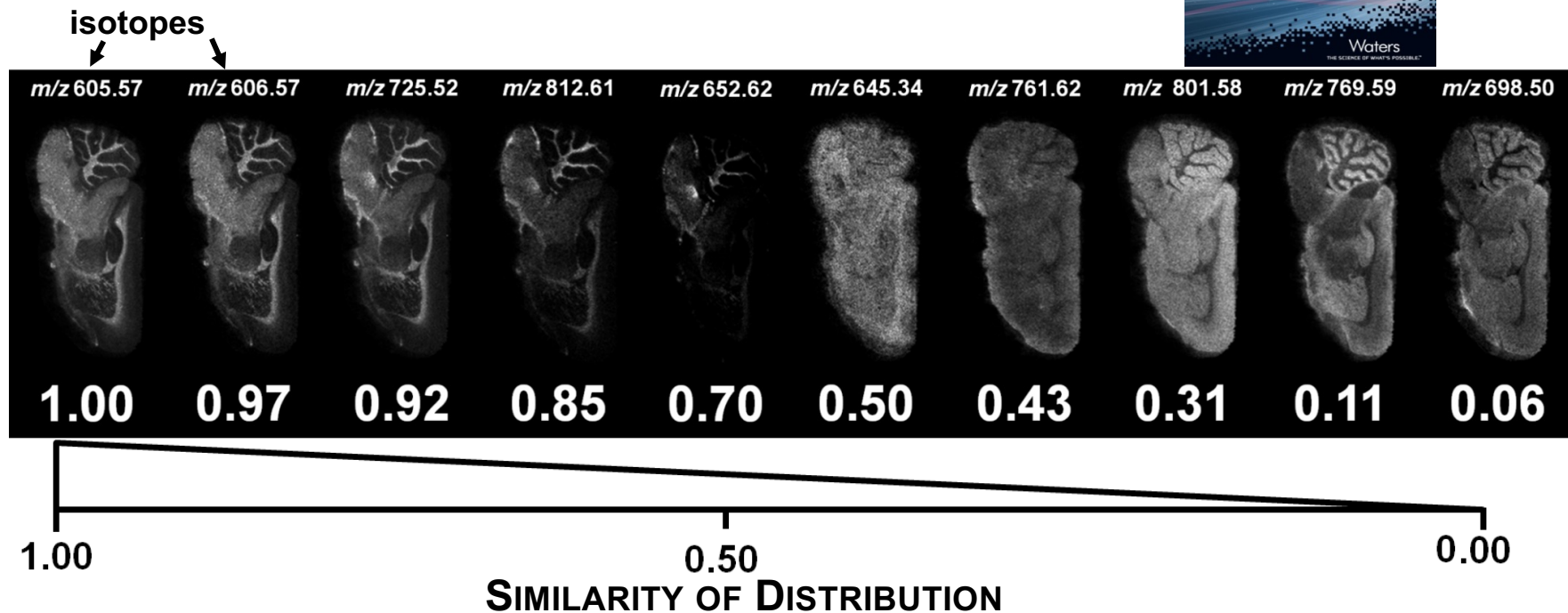
Digital Image Alignment



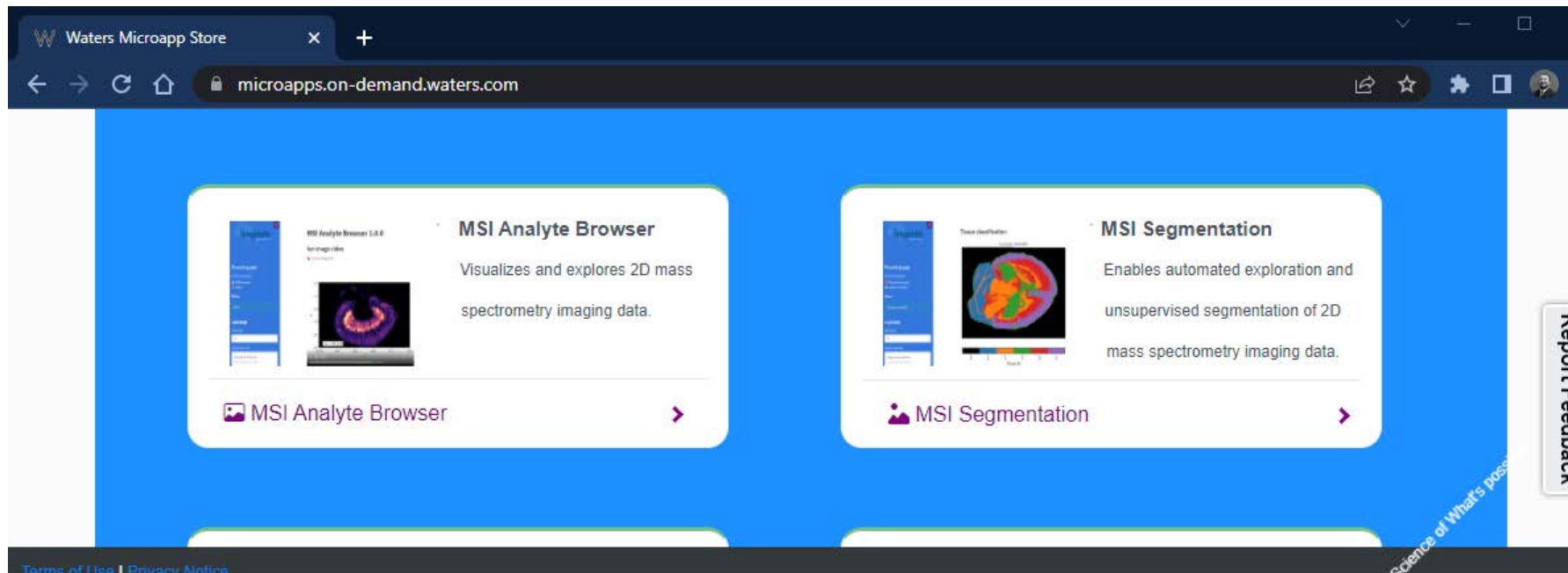
Spatial Correlation



Waters™



- Image correlation filter for an ion ranks other ions based their co-localization
