Forensic Science International 376 (2025) 112584

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Enhancing fingermark visualisation on pangolin scales and rhino horns using cyanoacrylate fuming and vacuum metal deposition techniques

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ARTICLE INFO

Keywords:
Fingermarks
Wildlife forensics
Trafficking
Vacuum metal deposition
Cyanoacrylate fuming

ABSTRACT

Fingermark analysis is a relatively underexplored area in wildlife forensics, partly due to the complex surfaces of evidence, which often have varying porosities and textures. There is a limited amount of literature exploring cyanoacrylate fuming (CAF) and vacuum metal deposition (VMD) for latent fingermark enhancement in wildlife crime. This research addresses the scarcity of current findings by applying VMD and cyanoacrylate fuming on pangolin scales and rhinoceros horns. Using different metal depositions, an optimal VMD method has been identified to enhance latent fingermarks on a rhinoceros horn and pangolin scales successfully. Findings demonstrate that manual deposition is significantly more efficient than automatic deposition for enhancing fingermarks. In addition, Gold/Zinc (Au/Zn) VMD coupled with sequential CAF was the most effective technique, within these investigations for fingermark enhancement on pangolin scales. Au/Zn VMD was found to be most effective on the interior areas of the rhino horn, though fingermarks grade ≥ 3 were also obtained from the exterior region of the rhino horn. CAF alone was found to be effective at visualising fingermarks on pangolin scales and rhino horn. A secondary dye staining step with BY40 or R6G was successful on the non-porous pangolin scales, but dye staining significantly reduced the quality of marks on the porous rhino horn. This study offers valuable insight into using VMD and CAF coupled with sequential techniques for fingermark analysis on wildlife samples. This novel area of research can assist in the identification of perpetrators and support criminal prosecutions to protect and preserve endangered wildlife.

1. Introduction

Wildlife forensics is a branch of forensic science that supports legal investigations related to detecting and preventing crimes associated with poaching, trafficking and exploitation of animals [1]. Poachers gain profit from the illegal trade of endangered wildlife, violating international laws and regulations that are designed to preserve and protect species. This, in turn, leads to unsustainable and detrimental impacts on ecosystems and animal welfare, resulting in a loss of biodiversity, animal extinction and the spread of disease [2–4]. Although international enforcement policies are in place, wildlife crime is not fully accounted for, possibly due to limited resources, ineffective deterrents and potential corruption, leading to low prosecution and conviction rates [5,6].

Wildlife crime mainly occurs to supply illicit market demands, commonly targeting tigers, elephants, pangolins, rhinoceroses and birds [7]. These species and their commodities serve as decorative items, meat, ornaments, clothing or key constituents in traditional medicines [8]. The trafficking of endangered animals involves the sale of their ivory [9], scales [10], horns [11], fur, skin, eggs and feathers [12,13]. Wildlife crime ranks as the fourth most profitable illegal trade globally, as poaching alone generates between $7 and $23 billion each year [14]. Those involved in wildlife crime are often associated with wider networks related to drug trafficking, money laundering and gun crime [15]. One example where wildlife trafficking is a threat is in China. Traditional Chinese Medicine (TCM) has been targeted by stricter legislative policies, but illegal trade is still profiting off deep-rooted cultural beliefs that animals and their goods have healing properties. A few examples of the endangered species covered under the 2020 TCM pharmacopoeia are rhinos, Reeve's turtles, tigers and pangolins [10,16].

In endeavours to assist law enforcement in detecting and preventing wildlife crime, experts employ a range of conventional forensic techniques such as DNA profiling and DNA barcoding [17]. Other forensic techniques include ballistics and fingermark analysis of wildlife samples

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https://doi.org/10.1016/j.forsciint.2025.112584
Received 17 March 2025; Received in revised form 18 June 2025; Accepted 26 July 2025
Available online 26 July 2025

to link suspects to the crime [18,19]. Fingermark analysis is a successful technique in traditional forensics which has been employed for over 120 years [20]. Still, it is less commonly employed in wildlife forensics. Currently, latent fingermarks are detected using alternate light sources and enhanced using a range of chemical and/or physical treatments [21]. Techniques are used alone if found efficient or sequentially to achieve better results. A few examples of common techniques include physical powdering, chemical treatments with ninhydrin or cyanoacrylate fuming (CAF), and vacuum metal deposition (VMD), which involves the use of vaporised metals to visualise fingermarks [22,23].

Both CAF and VMD have been employed in traditional forensics to visualise fingermarks effectively [24]. CAF can successfully visualise latent fingermarks on metal, glass and smooth and textured plastic surfaces. It can be used independently, in combination with another technique such as VMD, or sequentially with fluorescent dyes, as demonstrated Lam et al., when visualising latent fingermarks on polymer banknotes [25]. VMD is a more sensitive technique than CAF, [22] that can be used to visualise fingermarks on non-porous and semi-porous surfaces, such as metal cans and plastic bags [22,26]. VMD is particularly effective at recovering aged fingermarks and marks damaged by environmental exposure [26]. However, VMD is not routinely used in casework, which may be attributed to equipment costs and the specialised training required for the analysis [26].

There is limited literature exploring VMD and CAF for enhancing fingermarks on wildlife samples. Fairley et al. [27] and Downham et al. [28] discussed the challenges of using VMD with various leather samples. These included manufactured leathers (non-porous) and authentic leathers (porous), as leather products are considered a difficult surface for fingermark enhancement due to surface textures, colours and patterns. Findings indicated that VMD is an ineffective method for recovering latent marks from this range of leather samples. However, other enhancement techniques, such as powdering and CAF, proved more successful [27–29]. Additionally, fingermarks have been enhanced on the skin of reptiles using CAF. Reptiles are smuggled as exotic pets and are extensively trafficked for their valued skin in the fashion industry. Eveleigh successfully employed CAF on deceased reptiles and powdering on live reptiles to enhance latent fingermarks. The study found that variations in species affected the efficiency of the enhancement methods due to differences in skin texture [30].

Given the success of CAF and VMD with non-wildlife samples, they show great potential for improving fingermark analysis in wildlife forensic casework. This study aimed to use these techniques to enhance latent fingermarks on wildlife exhibits commonly associated with wildlife crime.

2. Experimental

2.1. Materials

The samples investigated in this study were Asian pangolin scales (Metropolitan Police, UK) and a rhino horn (Museum of Life Science, King's College London; species unknown). The rhino horn exhibit was received pre-cut into two halves, allowing the examination of both interior and exterior surface areas.

2.2. Fingermark collection

Collection and storage of fingermarks used within this study was conducted in accordance with ethical clearance granted by the King's College London Biomedical Sciences, Dentistry, Medicine and Natural & Mathematical Sciences Research Ethics Subcommittee (Reference Number: HR/DP-21/22-23072). All research was conducted in accordance with the Human Tissue Act 2004. Before depositing fingermarks, all donors were instructed to avoid washing their hands for at least 30 min after handwashing. They were also advised to continue their daily activities to allow sufficient accumulation of residues [31], which better represents real-life scenarios and adheres to the recommendations outlined in the Fingermark Visualisation Manual [22].

To enhance fingermarks on wildlife samples, 10 fingermark donors (five females and five males, over 18 years of age) deposited sets of four natural latent fingermarks using thumbs and index fingers. All fingermark deposition processes were supervised, ensuring a constant medium pressure (i.e. enough pressure to deposit a fingermark firmly) was applied for 5 s onto the sample's surface. Wildlife samples were left at room temperature for an hour before being processed.

