الخلاصة

الهدف من الدراسة هو تقييم دور تقنية رامان في الكشف عن العينات المختلفة في مسرح الجريمة حيث تم استخدام 11 عينة من الدم وتم فحص بقع الدم خلال فترات زمنية مختلفة (24 ساعة , 48 ساعة, 72 ساعة, 96 ساعة, 120 ساعة , 144 ساعة) بواسطة جهاز رامان , تم القياس وفق أطوال موجية للكشف عن بقع الدم وكانت النتائج ظهور أشارات مختلفة وحسب القمم المختلفة (742, 1001, 1123,1247,1341, 1368,1446,1576,1619 سم) وبواقع 11 عينة مختلفة.

أظهرت الدراسة امكانية تمييز بقع الدم عن بعضها وكذلك أمكانية أستخدام تحليل بقع العينات التى لايقل عمرها عن 3 أشهر في هذه الدراسة , حيث يمكن استخدام تقينة رامان في مسرح الجريمة لجمع البيانات من أجل تحقيق الطب الشرعي وأظهار ألأمكانات الكبيرة لتحديد هوية الدم ومقارنتها مع بيانات سابقة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية العلوم
قسم علوم الحياة

تطبيق تقنية الرامان في مسرح الجريمة

مشروع بحث مقدم الى

مجلس كلية العلوم – جامعة بابل

كجزء من متطلبات نيل درجة الدبلوم العالي في العلوم/ الأدلة الجنائية

من قبل

حسين علي يامر رحمن

(بكالوريوس علوم حياة، 2008)

إشراف

الاستاذ الدكتور أياد محمد المعموري

2021 م **1442هـ**

Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Science
Department of Biology

Raman Spectroscopy application in crime scene

A Research Project
Submitted to Council of the College of Science / University of Babylon in Partial Fulfillment to the Requirements for the Degree of High Diploma in Science /Forensic Evidence

By

Hussein Ali Yamer Rahman

B.Sc. Biology, 2008

Supervised by

Prof .Dr. Ayad Mohammed Jebur Almamoory

2021 A.D **1443 A.H**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

صدَقَ اللّٰه العليُّ العظيم

سورة ابراهيم، الآية ٤١

Certification

I certify that research is prepared under my supervision at the Department of Biology, College of Sciences, University of Babylon, as partial requirements for the Degree of Higher Diploma in Forensic Evidence.

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Data: / /2021

In view of available recommendation, I forward this research for debate by the Examination Committee.

Signature:

Name: Assistant Professor Dr. Adi Jassim Abdul Razzaq

Title: Chairman of Biology Dept.

Data: / /2021

Committee Certification

We, the examining committee, certify that we have read the research entitled (Raman Spectroscopy application in crime scene) and examined the student (Hussein ali yammer) in its contents at / /2021, and that in our opinion it is accepted as a research for the degree of High Diploma in Forensic Evidence with) estimation.

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Dean of Collage of Sciences –University of Babylon

Data: / /2021

Dedication

THE LAST OF THE PROPHETS AND MESSENGERS

MUHAMMAD, PEACE BE UPON HIM AND HIS FAMILY

TO THE SPIRIT OF MY FATHER

TO MY MOTHER

TO MY WIFE

HUSSEIN 2021

Acknowledgments

Praise be to Allah and peace and blessings be upon his Messenger the Prophet Muhammad peace be upon him and his family and followed them until the Day of religion.

I would like to thank my advisor :Prof .Dr .AyadM.J.Almamoori , University of Babylon for the supervision of the construction of this scientific effort that has been a great asset to me and the length of the continuous research of overseeing and careful supervision and valuable advice.

HUSSEIN 2021

Summary

The aim of this study to assess the role of Raman Spectroscopy in detection of different samples in crime scene.

10 Samples were collected and Respectively Blood stains were examined through different time period (24hr., 48hr., 72 hr., 96 hr.,120hr.,144hr.) by Raman instrument , The portable Raman spectrometer detected blood stain peaks (742,1001, 1123, 1247, 1341, 1368, 1446, 1576, and 1619 cm1) in all 11 stain samples.

Using principal component analysis, the human bloodstain could be discriminated from the each other. A bloodstain sample that is at up to 3 months old can be used for this study. The Raman spectrometer may be used at a crime scene to collect data for forensic investigation and demonstrates the great potential of for nondestructive, confirmatory identification of blood for forensic purposes.

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1. Introduction

The samples found at a crime scene can exist in trace amounts, and the analysis should be done with extreme care, or the sample might be lost to the surroundings and no conclusion of the scientific evidence for the investigation can be made for court testimony.

Raman spectroscopy is commonly used in chemistry to identify molecular structure. This technique is a nondestructive analysis, it needs no sample preparation, and the sample size can be in the order of picograms. Recently, Raman spectroscopy has been shown to be an effective multipurpose analytical method for forensic applications: identification of drugs,fibers, paints, and ink. A previous study reported the identification of body fluids (Boyd et al., 2010)

What is spectroscopy used for in forensics?

Spectroscopy for forensic toxicology

Raman spectroscopy serves the field of forensic toxicology as an analytical tool to detect and quantify drugs in biological specimens.

optimum analytical technique that does not destroy the small amounts of evidence is spectroscopy. The evidence, ranging from biological fluids, drugs to trace materials found at the crime scene can be examined as they are preserved with this technique. Furthermore, no sample preparation is needed for this particular technique, hence making it time-efficient compared to other analytical techniques. (Muehlethaler et,.2015)

What is the basic principle of Raman Spectroscopy?

What are the Basic Principles of Raman Spectroscopy. It is the shift in wavelength of the in elastically scattered radiation that provides the chemical and structural information. Raman shifted photons can be of either higher or lower energy, depending upon the vibrational state of the molecule under study.

Furthermore, automotive coatings consisting of both organic and inorganic chemical compounds can be identified by Raman and FTIR spectroscopy techniques. Whereas the additives for these compositions on the automotive coating can be analyzed through SEM/EDX and ICP-MS techniques.(Suzuki & Gresham,1986)

Raman spectroscopy is an optical technique based on inelastic scattering of light by vibrating molecules and can provide chemical fingerprints of cells, tissues or biofluids. The high chemical specificity, minimal or lack of sample preparation and the ability to use advanced optical technologies in the visible or near-infrared spectral range (lasers, microscopes, fibre-optics) have recently led to an increase in medical diagnostic applications of Raman spectroscopy(Kong etal.,2015).

Aim of study

Identify the role of Raman spectroscopy in detection of forensic samples.

Study Objectives

1-Collect Blood samples from proposed crime scene

2-divide the blood stain into different time period.

3- measured the samples by Raman instrument .

1. Review of Literatures

2-1 Raman Spectroscopy in Forensic scene

Raman microspectroscopy is a technique that uses a specialized Raman spectrometer to measure the spectra of microscopic samples. In general terms, a Raman spectrometer is integrated with a Raman microscope. Different exciting lasers may be used to excite a microscopic sample at different wavelengths so that the Raman microspectrometer can collect and analyze the vibrational spectra. Blood and body fluid samples are highly important to forensic science. They can place a person at a crime scene and even be used as evidence of a suspect's guilt.(Boyd etal.,2011)

What does Raman spectroscopy tell you?

Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorphy, crystallinity and molecular interactions. It is based upon the interaction of light with the chemical bonds within a material.

This analytical tool is further beneficial with its low detection level. To illustrate, D'Elia, et al. found that Raman spectroscopy can detect drugs to the lowest micrograms within 7 days of deposition.

Furthermore, the purity of the drug as well as the chain of custody, the two crucial things uphold in forensic laboratories, are preserved for the technique's non-destructive nature. However, future developments to improve fluorescence interference from the strong fluorescence background need to be implemented to efficiently identify drug samples.

Ferreira etal.,2017 stated that The use of Raman spectroscopy as a forensic tool for the examination of automobile paints was investigated with the goal of verifying measurement variability and studying spectral quality using parameters and circumstances and separated spectra library shall be developed for each laser wavelength as well as for each sample substrate. Further studies with a higher number of paint chips will enable the establishment of prediction models aimed to identify unknown samples involved in hit-and-run cases. Exposure period, Raman shift range, and baseline correction, on the other hand, had less of an impact on the characteristics of the acquired spectra. Intriguingly, samples taken from the bumper coating, which is made up of a plastic substrate, revealed distinct absorption band patterns than pieces taken from other components of the same car, which were made up of a metallic substrate.

Raman spectroscopy has been applied to a wide range of evidence types and has shown to be an important tool of examination for forensic investigators. Raman spectroscopy is defined as "a vibrational spectroscopy method concerned with inelastic scattering arising from rotational and vibrational transitions happening inside a molecule structure," according to this article.