- High spatial correlation coefficient → similar distribution



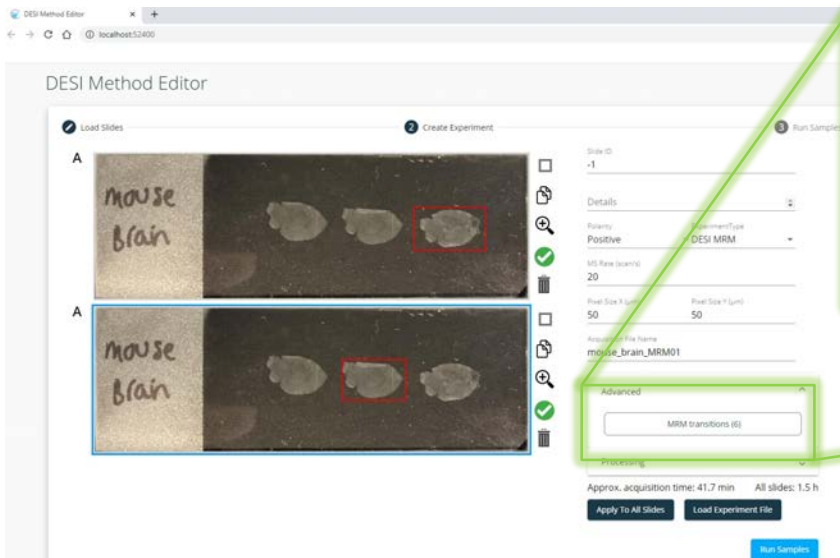
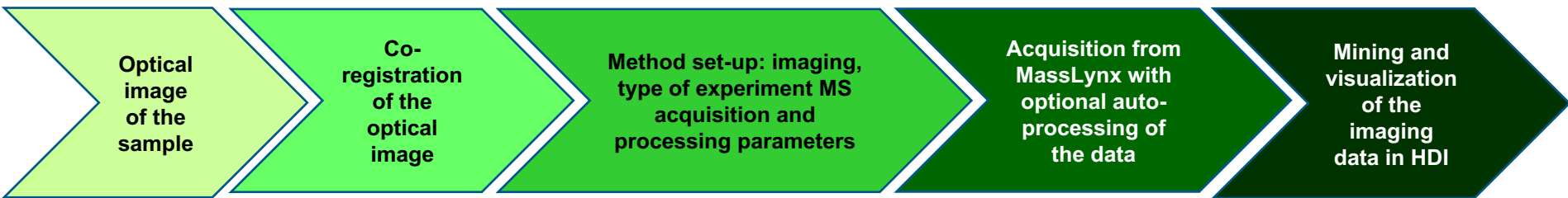
<https://microapps.on-demand.waters.com/>

