

MALDI Imaging of blood and blood provenance in old fingermarks

FP 216, ASMS 2021

Matthias Witt,¹ Cameron Heaton,² Katie Kennedy,² Glenn Langenburg,³ Simona Francese,²

¹Bruker Daltonics GmbH & Co. KG, Bremen, Germany
²Sheffield Hallam University, Sheffield, UK
³Bruker Elite Forensic Services, Saint Paul, MN, US

Introduction

Reliable confirmation of the presence of blood in stains and marks is crucial to reconstruct the dynamics of a violent crime. MALDI MS Profiling (MALDI MSP) and MALDI MS Imaging (MALDI MSI) have been shown to be suitable confirmatory tests to this effect [1-4]. However, additional and important information is based on the attribution of the source of blood (animal or human), as a few murder cases in the public domain demonstrate. The Fingermark Research Group at Sheffield Hallam University, recently published on the validation of a MALDI MSP method to detect and distinguish between human and animal blood (down to the animal species level) in enhanced blood marks and stains analyzed in a blind fashion [5] (Figure 1). Here we use a combination of bottom-up proteomics and MALDI MSI to visualize blood of bovine or human origin in 4-year-old fingermarks. These are unused marks originating from the batch of blind samples on which the Kennedy et al. publication [5] was based.