The general result of this research is that Raman spectroscopy has a significant and strong presence in forensic science. In the future, new and unusual forms of evidence will most likely offer themselves as possibilities for Raman spectroscopy examination(Khandasammy etal.,2018)

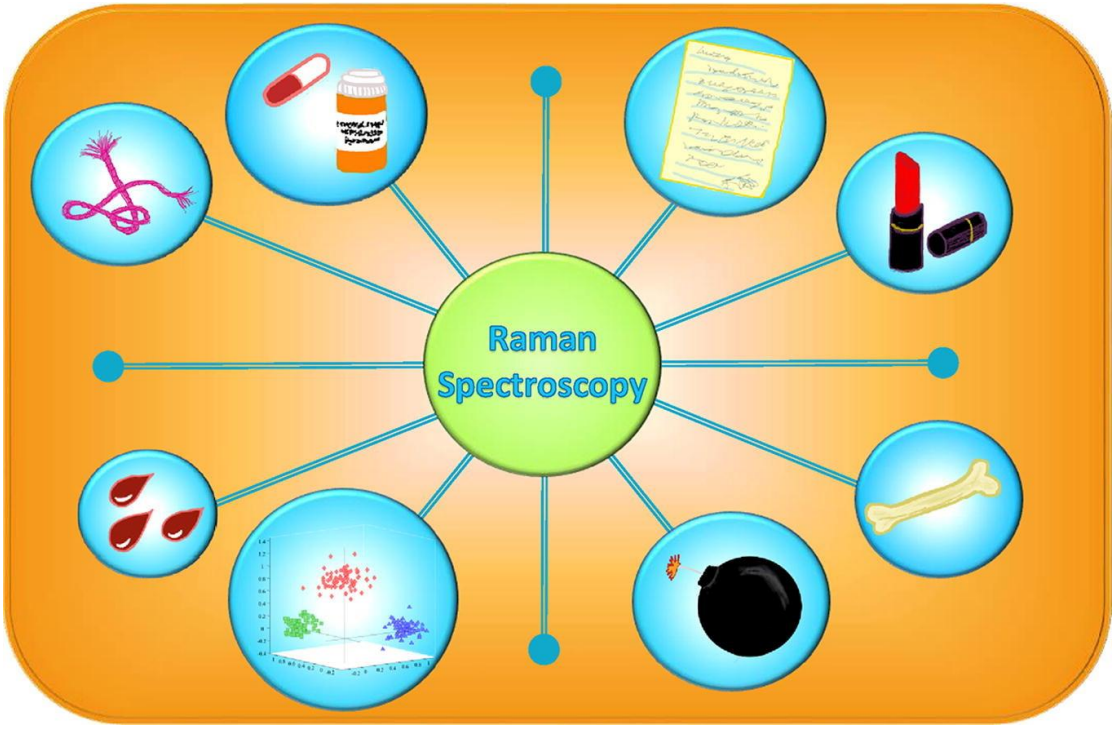

Figure1:Application of Raman Spectroscopy (Khandasammy etal.,2018)

2-2 Differentiation of Samples by Raman Spectroscopy

Lepot et al. established that Raman spectroscopy was a powerful method applicable to the routine analysis of fibers and recommended its use following MSP measurements. Buzzini et al. also compared the selectivity of UV-Vis MSP and Raman spectroscopy for the analysis of dyed fibers of acrylic, cotton and wool. In this case, UV-Vis MSP [Mass spectroscopy] was confirmed as the most efficient and discriminatory method for the analysis of textile fibers, whereas Raman provided a minor discrimination power and played a complementary role.

Taking into account that C-C bonds of the skeletal backbone structure of many polymeric fibers present a good Raman scattering and produce characteristic bands, Miller et al. studied the capacity of Raman spectroscopy for identifying subclasses of polymeric matrices. For this purpose, difficulties were only found to classify acrylic and saran fibers;

meanwhile the subclasses were more easily distinguishable in the Raman than in the IR spectra.

2-2 Factors influencing Raman measurements

Because of the generally flat surface that blood presents to the laser spot, as well as the methods used to prepare the blood samples, the quality of the analytical data is affected.

Other factors, such as the adsorbed water, the minor structural changes and the variations of the crystalline nature of the fibers also influenced the analytical results. According to Massonnet et al., similar spectra were obtained with aluminium foils, double side adhesive tapes, and glass slides. However, bands from the glass could alter the spectra when NIR lasers were used with glass cover slips.

Regarding the color, Raman scattering observed for blood with solid amorphous or crystalline pigment particles was higher than for individual molecules. The enhancement of the dye signal might be due to a higher local dye density or a great number of reflections (Miller etal., 2001).

Kyle &Lednev,2017 found The incorporation of chemometrics further enhances the specificity of RS, and offers the opportunity of automatic data analysis and estimation of error rates, which are important requirements for modern forensic tools. Applications of RS in forensic serology and for the analysis of gunshot residue (GSR) were chosen for this review since RS promises significant advancement of these areas for practical forensics.

It's easy to understand how Raman spectroscopy, one of the hottest analytical chemistry methods, may be useful. Raman spectroscopy is recognized for requiring little sample, being non-destructive, and being

extremely material specific, all of which make it excellent for assessing crime scene evidence.(Fikiet etal., 2017).

Suzuki etal.,2018,discussed how different analytical conditions can affect Raman spectra, and what bearing this and other factors may have on spectral interpretation; it also presents a review of the literature describing applications of Raman spectroscopy for the examination of various types of evidence.and found There have been several significant advances in Raman spectroscopy instrumentation during the past few decades, and this method is now a fully mature analytical technique on par with its counterpart, infrared spectroscopy.

Studies have shown that Raman scattering from human blood can indicate essential factors such as the identity of the donor, as well as its age and dilution. Research has also demonstrated that Raman scattering is more powerful than alternative luminescence methods that have lower sensitivities.

Other bodily fluids, such as tears, saliva, semen, sweat, urine, and vaginal secretion can also be analyzed with Raman scattering. Importantly, the Raman method is non-destructive, meaning that samples can be retained for future testing. This is vital in forensic studies because often only a small trace of the sample is left at the scene.(Boyd et al., 2010).

Enejder etal.,2002 demonstrates the feasibility of using Raman spectroscopy for quantitative measurements of biomolecular contents in highly light-scattering and absorbing media. Concentrations of multiple analytes were simultaneously measured in whole blood with clinical accuracy, without sample processing, using near-infrared Raman spectroscopy. Spectra were acquired with an instrument employing nonimaging optics, designed using Monte Carlo simulations of the influence

of light-scattering-absorbing blood cells on the excitation and emission of Raman light in turbid medium. Raman spectra were collected from whole blood drawn from 31 individuals. Quantitative predictions of glucose, urea, total protein, albumin, triglycerides, hematocrit, and hemoglobin were made by means of partial least-squares (PLS) analysis with clinically relevant precision (r(2) values >0.93).

The root mean squared error of prediction (RMSEP) for glucose in serum obtained with PLS is 21 mg/dL, and the RMSEP obtained with HLA is 17 mg/dL. In whole blood, the PLS RMSEP for glucose was 79 mg/dL, and HLA predictions had an RMSEP of 63 mg/dL. The measurement technique was robust over the 7-week period. HLA was shown to generate a lower prediction error than PLS. The predictions by both PLS and HLA were clinically acceptable. The result with whole blood requires further improvement (Koo etal., 1999).

Hanlon etal.,2000 have reviewed the biological and physical basis of Raman spectroscopy of tissue, to assess the current status of the field and to explore future directions. The principles of Raman spectroscopy and the molecular level information it provides are explained. An overview of the evolution of Raman spectroscopic techniques in biology and medicine, from early investigations using visible laser excitation to present-day technology based on near-infrared laser excitation and charge-coupled device array detection, is presented. State-of-the-art Raman spectrometer systems for research laboratory and clinical settings are described.

Raman spectroscopic probing of blood components and of whole blood has been on-going for more than four decades and has proven useful in applications ranging from the understanding of hemoglobin oxygenation, to

the discrimination of cancerous cells from healthy lymphocytes, and the forensic investigation of crime scenes(Atkins etal., 2017)

(Shaine etal., 2020) used An optimized procedure is described for the acquisition of 785 nm excited SERS spectra of dried bloodstains and shown to offer great potential for rapid, portable, highly sensitive and specific, confirmatory identification for forensic applications. Following extraction in 1 μL of 50% acetic acid, a robust, highly reproducible SERS spectrum is observed from dried bloodstains resulting from a hematin-like heme moiety (ferric, high spin). As anticipated, this blood signature can be classified with 100% specificity and sensitivity with respect to the SERS spectra of other body fluids. High quality SERS spectra can be observed from stains of blood diluted by as much as 105. Dried blood spectra acquired on Au and Ag SERS active substrates exhibit very different relative intensities at this electronically, non-resonant excitation wavelength (785 nm) indicating that a strong chemical effect contributes to the SERS enhancement of this body fluid. DFT calculations further confirm the vibrational band assignments of the features seen in these SERS spectra of dried blood.

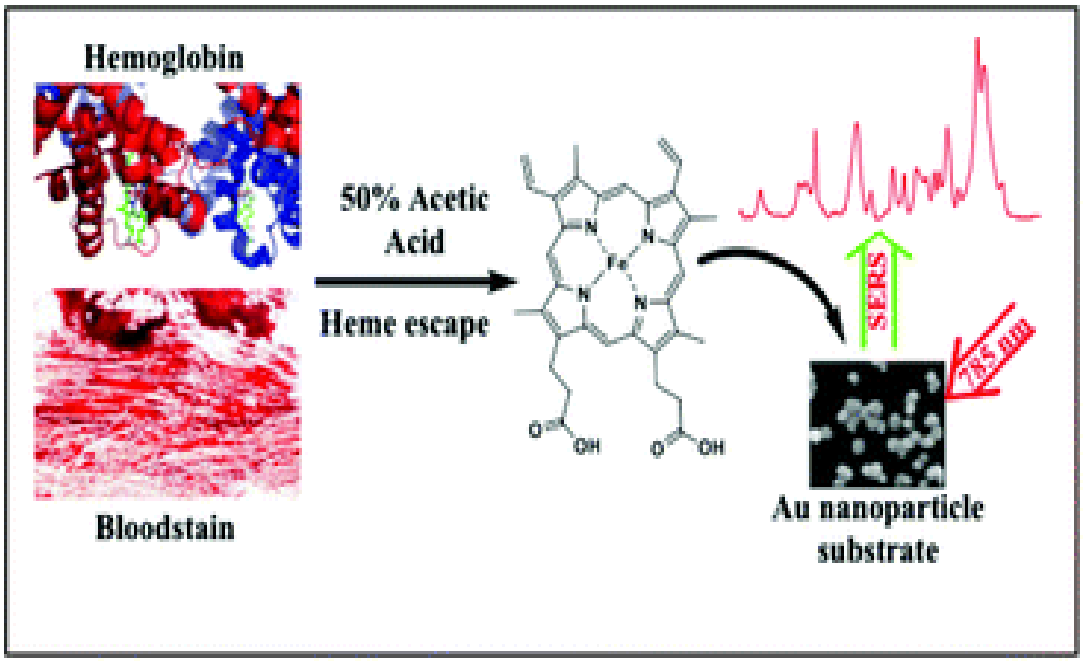

Figure2:Processing of Blood Stain for Raman Spectroscopy and further measurement (Shaine etal., 2020)