Full fingermarks were used in this study instead of split fingermarks, as it was not possible to cut the sample in two to halve the fingermark. Furthermore, there were concerns that attempting to cover half of the mark may smudge or damage the mark.

2.3. VMD procedure

All fingermark samples on pangolin scales were attached to the top of the vacuum chamber using magnets or placed on a shelf to stabilise the samples above the evaporation boats located at the base of the chamber. The rhino horn was processed on a wire shelf (West Technology Systems Ltd, UK) [32]. Each run included a positive control (i.e., a fingermark deposited on a 5 cm × 3 cm sheet of copier paper) to ensure the VMD chamber was effectively working.

Using the VMD560 chamber (West Technology Systems Ltd, UK), gold (Au) and zinc (Zn) deposition, followed by silver (Ag) deposition, was conducted. Conventional procedures initially evaporate Au, followed by Zn deposition. The Au deposits across the entire sample as a thin layer, accumulating onto the substrate's surface and diffusing within the fingermark ridges. The Au on the sample surface provides a foundation for sequential Zn deposition to adhere to (Fig. 1) [24,33–35]. Thin Au wire (0.25 mm dia.) was automatically evaporated at an approximate pressure of 1.5×10^{-4} mbar from the evaporation boat. Following Au deposition, Zn was evaporated either semi-automatically (by pressing the evaporation control button) or manually (by turning the evaporation control dial) at an increased pressure between 3 and 5×10^{-4} mbar until sufficient ridge detail was observed.

Ag deposition was conducted following Au/Zn deposition (Au/Zn/Ag) to enhance the latent fingermarks further. This method was employed due to successes in previous research with a range of materials such as fabrics, a knife blade and handle, and ammunition cartridges [36]. The Ag deposition is a manual procedure, evaporating thin silver wire (0.50 mm dia.) at an approximate pressure of 2×10^{-4} mbar. After every run, evaporation boat 1 was cleaned to ensure any remaining gold was vaporised, using the cleaning setting on the instrument to ensure that Au evaporated in subsequent runs is not contaminated with Zn.

Both semi-automated and/or manual control systems were employed until ridge detail of the latent fingermark was sufficiently enhanced. All processed wildlife samples were visualised under ambient light and then photographed using a Canon EOS 750D camera. Wildlife samples were graded using the standard Defence Science and Technology Laboratory (Dstl) and Home Office grading system (Section 2.5; Table 2) [22]. Graded marks from Au/Zn and Au/Zn/Ag VMD were compared to analyse any differences in the two metal deposition techniques [22,36,37].

2.4. CAF procedure

CAF was carried out in the Mason Vactron MVC3000 cyanoacrylate fuming chamber (Foster + Freeman, model number 3023, UK). The wildlife substrates were placed on a metal rack inside the cyanoacrylate fuming chamber.

Using recommendations provided by Dstl [38], an aluminium boat containing 2.5 g of room-temperature cyanoacrylate superglue (Orapi low viscosity Cyanoacrylate and Sureloc adhesive) was placed into the chamber with the wildlife samples. Fuming commenced on an automatic cycle, with a humidity of 80 %RH and a temperature of 120 °C. Each run

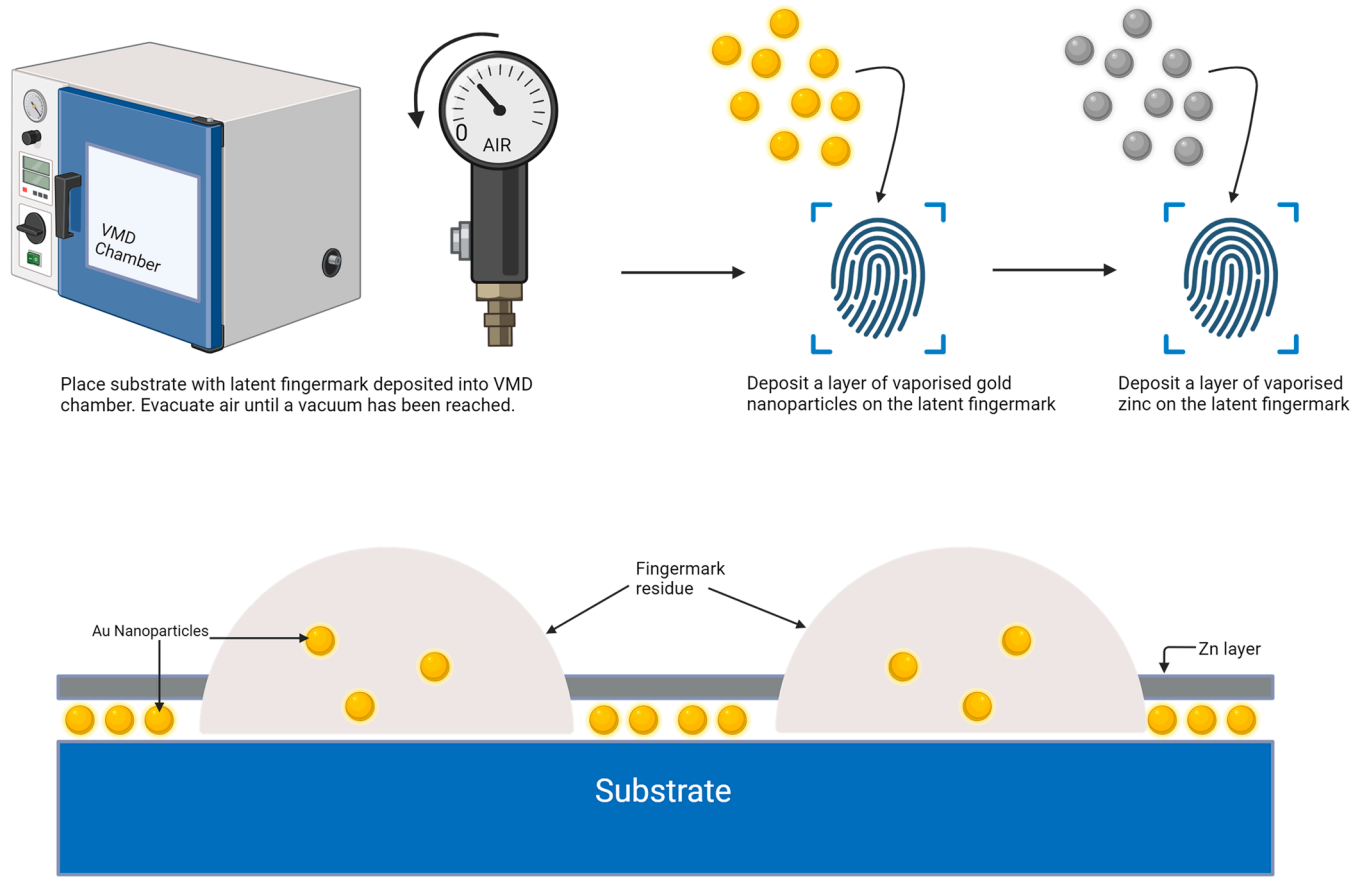

Fig. 1. Au/Zn VMD procedure used in this study. Vaporised gold nanoparticles diffuse inside the fingermark residues to form a thin layer on the surface of a substrate. Zinc is subsequently vaporised to bind to the gold nanoparticles between ridges of the fingermark on the substrate. Image **created with BioRender.com**.

took approximately 50 min under the automatic cycle. The fuming consisted of a relative humidity cycle (approx. 15 min), a superglue cycle (15 min) and a purge cycle (20 min).

