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Blue Ballpoint Pen Inks Differentiation using Multivariate Image Analysis of Digital Images Captured with PhotoMetrix PRO®

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Abstract. In Forensic Documentoscopy, it is frequently questioned if a particular document was written with one or more pens. Different methods have been developed to distinguish pen inks from each other, but some of these techniques require the ink extraction, destructing the document, and other techniques uses high cost instruments. PhotoMetrix PRO®, an app for mobile devices, is a qualitative and colorimetric analysis tool that applies uni- and multivariate analysis. Amongst them, Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Partial Least Squares Discriminant Analysis (PLS-DA) can be obtained from digital images' decomposing data. It is a non-destructive and a simple method, of easily use and low cost. Chemometric knowledge is important for results interpretation. This study aims to evaluate the PhotoMetrix PRO® capacity on blue ballpoint pens differentiation. Three experiments were performed with different ballpoint pens, including colorful pens as an app functionality control. The results showed appropriate differentiation between colorful ballpoint pens, and there was a satisfactory tendency of separation for different brands of blue ballpoint pens, most used in Brazil. This method is interesting to confirm subjective results, eliminating visual differences, intrinsic for each observer, which can be useful in places with an instrument deficiency, like the Video Spectral Comparator®. New studies to evaluate writing pressure's

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influence on data collection and the inks' age, as well as different mobile's camera quality, are recommended. This study introduces a new technology that might be further studied for practical application in Forensic Documentoscopy and other Forensic areas.

Keywords: Documentoscopy; Ballpoint pen ink differentiation; Multivariate statistical analysis; Digital images; PhotoMetrix PRO®.

1. Introduction

Forensic Documentoscopy aims to analyze information concerning a document history, its authenticity or inauthenticity and different kinds of alterations. Considering handwriting examination, sometimes it is necessary to demonstrate if a particular document was written with one or more pens, and this information may be related to fraud by writing in two different moments. In order to detect this kind of fraud, it is necessary to analyze the documents' pen inks[1].

Ink analysis has a great importance in Forensic Science. In Brazil, documentoscopy experts are frequently requested to solve questions regarding differentiation of pen inks, and about 80% of cases involve ballpoint pens[2].

There is not a pattern formula for pen inks fabrication. For this reason, the inks' components vary in their quantities between different brands and also between different regions in the world[1,3]. In this context, it is important to develop appropriate methods to differentiate pen inks from each other, and both the cost and the simplicity of the method should be evaluated. Sometimes, proving that two different pens were used in a document can provide answers in specific judicial cases, when a document has been scribed and re-written with a pen of similar color, or in a case with writing addition on the original document[4]. For example, a medical certificate can be altered on its days' quantity, a bank check could have additional numbers and text to increase its value, and work contracts might have altered information for retirement purposes.

In the past few years, many methods have been developed for ink characterization[5]. Some of those methods were designed as destructive for the document, when it is necessary to cut a part of the document to extract the pen ink, which is not the best option for Forensic Science. Some of the published papers describing pen inks differentiation using destructive techniques included HPLC (High performance liquid chromatography)[6,7], Orbitrap Mass Analyzer[8,9]; UV-Vis (UV-Vis Spectroscopy)[10,11]; Capillary Electrophoresis[12] and LDI-MS (Laser Desorption/Ionization Mass Spectrometry)[13]. Regarding non-destructive methods, in which the ink is analyzed directly on paper, without cutting the document, there are studies including FTIR (Fourier Transform Infrared Spectroscopy)[7,14,15], Raman Spectroscopy[16,17], LIBS (Laser Induced Breakdown Spectroscopy)[18,19], ToF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry)[20], PS-MS (Paper Spray Mass Spectroscopy)[21] and Video Spectral Comparator (VSC) - VSC®6000/HS, *manufactured by Foster + Freeman, UK*[22]. These studies demonstrated appropriate pen ink differentiation and characterization, providing different possibilities for ink analysis. However, those technologies require expensive instrumentations. Mass Spectroscopy (MS) and Spectroscopic Methods are among the most studied techniques for ink differentiation analysis, besides optical methods as VSC. MS approaches utilize different sources to induce the compounds ionization, and then the ions are separated according to their mass-to-charge ratio (*m/z*). Spectroscopic techniques are based on electromagnetic radiation, which can change a molecule`s energy level[23]. Those techniques generate chemical data that could be also visualized and separated as a pattern in a multivariate analysis, and Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are widely used techniques to explore this kind of data. PCA consists on data matrix transformation, which can represent the high number of variables into a small number of factors, reducing the experimental dimension. HCA is a useful analysis to determine objects' similarity and to identify anomalous samples[24]. Multivariate analysis has been successfully applied to pen ink analysis using data acquired from non-destructive methods such as UV–Vis-NIR spectroscopy[10], infrared spectroscopy[15] and Raman spectroscopy[16,17].

