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Hybrid Techniques in Fingermark Visualization: Combining Spectroscopic and Chromatographic Methods for Complex Substrates

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Abstract

Speculations have been made for some time as to what might happen if hybrid techniques were used in conjunctions to enhance fingermarks. The main consideration to date has been imaging, but there appears to have been no studies of the effects of using spectroscopic techniques on a fingermark that has been developed using a chromatographic technique. This is a significant omission as it is likely that the chemical additives inherent in some substrates would confound the spectral information obtainable from the sebaceous components of a fingermark. Four selected chromatographic techniques, Cobalt-2, Ninhydrin, Sticky Side Powder, and Vacuum Metal Deposition, were used to develop fingermarks on substrates containing chemical additives. Apart from Derek (Cobalt 2), a dark grey background, most of the chemical additives used were either at- or near-IR fluorescent. Fingermarks developed on substrates containing the chemical additives were subjected to three types of spectroscopy: IR, RAMAN, and SERS.

To the authors' knowledge, this is the first report of successful MALDI enhancement using VMD on marks which have also been enhanced on cellulose substrates. Previous reports have had success using VMD plate-based devices, although these marks had been developed on non cellulose substrates. MAT-irradiated marks and those treated with failed to enhance. This is likely due to the destruction or masking, respectively, of the peptide bonds that are essential for gold enhancement. Of the treatments that did enhance, a threshold was evident at 10 min, as this was the minimum time required for visible development. As discussed above, the signal was found to be on intensity dependent, which is likely a result of the concomitant obliteration of the at-IR fingerprint as the enhancement proceeded. Importantly, however, positive peaks indicative of the presence of sebaceous components were still seen in all but the darkest marks.

Keywords: Method development • Drug abuse • Chemistry • Thin-layer chromatography • Raman micro spectrometry • Sophisticated substrates

Abbreviations: VMD: Vacuum Metal Deposition; FTIR: Fourier Transform Infrared; SERS: Surface-Enhanced Raman Spectroscopy; CAF: Cyanoacrylate Fuming; RAMAN: Raman Spectroscopy; IR: Infrared; DNA: Deoxyribonucleic Acid; MALDI: Matrix-Assisted Laser Desorption/Ionization; ATR: Attenuated Total Reflectance

Introduction

Fingermark visualization techniques

Fingermarks are deposited impressions left by the ridges of the last joint of fingers and thumbs and the soles of bare feet. They consist of a mixture of excreted compounds from the sweat pores on the skin and environmental substances. The composition of the sweat is complex and differs widely among individuals and even within the same person, can vary depending on diet, emotions, medication and surrounding environment. Thus, the complexity of this material is one of the most challenging characteristics for forensic analysis. When a finger touches a surface, the contact can remove matter from the surface or deposit material on it, leading to impression fingermarks or to continuous or latent fingermarks, respectively [1-3].

The invention of powdering techniques in the last quarter of the nineteenth century allowed the first visualization of latent fingermarks under laboratory conditions. This discovery was rapidly applied to a wide variety of surfaces leading to their further development only a few years later. Since then, a plethora of methods have been reported that target different types of surface. However, the increasing variety of materials currently used in the production of objects for everyday life frequently yields unwanted fingerprints. This has inspired the research community to develop and adapt a number of biologically and physicochemically compatible techniques to a growing range of substrates to effectively reveal these covert marks. A hybrid approach combining spectroscopic and chromatographic visualization techniques was initially subjected to a series of model atmospheres under laboratory conditions. This led to evaluation of the approach on real substrates relevant to forensic case work.

These substrates were selected from a library of samples collected over several years of forensic investigation and comprised an array of materials common in the manufacturing of electronic goods. Such complex materials can cause problems in the detection of latent fingermarks, and procurement of the model atmospheres aimed to replicate and study the reasons for such issues. In forensic practice, a fingermark must be visualized on the substrate on which it is found. As a result, research has been dedicated to understanding of the nature of the fingermark residue and substrate interaction [4]. This technique was conducted on a wide selection of surface types. It was established that the NIR hyperspectral imaging technique could be successfully used with analog equipment on most smooth non-porous and some porous substrates, visualizing fingermarks as stains mostly by absorption. However, with both polychromatic white light and filtered xenon flash, fingermarks appear as localized points of scattered reflectance on many surfaces.

