# Forensic visualisation of blood and blood provenance in old fingermarks by MALDI MS Imaging

Blood can be often found at the scene of violent crimes. Whether visible or latent, it is critical that its presence is detected, confirmed and distinguished from other biofluids as well as determining its provenance.


## Abstract

This intelligence collectively aids in the reconstruction of the dynamics of the crime and indicates the nature of the crime itself. Over the years Matrix Assisted Laser Desorption/Ionisation (MALDI) Mass Spectrometry Profiling and Imaging (MSP and MSI) have demonstrated to be suitable analytical tools as confirmatory tests for blood stains and blood marks. MALDI MSP and MSI can prove or disprove the results of the currently applied presumptive tests deployed at the scene. Additionally, fingermark molecular images can be obtained through the mapping of human blood specific proteins in fresh fingermarks. Here, for the first time, we demonstrate the mapping of biomarkers of human and animal blood detected in four year old fingermarks.



*Keywords: Molecular fingerprinting, SCiLS, timsTOF fleX, Imaging*



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## 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 established 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 analysed in a blind fashion [5] (Figure 1).

Here we use a combination of bottom-up proteomics and MALDI MSI to visualise 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.

## Methods

The fingermarks investigated were generated by loading the fingertip with approximately 40-50 $\mu$L of either human blood or bovine blood and by contacting the fingertip with an aluminium 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 (visualisation) of blood marks.

