Raman Spectroscopy in Forensic Analysis of Blue Gel Inks on Paper

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Raman spectra of different brands of blue gel inks on A4 sheet were measured at uniform condition for all the samples. Spectral results were subjected to background correction and smoothening. The pre-processed spectral data were used for carrying out PCA (Principal Compound Analysis). Through the combination of Raman Spectroscopy (RS), Pearson's correlation calculation and statistical method (PCA) we were able to differentiate among very similar ink types in documents. The correlation value of (E,U)=0.995 and (D,U)=0.994 for pair wise combination of RS and similarities in loading plots in terms of PCA helped in identifying correctly the unknown brand of blue gel pen. Thus we demonstrate the effectiveness of Raman spectroscopy is identifying the usage of different inks in a document which could possibly be taken forward for forensic applications in the Indian context also.

Keywords: Raman Spectroscopy; Non-Destructive Analysis; Forensic Analysis; Legal Documents

Introduction

Forging and *malafide* alteration of legal and other essential documents are becoming increasingly sophisticated with the up gradation of technology. Identifying such malpractices is of utmost importance to various stakeholders. Among the various forms of forging a document, altering with a different ink or dye is commonplace. Preservation of the sample calls for a non-destructive technique of identification of such forgery. So distinction between inks is important for forensic document analysis where the document under investigation is suspected of forgery by ink. Raman spectroscopy (RS) is an optical technique that can be used in a non-destructive manner for the forensic investigation of documents in order to detect forgery.

S. E. J. Bell *et al.* from United Kingdom distinguished different inks based on their spectral variations (S. E. J. Bell *et al.,* 2013). In 2012 Raman Spectroscopy was used for the differentiation of old marker inks and new marker inks by A. Sodo *et al.* (A. Sodo *et al.,* 2012). Differentiated inks by thin layer chromatography (TLC) were examined by using

Raman Spectroscopy by Thomas Andermann from Germany (Andermann 2001). Recently Juan and co-workers from Spain reported the method for the differentiation of very similar ink types in documents by using the combination of Raman spectroscopy and chemometrics (Castro *et al.,* 2008, Prieto *et al.,* 2010). All the previously reported methods deal with document analysis of different inks available from different countries except India (Braz *et al.*, Grechukha *et al.,* 2017, Tãnase *et al.,* 2012, Lee *et al.,* 2016). Objective of this work is to distinguish the various commercially available inks from the Indian market by using Raman Spectroscopy. In order to achieve our objective, few commercially available blue gel inks were collected from Indian market and analyzed by using RS in combination with principal component analysis (PCA). Accuracy of distinction of inks can be increased by using mathematical tools like Pearson's correlation calculation and PCA on Raman data.

Principal Component Analysis (PCA) in combination with RS forms a very significant tool to analyse any heterogenous medium. There have been many reports ranging from the material analysis to

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biological applications in differentiating cells where the Raman data was analysed by PCA and extremely interesting revelations could be made possible (Berru *et al.,* 2007; Khan *et al*., 2017; Jason *et al.,* 2006; Umapathy *et al.,* 2015) transforms a dataset with the goal of representing the data variance in few uncorrelated axes called principal components. PCA provides a dimension reduction that has the advantage of facilitating the identification and visualization of the data structure. The PCs (Principal Components) are linear combination of the original variables and describe the data variance in decreasing order. The PC1 indicates direction of higher variability of the data; the PC2 indicates second highest variability in the data uncorrelated to the first one and so on.

Materials and Methods

Sample Collection

Five brands of blue gel pens Claro, Rey Water Proof, T-Max, Diffy and LinkMax labelled as A, B, C, D and E respectively were purchased from the local stationery storeand were used in this study. Few sentences were written with these pens on the A4 sheet of white paper (locally purchased) and individual Raman spectra of each of these inks were measured. A volunteer was requested to choose any one of the pens confidentially and write a few lines on the paper and the same was classified as unknown (U) for our measurement and subsequent analysis. The whole design of the work was to identify which brand of the pen was used by the volunteer through.

Raman spectra were measured using B&W TeK*i* Raman plus Spectrometer with 532 nm cw laser light as the source of excitation (shown in supplementary information as Figure S1). A single converging lens coupled with multimode fibre was used for both excitation and collection and the Raman photons (Stoke's) were dispersed using a grating (with groove density 2400 grooves/mm). These were finally detected with a CCD (14µm x 0.9mm: 2048 pixels). The measured spectra were subjected to background subtraction and smoothening (FFT filter).

