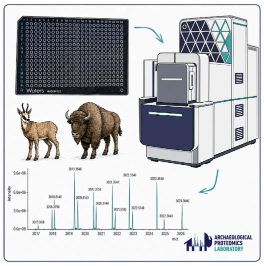
Jun 11, 2026

WATERS SELECT SERIES MRT MALDI Spot Analysis for Zooarchaeology by Mass Spectrometry (ZooMS) of collagenous material



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol gives guidance on how to set up and run a Waters™ Select Series Multi-Reflecting Time-of-Flight (MRT) with a MALDI source attachment for the purposes of Zooarchaeology by Mass Spectrometry (ZooMS) of collagenous material. ZooMS allows for taxonomic identifications to be made based on peptide mass fingerprinting of collagen type 1. The Waters MRT is an ultra-high resolution imaging instrument with MALDI spot analysis capabilities, allowing for high precision ZooMS to be carried out. This instrument has the potential to achieve sub-ppm mass accuracy, therefore, results can give peptide peaks accurate to at least one decimal place allowing for contaminants and non-diagnostic peptide markers to be distinguished.

Equipment

Select Series MRT	NAME
MALDI	TYPE
Waters	BRAND
https://www.waters.com/nextgen/us/en/products/mass-spectrometry/mass-spectrometry-systems/select-series-mrt.html?srsltid=AfmBOopwbKjMBGQ3lWwtUfEUhpYa8cC7Pwq8lRwSnPpKqvW61xH_cnuz#:~:text=Maximize%20MS%20imaging%20capabilities%20with%20DESI%20and%20MALDI&te	LINK
355 nm Nd:YAG laser, tightly focused laser beam profile; Quadrupole Multi-Reflecting Time of Flight (Q-ToF)	SPECIFICATIONS

Attachments

FILE	PNG	PNG	PNG	PNG	PNG	PNG
_extern.inf	MALDI_Values.png	StepWave_Values.png	MSProfile_Values.png	IonGuides_Values.png	Transfer_Values.png	RF_Values.png
12KB	259KB	134KB	189KB	197KB	155KB	107KB
FILE	FILE					
Sample_A.msd	Sample_B.msd					
2.3MB	2.2MB					

Guidelines

1. See "Materials" for reagent recipes
2. Mass Spectrometry is sensitive to contaminants eg. detergents, plastics etc.
3. This protocol can be performed in a standard wet chemistry laboratory.
4. Wear relevant PPE as designated by your local laboratory.
5. Follow local lab guidelines regarding sample handling and storage.
6. Follow country and facility specific guidelines regarding the disposal of chemical waste.
7. This protocol is intended to give guidance to mass spectrometry and/or Waters technicians - instrument settings should not be altered without specialist training.

Materials

Reagents

0.1% TFA/ 5% Acetonitrile (v/v) in HPLC-MS water: Add 940 µL **HPLC-MS** water to a 1.5 mL microcentrifuge tube. Add 50 µL ACN. Carefully add 10 µL 10% TFA. Secure cap and vortex briefly.

Conditioning Solution; 0.1% TFA in 50:50 Acetonitrile and HPLC-MS water: for 500ml, add 250 mL ACN to 250 mL LCMS water and add 500 µL 100% TFA.

α-Cyano-4-hydroxycinnamic acid (CHCA) | **VWR Catalog #**SIALC2020-10G : [M] 10 mg/mL in Conditioning Solution. Ensure powder is fully dissolved either through vortexing or heating (or sonicating if using a glass vessel) - hold up to the light to be sure. Store at Room temperature for no more than 24:00:00 . Store in the dark.

Red Phosphorous (RedP) | **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**343242-5G [M] 25 mg/mL finely ground suspension in ACN.

Waters™ MassPREP ADH Digestion Standard | **Waters Catalog #**186002328 Resuspend vial in 1 mL of 0.1% TFA/ 5% ACN (v/v) in LCMS water. This will give a concentration of [M] 1 pMol/µL . Aliquots should be stored -20 °C and only thawed immediately before use, do not continuously freeze-thaw.