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Methods

Overview of traditional methods

Fingermarks at crime scenes can be of significant evidential value. Despite a general improvement in the detection and management of fingermarks at crime scenes, there are still forensic submissions from casework where the marks remain invisible and undetectable by traditional fingermark treatments, or where background contaminants make their detection much more complex. These marks often require more time-consuming, complex treatments, potentially affecting the quality of the evidential information retrieved on other marks present. Therefore, there is a demand for further research and development in the area of new methods to improve the detection of a variety of fingermarks on both exposed and submerged substrates.

The growing appreciation of the need for more sensitive detection techniques has led to increased prominence of surface analysis techniques in fingermark deposition and development research in the last two decades. Some advances have been made in the detection of chemical compounds present in ridge detail by endeavors to preserve the integrity of the compounds and analysis of them with techniques such as Mass Spectrometry (MS). The goal of such methods is that, in addition to reproducibly enhancing fingermark ridge detail by the deposition of chemicals, the same treatment will also reveal the identity of those chemicals. Treatments that accomplish these aims are termed chemical ‘enhancement reagents’ [3].

Overview of new technologies

An exciting range of innovative techniques have been proposed for fingermark visualization within the last decade, including but not limited to ASL, LDI MS, and IRMPD-MS. The chemical composition of sweat resides, in turns, on the health and lifestyle of the individual, and thus an in-depth characterization of sweat buers is not feasible. These substances can react with the sebaceous residues present on the skin, which are themselves a mixture of triglycerides, wax esters, and squalene. When left behind after contact with a surface, these residues provide a substrate for sweat components, shedding light on the deposition age and composition of the mark.

Cocoa butter, as a representative model of hand creams and body butters, has been chosen for this study; cream has the advantage of remaining on the skin longer than other substances due to its greater viscosity. In this study, fingermarks were deposited on cocoa butter after an interval of two months from their detection. The pigmented components of the body butter and the chromophores induced in the light extraction process are detected in the harmony and finalized VMD mark image. Differently colored traces on the same substrate resulted in Cyanoacrylate (CAM) polymer traces shining in blue and violet, and black latent mark reproductions. This issue of substrate variability has been addressed in research studies extending to quantitative analysis of the enhancement process. As an example, denim cloth has been investigated using GC as a quantitative analysis method. Mark detections are classified on a four-point scale ranging from very poor, ridges barely discernible, to excellent, sharp ridges in the entire pattern. Processed marks retain their appearance for up to 2 days but fade thereafter [5].

The application of GC-MS to untreated marks yields a unique profile for each substrate, beyond the typical sebum profile observed on all marks. Additional substances detected entirely derive from background, the adhesives of which the denim cloth is composed. This study also sheds the light on the quantification of these extra peaks, ranging from 15 to 30% of the total chromatogram. matrix solid-phase dispersion for GC-MS detection of purple nitriles treated fingermarks on denim clothing; the mark is rarely detectable by eye on this material. Mark(s) were treated in either a purple nitrile solution or neat purple nitrile with either a stirring time of 5 minutes or 3 hours. The effect of purple nitrile treatment fades quickly, with most marks exhibiting a tan result within 72 hours. Marks treated in purple nitrile are most often detectable while untreated marks are less conspicuous. In general, less signal is observed from the majority of the compounds present in marks treated in solution compared to the same solvent applied in neat form [2].