The measured spectra were subjected to background subtraction and smoothening (FFT filter). The Pearson's correlation matrix calculations of all the possible pair wise combination were collected from XLstate 2018.1 version while the PCA was performed using UNSCRAMBLER software (trial version).

Results and Discussion

Raman spectra of all the five samples along with the one marked as U show no influence from the paper. This was confirmed by measuring the spectrum of the ink as well as the same ink on paper. The Raman response from the plain paper without the ink on it resembled only to the noise profile, thereby precluding any interference from the paper to the Raman response of ink on it. Spectra of all the samples looked almost similar whereby visual differentiation of the spectra was not possible. In Fig. 1 we show an illustrative Raman spectrum of the unknown sample labelled as U. The goal is to ascribe the spectrum to the correct brand using the spectrum as well as PCA.

In Fig. 1, the Raman spectrum of the unknown sample is depicted which shows peaks in the region between 500-1600 cm^{-1}. The spectrum exhibited good repeatability and reproducibility. Each Raman spectrum of a particular ink sample was measured 5 times and the average of the measurements were used for further analysis. Since the spectra were all measured in identical condition, we did not normalize them for the initial analysis using the spectra alone. All the spectra were recorded with 1 min integration time and 8.7 mW laser power.

Raman spectrum of the unknown sample was overlapped with that of the Raman spectrum of other brands and the same are shown in Fig. 2 and 3. The
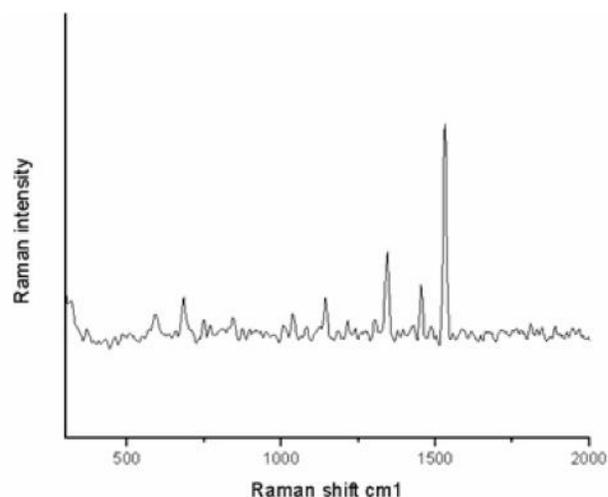

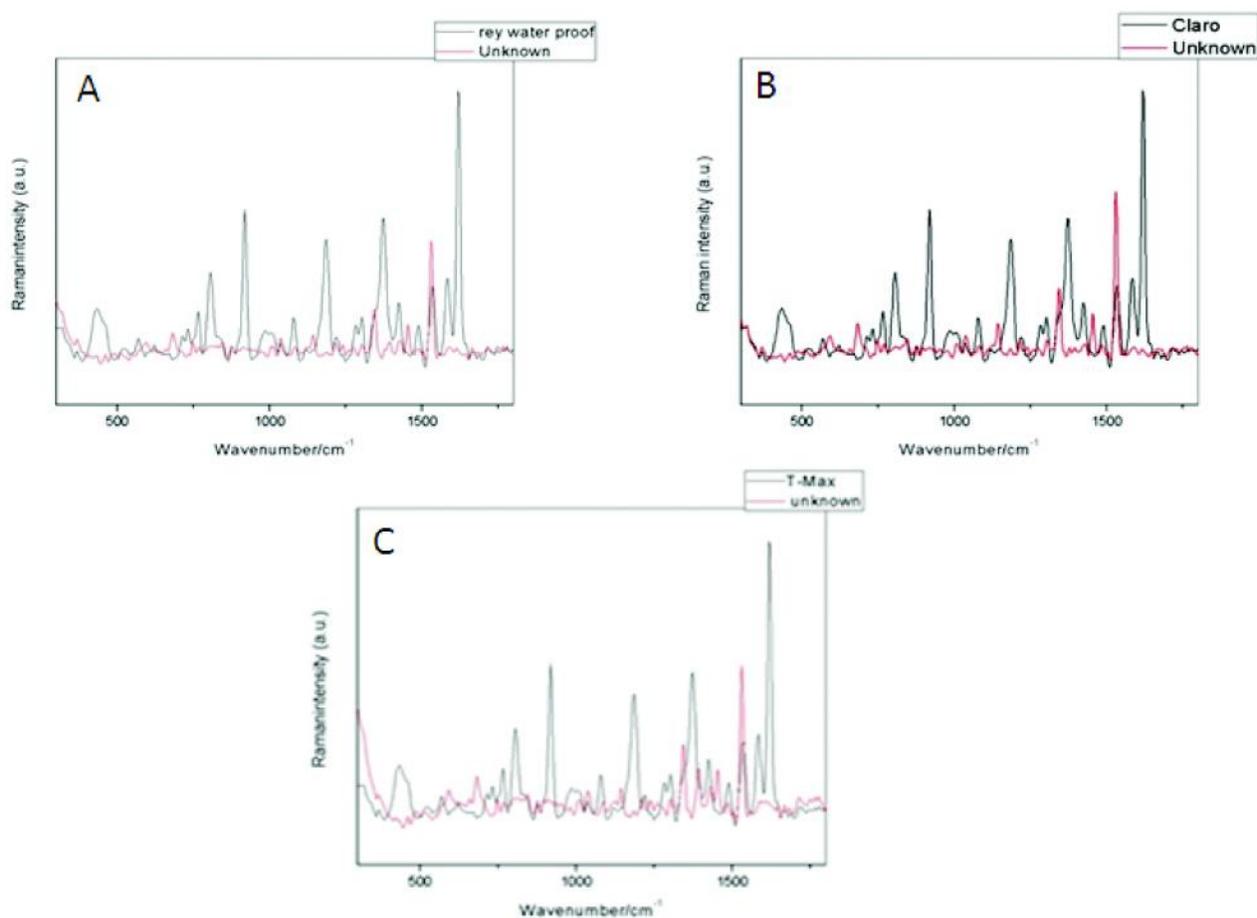**Fig. 1: Raman ink spectrumof the Unknown sample**