Multivariate image analysis (MIA) comprehends a set of tools that can be applied on characterization of many different samples, such as PCA and HCA, considering its pattern recognition power. Partial Least Squares Discriminant Analysis (PLS-DA) is another analysis that can be performed using MIA. PLS-DA method can be used to obtain linear correlation between different measured concentrations, but it can be also used to obtain possible congruence between different factors[25], such as different ballpoint pen brands.

PhotoMetrix PRO®[25] is a colorimetric analysis tool, developed for mobiles devices, which applies univariate and multivariate analysis, including PCA, HCA and PLS-DA. This app uses the mobile camera to capture digital images and, so that can proceed with multivariate image analysis, those images are decomposed into scores and loadings. This technique has been previously applied for environmental[26,27] and food analysis[28,29].

Therefore, the objective of this study is to evaluate the PhotoMetrix PRO® capacity on differentiating blue ballpoint pen inks, using common pens brands in Brazil. The app is a simple non-destructive method that can provide useful information to discriminate samples in the Forensic field; also, it is a fast, easy-handling and a low-cost tool.

2. Methods

2.1 PhotoMetrix PRO app for mobiles devices

PhotoMetrix PRO® App[25] is available free for Android, Windows Phone and iOS smartphones, and it can be downloaded on http://www.photometrix.com.br. PhotoMetrix PRO® is a qualitative analysis of digital images, which is constituted of pixels. An image is the result of light, absorbed, reflected and emitted by a surface, and the transmitted energy can be captured by a camera. RGB is a color model to represent the red, green and blue colors, captured and perceived in a different combination of colors by the human eye. Other types of color models include hue (H), used to differentiate red from yellow, saturation (S), for the distinction between red and pink, and intensity (I), which is a characteristic that can differentiate light from dark colors. Value (V) is a maximum measure of the RGB channel, while luminosity (L) is the minimum and the maximum averages of the RGB channel[25]. PhotoMetrix PRO® uses those channels for images acquisition and processing into scores and loadings when it computes PCA. The HCA embedded works through Euclidean distance with three options methods: average, complete and single linkage. This application offers two types of data matrix build: RGB histogram or channels averages. PLS-DA is based also with singular value decomposition using NIPALS algorithm.

2.2 Inks collection

Initially, all inks were applied on paper - blank paper sheets of same brand, with 75g of weight. The inks were applied by filling in squares, using lines of same pressure, and by writing a random word (“pen”), as represented in Figure 1a and Figure 1b (Figure 1). All inks were applied on paper by the same writing fist and then the inks were allowed to dry for one day. The ink deposition was set this way in order to uniform the ink quantity for all pen brands and types. The experiments were conducted on images collection from the squares (set as an uniform pattern for each ink) and from the graphisms (simulating real cases), and then compared.