The discrimination accuracy for human and nonhuman blood is important for customs inspection and forensic applications. Recently, Raman spectroscopy has shown effectiveness in analyzing blood droplets and stains with an excitation wavelength of 785 nm. However, the discrimination of liquid whole blood in a vacuum blood tube using Raman spectroscopy, which is a form of noncontact and nondestructive detection, has not been achieved. An excitation wavelength of 532 nm was chosen to avoid the fluorescent background of the blood tube, at the cost of reduced spectroscopic information and discrimination accuracy. To improve the accuracy and true positive rate (TPR) for human blood, a dual-model analysis method is proposed. First, model 1 was used to discriminate human-unlike nonhuman blood. Model 2 was then used to discriminate human-like nonhuman blood from the “human blood” obtained by model 1. A total of 332 Raman spectra from 10 species were used to build and

validate the model. A blind test and external validation demonstrated the effectiveness of the model. Compared with the results obtained by the single partial least-squares model, the discrimination performance was improved. The total accuracy and TPR, which are highly important for practical applications, increased to 99.1% and 97.4% from 87.2% and 90.6%, respectively (Bian etal., 2018).

(Morrison etal., 2018) indicated that the Field based forensic tests commonly provide information on the presence and identity of biological stains and can also support the identification of species. Such information can support downstream processing of forensic samples and generate rapid intelligence. These approaches have traditionally used chemical and immunological techniques to elicit the result but some are known to suffer from a lack of specificity and sensitivity. The last 10 years has seen the development of field-based genetic profiling systems, with specific focus on moving the mainstay of forensic genetic analysis, namely STR profiling, out of the laboratory and into the hands of the non-laboratory user. In doing so it is now possible for enforcement officers to generate a crime scene DNA profile which can then be matched to a reference or database profile. The introduction of these novel genetic platforms also allows for further development of new molecular assays aimed at answering the more traditional questions relating to body fluid identity and species detection. The current drive for field-based molecular tools is in response to the needs of the criminal justice system and enforcement agencies, and promises a step-change in how forensic evidence is processed. However, the adoption of such systems by the law enforcement community does not represent a new strategy in the way forensic science has integrated previous novel approaches. Nor do they automatically represent a threat to the quality control and assurance practices that are central to the field.

(Sikirzhytskaya etal., 2013) found that Body fluid traces recovered at crime scenes are among the most common and important types of forensic evidence. However, the ability to characterize a biological stain at a crime scene nondestructively has not yet been demonstrated. Here, we expand the Raman spectroscopic approach for the identification of dry traces of pure body fluids to address the problem of heterogeneous contamination, which can impair the performance of conventional methods. The concept of multidimensional Raman signatures was utilized for the identification of blood in dry traces contaminated with sand, dust, and soil. Multiple Raman spectra were acquired from the samples via automatic scanning, and the contribution of blood was evaluated through the fitting quality using spectroscopic signature components. The spatial mapping technique allowed for detection of "hot spots" dominated by blood contribution.

Samples were tested for DNA to determine whether the presumptive tests damaged or destroyed DNA. The DNA loci tested were D2S1338 and D19S433. Leuchomalachite green had a sensitivity of 1:10,000, while the remaining tests were able to detect blood to a dilution of 1:100,000. Substances tested include saliva, semen, potato, tomato, tomato sauce, tomato sauce with meat, red onion, red kidney bean, horseradish, 0.1 M ascorbic acid, 5% bleach, 10% cupric sulfate, 10% ferric sulfate, and 10% nickel chloride. Of all the substances tested, not one of the household items reacted with every test; however, the chemicals did. DNA was recovered and amplified from luminol, phenolphthalein, Hemastix, and Bluestar, but not from leuchomalachite green or Hemident.(Tobe etal., 2007)

(Suzuki&Carrabba,2001) mentioned that there are Several applications of Raman spectroscopy in the forensic sciences have recently been demonstrated, but few have involved the analysis of paints. Undoubtedly, this is a reflection of the sample degradation problems often encountered when a visible or near-infrared laser is focused on a light-absorbing matrix. In this study, a dispersive CCD Raman spectrometer (785 nm) was used in a configuration which collected scattered light from an excitation region 3 mm long and 80 microm wide, instead of from a focused spot. Sample degradation was not observed, and Raman spectra of automotive paints of all colors were readily obtained. Most of the paints analyzed were U.S. automobile original finishes (1974 to 1989) from the Reference Collection of Automotive Paints, and the inorganic pigments examined were those which had been identified previously by infrared spectroscopy in finishes from this collection. Prominent peaks of rutile were observed in Raman spectra of light-colored nonmetallic finishes for both monocoats and basecoat/clearcoat systems, and the rutile peaks are readily distinguished from those of anatase.

(Braz etal.,2015)presents a preliminary investigation on the applicability of Raman imaging for non-destructive and rapid analysis of blue crossing ink lines. The MCR method was used to facilitate visualization of the distribution of inks of the same colour and the most predominant Raman signature at the crossing was used to interpret the order of application of inks. Different pen ink types, different times separating the application of the two ink lines and different paper substrates were used. From the 90 Raman images examined, the correct order of application was determined in more than 60% by direct observation. The remainder cases were not as clear due to the uneven distribution of inks and the empty spaces similar to a net-like pattern observed at the crossing.

The heme compound found in deoxyribonucleic acid (DNA) extracted from bloodstains, which is regarded as a major inhibitor of polymerase chain reaction (PCR), was characterized in comparison with alkaline and acid hematin, histidine and ammonia hemochromogens, and globin and serum albumin hemochromogens digested by proteinase K. Alkaline and acid hematin were almost completely removed by phenol/chloroform treatment and ethanol precipitation, so as not to be copurified with DNA from the specimens. Spectrophotometric results indicated that the contaminant was likely to be the product of proteinase K digestion of some heme-blood protein complex, which was not completely extracted by organic solvents and remained in the ethanol precipitates of DNA. The results of polyacrylamide gradient gel electrophoresis and intensity of the inhibition of PCR suggested that the ligand of the contaminant was a somewhat large molecule, resistant to the proteolysis by proteinase K. The addition of bovine serum albumin to the reaction mixture prevented the inhibition of PCR by the heme compounds, probably by binding to the heme. This showed that the inhibition was not due to the irreversible inactivation of the enzyme (Akane etal., 1994)

3. Materials and Methods

Samples Collections

Human blood samples (n = 11) were taken intravenously from the proposed suspected persons. About 100 μL of the blood was dropped onto gauze to make a bloodstain After drying, all Raman measurements were performed within 48 h of sample preparation.

For the time course change study, bloodstains were left at room temperature for different period (24hr, 48hr, 72hr, 96 hr, 120 hr, 144 hr). The maximum sample dilution to detect Raman spectroscopy was investigated by

diluting the human blood (1:10, 1:50, 1:100, and 1:250) with saline. Raman measurement was performed By Raman Instrument (TakRam N1-541) Teksan company , IR.

Figure 3: Raman Instrument (TakRam N1-541)

Figure 4: Blood Stain Samples

Statistical Analysis principal component analysis (PCA) was performed.

4. Results and Discussion

In addition to the variation of the laser excitation wavelengths, there are other reasons that also explain the specific enhancement of some bands. Thus, Thomas et al. mainly observed bands from blue dyes when blue, yellow and red dyed fibers were analyzed. This was attributed to a significantly greater concentration of the blue dye. On the other hand, a localized heating effect from the laser increased the intensity of the Raman bands .

Moreover, the type of the polymeric matrix also influenced the observed signals. Thus, the analysis of acrylic fibers by Buzzini et al. (22) provided only signals about dyes; meanwhile, the influence of the matrix was also detected from wool and silk fibers. Another work observed that alterations on the molecular structure of azo dyes due to different solvents and pH modifications modified their initial spectral profile Figure 5.

