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CERTIFICAN:

Que el trabajo descrito en la presente memoria, titulado "**INVESTIGATING CURRENT CHALLENGES IN FORENSIC INK ANALYSIS BY RAMAN SPECTROSCOPY**" ha sido realizado bajo su dirección por D. André Filipe dos Ramos Martins Braz en el Área de Química Analítica del Departamento de Química Analítica, Química Física e Ingeniería Química de esta Universidad. Asimismo, autorizan su presentación para que sea defendido como Tesis Doctoral.

Y para que conste y surta los efectos oportunos, firman el presente en Alcalá de Henares a 28 de Noviembre de 2014.

Carmen García Ruiz　　　　María López-López

Universidad de Alcalá

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MINISTERIO DEL INTERIOR

ENRIQUE SANZ DELGADO, **Profesor Contratado Doctor de Derecho Penal y Director del IUICP,**

CERTIFICA:

Que el trabajo descrito en la presente memoria de tesis, titulado "**INVESTIGATING CURRENT CHALLENGES IN FORENSIC INK ANALYSIS BY RAMAN SPECTROSCOPY**" ha sido realizado en este Instituto Universitario por D. André Filipe dos Ramos Martins Braz bajo la dirección de las Dras. Carmen García Ruiz, Profesora Titular de la Universidad de Alcalá y María López-López, Personal Investigador Doctor de la Universidad de Alcalá.

Dicha tesis reúne los requisitos necesarios para su presentación y defensa.

Y para que conste y surta los efectos oportunos, firma el presente en Alcalá de Henares a 28 de Noviembre de 2014.

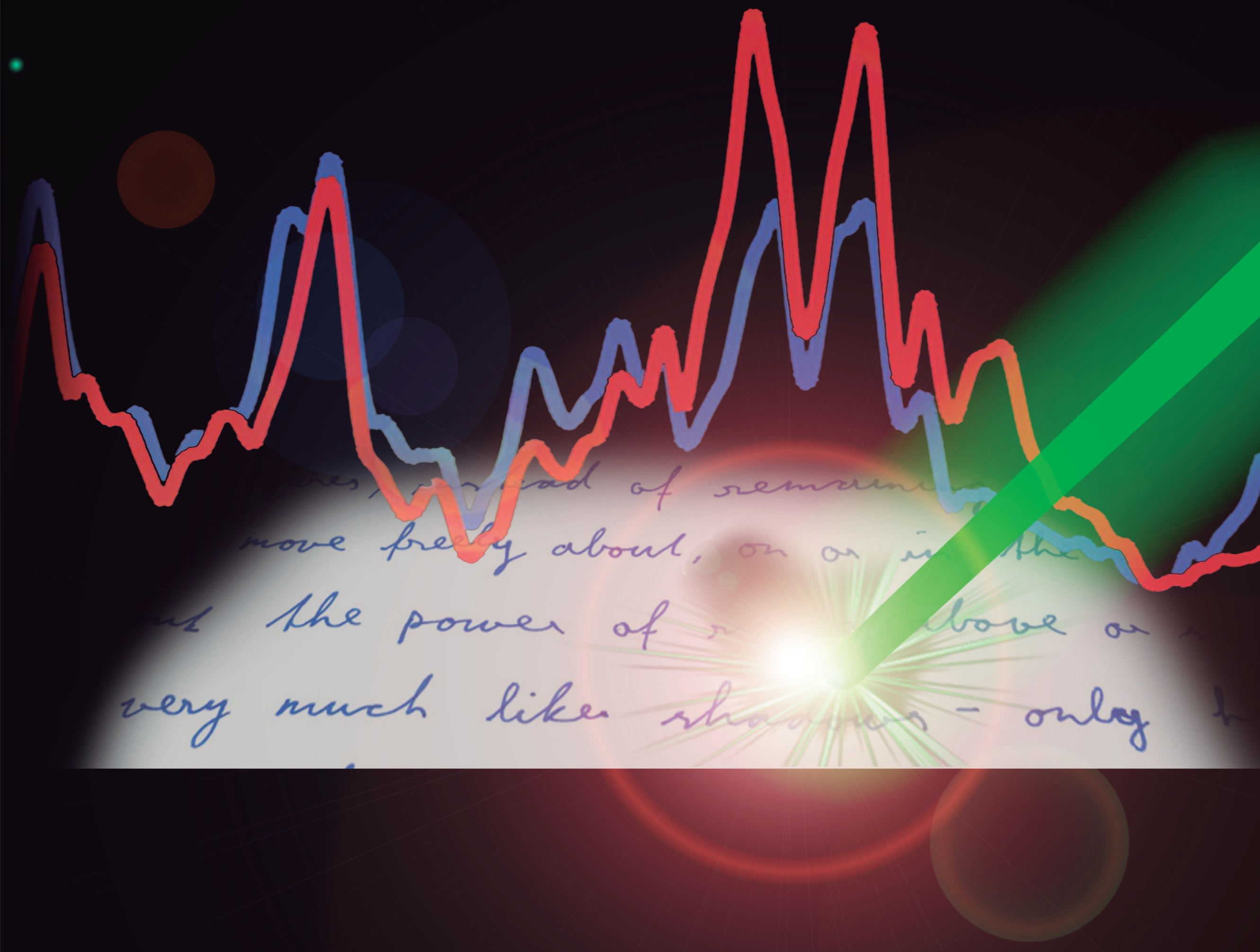
Enrique Sanz Delgado

UNIVERSIDAD DE ALCALÁ, PATRIMONIO DE LA HUMANIDAD

INVESTIGATING CURRENT CHALLENGES IN FORENSIC INK ANALYSIS BY RAMAN SPECTROSCOPY

ANDRÉ F.R.M. BRAZ

2014



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Summary

The methods for committing forgery and document alteration have become increasingly sophisticated. Scientific and technological advances have been playing an important role in Questioned Document Examinations but many challenges still remain, especially regarding the analysis of ink evidence. Thus, this Thesis develops scientific methodologies aiming to solve some current challenges in forensic ink analysis using Raman spectroscopy that can increase the fundamental knowledge regarding inks on paper and contribute to the improvement of ink examinations.

Chapter 1 contextualizes forensic ink analysis in the Forensic Science discipline and discusses the scientific and instrumental aspects of Raman spectroscopy that have furnished this vibrational technique with the great potential over other techniques for discriminating inks and identifying their possible source. The literature reviewed in this chapter showed that the main advantages of Raman include the chemical characterization of inks with little or no contributions from the paper, the analyses are fast and performed directly on the document and non-destructively, and the results obtained are robust and reproducible. Nonetheless, some limitations still exist, such as fluorescence interferences and low signals. Surface-Enhanced Raman spectroscopy (SERS) can be used to eliminate fluorescence and

simultaneously increase the signal's intensity through an intrusive sample pre-treatment. Additionally, the development of Raman techniques such as confocal Raman microscopy and Raman Imaging were considered prospective approaches to the future. This chapter also identifies the specific challenges that generally complicate ink examinations, such as i) the exact ink's chemical composition is proprietary and is usually kept secret; ii) the variety of ink formulations is remarkably large, as well as iii) of writing instruments and printing devices; iv) most non-destructive examinations used in routine casework provide limited information while more informative examinations involve damaging procedures which are not always allowed or possible to perform; v) many laboratories do not have available sophisticated instrumentation; vi) some basic premises and techniques are challenged in court due to lack of testing and validation. According to the gaps described in this chapter, three research goals are defined: to investigate pen inks, printing inks and intersecting ink lines.

Chapter 2 investigates the inter and intra brand, model and batch variability in the Raman spectral signature among blue pen inks found in the Spanish ink market in order to help forensic document examiners during the interpretation process. Chemical characterization of each pen ink was provided by identification of the main colorant. Principal Component Analysis (PCA) was used to visualize the structure and the associations in the data. Further discrimination of inks was based on small spectral differences. Results showed that most oil-based pen samples have very similar Raman signatures characteristic of the Crystal Violet dye and are difficult to discriminate with Raman. The Pilot pen samples were the only ones using the Victoria Pure Blue BO, which is a clear discriminant feature. The gel and liquid-based pen samples use several different colorants such as Rhodamine B, Copper Phthalocyanine, Ethyl Violet and Victoria Blue B, but no relation was found among their brands. Interestingly, the Victoria Blue B dye was only found in Pilot pen samples. Additionally, a large variability between and within the several models of gel-based Pilot pen samples was observed. Raman spectroscopy was also able to discriminate between samples of different batches of Bic pen inks but not within the same batch. Moreover, the signature pattern observed between batches suggested frequent changes in their chemical formula over the years.

Chapter 3 investigates the potential and limitations of a Raman spectrometer purposely designed for document examinations for the difficult task of discriminating between blue and black printed lines made by inkjet models of the same manufacturer of printers. Raman spectra of both ink lines showed low signals for most of the samples, especially black lines, due to fluorescence. SERS was performed to eliminate fluorescence interferences and the surface treatment, which consisted in the addition of a silver colloid prior to the aggregating agent (poly-l-lysine), resulted the less invasive treatment method. Results showed that SERS removed most fluorescent contributions and also improved the intensity of the bands for all blue and most black ink samples. However, the resulting profile was very similar to all samples possibly due to the enhancement of the signal of a common component. Notwithstanding, closer examination revealed minor spectral differences that allowed discriminating the Deskjet models from the professional models. Therefore, in spite of not being able to focus and analyse pure ink dots due to limitations with magnification, the Raman equipment used has the potential for fast and initial classification of printed ink lines and further discrimination can be accomplished using SERS.

Chapter 4 investigates how some variables such as the type of ink and printer, different papers, writing pressure and also time separating the application of inks can affect the distribution of two inks in an intersection, for a better understanding of the nature of crossing ink lines. Since determining the sequence of intersecting ink lines is frequently requested in many forensic laboratories and the current methods of examinations are unable to give a solution to many caseworks, this chapter also proposes two methods for determining the correct sequence of lines in a crossing. The first method was based on the microscopic examination of the cross-section of overlapping painted layers of blue and red inks. Results with pen/pen layers showed that liquid-liquid and gel-gel intersections tended to form a double layer but oil-oil intersections usually formed mixtures. Additionally, oil-liquid and oil-gel intersections tended to form a double layer whenever the oil ink was on top and liquid-gel intersections tended to form a double layer for almost all cases with the exception of those involving a particular gel pen ink from one manufacturer. Results from pen/printer layers showed the formation of a double layer only when the printer ink was on top of the pen ink, due to the printing process, while on the opposite crossing, the pen ink tended to penetrate through the printer ink producing the mixture of both inks. The time separating the application of layers was especially determinant when gel pen inks were involved, which is related to the drying of the first ink layer. Preliminary experiments with different papers and writing pressures showed no significant influence in the inks distribution. This methodology showed reproducible with overlapping layers but with real intersections, such as superimposed ink lines, illumination and focusing difficulties were encountered which led to a low rate of correct determinations in blind testing. The main limitations of this method are the fact that is destructive, depends on the cutting method, interpretation is visual and it can be influenced by light and focusing parameters. The second method was based on Raman Imaging to obtain fast chemical characterisation of the surface of crossings and identify the predominant ink line in a crossing between blue pens. Different pen ink types, different times separating the application of the two ink lines and different paper substrates were investigated. Results showed a partial skipping of the ink line applied last in most cases, illustrated by a net-like pattern, and even the complete skipping of one particular liquid-based pen ink. It was suggested that this occurred due to physical impediments (the first ink applied acted as a physical barrier) or chemical impediments (the two inks did not mix). That same pattern was also observed in the separate ink lines made with ballpoint pens, which was related to the controlled amount of ink applied by these pen's mechanism, and seemed to influence visualizing and determining the correct order of application of inks. Additionally, some mixing between inks occurred in all crossings but it was more accentuated when two inks were applied at shorter times than with longer times, suggesting that the drying time of the first ink is an important variable. The white and certificate papers used did not influence in the distribution of inks. Overall, the surface of the crossing area showed a non-uniform distribution of inks which highlights the need to use methodologies that measure representative areas for correct interpretations. Therefore, Raman Imaging is a very useful technique for fast and non-destructive chemical characterization of the entire surface of the crossing but the methodology used to interpret the sequence of ink lines still needs further development for obtaining results that are independent of the analyst interpretation.

Resumen

Los métodos para cometer falsificación y alteración de documentos son cada vez más sofisticados. Por ello, los avances científicos y tecnológicos juegan un papel importante en el examen forense de documentos pero hay muchos problemas aún por resolver, especialmente aquellos relacionados con el análisis forense de tintas. En los estudios descritos en esta tesis se desarrollan metodologías científicas para resolver los problemas actuales en el campo de análisis forense de tintas utilizando espectroscopia Raman, incrementando, de este modo, el conocimiento sobre las tintas en el papel y contribuyendo a una mejoría en el análisis forense de tintas.

El Capítulo 1 define el papel del análisis forense de tintas dentro de las Ciencias Forenses y describe los aspectos científicos e instrumentales de la espectroscopia Raman. Estas características otorgan a esta técnica vibracional un gran potencial para discriminar las tintas e identificar su posible origen en comparación con otras técnicas de análisis. La revisión bibliográfica detallada en este capítulo mostró que las mayores ventajas de la espectroscopia Raman en el análisis de documentos incluyen la posibilidad de caracterizar químicamente las tintas con poca o ninguna contribución por parte del papel, realización de análisis rápidos directamente sobre el documento de forma no destructiva, y una obtención de resultados robustos y reproducibles. Sin embargo, también presenta sus limitaciones como son la fluorescencia o la obtención de una señal débil. La espectroscopia Raman mejorada en superficie (SERS) se puede utilizar para eliminar la fluorescencia e incrementar la intensidad de la señal mediante un pretratamiento invasivo de la muestra. Además, el desarrollo de equipos de Raman confocal o Raman de imagen se consideran perspectivas de futuro. Este primer capítulo también identifica retos específicos que generalmente dificultan el examen de tintas, como son por ejemplo i) la composición química de las tintas es desconocida y se encuentra bajo patente; ii) una variabilidad extremadamente grande tanto en la composición de la tinta así como iii) en los instrumentos de escritura e impresión; iv) la mayoría de los exámenes no destructivos utilizados en los análisis policiales ofrecen información limitada mientras que para realizar análisis más informativos se necesitan procedimientos que dañan el documento los cuales no siempre se permiten realizar; v) muchos laboratorios no disponen de instrumentación sofisticada; vi) algunas premisas o técnicas se cuestionan durante el juicio debido a una falta de validación. Teniendo en cuenta los retos actuales descritos en este capítulo en el análisis forense de tintas, se definieron tres objetivos de investigación: investigar sobre tintas de bolígrafo, tintas de impresora y cruces de trazos.

En el Capítulo 2 se describe la variabilidad presente en los espectros Raman de bolígrafos azules procedentes del mercado español teniendo en cuenta sus marcas, modelos y lotes con la finalidad de ayudar al examinador forense de documentos en el proceso de interpretación de los resultados. La caracterización química de cada tinta de bolígrafo se realizó mediante la identificación de su colorante principal. Se utilizó el análisis de componentes principales (PCA) para visualizar la estructura y las asociaciones entre los espectros obtenidos y pequeñas diferencias espectrales sirvieron para una mayor discriminación de las tintas. Los resultados obtenidos mostraron que los bolígrafos de base de aceite tienen espectros Raman muy similares, característicos del tinte Cristal Violeta y, por ello, son difíciles de discriminar entre sí. Los bolígrafos Pilot fueron los únicos que presentaban el tinte Victoria Puro Azul BO en su composición, lo cual fue una clara característica discriminante. Aunque se observó que los bolígrafos de base gel y líquida utilizan diferentes colorantes, como por ejemplo el tinte Rodamina B, el pigmento Ftalocianina de Cobre, el tinte Violeta de Etilo y el tinte Victoria Azul B, no se encontró una relación entre los colorantes y sus marcas. Curiosamente, el tinte Victoria Azul B sólo se encontró en las tintas de los bolígrafos Pilot. Además, se observó una gran variabilidad entre y dentro de los diferentes modelos de los bolígrafos Pilot de base gel. La espectroscopia Raman también nos permitió discriminar entre bolígrafos Bic de diferentes lotes, lo que sugiere cambios en la composición de las tintas con el tiempo, pero no entre bolígrafos de un mismo lote.

En el Capítulo 3 se investiga tanto el potencial como las limitaciones de un equipo Raman especialmente diseñado para el examen de documentos, concretamente para la difícil tarea de discriminar líneas azules y negras impresas por diferentes modelos de impresoras de inyección de la misma marca. Los espectros Raman de ambas líneas mostraron una señal débil para la mayoría de las muestras; en especial para las líneas negras debido a efectos de fluorescencia. Para eliminar dichos efectos, se estudió la utilización de SERS. El método que resultó menos invasivo consistió en la aplicación de un coloide de plata antes del agente agregante (poli-L-lisina). Los resultados obtenidos mostraron que SERS eliminó gran parte de la fluorescencia y además mejoró la intensidad de las bandas para la mayoría de las muestras de tinta azules y negras. Sin embargo, los espectros SERS resultantes eran muy similares para todas las muestras, posiblemente debido al aumento de la señal de un componente en concreto presente en todas las tintas. No obstante, si se observaron pequeñas diferencias espectrales que permitieron diferenciar las tintas procedentes de los modelos Deskjet de los profesionales. Por ello, y a pesar de que el equipo no permite enfocar puntos de tinta individuales, cabe concluir que el equipo Raman utilizado permite una clasificación rápida inicial de las líneas impresas y debe considerarse una mayor discriminación mediante SERS.

Para comprender la naturaleza de los cruces de trazos, en el Capítulo 4 se investigó como algunas variables (tipo de tinta e impresora, papel, presión de escritura y tiempo de separación entre la aplicación de las tintas) pueden afectar a la distribución de dos tintas presentes en un cruce. La determinación de la secuencia de los trazos en un cruce es una pregunta ampliamente formulada a los laboratorios forenses, pero los métodos actuales no permiten dar respuesta en muchos de los casos. Por ello, este capítulo además propone dos métodos para determinar la secuencia de los trazos en un cruce. El primer método se basó en el examen microscópico de los cortes transversales de muestras realizadas aplicando dos capas superpuestas de tinta azul y roja. Los resultados obtenidos entre capas de bolígrafos mostraron que los cruces realizados con bolígrafos de base líquida-líquida o gel-gel tienden a formar una doble capa mientras que los cruces realizados entre bolígrafos de base de aceite normalmente dan lugar a mezclas. Por otro lado, los cruces realizados entre bolígrafos de base aceite-líquida o aceite-gel tienden a formar una doble capa siempre y cuando la tinta de base de aceite se aplique en segundo lugar mientras que los cruces realizados con bolígrafos de base líquida-gel tienden a formar una doble capa para la mayoría de los casos, exceptuando aquellos en los que se utilizó un bolígrafo de base de gel en concreto. Los cruces de capas entre bolígrafos e impresoras mostraron doble capa solo cuando la tinta de la impresora se aplicó en segundo lugar, mientras que cuando el bolígrafo se aplicó en segundo lugar este tiende a penetrar en la tinta de la impresora produciendo la mezcla de tintas. El tiempo entre la aplicación de las capas de tinta resultó especialmente relevante cuando se utilizaron bolígrafos de base de gel, lo cual se relaciona con el secado de la primera capa. Los experimentos preliminares con diferentes papeles y presiones de escritura no mostraron una influencia significativa en la distribución de las tintas. La metodología descrita resultó ser reproducible con capas superpuestas de tintas pero no así con cruces reales. Además, debido a problemas tanto de iluminación como de enfoque se obtuvo un bajo porcentaje de determinaciones correctas respecto al orden de aplicación cuando se hizo el ensayo a ciegas. Las mayores desventajas de este método son que es destructivo, depende del método de corte, la interpretación es visual y está sujeta a parámetros de luz y enfoque. El segundo método se basó en la utilización de un equipo Raman de imagen para una caracterización química rápida de la superficie de cruces de trazos y para identificar el trazo predominante en cruces realizados con bolígrafos azules. Se utilizaron diferentes tipos de bolígrafos, tiempos de aplicación entre trazos y papeles. En la mayoría de los casos, el segundo trazo aplicado cubrió soló de forma parcial la zona del cruce observándose en dicha zona un patrón con forma de red. Este efecto fue mucho más pronunciado para uno de los bolígrafos de base líquida, el cual no cubrió de tinta la zona del cruce. Este hecho puede deberse a impedimentos físicos (el trazo aplicado en primer lugar actúa como barrera física) o a impedimentos químicos (las dos tintas no se mezclan). El mismo patrón con forma de red también se observó en las líneas individuales realizadas con bolígrafos de esfera, lo que se relaciona con la cantidad de tinta aplicada por este tipo de bolígrafos y parece influenciar la visualización y determinación del orden de aplicación de los trazos. Además, se observó en todos los cruces de trazos cierta mezcla entre las tintas lo cual se acentuó cuando el tiempo de aplicación entre trazos era menor, sugiriendo que el tiempo de secado del primer trazo es una variable importante a considerar. La utilización de los papeles blanco de oficina y papel de certificado no influenció en la distribución de las tintas. En general, la superficie del área del cruce mostró una distribución no uniforme de las tintas en todos los casos lo que resalta la necesidad de utilizar metodologías que midan áreas representativas en vez de puntos concretos para dar lugar a interpretaciones correctas. Por ello, la espectroscopia Raman de imagen es una técnica no destructiva muy útil para la rápida caracterización química del área de un cruce de trazos, pero aún es necesario un mayor desarrollo de la metodología utilizada para interpretar la secuencia de aplicación de los trazos y obtener resultados independientes de la interpretación del analista.

Scope & outline of the Thesis

This Thesis emerged from the ongoing need to develop more scientifically based methodologies of investigation in Forensic Science in general, and Questioned Document Examinations in particular. Like in other fields of expertise, the methods for forensic ink analysis must be informative (i.e. add value to the forensic investigation), objective (i.e. independent of the examiner), robust (i.e. reproducible with respect to different examiners and institutions) and non-destructive of the documents evidential value. They also require to be validated under conditions reflecting those of the case under investigation and provide reliable results. Scientific and technological advances have been playing a crucial role in improving forensic investigations, but sometimes, the fundamental knowledge behind the nature of the evidence is overlooked. Therefore, the scope of the research described in this thesis was to build on the already existing knowledge and further investigate and develop the potential of Raman spectroscopy for current challenges related to forensic ink analysis. Considering this scope, this Thesis is organized into four chapters. The first chapter (chapter 1) aims to introduce the forensic ink analysis in the context of Forensic Science and Questioned Document Examinations with the objective of providing the reader a better understanding of the concepts used throughout the Thesis. Due to the fact that Raman spectroscopy is an emerging technique in forensic ink analysis, the fundamental and instrumental features of this technique are given prior to a revision of the state of the art of Raman spectroscopy in this specific area of application. After this literature revision, the several challenges concerning current forensic ink analysis are described, situating this way, the motivation of this Thesis. Consequently, the following three chapters aim at investigating pen inks, printing inks and intersecting ink lines. These chapters have been outlined according to the increasing complexity of the sample under study, the scarcity of studies published in the literature and the necessity for more advanced Raman spectroscopic approaches.

A basic approach in pen ink comparisons involves the search for differences between a questioned and a known ink sample; for this, document examiners perform physical and chemical analyses and comparisons with other ink entries in the same document or with ink reference collections. However, this approach does not consider the current ink market, which has become far more complicated (e.g. mass production of pens with similar ink formula). Considering this problematic, the second chapter of this Thesis (chapter 2) investigates the inter and intra brand, model and batch variability in the Raman spectral signature among blue pen inks with the final purpose of helping document examiners during the interpretation process of ink comparisons. To accomplish this, different ink types, brands, models and batches that were recently collected from the Spanish ink market of pens were examined with the following specific objectives: i) to identify the colorants that characterize each of the inks collected, ii) to compare the spectral similarities and differences in the chemical signature of the pen inks for establishing possible patterns and associations in relation to the type of ink, brand or model, and iii) to evaluate the potential of Raman spectroscopy to discriminate pen inks originating from different batches of the same model of pens as well as pens from the same batch.

An important forensic issue in document's examinations is the discrimination of blue and black printed lines produced by different models of the same brand of inkjet printers. Given the fact that this is also an issue for document examiners from the General Department for Forensic Science Police in Madrid, the third chapter of this Thesis (chapter 3) investigates the ability of their Raman spectrometer for discriminating printing inkjet inks directly on paper. More specifically, the objective of the research described in this chapter was to evaluate the potential and limitations of this simple, relatively inexpensive and purposely-designed equipment for discriminating a group of samples that originate from very similar cartridges of printing inks by conventional Raman and also by the chemically more advanced SERS technique. Since SERS requires an intrusive approach, this method was investigated further in order to obtain a less invasive procedure while maintaining the abilities of reducing the overwhelming fluorescence and enhancing the Raman signals.

Finally, the determination of the order of intersecting ink lines is an extremely complicated issue that is frequently required in forensic examinations to which the current methods of examination seem to give answer only to a limited number of cases. According to some research studies from other fields of application, the distribution of one ink in paper is a complex process that is affected by many variables. Thus, it is expected that the distribution of two inks applied on top of each other in paper will be an even more complex process. Understanding this process could provide the forensic document examiner more profound insights that could help developing better approaches to determine the correct sequence of intersecting ink lines. Nevertheless, this has been an understudied subject. For this reason, the research described in the last chapter of this Thesis (chapter 4) has two specific objectives. The first one was to build a more comprehensive understanding on how two inks distribute at the crossing, which involved investigating the several variables affecting the distribution of inks. The second was to develop innovative methodologies to determine the correct sequence of intersecting ink lines more effectively but also more objectively and whenever possible, non-destructively. To accomplish these objectives, the first study consisted in visualizing the cross-sections of intersections by microscopy. A gross approximation to real intersecting ink lines was investigated and involved two overlapping layers of painted inks made with several pens and printing inks. The type of ink, paper and the writing pressure were a few of the variables investigated in this study prior to determine the correct sequence of intersecting ink lines. Because this was a destructive method, the second study took on a non-destructive and more sophisticated approach. It consisted on analyzing the surface of the crossing area by Raman Imaging and utilizing the predominance of the Raman signature in that area to determine the correct order of crossings. Crossings between blue pen inks were examined because they present the most complex real case scenarios. The type of ink, paper and time separating the application of inks were the variables investigated in this study.

CHAPTER 1

Introduction

Part of this chapter has been published in A. Braz, M. López-López, C. García Ruiz, Raman spectroscopy for forensic analysis of inks in questioned documents, Forensic Sci. Int. 232 (2013) 206-212, http://dx.doi.org/10.1016/j.forsciint.2013.07.017

Forensic Science, Questioned Document Examinations and forensic ink analysis

Forensic Science is commonly referred as the field of science that uses the scientific principles and methodology to help solving criminal investigations and address legal issues. Similarly to the other sciences, such as Chemistry, Physics, Biology or Mathematics, it provides our best estimate of how the world works but the process involved in the scientific method is not quite the same. The natural sciences create models of the natural world that can be verified experimentally. This begins with observing the world followed by the formulation of hypotheses; falsification must then be tested and finally, it can only be established with a theory if they can be repeated. Over time, the scientific theories can survive to become scientific principles [1]. On the other hand, Forensic Science usually investigates past events which cannot be observed or replicated. At most, when trying to replicate the past with controlled experiments, the forensic scientist can only know whether certain events are possible or not to occur and how they can lead to specific observable findings under a set of defined circumstances; explanatory connections then replace scientific principles, and as such, it should not be modelled nor regulated in the same way as the other sciences. Furthermore, prior information from other sources is usually necessary in order to focus the laboratory investigation with specific experiments. The forensic scientist should assess the strengths and limitations of the evidence found while remaining ideologically separate and impartial. Once the information obtained is sufficient to make assessments, the forensic scientist can offer opinions in the form of a report but only to a reasonable degree of scientific certainty, keeping in mind the limitations of science in the context under investigation. Ultimately, their report will help answering the investigative questions like "what happened", "how it happened" and "who did it" which will then be used in court [2]. The nature of Forensic Science is as scientific as it is legal and social [3]. It is legal in nature because it must face the challenges in the legal arena, regarding the rules (of admissibility), the role that is supposed to play in the legal process, and the expectations created by the various participants like the judge, the prosecution, the defence and the jury. It is also the only science that plays a role of responsibility in the process of determining a person's guilt or innocence. Thus, Forensic Science is also social in nature because it emerges from society concerns. It often depends on the qualities of the forensic scientist and the criticism of other scientists and the public. In sum, Forensic Science applies the scientific methods that help forensic scientists develop reasoned opinions towards explanatory patterns emerging from evidences that, in spite of possibly being correct observations, they may not be proved conclusively true in court [4]. Figure 1.1 illustrates the overall phases of the investigation process and the roles that a forensic scientist may play in each phase.