Spectroscopic techniques in fingermark visualization

A number of spectroscopic techniques have been used in the study of latent fingermark residues including X-ray Photoelectron Spectroscopy, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy, Time-of-Flight Secondary Ion Mass Spectrometry and Laser Ablation Inductively Coupled Plasma Mass Spectrometry. Raman spectroscopy studies have been typically undertaken *in situ* on powder adhering to the ridges of fingermarks, with limited focus on such as skin cells, debris and latent reagent residues that are commonly found adjacent to the powder applications of interest. Case demonstrates the potential for extending the utility of Raman spectroscopy to visualise fingerprint deposits on a variety of surfaces in such a way that unwanted interference is efficiently minimised, enabling the generation of a detailed and usually high contrast image of a fingermark. There has been a recent increased interest in this area to take advantage of the high spatial resolution that allows chemical images to be captured from isolated particles [6].

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy and Raman Spectroscopy have previously been used to study the chemical composition of fingermark residues. Case is the first study to use a commercial micro-Raman system in such a way to visualise intact fingermarks on a variety of complex and problematic substrates, with the potential for wide adoption of the technique in this application. While similar in principle, the two techniques probe fingerprint residues in a different manner with differing depths of penetration and collection geometries. Studies of nose prints on copper have shown ATR-FTIR to have greater flexibility with regards to the secretor status of a donor compared with Raman [4].

Thanks to high brightness, ease of operation and greater availability, the case studies focus on ATR-FTIR and its potential for *in situ* studies of a variety of fingermark substrates. RF-Raman has a number of advantages that hold promise for future applications, including fluorescence mitigation, superior spatial resolution, greater depth of penetration. Other methods of *in situ* analyses are Optical Coherence Tomography, widely used in dermatology and ophthalmology for non-invasive visualization of tissue structure and an accurate determination of skin features [7].

Principles: Hybrid visualization techniques in illicit drug visualization are growing to allow detection in fingermarks of this illegal substance. The detection of illicit drug metabolites important for drug abuse investigation is also possible. Residual fingermarks can be difficult to enhance and visualize when present on complex substrates. Factors contributing to this are the morphology of the substrate and the aging of the mark itself. In this study, two hybrid chemometric techniques, each combining a spectroscopic and a chromatographic method, were applied to enhance and visualize fingermarks on paper aged for 24 h and on adhesive tape left on paper for 48 h. 2D maps with the distribution of salicylic acid were successfully retrieved. A preliminary comparison of the quality of 2D malic acid maps obtained by the different chemometric techniques was performed [8].

Illicit drugs are illegal substances whose detection in fingermarks can be of crucial importance for forensic investigations on cases of drug abuse or unauthorized possession. Moreover, it has been demonstrated that the detection of drug metabolites can give additional and important information in drug abuse cases where the drug itself cannot be detected due to fast metabolism and quick elimination of the drug. Surprisingly, it has been demonstrated that the detection and mapping of illicit drugs and their metabolites is still possible in artificially made fingermarks, even when the prior application of a forensic enhancement technique is performed. In particular, the detection and 2D mapping of ketamine, 4'-OH-ketamine, amphetamine, methamphetamine, MDMA, and MDA in latent fingermarks is reported. The method was also employed to effectively detect the presence of illicit drugs in the surface of the smooth, patterned, and rough substrates. The last step was aimed at the honoring of the hybrid chemometric technique. There, enhancement techniques were employed to improve spray deposition and powder visualization. 2D maps, with the distribution of salicylic acid, were successfully retrieved after enhancement [9].

Instrumentation: New hybrid techniques (Fingermark Enhancement Trading or FET 2.0) combining different post-mark treatment methods and various visualization techniques have been quantitatively tested. Suspended in drops of diethyl ether, specific substances (saccharides, amino acids, paracetamol, caffeine, testosterone and creatine) deposited as aqueous 5 µL droplet fingermarks onto complex substrates were analyzed. In addition to luminescence imaging following cyanoacrylate fuming or ethyl rhodamine B application, absorbance readings of visualized fingermarks were made with a UV-Vis spectrometer. Despite some evidence of compatibility issues between illumination techniques, instrumentation settings and fingermark enhancement sequences, the general methodological approach appeared successful.