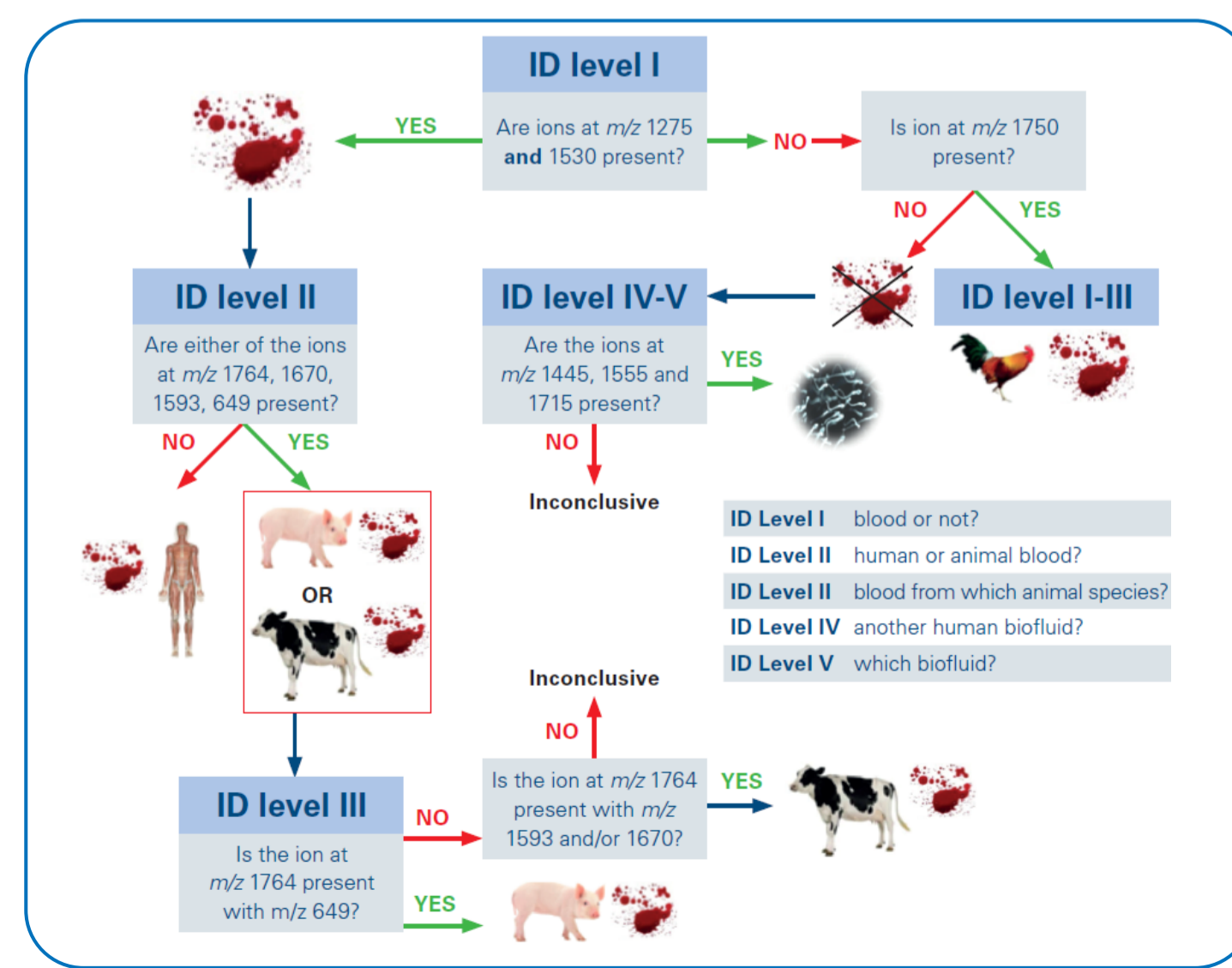


Figure 1: Blind sample spectra data interpretation strategy.