Methanol (MeOH) | **Fisher Scientific UK Catalog #**10653963

Water | **Fisher Scientific UK Catalog #**10777404

Acetonitrile (ACN) | **Fisher Scientific UK Catalog #**10616653

Equipment

MALDI 480 Target Plate	NAME
Waters	BRAND
405018182	SKU
Polished Stainless Steel	SPECIFICATIONS

Equipment

Select Series MRT	NAME
MALDI	TYPE
Waters	BRAND
https://www.waters.com/nextgen/us/en/products/mass-spectrometry/mass-spectrometry-systems/select-series-mrt.html?srsltid=AfmBOopwbKjMBGQ3lWwtUfEUhpYa8cC7Pwq8lRwSnPpKqvW61xH_cnuz#:~:text=Maximize%20MS%20imaging%20capabilities%20with%20DESI%20and%20MALDI&te	LINK
355 nm Nd:YAG laser, tightly focused laser beam profile; Quadrupole Multi-Reflecting Time of Flight (Q-ToF)	SPECIFICATIONS

Protocol materials

- Water | **Fisher Scientific UK Catalog** #10777404
- Methanol (MeOH) | **Fisher Scientific UK Catalog** #10653963
- Red Phosphorous (RedP) | **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #343242-5G
- Waters™ MassPREP ADH Digestion Standard | **Waters Catalog** #186002328
- α-Cyano-4-hydroxycinnamic acid (CHCA) | **VWR Catalog** #SIALC2020-10G
- Acetonitrile (ACN) | **Fisher Scientific UK Catalog** #10616653

Safety warnings

∗It is recommended that for MALDI analysis on the MRT, 50 volts is added to the detector voltage that has been obtained via the automatic detector gain setup. This should be done in consultation with the manufacturer.

Before start

1. We recommend using dedicated glassware where possible for preparation and storage of reagents.
2. Peptide extracts for troubleshooting this protocol were produced using an Acid Insoluble ZooMS protocol modified from Buckley et al. 2009 and Welker et al. 2015.
3. We do not recommend spotting the peptide extracts more than 24 hours in advance.
4. Ensure the MALDI laser window, mirror and octopole are clean.

Spotting

1 Spot Sample 1:1 with α-Cyano-4-hydroxycinnamic acid (CHCA) | **VWR Catalog** #SIALC2020-10G matrix (see Materials). Spot peptides first, then mix with CHCA by pipetting in a circular motion on the plate 1 µL of each

Note

Final volume in each target well should be 2 µL to cover full surface area

2 Spot the [M] 1 pMol/µL aliquots of Waters™ MassPREP ADH Digestion Standard | **Waters Catalog** #186002328 (see Materials) 1:1 with CHCA in the same manner as the peptide extracts. Spot 8 replicates. Each spot has a final concentration of [M] 500 fMol/µL

3 Allow the plate to dry on a heater block 35-50 °C to encourage crystallisation. Remove as soon as the last spot has dried.

4 The plate should be kept at room temperature in the dark if not running immediately.

Instrument Calibration

5 Ensure Red Phosphorous (RedP) | **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #343242-5G (see Materials) is resuspended before and during this step by shaking gently.

6 Spot 1 µL of RedP onto a calibrant spot 4-5 times, allowing 00:00:20 in between each dispense for the layer to dry.

7 Load the plate into the instrument and ensure the vacuum has been established.

8 Acquire data for the calibration using the following settings

Note

- Positive ; MRT ; Standard ; 1.0s ; 9600
- API Gas flow rate 350 mL/min
- Laser Rate 2500 Hz
- Laser Intensity 300
- Lens Focus 4.00 mm
- Step Size: X 1000 µm and Y 100 µm
- Scan Time 1.0 sec
- Decoding Threshold Automatic
- Function MS

9 Only accept the calibration if the Mean Prediction Error is ≤ 0.2 ppm.

Instrument Standard and Laser Optimisation

10 Test three laser intensities: 250, 300 and 350 to confirm optimum settings using the spotted ADH.

Note

The laser intensity needs to be optimised depending on variable environmental factors and laser wear and tear. This will also act as an instrument standard to ensure functionality before and after processing samples.