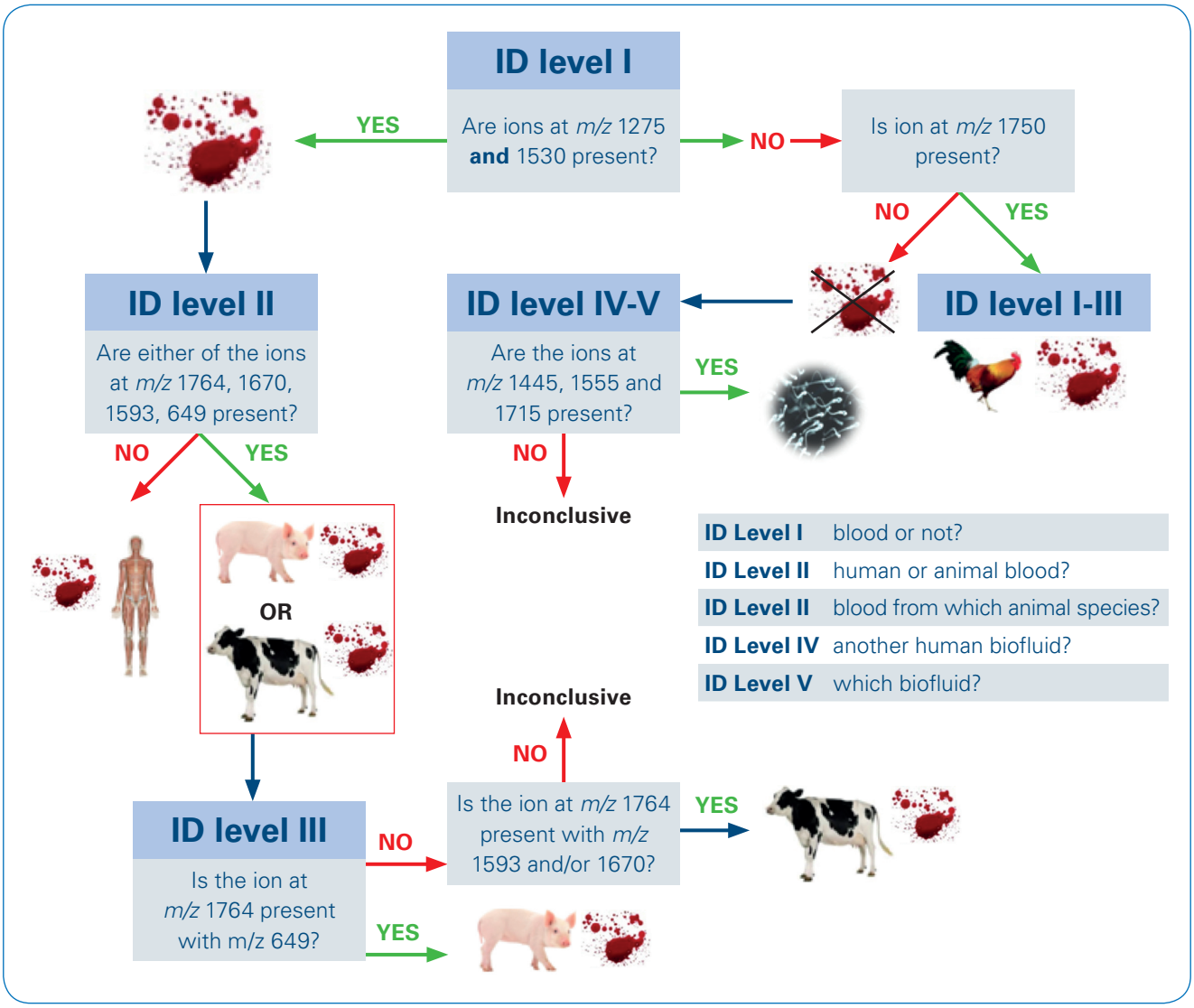


*Figure 1. Blind sample spectra data interpretation strategy. This strategy enables the determination of human and animal provenance down to species (as well as the determination of the presence of semen). The m/z values are nominal. Their presence is verified with a mass accuracy <15 ppm. (Reproduced from Kennedy et al. Scientific Reports, 2020 [5] under the Creative Common Attribution 4.0 International License, http://creativecommons.org/licenses/by/4.0/.)*

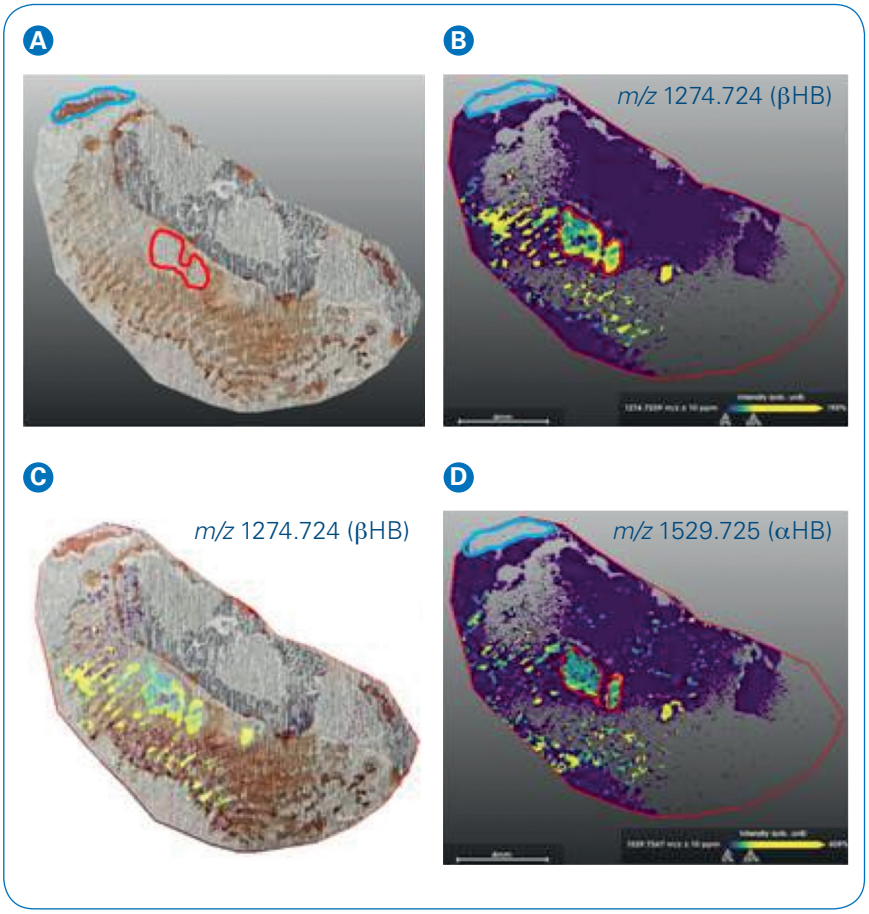


*Figure 2. MALDI MSI of a human blood fingermark of 4 years of age stored in uncontrolled ambient conditions. Ⓐ optical image; Ⓑ and Ⓓ MALDI MS image of the HB ions at* m/z *1274.724 (βHB) and 1529.725 (αHB) respectively, measured with a 10 ppm mass accuracy. Ⓒ Overlay of the image at* m/z *1274.724 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.*