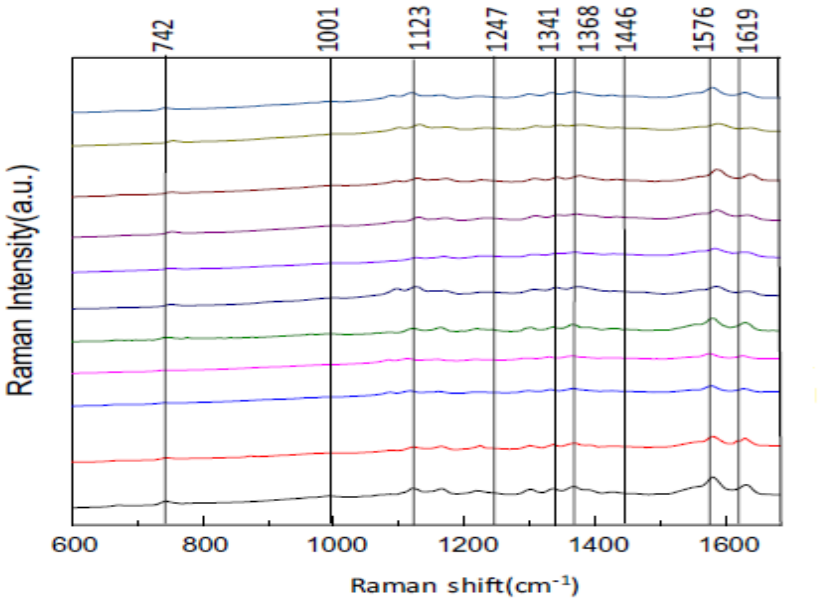

Figure 5: Raman spectra of 10 bloodstains in crime scene

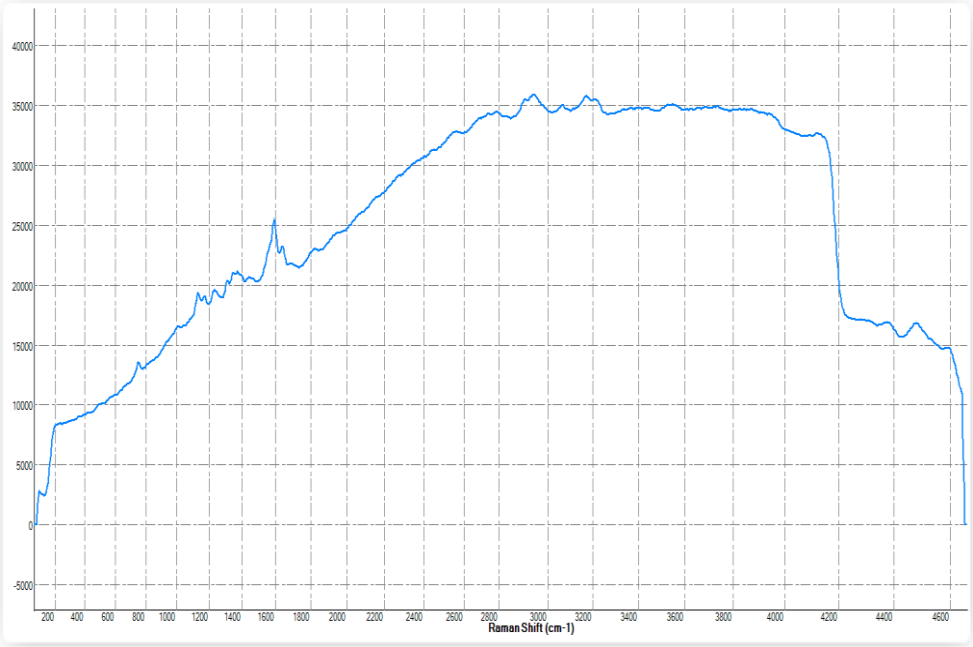

Figure 6: Raman spectra of Sample 1 in crime scene

Raman Technology offers several advantages over conventional Raman spectrometers and opens up enormous potential for the use of this new technology in different application areas.

The picosecond range laser excitation source and a time-gated single photon counting array detector create a totally new type of spectrometer which is able to acquire Raman spectra with real fluorescence suppression. The system rejects the fluorescence interference (which has a longer average delay) while capturing the instantaneous Raman scattering signal. It also enables the acquisition of time-resolved fluorescence spectra by sequentially sampling the emission pulses at different temporal positions. This approach simultaneously opens two windows for material characterization and provides valuable new information in several different application fields(Virkler& Lednev,2008)

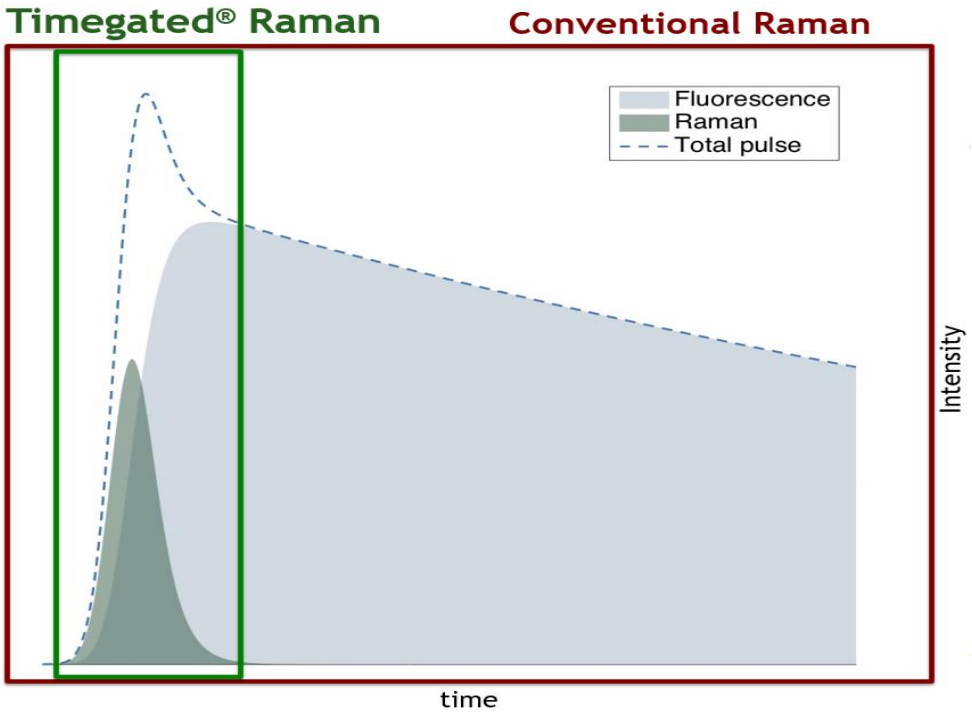

Figure 7: Relationship between time and Intensity for Raman Spectroscopy

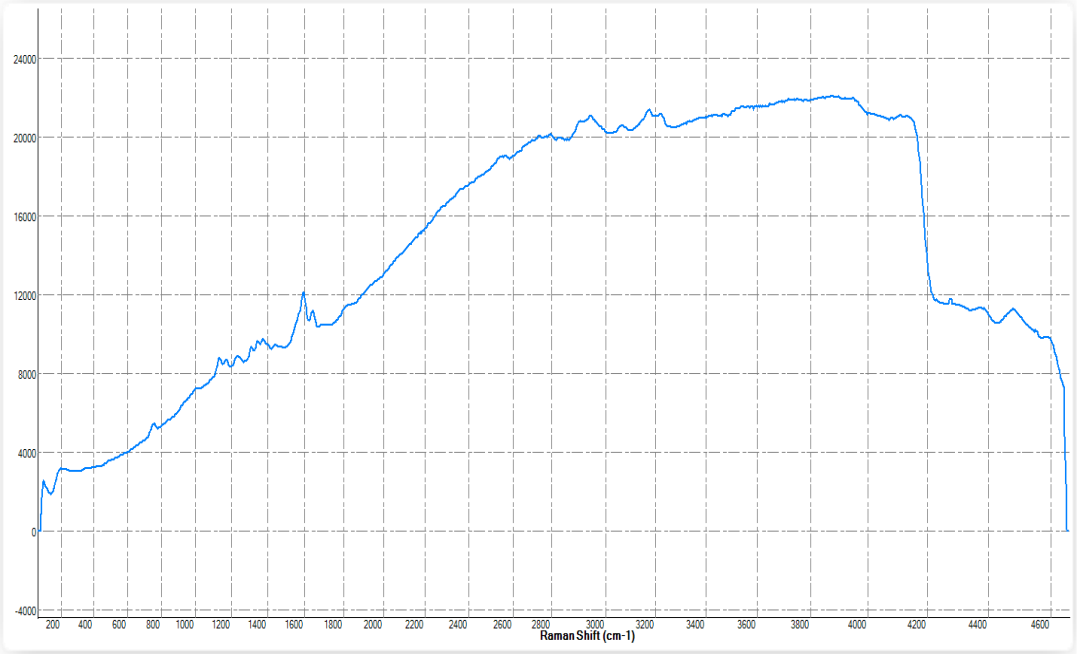

Figure 8: Raman spectra of Sample 2 in crime scene

The temporally short and spectrally multi-peaked Raman signal is clearly seen over the temporally and spectrally broad photoluminescence spectrum. In this figure, the origin of the time axis has no fundamental

physical significance (the values are just the settings of the electronic delay generator(Hackshaw etal.,2020).

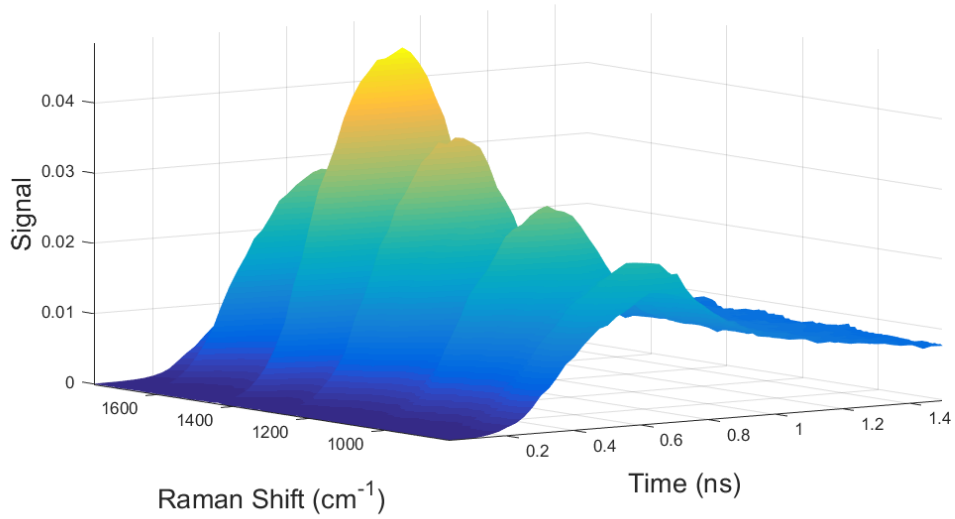

Figure 9: Correlation between time and Signal of Raman Spectroscopy

The change in the Raman spectra of bloodstains over time (up to 144 hours) was studied (Fig.10). Raman peaks for human blood (742, 1001, 1123, 1247, 1341, 1368, 1446, 1576, and 1619 cm1) were discovered in this investigation.