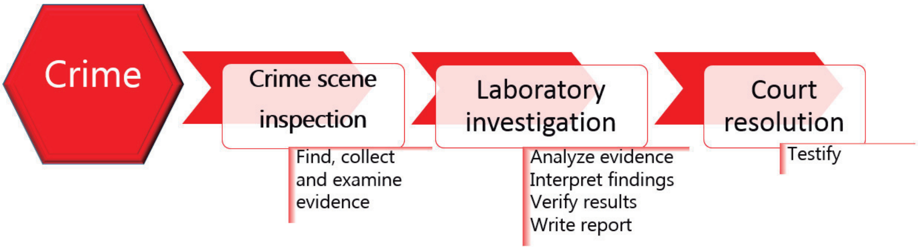
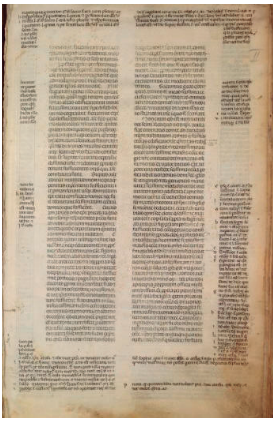


Figure 1.1. Schematic of a typical investigation process and the possible role of the forensic scientist.

The scientific and technological advances of our era, for example, the DNA typing and the fingerprint identification system, have certainly propelled considerable changes in the way Forensic Science has been applied to contemporary criminal investigations. Forensic Science evolved towards greater use of scientific approaches and techniques that not only permit to identify offenders but can also be used in routine cases and less serious crimes. It has grown into a multidisciplinary and independent scientific discipline that is developing to provide more objective and reliable results to court.

The forensic investigation of documents, or Questioned Document Examinations as it is most commonly referred to, is one of the earlier branches of Forensic Science and has been around since forgery was first practised and writing of documents had legal implications.

Historically speaking, references to forgery of documents date back to the ancient Egyptians and the Chinese [5] but it was only after the third century B.C. that protocols for examining documents and detecting forgery were first established by the Romans. Figure 1.2 shows part of Justinian's Constitution and specific translated excerpts concerning forgery.



Eightieth New Constitution, Chapter VII. Concerning forgery: *when anyone brings a complaint of forgery, or what is called false writing, the Quaestor [complaint] shall, under all circumstances, make an investigation.*

Constitution LXXVII. Concerning the penalty for forgery: *A forger who is guilty of the greatest degree of this crime shall be punished with death.*

Figure 1.2. Roman Emperor's Justinian Authentic or New Constitution (285 B.C.) in the left, and translated excerpts concerning forgery in the right [6].

From the sixteenth century on, with the increase of literacy in England, forgery was more frequent and in 1562 it became statutory, i.e., described by law and punished by a court [7].

Autodidactic and experienced-based professionals have set the empirical foundations for what has been a technical discipline for most part of its existence. Today, these professionals are generally referred to as forensic or questioned document examiners, and investigate all aspects related to a document [8]. A "questioned" document can be any written document whose source or authenticity is doubtful. Forensic document examiners handle different types of questioned documents, e.g. letters, checks, identity documents, contracts, wills, insurance claims, petitions, threatening letters, suicide notes, lottery tickets [9]. Documents have grown in value, number and complexity, due to the different writing instruments, the variety of papers available, the existence of printing technologies and the many uses that we give them. Consequently, the means for committing forgery have also increased, both in number and in skill. Hence, forensic document examiners recognize the need for more scientifically based approaches and comprehensive examinations of the several aspects contained in documents. Nowadays, these examinations cover a wide number of analyses, including handwriting and signature comparisons, detection of alterations, erasures, and possible inconsistencies as well as restoration of indented, obliterated, charred or torn documents, analysis of evidence produced by manual (e.g. stamps), mechanical (e.g. typewriters and cheque writers) and electronic (e.g. copier and printers) devices, the detection of counterfeits (e.g. currencies and identification documents), the analysis of inks and papers, and also determination of possible age of documents [9]; moreover, the linguistic content of the text can also be investigated but this is already a separate branch, also known as Forensic Linguistics.

Inks have been present for a great part of mankind's existence due to the perpetual need for painting, writing and more recently printing. They have influenced and revolutionised history in many ways, especially in the spread of knowledge. The history of inks is long and has been extensively described elsewhere [10]. In spite of the technological revolution of recent decades that changed our modern society, the use of ink and paper did not seem to lose much ground to the computerized and digital devices. In fact, how would we know the ingredients in the food, cosmetics or pharmaceutic packages without printed ink labels? Today, inks are divided into two classes: printing inks and writing inks. According to the Ink World Magazine [11], the ink business is an industry that involves billions worldwide and is evolving progressively, especially the printing sectors. The major end uses are advertising and packaging. There are also growth opportunities in research fields related to conductive inks, inkjet and energy curable inks. Therefore, inks and forensic ink analysis of documents should continue to be necessary for many years to come.

Inks are complex mixtures of chemical ingredients, such as dyes and pigments that provide colour, water and organic solvents that serve as a vehicle, resins that sustain the right viscosity and adhering properties, and also other agents like antioxidants, preservatives and trace elements. These are combined in a specific formulation which gathers the desired characteristics that serve the purpose of colouring [12]. The applications are countless and they determine the ingredients and formulation used. Table 1.1 illustrates four typical formulas for various usual applications [13].

Table 1.1. Typical formulations for printing and writing inks (values in percent) [13].

Fast-drying labels		Ballpoint pen	
Pigment Red 57	5.0	Methyl Violet 2B Base	2.6
Pigment Red 53	7.0	GG (Dye materials)	9.1
Chlorinated rubber	12.0	Victoria Blue Base	5.4
Phenolic resin	20.0	Victoria Blue Base BGO	8.6
Resinous plasticiser	4.0	A-2 Resin solution	35.9
Cioctyl Phthalante	4.0	Oleic acid	11.3
Polyethylene wax	1.0	Carbitol Solvent	12.5
Toluene	27.0	1,3 Butylene Glycol	11.6
Ethyl acetate	10.0	Polyvinylpyrrolidone K30	1.6
Aliphatic hydrocarbon	10.0	Yellow Tx	1.4
Solvent-based continuous jet ink		**Food wrapper**	
Aniline blue	3.0	Pigment Red 57	10.0
Phenol-formaldehyde polymer	6.0	Nitrocellulose	15.0
Alcohol	49.0	Maleic resin	5.0
Dimethyl formamide	41.0	Wax dispersion	2.5
Soluble electrolytes	1.0	Dioctyl Phthalate	5.0
		Ethyl acetate	59.0
		Glycol ether	3.5

The greater scientific and technological advances in Questioned Document Examinations occurred in the specialized field of forensic ink analysis, after 1960. Until then, visual examinations were preferred since they are non-destructive, simpler to perform, involved little or no manipulation of the document and could provide sufficient information without loss of the documents evidential value [14]. These examinations involved using the microscope, filters enhancing different contrast between inks and optic excitation under various light wavelengths [15]. Although these examinations are very sensitive to small quantities of ink, they only provide qualitative information and depend on the judgment of the examiner. During the same period, the development and commercialization of different writing instruments with varied ink formulations rendered these methods limited for detecting subtle differences in inks. Consequently, the development of more informative and effective tools for questioned document examinations became necessary and simultaneously, a challenge, as the methods for falsification and document alteration grew more sophisticated. In this sense, the analytical chemistry has played an important role with the application of separation and spectrometric techniques, as those described a recently published review on the analysis of questioned documents [16]. Thus, separation techniques such as Thin Layer Chromatography (TLC) [17-19], High Performance Liquid Chromatography (HPLC) [20], Capillary Electrophoresis (CE) [21, 22] and Gas Chromatography (GC) [23] have been applied for the forensic ink analysis. These techniques separate the individual chemical components of inks which can be identified or compared afterwards. In spite of their destructive nature, due to ink extractions and the sample preparation required that compromises the integrity of the document, these techniques revolutionized the forensic investigations of documents with great scientific contributions and with higher level of certainty achieved in the conclusions of ink and paper examinations [24]. Consequently, the American Society for Testing and Materials (ASTM) elaborated a standard guide with methods using these techniques for the comparison and identification of inks [25, 26]. Likewise, the technological advances in instrumentation in more recent years contributed to the potential application of spectrometric techniques for forensic ink analysis that were not suitable before. As an example, UV–vis spectrophotometry [27] and Mass spectrometry (MS) using various ionization methods have been reported in the literature [28-35]. Fourier Transform Infrared (FTIR) spectroscopy has also been reported for ink analysis [36-39]. The only problem that is still associated with this technique is that often, the strong absorption bands of the paper overlap with the inks signal. Alternatively, Raman spectroscopy has rapidly gained interest for ink analysis of questioned documents because the recent technological advances with instrumentation have allowed this technique to provide robust and reproducible results. Comparable to FTIR, it provides relevant chemical information about the ink composition. However, it has the advantages of having little or no contributions from the paper, the analyses are performed directly on the document without the need for sample preparation, it only requires small amount of ink, analysis times are fast and most importantly it is non-destructive. In spite of these advantages for Questioned Document Examinations, it is important to note that Raman spectroscopy is a technique that is still in development.

As consequence, this has been the technique of choice for this Thesis and the following section deals with the description of the fundamentals and instrumental aspects of Raman as well as the state of the art of the application of this spectroscopic technique to forensic ink analysis.

Raman spectroscopy for forensic ink analysis

Fundamental and instrumental aspects

The technological advances in Raman spectroscopy over the last two decades have put this technique in a vanguard position in the analysis of various evidential materials within the Forensic Sciences [40]. The contribution of Raman to ink examinations of questioned documents can be of great value, particularly over destructive analytical techniques. Raman spectroscopy is a vibrational technique that consists in focus samples with a monochromatic laser beam which interacts with the samples originating a scattered light. The scattered light with a different frequency than the incident one (also called inelastic scattering) is registered to construct a Raman spectrum that shows certain vibrations that are characteristic of the molecules, like a chemical fingerprint or signature, and allows compound identification [41]. This signature shows the frequency differences between the excitation radiation and the Raman scattered radiation, also called Raman shift, as a function of the intensity of the light detected. The Raman spectrum collected can represent either a wide range of functional groups of a complex molecule or a mixture of multiple components. As mentioned before, inks are complex mixtures of unknown components and for this reason, molecular identification of all specific ink components using Raman can sometimes be a hard task. Instead, spectral fingerprinting comparison is preferably used to differentiate inks in forensic contexts. Comparison involves examining the overall pattern or signature of the spectra, as well as differences in band positions (presence or absence at specific shift values), band intensities (broad or sharp) and relative intensities of adjacent bands. Comparison based on the bands absolute intensity is discouraged because it depends on a number of factors such as focus, intensity of the laser and other instrumental effects which cannot be reliably reproduced [40].

The main instrumental components of a Raman spectrometer are the laser, several mirrors and lenses, the filters, the grating and the detector. The actual configuration of these components can vary among manufacturers of commercial equipments and also with the application. Figure 1.3 illustrates a schematic of the Thermo Scientific DXR Raman microscope (Walthmam, USA), which was one of the Raman equipments used in this Thesis.

The laser sources available are in the UV (e.g. 248 and 364 nm), visible (e.g. 458, 514, 532, 633 and 685 nm), and near-IR spectral (785, 830 and 1064 nm) regions of the electromagnetic spectrum [42]. Several mirrors and lenses are placed to initially direct the laser beam towards the sample.

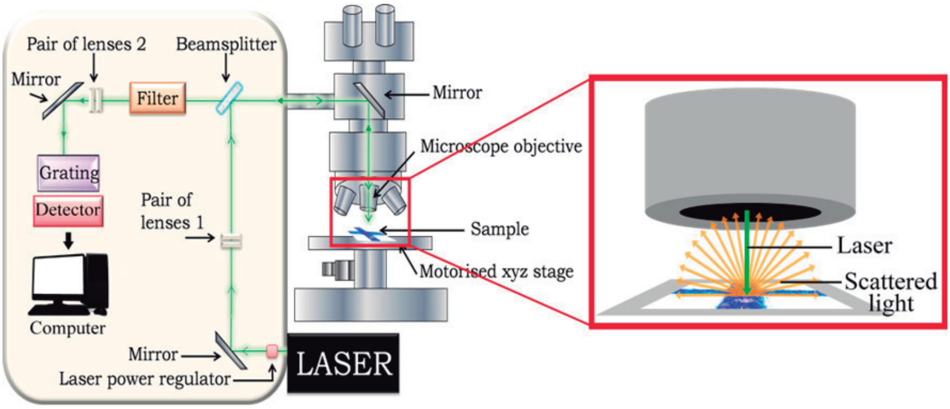


Figure 1.3. Raman spectrometer scheme. Figure modified from [42].

Many modern Raman spectrometers are equipped with a microscope with objectives of different magnification, which permits focusing and detecting specific and extremely small parts of the sample. The magnification of the objective determines the size of the laser spot incident on the sample. Furthermore, with a confocal microscope is possible to analyze the sample at different depths by moving the focal plane in the axial direction. The light scattered from the sample is reflected back to the microscope optics and is selectively filtered by either an edge or a notch filter, depending on the type of measurement and the part of the spectra to be excluded [43]. Then, an aperture in the shape of a pinhole or a slit from 10–20 µm to 50–100 µm excludes much of the undesired light that would otherwise saturate the detector. Pinhole apertures admit the least amount of light and are especially useful when measuring in the confocal mode. This light is polychromatic and must then be separated into its monochromatic components. The more efficient this process is, the better the spectral resolution obtained. This can be done with a dispersive monochromator or a Fourier transform (FT) interferometer [40]. The first consists on a grating that splits the light in various wavelengths and angles, depending on the wavelength, as schematized in Figure 1.4.

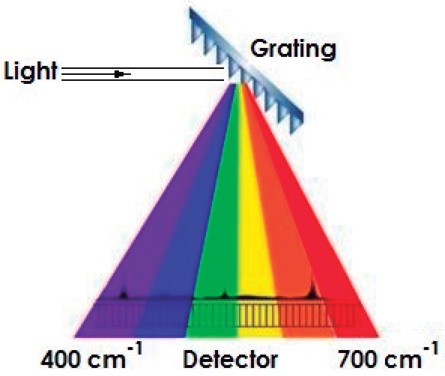


Figure 1.4. Schematic of the grating used in a dispersive monochromator. Figure modified from [44].

The second is commonly used with the near-IR laser (1064 nm), and consists of two mirrors set at right angles, one of them moves rapidly back and forth, and a beam splitter divides the light into two beams of approximately equal intensity [40]. The resulting scattered light is finally collected by a detector. Common detectors include the Charge Coupled Detector (CCD), where each element (or pixel) records a different spectral information, resulting in a multichannel advantage in the measured signal. CCDs are very sensitive to light and give low signal-to-noise ratios [45]. Additionally, another type of CCDs, the

Electron Multiplying CCD (EMCCD), has the potential for amplifying the measured signals up to a thousand times, which allows for shorter measurement times while maintaining the quality of the spectrum. This enhancement means faster measurements compared to conventional CCDs [46].

The key experimental parameters that can be optimized and altered by the operator are the type and intensity of the laser, the laser exposure time and the number of accumulations, the microscope objectives and, finally, the spectral range. The type of laser is chosen according to several factors, including sensitivity, sample degradation, fluorescence phenomena, and application [47, 48]. Laser sources in the UV region have the highest frequency and can provide the best Raman sensitivity. However, high frequencies may cause thermal degradation of some ink samples. Visible laser sources can be very useful but the paper and many inks fluoresce in the visible region originating broad signals that cover the much weaker Raman scattering. The near-IR largely avoid sample degradation and fluorescence but relatively high laser intensities must be used in order to obtain an acceptable sensitivity [40]. The intensity of the laser is given in milliwatts (mW) or percent and is directly proportional to the intensity of the Raman signal. Laser exposure times are given in seconds or milliseconds, depending on the equipment, and indicate the time that the sample is exposed to the laser. The number of accumulations refers to the amount of exposures that are combined in a single measurement. The measurement time is given by the combination of the laser exposure time and the number of accumulations. Longer measurement times usually increase the signal's intensity and the signal-to-noise ratio, but only to a limit, in which the detector becomes saturated and/or the sample suffers thermal degradation. The choice of magnifying objectives is related to the laser spot size incident on the sample. Higher magnifications give smaller laser spot sizes and allow for small specific areas to be measured. However, the intensity of the laser in a small sample area can rapidly cause sample degradation [49]. The intensity of the Raman signals is usually measured in counts per second (cps) and the Raman shift is measured in wavenumber (cm^{-1}) or its inverse equivalent, in wavelength (nm). The limits of the spectral range reached by many Raman spectrometers are from 4000 to 50 cm^{-1} and varies with the instrumental settings. However, this parameter can be adjusted to the specific spectral region of interest. Table 1.2 summarizes the experimental parameters that can be optimized and altered by the operator.

There are two main drawbacks associated with this technique: i) low sensitivity due to the naturally weak Raman scattering and ii) intense fluorescence signals exhibited by many substances, which covers the desired Raman signal [50].

Table 1.2. Summary of the experimental parameters that can be optimized and altered by the operator.

Experimental parameters	
Laser	Type
	Intensity
Measurement time	Laser exposure time
	Number of accumulations
Microscope objectives	
Spectral range	

Increasing the sensitivity can be achieved by performing Resonance Raman spectroscopy (RRS). It consists of using a laser wavelength similar to the maximum absorption of the molecules to induce an electronic resonance effect. Another technique that enhances the scattering signal and also quenches fluorescence is SERS. The signal enhancement is obtained when the sample is in contact with either a nanoscale roughened metal surface or metal colloids of gold or silver. It is caused by chemical and electromagnetic effects and depending on the excitation wavelength, one or the other effect may dominate the spectrum [51]. Ultimately, the combination of RRS and SERS techniques (i.e. Surface-Enhanced Resonance Raman spectroscopy (SERRS)) can increase the sensitivity up to ten orders of magnitude compared to conventional Raman [52].

The frequent combination of microscopy with Raman spectrometers have led to the development of imaging approaches. In Raman Imaging, the spectra of selected areas can be precisely measured using accurate positional devices. Measuring each spectrum from an entire area and gathering all the chemical information in an intensity map can be highly informative as well as time consuming. However, developments in instrumentation, particularly regarding detector systems such as the EMCCDs described before, have contributed to more rapid analysis while maintaining good quality spectra and spatial resolution. Additionally, the imaging setup usually requires a powerful software for fast visualization of the data. Thus, Raman Imaging is particularly useful for fast chemical and/or spatial characterization of complex samples [53]. The number of Raman based techniques is not limited to the ones mentioned before. In fact, many other techniques are presented in adequate literature with applications to the diverse fields of science. However, their application to ink analysis has never been described until now and will not be mentioned in this Thesis.

State of the art

The scientific developments in forensic ink analysis by Raman spectroscopy are relatively recent and started at the beginning of the last decade. Overall, forensic ink analysis aims at discriminating inks and also at identifying the possible source of the ink. The identification of the possible source of the ink is rather a class identification (e.g. writing instrument, ink manufacturer, chemical formulation, brand and model or production batch) and is usually done by comparing the questioned ink sample with an ink of known origin stored in a reference database or another ink sample present on other parts of the same document or on other documents. Another aim of forensic ink analysis is assessing the ink's age and time of application, which is also known as ink dating. However, it falls out of the scope of this dissertation and will not be addressed any further. Notwithstanding, for further reading see [54]. Most studies on Raman spectroscopy seemed to focus mainly in discriminating pens and printers while some studies also introduced interesting approaches to determine the order of two or more ink lines that intersect each other. For this reason, the next section, which has been divided in three subsections respectively deals with pen inks, printing inks and intersecting ink lines, intends to give a review of the literature published till date on the Raman spectroscopy approaches in the forensic analysis of inks.

Pen inks

Forensic laboratories routinely encounter ink evidence originated from pens during their investigations. Ballpoint and gel pens are the most common type of pens commercially available, thus it is not surprising that most of the studies on ink analysis by Raman referred to discrimination between different brands of ballpoint and gel pens written on white paper. Claybourn & Ansell [55] compared and discriminated several black inks using 514 and 782 nm laser excitation wavelengths. They observed that black inks were easily burned unless very low laser intensities were used and fluorescence was also encountered with the 514 nm laser but not with the 782 nm. Four forensic cases were also described to exemplify the potential of Raman spectroscopy for proving document alterations but also its limitations, particularly when more corroborating data is needed to reach conclusive results. Mazzella & Buzzini [56] focused on the discrimination of blue gel pen inks manufactured in different geographical regions, as a sequence of their previous work [57]. The Raman spectra of pens from the same brand and model obtained in different geographical origin showed no differences. Additionally, combining the discriminating performance obtained by the two laser excitation wavelengths (514 and 830 nm) allowed for better differentiation of gel pen inks than if they were considered separately. Savioli et al. [58] were also able to univocally discriminate black and other different colored ballpoint pen inks using the 633 and 785 nm laser excitation wavelengths. The authors further used SERS to reduce fluorescence problems and reported that the bands definition was improved as well as the quality of entire spectra. Kalantzis [59] however, focused on possible paper contributions to ink analysis in documents. The author analyzed blue and black ballpoint and gel pen inks in different white, colored and lined papers by Raman spectroscopy and SERS. Results showed that white and transparent papers have no characteristic Raman signals, but that the colored papers and their border lines have a characteristic Raman spectrum. SERS only intensified the Raman signal and enhanced some paper contributions that were not visible with conventional Raman spectroscopy. This study exemplifies that some papers have more contributions than others to the Raman spectra (especially SERS spectra), and that such contributions can interfere with the inks signal. Seifar et al. [60] tested SERRS performance on blue and black ballpoint pen inks. Most ink samples were discriminated by SERRS, except the blue inks. The authors suggest that because the cationic methyl violet derivatives suffered a resonance enhancement at 458 and 514 nm, they had preferential affinity to the silver colloid and the anionic derivatives of the dyes could not be observed. As a solution, they proposed using a laser excitation wavelength close to 700 nm to reduce this effect and improve discrimination. SERRS repeatability was good, as seen in Figure 1.5, from the consecutive spectra of the same spot (1-1, 1-2, 1-3) and at different places on the same line (1-1, 2, 3).

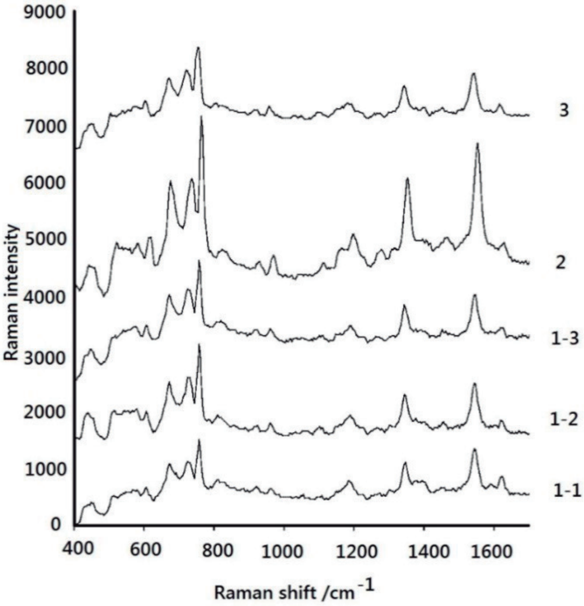


Figure 1.5. Repeatability of *in situ* SERRS (λ_{exc}= 685 nm) of blue ink line 1. 1-1, 1-2 and 1-3 corresponds to three spectra on the same spot and 2 and 3 correspond to spectra on different spots on the same line [60].

The major intensity changes observed in the spectra were attributed to the irregularities of the papers surface that affected the colloids adherence to the ink and therefore the total intensities of the bands. The authors recommended that the absolute intensity differences should not be used for discrimination purposes because there were two other factors affecting the resulting spectra: i) the time elapsed between the colloids application and measurements and ii) the constant laser incidences on the same spot that could cause burning of the ink components.

White [52] disagreed and showed that once a silver colloid is applied to the ink, the treatment becomes quite stable and that the loss of signal between the colloid application and measurements observed by [60] was probably due to the order that the reagents were being applied in the colloids application method. The author suggested that the method providing stronger SERS signals required the addition of the aggregating agent (poly-l-lysine) prior to the colloid. Additionally, this intrusive sample pre-treatment could be improved by applying a very fine mapping pen nib allowed for reagents to spread uniformly along the ink stroke instead of a drop addition performed by [60]. Andermann [61] examined ballpoint and gel pen inks of different colors by Raman spectroscopy and SERS using 514, 633, 685 and 786 nm laser excitation wavelengths. The SERS performance was overall much better than conventional Raman spectroscopy for all samples except for black ballpoint inks, because fluorescence, which was used as a criterion for discriminating these inks from the rest by Raman, was eliminated with SERS and discrimination of those inks was no longer possible. Additionally, the authors reported that adding ascorbic acid to the colloid induced the aggregation process and produced slightly better results.

Some studies have compared the performance of conventional Raman spectroscopy and SERRS to discriminate inks with other analytical techniques. For example, Morsy et al. [62] compared Raman and SERS (λ_{exc}= 685 nm, silver colloid) with filtered light examinations, microspectrophotometry, TLC and Energy-Dispersive X-ray spectroscopy (EDX) for the analysis of blue and black ballpoint pen inks. Raman differentiated one blue and one black ink sample from the rest of the inks based solely on fluorescence and SERS discriminated two other black inks when other techniques failed. Although the quality of the spectra was improved by SERS, it did not discriminate the blue inks. The authors concluded that Raman and SERS should be used as complementary with other non-destructive techniques. Kunicki [63] also agreed to the great potential of complementing Raman spectroscopy (λ_{exc}= 685 nm) with other techniques, in this case, Luminescence, Absorption spectroscopy (vis and IR) and TLC, to the differentiation of writing materials in forensic casework. The author also noticed that performance of Raman spectroscopy could be increased after baseline correction but in fact, this and other types of data manipulation can cause the loss of spectral information. For example, two pairs of pens that were differentiated before baseline correction, showed no spectral differences after baseline correction. Thus, forensic document examiners must be cautious when interpreting the results obtained by manipulated data. Zięba-Palus & Kunicki [37, 64] reported on the complementary use of FTIR and X-ray Fluorescence spectroscopy (XRF) for providing information on the chemical composition of blue and black ballpoint and gel pen inks, when Raman spectra resulted complex. FTIR provided information about the main dyes, resins and oily liquids while XRF provided the inks elemental composition. Hoehse et al. [65] combined Raman spectroscopy (λ_{exc}= 488, 633 and 785 nm) with Laser-Induced Breakdown spectroscopy (LIBS) for elemental composition and multivariate data analysis for classification. They reported that using both techniques separately did not allow for the classification or identification of some of the inks but combining the two techniques allowed for more correct classifications. Chemometric methods such as PCA, soft independent modeling of class analogy (SIMCA), partial least-squares discriminant analysis (PLS-DA) and support vector machine (SVM) were proposed for the classification and individualization of different groups of inks in questioned document examinations.

Besides written ink, other systems are also likely to be found in question documents examinations, for example the ink found in seals and stamps, which can be used alone or along with signatures. Wang et al. [66] have recently examined the dye components of red ink seals on documents with Raman spectroscopy and FTIR. They found that the

1064 nm laser was more suitable for organic dyes, while the 780 nm laser showed strong fluorescent contributions and was more suitable for inorganic dyes. Four common dyes were found to be present in all samples and based on different spectral characteristics, the ink seals were classified in four different groups and several sub-groups. Combining the information obtained by FTIR allowed for discrimination of almost all samples. Also recently, Raza & Saha [67] investigated the performance of Raman spectroscopy (λ_{exc}= 514 nm) for discriminating blue stamping inks and compared these results with High Performance-TLC (HPTLC). Besides other spectral patterns, they also used differences in the bands intensity (more than 10% variation) to discriminate and classify the samples in different groups. However, the consequence for considering such small differences is the possibility for erroneous classification. Raman was considered a better discriminating tool than HPTLC but their complementary use was valuable to group the inks based on their chemical compositions. Geiman et al. [68] investigated synthetic dyes that were deposited on microscopic slides and on TLC plates by Raman spectroscopy and SERS. For dyes deposited on microscopic slide, the 1064 nm laser provided good Raman spectra and allowed differentiating all dyes, even those with similar molecular structure, while the 633 and 785 nm lasers showed fluorescence and poor quality spectra. SERS greatly reduced fluorescence contributions and improved the spectral quality, as illustrated in Figure 1.6.

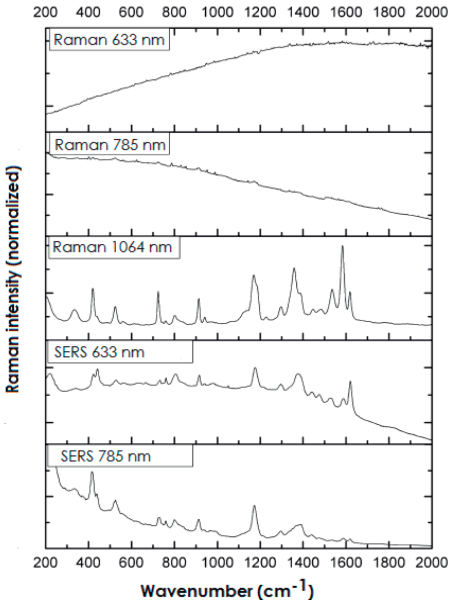


Figure 1.6. Raman and SERS spectra of methyl violet dye at different laser excitation wavelengths (633, 785 and 1064 nm) [68].

This study demonstrated the useful combination of TLC and SERS because TLC plates can be used directly as SERS substrates by applying the silver colloid after chromatographic separation.