3 imaging MicroApps free to download and use via website above.

An abstract graphic consisting of a network of interconnected nodes and lines, resembling a molecular structure or a data network. The nodes are represented by small circles of varying sizes and colors (light blue, dark blue, and grey), and the lines are thin, light blue lines connecting these nodes. The background is a gradient of blue, transitioning from a lighter shade on the left to a darker shade on the right. A horizontal blue bar is overlaid across the middle of the image, containing the word "Quantitation" in white, italicized text.

Quantitation

Targeted MS Imaging Software workflow



- Experiment types: MRM, MS, MS/MS (one transition) and SIR
- Up to 32 MRM and SIR transitions
- Addition of transition from library and from file

DESI MSI LODs of theophylline, atenolol and ranitidine in high resolution untargeted full scan MS and targeted MRM modes

MSI Quantify microapp dilution curve for ranitidine (+)

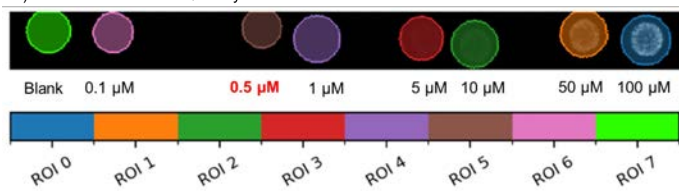
Courtesy of
Janssen, R&D,
Belgium

1) High resolution untargeted full scan MS

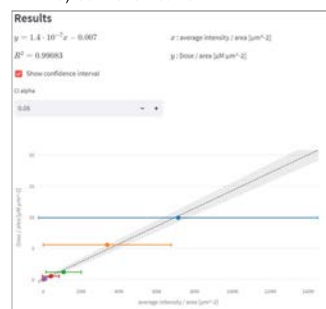
A) Ion image in HDI software



B) ROIs defined in MSI Quantify software



C) Calibration curve

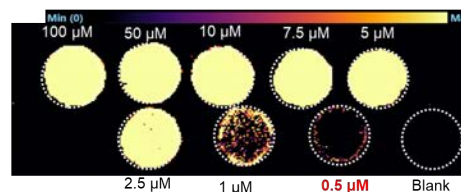


D) Evaluation of the predicted unknown on the dilution series

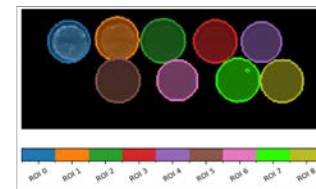
Label	Predicted dose [μM]	CI ($\alpha=0.05$) [μM]
ROI 0	102.8824	(95.0631, 110.7017)
ROI 1	43.5086	(40.0536, 46.9637)
ROI 2	13.0222	(10.4198, 15.6246)
ROI 3	5.3818	(2.2584, 8.5052)
ROI 4	1.2238	(-2.1060, 4.5536)
ROI 5	0.5948	(-2.3198, 3.5095)
ROI 6	0.1045	(-3.0615, 3.2705)
ROI 7	0.0222	(-3.3316, 3.3759)