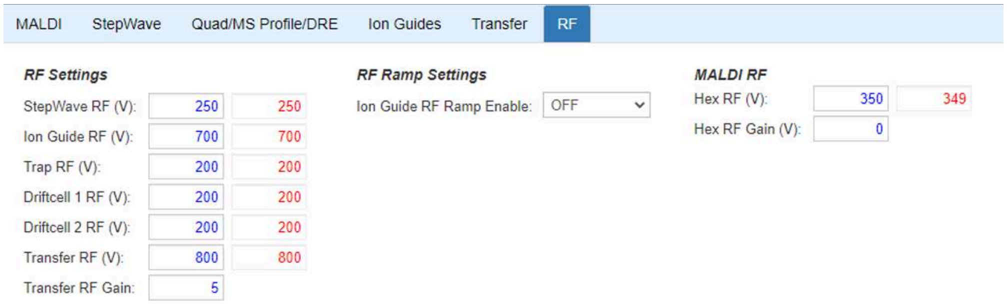
11 Acquire data using the following recommended settings

Note

- Positive ; MRT ; Standard ; API Gas ; 1.0s ; 9600
- Laser Rate 2500 Hz
- Lens Focus 4.00 mm
- Well Diameter: 2.15 mm ; Step Size: X 500 µm and Y 150 µm
- Scan Time 1.0 sec
- m/z range 1000 - 3500
- Decoding Threshold Low
- Function MS

Manual Quad Profile:
- Dwell time 20% at 1000 m/z
- Dwell time 40% at 1500 m/z
- Top end mass 3000 m/z
- Ramp time 20%

The RF controls below were applied to allow for higher peptide markers to pass through the quadrupole:



RF Settings

StepWave RF (V):	250	250
Ion Guide RF (V):	700	700
Trap RF (V):	200	200
Driftcell 1 RF (V):	200	200
Driftcell 2 RF (V):	200	200
Transfer RF (V):	800	800
Transfer RF Gain:	5	

RF Ramp Settings

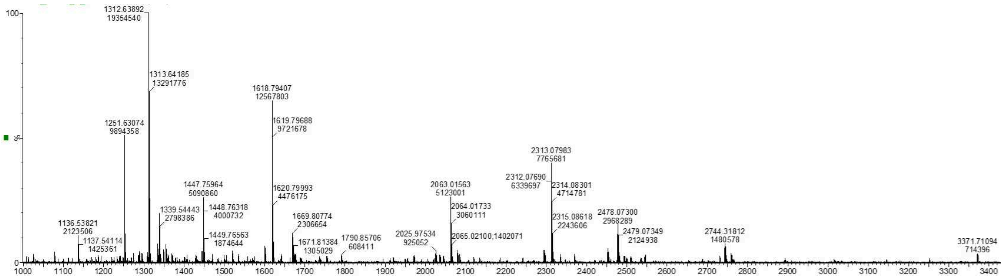
Ion Guide RF Ramp Enable: OFF

MALDI RF

Hex RF (V):	350	349
Hex RF Gain (V):	0	

See attachments for full settings and an example run file.