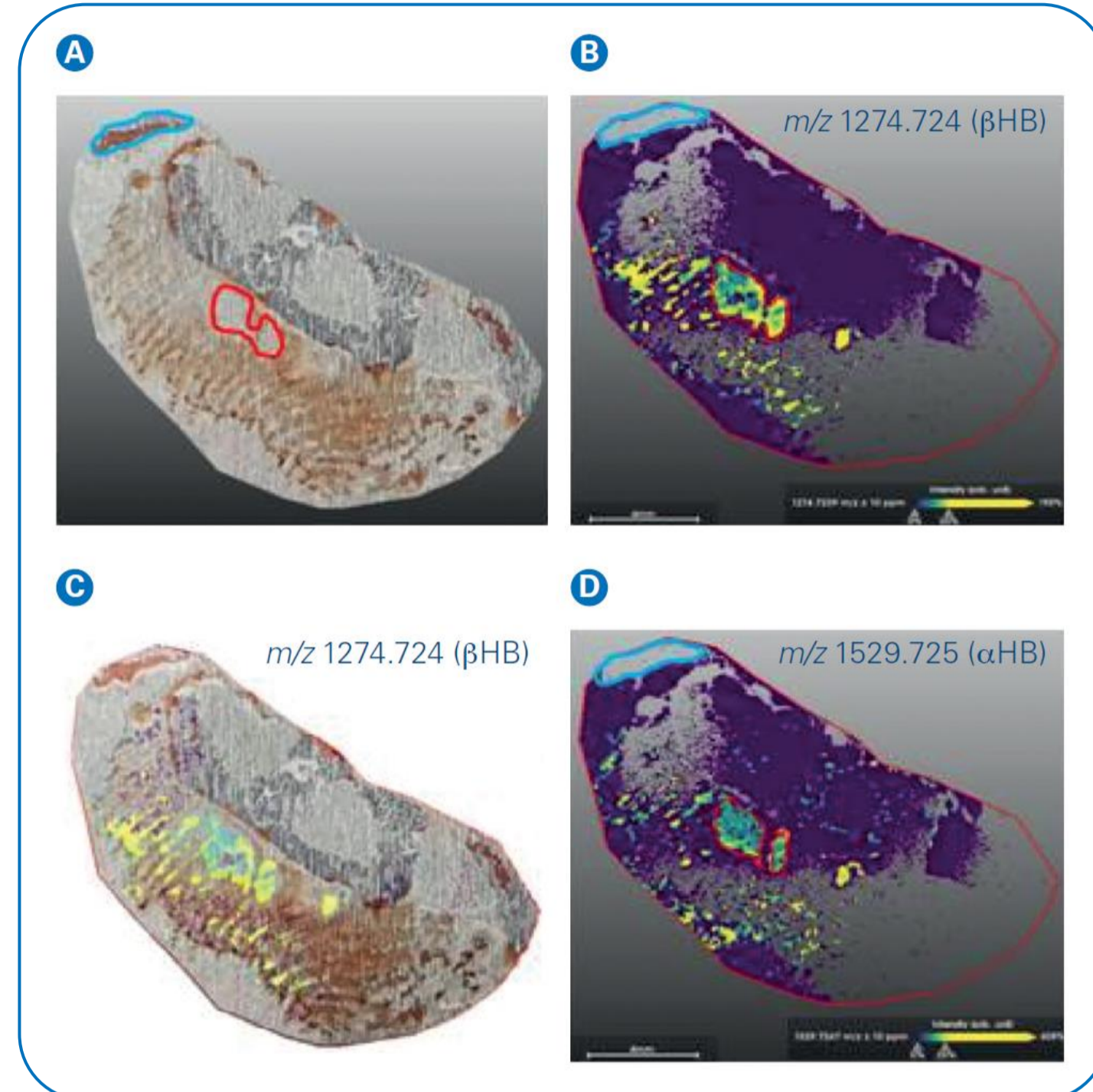


Figure 2: MALDI MSI of a human blood fingermark of 4 years of age stored in uncontrolled ambient conditions. (A) optical image; (B) and (D) MALDI MS image of the HB ions at m/z 1274.724 (β HB) and 1529.725 (α HB) respectively, measured with a 10 ppm mass accuracy. (C) Overlay of the image at m/z 1274.724 with the optical image. Blue and red framed highlight: regions of the mark where blood is visible or not visible respectively, on the optical image.

Methods

Sample preparation

The fingermarks investigated were generated by loading the fingertip with approximately 40-50 μ L of either human blood or bovine blood and by contacting the fingertip with an aluminum slide after 10-15 seconds. Once dried, in the case of the bovine blood mark, Acid Black-1 (AB-1) was applied using the same protocol employed by crime labs for the enhancement (visualization) of blood marks. The fingermarks marks were digested *in situ* by spraying trypsin using the SunCollect (Sunchrom, Germany). After incubation at 37°C, HCCA in 70/30 acetonitrile/TFA (0.5%)*aq* was sprayed for a total of 5 layers. The blood fingermarks were imaged on a timsTOF flex mass spectrometer (Bruker Daltonics GmbH & Co. KG, Germany) at a lateral resolution of 50 μ m in the mass range m/z 100-3000. Images were processed using SciLS™ Lab software using RMS normalization.

References

[1] Bradshaw R et al. (2014), Sci. Just. J. Forensic Sci. Soc. 54, 110-117; [2] Patel E et al. (2015), Analyst 141, 191-198
 [3] Kamanna S et al. (2017), Rapid Commun. Mass Spectrom. 31, 1927-1937; [4] Deininger L et al. (2016), Proteomics 16, 1707-1717; [5] Kennedy et al. (2020), Scientific Reports 10, 17087-17104