Fig. 2: Overlap of the Raman spectrum of the unknown sample (U) with the Raman spectrum of samples A, B and C in a, b and c respectively

overlap between the Raman spectrum of the unknown and those of samples A, B and C are not significant as seen in Fig. 2 a, b, and c respectively. Whereas, we can observe that the Raman spectrum of the unknown sample significantly overlap with that of sample E's Raman spectrum as seen in Fig. 3b. It is not possible to right away conclude that the unknown sample is Link-Max because there is an appreciable overlap with the Raman spectrum of sample D as well, as seen in Fig. 3a. Hence by visual inspection of the Raman spectra we can only conclude that the sample marked as unknown could either be sample D or E which correspond to the brands Link-Max or Diffy respectively. This necessitates us to undertake PCA methods for further differentiation.

Pearson's Correlation Matrix Calculation

As mentioned earlier, Pearsons's correlation matrix calculation helps in assessing linear relationship between two variables. For this, we had taken the intensity values of all the data points obtained from the spectrometer between 300 and 1600 cm^{-1}. This intensity data from 6 different samples (A to E with unknown U) served as 6 vectors and their Pearson's correlation was calculated and the same is shown in Table 1 below. Correlation values approaching unity indicates strong correlation and vice versa.It is to be noted that even though we observed that the Raman spectrum of the unknown does not overlap with samples A, B and C, yet we have considered them for the correlation analysis in order to validate conclusion which was driven by visual inspection with a quantifiable method. We wish to highlight here that all the measurements were carried out under identical conditions. Hence the intensity values were not subject to any normalization for this analysis, initially. Later we repeated the same analysis after normalizing each of the vectors and the correlation matrix was identical to Table 1.
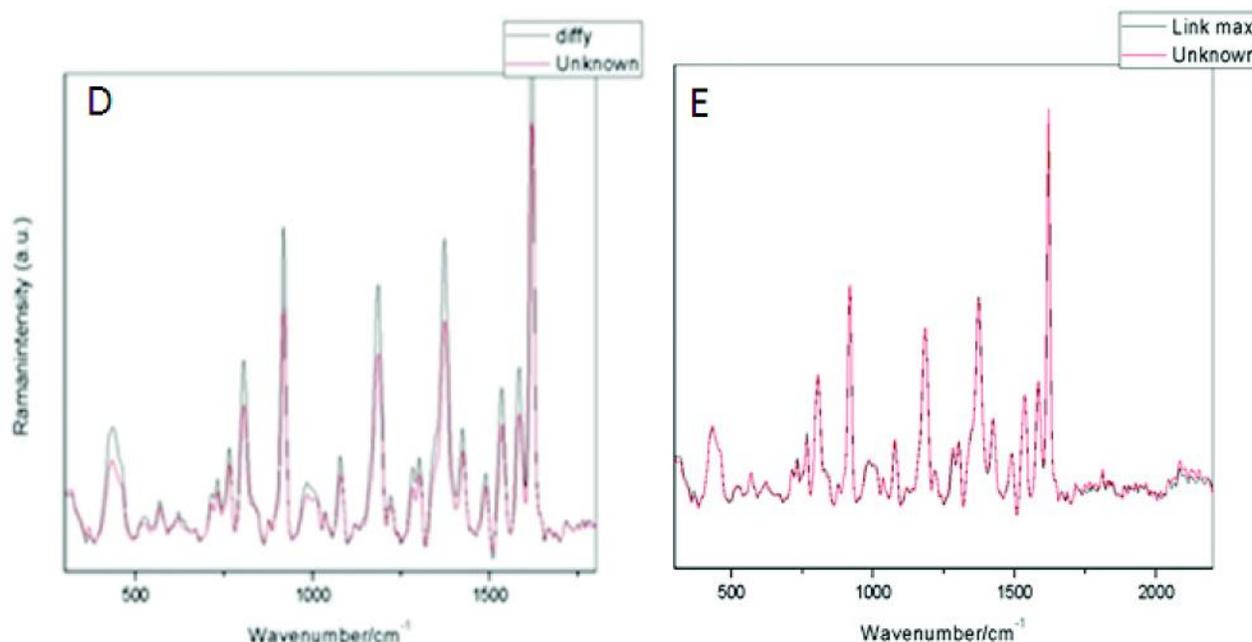
Fig. 3: Overlap of the Raman spectrum of the unknown sample (U) with the Raman spectrum of samples D and E in d, and e respectively