12



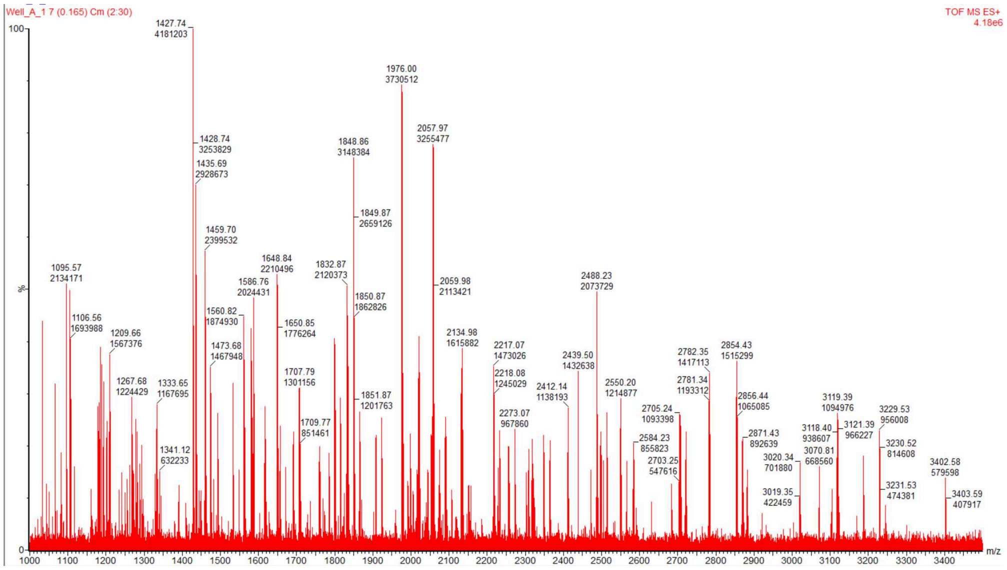
ADH Spectrum

Expected result

Key peptide peaks that should be observed are 2744 m/z and 3369 m/z to ensure higher mass peptides are being appropriately measured.

Sample Data Collection

13 Use the same settings as for the ADH instrument standard. Set the laser intensity to the optimised setting based on spectral quality of signal-to-noise and TOF MS ES+ counts.



Sample Spectrum

14 Ensure you set the instrument to acquire all sample data, followed by one ADH spot to confirm consistency throughout the run.

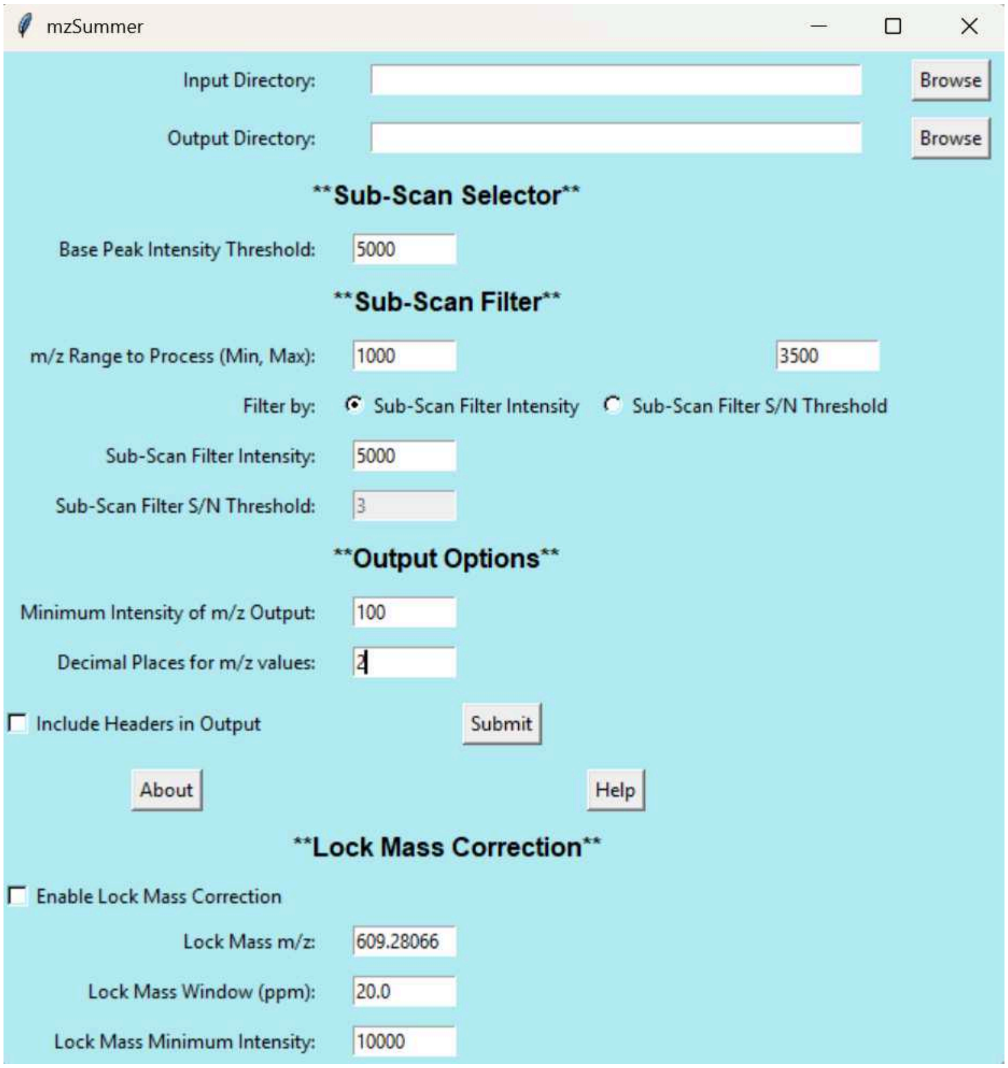
15 Each spot receives 30 scans which will be summed.