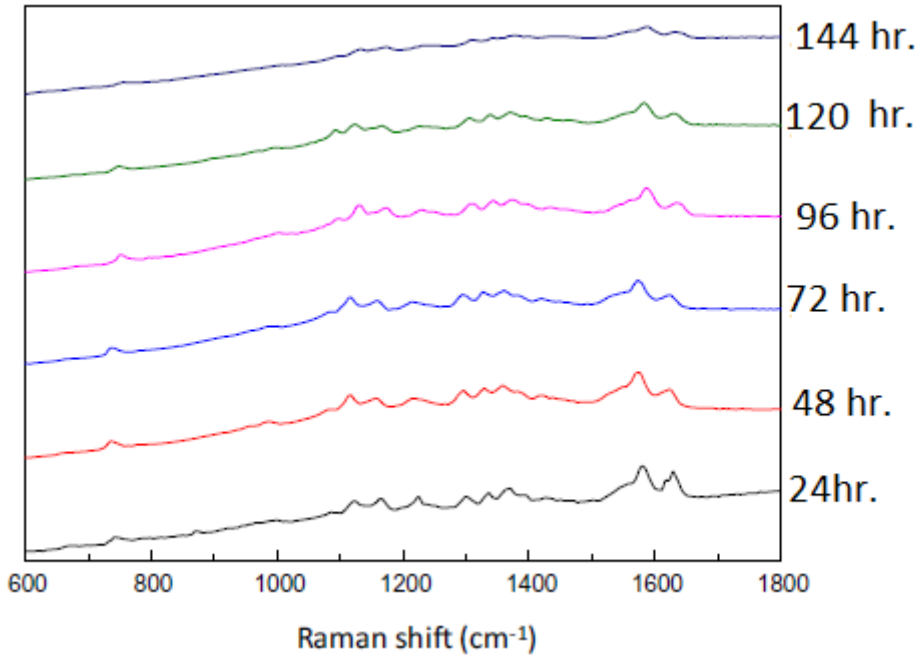

Figure 10:Changes in the Raman spectra of human bloodstains over time

The Achilles 'heel of conventional Raman technology is photoluminescence (including fluorescence and phosphorescence) interference. Photoluminescence is a competing phenomenon with Raman scattering and it can overlap or "swamp" Raman signals making the identification and quantification of materials impossible by achieve real fluorescence suppression and redefine the ways Raman spectroscopy is exploited.

Time-resolved total emission spectrum produced by a 532 nm pulsed excitation laser. This spectrum is the sum of the Raman and photoluminescence emission.

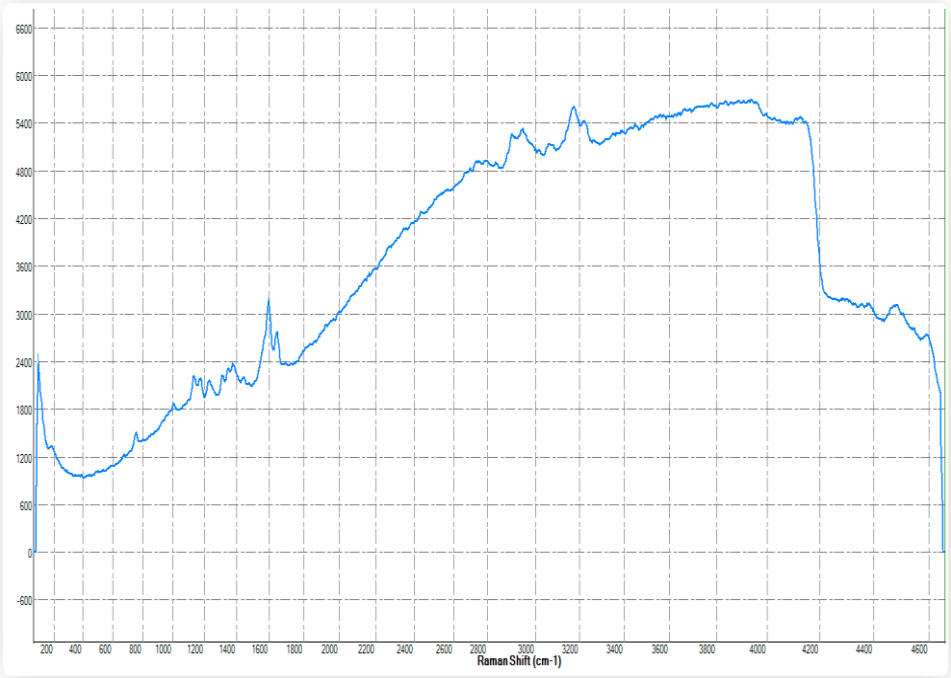

Figure 11: Raman spectra of Sample 3 in crime scene

Time-resolved background i.e. fluorescence surface contains fluorescence decay information and no Raman signals. Note that the temporally long "tail" of emission occurring well after the excitation. The 2D Raman spectrum is obtained by subtracting this fluorescence contribution from the overall signal. Fluorescence suppression is based on only collecting data from a time range containing a significant amount of Raman information while containing a minimum amount of the interfering background intensity.

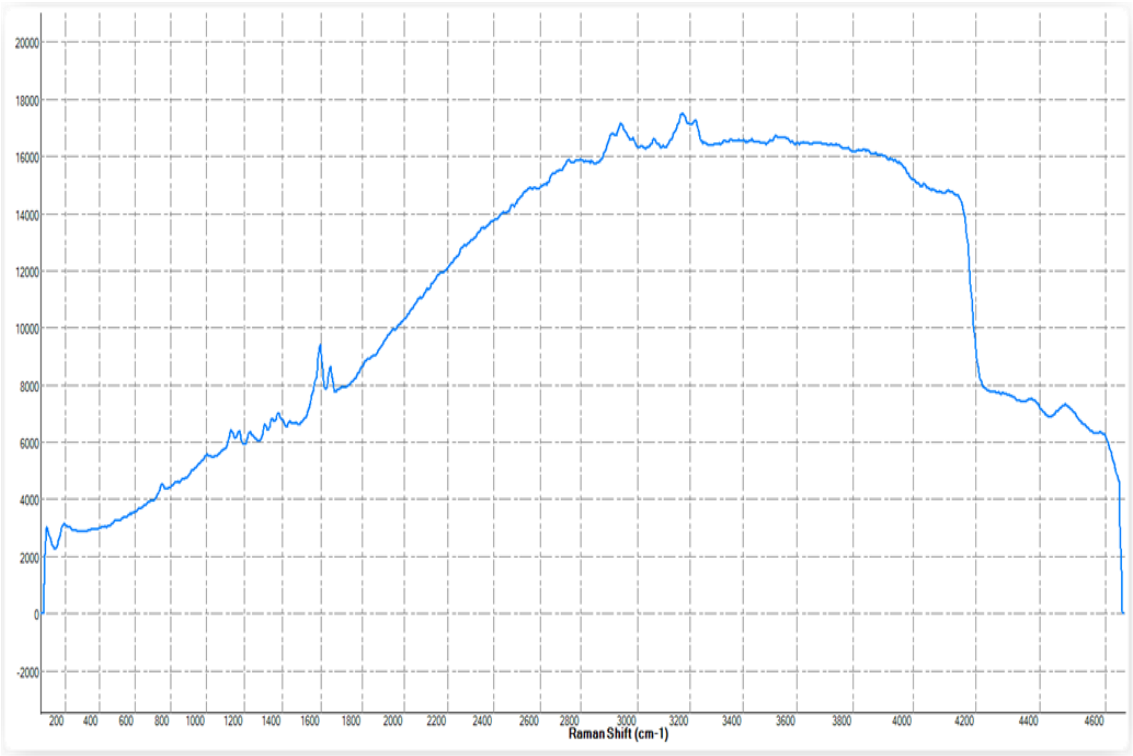

Figure 12: Raman spectra of Sample 4 in crime scene

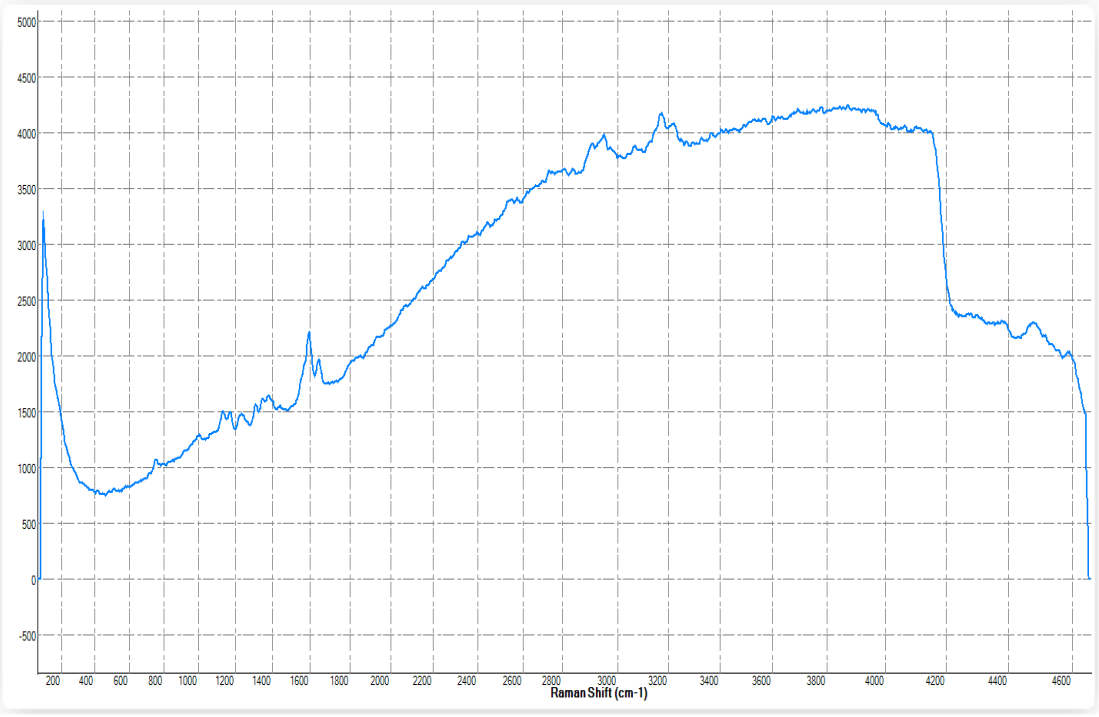

Figure 13: Raman spectra of Sample 5 in crime scene

(Sikirzhytski etal., 2010) demonstrated the capability of Raman spectroscopy to identify an unknown substance to be semen, blood or saliva with high confidence through Detection and identification of blood,

semen and saliva stains, the most common body fluids encountered at a crime scene, are very important aspects of forensic science today. This study targets the development of a nondestructive, confirmatory method for body fluid identification based on Raman spectroscopy coupled with advanced statistical analysis. Dry traces of blood, semen and saliva obtained from multiple donors were probed using a confocal Raman microscope with a 785-nm excitation wavelength under controlled laboratory conditions.