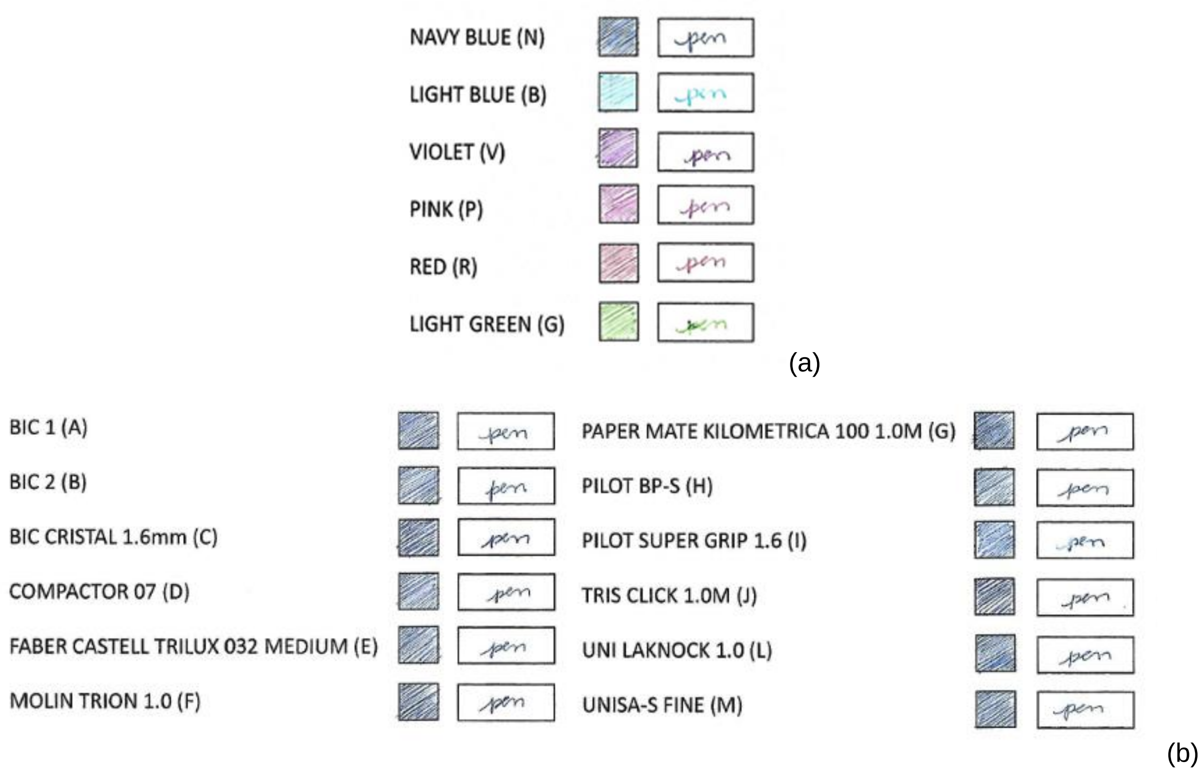


Figure 1. Pen inks application on paper, so that images could be captured with the PhotoMetrix PRO® app. All the different brands are also described, as well as their codes for PCA and HCA graphics. a) colorful ballpoint pens; b) blue ballpoint pens.

First, six ballpoint pens of different colors (navy blue, light blue, violet, pink, red and light green) were analyzed as a preliminary study, to evaluate the app functionality and to define the images capturing conditions. Figure 1a shows all the colors, the codes used for data display, and the template used for inks collection.

After establishing the app capability of distinguish different visual colors, twelve blue ballpoint pens, of nine different brands, were tested. The intention of this work was to evaluate different blue ballpoint pen brands, and those pens were chosen considering their availability in the Brazilian market (specifically stores located in Porto Alegre city, state of Rio Grande do Sul). For Bic® and Pilot® brands, more than one type of pen was found. Also, two pens of identical types of Bic® pens were used to evaluate the behavior of inks from the same brand and type. Figure 1b shows all pen brands and types used in this study, their codes for data display and the template utilized for inks collection.