2.5. Post-CAF dye staining and DCS®5 visualisation

Following cyanoacrylate fuming, marks were photographed under white light on the DCS®5 imaging system (Foster + Freeman). Fingermarks were then dye stained with either Basic Yellow 40 (BY40) or Rhodamine 6G (R6G). The BY40 working solution was prepared using Basic Yellow 40 dye powder (Scenesafe, batch code 71717) dissolved in ethanol (Sigma-Aldrich, Dorset, UK) at a ratio of 2 g: 1 L. The Rhodamine 6 G working solution was made by mixing Rhodamine 6G powder and methanol (both from Sigma-Aldrich, Dorset, UK) (0.010 g: 1 L) [39].

Marks were dye-stained under a fume hood. Pangolin scales were dipped in a glass beaker of the dye-working solution, rinsed with water, and dried. The fluorescent dyes were applied to the rhino horn using a paintbrush, followed by a subsequent rinse with water.

All fluorescently dyed marks were imaged using the DCS®5 imaging system (Foster + Freeman). The DCS®5 is a comprehensive imaging system that can visualise marks enhanced with fluorescent dyes that require illumination in the non-visible light spectrum—namely UV (300–400 nm) and IR (700 nm to 1000 nm). The marks were visualised under the conditions summarised in Table 1 and in accordance with the manufacturer's recommendations.

The images on the DCS®5 were automatically balanced to correct the contrast, brightness, and gamma of the image. The images were then calibrated against a validated ruler and graded (as per Table 2).

Table 1
Settings used for visualising fluorescently dyed marks on the DSC®5.

Powder	Excitation Wavelength	Filter
Basic Yellow 40	470 nm	GG495
Rhodamine 6G	520 nm	590AG

2.6. VMD with sequential cyanoacrylate fuming (CAF)

To further enhance latent fingermarks, CAF was applied sequentially 24 h after VMD enhancement, utilising the Mason Vactron cyanoacrylate fuming chamber. This was conducted in accordance with the method detailed in Section 2.4.

2.7. Fingermark ageing

To investigate whether the ageing of fingermarks affects the quality of ridge detail and efficiency of the VMD on wildlife samples, three fingermark donors each deposited two latent thumb marks on the inside

Table 2
Commonly used fingermark grading chart recommended by the Home Office [22]. This grading scheme is commonly adopted by researchers and academics in scientific studies [40].

Grade	Level of Detail
0	No Mark Evidence Found
1	Evidence of Contact but no Mark Detail
2	Less than 1/3 of Print Showing Clear Ridge Detail
3	Between 1/3 and 2/3 of Print Showing Clear Ridge Detail
4	More than 2/3 of Print Showing Clear Ridge Detail

of the rhino horn and two pangolin scales. This was repeated in triplicate, where each donor deposited the two thumb marks in three separate locations on the rhino horn and on a total of six pangolin scales. Fingermarks were allowed to age for 3, 7 and 10 days before being enhanced with the VMD. All samples were stored in a breathable evidence box at ambient room temperature for the duration of the ageing process.

2.8. Grading and statistical analysis

The Dstl grading recommendations (Home Office, UK) were used to assess all developed latent fingermarks [22]. Under the grading system, fingermarks on wildlife samples were graded 0–4 depending on the level of ridge detail observed (Table 2).

Three fingermark assessors were involved in grading the fingermarks. Assessor one had extensive experience working operationally as a fingermark examiner. Assessors two and three were academics who had been trained with the Dstl grading scheme in mind. Assessors two and three graded all fingermarks between them, and a sample size of approximately 12.5 % of marks were verified by assessor one.

To analyse results from fingermark development on wildlife samples using VMD and CAF, the Chi-square, Wilcoxon signed rank and Friedman statistical tests were conducted using SPSS (SPSSv29.0.2.0).

2.9. Cleaning wildlife samples

A cleaning step was required as the 40 pangolin scales and one rhino horn (which was cut in half) were reused in this study. Pangolin scales and rhino horn were cleaned with mild dish soap and lukewarm water prior to treatment. This method was previously used by The Canadian Conservation Institute to clean and care for wildlife samples [41]. Soft paper was used to dry the excess water from the scales. Wildlife samples were cleaned after VMD treatment with Hydra Sprint V2 tar & chemical stain remover (Hydra Int Ltd, Milton Keynes, UK), a solution recommended by West Technology Forensics for cleaning surfaces that have been coated with metal from the VMD process [36]. Cyanoacrylate fumed substrates were cleaned with magic cleaning sponges (BSJCLEAN, China) as per a standard operating procedure used by forensic fingermark analysts at the City of London Police to remove cyanoacrylate [42].

3. Results

3.1. Automatic vs manual deposition of zinc

Optimisation was first conducted to determine if automatic or manual zinc deposition methods, following Au deposition, affected the visualisation of ridge detail developed on pangolin scales (Fig. 2). The optimal manual deposition for Zn was found by gradually turning the potentiometer clockwise to 75 % of its full rotation and the time taken for each fingermark to develop ridge detail was recorded. The same recorded times were then used for automatic deposition.

Manual deposition was more effective, with 21 out of 40 fingermarks graded as 3 or above, in comparison to 0 deposits achieving a grade 3 or above with automatic deposition (Fig. 2) (χ^2 = 28.745, df = 1, p = <0.001).

The notable difference between manual and automatic deposition lies in the voltage applied to the Zn evaporation boats, as manual deposition requires the user to adjust the potentiometer until ridge detail is developed. Increasing the voltage accelerates the evaporation of thin Zn vapour, which results in faster enhancement of ridge detail. Conversely, automatic Zn deposition using the unit's remote control applies a much lower voltage to the evaporation boat, preventing the overdevelopment of latent fingermarks but consequently extending the time required to visualise ridge detail [36]. Even after several minutes of zinc evaporation, no ridge detail exceeding a grade 2 was obtained using automatic evaporation of zinc.

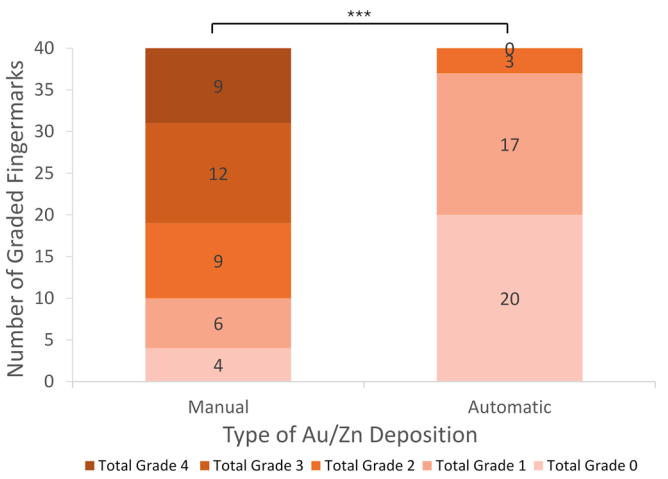

Fig. 2. A stacked bar chart displaying the number of fingermarks visualised with automatic and manual zinc deposition at each grade. The total number of fingermarks for each technique is 40.