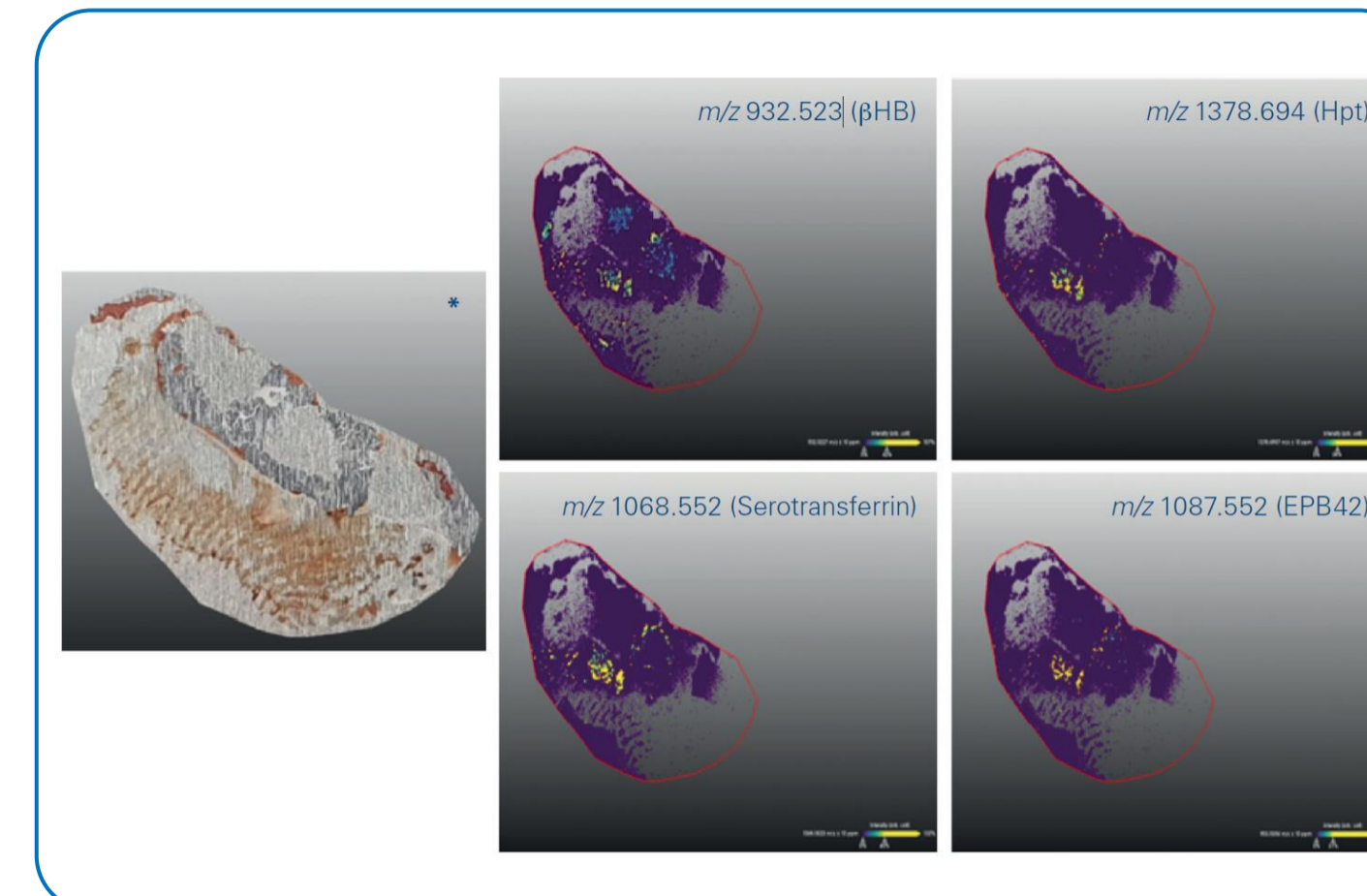


Figure 3: MALDI MS images of blood specific protein deriving peptides in a human blood fingermark. Panel* shows again the optical image of the human blood mark that was imaged. Many ion signals indicate the presence of blood through the detection of HB, Hpt, Serotransferrin and EBP42.