Table 1: Pearsons correlation values of Raman scattered intensity values of different pens and unknown sample

Variables	A	B	C	D	E	U
A	**1.000**	0.845	0.941	0.103	0.113	0.108
B	0.845	**1.000**	0.962	0.128	0.109	0.122
C	0.941	0.962	**1.000**	0.127	0.120	0.126
D	0.103	0.128	0.127	**1.000**	0.992	0.994
E	0.113	0.109	0.120	0.992	**1.000**	0.995
U	0.108	0.122	0.126	0.994	0.995	**1.000**

From the correlation values of the five gel pen inks calculated against the unknown ink, it is interesting to note that the pair wise combination of pen D and E with U gives the values r = 0.994 and 0.995 respectively. This indicates that the unknown could either be pen D or pen E The correlation analysis indeed validates our earlier conclusion drawn by mere visual inspection of the Raman spectra that the unknown could not be samples A, B or C. Hence from this pairwise correlation analysis, it cannot be conclusively declared which one of the pens D or E was used as the unknown.

Principal Component Analysis (PCA)

PCA through its score plot and loading score allows all samples (which appear as clusters of all variables) to be assessed for similarities using the principalcomponent scores associatedwith the samples. In this case the "closeness" of PC scores oftwo samples indicates that they are similar and vice versa. This is used to identify and magnify differences and similarities among the ink samples.

The loading plots obtained from PCA analysis of RS dataset are shown in Fig. 4. Each dataset explains the variation of all the variables of the samples. We see four clusters labelled as K, L, M, and N in the loading plots. The cluster labelled as K in the above figure is representative of samples D, E and the unknown U. The other clusters, namely L, M
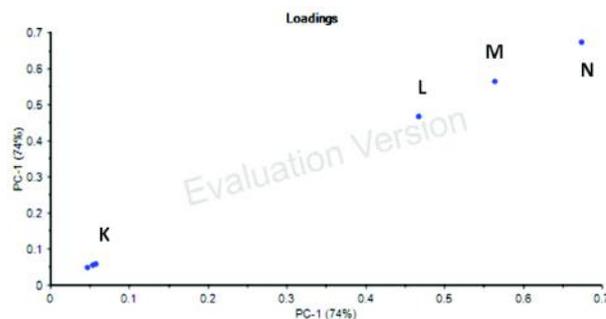

Fig. 4: Loading plot from the PCA of the Raman data of all the samples

and N are quite distinct from each other and also are far from the location of U in the loading plot. These finding indicate that the unknown ink could have originated from brand D or E. Going by the results from overlapping the Raman spectrum of U with D and E, from the correlation analysis and PCA, we can conclude that the unknown sample originates from sample E. The analysis also shows that sample D could also be a probable candidate indicating that the molecular constituents of inks in the brands Link-Max and Diffy are approximately similar.

Conclusion

The combination of direct visual examinations of RS with chemometric analysis using Pearson's correlation and PCA for source determination of the unknown blue gel pen inks was successfully accomplished. The PCA had successfully linked the unknown ink with sample E (Link-Max) which was the actual pen used to write the text on paper. This is a promising methodology to analyse questioned documents with Raman spectroscopy and PCA to resolve any issues related to forensic analysis.