For sugar and amino acid marks, VMD was significantly more effective than CAF or ERB. Male marks were generally treated more effectively by the visualization methods employed, possibly due to a higher specific concentration in the ridge region. Marks treated with ERB following CAF were typically most effectively visualized, due to a specific match between the biomolecular composition and the chosen enhancement procedures [2]. Absorbance reading of visually enhanced marks provides a valuable additional source of comparative data for forensic intelligence purposes. All of established hybrid techniques worked successfully and potentially offer greater flexibility for the subsequent processing of biologically and forensically interesting marks than currently used singular approaches [10,11].

Chromatographic techniques in fingermark visualization

Experiments were conducted to extend the potential of imaging spectroscopic techniques in combination with chromatography to visualize drugs/fringe substances after fingermarks evaporate. Initially, the focus laid on dissolved fingermark material, and then an experiment was conducted with a donor pendant transfer of skin cream carrying nicotine and cocaine. Direct IVIS spectroscopic imaging of substances after fingermarks have evaporated and after a transfer has been feasible for both water and isopropanol. Moreover, it has been shown for the first time that substances can be visualized after a pendant transfer process carrying skin cream components [12,13]. New experiments have extended current knowledge about substances that can be visualized with direct IVIS spectroscopic imaging to small drugs such as nicotine and cocaine. It was possible to visualize residues of non-volatile components or specific compounds in evaporated fingermark material. Furthermore, it was demonstrated for the first time that the residual substances from complex mixtures such as skin cream components can be visualized with IVIS. A limitation is the precise visualization of single substances with identical excitation or emission maxima.

Thus, special staining or labelling could be necessary in practice to restrict the transfer of a distinct substance of interest. It is important to consider that interaction of compounds with a substrate may also have an impact on the measured fluorescence distribution and intensity. To enhance the visualization of substances, complementary chromatographic techniques, such as thin layer chromatography and direct analysis in real time, have been investigated and preliminary results for the latter are also included. Furthermore, experiments with evaporated substances onto surfaces were performed to use alternative visualization techniques after the samples have undergone forensic vacuum metal deposition [2].

Types of chromatography: Most current developments regarding the detection of fingermarks on complex substrates are primarily focused on the detection itself. As most common techniques are applied, the detection of illegal drugs is focused upon. On the other hand, the applicability of hybrid detection techniques is considered utilizing the following inlet systems: SPME, FET, and Pepsin Inlet. An assumption is this setup may promote hybrid detection techniques more feasible for detection enhancement of several other different compounds, as nearly all of the existing compounds already have different commercial kits for their extraction and analysis [5].

Chromatography detection, in contrast to current practices, enables the simultaneous detection of large groups of compounds, still being selective, sensitive, and characterized by high quantitative accuracy. Current practices focus primarily on enhancement and detection of ridges revealing different type of compounds, subsequently detected either by Raman spectroscopy or chromatographic analysis. In the case of offered hybrid techniques, a lipophilic mixture is used which is formulated to cover and dissolve a diverse range of drug types. Consequently, existence of other drug types and/or compounds, and not only those which are commonly attempted to be enhanced, can be pinpointed within the fingermark. On the other hand, using alternative chromatography setups can indicate the presence of additional illicit substances covering the drug class. Obtained compound profiles from Chromatography testing can be easily crossmatched among other criminal evidence or crime scene findings for possible subject identification. This paper presents numerous complex substrates including different fibres, skin, and paper with the application of various popular enhancement agents and testing for most common illegal drug types [14].

Chromatography applications: This group reported the first ever combination of CAF with VMD on fresh prints. Using spectromicroscopic imaging we show that CAF enhances Raman signal on untreated fingermarks similar to monochromatic illumination. In contrast, VMD does enhance Raman signal. A higher number of species are visible with VMD as compared with CAF, and the adhesives within cyanoacrylate treatments are also exploited. An increase of the efficiency is shown by FCA/EV<VC, the currently most effective techniques in practice. Attention in this study is focused on marks collected from challenging sources after routine exposure to CAF of variable quality. Using two completely different systems, not intended for the same purpose, however it is shown how one can assist in this comparison. The developed methodology is promising for the benchmarking of enhancement methods. It provides a new means to evaluate novel or existing physique-chemical enhancement procedures, either on the basis of trace intensity, or chemometrically with the consideration of spectra [15].