Data processing

16 The Waters raw data files (.raw) can be converted to mzML format using MSConvert from ProteoWizard (Chambers et al., 2012)

17 The individual scans can be summed into .txt files using an in-house developed Python script (MZSummer), which we make publicly available here:

https://github.com/nm303909/MZSummer/tree/main



Input Directory:

Output Directory:

Sub-Scan Selector

Base Peak Intensity Threshold: 5000

Sub-Scan Filter

m/z Range to Process (Min, Max): 1000 3500

Filter by: ● Sub-Scan Filter Intensity ○ Sub-Scan Filter S/N Threshold

Sub-Scan Filter Intensity: 5000

Sub-Scan Filter S/N Threshold: 3

Output Options

Minimum Intensity of m/z Output: 100

Decimal Places for m/z values: 2

☐ Include Headers in Output

Lock Mass Correction

☐ Enable Lock Mass Correction

Lock Mass m/z: 609.28066

Lock Mass Window (ppm): 20.0

Lock Mass Minimum Intensity: 10000

MZSummer user interface

See our standard settings in the screenshot above - these settings can be adjusted to suit your needs. (We recommend running this programme overnight as it can take several hours if running a full plate on a laptop)

18 After summing, any technical replicates can be merged in R using the MALDIquant and MALDIquantForeign packages and published scripts (Gibb and Strimmer, 2012; Mylopotamitaki et al., 2024)

19 Once you have fully processed the raw data into a workable file, we advise following this separate guide for completing spectral identifications:

https://www.protocols.io/view/identifying-zooms-spectra-mammals-using-mmass-kqdg36rppg25/v1. Some modifications may be necessary due to the ultra-high resolution of the spectra (e.g. you may consider unselecting the deisotoping panel for peak picking due to the narrow peaks).

See attachments for example summed and merged MRT spectra: Sample_A and Sample_B

Target Plate Cleaning

20 Rinse the plate Methanol (MeOH) | **Fisher Scientific UK** **Catalog #**10653963 .

Visibly check the plate and use a cotton bud, damp with MeOH, to manually wipe any residue that was not easily rinsed away.

21 Sonicate the plate in 50:50 MeOH and Water | **Fisher Scientific UK** **Catalog #**10777404 for 00:15:00

22 Dry in the fume hood either under regular extraction or with a Nitrogen gas tube.

Protocol references

Buckley, M., Collins, M., Thomas-Oates, J., & Wilson, J. C. (2009). Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry: RCM, 23(23), 3843–3854. https://doi.org/10.1002/rcm.4316

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Acknowledgements

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