2.3 Images collection

All the images were obtained using two different mobiles devices: a G5 Motorola® smartphone operating with Android 8.1.0 system, with a 13MP rear camera, and a Samsung® Galaxy A8 smartphone, operating with Android 9 system, and a 16MP rear camera. Figure 2 shows the established parameters for images capturing. After programing the number of samples and the region of interest size (defined at 32x32 in this study) at Settings, we chose Multivariate Analysis, PCA Analysis, Sampling, and then mean method. Concerning single channels parameters, using all channels but luminosity was the best condition in this study. All images were collected in triplicates in the exact same spot on paper, as an internal control for each pen brand. In order to collect three images of the same spot, the mobile device was positioned on a stable platform. Different heights (6 and 8cm) were tested for image capturing, and no influence of height was observed. However, the same height (8cm) was established for both mobile devices to maintain a pattern, and this distance was appropriated to focus the camera for taking the pictures. All the images were collected under natural ambient luminosity. After collecting all the pictures, PCA graphs scores are provided and it is possible to take a screen shot of the results, for both PCA score graphs and PCA loadings graphs. HCA analysis can be performed with the same data, by choosing HCA Analysis and Re-Processing.

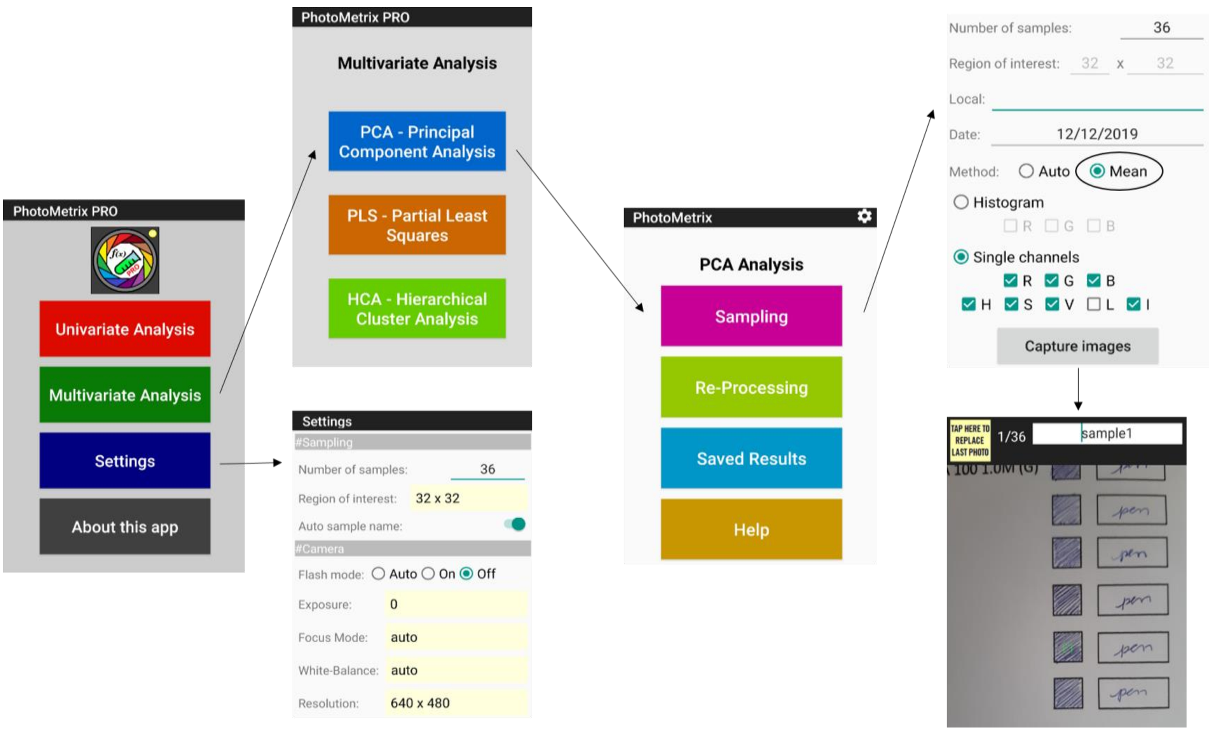


Figure 2. Schematic PhotoMetrix PRO® configurations for this study.