Pangolin scales and rhino horn are, in terms of surface topography, complex substrates. Even though pangolin scales are considered to be smooth and non-porous, SEM examination has revealed keratinous overlapping sheaths [43,44]. A higher voltage leads to a higher quantity of zinc particles vaporised, which could be necessary for complex, dark coloured samples with an irregular topography, in order to enhance the contrast between the fingermark and the substrate.

3.2. VMD and sequential processing on pangolin scales

Latent fingermarks deposited on pangolin scales were developed with Au/Zn VMD, Au/Zn/Ag VMD and Au/Zn VMD with CAF treatment. The optimised manual deposition of zinc described in Section 3.1 was used. A comparative study was conducted among the three independent methods to evaluate which enhancement technique was most effective at visualising fingermark ridge detail on pangolin scales (Fig. 3).

Au/Zn VMD was chosen as it is generally the most effective VMD option for enhancing latent fingermarks on non-porous (such as pangolin scales) and semi-porous samples [22]. Silver VMD offers an extremely sensitive enhancement, and it is recommended to be used in sequence after Au/Zn VMD. This combination helps to fill in any ridge detail that is unable to be visualised by Au/Zn VMD alone, such as in the case of grease contaminated marks [22] and can result in additional mark detail being visualised [45]. CAF is a category A (non-destructive) technique that can be used following VMD [22]. It was selected to be used after VMD because the milky-white cyanoacrylate may enhance the colour contrast between the sample and the mark, following the grey Au/Zn VMD metal deposition.

Findings indicate that the number of identifiable enhanced fingermarks significantly differs among Au/Zn VMD, Au/Zn/Ag VMD and Au/Zn VMD with sequential CAF treatment ($\chi 2$ = 19.596, df = 1, p = <0.001). Fig. 3**a** shows that Au/Zn VMD with sequential CAF treatment yielded the highest number of identifiable fingermarks graded 3 or above out of all three treatments, with 26 out of 40 successful enhancements (65 %), compared to only 7 out of 40 fingermarks (17.5 %) graded 3 or above using Au/Zn/Ag VMD.

There is a statistically significant difference ($\chi 2$ = 10.769, df = 1, p = < 0.001) between Au/Zn VMD and Au/Zn/Ag VMD in successfully developing identifiable fingermarks on pangolin scales. Similarly, a significant difference was found between the Au/Zn/Ag VMD and Au/Zn VMD with sequential CAF treatment ($\chi 2$ = 18.620, df = 1, p = < 0.001). However, no significant difference was seen between the Au/Zn VMD and Au/Zn VMD with sequential CAF treatment in developing

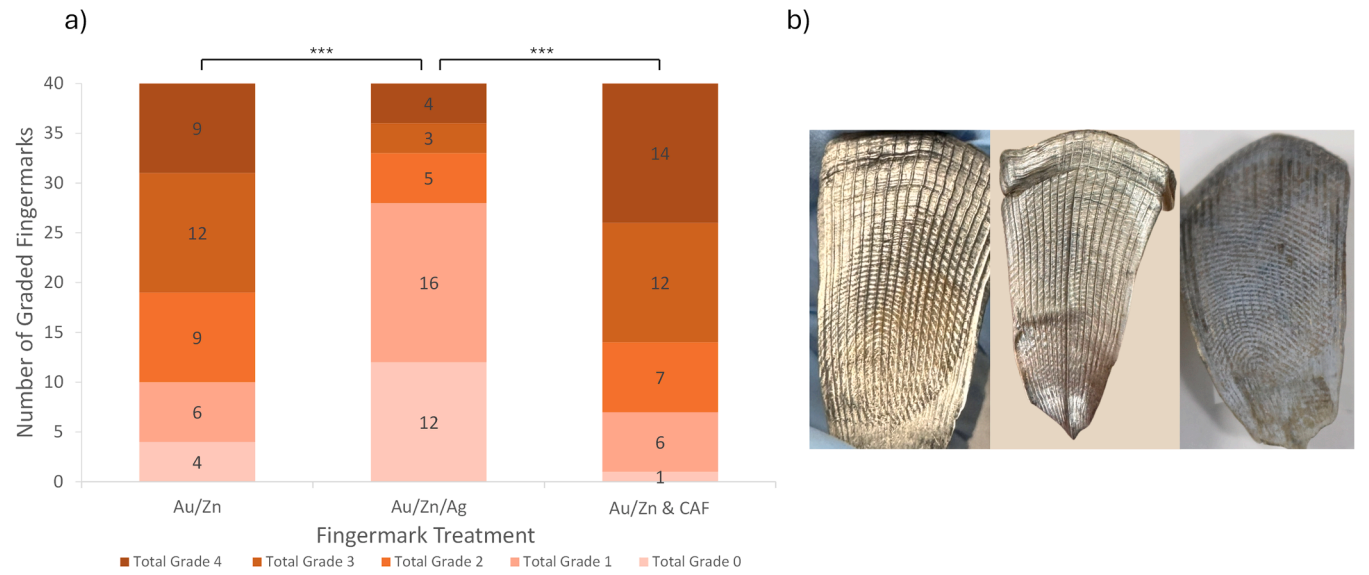

Fig. 3. a) A stacked bar chart displaying the number of fingermarks visualised on pangolin scales with manually deposited zinc when conducting Au/Zn VMD, Au/Zn/Ag VMD and Au/Zn VMD with sequential CAF, at each grade. The total number of fingermarks for each treatment is 40. b) An example of a fingermark (from the same donor) visualised using Au/Zn VMD (**left**), Au/Zn/Ag VMD (**middle**) and Au/Zn VMD with sequential CAF (**right**) on a pangolin scale.

fingermarks suitable for comparison ($\chi 2 = 1.289$, df = 1, p = 0.256). These findings suggest that either of these techniques could be applied to enhance fingermaks on pangolin scales. In contrast, Au/Zn/Ag VMD was found to be the least effective of the three techniques explored in this study, yielding the fewest successfully enhanced fingermarks on pangolin scales, with 7 out of 40 graded 3 or above (Fig. 3**a**).

Additionally, the Au/Zn VMD fingermarks graded at a 1 were developed as "empty marks", marks where there is evidence of a mark being deposited, but no ridge detail. These issues can occur when deposited natural fingermarks contain excessive sebaceous sweat or greasy external contaminants [22]. The gold deposited sinks into the grease and cannot deposit onto the background of the substrate, due to the extra sebaceous sweat leading to minimal space between the ridges of the fingermark. Subsequent zinc binding therefore cannot be initiated, leading to no discrimination between the ridges and the furrows [22,46]. In these situations, the DSTL Fingermark Visualisation Manual recommends retreating the marks with Ag VMD to enhance any missing regions of the ridge detail, as Ag deposits similarly to Au but produces enhanced colour contrast [21]. This method was applied to Au/Zn VMD processed pangolin scales with empty and partial marks however, it proved unsuccessful in producing an identifiable fingermark and led to over-development (Fig. 4).