Printing inks

Printing inks are used in almost all offices and households. The most common types are inkjet printers, that use cartridges of liquid acidic dyes of four different colors (Cyan, Magenta, Yellow and blacK - CMYK) that are combined to form the rest of the other colors and laser printers, that use toners of liquid or dry resinous ink particles (μm range) [69]. Savioli et al. [58] reported the use of Raman spectroscopy and SERS (λ_{exc}= 633 and 785 nm, silver colloid) to examine different black and colored inks printed by different inkjet and laser printers. The authors noticed no significant differences for SERS over conventional Raman for both types of printers. The Raman spectra of different brands of cartridges printed by the same inkjet printer showed different results and the same cartridge printed by different printers presented the same Raman spectra, which indicated that the printing process did not influence Raman analyses. On the contrary, small differences in the Raman spectra were observed when the same toner was printed by different laser printers, which the authors attributed to the toners deposition temperature. Rodger et al. [70] compared the Raman spectra (λ_{exc}= 514, 633 and 1064 nm) of CMYK dyes dispersed in solution and printed onto five different papers by five different inkjet printers. The spectra obtained at 1064 nm showed better quality for all dyes, mostly because fluorescence was reduced. This reduction is due to the fact that this excitation wavelength is so far from the resonance effect of the visible chromophores used in dyes and most of the fluorescent materials found in the papers. The only inconvenience was the long measurement times required (2-30 minutes) compared to the 10 seconds required with the 514 and 633 nm laser excitation wavelengths. For these, SERRS had to be used to reduce the fluorescence contributions. The authors found no differences in the Raman spectra of the samples printed by the same printer on different papers from the Raman spectra of the samples printed by different printers using the same paper. SERRS spectra showed some signal variations to which the authors attributed to the ineffective attachment of the colloid to some of the surfaces. Therefore, the printing process did not influence analyses. This was also confirmed by Udriştioiu et al. [71] in a study that compared powder and printed samples of cyan, magenta and yellow inks by different brands and models of laser printers. The Raman spectra showed great reproducibility and the minor intensity differences observed in the spectra by the powder and the printed samples were attributed to the different concentration of the toner components. Božičević et al. [72] proposed that the Raman spectra of yellow toners at 514 nm excitation wavelength was so unique that it could be used to determine whether counterfeit banknotes were printed with the same toner or at least with the printers coming from the same manufacturer. However, the same conclusions were not drawn about cyan and magenta, which leaves a major gap in printing ink examinations. Littleford et al. [73] investigated the interaction between the silver colloid with two black inkjet azo dyes with similar structures but one different sulfonic acid group in two different systems: dispersed in solution and printed on white office paper. A comparison of the SERRS spectra at different laser excitation wavelengths (λ_{exc}= 488, 514, 633 and 785 nm) from the dyes adsorbed onto silver particles in suspension and from the dyes on paper demonstrated the azo form suffered some electronic changes as the band shifted further into the red region which indicated that there were interactions with the paper surface. The identification of these changes clearly indicated the ability of SERRS to provide specific electronic and vibrational information from dyes adsorbed onto paper. Vikman & Sipi [74] also investigated the interaction between ink and paper in inkjet and laser printed samples by Raman spectroscopy (λ_{exc}= 785 nm). The Raman spectra from the inkjet samples showed strong fluorescence contributions from the colorants and the paper which was only reduced by confocal Raman measurements. Depth-profiling analysis showed that the presence of these dyes at the papers surface was rather poor, suggesting that dye-based inkjet inks do not form a separate layer on the papers surface but instead, penetrate into the paper coating. Regarding the toner samples, there was no fluorescence but contrary to what was observed with inkjet samples, depth profiling analysis of toner samples revealed that the toner formed a fairly compact layer of 8 µm, and around 12 µm, ink and paper started to mix together and at a depth of 16 µm the Raman signal detected was mainly from the paper. In Figure 1.7 it can be observed the NH and CH deformations (1591 cm^{-1} and 792 cm^{-1}, respectively) and the aromatic ring vibrations (662 cm^{-1} and 1110 cm^{-1}) of the toner pigment and also the paper contribution (1084 cm^{-1} from calcium carbonate, which is used as filler in paper).

Much like pen inks, the spectral information obtained by Raman for the analysis of printing inks sometimes require the complementary information provided by other analytical techniques to help discriminate between samples even further.

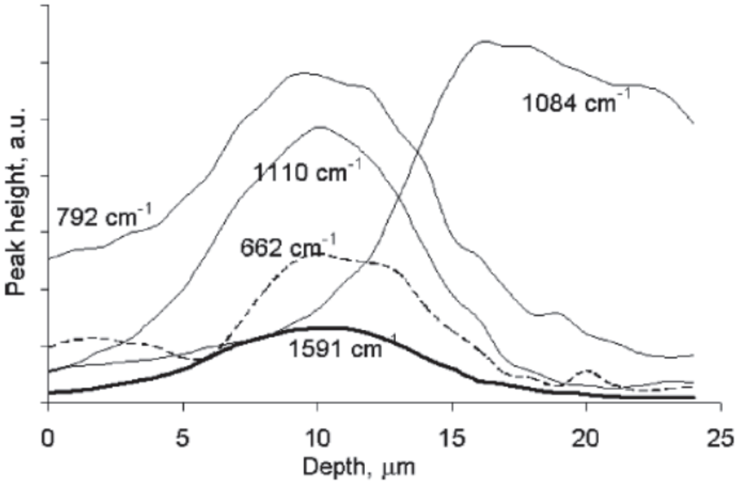


Figure 1.7. Confocal Raman spectrum of a calendered sample [74].

This was the case in the study reported by Heudt et al. [75] that combined Raman and two different ionization methods of MS, laser desorption and matrix-assisted laser desorption, to discriminate printer inks from different cartridges printed by different inkjet printers. Carbon black was identified as the main component in all black ink samples by different laser excitation wavelengths (532, 633 and 785 nm) and other minor differences observed in the spectra were unclear and considered unreliable to discriminate between manufacturers. Raman spectra of magenta and yellow inks allowed good discrimination of these samples at longer excitation wavelengths but cyan inks required the combined spectral information from various lasers. The mass spectra provided additional information about the ink additives and composition which lead to further discrimination of the samples. Another study [76] examined the authenticity of two doubtful court fee stamps by Raman (λ_{exc}= 514, 633 and 785 nm), IR, visible spectrometry, and XRF. The ink present in three different graphic elements of the two questioned court stamps was compared with the same graphic elements from two genuine stamps provided by the manufacturer. The Raman spectra obtained at 785 nm showed similarities between the inks from the doubtful stamps and the comparative stamps, which was sufficient to prove their authenticity. Some discrepancies were observed at 514 and 633 nm but since they were not repeatable, the authors attributed those to the presence of sweat or fat. Combining the information obtained by Raman spectroscopy with the one obtained by IR, visible spectrometry and XRF allowed discrimination of these inks.

Intersecting ink lines

Very often forensic document examiners are asked to determine whether or not a document has been altered at a later stage, or the signature that appears in a contract has been made in a blank page. This usually requires that areas where lines intersect are examined and determine which line was written last. While most of the intersections involve writing inks, they may also involve typewriting, computer-printed text, paper folds, and indentations [77]. In principle, two lines originating from different inks intersecting each other may show different Raman spectra. Therefore, it is simple if both inks form a distinct layer on top of each other; this way, at the point of intersection, the Raman spectrum at the surface will show more features of the ink on top and the Raman spectrum at a deeper layer will show more features of the ink underneath. However, when two inks are partially or even completely diffused in one another, forensic document examiners have a great difficulty discerning the order of the two inks. Additionally, microscopic visualization of darker colors cause the optical illusion that they are on top of lighter ones, whether this is really the case or not [55]. Fabiańska & Kunicki [78] analyzed many homogeneous and heterogeneous intersections made by different pens (ballpoint, fiber tip and roller ball) and different inkjet printers by Raman spectroscopy (λ_{exc}= 685 nm). The methodology followed for obtaining the Raman spectra of the upper layer consisted of measuring the samples with 25 % of the laser's intensity and for obtaining the Raman spectra from the bottom layer consisted of burning the upper layer with maximum laser incidences. Given the fact that the Raman spectrometer used only allowed for surface measurements, this methodology was not suitable since only half of the heterogeneous intersections and one third of the homogeneous intersections were unequivocally determined. On the other hand, Claybourn & Ansell [55] examined the intersection of two black ballpoint pens inks using a confocal Raman spectrometer that could perform surface and depth analyses. The authors assumed that the two inks formed a distinct layer on top of each other and therefore, given the greater contribution of the ink at the surface (b) and a greater contribution of another ink at a deeper level (a), then it could be concluded that ink (b) was on top of ink (a). Figure 1.8 shows the Raman spectra (λ_{exc}= 514 nm) of the two lines alone (a) and b)), the spectrum from the top surface (c)) and the spectrum at 5 μm deep at the intersection (d)).

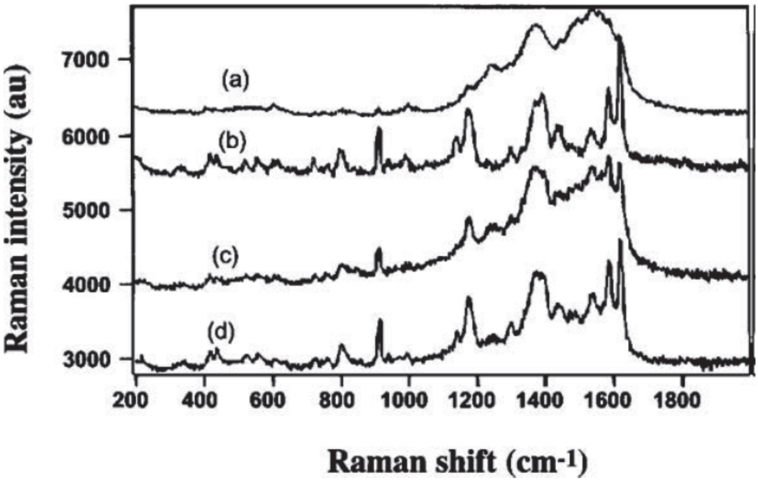


Figure 1.8. Raman spectra taken through the intersection of a crossed ink line; (a) and (b) spectra from isolated lines of the original black inks, (c) spectrum from the top surface, and (d) spectrum taken 5 microns focused into the intersection [55].

In another case study, the analysis of the intersection of two blue ballpoint pens in an insurance claim proved that the document was possibly altered at a later time. The Raman spectrum of the surface layer at 782 nm excitation wavelength was dominated by fluorescence, which differed from the Raman spectrum of the 5 μm deep layer. In spite of the data shown in this study, the authors did not mention whether the results were reproducible in different points of the intersection and whether this method also applies to other types of intersections. Raza &

Saha [67] also examined the intersection between red seals and various writing materials (pencil, gel and ballpoint pen, toner) by Raman spectroscopy. Results regarding pencil intersections showed that the spectrum characteristic of the ink at the top dominated the spectrum of the intersection, similar to the methodology followed by Claybourn & Ansell [55]. However, the intersections involving toner, gel and ballpoint pen inks did not yield any conclusive results. In the case of gel pen inks, the authors suggested two explanations: i) presence of highly Raman scattering pigment that dominated the entire spectra or ii) the low viscosity of gel inks which prompted inter-diffusion and mixing of inks. Notwithstanding, the authors did not explain the inconclusive results obtained by ballpoint pen inks and toner intersections. Gomes & Sercheli [79] investigated different intersections of blue and black ballpoint inks following a similar methodology as the studies mentioned above. Yet, every intersection was analyzed at the surface and at three different depths. The authors reported that although visual comparison of the Raman spectra suggested the correct order of the inks for most of the cases, this methodology failed in some cases. Furthermore, they measured the Raman spectra of the intersections at six different depths but compared only the variation of specific bands that were present in the spectrum of one ink but not the other. By plotting the ratio of the normalized intensities of the specific bands as a function of the depth provided allowed for a more simple graphical view of the correct order of the intersecting lines for both line permutations. However, the authors did not mention whether these results were reproducible and repeatable in different points of the same intersection, which would eliminate possible non-uniform distribution of the inks throughout the paper surface. Raman Imaging of the intersections was also reported in this study. The PCA correlation analysis between the individual Raman spectra of the two inks and the paper with the twenty thousand Raman spectra measured by a two dimensional mapping of the intersection resulted in a 2D-color map of the intersection area, where each color corresponded to the individual contributions of the inks and the paper at the intersection, as shown in Figure 1.9.

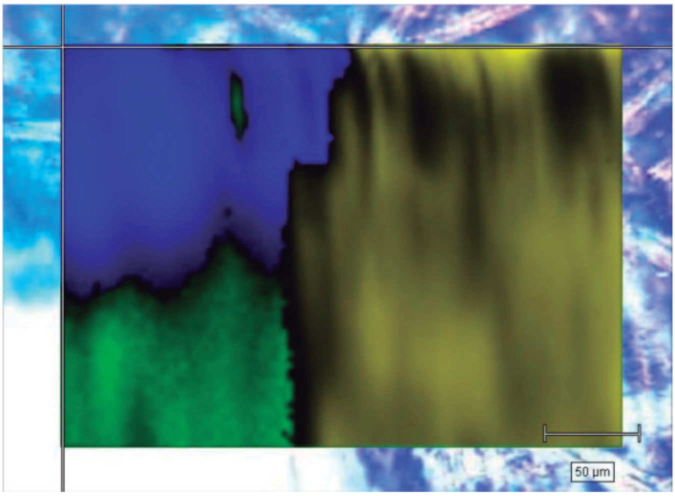


Figure 1.9. 2D color map of the PCA correlation analysis of the individual Raman spectra at the intersection. The green color represents the paper contribution and the blue and yellow colors represent the contributions by the two pen inks [79].

The application of Raman Imaging for determining the correct order of two crossing lines was also reported in a technical note by WITec [80]. However, it was not shown whether the method could be successfully applied to other intersections. Fieldhouse et al. [81] investigated another type of intersections, the ones between a pen or printer ink and a latent fingermark. The fingermarks were enhanced with appropriate development techniques and the sequence of application was examined by SERS (λ_{exc}= 685 nm, gold colloid) as well as by filtered light analysis and Electrostatic Detection Apparatus (ESDA). There were initial difficulties visualizing the intersection area and SERRS spectra of the fingermark placed on the paper before and after the ink were similar. On the other hand, the combination of ninhydrin treatment and ESDA made it possible to determine the sequence of application in some of the cases studied. The investigation of the chorological order of two ink entries becomes more difficult to determine when the inks do not intersect each other. Yet, one application note by Horiba Scientific [82] reported a methodology to determine the sequence of blue ballpoint ink signatures and black printer toner particles on documents, even if there are no intersecting lines. It consisted on localizing the dry toner microparticles that copiers and laser printers randomly spread throughout the paper and determine whether they were on top of the pen ink. Although this is a promising methodology, no further research has been published on this subject.

Challenges surrounding forensic ink analysis

There are still many challenges to overcome regarding forensic ink analysis. One is not knowing the exact ink's chemical composition, which is proprietary and usually kept secret. This complicates investigations since forensic document examiners have to determine the compositions, if needed, using their own resources. Another is the variety of possible ink formulations that is remarkably large, considering the great number of chemical substances that can be used for the manufacturing of inks, including materials from natural or synthetic sources. Also, the wide variety of writing instruments available in the market and printing devices also complicate analyses because each require those substances to be combined in a specific manner in order to satisfy the desired properties of viscosity, flow and appearance, among others. For example, ballpoint pens use a thick oil-based ink, while rollerball pens, in spite of having a similar mechanism, use a water-based ink. On the other hand, gel pens use a water-based gel ink that has an intermediate viscosity compared to the other pens. Similarly, printing inks can differ, depending on the type of printing process. For example, toner inks usually use dry pigments and resins while inkjet inks normally use liquid dyes [9, 12]. With such a great variety of ink formulations, it is difficult to identify a brand or a manufacturer from ink analysis. The collection of reference databases of inks could help in this process. The only reference databases of inks officially known are the one of the Bayern

Landeskriminalamt in Germany and the International Ink Library database of the USA Secret Service, which is known for being the largest in the world, containing a collection of nearly 10.000 ink samples that have been manufactured since 1920 [83]. However, keeping the collection updated and complete is a laborious and everlasting task that may result impractical. Moreover, the mass production of pens observed in recent years have originated batches with thousands of pens with similar ink formula. Thus, attempts to individualize a specific pen is certainly impossible without the identification of specific markers. To complicate even further the forensic analysis of inks, the constant changes inserted in the global market of inks due to economic demands caused for many pen producers to have their inks made by the same manufacturer, and as such, many pens from different brands may have similar ink formula. Also, ink manufacturers may substitute some ink chemical components with cheaper ones and sell them as the same, provided that the ink properties remain unaltered. Consequently, pens from the same brand/model may have different ink formula [17]. Similarly, printer cartridges can be disposable and refilled with ink from suppliers other than the printer manufacturer, which are often less expensive and contain different chemical formulation [84]. Therefore, it is difficult for the forensic document examiner to know the exact variations inserted in the inks composition and to follow the tendencies in the ink market. The challenges in the forensic analysis of inks are certainly not limited to the variety of ink formulas available. There are also difficulties regarding the use of methodologies and techniques. For example, most examinations used for routine casework are visual-based, offer only qualitative information and involve a subjective interpretation. Thus, the capacity to differentiate inks with very similar characteristics is limited as well as the risk for bias. This is possibly related to the fact that questioned document examinations has been, and many of the examinations still are, more based on technical approaches rather than scientific. Many forensic laboratories and courts have accepted the use of chemical analysis, provided that the information gained from these examinations result helpful to the detection of fraudulent documents and that they do not destroy the legibility of the writing. In other occasions, the amount of ink evidence is simply insufficient to carry those examinations or the forensic document examiner has no legal permission to perform invasive analysis. The ASTM standard guide for ink analysis recommends conducting non-destructive optical examinations first, but some inks, like the gel inks for example, are difficult to analyse by the recommended methods [56, 85, 86]. Consequently, many forensic laboratories are now turning to other more modern technologies such as Attenuated Total Reflectance (ATR)-FTIR, Raman spectroscopy and MS based techniques that allow an *in situ* non-destructive ink analysis [9]. Needless to say, the instrumental cost of some techniques is still high and might not be readily available in many forensic laboratories. Additionally, some techniques have limited sensitivity to detect relatively small differences in ink compositions or selectivity to isolate the paper contributions from the inks. Nonetheless, the constant technological advances allows for more laboratories to have access to that instrumentation which increase the number of possible forensic examinations that can be performed in a document. Finally, Questioned Document Examinations was one of the first sub-disciplines in Forensic Science to receive repeated challenges in court because of the little research testing and validation of some of the field's basic premises and techniques. Nowadays, the Scientific Working Group on Document Examination (SWGDOC) [87] and the European Document Experts Working Group (EDEWG) [88] work to exchange information and expertise, set quality guidelines and standards as well as to promote cooperation in research and development among law enforcement institutions and research centres. However, a great deal of work remains to be done to incorporate the scientific advances to this forensic area. Figure 1.10 resumes the major challenges in forensic ink analysis.

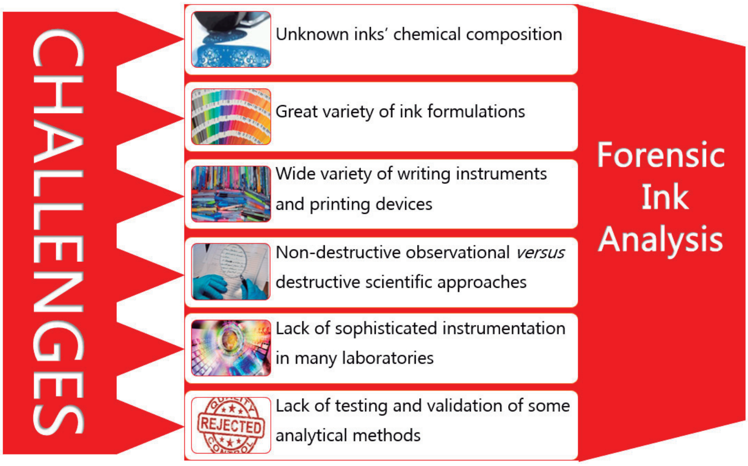


Figure 1.10. Major challenges in forensic ink analysis.

Retrospective and goals of the Thesis

In summary, the technological advances of our time contributed to a tremendous increase of usage of inks and paper and originated more sophisticated methods for perpetrating forgery and alteration of documents. Questioned Document Examinations has been a technical sub-discipline of Forensic Science for most of its existence but currently needs to embrace the scientific methods of analysis in order to obtain more informative results. The forensic ink analysis, which mostly aims at discriminating inks and identifying the possible source of the ink is one specialized field where the observational based examinations are being replaced with more scientific approaches such as Raman spectroscopy. Raman has shown in the last decade to be a very propitious technique for discrimination of pen and printing inks in questioned documents because it provides a chemical signature for written and printed ink, mostly from the inks' colorants (dyes and pigments) as they are more active than most of the other components. The analyses are fast, do not require sample preparation nor leads to destruction of the document. Several laser excitation wavelengths are available and they can provide complementary information about the inks' composition. However, some drawbacks still remain, especially the fluorescence exhibited by the paper or many inks. Although it can be a helpful characteristic that allows discrimination, most of the time it is an undesired effect that covers the relevant chemical information in the spectra. SERS seems to eliminate fluorescence and greatly increase the bands' intensity but it requires a slight alteration of the sample by the addition of a small amount of a metallic colloid. However, in this process, paper contributions can also be enhanced, a greatly unwanted effect in questioned document examinations.

Determining the order of intersection between two ink lines is yet another matter that is frequently encountered in forensic casework but in many cases, remains unsolved. The literature applying Raman spectroscopy to this matter is still very scarce and in spite of the potential of confocal Raman microscopes and Raman Imaging, only further research will assure the significance of the findings. Besides, the phenomena occurring between the two inks and the paper have been understudied by document examiners and undeniably will affect interpretation of the results obtained in their examinations.

Examinations and interpretations of ink analysis are further affected by the fact that ink compositions are unknown, modern ink formulations are complex and varied, and commercialized in a wide variety of writing instruments and printing devices. Also, the methods of examination are either non-destructive but observational or scientific but destructive of the document. Then, the scientific examinations usually require sophisticated and expensive instrumentations which may not be available in many laboratories. In addition to this, some of these methods still lack testing and validation in order to obtain full acceptance in the court of law.

Thus, considering what has been discussed in this chapter, the following chapters of this Thesis aim at investigating Raman spectroscopic approaches that can give solution to three particular aspects of forensic ink analysis: pen inks (chapter 2), printing inks (chapter 3) and intersecting ink lines (chapter 4). More specifically, the objective of chapter 2 is to investigate the variability in the Raman chemical signatures of modern blue pen inks from various brands, models and batches and identify spectral patterns that could indicate how common or characteristic the chemical compositions of pen inks really are. It is expected that the information obtained will give an understanding about the sort of variations mostly occurring, especially in respect to the type of ink, brand, model and batch, and whether they are systematically or arbitrary, for better assessments of the evidential value obtained from ink examinations.

The objective of chapter 3 is to investigate the potential and limitation of a specific Raman system available at the General Department for Forensic Science Police in Madrid to discriminate blue and black lines printed by different models of the same brand of inkjet printers, directly on paper. It is expected that the methodology and the information provided can assist forensic document examiners in better discriminating strategies of very similar printing samples.

The objective of chapter 4 is to investigate intersections of ink lines and their distribution in the paper in order to gain a more comprehensive understanding on some of the variables affecting this interaction. It is expected that this information can help with the determination of which line was written last. An additional objective is to develop methodologies that can determine that more effectively and non-destructively.

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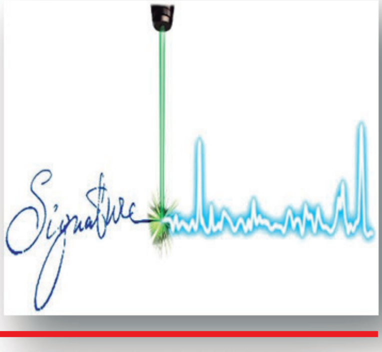
CHAPTER 2

Investigating pen inks

STUDYING THE VARIABILITY IN THE RAMAN SIGNATURE OF WRITING PEN INKS



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Abstract

This chapter investigates the inter and intra brand, model and batch variability in the Raman spectral signature among modern pen inks that will help forensic document examiners during the interpretation process. Results showed that most oil-based samples have similar Raman signatures that are characteristic of the Crystal Violet dye, independently of the brand. Exception was the Pilot samples that use Victoria Pure Blue BO instead. This small inter-brand variability makes oil-based pens difficult to discriminate by brand. On the contrary, gel and liquid-based samples use different colorants such as Rhodamine B, Copper Phthalocyanine, Ethyl Violet and Victoria Blue B. No particular pattern was observed regarding the colorants used by each brand, except the Pilot samples that were the only brand using the Victoria Blue B dye, which is a clear distinct feature. Additionally, the intra-brand variability was also large among gel-based Pilot samples. The small spectral differences observed among several batches of Bic Crystal Medium samples demonstrated that changes were introduced in their chemical formula over the years. The intra-batch variability was small and no spectral differences were observed within batches. This research demonstrates the potential of Raman spectroscopy for discriminating pens inks from different brands and models and even, batches. Additionally, the main colorants used in modern pens were also identified.

Introduction

When a certain ink entry is in question, examiners must perform physical and chemical analyses and comparisons with other entries in the same document as well as with ink libraries [1]. Their main interest is the verification that the components in the questioned ink are the same as a control ink sample, which is representative of a certain brand/model of pens [2]. However, this simplistic approach does not consider the current ink market, which has become far more complicated. These complications derive from the mass production of pens that originates batches with thousands of pens with similar ink formula, and also from the constant variations inserted in the global market by ink producers and pen manufacturers that, in compliance to market and economic demands, originate pens from different brands to have similar ink formula, and also pens from the same brand/model to have different ink formula [3]. Moreover, some companies are starting to recycle writing instruments so it will become more frequent to have mixtures of chemical compositions. The techniques on non-destructive ink examinations that are mostly published in the literature are useful to distinguish among different types of inks but do not provide individualization information to characterize the varied ink formulations. Other techniques, based on chromatography (e.g., HPLC or GC), offer great discrimination performance in the identification process of the source of a questioned ink, but they are destructive, time consuming and require cost-intensive sample preparations [4]. Even so, none of the studies published to date considered the different sources of variations present in pen inks: variation between groups and variation within a group (i.e. differences between individual pens of a single group). A study describes the statistical variations in the infrared spectra between brands and models of black pen inks [5]. The authors showed that the inter-brand/model variability was overall larger than the intra-brand/model variability but within groups varied, depending on the manufacturer. However, only three brands of pens were studied and the authors failed to report more detailed information about the pens studied. They also found that the paper substrate greatly interfered in the inks infrared spectra.

For forensic purposes, it is important to follow the market tendencies to know when and what sort of variations mostly occur and whether they are systematically or arbitrary, in order to have an indication on how common or characteristic the chemical composition of a questioned ink is, and thus its evidential value. Therefore, considering the lack of information regarding the variability in the Raman chemical signatures of modern inks, this study investigates if the Raman spectra of ink lines produced by a group of representative pen samples from various brands, models and batches allow to establish patterns and identify particular spectral differences and similarities among them for a better assessment of the evidential value of ink examinations in questioned document cases. More specifically, the variability was investigated among pens with different ink formula types (oil, liquid, gel-based and other combinations), between brands of the same ink formula type (inter-brand), between models of the same brand (intra-brand) and between and within batches of the same model (inter and intra-batches variability, respectively). Raman spectroscopy was chosen for this study because it is becoming an invaluable and increasingly frequent technique in many forensic laboratories that is non-destructive and has the ability for detecting the inks major chemical components, such as dyes, pigments and resins as well as other minor organic and inorganic components used in their manufacture. Additionally, Raman technique was preferred over infrared because paper has scarce influence in measurements [6, 7].

Experimental section

Samples

The initial sampling set consisted of 190 blue pens of 12 known brands and 38 models (Table 2.1) that were acquired from several sources (privately, on several local shops and directly from the manufacturer). Additionally to this set, 120 blue Bic Crystal pens (Societé Bic, France) of twelve different batches manufactured in 2007, 2008, 2010, 2011, 2012 and 2013 (two batches per year, ten pens per batch) were obtained for inter-batch variability studies. For each pen, one straight parallel line was drawn on the same sheet of common office paper (white, A4 80 g·kg^{-1} density).

The standard references Victoria Blue B, Solvent Blue 38, Rhodamine B, Victoria Pure Blue BO, Ethyl Violet, Victoria Blue R and Copper (II) Phthalocyanine were purchased from Sigma-Aldrich.

Table 2.1. Detailed information of the pen samples studied.