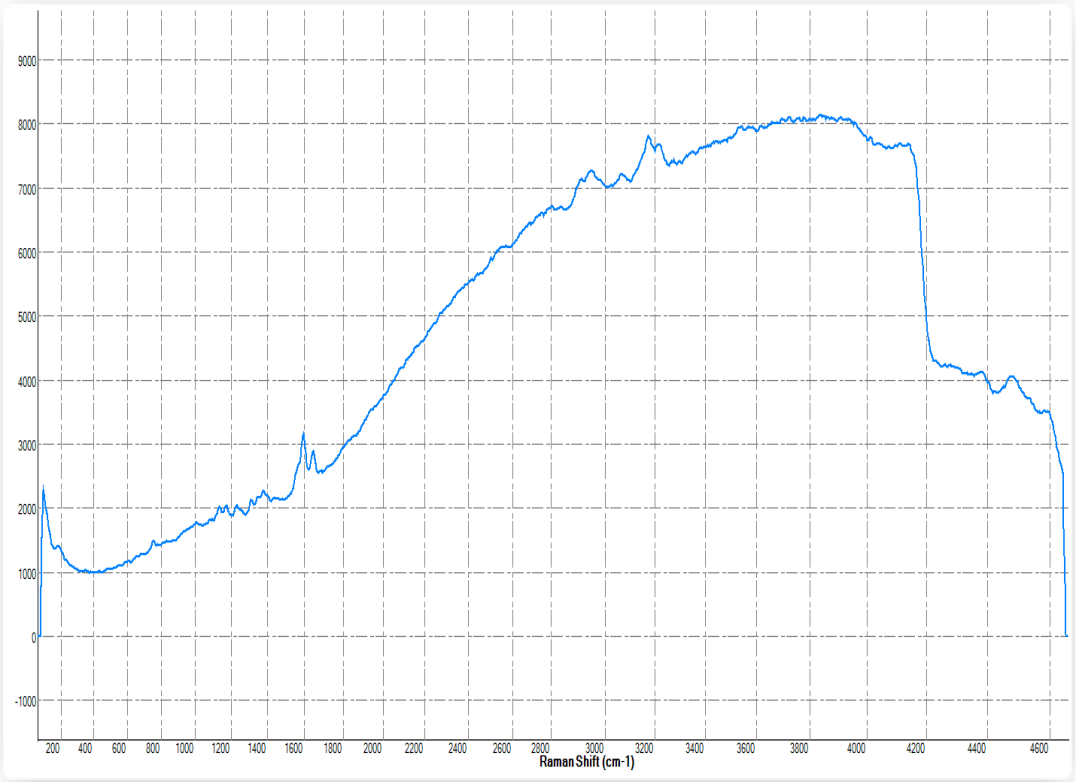

Figure 14: Raman spectra of Sample 6in crime scene

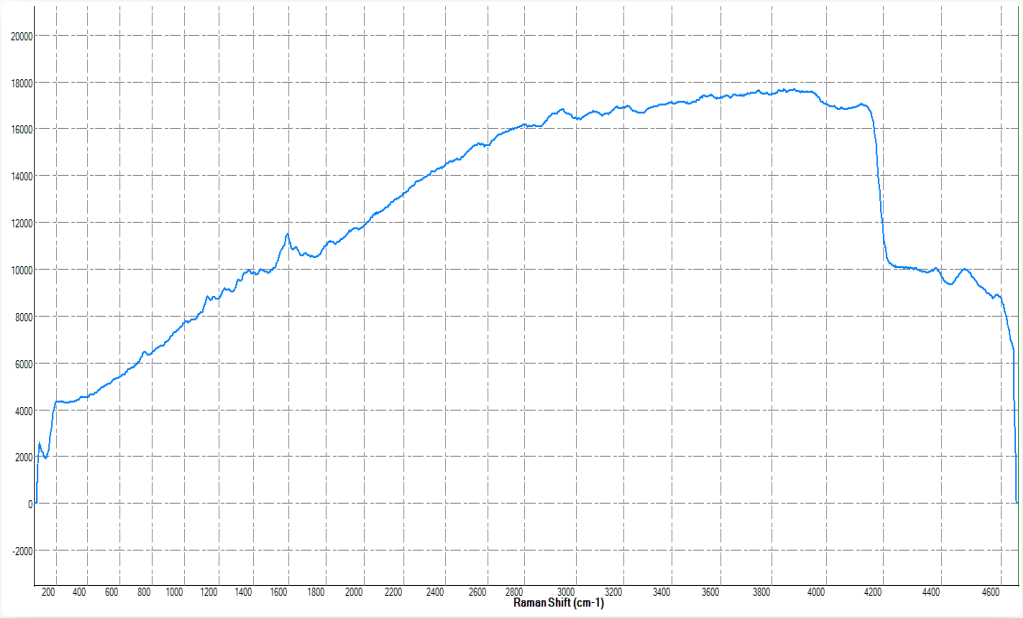

Figure 15: Raman spectra of Sample 7 in crime scene

In controlled laboratory circumstances, Raman spectroscopy has showed amazing skills in detecting blood. However, substrate interference poses a substantial problem when using Raman spectroscopy to characterize bodily fluid traces at a crime scene(McLaughlin eta., 2013), used micro-Raman spectroscopy with high-resolution analysis to discriminate between bloodstains from infants and bloodstains from adults. Raman peaks were detected at 674, 754, 976, 1002, 1105, 1127, 1176, 1248, 1340, 1368, 1390, 1560, and 1611 cm-1 ; these peaks were derived from hemoglobin, albumin, and glucose. However, a peak was obtained at 1105 cm-1 , which was assigned to histidine; this peak was observed only for bloodstains from adults. Human adult hemoglobin (HbA) is composed of an α2 β2 tetramer structure, whereas human fetal hemoglobin (HbF) is composed of an α2 γ2 . Therefore, the lack of a Raman peak at 1105 cm-1 in bloodstains from infants indicates the possibility of two histidine substitutions (His116Ile and His143Ser) in the γ chain of HbF(Fujihara etal., 2019) , The removal of exogenous salts and matrix

components from biological samples is a critical problem for Raman analysis and It was shown by (Doctor&McCord,2015) that at a pH 5.0 many drugs that are prevalent in urine samples can be removed, permitting a selective detection of the benzodiazepine of interest. This technique has been shown to provide rapid (less than 20 min), sensitive, and specific detection of benzodiazepines with limits of detection between 32 and 600 ng/mL and dynamic range of 32-25,000 ng/mL. It provides the forensic community with a sensitive and specific screening technique for the detection of benzodiazepines in drug facilitated assault cases.