In order to examine if CAF improved the mark quality in comparison to Au/Zn VMD alone, a further experiment was conducted with sequential CAF on the same fingermark, graded before and after additional cyanoacrylate fuming (Fig. 5). The data shown in Fig. 3 includes graded marks from independent samples, whereas Fig. 5 displays paired data. Out of the 40 enhanced fingermarks, the sequential CAF elicited an improvement in developed ridge detail and overall visualisation for 21 fingermarks (graded 3 or above) compared to the Au/Zn VMD technique. There is a statistically significant difference before and after applying CAF (Z = 2.94, df = 1, p = 0.003), suggesting that employing VMD with sequential CAF was more effective than using Au/Zn VMD alone on pangolin scales.

While VMD is a promising enhancement method to be used on wildlife exhibits in operational casework, certain limitations should be acknowledged with recommendations to assist future studies in this area. It is common for enhanced fingermarks to fade over time post-VMD treatment due to surface oxidation [22]. Oxidation of zinc results in the formation of zinc oxide, which is transparent, therefore, marks coated

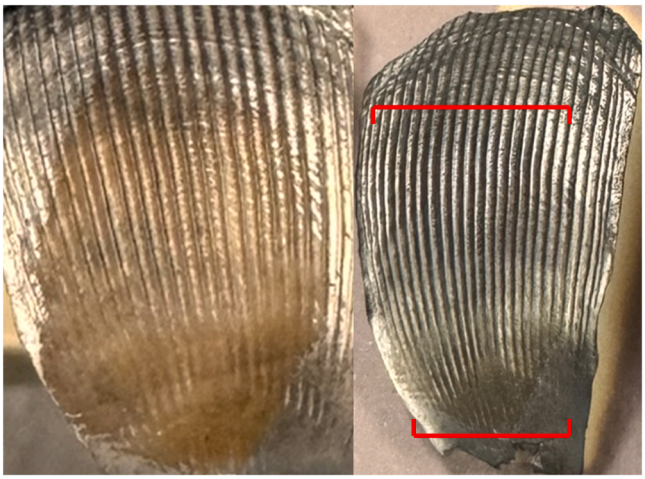

Fig. 4. An example of a fingermark on a pangolin scale developed with Au/Zn VMD, producing a partial mark of grade 2 (**left**). Following Au/Zn deposition, the same mark was sequentially developed with Ag, resulting in a loss of ridge detail and overdevelopment (**right**).

with zinc may fade over time. The DSTL Fingermark Visualisation Manual suggests that this oxidation of zinc could be accelerated by the substrate's surface and environmental conditions such as higher temperatures and humidities, and could be a limitation in hotter climates where pangolin poaching and trafficking are prevalent [22]. As oxidation cannot be prevented, therefore enhanced fingermarks with VMD alone are recommended to be photographed as soon as they are removed from the chamber [22]. Sequential CAF could be used to re-visualise fingermarks if they have faded due to zinc oxidisation.

3.3. *VMD on rhinoceros horn*

Latent fingermarks were deposited on a rhino horn by 10 donors, with each fingermark donor depositing four fingermarks on both the interior and exterior surfaces. All fingermarks were developed using Au/Zn VMD. Fig. 6 displays the differences in VMD enhancement between the exterior and interior surfaces of the rhino horn. Findings show that

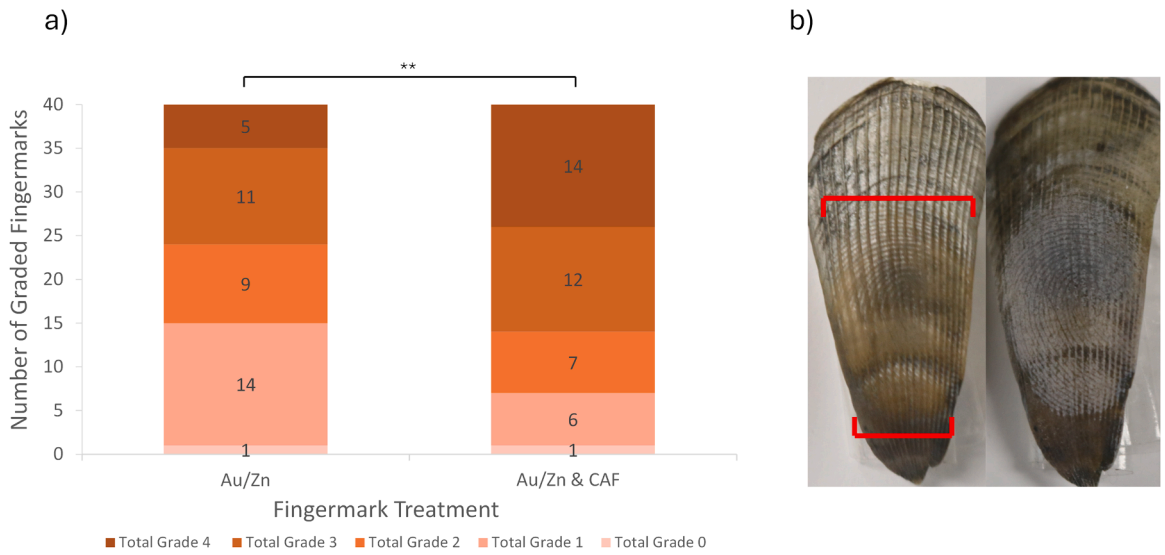

Fig. 5. a) A stacked bar chart displaying the number of fingermarks visualised using CAF following manual Au/Zn VMD on the same fingermark, highlighting the increase in successfully enhanced fingermarks achieved after the use of CAF. The total number of fingermarks for each treatment is 40. b) An example of a pangolin scale before CAF enhancement, using Au/Zn VMD to develop a fingermark of a grade 2 (**left**). The same scale was enhanced further, applying sequential CAF (**right**).

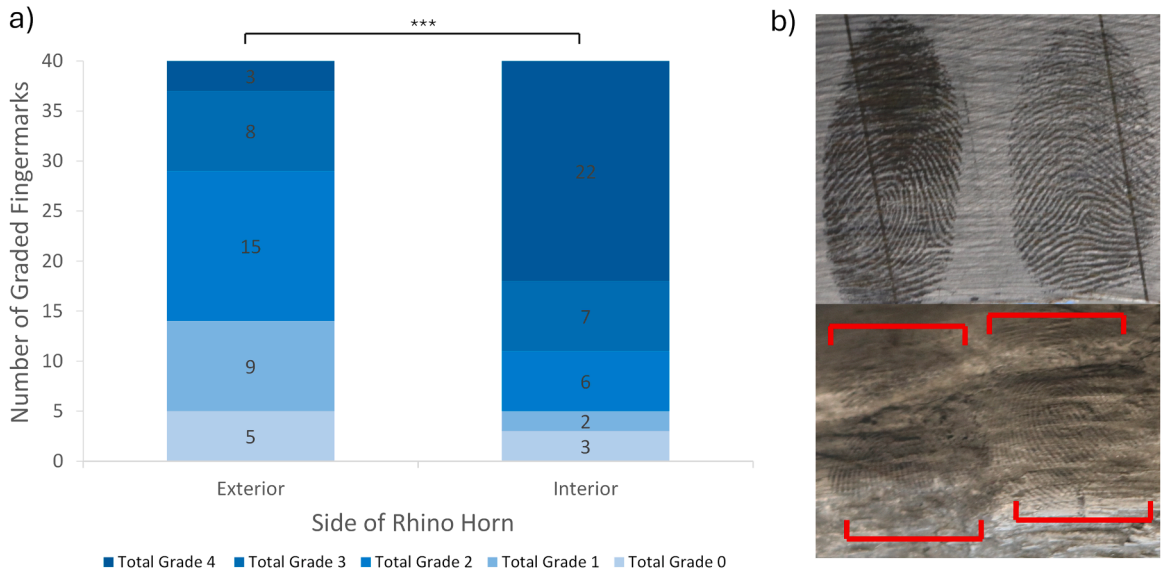

Fig. 6. a) A stacked bar chart displaying the number of fingermarks visualised on the exterior and interior regions of a rhino horn using Au/Zn VMD. The total number of fingermarks for each treatment is 40. b) Examples of latent fingermarks (from the same fingermark donor) developed on the interior (**top**) and exterior (**bottom**) regions of the rhino horn.