2) Targeted MRM

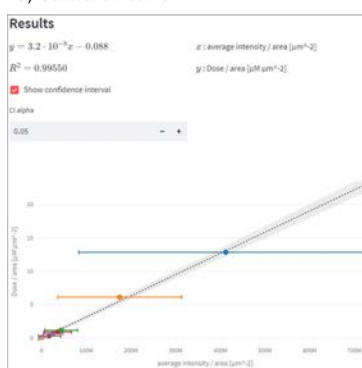
A) Ion image in HDI software



B) ROIs defined in MSI Quantify software



C) Calibration curve



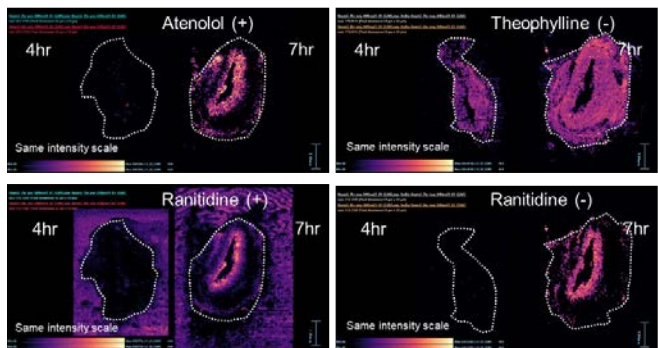
D) Evaluation of the predicted unknown on the dilution series

Label	Predicted dose [μM]	CI ($\alpha=0.05$) [μM]
ROI 0	101.5878	(96.6185, 106.5571)
ROI 1	44.9799	(42.6350, 47.3248)
ROI 2	11.9173	(9.7721, 14.0626)
ROI 3	9.1655	(7.1330, 11.1980)
ROI 4	5.9596	(3.9974, 7.9219)
ROI 5	4.3805	(1.9994, 6.7616)
ROI 6	0.1937	(-2.0403, 2.4276)
ROI 7	-0.6402	(-2.8218, 1.5414)
ROI 8	-0.671	(-2.7332, 1.3911)

DESI of dosed tissue of theophylline, atenolol and ranitidine in high resolution untargeted full scan MS and targeted MRM modes

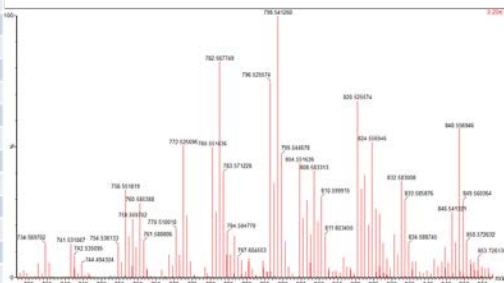
Courtesy of Janssen, R&D, Belgium

1) High resolution untargeted full scan MS

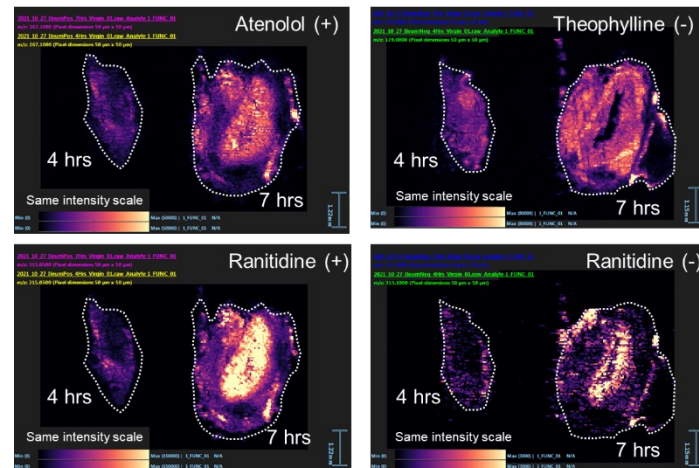


- Theophylline, ranitidine and atenolol were IV administration at 2.5 mg/kg tp Sprague-Dawley rats.
- Gastrointestinal (GI) track (ileum) were collected at 4 and 7 hours post dosed.