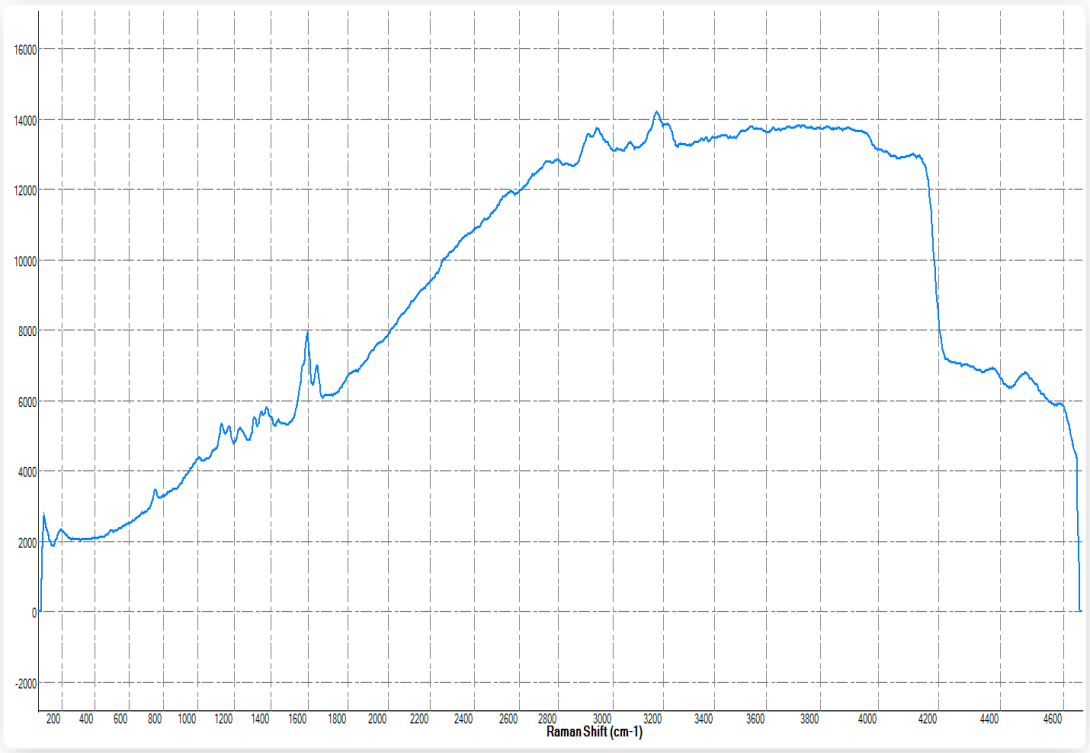

Figure 16: Raman spectra of Sample 8 in crime scene

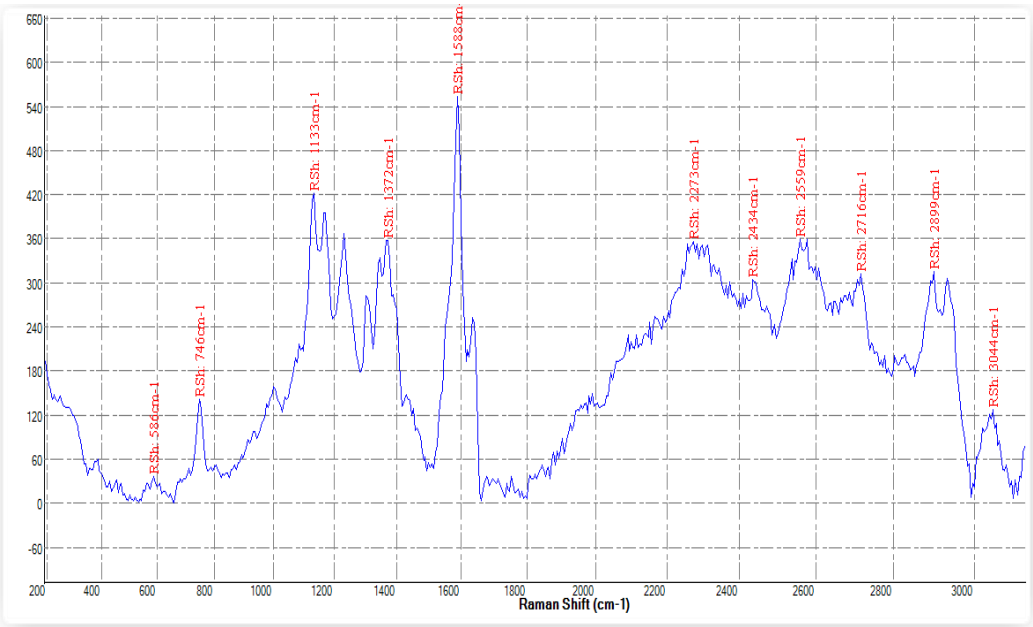

Figure 17: Raman spectra of Sample 9 in crime scene

Blood cellular components (red blood cells, white blood cells, and/or platelets) are releasing hypoxanthine into the plasma over this time interval. The SERS spectrum of red blood cells (RBCs) excited at 785 nm is reported for the first time and exhibits well-known heme group marker bands as well as other bands that may be attributed to cell membrane components or protein denaturation contributions. SERS, as well as normal Raman spectra, of oxy- and met-RBCs are reported and compared. These SERS results can have significant impact in the area of clinical diagnostics, blood supply management, and forensics.(Premasiri etal., 2012), The supramolecular bonding enhances the probability of through-space interactions between the transition dipoles from electronic transitions of extended π systems. Our results indicate that the intensity of ν(4) is in part strongly affected by C-H···X hydrogen bonding interactions when X is an electron-donating entity this conclusion was indicated by (Puntharod etal., 2010) when study to gain more understanding into the mechanism that enables the dramatic resonant Raman enhancement of totally symmetric

modes observed in hemozoin (malaria pigment) and other related heme supramolecular arrays when applying near-infrared excitation wavelengths, the iron(III) porphyrins Fe(TPP)Cl, [Fe(TPP)](2)O, Fe(OEP)Cl, and [Fe(OEP)](2)O along with β-hematin (synthetic hemozoin or malaria pigment) were analyzed in the solid state using resonance Raman spectroscopy. The critical finding was that from the model compounds investigated, all except [Fe(OEP)](2)O exhibited the enhancement of the totally symmetric mode ν(4) when exciting the molecules with 782 and 830 nm laser lines. Through a detailed comparison of X-ray crystallographic structures, it is proposed that intermolecular noncovalent interactions play an integral role in enabling excitonic interactions to occur in these heme supramolecular systems.

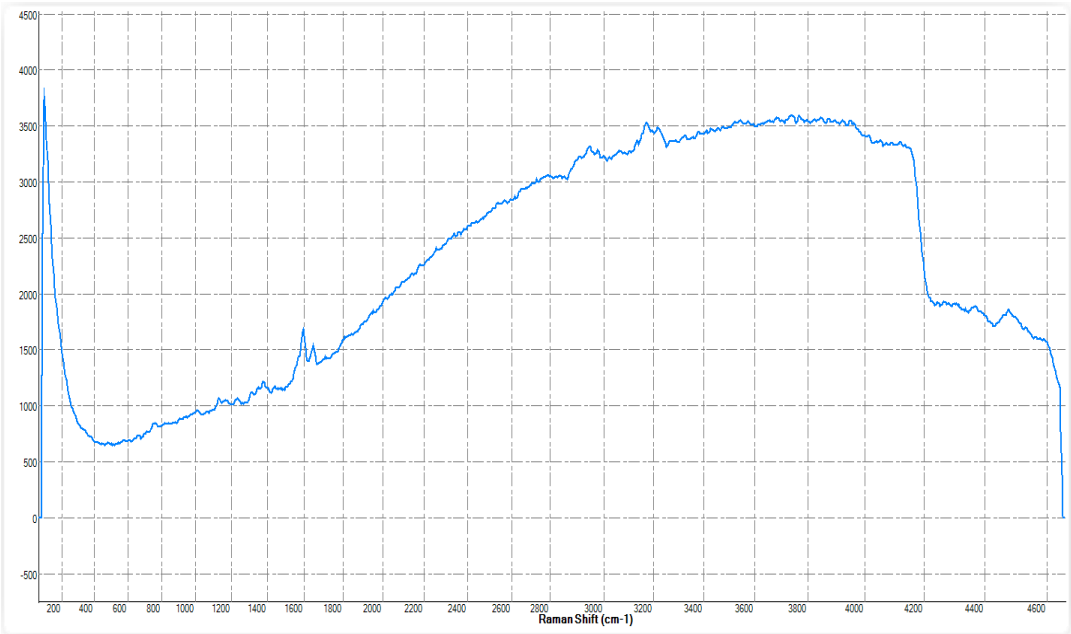

Figure 18: Raman spectra of Sample 10 in crime scene

Conclusions

1. The optimal method for detecting blood is demonstrated to be dependent on the substrate and the sort of interference encountered..

2-The suggested technique has a lot of promise for identifying blood in heavily polluted samples.

Recommendations

1-In a future work, the effects of the preservation time and conditions will be studied and discussed.

2-Differentiate between human blood stain and non-human blood stain by Raman spectroscopy

References

- Atkins CG, Buckley K, Blades MW, Turner RFB. Raman Spectroscopy of Blood and Blood Components. Appl Spectrosc. 2017 May;71(5):767-793. doi: 10.1177/0003702816686593. Epub 2017 Apr 11. PMID: 28398071.
- Bender, E. C. Analysis of low explosives. In: Forensic Investigation of Explosives. A. Beveridge, ed. Taylor and Francis, London, 1998, pp. 343-388.
- Bender, E. C. Analysis of smokeless powders using UV/TEA detection. In: Proceedings of the International Symposium on the Analysis and Detection of Explosives. U.S. Government Printing Office, Washington, DC, 1983, pp. 309-320.
- Beveridge, A., ed. Forensic Investigation of Explosives. Taylor and Francis, London, 1998.
- Bian H, Wang P, Wang N, Tian Y, Bai P, Jiang H, Gao J. Dual-model analysis for improving the discrimination performance of human and nonhuman blood based on Raman spectroscopy. Biomed Opt Express. 2018 Jul 5;9(8):3512-3522. doi: 10.1364/BOE.9.003512. PMID: 30338136; PMCID: PMC6191633.
- Boyd, S., Bertino, M. and Seashols, S. (2011). Raman spectroscopy of blood samples for forensic applications. Forensic Science International, 208(1-3), pp.124-128. https://www.sciencedirect.com/science/article/pii/S037907381000513X
- Bureau of Alcohol, Tobacco and Firearms, Arson and Explosives Incidents Report (1994). ATF P3320.4, Department of the Treasury, Washington, DC, 1994.
- Doctor EL, McCord B. The application of supported liquid extraction in the analysis of benzodiazepines using surface enhanced Raman

spectroscopy. Talanta. 2015 Nov 1;144:938-43. doi: 10.1016/j.talanta.2015.07.036. Epub 2015 Jul 15. PMID: 26452911.