Integration of spectroscopic and chromatographic methods

Fingermarks can be found on sophisticated drug packages as part of the new evidence found by the police at crime scenes; especially if cleaned prints on complicated surfaces are studied. As part of an investigation on potential visualization techniques that do not contaminate subsequent chemical analysis, the compatibility of detection of sebum, in fingermarks on CDs, using surface enhanced Raman spectroscopy with subsequent GC-MS analysis was studied. 10 drugs common in society, in widespread illicit and medicinal use, were chosen. Analysis of these, if found, is performed routinely in forensic investigations [16].

Using a fast 785 nm BODIPY painted Raman hand held spectrometer, SERS was performed on DVDs, CDs, and 2 different thermal printable CDs. Spectra showed the polymer coating and the silver layer underneath. SERS enhancement of the 10 drugs was not observed. The limit of detection for pure sebum on these substrates was determined. GC-MS analysis of the drugs in pure sebum gave LODs of between 0.01 and 0.5 mg/cm^2 depending on the drug. Then CDs were handled for 48 h prior to SERS analysis at concentrations between the LOD for sebum GC-MS and the cleaned substrate LOD, and sebum discovery was made for all at 10 mg/cm^2. The SERS enhancement of cocaine, a common ampiphilic drug, was attempted after removal with methanol. This was unsuccessful suggesting the drug can penetrate the substrate [16].

Few of the techniques that are commonly applied to visualizing fingermarks are compatible with following analysis. The chemical composition of fingermark residues is known its primary component is sebum, secreted by sebaceous glands in the skin. With this as a basis of their comparison, a large number of illicit and medicinal drugs have been detected as excreted in sebum in the mg/cm^2 range. This facet of fingermarks has been exploited in the recent years in medical and analytical studies. Mass spectrometry imaging on fingermarks has been reported, as have studies looking at the detection of exogenous substances in sebum and the absorption of natively excreted substances using fingermark substrate mimics. There are two other studies on the detection of sebum within the context of fingermark enhancement; FTIR and GC-MS. MED detection of sebum was possible as a secondary effect of enhancement of fatty acids within the sebum, at higher quantities than present in sebum. Later, GC-MS within latent prints was also reported. Prior to this investigation, as part of a project on the elaboration of new evidential items for forensic applications, surface enhanced Raman spectroscopy was used to detect pure sebum on questioned CDs with high sensitivity [17].

Advantages and challenges: The significant scope for improving the visibility of fingermarks by the application of a variety of enhancement procedures has been outlined. However, irrespective of the methodology selected, the visualization will display certain intrinsic features depending on the detail of the surface where the fingermark is deposited. It is also emphasized that fingermark analysis should be part of the scene examination and additional physical or chemical substances that may aid the visualization process prior to their subsequent forensic processing [18].

A study of Electron-Induced Photon Emission (EIPE) of fingermarks has been described, but the provision of a detailed and comprehensive investigation of these decay processes has not been addressed. Electron-like atomic emissions from fingermark ridges resulting from the intrinsic X-ray effect of Funning radiation have been described. Here, the use of an additional annular gold foil to suppress the X-ray continuum and identify atomic emissions provides a novel and potentially useful result. This work mainly reports on the EIPE imaging of retention of the ridge detail in surface potential contrast and an associated post-methodology atmospheric dependence study [2].

Best practice solutions: Nowadays a crime scene investigation routine is unthinkable without the detection and development of fingermarks and here, up to 16 different enhancement agents, modes and physical developers can be used with Leuco Crystal Violet (LCV) being the commonest between them. Despite the many advantages of Raman spectroscopy, until now it has not been used as an enhancement agent because of the difficulty in adapting lasers to the microscope. At the same time, Surface Enhanced Raman Spectroscopy (SERS) has been received significant attention as a measurement science because of its ability to allow high sensitivity measurements, even at the single molecule level.