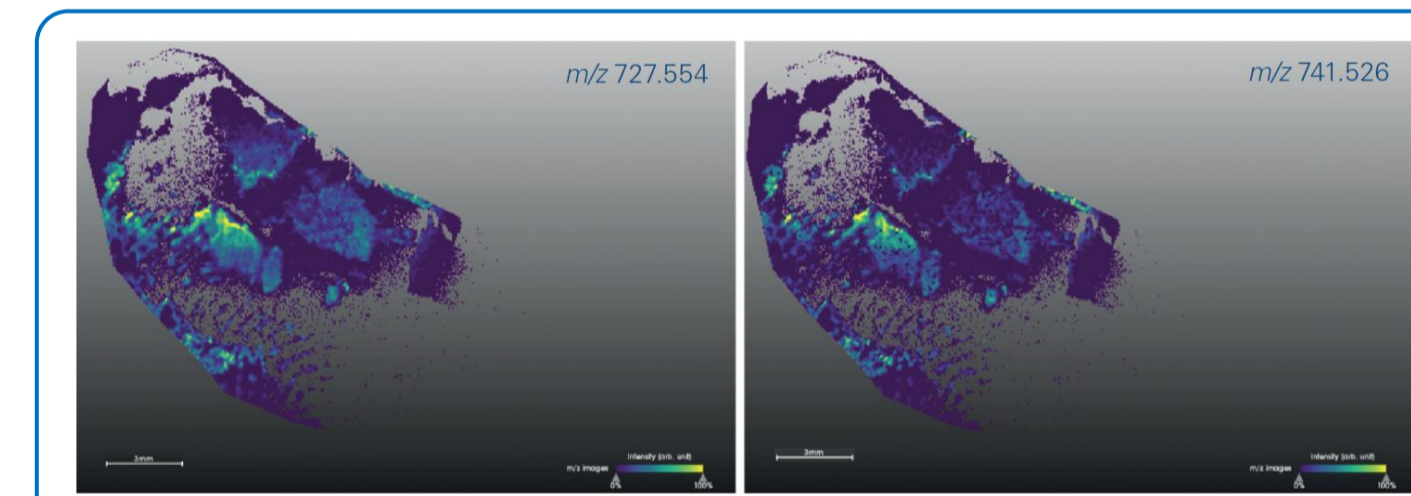


Figure 4: MALDI MS images of lipids at m/z 727.554 and 741.526 in a human blood fingermark. These ion signals show additional ridge coverage with respect to the blood specific protein-deriving peptides.

Results

The investigated human blood mark exhibits poor ridge detail (Figure 2). This is somewhat common in many violent crime scenes, where blood marks are generated through careless contact of the bloodied fingertip with the surface. Although no imaging technique can improve the quality of an originally poor/smudged ridge pattern, the MALDI MSI analysis on the timsTOF flex instrument was able to accurately visualize Haemoglobin (HB) onto the visible ridge flow in this 4-year-old human blood mark (Figure 2). In addition, some ridge flow was reconstructed and found to be superimposable with that exhibited by the optical image. It is important to note that MALDI MSI revealed the presence of blood (through HB) where this was not visible on the optical image (red frame highlight, Figure 2).

However, in the region where blood was visible by the naked eye (blue framed), MALDI MSI did not yield the HB signals. These occurrences reflect the delicate nature of the optimal enzyme : substrate ratio. This observation indicates that, as the presence of Haemoglobin may vary greatly across the blood mark itself, visualization of this biofluid will be dependent on its local ratio with trypsin

The simultaneous detection of the ion signals at m/z 1274.724 (β HB) and 1529.725 (α HB) indicates the presence of human blood, according to the findings of Kennedy et al. [5] and within the system that they investigated. Additional (and aspecific) HB signals and those from other blood specific proteins previously detected [4] were also visualised. These are: Erythrocyte Band Protein 4 (EBP42), Haptoglobin (Hpt) and Serotransferrin (Figure 3) detected with a mass accuracy ranging between -3.1 to 0.9 ppm.

However, in some cases, the distribution maps were weak, reflecting the low abundance of these proteins and the lower ionization yield of the corresponding peptides (compared to those of Haemoglobin).

In order to improve the quality of the biometric information, lipids can also be imaged within the same analysis. Figure 4 illustrates an example for two lipids at m/z 727.554 and 741.526 respectively, yielding molecular images of the mark in which additional ridge coverage can be observed. A bovine blood fingermark has been subsequently imaged to assess the possibility to map the bovine blood biomarkers discovered by Kennedy et al. [5] using MALDI MSI. Once again, the mark has been produced with no specific intention to generate ridge detail but simply mimicking a crime scene scenario in which a fingertip containing much blood is contacted carelessly with a surface.