- Enejder AM, Koo TW, Oh J, Hunter M, Sasic S, Feld MS, Horowitz GL. Blood analysis by Raman spectroscopy. Opt Lett. 2002 Nov 15;27(22):2004-6. doi: 10.1364/ol.27.002004. PMID: 18033426.
- Ferreira ,K. B., A. G. G. Oliveira & J. A. Gomes (2017) Raman spectroscopy of automotive paints: Forensic analysis of variability and spectral quality, Spectroscopy Letters, 50:2, 102-110, DOI: 10.1080/00387010.2017.1288635
- Fikiet, Marisia A., Khandasammy, Shelby R., Mistek, Ewelina, Ahmed, Yasmine, Halámková, Lenka, Bueno, Justin and Lednev, Igor K.. "Forensics: evidence examination via Raman spectroscopy" Physical Sciences Reviews, vol. 4, no. 2, 2019, pp. 20170049. https://doi.org/10.1515/psr-2017-0049
- Fujihara J, Nishimoto N, Yasuda T, Takeshita H. Discrimination Between Infant and Adult Bloodstains Using Micro-Raman Spectroscopy: A Preliminary Study. J Forensic Sci. 2019 May;64(3):698-701. doi: 10.1111/1556-4029.13904. Epub 2018 Aug 31. PMID: 30170335.
- Hall, K. E. and McCord, B. R. The analysis of mono- and divalent cations present in explosive residues using ion chromatography with conductivity detection, Journal of Forensic Sciences (1993) 38:928-934.
- Hanlon EB, Manoharan R, Koo TW, Shafer KE, Motz JT, Fitzmaurice M, Kramer JR, Itzkan I, Dasari RR, Feld MS. Prospects for in vivo Raman spectroscopy. Phys Med Biol. 2000 Feb;45(2):R1-59. doi: 10.1088/0031-9155/45/2/201. PMID: 10701500.
- Khandasammy ,Shelby R., Marisia A. Fikiet, Ewelina Mistek, Yasmine Ahmed, Lenka Halámková, Justin Bueno, Igor K. Lednev, Bloodstains, paintings, and drugs: Raman spectroscopy applications in forensic

science, Forensic Chemistry, Volume 8, Pages 111-133, ISSN 2468-1709,

- Premasiri WR, Lee JC, Ziegler LD. Surface-enhanced Raman scattering of whole human blood, blood plasma, and red blood cells: cellular processes and bioanalytical sensing. J Phys Chem B. 2012 Aug 9;116(31):9376-86. doi: 10.1021/jp304932g. Epub 2012 Jul 31. PMID: 22780445; PMCID: PMC3704210.
- Kong K, Kendall C, Stone N, Notingher I. Raman spectroscopy for medical diagnostics--From in-vitro biofluid assays to in-vivo cancer detection. Adv Drug Deliv Rev. 2015 Jul 15;89:121-34. doi: 10.1016/j.addr.2015.03.009. Epub 2015 Mar 22. PMID: 25809988.
- Koo TW, Berger AJ, Itzkan I, Horowitz G, Feld MS. Reagentless blood analysis by near-infrared Raman spectroscopy. Diabetes Technol Ther. 1999 Summer;1(2):153-7. doi: 10.1089/152091599317369. PMID: 11475287.
- Kyle C. Doty, Igor K. Lednev,(2018) Raman spectroscopy for forensic purposes: Recent applications for serology and gunshot residue analysis, TrAC Trends in Analytical Chemistry, Volume 103, Pages 215-222, ISSN 0165-9936,
- Makers. National Academy Press, Washington, DC, 1998.
- Martz, R. M. and Lasswell, L. D. Identification of smokeless powders and their residues by capillary column gas chromatography/mass spectrometry. In: Proceedings of the International Symposium on the Analysis and Detection of Explosives. U.S. Government Printing Office, Washington, DC, 1983, pp. 245-254.
- Massonnet, G., Buzzini, P., Monard, F., Jochem, G., Fido, L., Bell, S., Stauber, M., Coyle, T., Roux, C., Hemmings, J., Leijenhorst, H., Van

Zanten, Z., Wiggins, K., Smith, C., Chabli, S., Sauneuf, T., Rosengarten, A., Meile, C.,

- McLaughlin G, Sikirzhytski V, Lednev IK. Circumventing substrate interference in the Raman spectroscopic identification of blood stains. Forensic Sci Int. 2013 Sep 10;231(1-3):157-66. doi: 10.1016/j.forsciint.2013.04.033. Epub 2013 May 25. PMID: 23890631.
- Ketterer, S., and Blumer, A. (2012) Raman spectroscopy and microspectrophotometry of reactive dyes on cotton fibres: Analysis and detection limits. Forens. Sci. Int. 222: 200–207.
- McCord, B. and Bender, E. C. Chromatography of explosives. In: Forensic Investigation of Explosives. A. Beveridge, ed. Taylor and Francis, London, 1998, pp. 231-265.
- Meyer, R. Explosives. 3rd rev., Weinheim, New York, 1987.
- Miller, J.V. and Bartick, E.G. (2001) Forensic analysis of single fibers by raman spectroscopy. Appl. Spectros. 55: 1729–1732.
- Miyauchi, H., Kumihashi, M., and Shibayama, T. The contribution of trace elements from smokeless powder to post-firing residues, Journal of Forensic Sciences (1998) 43:90-96.
- Muehlethaler, C., Leona, M. and Lombardi, J. (2015). Review of Surface-Enhanced Raman Scattering Applications in Forensic Science. Analytical Chemistry, 88(1), pp.152-169. https://pubs.acs.org/doi/abs/10.1021/acs.analchem.5b04131
- Morrison J, Watts G, Hobbs G, Dawnay N. Field-based detection of biological samples for forensic analysis: Established techniques, novel tools, and future innovations. Forensic Sci Int. 2018 Apr;285:147-160. doi: 10.1016/j.forsciint.2018.02.002. Epub 2018 Feb 21. PMID: 29518713.

- National Research Council, Committee on Smokeless and Black Powder. Black and Smokeless Powders: Technologies for Finding Bombs and the Bomb
- Northrop, D. M., Martire, D. E., and MacCrehan, W. A. Separation and identification of organic gunshot and explosive constituents by micellar electrokinetic capillary electrophoresis, Analytical Chemistry (1991) 63: 1038-1042.
- Puntharod R, Webster GT, Asghari-Khiavi M, Bambery KR, Safinejad F, Rivadehi S, Langford SJ, Haller KJ, Wood BR. Supramolecular interactions playing an integral role in the near-infrared Raman "excitonic" enhancement observed in β-hematin (malaria pigment) and other related heme derivatives. J Phys Chem B. 2010 Sep 23;114(37):12104-15. doi: 10.1021/jp102307s. PMID: 20804182.
- Radford Army Ammunition Plant. Processing Manual. Radford, Virginia, 1987.
- Saferstein, R. Criminalistics: An Introduction to Forensic Science. 6th ed., Prentice Hall, Upper Saddle River, New Jersey, 1998.
- Scott, L. Pipe and Fire Bomb Designs: A Guide for Police Bomb Technicians. Paladin Press, Boulder, Colorado, 1994.
- Selavka, C. M., Strobel, R. A., and Tontarski, R. E. Systematic identification of smokeless powders, an update. In: Proceedings of the Third Symposium on the Analysis and Detection of Explosives. Berghausen, Fraunhofer Institute fur Chemische Technologie, 1989, Chapter 3, pp. 1-27.
- Shaine ML , Premasiri WR , Ingraham HM , Andino R , Lemler P , Brodeur AN , Ziegler LD . Surface enhanced Raman scattering for robust, sensitive detection and confirmatory identification of dried bloodstains.

Analyst. 2020 Sep 14;145(18):6097-6110. doi: 10.1039/d0an01132k. PMID: 32840504.

- Sikirzhytski V, Virkler K, Lednev IK. Discriminant analysis of Raman spectra for body fluid identification for forensic purposes. Sensors (Basel). 2010;10(4):2869-84. doi: 10.3390/s100402869. Epub 2010 Mar 29. PMID: 22319277; PMCID: PMC3274205.
- Sikirzhytskaya A, Sikirzhytski V, McLaughlin G, Lednev IK. Forensic identification of blood in the presence of contaminations using Raman microspectroscopy coupled with advanced statistics: effect of sand, dust, and soil. J Forensic Sci. 2013 Sep;58(5):1141-8. doi: 10.1111/1556-4029.12248. Epub 2013 Jul 30. PMID: 23898809.
- Smith, K. D., McCord, B. R., MacCrehan, W. A., Mount, K., and Rowe, W. F. Detection of smokeless powder residue on pipe bombs by micellar electrokinetic chromatography, Journal of Forensic Sciences (1999) 44:789-794.
- Stoffel, J. Explosives and Homemade Bombs. Charles C. Thomas, Springfield, Illinois, 1972.
- Suzuki EM, Carrabba M. In situ identification and analysis of automotive paint pigments using line segment excitation Raman spectroscopy: I. Inorganic topcoat pigments. J Forensic Sci. 2001 Sep;46(5):1053-69. PMID: 11569543.
- Suzuki EM, Buzzing P. Applications of Raman spectroscopy in forensic science. II: Analysis considerations, spectral interpretation, and examination of evidence. Forensic Sci Rev. 2018 Jul;30(2):137-169. PMID: 30064967.
- Tobe SS, Watson N, Daéid NN. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular-weight

DNA. J Forensic Sci. 2007 Jan;52(1):102-9. doi: 10.1111/j.1556-4029.2006.00324.x. PMID: 17209919.

- Virkler K, Lednev IK. Raman spectroscopy offers great potential for the nondestructive confirmatory identification of body fluids. Forensic Sci Int. 2008 Oct 25;181(1-3):e1-5. doi: 10.1016/j.forsciint.2008.08.004. Epub 2008 Sep 24. PMID: 18818034.
- Wissinger, C. and McCord, B. R. A reversed phase HPLC procedure for smokeless powder comparison, Journal of Forensic Sciences (2002) 47:168-174.
- Yinon, J. and Zitrin, S. Modern Methods and Applications in Analysis of Explosives. John Wiley, Chichester, United Kingdom, 1993.
- Zitrin, S. Analysis of explosives by infrared spectrometry and mass spectrometry. In: Forensic Investigation of Explosives. A. Beveridge, ed. Taylor and Francis, London, 1998, pp. 267-314.