11 (27.5 %) latent fingermarks on the exterior surfaces were graded 3 or above, in comparison to 29 (72.5 %) fingermarks on the interior surfaces graded 3 or above (Fig. 6a).

There was a statistically significant difference between the two sides ($Z = 87$, $df = 1$, $p = < 0.001$), where Au/Zn VMD enhanced a significantly higher number of fingermarks graded 3 or above on the interior areas than the exterior areas. The findings indicate that VMD remains an effective enhancement technique for both the interior and exterior surfaces of the rhino horn, with greater enhancement observed on the interior regions, likely due to the smoother topography of the interior structure.

Rhino horn is mainly composed of keratin, melanin, and calcium [47]. The structure of the horn is a solid, dense matrix possessing a hard structure due to the calcification of melanin. Rhino horn is constructed from keratinocyte fibres. The interior region is smooth and differs from the rough, textured exterior of the horn. The surfaces vary as calcified melanin is highly concentrated within the inner surfaces, while UV damage from the sun weakens and degrades keratin within the exterior surfaces [47]. The structure of rhino horn is notably similar to pangolin scales as both are primarily made of keratin. While the interior of the rhino horn is made up of dense compact lamellae made from keratinocyte fibres similar to pangolin scales [48], Roth et al. suggested that the rhino horn has porous properties, as horn samples have the ability to absorb minerals present in the soil [49].

These findings hold evidential value to wildlife forensics as rhino horn is often cut in half to generate more profit, or into discs or blanks during the trafficking process to make transport of the exhibits easier and less conspicuous [11]. Altering the size and shape of the rhino horn exposes the interior regions. Thus, VMD would be a valuable technique for the development of latent fingermarks in rhino horn seizures of cut samples.

It is worth mentioning that the manual Au/Zn and Ag VMD processes

require the operator to monitor the development of latent fingermarks closely and stop the process once sufficient ridge detail is enhanced to prevent over-development. The temperature of the evaporation boat and metal deposition times vary depending on the type of substrate being examined. While the present study demonstrated that 75 % of the potentiometer's rotation was effective for enhancing fingermarks on pangolin scales and rhino horns, future studies are required to investigate optimal temperatures and time for other wildlife exhibits [38].

3.4. Cyanoacrylate fuming on pangolin scales

Latent fingermarks were deposited on pangolin scales by 10 donors, with each fingermark donor depositing four fingermarks per treatment. All fingermarks were developed using cyanoacrylate fuming, with subsequent dye staining treatments being Basic Yellow 40 (BY40) or Rhodamine 6G (R6G). Fig. 7 displays the differences in fingermark visualisation between CAF fuming alone, CAF enhancement coupled with BY40, and CAF enhancement coupled with R6G.

When visualising latent fingermarks on pangolin scales, grade 3 and 4 fingermarks were found with cyanoacrylate fuming alone and when performing CAF with subsequent dye staining with BY40. With CAF alone, ridge detail greater than 1/3rd of total mark area was obtained 17.5 % of the time. With subsequent dye staining, this increased to 25 % and 27.5 % of the time with BY40 and R6G respectively.

There was no significant difference found between the two dye stains ($x^2 = 0.07$, df = 1, p = 0.799). There was a significant improvement after dye staining with BY40 in comparison to CAF alone (Z = − 2.236, N = 40, p = 0.025), but not when dye staining with R6G, in comparison to CAF alone (Z = − 1.000, N = 40, p = 0.317). This suggests that adding a subsequent dye staining step with BY40 would enhance the workflow. BY40 is also recommended over R6G for safety reasons [39] as there are concerns about R6G being carcinogenic [39,50].

Dye stains are effective on pangolin scales as they are considered to be a non-porous surface [40]. Pangolin scales have some exterior layers of loosely flattened keratinised cells. However, the interior structure is comprised of densely packed lamellae of keratin [44]. This dense structure allows for minimal absorption of dye stains. Only the cyanoacrylate ester bound to the residue of latent fingerprints absorbs the dye, therefore enhancing the contrast between the print and the surface.

3.5. Cyanoacrylate fuming on rhinoceros horn

Cyanoacrylate fuming is not typically recommended on highly textured, rough and porous surfaces, such as the outside surface of rhino horns [22]. Therefore, a preliminary investigation was conducted using four fingermark donors, (two males and two females) to attempt to visualise fingermarks deposited on the outside surface of rhino horn using CAF alone. When donors deposited two thumbprints and two forefinger prints on the outside surface of the rhino horn, the CAF alone yielded poor results. Fingermarks graded 3 or above were obtained for only two (12.5 %) out of 16 prints. In all the other cases, no evidence of a fingermark was found (grade 0) (Table 3). Therefore, using CAF to visualise fingermarks on the outside surface of the horn was not taken forward for further experiments.

Latent fingermarks were deposited on the interior surface of a rhino horn by 10 donors, with each fingermark donor depositing four fingermarks per treatment. All fingermarks were developed using cyanoacrylate fuming, with subsequent dye staining treatments being Basic Yellow 40 (BY40) and Rhodamine 6G (R6G). Fig. 8 displays the differences in fingermark visualisation between CAF fuming alone, CAF enhancement coupled with BY40, and CAF enhancement coupled with R6G.

On the interior surface, marks graded 3 and above were obtained with all treatments. CAF alone resulted in grade 3 or above marks 43.75 % of the time, and R6G and BY40 yielded them 20 % and 27.5 % of the time, respectively.

There was no significant difference between marks dye stained with R6G and BY40 (x2 = 0.621, df = 1, p = 0.431); however, the best results were obtained using CAF alone. Dye staining with R6G significantly decreases mark quality (Z = -3.162, df = 1, p = 0.002), and the same applies to dye staining with BY40 (Z = -2.449, df = 1, p = 0.014). This is due to the nature of the surface of the horn. Rhino horn is a porous surface and as such absorbs the fluorescent dyes, causing poor contrast between the print and the surface. Therefore, for the enhancement of

Table 3
The number of fingermarks deposited on the exterior surface of rhino horn visualised at each grade using CAF. Fingermarks were graded using the grading scheme outlined in Table 2.