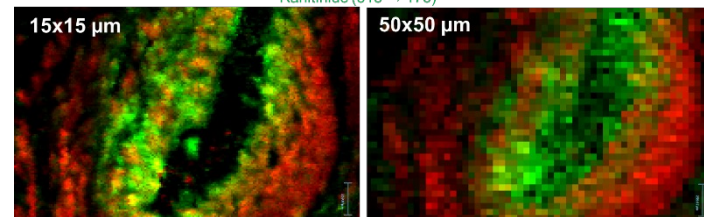
ID	Formula	Adduct	Expected mass	Observed mass	mDa error	ppb error
PC (32:0)	C ₄₀ H ₈₀ NO ₈ P	H ⁺	734.5694315	734.569702	0.271	368
PC (34:2)	C ₄₂ H ₈₀ NO ₈ P	H ⁺	758.5694315	758.569702	0.271	357
PC (34:1)	C ₄₂ H ₈₂ NO ₈ P	H ⁺	780.5850815	780.585388	0.306	403
PC (34:2)	C ₄₂ H ₈₀ NO ₈ P	Na ⁺	780.5513759	780.551636	0.260	333
PC (36:2)	C ₄₄ H ₈₄ NO ₈ P	H ⁺	786.6007316	786.601013	0.281	358
PC (34:2)	C ₄₂ H ₈₀ NO ₈ P	K ⁺	796.5253131	796.525574	0.281	328
PC (34:1)	C₄₂H₈₂NO₈P	K⁺	798.5409632	798.541126	0.297	372
PC (36:4)	C ₄₄ H ₈₀ NO ₈ P	Na ⁺	804.5513759	804.551636	0.280	323
PC (36:4)	C ₄₄ H ₈₀ NO ₈ P	K ⁺	820.5253131	820.525574	0.261	318
PC (36:3)	C ₄₄ H ₈₂ NO ₈ P	K ⁺	822.5409632	822.54126	0.297	361
PC (36:2)	C ₄₄ H ₈₄ NO ₈ P	K ⁺	824.5566133	824.556946	0.333	404
PC (38:5)	C ₄₆ H ₈₆ NO ₈ P	K ⁺	846.5409632	846.541321	0.358	423
PC (38:4)	C ₄₆ H ₈₄ NO ₈ P	K ⁺	848.5566133	848.556946	0.333	392
		Mean			0.291	364
		SD			0.033	34
		RMS			0.293	366



2) Targeted MRM



PC 34:1, K⁺ (798.5 → 163)
Ranitidine (315 → 176)



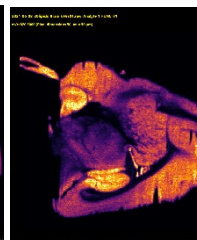
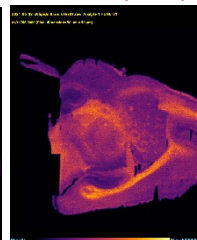
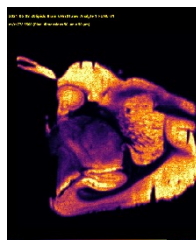
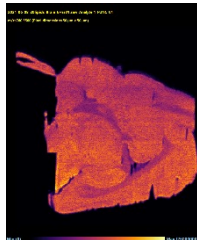
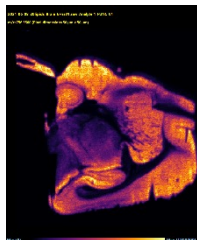
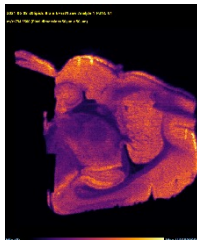
Targeted MS Imaging for endogenous biomarkers

lipid analysis (+) in mouse brain

734.5: PC (32:0),H⁺

760.5: PC (34:1),H⁺

786.5: PC (36:2),H⁺

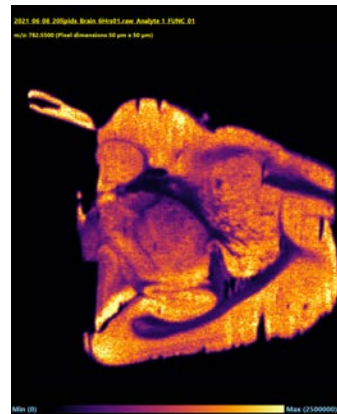
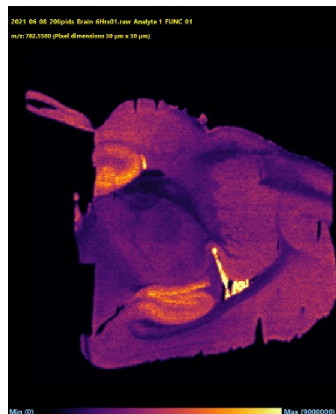