All the experiments were performed at least five times, at the same day and at different days for both devices, to evaluate the app repeatability. There is a small variability concerning each sample position in the PCA graph, considering a possible variability for image capturing. The best graphs scores were chosen considering the triplicates proximities, demonstrating adequate images collection for each pen brand.

Qualitative pen brands differentiation is evaluated considering the distances between each brand triplicates in the PCA graph score.

2.4 Method confirmation

After PCA and HCA analysis, the PLS-DA method was applied to confirm PhotoMetrix PRO® capability to discriminate different pen brands, separated at the PCA graph score. The PLS-DA method was conducted as follows (Figure 3): a) calibration model: two sets of five images of each pen brand were collected, using "0" for images not belonging to the class and "1" for images belonging to the class in question; b) validation: sample collection with known classes, using "0" and "1" in the same way as above; c) blind samples: collecting the same samples without informing their classes. Both validation and blind samples collection should also be positioned as "0" and "1" in the plot graph, as the calibration model. Sample sets distance and its calculated errors will show the difference between the two analyzed colors. All confirmation tests were performed using image capturing from the squares, described at Figure 1b.

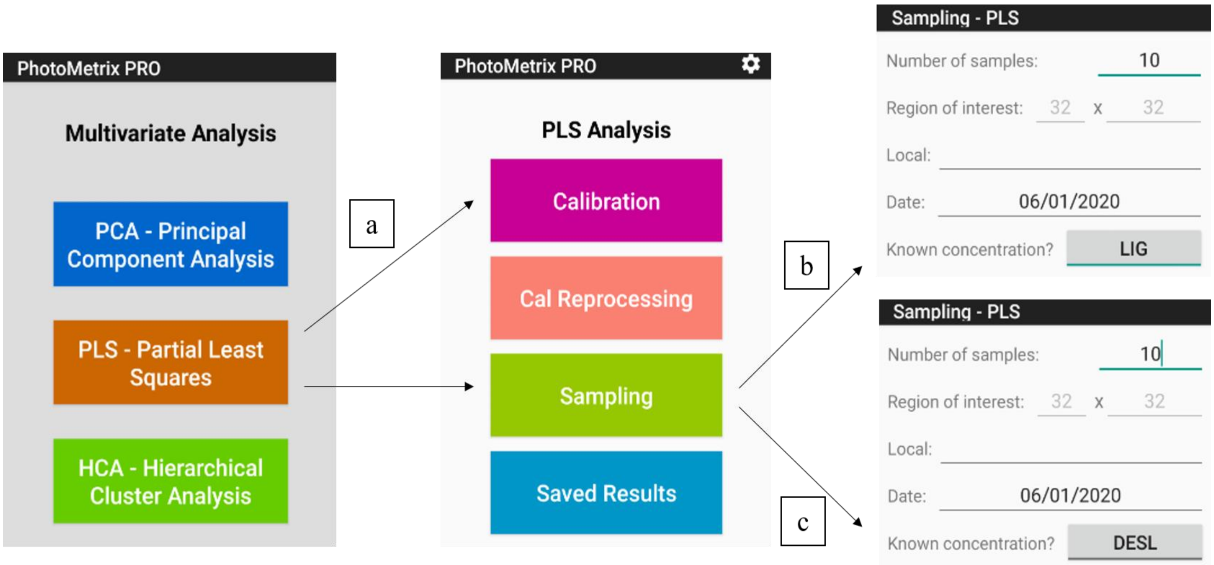
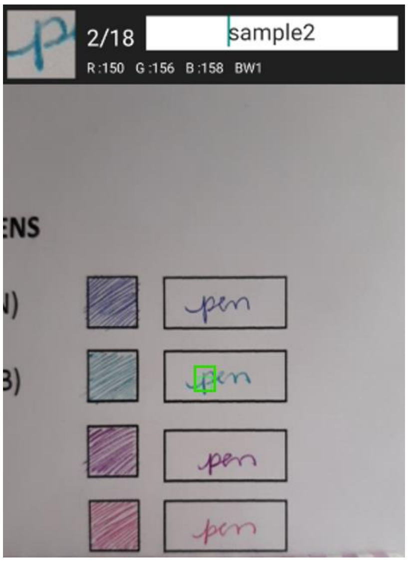


Figure 3. Schematic validation model using PLS with PhotoMetrix PRO®.