Brand and Model		Sample ID	Ink Type	#Pens
Bic	Crystal Medium	B_1	Oil	5
	Ecolutions	B_2	Oil	1
	Rondo Style	B_3	Oil	1
	Cristal Gel	B_4	Gel	1
	Atlantis Gel	B_5	Gel	1
	Atlantis	B_6	Other (Easyglide)	4
Pilot	SupergripM	P_1	Oil	5
	BPS-GP	P_2	Oil	5
	Rexgrip EF	P_3	Oil	1
	G1	P_4	Gel	3
	G2	P_5	Gel	5
	G-Tec	P_6	Gel	5
	Frixionball	P_7	Gel	2
	V5	P_8	Liq	4
	Vball	P_9	Liq	5
	Greenball	P_10	Liq	1
	Acroball	P_11	Other (Oil-Gel)	5
Papermate	Stick 2020	PM_1	Oil	2
	Gel 2020	PM_2	Gel	1
	Flexigripultra	PM_3	Other (Lubriglide)	1
	Flexgrip Elite 1.4	PM_4	Oil	1
Uniball	Jetstream	U_1	Gel	2
	Eye Fine	U_2	Liq	1
	Insight UB-211	U_3	Other (Liq-Gel- Unisuperior)	2
Inoxcrom	Medium	I_1	Oil	5
	Roller Smooth Writing	I_2	Liq	4
Staedtler	Stick 430	ST_1	Oil	5
	Ball 432	ST_2	Oil	5
	Triplus Fineliner	ST_3	Liq	1
Pelikan	Stick	PK	Oil	5
Pentel	BK 77	PE_1	Oil	1
	BK 77C Superb	PE_2	Oil	5
	Energel	PE_3	Other (Liq-Gel)	1
Unipapel	Unipen	UP	Oil	1
Milan	P1 Touch	M	Oil	1
Faber-Castell	CX 7	FC	Oil	5
Stabilo	Pointvisco	S_1	Other (Liq-Gel)	1
	Cultdocusafe	S_2	Oil	1

Instrumentation

Raman measurements were performed using a Thermo Scientific DXR Raman microscope controlled by the Omnic for dispersive Raman 8.3 software (Thermo Fisher Scientific Inc., Madison, USA). A diode-pumped solid state laser of 532 nm wavelength was used, with an intensity below 1.0 mW on sample. The microscope objective was set to 50× magnification, which resulted in an estimated spot size of 1.1 μm. Spectra were measured in the 300-2000 cm^{-1} range and a spectral resolution of 2.7-4.2 cm^{-1} was obtained. Spectral acquisitions were 1 s × 10 scans for all samples. Each sample was measured five times at different points of the same line. Cosmic spikes filter was systematically applied for all measurements as well as automatic fluorescence corrections (polynomial of order 6) to scale-remove the slowly variations of background.

Data treatment

The Raman spectra were imported into Unscrambler X 10.2 (CAMO, Norway) for preprocessing and statistical analysis. Baseline correction of each spectrum was used to remove the gradual curvature from both the sample and background that is not due to the Raman effect, followed by smoothing with the Savitzky-Golay algorithm (polynomial order 2, section size nine points) in order to reduce spectral noise and unit vector normalization. The spectral range was reduced to 400-1700 cm^{-1} in order to eliminate the non-informative parts of the spectra as well as to reduce the initial number of variables. In PCA, the data was organized in a 3D scores plot using the first three Principal Components (PCs) according to spectral similarities and differences among samples. From the PCs used, a value of explained variance is given that quantifies the variation in the data and expresses the proportion of structure found in the data by the model.

Results and discussion

A large population of pens was chosen as representative of the Spanish market of pens and the different brands and models represent sub-populations from which each ink line is an individual sample. Initially, a blank sample (paper without ink) was measured and no bands were observed, thus the paper had no influence in the inks spectra, as previously reported [6, 7]. In order to examine the spectral response along the ink lines, five different points along the same line were measured. The spectra were reproducible and only small absolute intensity variations were observed between measurements (data not shown). Therefore, the most intense spectrum that also showed the best S/N ratio out of the five measurements was considered as the characteristic Raman signature for each sample.

Raman spectroscopy is a technique that can identify many of the ink components but dyes and pigments are usually the components with the most intense Raman signal, dominating most of the spectra.

Notwithstanding, minor spectral contributions from other components can also be visible [8]. The methodology used in this study was based on spectral comparison of an informative region of the spectra, followed by the identification of the dye or pigment present through band assignation, to finally use the minor spectral differences to discriminate among samples. Although it was not possible to identify those minor components in this study since the complete ink composition is very complex and unknown, they were used to interpret and explain the variability observed.

There is a general confusion when defining and assigning a pen to an ink formula type because companies either omit what makes up their ink or advertise it differently for commercial reasons. Furthermore, manufacturers tend to combine formulations in order to obtain inks with varied properties, introducing more ink type variants in the market. For clearance, the most common forms of modern pens are ballpoint and rollerball pens (also fountain and marker pens but to a lesser extent) and each consist of a different tip or point mechanism that requires a particularly adapted type of ink for best functioning. Categorically, ink formula types can be divided in oil, liquid or gel, whether their main solvent is an oil, water or a water-based mixture with high viscosity (i.e. a gel type), respectively. Usually, ballpoint pens use oil-based inks but rollerball pens can either use liquid or gel-based inks. Consequently, the main solvent also depends on whether the ink contains a certain pigment or a dye. Although in questioned document examinations, comparison of the Raman signatures of inks with different ink types is not valuable, as inks can be distinguished by their appearance using a microscope, we considered interesting nonetheless to visualize the clustering pattern of writing inks with the same ink formula type (at least commercially advertised as so) in order to know whether they tend to group independently of their brand or exhibit another pattern. For this study, the information regarding the ink formula type labelled for each pen was obtained from each manufacturer and is presented in Table 2.1 Other ink type variations such as lubriglide or easyglide were labelled as "other" to simplify.

PCA was used to visualize the general structure of the data, since it allows the differentiation of a large group of interrelated variables that cannot be so easily observed by the human eye and has shown to be especially helpful in the forensic examination of inks by Raman spectroscopy [9, 10]. However, PCA should not be used alone since it is an unsupervised pattern recognition tool and so, spectral examination is also required or the use of more advanced chemometrics. Samples were labelled according to their brand in order to visualize the variability between brands (inter-brand variability), i.e., whether the Raman signatures of writing inks with the same brand are more similar than other brands or exhibit another pattern. Given the large amount and variety of brands and models of pen samples, it was initially assumed that differences among pens within the same model were not significant enough. Therefore, the spectra of pen samples within the same model sub-population were averaged and a single spectrum per model was used. Samples were also colour-labelled according to their ink formula type in order to simultaneously visualize the variability among ink formula types. The Pentel Energel (PE_3) and Stabilo Pointvisco (S_1) stood out as they showed no spectral information, possibly due to the strong fluorescence that masked the entire signal of the ink components. Since this would greatly influence the PCA model, those samples were excluded. The PCA scores plot of the first three PCs, which explained 79.18 % of the variance among all samples, showed a large variability among the population of pen samples used (Figure 2.1).

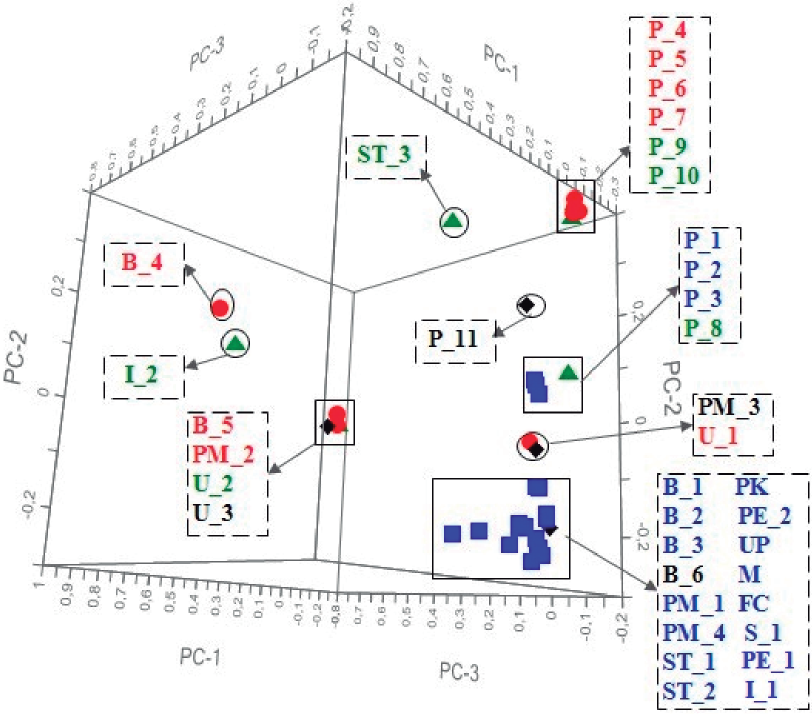

Figure 2.1. 3D PCA scores plots for the different ink types: oil (blue squares), gel (red circles), liquid (green triangles) and “other” variations (black diamonds). PC1 (45.55%); PC2 (17.62%); PC3 (16.01%).

Most of the oil-based samples (blue squares) formed a cluster but no particular pattern could be depicted in relation to their brand. All of the Pilot pen samples stood out as they clustered apart from the rest of pen samples. As for the remaining samples, there was no clear connection regarding either ink type or brand. For a better understanding of the variability in the Raman signature among pen inks, what follows is a more thorough analysis of the population of samples providing also spectral examination.

Inter and intra-brand variability

For the assessment of the inter-brand variability, the various brands of pen samples with the same ink formula type were selected and the Raman spectra were compared. Regarding the oil-based samples, Figure 2.2 shows the Raman spectra for the different brands and models. Most samples exhibited very similar Raman signatures characteristic of the Cristal Violet dye (main bands, according to literature [11]: 442, 724, 809, 916, 1175, 1372, 1533, 1584 and 1617 cm^{-1}). The only exceptions were the Unipapel (UP) sample that showed two additional bands at 622 and 1648 cm^{-1} (pointed out with asterisk in the figure), possibly due to contributions from another non-identified component, and the Pilot samples (P_1, _2 and _3) that showed a different Raman signature, characteristic of the Victoria Pure Blue BO dye (main bands: 442, 655, 681, 710, 728, 761, 804, 863, 917, 1017, 1075, 1160, 1182, 1203, 1286, 1368, 1447, 1486, 1534, 1566, 1584 and 1615 cm^{-1}).

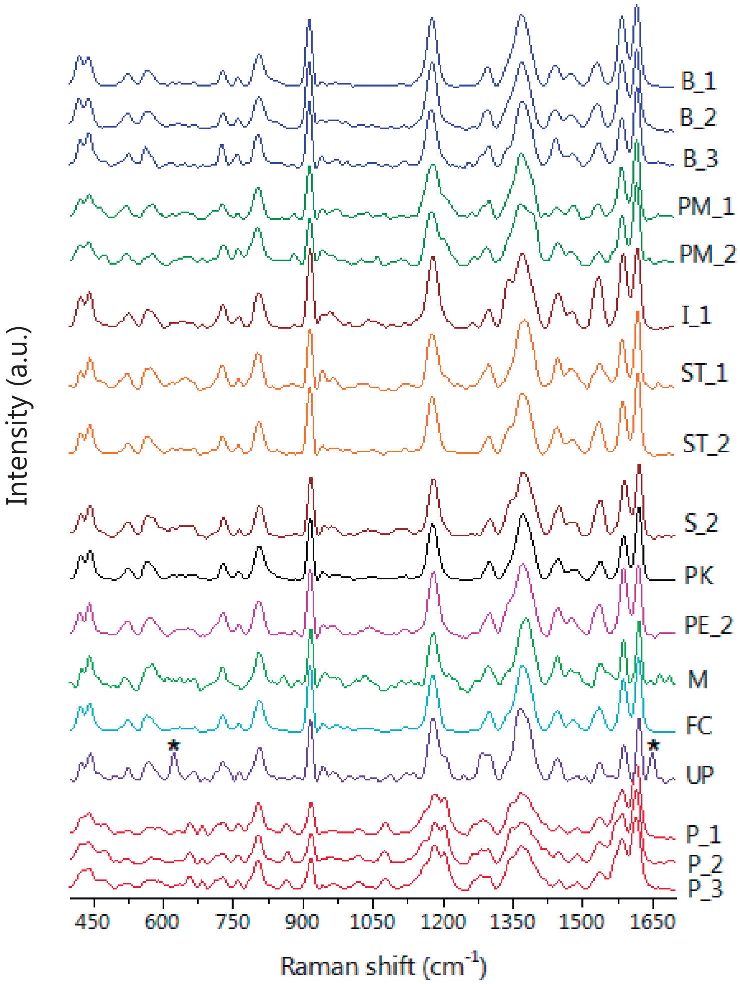

Figure 2.2. Raman spectra for the different brands and models of oil-based ink samples.

Regarding the gel-based samples, Figure 2.3 shows the Raman spectra for the different brands and models. The Bic sample (B_4) showed some major bands characteristic of Rhodamine B (1362, 1508, 1575, 1598 and 1650 cm^{-1}).

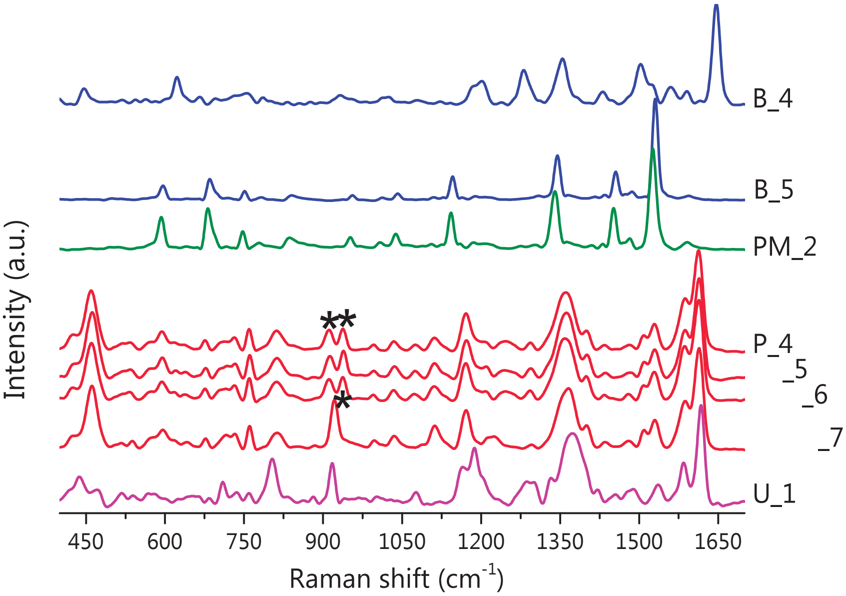

Figure 2.3. Raman spectra for the different brands and models of gel-based ink samples.

However, other minor bands characteristic of this dye were not observed (612, 773, 1184 and 1311 cm^{-1}) and instead, other bands were more noticeable (446, 622, 1201, 1280 and 1430 cm^{-1}) but these could not be assigned. The Bic (B_5) and the Papermate (PM_2) samples showed a Raman profile similar to the Copper Phthalocyanine pigment (major bands: 593, 678, 746, 833, 950, 1034, 1141, 1336, 1466, 1478, 1521 and 1586 cm^{-1}). No further differences were visible between these two samples. The Pilot samples (P_4, _5, _6 and _7) showed identic Raman profiles characteristic of the Victoria Blue B dye (major bands: 426, 459, 533, 676, 731, 759, 911, 938, 1035, 1074, 1111, 1171, 1208, 1247, 1293, 1360, 1400, 1434, 1481, 1529, 1586 and 1612 cm^{-1}). There were also some additional bands observed (593, 811, 995 and 1508 cm^{-}

^{1}) that are possible contributions from other components. Moreover, the sample (P_6) exhibited a band at 921 cm^{-1} instead of two bands at 911 cm^{-1} and 937 cm^{-1} like in the spectra of the remainder gel-based models (pointed out with asterisk in the figure). The Uniball sample (U_1) showed a Raman profile similar to the Ethyl Violet dye (major bands: 441, 569, 707, 799, 916, 1075, 1159, 1186, 1373, 1420, 1491, 1536, 1586 and 1619 cm^{-1}). Additionally, other non-identified bands were also observed in this spectrum (471, 517, 538, 735, 759, 881, 1285, 1300, 1332 and 1455 cm^{-1}). Regarding the liquid-based samples, Figure 2.4 shows the Raman spectra for the different brands and models. All Pilot samples (P_8, 9, 10) showed a Raman signature very similar to the spectrum of the Victoria Blue B dye. However, the sample (P_8) showed two bands at 911 and 938 cm^{-1} instead of a band at 921 cm^{-1} (pointed out with asterisk in the figure), exactly the opposite of what was observed with the gel-based sample (P_6).

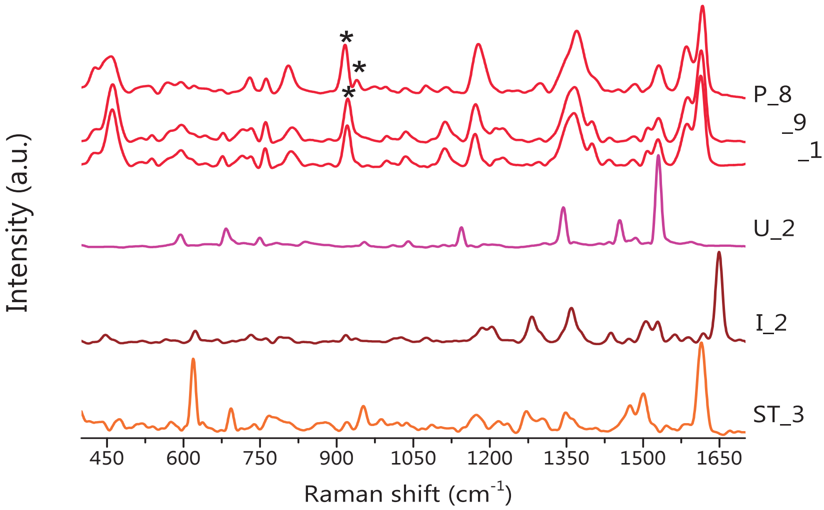

Figure 2.4. Raman spectra for the different brands and models of liquid-based ink samples.

This is an indication that the Raman signature of the gel-based sample (P_6) is more similar to the other liquid-based Pilot samples and the Raman signature of the liquid-based sample (P_8) is more similar to the other gel-based Pilot samples measured. The Uniball sample (U_2) showed a Raman signature similar to the Copper Phthalocyanine pigment, similarly to the Bic (B_5) and Papermate (PM_2) samples. The Inoxcrom sample (I_2) showed the major bands of Rhodamine B (1362, 1508, 1575, 1598 and 1650 cm^{-1}) but some minor bands characteristic of this dye were not observed (612, 773, 1184 and 1311 cm^{-1}) and other non-identified bands were noticeable (446, 622, 1201, 1280 and 1430 cm^{-1}), similarly to the Bic sample (B_4). The Staedtler (ST_3) exhibited a different Raman signature from the remainder liquid-based samples but the main bands could not be identified with none of the reference colorants analysed or reported in the literature.

Regarding the “other”-based samples (Figure 2.5), the easyglide Bic sample (B_6) exhibited a Raman signature very similar to most of the oil-based samples and characteristic of the Crystal Violet dye. The Pilot sample (P_11) exhibited a Raman signature similar to the oil-based Pilot samples and typical of Victoria Pure Blue BO but some bands (436, 714, 1203 and 1343 cm^{-1}, pointed out with asterisk in the figure) differed in relative intensity of adjacent ones, which indicates additional contributions by other chemical components.

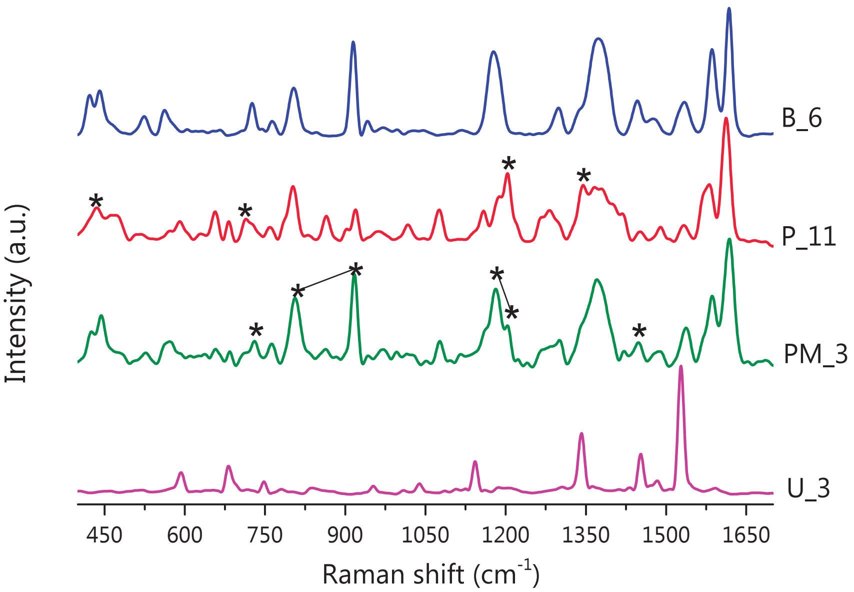

Figure 2.5. Raman spectra for the different brands and models of "other"-based pen samples.

In a similar fashion, the lubriglide Papermate sample (PM_3) showed a profile very similar to the Raman spectrum of the Ethyl Violet dye but with minor differences due to the presence of two additional bands and some differences in relative intensity of adjacent bands were observed at 916 and 1186 cm^{-1} (pointed out with asterisk in the figure) The Uniball sample (U_3) showed a Raman signature identical to Copper Phthalocyanine pigment.

To study the intra-brand variability, a smaller set of samples was chosen with different models of the same brand. Consequently, the gel-based Pilot pen samples were chosen since this sub-population contained the larger number and variety of models. The spectra of each pen, which had been averaged in the previous sections, were now treated as individual samples. Similarly, the five replicate measurements for each pen were considered as individual samples since differences among pens within the same model may be significant. In fact, the PCA scores plot of the first three PCs, which accounted for 72.98% of the variance, showed the separate clusters formed by each model of pen samples and by some pens within each model (Figure 2.6), suggesting not only intra-brand but also within model variability in the Raman signature of the gel-based Pilot samples studied.

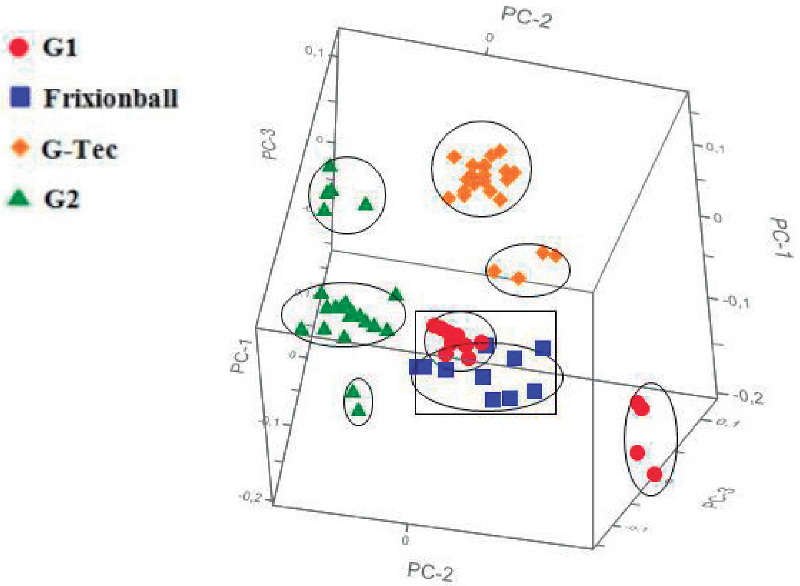

Figure 2.6. 3D PCA scores plot of the different models of gel-based Pilot pen samples. PC1 (44.64%); PC2 (19.98%); PC3 (8.36%).

Since there was no further information regarding the manufacturing batch for each pen, it was not possible to interpret any further. Moreover, the number of pen samples per model was not equal (five G2, five G-Tec, three G1 and two Frixionball pens), leaving the possibility that the pens used in some models may be more similar than in others.

Inter and intra-batches variability

The ink manufacturing is now a well-established process, so it is expected that inks produced using the same major and minor components will have the same spectral signature. For a comprehensive study of the variability within the same model of pens, twelve batches from six different years (two batches per year, ten pens per batch) of the Bic Crystal model were obtained directly from the manufacturer. All samples were measured in the same day, under the same experimental conditions in order to reduce instrumental fluctuations or anomalies that could influence the PCA model. The PCA scores plot of the first three PCs, which accounted for 85.20 % of the total variance, showed six clusters (Figure 2.7), from which several results can be depicted: samples manufactured in 2007 and 2008 formed separate clusters, different from the rest; the two sampling batches manufactured in 2010 formed separate clusters, which suggests significant differences in the Raman signature of the batches manufactured during this year; also, samples manufactured in 2011 and 2013 formed separate clusters but each of the sampling batches manufactured in 2012 was clustered to either the samples manufactured in 2011 or 2013.

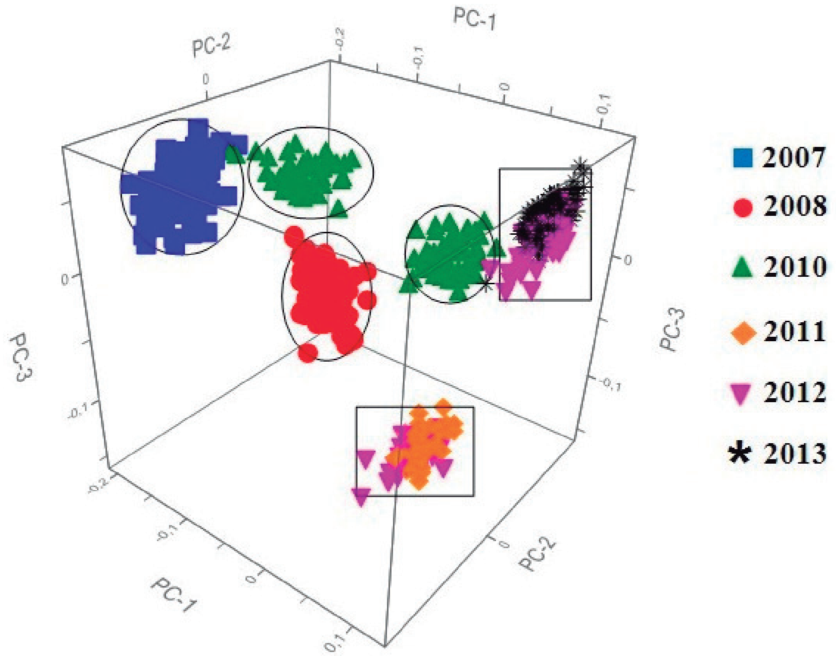

Figure 2.7. 3D PCA scores plot of the different batches of Bic Crystal Medium samples. PC1 (57.33%); PC2 (15.07%); PC3 (12.80%).

This suggests that the Raman signature of one of the batches produced in 2012 was similar to the batches manufactured in 2011 and during that year some changes were made in the chemical formula which resulted in a Raman signature more similar to the batches manufactured in 2013. The differences between the two batches manufactured in 2012 can either be systematic or punctual (for example correction of tint). Only further analysis of other batches could corroborate either occurrences. The Raman scattering of the Crystal Violet dye dominated the spectra of all Bic Crystal samples (Figure 2.8) and the spectral differences which allowed for PCA clustering were possibly due to other minor components, e.g. batches from 2007, 2008 and one from 2010 contained several bands that were not present in the remainder batches (i.e. 878, 1042, 1211, 1261, 1279 and 1422 cm^{-1}); the batch 2013 and one of 2012 presented a band at 464 cm^{-1} that was not present in the remainder samples and a band at 744 cm^{-1} presenting a relative intensity different to adjacent bands.

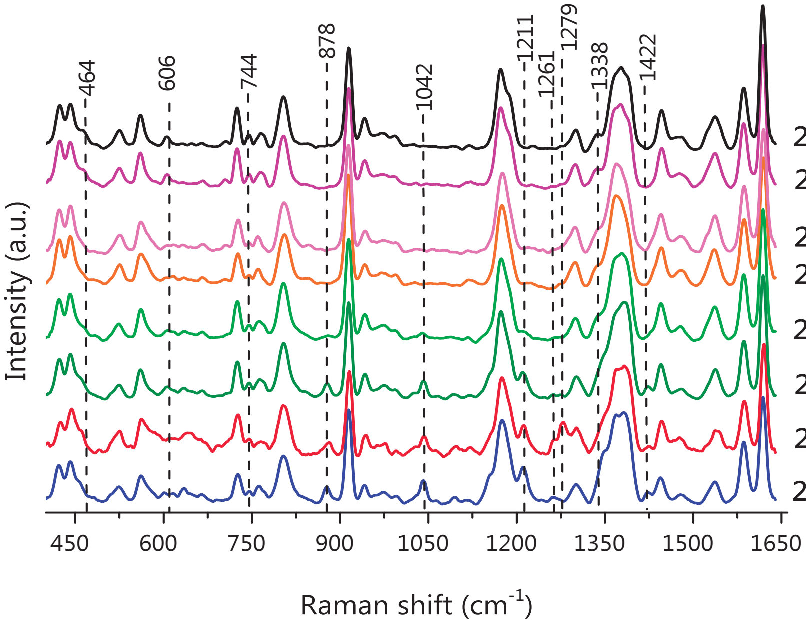

Figure 2.8. Averaged Raman spectra of the different batches of Bic Crystal Medium samples.

These results show that the inter-batch variability is large between batches manufactured in different years and in some cases also between batches manufactured in the same year. The reasons that could explain this variability are varied, for example, some major variations may occur when manufacturers substitute certain components with different and cheaper ones or introduce a similar component that have been produced elsewhere. Additionally, small variations are likely to occur during production, mostly due to variations in the amount of a component added or in their chemical purity, or even fluctuations in the speed of agitation, temperature, order of incorporation of ink components and duration of the manufacturing process [12].