	Precursor ion		Product ion	Dwell (ms)	Collision energy
1	734.55	>	184	6	35
2	756.55	>	147	6	35
3	758.55	>	184	6	35
4	760.55	>	184	6	35
5	772.55	>	163	6	35
6	782.55	>	147	6	35
7	782.55	>	184	6	35
8	786.6	>	184	6	35
9	792.2	>	147	6	35
10	798.55	>	163	6	35
11	806.55	>	184	6	40
12	806.6	>	147	6	40
13	810.6	>	147	6	35
14	820.5	>	163	6	40
15	828.55	>	147	6	40
16	828.6	>	163	6	40
17	830.55	>	163	6	40
18	834.6	>	147	6	40
19	848.6	>	163	6	40
20	872.6	>	163	6	40

756.5: PC (32:0),Na⁺

772.5: PC (32:0),K⁺

820.5: PC (36:4),K⁺



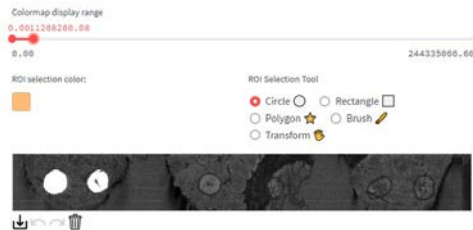
782.5: PC (34:1),Na⁺

782.5: PC (36:4),H⁺

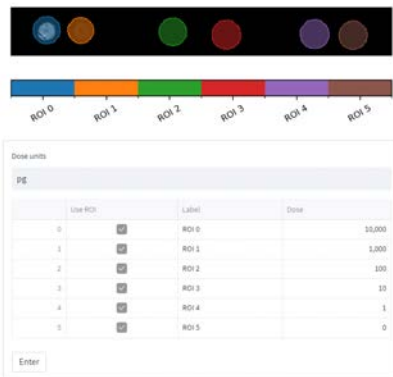
- Obtain comprehensive and detailed spatial information with the low sample prep requirements of DESI
- Visualize and quantify known biomarkers, APIs, and metabolites at therapeutic and low biological concentrations
- Combine high sensitivity with higher throughput to handle imaging studies more efficiently
- Access quantification workflows for MS Imaging
- Reduce the data size and interpretation burden of complex, full scan MS imaging data sets
- Add MSI analysis into a multi-modal imaging workflow with ease
- Achieve improved environmental sustainability and lower laboratory operational costs with an MSI system that uses 50% less electricity and gas and produces 50% less heat than most other QQQ systems