Grade	Number of Fingermarks Visualised
0	12
1	1
2	1
3	2
4	0

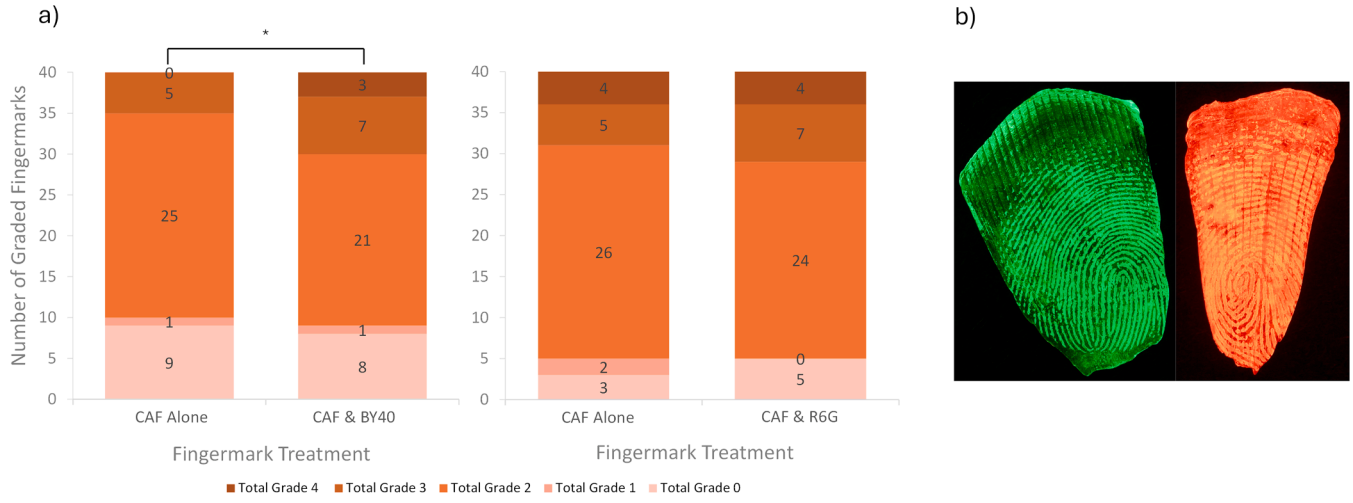

Fig. 7. a) Stacked bar charts displaying the number of fingermarks visualised on pangolin scales using CAF alone, and with sequential dye staining with BY40 (**left**), and R6G (**right**). The total number of fingermarks for each treatment is 40. b) An example of fingermarks from the same donor deposited onto pangolin scales and enhanced using BY40 (**left**) and CAF with R6G (**right**).

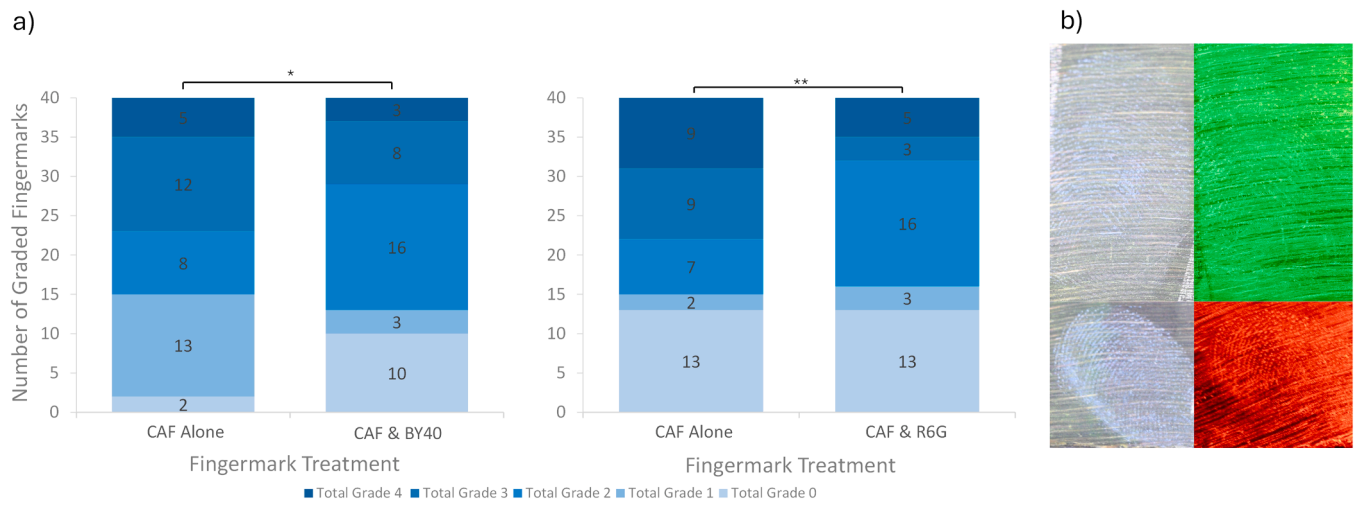

Fig. 8. a) Stacked bar charts displaying the number of fingermarks visualised on the interior surface of rhino horn using CAF alone, and with sequential dye staining with BY40 (**left**) and R6G (**right**). The total number of fingermarks for each treatment is 40. b) An example of fingermarks from the same donor deposited onto rhino horn and enhanced using CAF alone with a BY40 dye staining step (**top**), and CAF alone with an R6G dye staining step (**bottom**). Subsequent dye staining significantly reduces fingermark quality.

fingermarks on rhino horns, it is not recommended to incorporate dye staining into the fingermark visualisation workflow.

Cyanoacrylate fuming was previously conducted on latent fingermarks deposited on elephant ivory, another hard, porous wildlife sample [51]. The authors found cyanoacrylate fuming to be largely ineffective, and it was deemed to be an inferior fingermark visualisation method in comparison to powdering with SupraNano black magnetic powder [51]. Furthermore, a study using cyanoacrylate fuming to visualise fingermarks on deer antlers also reported cyanoacrylate fuming to be unsuccessful [52]. Both the pangolin scales and the rhino horn used in this study are darker-coloured substrates, which provide better contrast against the milky, white-coloured cyanoacrylate than pale-coloured ivory. While some success was found using cyanoacrylate fuming to visualise latent fingermarks on the cut surface of rhino horn, the results obtained with VMD were superior, yielding 30 % more fingermarks graded 3 or above.

3.6. VMD for visualising fingermarks over ageing periods

VMD was chosen as the technique to take forward for ageing experiments, as it had an overall higher success rate than CAF. CAF coupled with dye staining led to a 40 % success rate on pangolin scales, and CAF alone yielded a 43.75 % success rate on the interior of the rhino horn. Au/ Zn VMD led to a 72.5 % success rate on the interior surface of the rhino horn, and Au/Zn VMD alone yielded a 52.5 % success rate on pangolin scales, although sequential treatment with CAF increased this to 65 %.

Controlled ageing periods are recommended by the International Fingerprint Research Group (IFRG) when conducting research to reflect casework investigations [53]. Fingermark residue deposited on exhibits immediately ages once exposed to the substrate surface and environment, where gradual changes occur in the chemical composition of the fingermark residue, and enhanced ridge detail may become distorted [54]. Fingermarks were deposited on pangolin scales and on the interior areas of the rhino horn. The interior of the horn was selected for ageing investigations as it is a smoother surface in comparison to the outside of the horn; VMD is most effective at visualising marks on smooth surfaces.

Latent fingermarks on the wildlife samples were aged for 3, 7 and 10 days before being enhanced using Au/Zn VMD. Findings indicate that fingermarks on the rhino horn were less affected by these ageing periods, as 55.6 % of the fingermarks were graded 3 or above, compared to only 16.7 % achieving a grade 3 on the pangolin scales across all ageing periods (Fig. 9). While previous research has investigated the impact of ageing when using VMD for enhancing fingermarks [55–57], in this study no significant ageing effect was observed for the latent fingermarks enhanced on pangolin scales using Au/Zn VMD across the three ageing periods ($\chi 2 = 1$, df = 2, p = 0.607). Additionally, there were no significant ageing effects observed for the enhanced fingermarks deposited on the interior of the rhino horn using Au/Zn VMD across the ageing periods ($\chi 2 = 1.333$, df = 2, p = 0.513), suggesting that ageing over a period of 10 days did not affect fingermark visualisation using VMD.