Regarding the intra-batch variability (or within batches), results indicated very similar Raman signatures of the pen samples from each batch (data not shown), which could either suggest that each of the batches analysed have a similar chemical composition or that if chemical differences do exist between pens from the same batch, Raman spectroscopy was not able to detect and other more specific analytical techniques will be required.

Concluding remarks

This chapter investigated the variability in the Raman signature between and within several brands, models and batches of writing pen inks. Although Raman spectroscopy only detects the main colorant and some minor components, it was established that some models of pens can have characteristic Raman signatures, distinct from the rest, and also that most of the oil-based pens studied use Crystal Violet as their main colorant except for the Pilot pens that use Victoria Pure Blue BO. From a forensic perspective, the small inter-brand variability observed in the Raman signature of oil-based pens means a small discrimination potential. On the contrary, the different brands of gel and liquid-based pen inks studied use different colorants in their formulations, such as

Rhodamine B, Copper Phthalocyanine, Ethyl Violet and Victoria Blue B. Although the Raman signature exhibited no particular pattern regarding the use of a particular colorant by most brands, the Pilot pen samples were the only brand that used Victoria Blue B dye in their chemical formula. This is a clear distinct feature to account in forensic ink examinations. Likewise, the “other”-based pen inks used the same colorant as the other types of pen inks but some of these pens showed additional spectral contributions by other chemical components, resulting in distinctive Raman signatures.

The intra-brand variability observed among the gel-based Pilot pens studied was overall large and some spectral differences were observed in the Raman signatures for each of the models examined. However, the large dispersion within each model suggested within model variability.

The within model (inter-batch) variability observed with the oil-based Bic Crystal Medium pens studied showed clear spectral differences between batches manufactured in different years, and only in two cases, between batches manufactured in the same year. This illustrates that changes were introduced in the chemical formula of those pens over the years. Furthermore, the intra-batch variability was small, which indicates that either the manufacturing process of each of those batches produced very similar ink compositions or Raman spectroscopy was not able to detect possible chemical differences, for which another analytical technique may be more revealing. Nonetheless, it is relevant for the forensic ink examiner to know that Raman spectroscopy has the potential to discriminate between pens from different batches, regarding that some changes were introduced in their chemical formula. Additionally, knowledge of these changes will certainly help the examiner with more informed interpretations.

The authors recommend that these results should not be extrapolated nor generalized beyond the population of pens studied. Factors such as aging or the document's storage conditions should be studied further in order to determine the influence in the Raman signature of inks and thus, the impact in forensic discrimination cases.

Finally, this study has a great utility for forensic document examiners when visually comparing the Raman spectra of different inks, however, the application of more powerful chemometrics should be further studied to help with the classification performance, for example, obtained by discriminant analysis or class modelling tools (i.e. SIMCA).

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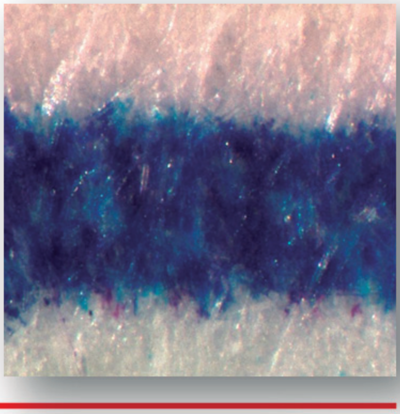
CHAPTER 3

Investigating printing inks

FORENSIC DISCRIMINATION OF INKJET PRINTED LINES BY RAMAN SPECTROSCOPY AND SURFACE-ENHANCED RAMAN SPECTROSCOPY



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Abstract

This study investigates the potential and limitations of one Raman instrument especially designed for document casework to the complex discrimination of samples printed by different models of the same manufacturer of inkjet printers. Results showed low signals for most of the samples, especially black lines, due to fluorescence. To eliminate this, SERS was performed. The surface treatment method with a silver colloid was first studied and optimized to a less invasive one. SERS spectra resulted more intense and well-shaped bands but the same profile overwhelmed the entire spectra for blue and black ink lines. Notwithstanding, closer examination revealed smaller spectral differences which allowed discriminating samples in two groups: samples printed by the Deskjet models and samples printed by professional models like Photosmart, Business and Officejet.

Introduction

Printing is an ubiquitous part of our lives, in constant technologic development. With it, emerges more frequent and sophisticated fraud and counterfeit activities. Establishing a documents authenticity or fraudulent nature requires, on the one hand, physical examinations of documents, that look into intrinsic features left by the printing process or embedded at the hardware level of the printer [1], and on the other hand, discrimination based on comparison of inks chemical composition and properties. Other times, it is also necessary to identify the manufacturer and model of the machine used, ascertain whether or not a specific machine has been used to produce a particular document, or even establish a date before which a certain document could not have been produced. There are several challenges regarding chemical examinations of printing documents: first, there is great variety of brands and models of printers accessible by anyone; second, the specific chemical composition of each ink cartridge is unknown; additionally to this, manufacturers often utilize very similar ink compositions and big changes among cartridges can be difficult to find; third, although each ink cartridge is manufactured to a particular model of printer and physical or electrical incompatibilities are expected with other printers, notwithstanding, the same ink cartridge can be used by several models of printers; finally, cartridges can be refilled with any ink formulation [2, 3].

In routine casework, examinations using optical microscopy and a combination of infrared, ultraviolet and fluorescence enhancements result adequate but in more challenging cases, they may be insufficient, particularly when chemical selectivity is required [4]. For these cases, Raman spectroscopy has been applied for the discrimination of inks due to the possibility for displaying a unique spectrum of the inks molecular formulation. It is also non-invasive, non-contact, rapid and simple to use [5]. Mazzella et al [6] were able to discriminate and classify 22 inkjet inks in 11 groups based on the Raman spectra of magenta dots but not with cyan and yellow inks. Udrıştioiu et al [7] analyzed colored toners and reported that yellow pure dots showed better Raman spectra and thus better discrimination. Božičević et al. [8] obtained good discrimination between manufacturers of printers based on the Raman spectra of magenta and even better results, of yellow pure toner dots. Heudt et al [9] combined the information of several lasers for best discrimination of colored inkjet dots; while magenta and yellow inks exhibited the best discriminating spectra, cyan samples only strongly scattered its major dye, Copper Phthalocyanine, which lead to poor discrimination. Similarly, in black samples, only the signal of the carbon black pigment was observed.

All of these studies described analysis of pure ink dots using sophisticated Raman spectrometers capable of providing spectral resolutions of less than 3 cm^{-1}, laser spots down to 1 µm and equipped with microscope magnifications up to 100×. These instruments are oriented for academic research and sometimes result very expensive and complex to apply in routine forensic cases. However, some forensic laboratories are able to invest in simpler, cheaper and purposely-designed instruments, such as the Foram 685-2 Spectral Comparator® (Foster & Freeman, England). This instrument has a sample stage especially designed for document analysis and it provides a spectral resolution of 8 cm^{-1}, laser spots down to 5 µm and magnification up to 20×. Some studies have reported the successful discrimination of writing inks using this instrument [10-15] but as for printing inks, only Foster & Freeman described its application to inkjet and toner inks discrimination [16, 17]. Notwithstanding, those investigations involved ink extractions from the paper, so no study has been reported in the literature up to date regarding printing inks discrimination directly on paper using this instrument.

Considering that many law enforcement institutions have available the Foram 685-2 spectrometer, the aim of the current study was to investigate the potential and limitations of this Raman system for discrimination of printing inkjet inks directly on paper. For this, black and blue lines, which are the most frequently encountered printed lines in forensic investigations involving questioned documents, were printed by different commercially available models of Hewlett Packard (HP) inkjet printers and analyzed with the Foram 685-2 spectrometer. The main limitation of Raman is fluorescence that sometimes obscures the Raman signal. Fluorescence emissions manifest as a sloping or curved background extending over most of the Raman spectra and can be so intense that it covers the relatively much weaker Raman bands [18].

Utilizing longer laser excitation wavelengths for printing inks usually reduces significantly the fluorescence background but the Raman scattering result weaker and ink samples tend to burn with long measurement times [19]. SERS has been reported in the literature for quenching fluorescence in printing ink analysis [19, 20]. In SERS, samples are treated with a nanostructured metallic colloid, usually made of silver or gold, and once it has been effectively adsorbed to the sample, the surface becomes SERS active and fluorescence can be quenched almost completely. Additionally, enhancements in scattering efficiency can rise up to about 10^6 over normal Raman scattering [21]. Therefore, SERS method was also applied, optimized and compared to the discrimination of the inkjet printing inks.

Experimental section

Samples

The sample set consisted of black and blue lines printed out, on normal quality, by nine inkjet printers of different models from the HP manufacturer (Table 3.1) on the same white office paper. Only original equipment manufacturer cartridges have been used.

Instrumentation

A Foram 685-2 spectrometer from Foster & Freeman (Evesham, England) equipped with a 685 nm diode laser, an integrated microscope, and a thermoelectrically cooled CCD camera was used. This instrument permits a maximum laser intensity of 15 mW, measures in the range of 400-2000 cm^{-1} at 8 cm^{-1} resolution and was equipped with 5, 10 and 20× microscope magnifications. The measurement times depended on each sample and ranged from 0.1 to 100 seconds. 100% of the laser's intensity was used almost every time except when sample burning occurred, for example with most black lines. In these cases, the laser's intensity was reduced to 25%. Each sample was measured in five different points along the same line. Spectra baseline was corrected using offset and linear methods and then normalized using unit vector normalization. The spectral region of interest was reduced to 400-1700 cm^{-1}.

SERS preparation

A silver colloid solution was synthesized according to Lee & Meisels' method [22]. Then, the colloid was concentrated by centrifugation for 30 min at 4000 rpm followed by removal of the supernatant. A drop of the concentrated colloid was applied to the inks surface and let it dry prior to the addition of a similar amount of an aqueous solution of poly-L-lysine (0.01 %, v/v). The reverse order of application of the colloid and the poly-L-lysine was also investigated. Spectra were collected directly on paper once the surface was dry.

Table 3.1. Information regarding the cartridges used with each model of inkjet printer and compatibility with other models, according to the manufacturer.

Model	Black Cartridges	Colour Cartridges	Compatibility with other models
Business Inkjet 2800	C4810A/10	Cyan (C4811A/11) Yellow (C4813A/11) Magenta (C4812A/11)	Business (including 2280TN), Color Inkjet, CP Designjet, Deskjet 2000 series, Officejet, Officejet Pro
Business Inkjet 2280TN	C4844ae/10	Cyan (C4836ae/11) Yellow (C4838ae/11) Magenta (C4837ae/11)	Business (including 2800), Color Inkjet CP, Designjet, Deskjet 2000 series, Officejet, Officejet Pro
Deskjet 950C	51645ae/45	C6578ae/78	Color copier, Deskjet 900, 1000, 6000, 9300 series, Fax, Officejet G and K series, Photosmart 1000 and P series
Deskjet 3745	C8727ae/27	C8728ae/28	Deskjet 3000 series, Officejet 4100 to 4200 series, PSC
Deskjet 3845	C8727ae/27	C8728ae/28	Deskjet 3000 series, Officejet 4100 to 4200 series, PSC
Deskjet 5600 All-in-one	C6656ae/56	C6657ae/57	Deskjet 5000 and F series, Officejet 4100 to 6000 series, PSC, Photosmart 7000 to 8000 series
Deskjet F2180 All-in-one	C9351ae/21	C9352ae/22	Deskjet D, F and 2100 to 4000 series, Officejet J and 4000 series, PSC, Fax
Officejet 4500 wireless	CC653A/901	CC654A/901	Officejet J and “all-in-one” series
Photosmart C4480	CB335EE/350	CB3375EE/351	Deskjet C and D series, Officejet J series, and Photosmart C series

Results and discussion

Blue and black inkjet printed lines are combinations of Cyan, Magenta, Yellow and black (Key) inks, as a result of the CMYK subtractive color model. Computer screens operate with the RGB (red, green and blue) model and printer drivers are used to convert the data to the CMYK model that is specific of the printer. Therefore, the conversion process depends on the printer [23]. As described in the literature (see introduction), better discrimination was obtained when pure yellow and magenta ink dots were analyzed. This comes as a limitation for the Foram 685-2 because the 20× objective does not allow single ink dots analysis in a document. Notwithstanding, our preliminary measurements showed that the same Raman signal was observed in every point of one blue line, independently whether the laser was focused in areas covered with more cyan or magenta inks (data not shown).

Printing inks are complex mixtures of unknown chemical composition and identification of specific molecular components by Raman is extremely difficult. In forensic contexts, spectral fingerprinting comparison is preferred for the differentiation of inks, which consists of comparing the entire spectra considering patterns like the positioning (presence or absence at specific shift values) and broadening (broad or sharp) of bands and also the relative intensity of adjacent bands. However, the presence of strong scattering compounds can overwhelm the entire spectra, the spectral signals from other compounds of the ink mixture might be harder to detect. Additionally, comparison based on the bands absolute intensity is discouraged because it depends on a number of factors which cannot be reliably reproduced, such as focusing, the intensity of the laser and other instrumental effects [24].

The Raman spectra of the paper were initially measured in order to examine any possible interference to the inks profiles. Results confirmed no interfering bands from the paper.

The results obtained for the blue ink lines showed poor Raman signals with only few Raman bands of low intensities for almost all samples except for the ones printed by the HP Photosmart C4480, the HP Officejet 4500 wireless and the HP Business models 2800 and 2280TN, whose signals were well-defined and profiles showed overall good quality (Figure 3.1). Apart from the intensity differences observed, it was not possible to further differentiating these samples.

Regarding black ink lines, only the spectrum of the sample printed by the HP Photosmart C4480 (Figure 3.2) showed a few bands of low intensity. The remainder samples showed no spectral information possibly due to fluorescence, so SERS was performed next in order to remove the fluorescence.

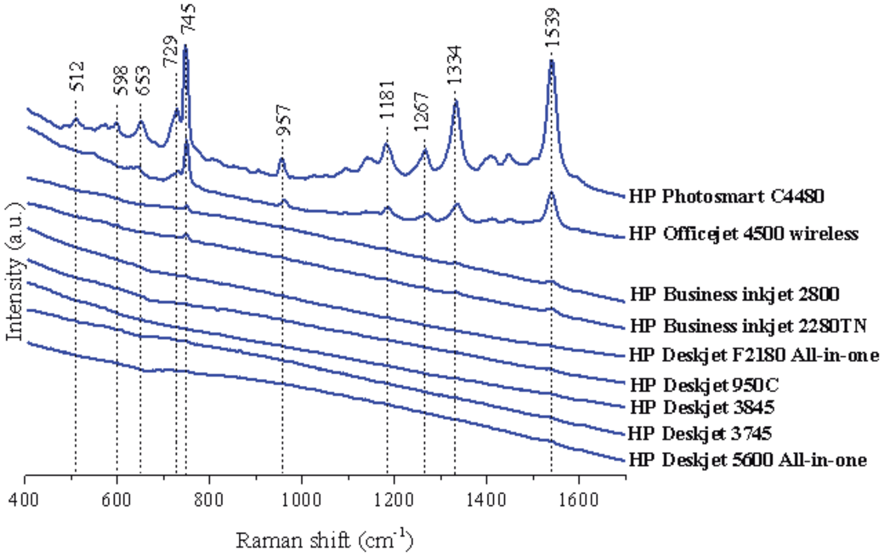

Figure 3.1. Raman spectra of blue inkjet lines.

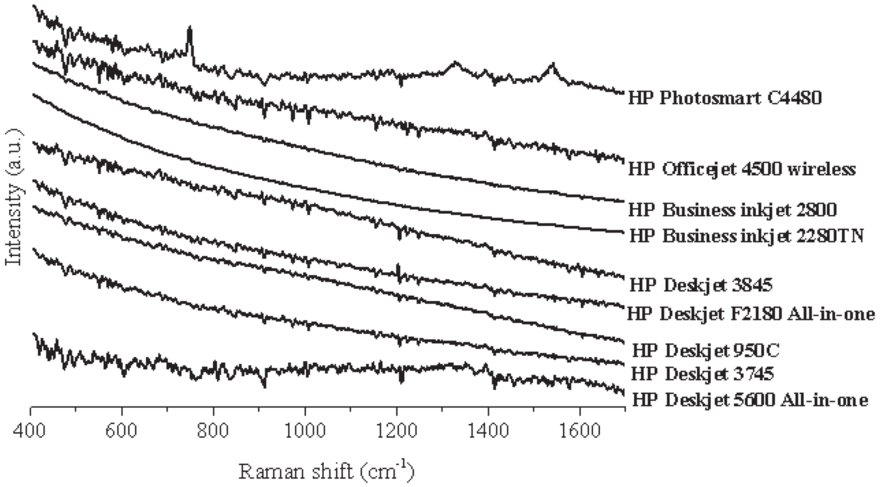

Figure 3.2. Raman spectra of black inkjet lines.

Typical SERS sample preparations for questioned documents involve treating ink surfaces with a very small amount of a silver or gold concentrated colloid and an aggregating agent, usually poly-L-lysine. Rodger et al [20] argued that greater SERS enhancement can be obtained when the colloid is concentrated before it is applied. In this study, we have tested the effect of four degrees of concentrated colloid (concentrated, 10, 100 and 1000 fold diluted) in the inks SERS spectra. Our preliminary testing showed that the more diluted the colloid, the lower were the intensity signals obtained. This might be related to the colloids aggregation efficiency to the ink in that, the more concentrated the colloid, the more particles to promote aggregation, thus the more fluorescence quenched and the higher the SERS signals obtained. The order that these reagents are added was also found to influence SERS enhancement, as reported in the literature [25]. However, our preliminary testing showed that there were no significant spectral differences whether the concentrated colloid was added prior or after the aggregating agent. SERS spectra were exactly the same, independently of the order that the reagents were added. The only differences concerned practical matters: on the one hand, when the aggregating agent was applied first, the treated areas resulted larger and in some cases (the dye based inks), the ink diffused through the paper. This occurred because the aggregating agent is an aqueous solution which facilitates the ink spreading. On the other hand, when the concentrated colloid was applied first, the treated surface was contained to a smaller area since the colloid particles functioned as a barrier to the diffusion of the aggregating agent which resulted in “cleaner” surfaces. Thus, the latter procedure resulted less invasive and it was used throughout this study. Figure 3.3 illustrates the SERS preparation procedure performed in this study.

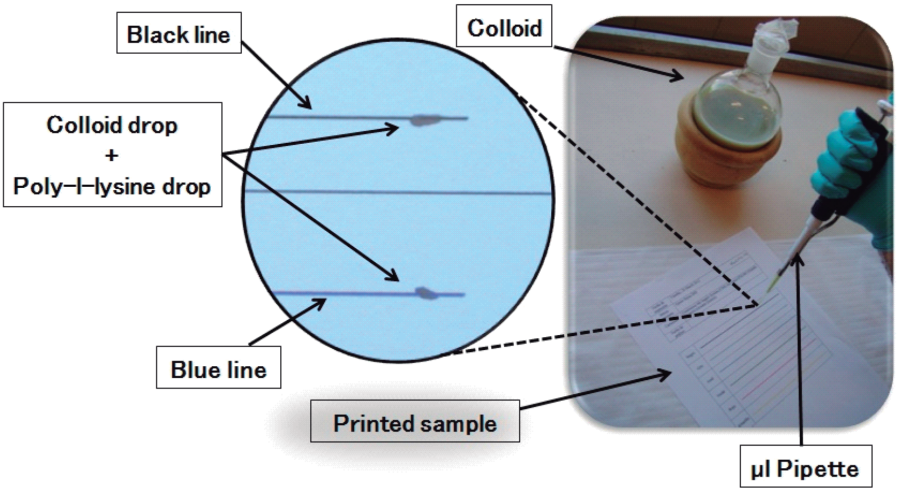

Figure 3.3. SERS sample preparation procedure.

Additionally, SERS signal enhancements were found to be reproducible in every measured point, only varying in small intensity levels, probably due to differences in the aggregation of the colloid to the ink.

The SERS spectra of the blue inkjet samples were greatly improved compared to their respective Raman spectra (Figure 3.4). However, the profiles resulted very similar with each other.

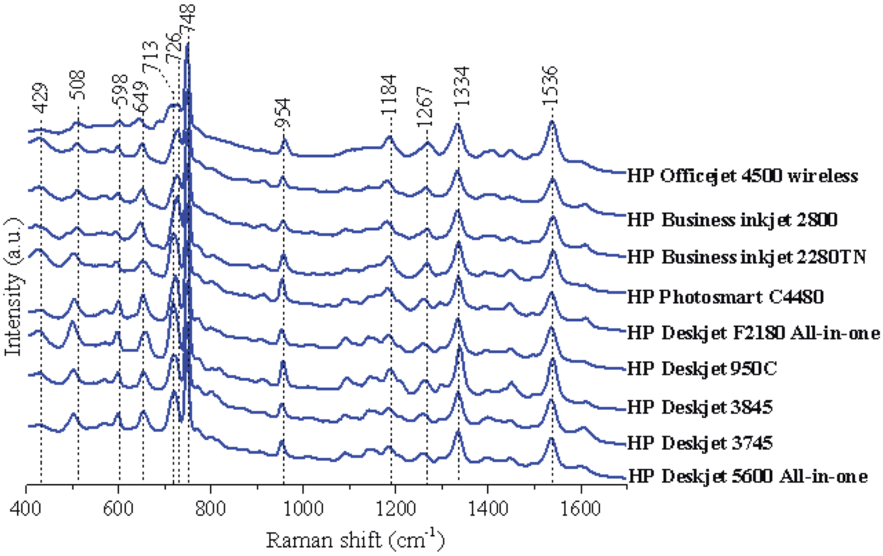

Figure 3.4. SERS spectra of blue inkjet lines.

After closer examination of specific regions (from 1000 to 1700 cm^{-1} and from 400 to 800 cm^{-1}), small differences were observed, as depicted in Figure 3.5. These small differences were reproducible in different points along the same ink. The presence and absence of small bands at specific Raman shift values (as indicated by the arrows in Figure 3.5) allowed discriminating the blue inkjet samples in two groups of printers, according to spectral similarities: the group containing the HP Officejet, the Business inkjet models and the Photosmart, and another group containing all of the remainder Deskjet models. From this classification, we can interpret that the HP Deskjet models may use a slightly different from the professional models. Further discrimination between groups was not possible.

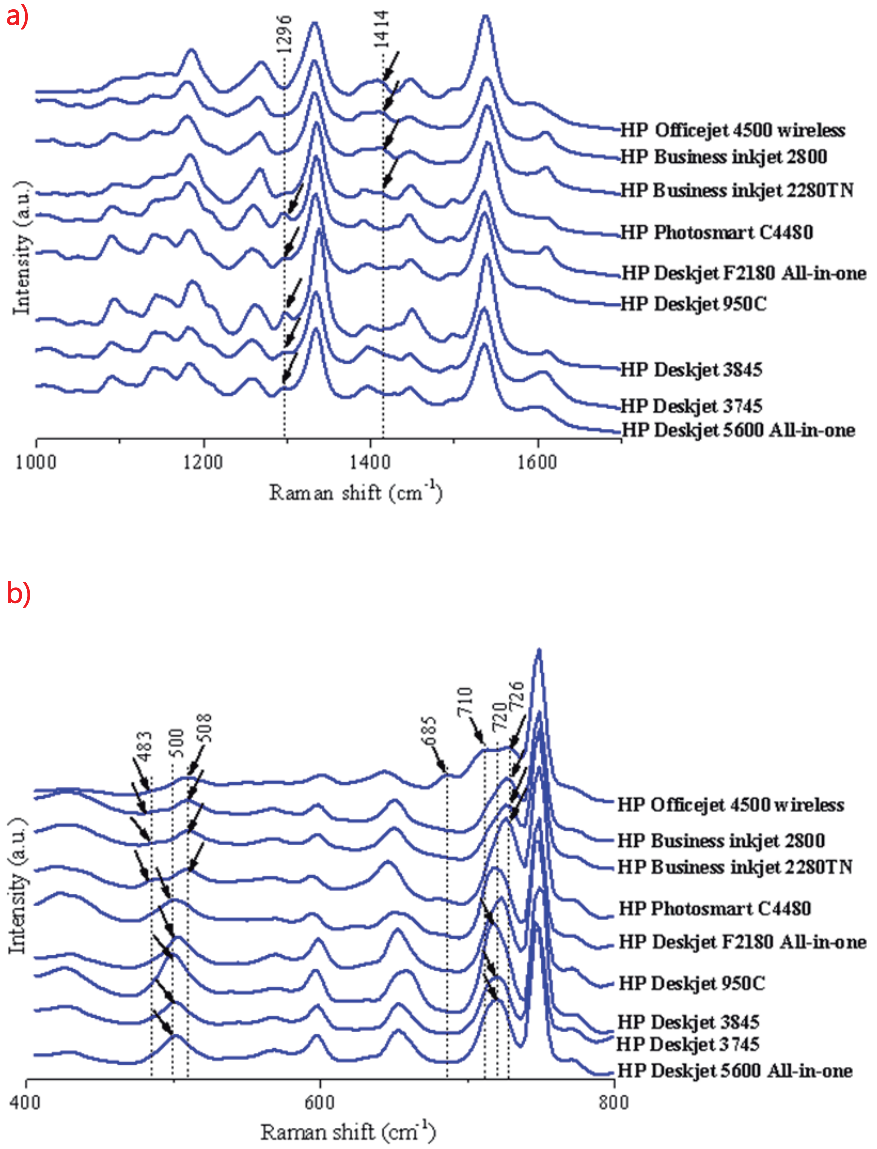

Figure 3.5. SERS spectra of blue inkjet lines of two spectral regions: a) 1000-1700 cm^{-1}; b) 400-800 cm^{-1}.

These small differences were reproducible in different points along the same ink. The presence and absence of small bands at specific Raman shift values (as indicated by the arrows in Figure 3.5) allowed discriminating the blue inkjet samples in two groups of printers, according to spectral similarities: the group containing the HP Officejet, the Business inkjet models and the Photosmart, and another group containing all of the remainder Deskjet models. From this classification, we can interpret that the HP Deskjet models may use a slightly different ink composition from the professional models. Further discrimination between groups was not possible.

Regarding SERS spectra of black ink lines, results showed overall great signal enhancements (Figure 3.6) and the curve from fluorescence contributions was almost entirely eliminated for almost all samples, except the samples printed by the HP Deskjet 5600 and F2180 printers.

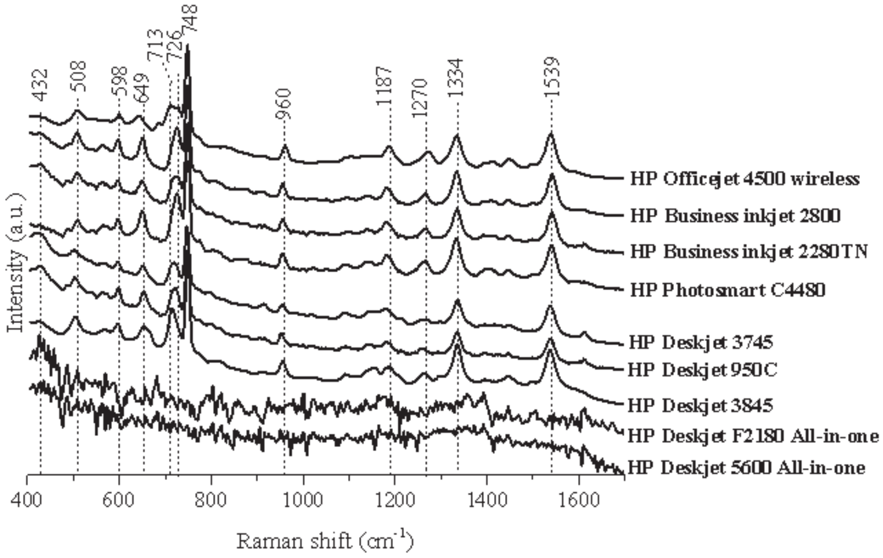

Figure 3.6. SERS spectra of black inkjet lines.

The “lack of Raman signal” could be a possible discrimination criterion for these samples, as Heudt et al. also argued [9]. These spectral differences was due to the fact that the lines printed by these two printers contained only black ink, while the remainder black lines contained all CMYK inks, as far as the light microscope could corroborate. Additionally, the cartridges used by the HP Deskjet 5600 series is compatible with the HP Deskjet F series (Table 1), and so, it is very likely that similar black ink compositions are manufactured for the cartridges used by these two printers. Another interesting result was the SERS signals resulted very similar to the blue inks. So, it is very likely that the components that are common to both ink lines scattered more strongly than the rest of the components. Still, no further discrimination was possible from SERS spectra of black inks.

Concluding remarks

This study exemplifies the contribution of Raman and SERS for discrimination of blue and black ink lines printed by different models of the same inkjet HP manufacturer, using the Foram 685-2 spectrometer. The Raman signals were overall very weak, strongly affected by fluorescence. SERS quenched the fluorescence effects and greatly improved the scattering signals of all samples. Components that are present in both blue and black inkjet lines scattered more strongly than other compounds and overwhelmed the entire SERS spectra of both ink lines. The presence/absence of smaller bands or also the absence of cyan ink in the case of two black ink lines allowed further discrimination between samples. Although it is very complex to discriminate ink lines produced by printers of the same manufacturer, as it is likely they were printed using similar ink, Deskjet models and other professional type models (including the Photosmart, Officejet and Business models) have different Raman and SERS signatures, which is an indication of different ink formulation. Notwithstanding, a larger group of samples covering a bigger portion of the market must be studied in the future in order to reach more generalized conclusions.