MSI Quantify MicroApp

Draw ROI



Select ROIs



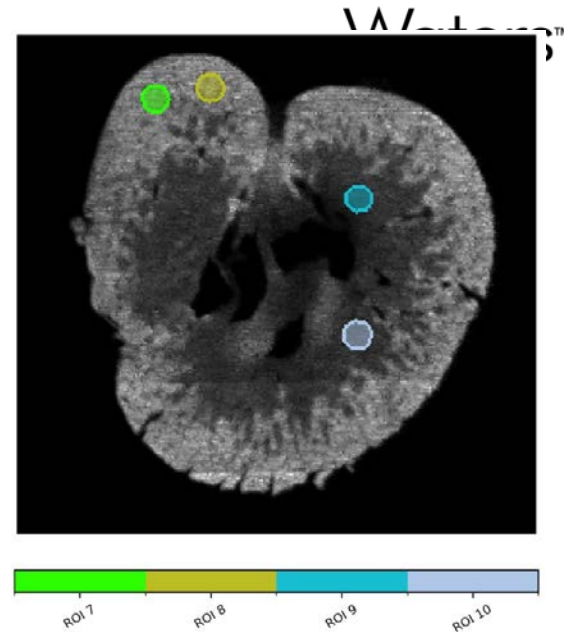
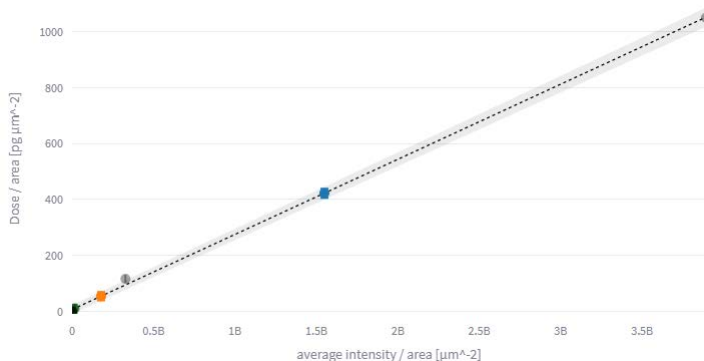
- Quantitative workflow
- Dilution series + dosed tissue data loaded in a single project
- Define Regions of Interest (ROIs)
- Generate standard curve from the dilution series data
- Apply the standard curve to dosed tissue data

Results

$$y = 6.5 \cdot 10^{-6}x - 20.814$$

$R^2 = 0.99967$

x : average intensity / area [μm^2]
 y : Dose [pg]



	Label	Predicted dose [pg / μm^2]	CI ($\alpha=0.05$) [pg / μm^2]
0	ROI 7	68.6337	(54.0290, 83.2383)
1	ROI 8	66.6941	(52.0972, 81.3210)
2	ROI 9	28.5759	(13.4530, 43.6988)
3	ROI 10	32.3397	(17.2706, 47.4088)



Wrap Up

MS IMAGING

Waters™

DISCOVERY IMAGING

TARGETED DESI IMAGING

- DESI XS Xevo Absolute



Benchtop DESI MS

- DESI XS Xevo G2-XS
- Perfect for entry level imaging & discovery platform with SONAR



Full Spectrum Molecular IMAGING (FSMI) ie DESI XS & MALDI

DESI with IMSⁿ

- DESI XS SELECT SERIES DESI XS Cyclic IMS
- Scalable IMS with unique IMSⁿ capability
- 100K Mass Resolution



High Performance

- DESI XS and SELECT SERIES MALDI MRT
- PPB mass accuracy
- 200K mass resolution independent of scan speed



Flexibility

- DESI XS and MALDI SYNAPT XS
- Complementary IMS, SONAR & DDA for complex mixtures



NextGen MSI Webinar Series
Overview of the Latest High Resolution
Mass Spectrometry Technology
All 4 Sessions
Available On Demand

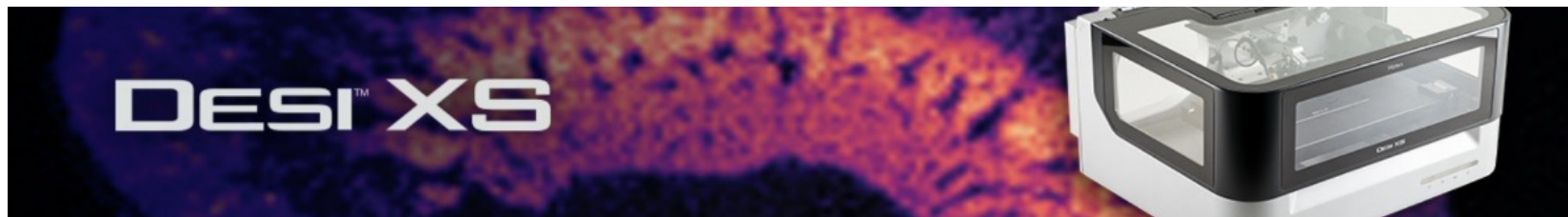


[MSI WEBINAR SERIES]