As it is unlikely fingermarks will be enhanced immediately after deposition in a wildlife crime setting, ageing is an important consideration to account for when processing marks of wildlife exhibits with VMD. VMD is extremely sensitive and can withstand degradation of marks that have been exposed to high temperatures and humidities as the process targets the oily components within fingermark residue, even when water content decreases [22]. Therefore, a notable advantage of this enhancement technique is that it may also be successfully applied to wildlife exhibits in hot climates where wildlife crime is a problem.

3.7. Limitations of the study

Before the findings of this study can be fully implemented in the field, further investigations are needed to evaluate the robustness of the proposed techniques. A larger validation study using VMD and CAF to visualize fingermarks on pangolin scales should include additional variables, such as whether the orientation of the deposited fingermarks affects the quality of their development. It should also examine the deposition of fingermarks on the underside of the pangolin scales.

Access to wildlife samples was a limitation of this study, and wildlife samples used in this work were cleaned and reused. More validation work should be done using a larger quantity of pangolin scales and rhino horn to confirm the efficacy of these methods.

Regarding the visualisation of fingermarks over time, supplementary experiments should be conducted over longer ageing periods with a larger sample size of fingermarks. This will allow for a comprehensive exploration of how the ageing of marks over weeks and months impacts the quality of visualised fingermarks. Investigations extending over these longer periods could better represent how samples are found in actual wildlife crime scenes.

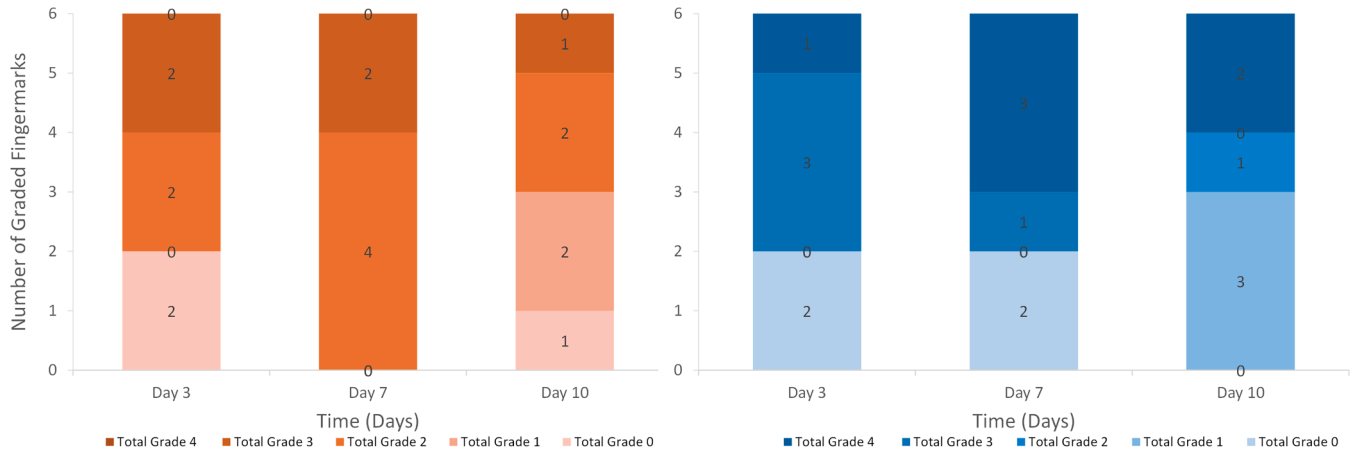

Fig. 9. The number of grade 3 and grade 4 fingermarks visualised using Au/Zn VMD over time, on pangolin scales (**left**) and the interior surface of a rhino horn (**right**). The total number of fingermarks for each ageing treatment is six.

4. Conclusion

This is the first study to demonstrate the effectiveness of CAF and VMD (both alone and in sequence) in visualising fingermark evidence on two of the most commonly targeted samples of wildlife trafficking: pangolin scales and rhino horn. The significant findings discussed in this study lay the foundations for applying VMD and CAF in fingermark analysis for wildlife crime investigations. Using both of these methods can reduce workloads for fingermark practitioners, as multiple items can be processed at once. Furthermore, these methods are more automated than other fingermark visualisation methods, such as powdering.

Manual deposition of VMD was found to be a rapid and efficient method for the enhancement of latent fingermarks on pangolin scales in comparison to automatic deposition. When investigating different variants of VMD, Au/Zn VMD was deemed most effective on pangolin scales when compared to Au/Zn/Ag VMD. When investigating VMD with sequential CAF, it was observed that sequential CAF significantly improved the enhancement of fingermarks on pangolin scales and was deemed as the most effective method overall for this wildlife exhibit. One potential limiting factor of VMD is that the system is large and expensive, however, portable systems have now been developed and can be implemented within the field. When investigating CAF with sequential dye staining, it was found that CAF could visualise high-quality fingermarks across all the wildlife samples used. Still, sequential dye staining only improved marks on pangolin scales; rhino horn is a porous substrate that absorbs dye stains, leading to poor contrast between the sample and the mark.

This study demonstrates the effectiveness of these techniques when used under relatively controlled conditions, however, pseudo-operational trials should be conducted to fully validate proposed techniques. Efforts to examine how effectively they would work on exhibits from a simulated wildlife crime scene will be of great interest.

The methods investigated in this study should be deployed in police laboratories and employed on confiscated trafficked wildlife items after further testing has been done. Fingermark detail visualised on wildlife samples can not only link individuals to specific wildlife crime scenes but also link individuals to other criminal activities including gun crime, fraud and corruption, thereby assisting with other challenges intertwined with poaching.

Authors' contributions

All authors contributed to the study conception and design. Initial manuscript preparation and laboratory work was performed by Lauren Woodcock and Nicole Coogan. Critical analysis and final approval of the manuscript was provided by Julia Ringe and Nunzianda Frascione.

CRediT authorship contribution statement

Nunzianda Frascione: Writing – review & editing, Supervision, Methodology, Conceptualization. **Woodcock Lauren Danielle:** Writing – original draft, Supervision, Methodology, Investigation, Data curation. **Nicole Coogan:** Writing – original draft, Methodology, Investigation, Data curation. **Julia Ringe:** Writing – review & editing, Supervision, Methodology.

Ethics approval

Ethical clearance for the collection and storage of fingermarks was conducted in accordance with ethical clearance granted by the King's College London Biomedical Sciences, Dentistry, Medicine and Natural & Mathematical Sciences Research Ethics Subcommittee (Reference no.: HR/DP-21/22-23072).

Consent to participate

Adult donors were recruited (five females and five males), with no restrictions on sex, ethnicity or age (outside of being aged over 18), all donors signed consent forms prior to donating fingermarks.

Consent for publication

This article does not include any content for which informed consent would need to be obtained.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Code availability

N/A.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to thank Tracy Alexander, Director of Forensic Services for the City of London Police for their advice and input on this work.

Availability of data and material

N/A.

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