Additionally, it was found that applying the concentrated colloid before the aggregating agent resulted in cleaner and therefore less invasive procedure for ink analysis of documents. All of the SERS signals were found to be reproducible.

This study also revealed that the Foram 685-2 Raman spectrometer was able to measure small spectral differences of complex printing ink samples even though it has lower resolution and lower magnification than other more sophisticated spectrometers. This instrument could be useful for an initial classification of blue inks based on the presence or absence of cyan dyes and other minor components but greater discrimination cannot be achieved since it requires higher resolution for analyzing the pure dye spots. Finally, forensic document examiners must take into consideration that the same cartridges can be compatible to several printers, and so, crossing this information in a database can result important when establishing links between unknown printing evidence and database references.

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CHAPTER 4

Investigating intersecting ink lines

A STUDY TO VISUALIZE AND DETERMINE THE SEQUENCING OF INTERSECTING INK LINES

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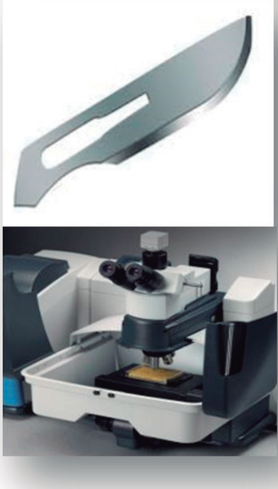

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Abstract

The way two inks will distribute and interact with each other and the paper at the crossing is a dynamic process that can be affected by many variables. The main purpose of this research is to visualize and have a more comprehensive understanding of this process as well as study a methodology for determining the correct order of intersecting ink lines. For this, overlapping layers of different types of inks from writing instruments and printers were cross-sectioned and examined with a microscope. Results from pen/pen crossings showed that liquid-liquid and gel-gel intersections tended to form a double layer but oil-oil intersections usually formed mixtures. Additionally, oil-liquid and oil-gel intersections tended to form a double layer whenever the oil ink was on top and liquid-gel intersections tended to form a double layer for almost all crossings with exception of the ones involving a gel pen ink from one manufacturer. Results from pen/printer crossings showed the formation of a double layer only when the printer ink was on top of the pen ink. On the other permutation, the pen ink tended to penetrate through the printer ink producing the mixture of both inks. The inks drying time was found to be an important factor affecting the interaction between two inks in a crossing, particularly crossings involving gel pen inks. On the contrary, the type of paper and the writing pressure showed no significant influence on the inks distribution at the crossing. The methodology developed was reproducible with overlapping layers but there were many experimental difficulties during the validation process of intersections representing real crossings.

Moreover, interpretation was dependent on the operator’s eye which was a limiting factor.

Introduction

Ink analysis is an important type of examination involved in civil and criminal cases of fraud and counterfeit activities of documents and currency. The research methods that have been developed over the years have certainly helped proving or disproving most of the judicial claims, but there are still some challenges yet to overcome, for example, the sequencing of intersecting ink lines. Forensic document examiners agree that some methods result better than others and that this subject should be approached with caution. The combination of optical and microscopic methods which examine some ink properties such as color, absorption, luminescence and the gloss of the ink lines, result effective in certain cases, and also advantageous, as they are fast and non-destructive [1]. However, particularly in cases when dark colors are used or when inks are partially or completely dispersed in one another or even when inks have very similar compositions and properties, examinations with these methods can be misleading and highly dependent on human interpretation. Other more objective methods have been reported which examine more specific chemical properties of inks [2-5]. Notwithstanding, these methods have limitations and the combination of several techniques is often required for higher accuracy examinations [6].

The intersecting of ink lines is a dynamic process that is affected by many physical and chemical variables which determine how the two inks will distribute and interact with each other and the paper. In the case of pens, the writing pressure, the type of pen and ink used, as well as the ink's chemical composition and properties greatly determine this interaction; the time between application of the ink lines, which is directly correlated to the inks drying process, is also an important factor; the papers properties (structure and surface) will obviously affect how the two inks will distribute across the paper [7]; finally, external factors such as heat, humidity and light can also be accountable. In the case of printers, the printing process greatly affects the interaction of intersecting lines and so, the inks chemical properties such as viscosity and drying efficiency will be determinant factors.

There have been some misconceptions regarding the ink's distribution at intersections that can lead scientists to reach wrong interpretations during their investigations: i) that inks distribute uniformly and ii) that inks usually form a compact layer at the crossing point [6]. Therefore, the main purpose of this study is to have a more comprehensive understanding on how inks distribute at intersections and penetrate in the paper. For this, we have painted two overlapping layers with different types of inks, including printer inks and then a transversal cut was made and the cross-sections were examined under a light microscope. The overlapping layers of ink represented the intersection of ink lines. The method used for cutting involved a surgical blade and it is the most popular method for ink penetration studies [8], and although it is a very simple method to execute, sectioning requires precise manipulative skills, that otherwise can alter the paper fibers during the cut. Several other methods have also been proposed to clarify ink penetration, such as Focused Ion Beam (FIB) techniques [10], Scanning Electron Microscopy (SEM) [10], Confocal Laser Scanning Microscopy (CLSM) [11] and X-ray Photoelectron spectroscopy (XPS) [12] but these result more complicated, time-consuming, require more expensive instrumentation and have been focused at analyzing the extent of inkjet dye inks penetration in paper for studies of printing quality.

Experimental section

Samples

Pen sample crossings were prepared using eight blue and eight red pens of different brands purchased at local markets in Madrid (Spain): BIC Cristal Ballpoint (BIC Cristal), BIC Atlantis Gel (BIC Atlantis), PILOT Super Grip M Ballpoint (PILOT Super Grip), PILOT G2 gel (PILOT G2), PILOT V5 Hi-Tech Point Needlepoint Rollerball (PILOT V5), Paper Mate Flex Grip Ultra-Retractable Ballpoint (Paper Mate), Uniball Eye Micro Rollerball (Uniball Eye), and Uniball Jetstream Ballpoint (Uniball Jetstream). Black printer ink samples were prepared using a HP Deskjet D4260 inkjet printer and a HP Color Laserjet CP2025 toner printer. The same UPM office copy/print white paper (80 $g.m^{-2}$) was used for all samples. Only for studying the inks drying process, Natur recycled paper

(80 g.m^{-2}) and Conqueror laid brilliant white paper (100 g.m^{-2}) were used.

Sample Preparation

A total of 192 different crossings involving two different writing systems (pen/pen and pen/printer, in all permutations) were prepared for investigation and stored under similar climatic conditions. On a typical preparation, a first layer of ink was painted covering a paper area of 2 cm^2 and left to dry for two days. The second layer was then superimposed, covering the entire first layer, and it was left to dry for one hour. Afterwards, 1 cm cross-sectioning was made with a carbon steel surgical blade (Swann-Morton, England) in parallel to the writing direction. The sample was then immobilized between two glass slides, leaving the side of the cut out of the glass slides. This side of the sample was cut once more against another glass surface, to obtain a more precise cut and to prevent deformation of the paper fibers. Samples were mounted in a homemade carton slide holder, as shown in Figure 4.1, especially designed for the microscope stage.

For reproducibility study, a triplicate of several samples was prepared under the same experimental conditions and examined in five different regions of the cross-section. Several samples were also prepared using two other different types of papers (under the typical conditions described previously).

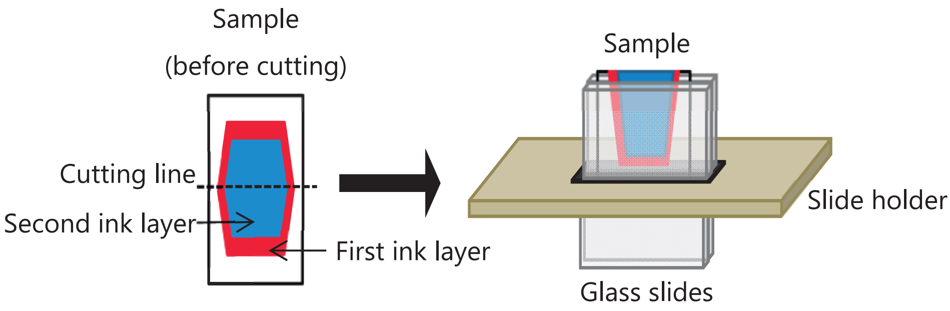


Figure 4.1. Scheme for the sample preparation.

For testing of different writing pressures, several samples were prepared in the following way: the first ink layers were painted by the same person (under typical conditions described previously) while the second ink layers were painted by four different persons, unrelated to this study. The same paper sheet was used and inks were allowed to dry for two days. In order to test the effect of the drying time in the ink's distribution, several samples were prepared where the second ink layer was painted immediately after the first ink layer.

Instrumentation

Samples were examined with a Thermo DXR Raman Microscope (Thermo Scientific, USA), with 10 × and 20 × magnifications under bright and dark field illumination.

Validation study and blind testing

To study the validity of this methodology in real crossings, several samples were prepared where a 2 cm straight ink line which was left to dry for two days and then the second ink line was applied in two different ways: 1) drawn precisely along and on top of the first line; 2) drawn at a 90º angle with the first line. In both preparations, the second ink line was left to dry for one hour before cross-sectioning. Cuts were made in a 45º angle between the two lines. The same paper sheet was used for all samples.

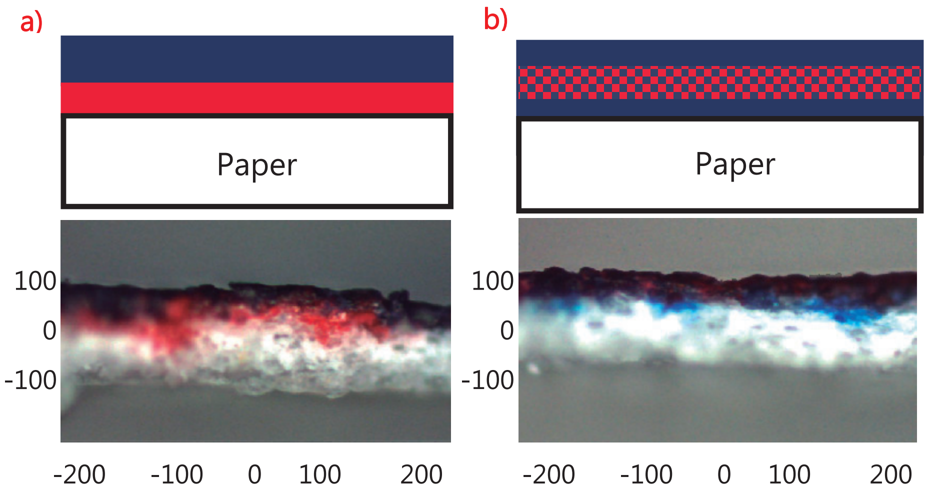
For blind testing, 25 pen ink crossings were prepared in duplicate by two different persons. In these crossings, the second ink line was drawn precisely along and on top of the first line. Two of the four authors examined the same set of blind samples and reported on the distribution of the inks at the crossing, i.e. whether a double layer or a mixture was observed, and also, whenever possible, the order of ink lines observed.

Results and discussion

The pen/pen crossings from the same and different ink types were examiner first. Three writing ink types were used, according to the pen manufacturers, they were oil-based, liquid-based and gel-based. Noteworthy, some pen inks can behave differently from the classification type provided by the manufacturer. For example, the PILOT G2 gel-based pen behaves like a liquid ink as it contains dyes instead of pigments; also the Paper Mate oil-based pen exhibits a smoother writing than other oil-based pens, which resembles more liquid-based pens.

Preliminary studies showed that crossings with the same ink color or dark colors were difficult to distinguish visually under the experimental conditions followed in this investigation. Therefore, blue and red inks were used to facilitate the visual distinction of the inks at the crossing. However, red inks are not as often encountered in caseworks as blue or even black inks. Cross-sections of the individual blue and red inks from each pen were first prepared according to the methodology described above and examined under the microscope. The different inks exhibited different penetration in the paper as well as specular reflection and gloss (results not shown). However, quantification of the inks' penetration for comparison purposes was not possible because the amount of ink applied was not controlled.

The methodology described in this study consisted of painting overlapping layers of ink and cuts of 1 cm^2, which were then examined under microscope. However, real ink crossings consist of 1 mm^2 size and have less amount of ink. Therefore, the methodology described might not entirely reflect real ink crossings but it was chosen nonetheless in order to facilitate visualization of the inks distribution at the crossing. Although some deviations were assumed, for comparison purposes, the results obtained with the methodology described were extrapolated to real crossings. From all crossings investigated, three different arrangements were observed, as exemplified in Figure 4.2



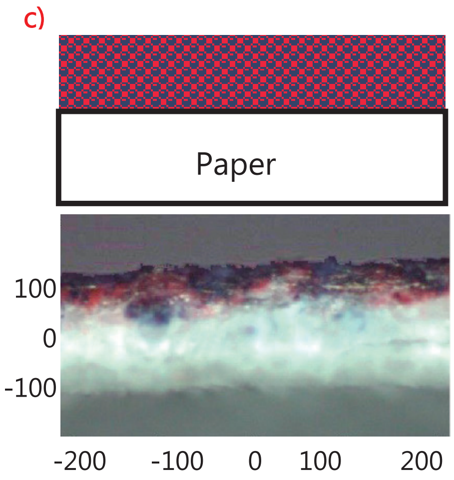


Figure 4.2. Scheme (above) and microscopic image (under) of the different arrangements observed under 10 × magnification and bright field; a) double layer formed by the blue liquid Pilot V5 pen applied on top of the red liquid Pilot V5 pen ink; b) partial mixing and penetration of the blue liquid Pilot V5 pen ink applied on top of the red oil Pilot Super Grip pen ink; c) complete mixing of the blue oil Paper Mate pen ink applied on top of the red liquid Uniball Eye pen ink. Scale in micrometers.

In the first arrangement (a)), the two inks formed a distinct layer on top of each other causing a double layer. However, in the second and third arrangements, the ink at the top has partially or completely dispersed in the ink at the bottom, causing the mixture of the two inks (b) and c)). This mixing may be caused by different factors, such as differences in the inks' viscosity, differences in the amount of ink applied due to different pressures and types of writing device or differences in the amount of time needed for each type of ink to dry.

Tables 4.1 and 4.2 summarize all of the crossing permutations examined. To facilitate, only the crossings that formed double layers were marked with a full dot (about 49 % of the crossings). Since differentiating partially and completely dispersed ink samples was extremely difficult, the remainder samples were referred as mixtures.

The crossings using pens with the same ink type were first examined. Regarding the crossings with oil-oil inks using pens of the same manufacturer, it was observed that whenever the blue ink was under the red ink there was a double layer. However, when the red ink was under the blue ink, only the crossing with the Paper Mate pen ink formed a double layer. The greater penetration of the blue oil inks compared to the red oil inks means that there are differences in the chemical composition of blue and red inks from oil pens of the same manufacturer.

Table 4.1. Overlapping pen/pen layer combinations regarding red pen inks applied on top of blue pen inks. Full dots represent the crossings where the formation of a double layer was visualized.

RED TOP LAYER BLUE BOTTOM LAYER		OIL			LIQUID		GEL		
		BIC Cristal	PILOT Super Grip	Paper Mate	PILOT V5	Uniball Eye	BIC Atlantis	PILOT G2	Uniball Jetstream
OIL	BIC Cristal	●	●				●		
	PILOT Super Grip		●						
	Paper Mate	●		●					
LIQUID	PILOT V5	●	●	●	●	●	●	●	
	Uniball Eye		●		●	●	●	●	
GEL	BIC Atlantis	●	●	●	●		●	●	●
	PILOT G2	●	●	●	●	●	●	●	
	Uniball Jetstream	●	●	●			●	●	●

Table 4.2. Overlapping pen/pen layer combinations regarding blue pen inks applied on top of red pen inks. Full dots represent the crossings where the formation of a double layer was visualized.

BLUE TOP LAYER RED BOTTOM LAYER		OIL			LIQUID		GEL		
		BIC Cristal	PILOT Super Grip	Paper Mate	PILOT V5	Uniball Eye	BIC Atlantis	PILOT G2	Uniball Jetstream
OIL	BIC Cristal								
	PILOT Super Grip								
	Paper Mate	●		●					
LIQUID	PILOT V5			●	●	●	●	●	●
	Uniball Eye				●	●	●	●	
GEL	BIC Atlantis		●		●		●	●	
	PILOT G2				●	●	●	●	
	Uniball Jetstream	●	●	●				●	●

Regarding the crossings with oil-oil inks using pens from different manufacturers, results differed with every crossing but, in general, inks showed the tendency for mixing, with exception of three crossings which involved the BIC Cristal pen ink.

When examining the results from the crossings with liquid-liquid inks using pens from the same manufacturer, it was observed the formation of a double layer for all crossings. Similar results were observed with crossings with liquid-liquid inks using pens from different manufacturers.

Results involving crossings with gel-gel inks using pens from the same manufacturer showed the formation of a double layer for all samples examined. However, in crossings with gel-gel inks using pens from different manufacturers, more cases of double layer were observed when the blue was under the red gel ink than when the red was under the blue gel ink. Consequently, blue gel inks showed greater penetration than the red inks, similar to what occurred with oil pens. Only the crossings between the PILOT G2 and BIC Atlantis pen inks formed double layers in all four possible combinations.

When examining the crossings with oil-liquid pen inks, a double layer was observed when the blue liquid PILOT V5 was under every red oil ink, when the red liquid PILOT V5 was under the blue oil Paper Mate pen ink and also when the blue liquid Uniball Eye was under the red oil PILOT Super Grip pen ink.

In respect to crossings with oil-gel pen inks, it was observed a double layer for all the cases whenever the blue gel pen ink was under the red oil pen ink. Other crossings that also formed a double layer included the red gel Uniball Jetstream under all of the blue oil pen inks, and also the crossings involving the red gel BIC Atlantis under the red oil PILOT Super Grip pen ink and the blue oil BIC Cristal under the red gel BIC Atlantis pen ink.

Concerning the crossings with gel-liquid pen inks, it was observed that most crossings with the gel BIC Atlantis and the gel PILOT G2 pen inks formed a double layer with liquid pen inks while the crossings involving the gel Uniball Jetstream pen ink always formed mixtures.

To study the reproducibility of the results, some of the crossings previously examined that formed a double layer in all possible permutations were reproduced again three times under the same conditions and examined in five different points. Thus, the following crossings were examined: PILOT V5 with PILOT V5, BIC Atlantis with BIC Atlantis, PILOT G2 with PILOT V5, PILOT G2 with BIC Atlantis, and Uniball Eye with BIC Atlantis. The formation of a double layer was observed in all cases studied, which showed good reproducibility of the method.

To study the influence of different papers on the inks absorbency and dispersion at the crossing, several samples were prepared in two other different types of papers (recycled and certification paper) and the crossings were compared with the crossings that were prepared in the white office paper that has been used during the entire study. The following crossings were examined: for double layers, blue BIC Cristal under red BIC Cristal, blue PILOT V5 under red BIC Cristal, blue BIC Atlantis under red BIC Cristal, and for mixtures, red PILOT G2 under blue Uniball Jetstream, red PILOT G2 under blue BIC Cristal and red BIC Cristal under blue BIC Cristal. There were no significant differences observed among the same samples prepared in different papers. This means that either the inks absorbency and dispersion is similar in the different papers used in this study or that significant differences were not visible with the magnification used.

To study the influence of the writing pressure, i.e., the amount of pressure exerted over the paper during writing, two samples that previously formed a double layer and two samples that previously formed mixtures were prepared by four different persons on the same paper sheet and table surface. For closer approximation to reality where line intersections are often made by two different persons, the first ink layer was painted by the same person while the second ink layer was painted by different persons. Each person was told to cover abundantly the first ink layer. The following crossings were examined: the double layers, blue BIC Cristal under red BIC Cristal, blue PILOT V5 under red BIC Cristal, and for mixtures, red PILOT G2 under blue Uniball Jetstream and blue BIC Cristal under red PILOT V5. The formation of double layers and mixtures was observed in each corresponding sample, which means that the writing pressure had no significant influence on the distribution of inks at the crossing. The only influence of the writing pressure was on the ink penetration of the second layer but it was not possible to quantify the differences observed.

The results discussed so far concerned crossings where the top ink was applied two days apart of the ink at the bottom. To study the effect of the inks drying time, the set of crossings previously examined for reproducibility studies were reproduced again, but here the top layer was applied immediately after the first layer, without drying. The same paper was used and differences within the same batch were considered insignificant. The inks drying process varies with the absorbency of the paper and the ability for the inks solvent to evaporate and absorb into the paper. In theory, when ink layers are applied immediately without drying, the inks would have the tendency to form mixtures. However, results showed the formation of a double layer for the crossings PILOT V5 with PILOT V5, PILOT G2 with PILOT V5 and Uniball Eye with BIC Atlantis. This can be explained by the fact that liquid inks, which are less viscous than gel inks, are more readily absorbed into the paper than gel inks, which are less prone to being absorbed. Consequently, the drying time is an important parameter that affects the interaction between two inks in a crossing, particularly crossings involving gel-gel pen inks.

The pen/toner printer crossings were examined next. Laser printers use powdered ink particles, also known as toner, which are electrically charged by a drum, heated and finally fused onto paper. Since the ink is fused, there will be little penetration in the paper; instead the ink cools and bonds to the cellulose fibers. The crossings of eight blue and eight red pens with black toner ink were prepared in all possible permutations (total of 32 samples). Figure 4.3 shows the two arrangements depicted from examining all samples. In the first arrangement (a)), the toner ink formed a layer on top of the pen ink, which penetrated deeply into the paper. On the second arrangement (b)), some of the pen ink penetrated through the toner ink spaces and into the paper, and so, the pen ink was visible on top and also under the toner ink.

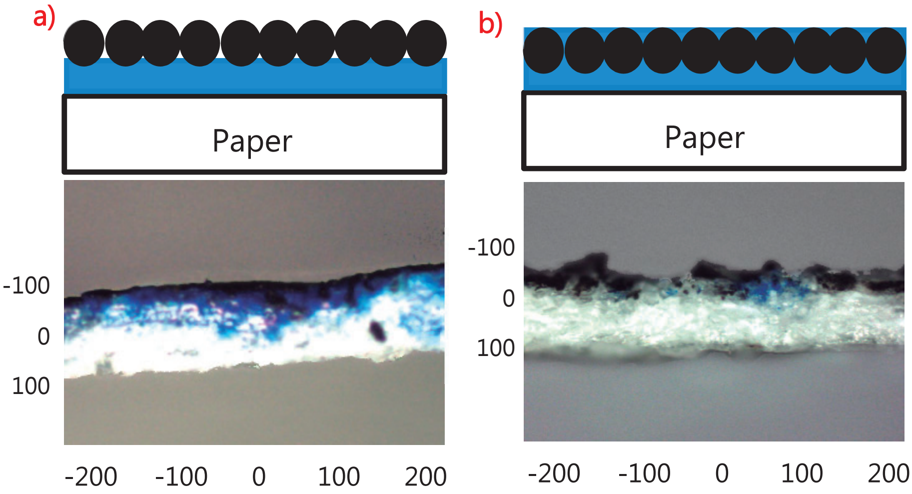


Figure 4.3. Scheme (above) and microscopic image (under) of the different arrangements observed under 10 × magnification and bright field. a) double layer formed by the black toner ink applied on top of blue BIC Atlantis pen ink and b) partial mixing and penetration of the blue BIC Atlantis pen ink applied on top of black toner ink. Scale in micrometers.

Inkjet printers usually use liquid ink that is transferred onto the page through tiny nozzles, creating microscopic dots. Since the inkjet ink is liquid, there will be evaporation and penetration in the paper, which causes bleeding and blurring of the edges of the printed text. 32 crossings between inkjet ink and pen inks were prepared, similarly to the crossings with the laser printer. Figure 4.4 shows the two arrangements depicted from examination of all samples. In the first arrangement (a)), the pen ink penetrated deeply into the paper, while the inkjet ink formed a layer on top of the pen ink. It was also visible some penetration of the inkjet ink into the paper. In the second arrangement (b)), the inkjet ink penetrated deeply into the paper and some of the pen ink penetrated through the inkjet ink spaces and into the paper as well, favoring the mixture of the two inks.

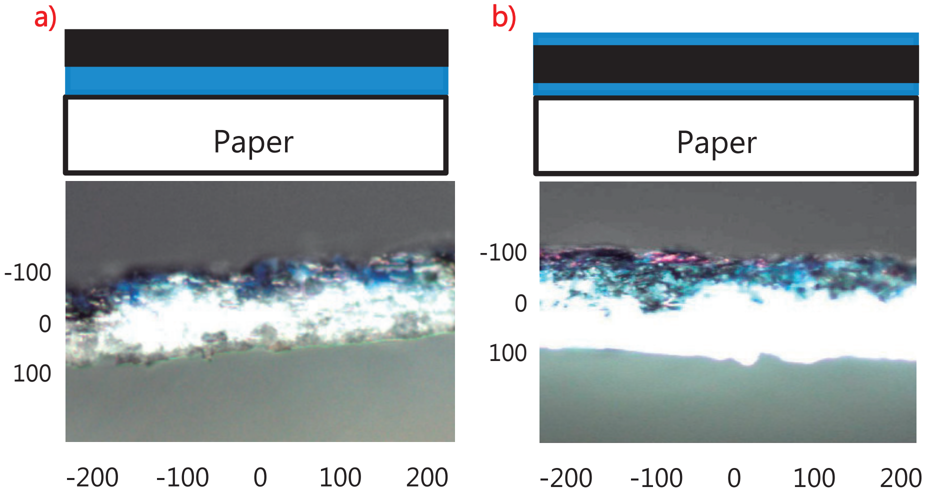


Figure 4.4. Scheme (above) and microscopic image (under) of the different arrangements observed under ×10 magnification and bright field. a) double layer formed by the black inkjet ink applied on top of blue BIC Atlantis pen ink and b) partial mixing and dispersion of the blue BIC Atlantis pen ink applied on top of black inkjet ink. Scale in micrometers.

Table 4.3 summarizes the results obtained for all pen/printer crossings. The double layer was observed in all cases when the printer ink was on top of the pen ink (Table 4.3 a)). Additionally, the inkjet ink showed more penetration than the toner ink, which was probably due to the different printing process of laser and inkjet printers and to the fact that inkjet ink is liquid while toner ink is solid. However, when the pen ink was on top of the printer ink, the pen ink penetrated in the printer ink causing mixtures in all the cases studied (Table 4.3 b)).

Table 4.3. Overlapping pen/printer layer combinations when a) printer inks were on top of pen inks, and b) pen inks were on top of printer inks. Full dots represent the crossings where the formation of a double layer was visualized.

a)

BOTTOM LAYER TOP LAYER		OIL			LIQUID		GEL		
		BIC Cristal	PILOT Super Grip	Paper Mate	PILOT V5	Uniball Eye	BIC Atlantis	PILOT G2	Uniball Jetstream
PRINTER	Laser	●	●	●	●	●	●	●	●
	Inkjet	●	●	●	●	●	●	●	●

b)

TOP LAYER BOTTOM LAYER		OIL			LIQUID		GEL		
		BIC Cristal	PILOT Super Grip	Paper Mate	PILOT V5	Uniball Eye	BIC Atlantis	PILOT G2	Uniball Jetstream
PRINTER	Laser								
	Inkjet								

To test the validity of the method to real casework, several samples were prepared involving crossings between two ink lines. Two methods for preparation of the crossings were studied: the first involved superimposing two straight ink lines with no angle of intersection between the two lines. This method intended to replicate more closely the methodology applied throughout the entire study; the second involved crossing two straight ink lines with a 90º angle of intersection. Other angles of intersection between the two lines were not studied because they were considered close approximations to either of the two intersecting angles studied. The cross-sections of the samples prepared by the two methods were then examined with the microscope and compared. The following crossings were examined: for double layers, red BIC Atlantis under blue BIC Atlantis, blue BIC Atlantis under red BIC Atlantis, blue Uniball Eye under red BIC Atlantis, red Uniball Eye under blue BIC Atlantis, and for mixtures, red BIC Atlantis under blue Uniball Eye and blue BIC Atlantis under red Uniball Eye. The results from the crossings prepared by the first method were consistent with the expected distribution of the inks. However, regarding the results from the crossings prepared by the second method, it was difficult to visualize those crossings, mainly due to focusing and lightening problems. Therefore, for these crossings, it was not possible to obtain any results regarding the inks distribution.

Given the difficulties observed with visualizing small crossing areas with a 90º angle of intersection, only the crossings of superimposed ink lines with no angle of intersection were used for double blind testing. Results obtained revealed that the two involved operators were able to determine the correct distribution of the inks at the crossing in 40 % and 52 % of the cases, respectively. Regarding the determination of the order of ink lines, the two operators only reported a 20 % and 24 %, respectively, of correct results. In this case, almost 50 % of samples were undetermined due to difficulties in visualizing such a small area of intersection. Additionally, authors experienced some complications regarding insufficient illumination and focusing due to the different planes at the crossing area. Consequently, images resulted blurry which made it difficult to distinguish inks distribution at the crossing.