In contrast to Raman spectroscopy, SERS allows for the monitoring of solutions at very low levels of analyte concentration because of the strong enhancement of the spectral response experienced by molecules adsorbing on specific nano-structured metallic surfaces. Consequently, if the position of a stolen metallic substrate containing developed fingermarks is recovered from a suspect, the most straightforward link between the individual and the whole crime can become unavailable if the enhancement is imperfect [4]. The recent technological development allows the combination of different types of spectroscopies or the coupling of spectroscopic methods with Extra Techniques (ET) to enhance the detection ability of fingermark residues, representing a crossover between analytical chemistry, forensic sciences and materials science.

This approach has the consequence of a more in-depth study of the same object giving rise to the fascinating problem of how to coordinate and take advantage of the specific capabilities and peculiarities of the different techniques. In this respect, the enhancement techniques already present on the mark might be compatible or otherwise influence the beneficial use of the spectroscopic approach leading to an optimization and the selection of the best enhancement technique was necessary for the effectiveness of a subsequent thin layer of gold deposition on both the background and the fingermark [2].

Applications for hybrid techniques in complex substrates

The development of fingermark enhancement methods and the search for improvement of the processes and materials available are still at the forefront of research on latent fingermark visualisation. This focus is also influenced by the fact that it is one of the oldest forensic disciplines, and so the pressure to improve existing processes, or to develop alternative ones, is significant. In this context, hybrid techniques carrying complementary analytical power appear to be particularly suitable for the study of both enhancement materials and the development of archetypal marks.

Despite the enormous attention to fingermark analysis and the numerous strategies proposed to date, there are still cases when the development of clear and detailed fingermark images on non-traditional substrates is particularly challenging. Herein, the combined use of advanced mass spectrometric techniques, infrared spectral mapping, and chromatic and elemental analysis is investigated for the visualisation of latent fingermarks in selected complex matrices. Offering the identification of the substance generating the aged fingerprints, in addition to the donor's identity through DNA analysis, may lead to scenario reconstruction and suspect's actions tracking in criminal investigations [19].

Conclusion

After 3.5 hours post deposition the conditioned marks revealed an excellent development which is in concordance with previous observations of the uptake and release of eccrine components into Fps in dependence on the conditions of contact. In general the uptake of endogenous molecules into the Fp is excreted again within a few hours. However, there are also prevailances, e.g. biogenic amines which are well-known substrates for canine detection dogs that have been detected for more than 2 weeks post print deposition. It was also shown for phenol that the detection within fingermarks is possible for at least 2 weeks. Myristic acid and sebum-related features show a similar degradation pattern within the on skin sebum signal. Besides those features, many other lipidic substances are contained in the human sebum the exact molecular profile of those substances may vary interindividually as well as intraindividually over time and was so far not completely unveiled. In addition, well detectable substances decayed already after 1 day.

The collection of on skin sebum samples with glass plates can be considered as reference for the effective sebum signal quantification. The standardized sampling allowed to study the molecules on the glass plate statistically which is not possible with respect to human fingermarks. The results are in the same range as determined herein. The fact that the analysis of real SEs contained on average 2.25 times higher signal intensity compared to the glass plate samples could be attributed to the much higher area of the considered SE sample. Two gender related species (1 unknown) were detected in the investigation of male and female SEs on glass plates, whereas the sebum measurements with respect to gender differentiation are highly ambiguous and could not indicate useful information.

Highlights

- Hybrid techniques combine spectroscopic and chromatographic methods for improved visualization of latent fingermarks.
- Four key chromatographic techniques include Cobalt-2, Ninhydrin, Sticky Side Powder, and Vacuum Metal Deposition.
- Spectroscopic techniques like IR, Raman, and SERS are effective for analyzing chemical residues in fingermarks.
- Hybrid methods are particularly useful for complex substrates, overcoming traditional visualization limitations.
- Techniques like MALDI enhancement and ATR-FTIR expand forensic applications, enabling chemical and DNA profiling.

Ethical Considerations

All ethical considerations, including cultural sensitivity, data privacy, and equitable access to resources, have been rigorously observed throughout the study.

Conflict of Interest

The author declares no conflict of interest.

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