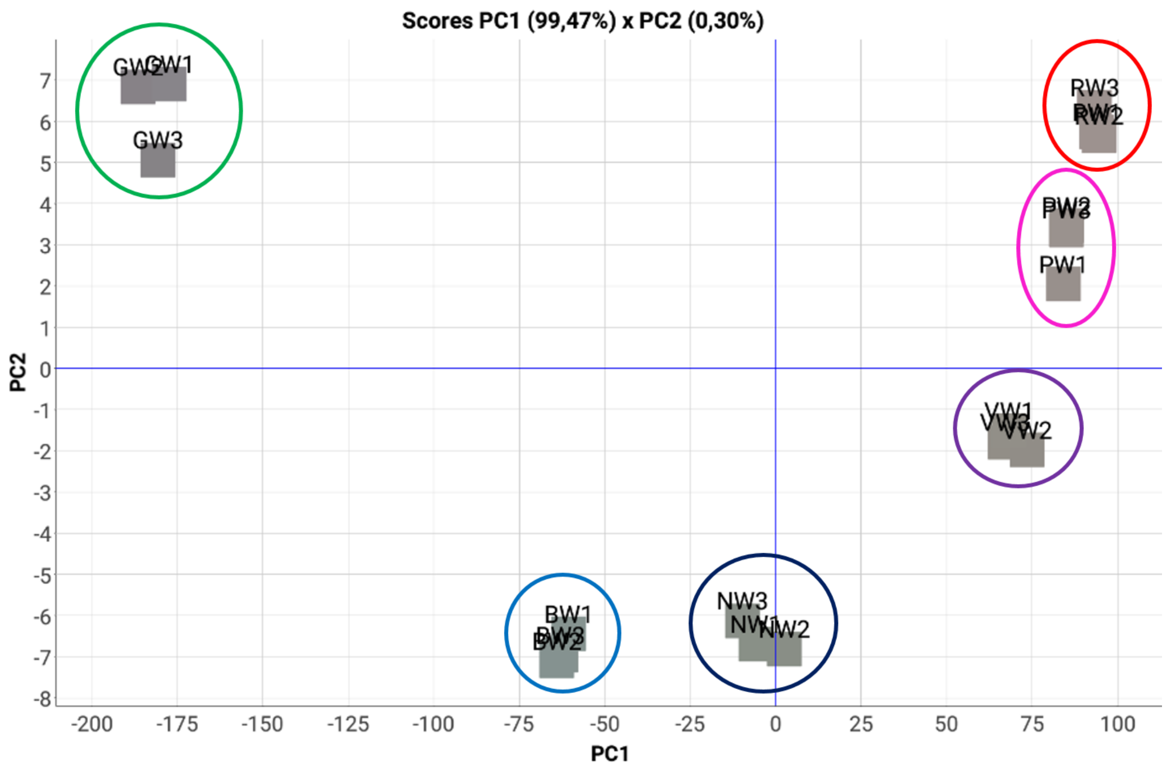
3. Results

For colorful pens, both squares and graphism images showed similar results. Figure 4 shows the results for image collection from the graphism "pen", using three images from the same region represented in Figure 4a, for the colorful pens (figure 1a). We can observe a great differentiation between those pens in the score chart, which was expected, and it is also shown that inks of similar color grouped together (red, pink and purple close to each other; navy blue, light blue and green separated). Those results were obtained using both mobiles devices, Motorola® and Samsung®, even though some differences can be observed between them. Both graphics of two principal components (PC1xPC2) explains 99% of the experimental variation (figure 4b and 4c). Through the analysis of PC1 loading graph (figure 4d), we can also observe that this differentiation occurred in function of hue (H), and for PC2 loading graph (also on figure 4d), the RGB channels are more representatives for colors discrimination, especially red (R) and green (G) channels. HCA analysis also demonstrated perfect differentiation of the colorful ballpoint pens, grouping the same color triplicates for both devices (Figures 4e – Motorola® - and 4f - Samsung®).