Acknowledgements

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Supplementary Information:
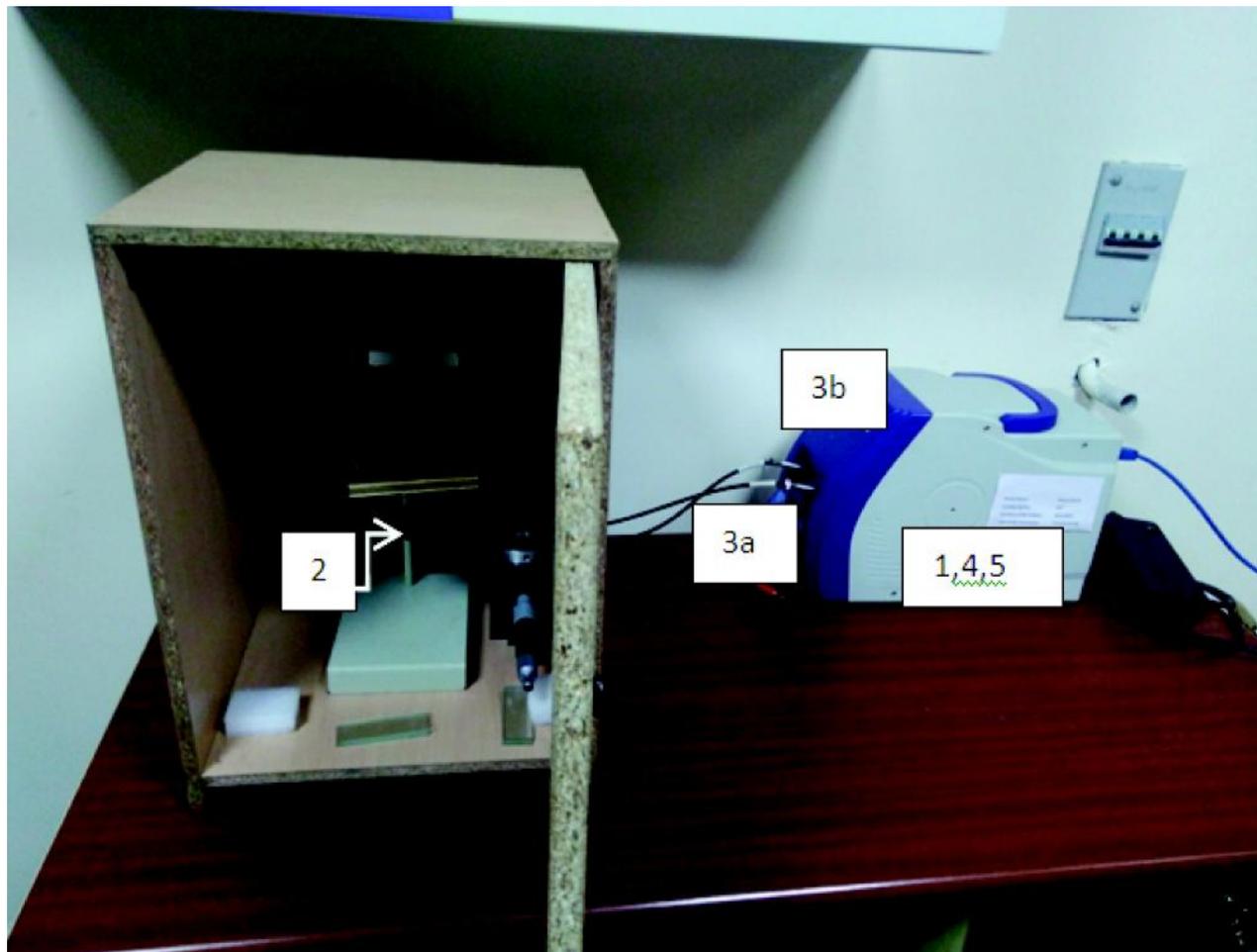
Figure S1: Photograph of the B&W TeK*i*Raman plus Spectrometer

Thisis the photograph of the Raman spectrometer that was used for the study reported in the main manuscript. In this spectrometer 532 nm cw laser light is the source of excitation (labelled as 1 in the figure). A single converging lens (2 in the figure) coupled with multimode fibre was used for both excitation (3a in the figure) and collection (3b in the figure) and the Raman photons (Stoke's) were dispersed using a grating (with groove density 2400 grooves/mm) (4 in the figure). These were finally detected with a CCD (14μm x 0.9mm: 2048 pixels) (5 in the figure). Components 1,4 and 5 are all housed inside one unit that is seen in the figure