Waters
THE SCIENCE OF WHAT'S POSSIBLE™

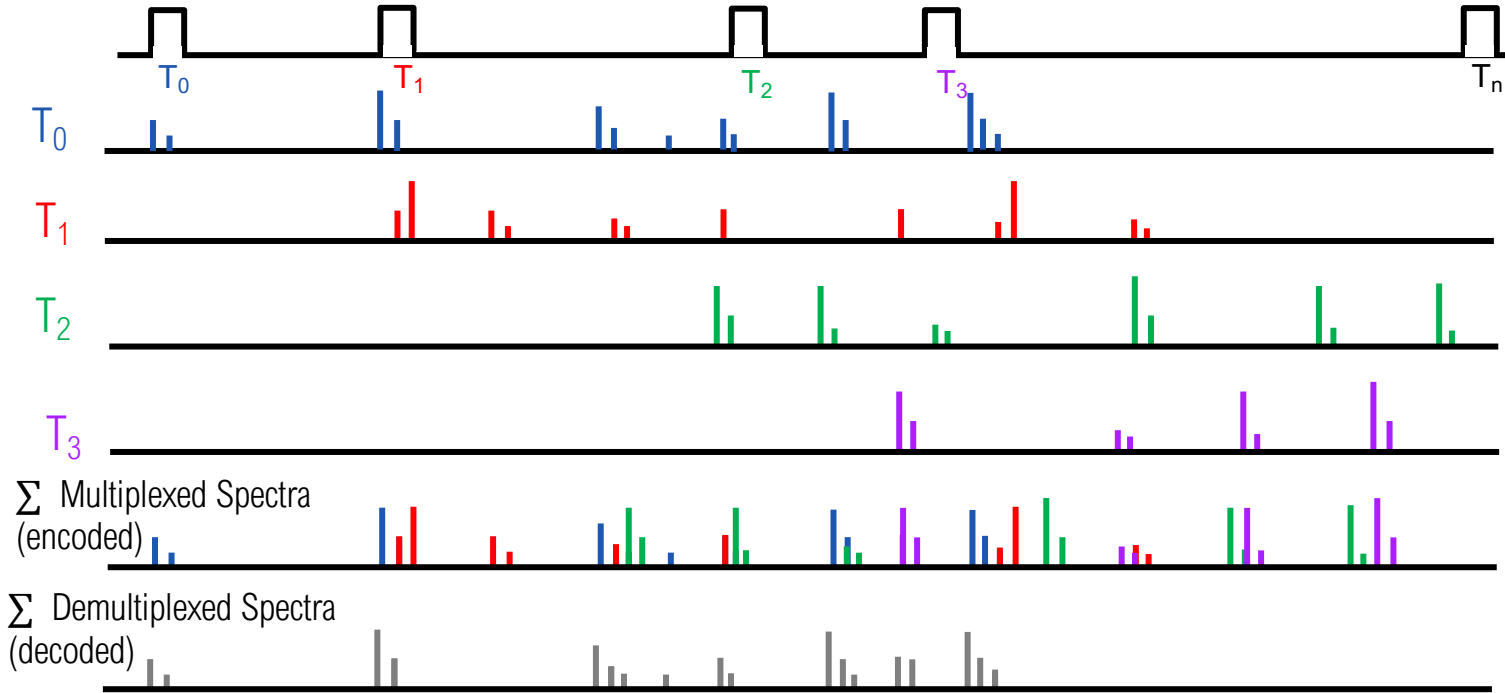
Thank you for your attention!
Any questions?

Thoughts to take away...



- Mass Spec Imaging with DESI XS requires limited sample preparation and is now a user-friendly technique
- It determines where things are in samples (localisation) and show compound differences between samples
- DESI XS can be combined with a variety of different Waters mass spectrometers to address a range of challenges

Encoded Frequent Pushing (EFP) – Conceptual Framework



- Duty cycle is improved by pushing ions into the TOF before ions from previous pushes have reached the detector
- Ions are pushed in multiple defined (encoded) pushes that overlap and decoding with statistical method to demultiplex final spectra
- Benefits are an increase in sensitivity, better mass accuracy (more ions per signal = better peak shape), reduced background noise (decoding is statistical and random noise is classed as 'incoherent' and removed)