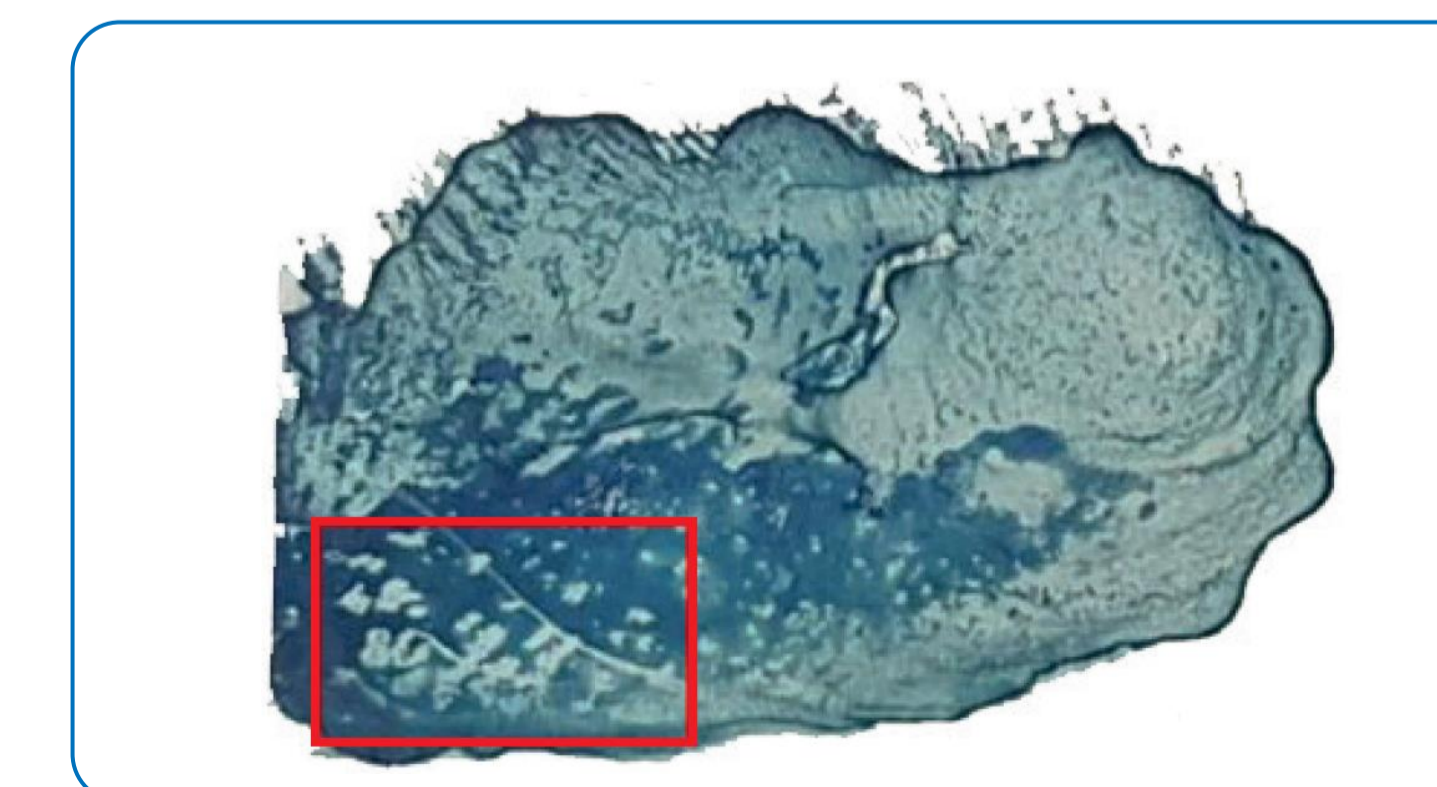


Figure 5: Optical image of an Acid Black 1 (AB-1) enhanced bovine blood fingermark. The red frame shows partial ridge pattern.