The unenhanced human blood fingermark and the enhanced bovine mark were digested *in situ* using the SunCollect (Sunchrom, Germany) by employing trypsin at a concentration of 200 µg/mL containing 0.1% RapigestSF (Waters Corp, UK). Nine layers of trypsin were sprayed at a flow rate of 2 µL/min. After incubation at 37°C, 5 mg/mL of α cyano-4 hydroxy cinnamic acid (HCCA) in 70/30 acetonitrile/TFA (0.5%)aq were sprayed at a flow rate of 4 µL/min for a total of 5 layers.

The blood fingermarks were imaged on a timsTOF fleX mass spectrometer (Bruker Daltonik GmbH, Germany) at a lateral resolution of 50 µm in the mass range *m/z* 100-3000. Images were processed using SciLS™ Lab software (Bruker Daltonik GmbH, Germany) and were all RMS normalized.

## Results and discussion

The investigated human blood mark exhibits poor ridge detail (Figure 2). This is somewhat common in many violent crime scenes, where blood marks have a scarce amount of *minutiae* permitting an identification. 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 pattern 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, visualisation of this biofluid will be dependent on its local ratio with trypsin.

The simultaneous detection of the ion signals at *m/z* 1274.724 (βHB) and 1529.725 (αHB) 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 namely: Erythrocyte Band Protein 4 (EBP42), Haptoglobin (Hpt) and Serotransferrin (Figure 3) 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 ionisation 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. 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.