Concluding remarks

Most of the pen/pen crossings with inks from the same manufacturer formed a double layer, with exception of the crossings with the oil BIC Cristal and the PILOT Super Grip pen inks. Double layers in all possible permutations were also observed with the liquid-liquid crossings involving the PILOT V5 and the Uniball Eye pen inks, the gel-gel crossings involving the BIC Atlantis and the PILOT G2 pen inks, and the liquid-gel crossings involving the BIC Atlantis and the PILOT V5 pen inks as well as the PILOT G2 and the PILOT V5 pen inks and also the PILOT G2 and the Uniball Eye pen inks.

Regarding the printer/pen crossings, results showed the formation of a double layer whenever the printer ink was on top of the pen ink, for crossings involving both laser and inkjet printers. On the contrary, when the pen ink was on top of the printer ink, the pen ink showed the tendency to penetrate through the printer ink leading to the inks mixture. Furthermore, the inkjet ink showed greater penetration than toner ink, which can be explained by the printing process and the liquid nature of the inkjet ink.

The inks drying time was found to be an important factor affecting the interaction between two inks in a crossing, particularly crossings involving gel pen inks. Other drying times should be studied in future investigations as well as the effect of the inks ageing once the crossing has been made. Other factors studied, such as the type of paper and writing pressure showed no significant influence on the inks distribution at the crossing, even though some variations were observed in the penetration of the second ink layer with different pressures. However, it was not possible to quantify such variations. The only factor that was not controlled during this study was the amount of ink applied by each pen and printer, so further research must be performed in this sense in order to evaluate its real contribution to the inks distribution at the crossing.

This research gave a more comprehensive understanding on how inks distribute at the crossing and penetrate in the paper. From the 128 pen/pen crossings studied, 49 % of the intersecting lines formed a double layer while the rest mixed; in the case of the pen/printer crossings studied, the formation of a double layer was only observed when the printer ink was on top of the pen ink. This information is extremely relevant to consider in future investigations on intersecting ink lines but the results should not be extrapolated to other models and manufacturers of pens and printers since the number of pens and printers was reduced in this study. Additionally, in real cases it would be important to know which pens were used before cutting, in order to know if a double layer can be created, otherwise, the cutting of the document is not necessary.

Although this methodology showed promising results with intersections that represent real crossings, there were some experimental difficulties in the validation process. Thus, this study provided a preliminary insight at the problematic of the sequencing of intersecting ink lines, to which further research is necessary for applications in real casework. Further research must be developed which will, on the one hand, provide more objective examination and on the other hand, be able to visualize and distinguish the distribution of inks with the same color (and dark colors). For this, the use of other microscopes with better definition and lightening are recommended and the combination with other techniques, for example CLSM, Hyperspectral Imaging (HSI), FTIR or Raman spectroscopy, will be useful for interpretation.

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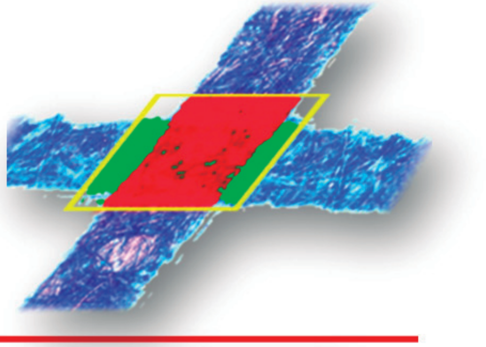
RAMAN IMAGING FOR DETERMINING THE SEQUENCE OF BLUE PEN INK CROSSINGS



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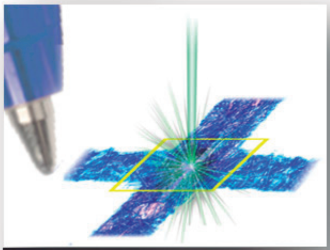
María López-López
Carmen García Ruiz



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Abstract

This study investigates the applicability of Raman imaging for non-destructive examination of real crossing lines made with blue pen inks when different pen ink types, different times separating the application of the two ink lines and different paper substrates are used. Raman imaging allowed analysing the entire crossing area under short measurement times and also identified the inks colorants. The correct sequence of crossings was determined for many of the samples examined. One exception included the crossings involving the U. Eye pen ink, where this ink line was always seen under the other ink. The effect visualized suggested the complete skipping of this ink line in the crossing area when it was applied over another ink. Additionally, a net-like pattern was observed in the ink line applied last at the crossing area of many samples, suggesting the partial skipping of those ink lines. However, it did not influence observing the correct distribution of inks in most crossing samples. Exceptions included some crossings involving the ballpoint pens where the net-like pattern was observed in the crossing area as well as the ink lines separately. The amount of ink applied by these pens, which is controlled by the ballpoint mechanism, seemed to influence visualizing and determining the correct order of application of inks. Moreover, most crossings showed some mixing between the two inks and it was more accentuated when the time separating the application of the inks was shorter than with longer times, suggesting that it is influenced by the drying time of the first ink

line. The different papers used did not seem to influence in the inks distribution nor determining the order of the inks.

Introduction

Determining the sequence of crossing ink lines is a “hot” topic in Questioned Document Examinations that has been around for many years and it is often related to cases regarding the authenticity of a document or simply to confirm or disprove the credibility of a suspected claim related to another dispute. Examination of such cases must be non-destructive in order to undergo multiple analysis and to preserve the documents evidential value. Several methods have been proposed in the past, some being more invasive than others, such as lifting techniques, chemical reactions, scanning electron microscopy, spectrophotometry as well as microscopic, photographic, indented impression and electrostatic techniques [1]. Nowadays, high-powered microscopic examinations with different magnifying lenses and filters that rely on differences in gloss and specular reflection are the easiest and most rapid methods that can give solution to a great number of cases [2-5]. However, these methods remain subjective to human interpretation which may lead to inconclusive or incorrect results, especially with inks that have similar optical properties or are optically deceptive [6]. The use of absorption and reflection microspectrophotometers that have been especially designed for document analysis have contributed to more objective examinations, particularly for crossings between pens and printing inks. However, they have failed with crossings between pen inks, especially gel-based, since the technique lacks chemical specificity [7, 8]. One of the vibrational techniques, ATR-FTIR showed to be useful in some studies [9-11]. Some authors also reported improved results when additional spatial information was obtained using the imaging configuration, especially with crossings between ballpoint pens and toners [9]. Nevertheless, this technique failed with many crossings, like those involving ballpoint, gel, roller ball and felt-tip pen inks. Moreover, the authors reported that paper contributions greatly obstructed the measurements. Alternatively, Raman spectroscopy has shown to be a very promising technique for crossing ink lines [12] and more advantageous than FTIR because paper substrates have little or no interferences in the inks spectra. Similarly to FTIR, the Raman technique provides chemical and spatial information from the surface, but also in profundity, when equipped with the confocal mode that allows analysing inner parts of the intersection. The literature reported some successful cases [13-15] but most studies only analysed a few points in the crossing area, which is not representative of the crossing area and the authors failed to demonstrate the repeatability and reproducibility of the results to other intersections. In sum, all of the methods referred before have shown great analytical performance for solving many cases regarding crossing ink lines, some being more informative and objective than others and each having their advantages and limitations. However, they fail in a great number of cases, especially when two very similar ink lines are present.

The interaction between an ink and a paper substrate, especially printing inks, has been covered in the literature from different standpoints and applications [16-20]. It is a complex process, where physical and chemical reactions take place, such as evaporation of volatiles, polymerization, oxidation, cross-linking and even paper corrosion [21]. Concerning writing pens, when an ink line is applied, it disperses throughout the paper and is absorbed into it. Oil-based inks have the tendency for being only partially absorbed and most of the ink stays adherent to the surface of the paper, due to its high viscosity. Water-based inks on the other-hand, have the tendency for soaking into the paper fibres, like water into a sponge [6]. In contrast, the interaction between two crossing ink lines in a paper substrate has been mostly understudied. In principle, when a second ink is applied over another, it can disperse across the void spaces and in the other ink. Nevertheless, the distribution resulting from the crossing between two ink lines has never been visualized *per se*. The previous study in this chapter [22] has shown cross-sections of painted overlapping layers of inks for the purpose of visualizing and understanding a process that is very similar to real crossing ink lines. Three different configurations were observed: the two ink layers formed a double layer distinctly on top of each other and the ink layer at the top was dispersed in the ink layer at the bottom, forming either a partial or a complete mixture. Several factors could determine the formation of either of these configurations: i) the ink's chemical composition, which is patented and usually unknown to the forensic examiner; ii) the amount of ink applied, which mostly depends on writing pressures as well as the type of point and pen used; iii) the time that separates the application of both inks, which is directly related to the inks drying processes; iv) the structure and surface of the paper substrate, that determines the hydraulic conductivity and absorption of the ink; and v) climatic factors such as heat, humidity and light, which may also play a role. In the previous study [22], red and blue overlapping layers of painted ink of 1 cm^2 were used to facilitate microscopic visualization. Although this might not entirely reflect the size of real ink crossings, nor should be reproducible to crossings involving other ink colours, it was concluded that the paper and the writing pressure showed no significant influence on the inks distribution and that the type of ink as well as the drying times were very important factors that should be studied further with real crossing ink lines. Surprisingly, little is known about the influence of each of these factors in the distribution of crossing ink lines. This study focus on investigating the applicability of Raman imaging for non-destructive examination of real crossing lines made with blue pen inks when different pen ink types, different times separating the application of the two ink lines and different paper substrates are used. These parameters have been studied to understand the distribution of the two inks at the crossing as well as their influence in determining the correct order of crossings.

Experimental section

Sampling

Six blue pens were purchased at local markets in Madrid (Spain): a Bic Cristal Medium ballpoint pen (B. Cristal), writes a 0.4 mm width line, a Bic Atlantis Gel rollerball pen (B. Atlantis), writes a 0.4 mm width line, a Pilot Super Grip M ballpoint pen (P. SGrip), writes a 0.31 mm width line, a Pilot G2 gel rollerball pen (P. G2), writes a 0.39 mm width line, a Pilot V5 Hi-Tech Needlepoint rollerball pen (P. V5), writes a 0.3 mm width line, and a Uniball Eye Micro rollerball pen (U. Eye), writes a 0.3 mm width line. Crossings were prepared by the same person in the following manner: the first ink was always a horizontal line applied from left to right and the second ink line was crossed vertically to the first from top to bottom. The second ink line was then applied at different times: immediately (zero minutes), thirty minutes, five hours, two days and one week. The same UPM office copy/print white paper (80 $g{\cdot}m^{-2}$) was always used, except in the study of the effect of different papers, where all samples were prepared in a Natur recycled paper (80 $g{\cdot}m^{-2}$) and a Conqueror laid brilliant white paper (100 $g{\cdot}m^{-2}$). To test the effect of the amount of ink in oil-oil crossings, three superimposed lines were applied for extra amounts of ink. All crossings were allowed to dry at least for a few hours under the same laboratory climatic conditions before analyses.

Instrumentation

Samples were examined with a Thermo Scientific DXR™xi Raman Imaging Microscope (Waltham, United States) with an EMCCD detector and controlled by the Thermo Scientific OMNICxi Raman Imaging 1.0.0.2427 software. The green laser emitting at 532 nm was used for all measurements with an intensity set at 1.0 mW on the sample. A grating of 1200 lines per mm and a confocal pinhole of 25 µm were used. The microscope was set to 10× magnification under bright field illumination. Laser exposure times were 14.29 milliseconds (i.e. 70 spectra acquired per second) in a total of 30 scans. The mapping areas were defined in a "mosaic" image tile of approx. 600 µm × 600 µm including the intersection area and part of each individual ink line, depending on each crossing dimensions. With a step size between two successive measurements of 8 µm, approximately 5000 – 7000 spectra were registered for each sample, which resulted in measurement times of less than 50 minutes. The wavenumber range measured was from 85 to 3500 cm^{-1}. The Multivariate Curve Resolution (MCR) method with two components and a 4th order polynomial baseline correction was automatically applied to the spectral data using the equipment's software.

Results and discussion

The Raman imaging technique was chosen for this investigation because it can analyse the entire crossing area under short measurement times due to fast-readout detectors and fast automatic stages, in addition to identifying some of the inks chemical components like in conventional Raman spectroscopy. The imaging configuration delivers a chemical image from thousands of spectral locations (pixels) of the crossing which shows the spatial distributions of the inks chemical components at the crossing [23]. For successful Raman imaging of the crossing, the criterion for selecting the samples in this study was that the spectrum of each pen ink was different from each other and from the paper substrate in at least one band. A total of six pens satisfying this criterion were selected. Figure 4.5 shows the spectra of the blue pen inks selected at the specific region of interest, 400-1650 cm^{-1}. In Raman spectroscopy, the ink`s colorants (dyes and pigments) are more active than most of the other components, thus they are the components typically detected. According to the literature, the main colorants identified for each pen ink sample analysed were: Cristal Violet dye (B. Cristal), Victoria Pure Blue BO dye (P. SGrip), Copper Phthalocyanine pigment (B. Atlantis and U. Eye) and Victoria Blue B dye (P. G2 and P. V5) [24].

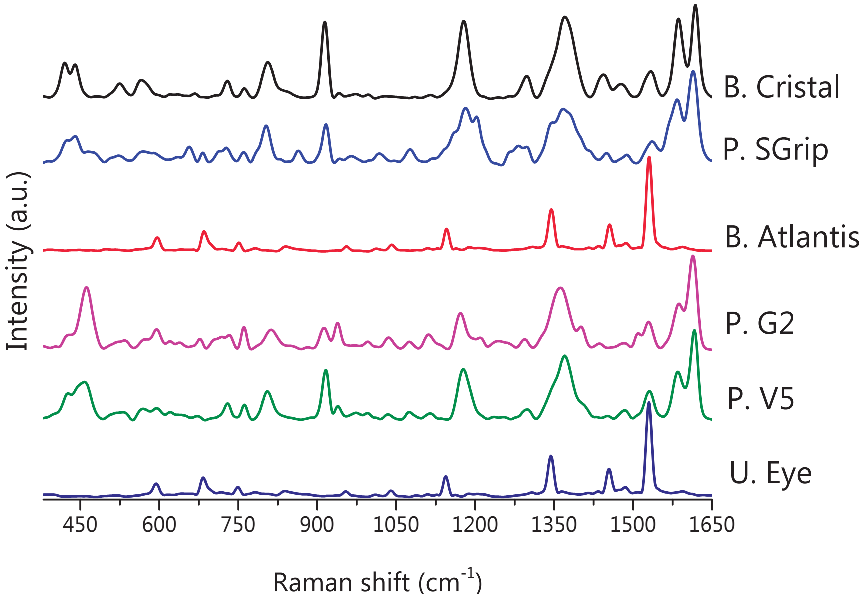

Figure 4.5. Raman spectra of the blue pen ink samples.

The B. Atlantis and the U. Eye showed similar spectra and no differences were identified. For this reason, crossing combinations involving these two pens were not performed. That being said, several combinations of crossings were prepared with these pens and each crossing was also prepared in the opposite permutation, originating a total of eighteen combinations. To facilitate the interpretation, the first line applied was always horizontal and the last line was always vertical. The MCR method was used to decompose the Raman image into the spectral signatures of the pure components, providing this way a reliable distribution map and chemical characterization of the colorants detected at the surface of the crossing. The great advantage of this method is that it does not require priori information about the system nor uses any behaviour model. Thus, the data visualized will follow physically or chemically meaningful constraints, rather than mathematical or statistical constraints [25]. The area selected for Raman imaging included the crossing of the two inks and also part of the ink lines separately. The spectral contributions of the ink lines separately facilitated the MCR model obtaining better resolutions of the crossing area because they represent pure ink contributions. The two pure components found in the images were given a colour. The green colour was given to the pure component detected in the horizontal ink line and red was given to the pure component detected in the vertical ink line. The paper substrate, which did not show any bands, was seen in black, by default. The methodology used for determining the sequence of the crossing consisted in identifying the most predominant Raman signature in the crossing area, which could give indication of the ink that was applied last; additionally, examination of the continuity or discontinuity of that signature in the ink lines separately and through the crossing area could give indication of the physical and chemical distribution of the inks at the crossing.

From the several factors that can affect the inks' distribution at the crossing, the writing pressure, the amount of ink applied in a single line and climatic conditions were not investigated in this study. Notwithstanding, all samples were prepared by the same operator, so little variations in the writing pressure were expected, the line widths of all pen samples were in a similar range (from 0.3 to 0.4 mm), and the laboratory climatic conditions did not vary significantly during experiments. On the contrary, the time separating the crossing of the two ink lines was investigated comprehensively. It was hypothesized that the fresher the first ink line at the moment of crossing of the second ink line, the more likely that the two inks mix. By contrast, the longer the time separating the crossing of the two ink lines, the dryer the first one, thus the more likely that the two inks do not mix. In order to investigate this, all crossings were prepared with different times separating the crossing of the two ink lines. The times chosen were immediately (zero minutes), thirty minutes, five hours, two days and one week, resulting in a total of 90 crossing samples. The results with the crossings using pens with the same ink type were first examined, and the Raman images of all crossings at different times and in both permutations were obtained. Regarding the gel-gel crossing P. G2 over B. Atlantis (Figure 4.6), results showed a predominance of the Raman signature of the P. G2 ink at the surface of the crossing (in red) for the several times measured.

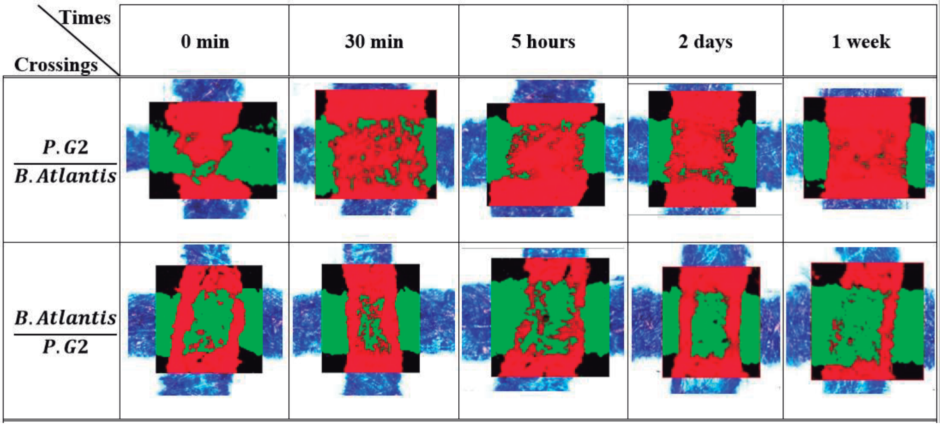

Figure 4.6. Raman images of the crossings using gel-gel blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

In these crossings, some occasional gaps similar to a net-like pattern were visible in the crossing area, in which the spectral contributions of the B. Atlantic ink underneath (in green) were also visible. This pattern was not observed in the ink lines separately. Additionally, some darker shades in the crossing areas of all images can be seen, and their spectral examination showed that they contained contributions from both inks, as exemplified in Figure 4.7. The dashed lines indicates the spectrum of the mixture (in grey) and it can be depicted band contributions from both inks, suggesting the mixing of both inks occurred in those areas.

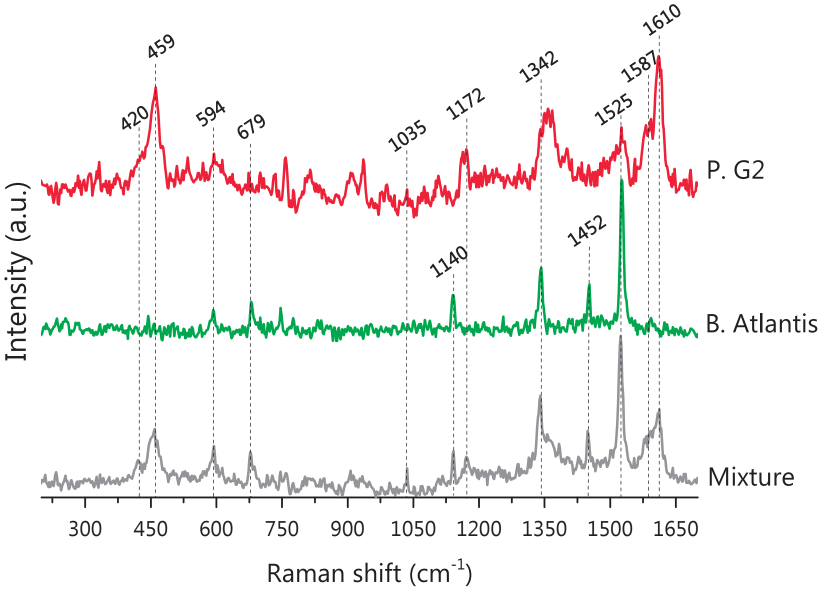

Figure 4.7. Raman spectra of single points in the P. G2 (red) and the B. Atlantis (green) ink line separately and of a dark shade point at the crossing area (grey), of the crossing sample P. G2 over B. Atlantis.

Comparing the images obtained at different times, the net-like pattern and the dark shades became less notice as the times separating the application of inks were larger. In fact, the crossing image of 1 week showed a dense predominance of the P. G2 ink (in red) and very little net-like pattern and dark shades were observed. In the opposite crossing (B. Atlantis over P. G2), it was observed a predominance of the Raman signature of the P. G2 ink (in green) in the central part of the crossing, while the lateral parts were dominated by contributions of the B. Atlantis ink (in red). The continuity of the B. Atlantis ink line separately and throughout the crossing area suggests that this ink line was distributed over the P. G2 ink, in spite of the predominance of the P. G2 ink in the crossing area. This is in agreement with the correct order of application of inks. Additionally, the few gaps that were observed in the B. Atlantis ink line at the crossing area as well as separately may suggest a possible failure of that pen's mechanism.

Regarding the oil-oil crossings involving the B. Cristal and the P. SGrip (Figure 4.8), results showed a net-like pattern in the crossing area that influenced a clear visualization and determination of the predominance of either inks. The net-like pattern, which could also be found in both pen ink lines separately, is typical of oil-based ballpoint pens, where the ink is controlled and applied by frictional forces of a ball as it rolls on the paper surface; given the fact these inks are very viscous, only a controlled amount gets out, resulting in occasional empty spaces in the lines, like the net-like pattern observed. Although rollerball pens use a similar mechanism, the type of ink used in these pens is more fluid and releases a greater amount of ink.

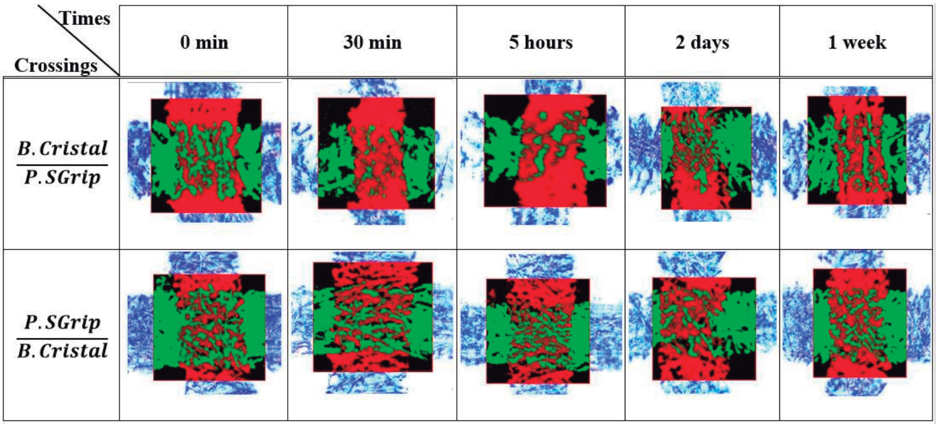

Figure 4.8. Raman images of the crossings using oil-oil blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

Thus, the net-like pattern is not observed with ink lines made by liquid and gel pen inks. In order to test whether the amount of ink applied at the crossing could influence the physical distribution of the oil-oil inks in that area, an additional experiment was carried out in which the same crossings were prepared again, in both permutations, but instead of a single line, three superimposed lines representing more amount of ink. The time separating ink applications was 2 days. Different combinations were prepared: three superimposed lines of both inks, three superimposed lines only of the ink applied first and three superimposed lines only of the ink applied last, resulting in a total of six crossing samples. These crossings were then compared with the crossings made with only a single line of both inks.

In Figure 4.9, the Raman images obtained showed that when there was more ink in both lines, the net-like pattern was no longer visible in the ink lines separately but it was still present in the crossing area, in both crossings.

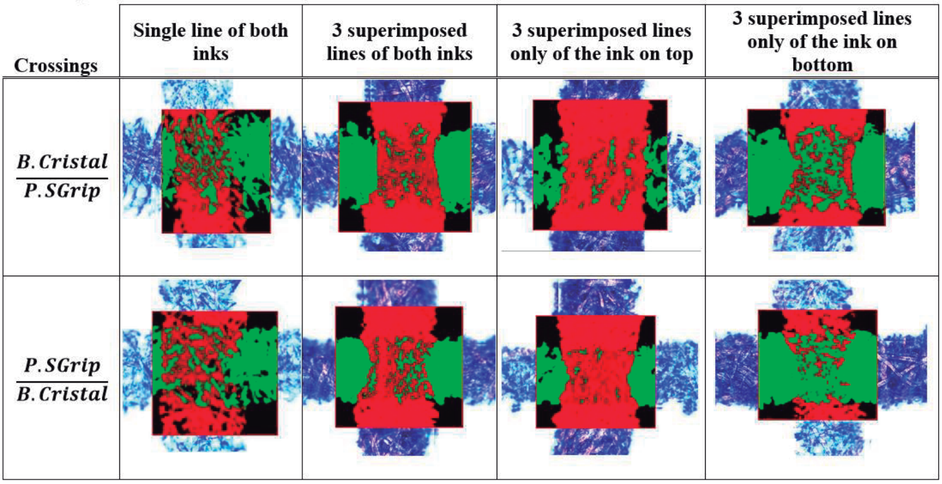

Figure 4.9. Raman images of the crossings using different amounts of oil-oil pen inks and using two days separating the application of each ink line, where the horizontal line was applied first (green).

In that area, the predominance of the Raman signature of the ink applied last (in red) at the surface was more evident. Similar results were observed when there was more ink only in the line applied last (in red). When there was more ink only in the line applied first (in green), it was still possible to visualize the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing in the crossing B. Cristal over P. SGrip in spite of the reduced amount of this ink at the crossing. However, in the opposite crossing, the predominance of the

Raman signature of the ink applied first (in green) could induce in error by mere visual examination. Therefore, these results indicate that the amount of ink in the oil-based ink samples studied can determine the degree of net-like pattern that can be observed in those ink lines separately and also at the crossing area, which influences visualization the most predominant Raman signature at the crossing area.

Regarding the liquid-liquid crossings involving the P. V5 and the U. Eye pens (Figure 4.10), results showed a predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing, for the several times of crossing but only in the crossing P. V5 over U. Eye.

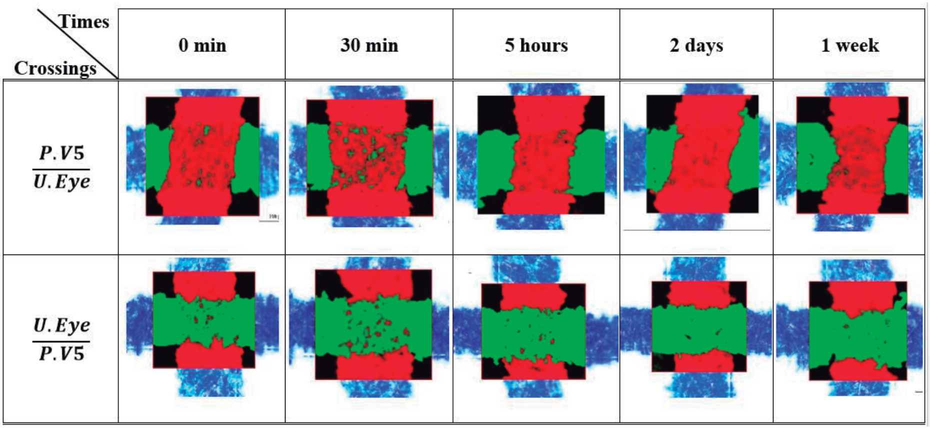

Figure 4.10. Raman images of the crossings using liquid-liquid blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

In this case, the correct order of application of inks was obtained for the several different times separating the application of the ink lines. Additionally, many dark shades and a net-like pattern similar to what was described previously were depicted in every image in both crossings, indicating the tendency for these inks to mix. This pattern became less noticeable as the times separating the application of the inks were longer. Interestingly, in the opposite crossing (U. Eye over P. V5), the Raman signature of the U. Eye pen ink (green) was seen over the other ink, suggesting the inverse order of application. In order to assure that this was not an isolated occurrence, these crossings were repeated and analysed once again. The same results were consistently obtained with each time separating the application of the ink lines, indicating that the distribution of these two inks is seen in the inverse order and that the time separating the application of these two ink lines had no significant influence in their distribution at the crossing (data not shown). These results suggest the complete skipping of the U. Eye pen ink line at the crossing. The results obtained with the crossings using pens with different ink types were examined next. In Figure 4.11, the Raman images of the gel-liquid crossings involving the P. G2 and the U. Eye pens showed the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing for the several times of crossing but only in the crossing P. G2 over U. Eye. This is in agreement with the correct order of application of inks and the times separating the application of the ink lines showed no significant influence in the inks distribution at the crossing. However, in the opposite crossing (U. Eye over P. G2), the order of the inks was the inverse, similarly to the crossings described before with the U. Eye pen.