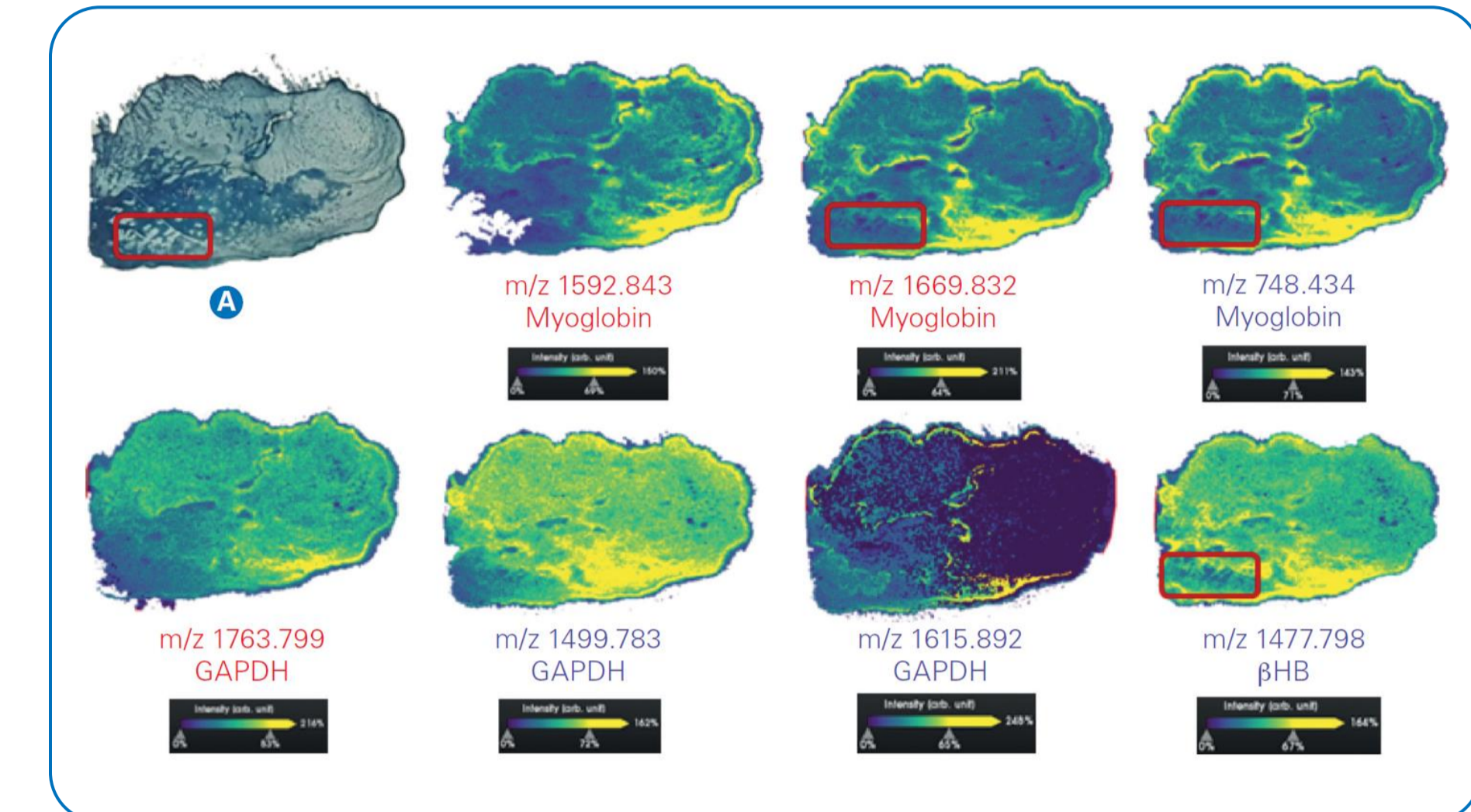


Figure 6: (A) shows the optical image of the AB-1 enhanced bovine blood mark. The red rectangle indicates the presence of ridge flow in both the optical image and some of the MS images. The m/z values in red indicate the proteotypic bovine blood peptide biomarkers within the system investigated by Kennedy et al. [5].

Conclusions

This short investigation confirmed the ability of MALDI MSI to image blood fingermarks and yield ridge detail in old fingermarks. This capability was contextual to detecting and imaging, for the first time, biomarkers enabling discrimination of non-human blood with the clear indication of bovine blood. As the marks were 4 years old by the time they were imaged, these results show that, by using a high end mass spectrometer capable of high sensitivity, spatial and mass resolution, it may be possible to perform MALDI based confirmatory tests for blood marks recovered at the scene of violent crimes that can sometimes be accessed at a much later time after the crime has been committed.

MALDI Imaging