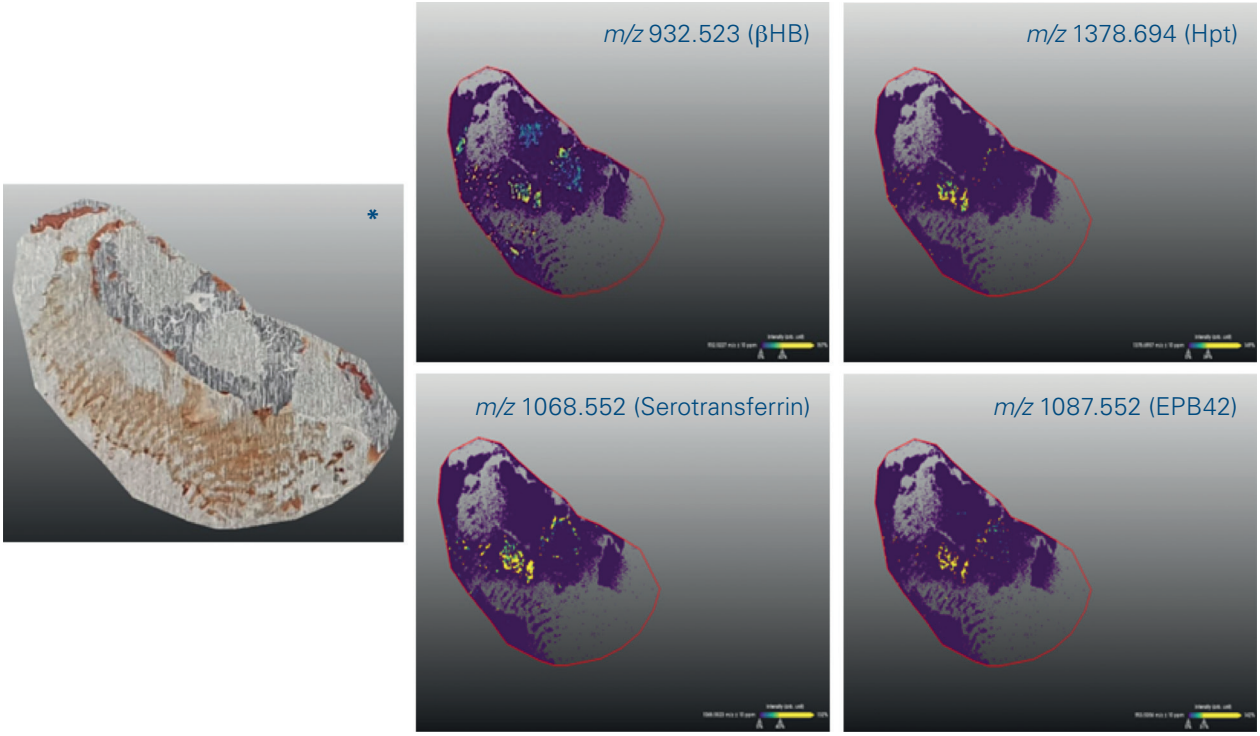


*Figure 3. MALDI MS images of blood specific protein deriving peptides in a human blood fingermark. Panel* shows again the optical image of the human blood mark that was imaged. Many ion signals indicate the presence of blood through the detection of HB, Hpt, Serotransferrin and EBP42.*

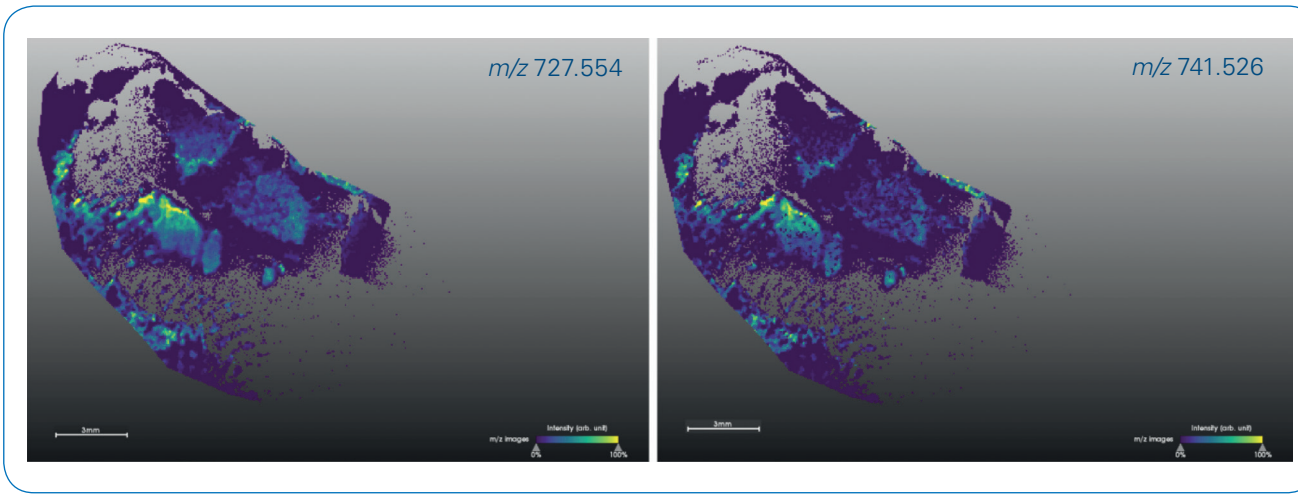


*Figure 4. MALDI MS images of lipids at m/z 727.554 and 741.526 in a human blood fingermark. These ion signals show additional ridge coverage with respect to the blood specific protein-deriving peptides.*

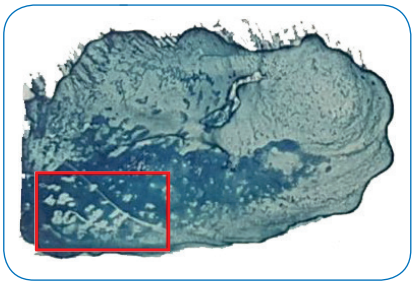

*Figure 5. Optical image of an Acid Black 1 (AB-1) enhanced bovine blood fingermark. The red frame shows partial ridge pattern.*

Figure 5 shows such a bovine blood mark in which blood has irregularly pooled. The red frame in the figure highlights the presence of partial ridge detail.