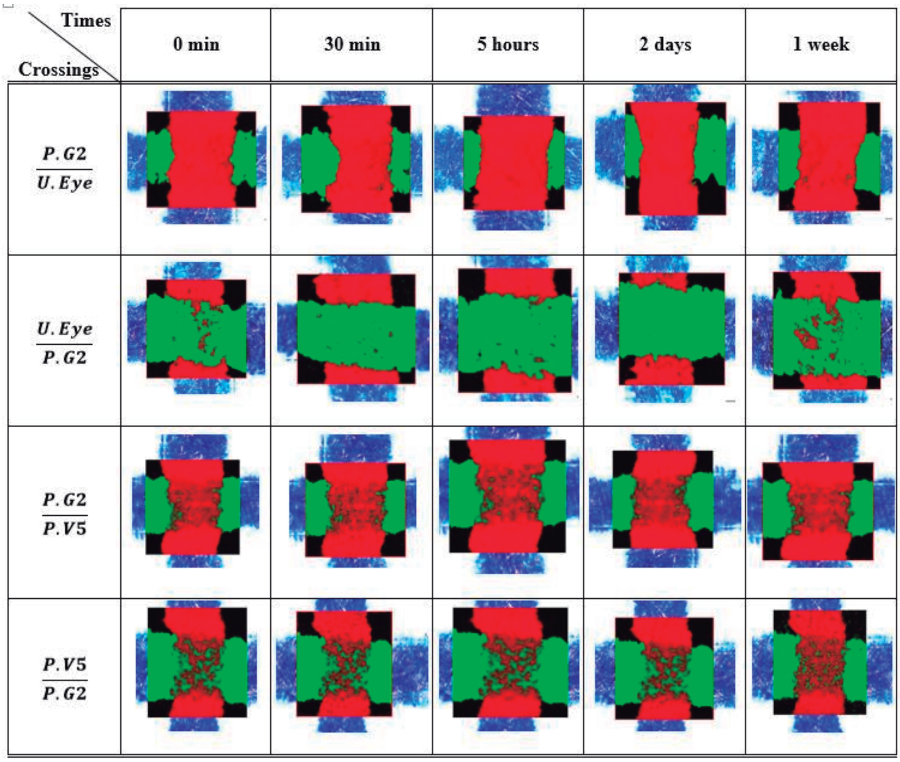

Figure 4.11. Raman images of the crossings using gel-liquid blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

No significant differences were observed in the crossings with different times, apart from some net-like patterns observed in the crossing area. The Raman images of the gel-liquid crossings involving the P. G2 and the P. V5 pens showed the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing, for the several times of crossing and in both crossings. Many dark shades corresponding to the mixing of the two inks can be seen, as described previously. Additionally, the net-like pattern was observed in every crossing area but not in the ink lines separately. Nonetheless, the continuity in the ink line applied last (in red) separately and throughout the crossing areas evidences the correct order of application of inks, contrary to the crossings involving the other liquid-based, the U. Eye pen.

Regarding the gel-oil crossings involving the P. G2 and both oil-based pens, B. Cristal and P. SGrip (Figure 4.12), results showed the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing for the several times and in both crossings.

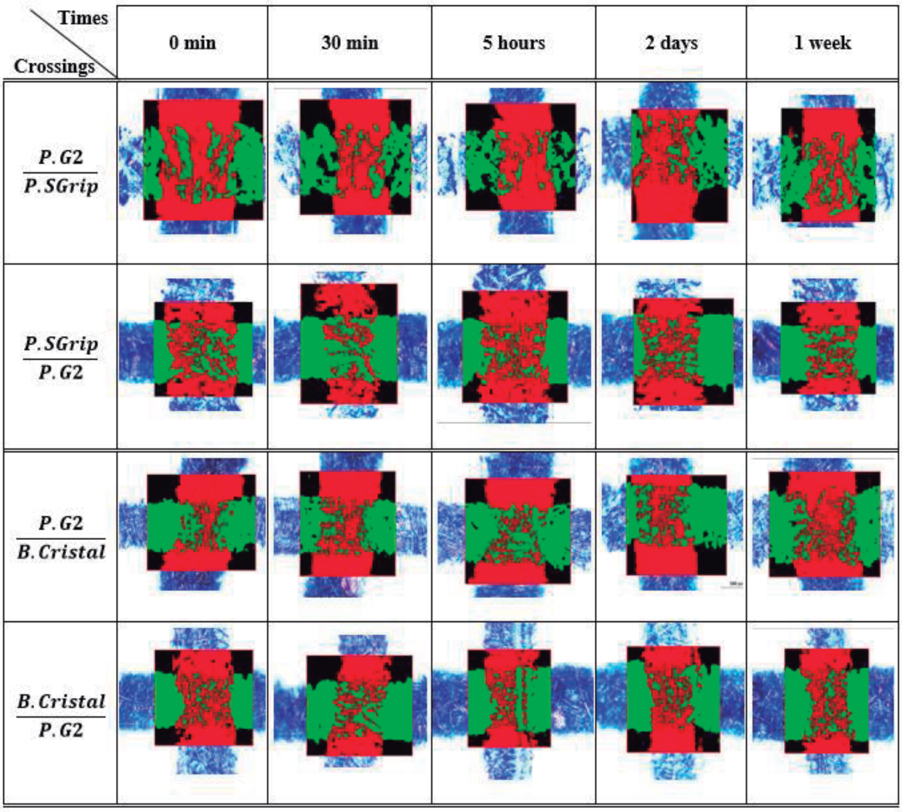

Figure 4.12. Raman images of the crossings using gel-oil blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

Although the net-like pattern observed could greatly influence the visualization of the predominance of the inks at the crossing, examination of the continuity in the ink lines separately and throughout the crossing area indicated more clearly the ink that was applied last. The only exception was observed in the crossing P. G2 over B. Cristal with 5 hours separating the application of inks where the amount of P. G2 ink appreciated in the crossing area was reduced and could induce visualization of the inverse order of application of inks. Furthermore, some mixing of the two inks also occurred in both permutations as the dark shades indicate in all of the crossing images.

In Figure 4.13, the Raman images of the liquid-oil crossings involving the U. Eye and both oil-based pens, B. Cristal and P. SGrip, showed the predominance of the Raman signatures of the oil-based inks at the surface of the crossing, for the several times of crossing and in both crossings, even when they were applied first. This result is similar to the ones previously observed with the U. Eye pen ink. However, in this case, the spectral contributions of the U. Eye were still observed since the oil-based pens left empty spaces in the paper to which the U. Eye ink absorbed to the paper fibres. Overall, no significant differences were observed in the crossings with the different times separating the application of the ink lines. These results were comparable to the ones obtained with the gel-oil crossings.

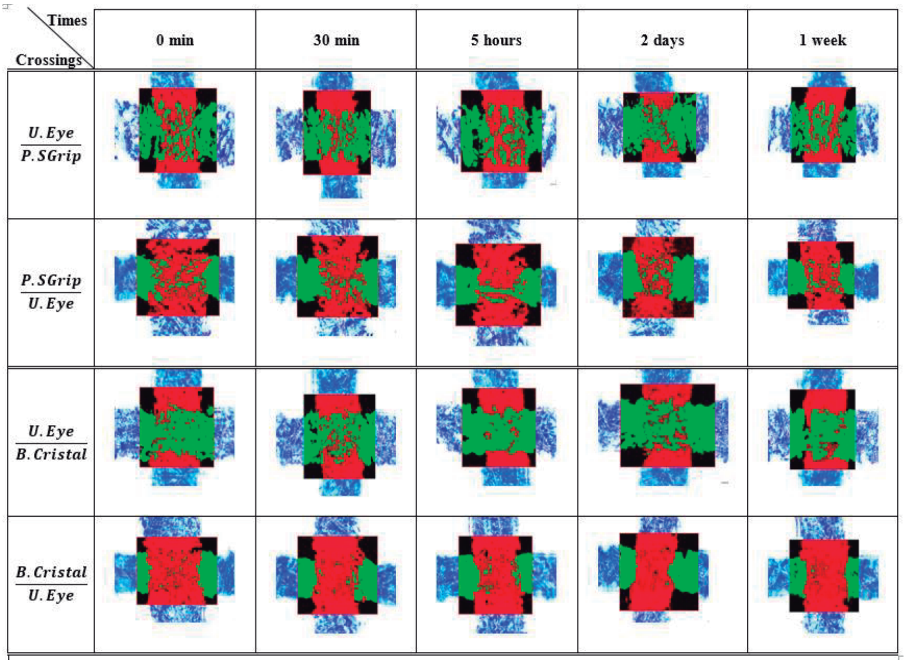

Figure 4.13. Raman images of the crossings using liquid-oil blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

To study the influence that the use of different papers can have in the distribution of crossing ink lines and in the determination of the correct order of application of the inks, all crossing samples were prepared in two other different papers because the type of paper can influence both the inks' penetration and drying process. A recycled office paper and a white paper mostly used to print certificates and official documents were used and so, the three papers differed in terms of colour, thickness and roughness. The inks absorption to the paper is regulated by capillary effects and also depends on the papers porosity [26] but since only a qualitative assessment was intended in this study, additional information regarding the papers porosity was disregarded.

The time separating the application of the ink lines was two days and these results were compared with the same crossings described before in regular white office paper. Regarding the crossings in recycled paper, the Raman signal of the inks separately was very low and at the crossings, all samples gave signals of fluorescence, possibly from the paper (data not shown). Consequently, the results obtained with this paper rendered bad quality spectra and were discarded from this study. Regarding the crossings in certificate paper, results obtained were identical to the ones obtained with white office paper, in terms of predominance of the Raman signature, the net-like pattern in some crossings and the inverse order observed with the U. Eye pen ink.

Concluding remarks

This study showed the use of Raman imaging to determine the inks distribution at the surface of a crossing between two ink lines. All possible crossing combinations using several blue pens of different ink compositions were investigated as well as different times separating the crossing of the lines and different papers.

The correct sequence of crossings was determined for many of the samples examined. One exception included the crossings involving the U. Eye pen ink, where this ink was always seen under the other ink, even when it was applied last. The images indicated the complete skipping of this ink line at the crossing which possibly could have been caused by physical impediments (the first ink applied acting as a physical barrier) or chemical impediments (the two inks did not mix). In most of the other crossings, a net-like pattern was observed only in the crossing area suggesting the partial skipping of the ink lines applied last. In those areas, some mixing between the two inks also occurred and it was more accentuated in shorter times than with longer times since the first ink line was fresher, corroborating the initial hypothesis proposed. Nevertheless, the inks distribution at the crossing was not influenced by the net-like pattern in the crossing area in most of the cases and the order of crossing was consistently the same for the different times. The net-like pattern was also visible in the oil-based pen ink lines separately, which is due to their ballpoint mechanism. Hence, the amount of ink applied by these pens is uneven and leave empty spaces in the line which complicate the examinations and can influence determining the correct order of application of inks with the methodology used in this study. The white and certificate papers used also did not seem to influence in the inks distribution nor determining the order of the inks. Overall, the ink's distribution in the crossing area was non-uniform. Thus, other methodologies that are based on measurements of a few points at the crossing will not be representative and can actually induce the forensic document examiner in making erroneous interpretations.

One limitation of the methodology used in this study was the visual interpretation of the most predominant Raman signature at the crossing area, which resulted, in many cases, unclear to determine the order of application of inks. To overcome this, the development of objective methodologies, able to quantify the predominance of the Raman signatures by probabilistic approaches are necessary in future research. Additionally, the investigation of the nature of the interaction between two crossing ink lines is also required for a better understanding of the physical and chemical processes taking place at the crossing and the influence of the several factors affecting it. Thus, the combination of Raman imaging with other analytical and chemometric techniques is a future trend.

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Conclusions & future outlook

This Thesis described several scientific approaches that can be especially useful in the field of forensic ink analysis of questioned documents. The research studies here described addressed several real issues in this specific field and provided scientific methodologies, mostly based on Raman spectroscopy, that can increase the fundamental knowledge regarding inks on paper and contribute to the improvement of pen inks, printing inks and intersecting ink lines examinations. The introductory Chapter 1 briefly contextualized forensic ink analysis in the field of Forensic Science and Questioned Document Examinations and defined the type of analyses involved, such as discrimination and identification of any of the possible sources (that being the instrument, the manufacturer and the brand, model or batch production) through ink comparison. The discussion of the state of the art regarding Raman spectroscopy for ink analysis of questioned documents remarked the several advantages of this technique over other examinations routinely used, particularly great characterization of inks, chemical selectivity, non-destruction of the evidence as well as instrumental versatility which increase the potential for developing several methodologies based on confocal Raman microscopy and Raman Imaging. This chapter also showed the major challenges that document examiners face when examining inks: on the one hand, the complexity and immense variety of ink formulas available in the modern market of inks and the exact variations and tendencies which are difficult to know. On the other hand, the examinations routinely used seem to provide limited information and/or greatly depends on the examiners knowledge and experience.

The study described in Chapter 2 showed the inter and intra brand, model and batch variability in the Raman spectral signature among blue pen inks found in the Spanish market and identified the main colorants for all pen ink samples with exception of one. It was also found that most oil-based pens have very similar Raman signatures and are difficult to discriminate with Raman, with exception of the Pilot pen ink samples that stood out with a distinct colorant. Different colorants were identified in the gel and liquid-based pen inks but no relation was found among their brands. Again, exception included the Pilot pen inks, where the colorant identified was distinct from the other pens. Additionally, when discriminating several models of gel-based Pilot pens, it was found a large variability between and within the various models analysed, suggesting differences among batches. The potential of Raman for discriminating between batches of pens was finally demonstrated with Bic pen inks, where small spectral differences suggested frequent changes in their chemical formula over the years. In spite of this, the methodology used did not allow further discrimination between pens from the same batch. Although PCA was a simple but useful tool to visualize the spectral relations among a large group of samples, other more powerful chemometric tools may improve discrimination.

The study described in Chapter 3 showed the potential and limitations of the Foram 685-2 Raman spectrometer used by the General Department for Forensic Science Police in Madrid to face the difficult task of discriminating between printed lines made by inkjet models of the same manufacturer of printers. It was found that the main limitation of that equipment for analyzing printing inks was the limited magnification range, which did not allow focusing and analyzing pure ink dots. Nevertheless, this instrument allows a fast and initial classification of printed ink lines. Discrimination was further improved with SERS that reduced the strong fluorescence coming from the samples. SERS spectra were reproducible, and based on minor spectral differences, it was concluded that Deskjet models have different signatures than other professional type models such as Photosmart, Officejet and Business models. Finally, the SERS pre-treatment method that resulted in a less invasive procedure consisted in applying the concentrated colloid before the aggregating agent.

The studies described in Chapter 4 gave a better understanding of the different variables affecting the distribution of inks in a crossing, such as the type of ink and printer, different papers, writing pressure and also time separating the application of inks. Through microscopy examination of cross-sections of overlapping layers of painted inks, the first study of this chapter showed that pen/pen ink samples formed a double layer only in half of the cases studied, such as those involving inks from the same manufacturer and many involving liquid and gel-based inks. Conversely, most samples involving oil-based inks formed mixtures. Regarding the pen/printer ink samples studied, the formation of a double layer was observed whenever the printing ink was applied last. In this case, the printing process was found to be an important variable. Another important variable was the time separating the application of inks, especially with samples involving gel pen inks. This was especially influenced by the drying of the first layer of ink. On the other hand, the different papers and the writing pressure examined in this study showed no significant influence in the inks distribution. Through examination of the Raman images of the surface of crossings, the second study of this chapter showed a partial skipping of the ink line applied last in most cases and even the complete skipping in crossings involving one particular liquid-based pen ink. It was argued that physical impediments (the first ink applied acting as a physical barrier) or chemical impediments (the two inks did not mix) could be the possible reason causing the partial or complete skipping of the ink lines. Additionally, some mixing between the two inks was evident in all crossings and it was more accentuated with shorter times separating the application of inks, which indicated that the first inks drying time was a key variable. Also, the white and certificate papers used also did not seem to influence in the inks distribution. This study further showed that the ink’s distribution in the crossing area was non-uniform which means that methodologies that are based on measurements of a few points at the crossing will not show representative distributions of the inks and can actually induce the forensic document examiner in making erroneous interpretations.

This chapter also proposed two methodologies for determining the correct sequence of intersecting ink lines. The methodology described in the first study showed promising results with superimposed ink lines but there were instrumental difficulties regarding illumination and focusing that lead to a low rate of correct determinations in the blind testing. It is expected that improving illumination and focusing as well as the cutting method could result in more successful determinations. Nevertheless, this method suffers from inherent limitations, such as the fact that it is destructive, the interpretation is visual and dependent of the examiners and influenced by light and focus parameters. Additionally, it should require previous information of the pens used before cutting, in order to know if a double layer can be created in real cases. On the other hand, the methodology described in the second study showed many instrumental advantages, such as non-destructive chemical characterization of the surface of the crossing, the results obtained were reproducible even with different times separating the application of inks, the papers used did not influence the result and the entire crossing area was examined, which was a more representative approach. Finally, the speed of analysis was found to be an interesting advantage for routine examinations. The one limitation found with this methodology was the visual interpretation of the most predominant ink at the crossing area. This limitation can be exceeded with further developing objective methodologies that quantify the predominance of the signature such as using probabilistic approaches. An interesting methodological approach for future investigations could consist in Raman Imaging of the cross-sections of intersecting ink lines for characterizing the inks distribution at the crossing.

This Thesis offered several scientific methodologies to investigate real issues concerning forensic ink analysis of questioned documents but further research is still needed, as it was remarked in every chapter. A future trend is to continue investigating the use of Raman based techniques for ink analysis, not exclusively from a problem-solving perspective but also for increasing the fundamental knowledge about inks, papers and their interaction. Another upcoming tendency is the use of imaging systems such as IR HSI cameras that also analyse the ink’s components and give complementary information to Raman spectroscopy. The main value of imaging systems to forensic ink analysis is the non-destruction nature and the possibility for rapid application to routine casework. Finally, the development of more powerful statistical treatments is required in order to transform the complex chemical data into information easier to interpret.

Conclusiones & perspectivas de futuro

En esta tesis se describen varios estudios científicos que pueden ser de especial utilidad en el campo del análisis forense de tintas en documentos. Los estudios de investigación descritos abordan varias problemáticas reales en este campo y ofrecen metodologías científicas, principalmente basadas en la espectroscopia Raman, que aumentan el conocimiento básico sobre las tintas en el papel y contribuyen a la mejora de los análisis forenses de tintas de bolígrafo o impresora y de los cruces de trazos. El Capítulo 1 contextualizó brevemente el análisis forense de tintas en el campo de las Ciencias Forenses y el análisis de documentos y definió los diferentes análisis forenses realizados, como es la identificación y discriminación de las posibles fuentes de las que proviene la tinta (p. ej. el instrumento, el fabricante, la marca, el modelo o el lote) mediante comparación de tintas. El estado del arte de la espectroscopia Raman para el análisis de tintas en documentos remarcó las diferentes ventajas de esta técnica frente a otros exámenes de rutina, en concreto la espectroscopia Raman permite la caracterización de las tintas, presenta selectividad química, es una técnica no destructiva, presenta una gran versatilidad instrumental dando lugar a, por ejemplo, la espectroscopia Raman confocal y la espectroscopia Raman de imagen, lo que incrementa su potencial para desarrollar diferentes metodologías. Este capítulo, además, describió los mayores retos a los que se enfrentan los peritos de documentos al realizar el análisis forense de tintas: por un lado, la composición de las tintas es muy compleja y existe una inmensa variedad en la composición de las tintas disponibles en el mercado, lo que se complica al desconocer las variaciones exactas y las tendencias en la fabricación de las mismas. Por otro lado, los exámenes de rutina actualmente utilizados parecen ofrecer información limitada y/o dependen en gran medida del conocimiento y experiencia del perito.

El estudio descrito en el Capítulo 2 demostró la variabilidad en los espectros Raman de diferentes bolígrafos azules procedentes del mercado español en términos de marca, modelo y lote. A su vez, se identificaron los colorantes principales de todos los bolígrafos estudiados a excepción de uno. Además, se observó que la mayoría de los bolígrafos de base de aceite ofrecían un espectro Raman similar no pudiéndose discriminar entre sí a excepción de los bolígrafos Pilot, los cuales presentaban un colorante distinto. Se identificaron diferentes colorantes en los bolígrafos de base gel y líquida pero no se observó ninguna asociación entre los colorantes y sus marcas. De nuevo, la excepción fueron los bolígrafos Pilot, que presentaron un colorante distinto al de los otros bolígrafos. Además, cuando se llevó a cabo la discriminación entre los diferentes modelos de bolígrafos Pilot de base de gel se encontró una gran variabilidad entre y dentro de los diferentes modelos analizados, sugiriendo diferencias entre lotes. El potencial de la espectroscopia Raman para discriminar entre lotes de bolígrafos se demostró finalmente comparando los espectros obtenidos de los bolígrafos Bic procedentes de diferentes lotes, donde pequeñas diferencias espectrales sugirieron cambios frecuentes en la composición química de las tintas con el tiempo. A pesar de ello, la metodología utilizada no permitió la discriminación de bolígrafos dentro del mismo lote. Aunque el PCA demostró ser una herramienta simple y útil para visualizar asociaciones entre las muestras de tinta de bolígrafos, otras herramientas quimiometricas más potentes deberían mejorar la discriminación.

El trabajo descrito en el Capítulo 3 estudió el potencial y las limitaciones del equipo Raman Foram 685-2 utilizado en la Comisaría General de Policía Científica de Madrid para hacer frente a la difícil tarea de discriminar trazos impresos con impresoras de inyección de un mismo fabricante. La mayor desventaja del equipo encontrada fue su limitado intervalo de magnificación, por lo que no se pudieron enfocar y analizar puntos de tinta puros. Sin embargo, este instrumento permitió una clasificación inicial rápida de las líneas impresas. La utilización de SERS permitió aumentar la discriminación de las muestras y redujo la fluorescencia proveniente de las muestras. Los espectros SERS fueron reproducibles, y se concluyó que, debido a pequeñas diferencias en los espectros, las tintas procedentes de impresoras Deskjet presentaban diferentes espectros de otros modelos profesionales como son Photosmart, Officejet y Business. Finalmente, un pretratamiento con

SERS menos invasivo consistió en añadir el coloide concentrado antes del agente agregante.

Los estudios descritos en el Capítulo 4 ofrecen una mayor comprensión de las diferentes variables (tipo de tinta e impresora, utilización de diferentes papeles, presión de escritura y tiempo de separación entre la aplicación de las tintas) que afectan a la distribución de las tintas en un cruce de trazos. En el primer estudio de este capítulo se visualizaron cortes transversales de capas de tintas superpuestas mediante microscopia. Las muestras realizadas entre bolígrafos mostraron una doble capa en aproximadamente la mitad de los casos estudiados, por ejemplo, en los casos en que se utilizaron bolígrafos del mismo fabricante y bolígrafos de tinta con base líquida y gel. Por otra parte, la mayoría de las muestras realizadas con bolígrafos con base de aceite dio lugar a mezclas. En lo referente a las muestras realizadas entre bolígrafos e impresoras, se visualizó una doble capa cuando la tinta de impresora se aplicó en segundo lugar. En este caso, el proceso de impresión resultó ser una variable importante. Otra variable importante fue el tiempo de separación entre la aplicación de las tintas, especialmente en las muestras en las que se utilizaron bolígrafos de gel. Por otro lado, los diferentes papeles estudiados y la presión de escritura no mostraron influencias significativas en la distribución de las tintas. El segundo estudio de este capítulo, se centró en el análisis de las superficies de los cruces de dos trazos mediante espectroscopia Raman de imagen. Se observó que en la mayoría de los casos, el segundo trazo aplicado cubrió la zona del cruce de forma parcial, e incluso en las muestras con uno de los bolígrafos de base líquida, no se observó la presencia de esta tinta en la zona del cruce. Esto puede ser debido a posibles impedimentos físicos (la primera tinta aplicada actúa como barrera física) o a impedimentos químicos (las dos tintas no se mezclan). Además, se observó cierta mezcla de las dos tintas empleadas en todos los cruces, lo cual era más acentuado en tiempos menores de aplicación entre tintas. Esto indicó que el tiempo de secado de la primera tinta es un factor clave. Además, tanto el papel de oficina blanco como el papel de certificado utilizados no influenciaron en la distribución de las tintas. Este estudio mostró, además, que la distribución de las tintas en el área del cruce no es uniforme, lo que implica que aquellas metodologías basadas en medidas puntuales en la zona del cruce no ofrecen información representativa sobre la distribución de las tintas, lo cual puede dar lugar a una interpretación errónea por parte del perito.

En este capítulo también se propusieron dos metodologías para determinar la secuencia de los cruces de trazos. La metodología descrita en el primer estudio mostró resultados prometedores utilizando capas de tinta superpuestas pero, debido a problemas de iluminación y enfoque, se obtuvo un porcentaje bajo de determinaciones correctas en los ensayos a ciegas. Cabe esperar que una mejora en la iluminación y el enfoque así como el método de corte den lugar a mejores resultados. Sin embargo, este método tiene sus propias limitaciones ya que es destructivo, la interpretación es visual y subjetiva al analista, además de estar influenciado por parámetros de luz y enfoque. Igualmente, el método requiere de información previa sobre los bolígrafos empleados para una previsión de si una doble capa podría estar presente en los casos a estudiar. Por otro lado, la metodología descrita en el segundo estudio mostró multitud de ventajas como, por ejemplo, la posibilidad de una caracterización química no destructiva en el área del cruce y la obtención de resultados reproducibles incluso cuando se utilizaron diferentes tiempos de aplicación entre las tintas. Además, los papeles blancos de oficina y de certificado utilizados no influenciaron los resultados y el área completa del cruce pudo examinarse, lo que permitió un análisis más representativo. Finalmente, la rapidez de los análisis se considera una ventaja de gran interés para análisis de rutina. No obstante, esta metodología presenta como limitación el hecho de que la correcta determinación de la secuencia de los trazos está basada en la interpretación visual de la tinta más predominante en el área del cruce. Esta limitación podrá superarse mediante el desarrollo de metodologías objetivas que cuantifiquen la predominancia de una tinta, por ejemplo, utilizando métodos probabilísticos. Un enfoque metodológico interesante para futuras investigaciones podría ser el análisis de los cortes transversales de los cruces de trazos mediante Raman de imagen para alcanzar la caracterización de las tintas en el cruce.

Esta tesis presenta diferentes metodologías científicas para abordar problemas reales en el campo del análisis forense de tintas en documentos pero requiere de estudios adicionales, como se ha remarcado en cada capítulo. Una tendencia de futuro es continuar investigando el potencial de la espectroscopia Raman para el análisis de tintas, no solo con la finalidad de dar solución inmediata a casos forenses, sino también para aumentar el conocimiento sobre las tintas, los papeles y sus interacciones. Otra tendencia de futuro es la utilización de sistemas de imagen como son las cámaras IR HSI que permiten analizar los componentes de la tinta y dar información complementaria a la espectroscopia Raman. El valor principal de estos sistemas de imagen para el análisis forense de tintas es su carácter no destructivo y la posibilidad de una aplicación rápida. Finalmente, otra perspectiva de futuro consiste en el desarrollo de tratamientos estadísticos más potentes para transformar la compleja información química en una información más fácil de interpretar.

Acronyms & abbreviations

AD	Array Detectors
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflectance
CCD	Charge Coupled Detector
CE	Capillary Electrophoresis
CH	Carbon-Hydrogen bond
CLSM	Confocal Laser Scanning Microscopy
CMYK	Cyan, Magenta, Yellow and Key (blacK)
Cps	Counts per second
EDEWG	European Document Experts Working Group
EDX	Energy-Dispersive X-ray spectroscopy
EMCCD	Electron Multiplying Charge Coupled Detector
ESDA	Electrostatic Detection Apparatus
FIB	Focused Ion Beam
FT	Fourier Transform
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
HP	Hewlett Packard
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
HSI	Hyperspectral Imaging
IR	Infrared
LASER	Light Amplification by Stimulated Emission of Radiation
LIBS	Laser-Induced Breakdown Spectroscopy
MCR	Multivariate Curve Resolution

MS	Mass spectrometry
mW	milliWattz
NH	Nitrogen-Hydrogen bond
PC	Principal Component
PCA	Principal Component Analysis
PLS-DA	Partial Least-Squares Discriminant Analysis
Rpm	Rotations per minute
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman spectroscopy
SERRS	Surface-Enhanced Resonant Raman spectroscopy
SIMCA	Soft Independent Modelling of Class Analogy
SVM	Support Vector Machine
SWGDOC	Scientific Working Group on Document Examination
TLC	Thin Layer Chromatography
UV	Ultraviolet
Vis	Visible
XPS	X-ray Photoelectron Spectroscopy
XRF	X-Ray Fluorescence Spectroscopy
λ	Wavelength
λ_{exc}	Laser Excitation Wavelength
S/N	Signal to noise ratio
v/v	volume/volume

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Relevant author's contributions

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