As it can be seen in Figure 6, the three peptide markers indicating the presence of *bovine blood* at *m/z* 1592.843 (Myoglobin), 1669.832 (Myoglobin) and 1763.799 (Glyceraldehyde-3 phosphate dehydrogenase) have been, for the first time, successfully imaged showing coherent distributions.

The ions were imaged with a mass accuracy of 20 ppm. Additional (aspecific) Myoglobin and GAPDH ion signals were also mapped. Despite being aspecific, these ion signals support the putatively identified presence of these bovine blood biomarkers.

Particularly the images of the ions at *m/z* 1669.832, 1784.434 and 1477.798 depict the partial ridge detail that was observed in the optical image (red rectangle).

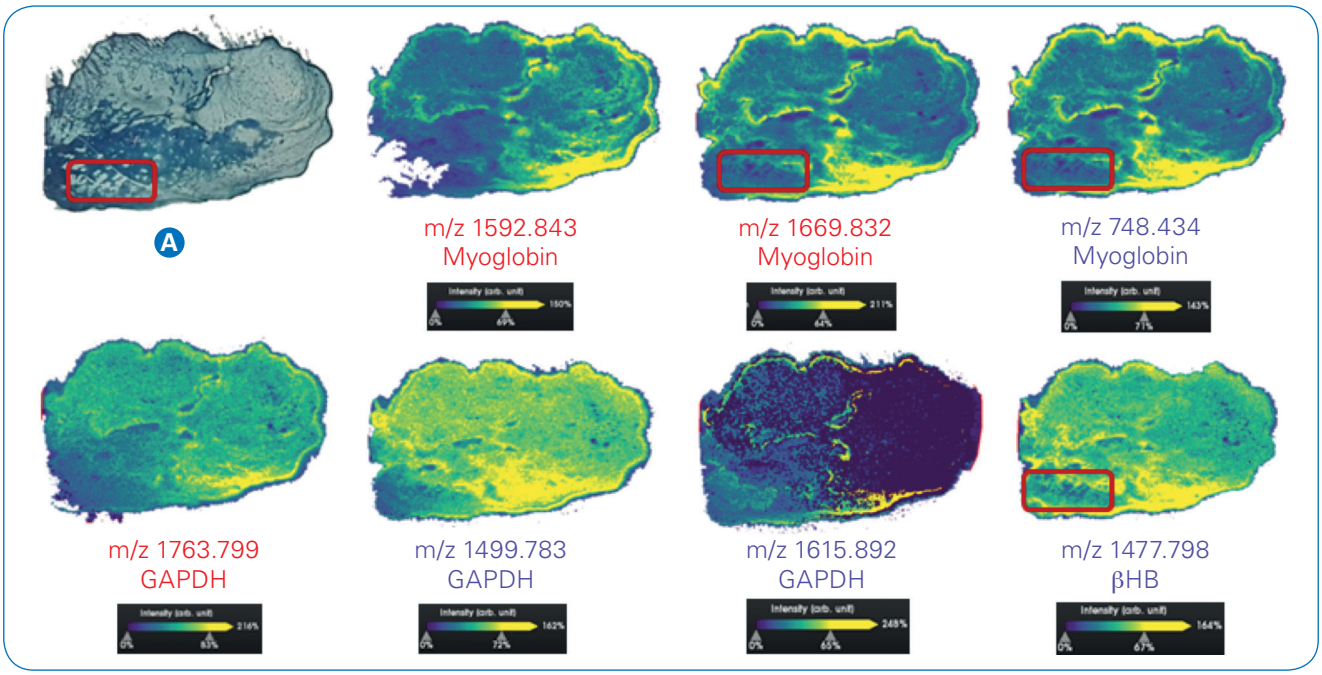


*Figure 6. Many ion signals were recovered indicating the presence of blood through Haemoglobin, Myoglobin and GAPDH. A selection of such images is presented here. (A) shows the optical image of the AB-1 enhanced bovine blood mark. The red rectangle indicates the presence of ridge flow in both the optical 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].*

## Conclusion

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 blood from different origin. 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.

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