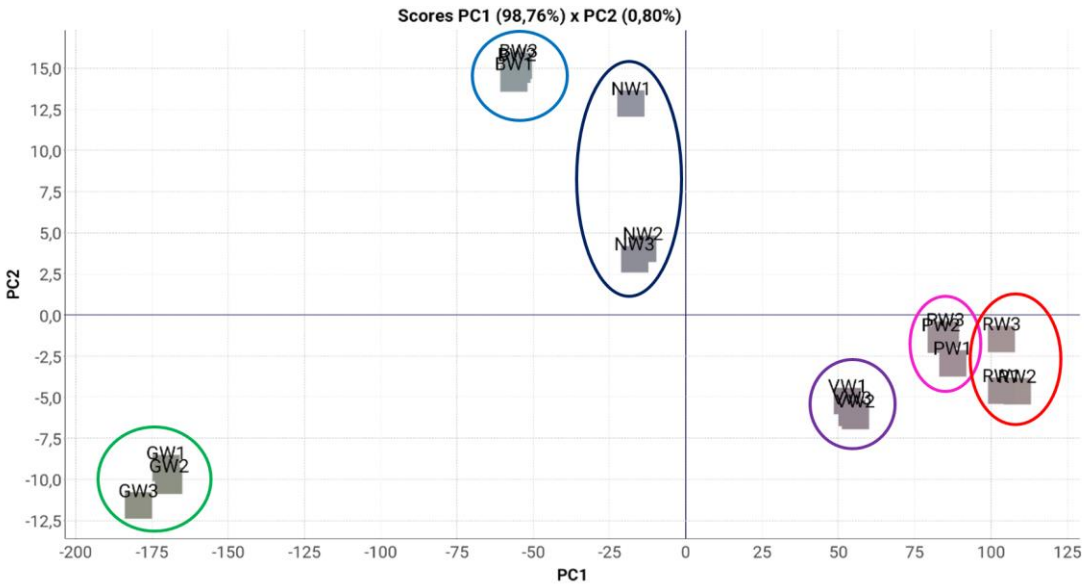
Figure 5 shows PCA (5a, 5b and 5c) and HCA (5d and 5e) for the first experiment with blue ballpoint pens, proceeding with three images of the exact same region of each square (center) for the 12 blue pens (figure 1b). Figure 5a shows PCA analysis using images collection with the Motorola® device, and Figure 5b shows PCA analysis using images collection with the Samsung® device. With both mobiles' devices, the two identical Bic® pens and two pens from Pilot® brand grouped together in the PC1xPC2 graph scores, demonstrating its composition similarities. Although we can observe some differences on PC1xPC2 graphs scores for Motorola® and Samsung® devices, it is possible to notice a good differentiation between Bic®, Pilot® and Tris® brands, using both devices. Faber Castell® and Molin® brands could be also distinguished using Samsung® device, suggesting that a higher quality camera is important for blue ballpoint pens analysis with PhotoMetrix PRO®. We can also observe that Paper Mate®, Unisa-S® and Uni Laknock® brands group together, as well as the Compactor® brand groups with Bic® brand, for both devices. Loadings graphs shows the impact of RGB (PC1 – Figure 5c) and hue (PC2 – Figure 5c) channels in the pen inks differentiation, for both Motorola® and Samsung® devices. PC1 and PC2 positive and negatives scores are different for Motorola® and Samsung® devices. However, the app used the same channels to differentiate those blue ballpoint pens, with very similar results between the devices. For HCA analysis, Motorola® device data shows a pattern of differentiation for the pens, but some of the triplicates disperse from each other, like the PCA analysis (Figure 5d). However, HCA analysis performed with data from the Samsung® device shows a very good differentiation for those pen brands, also corroborating its PCA analysis (Figure 5e).



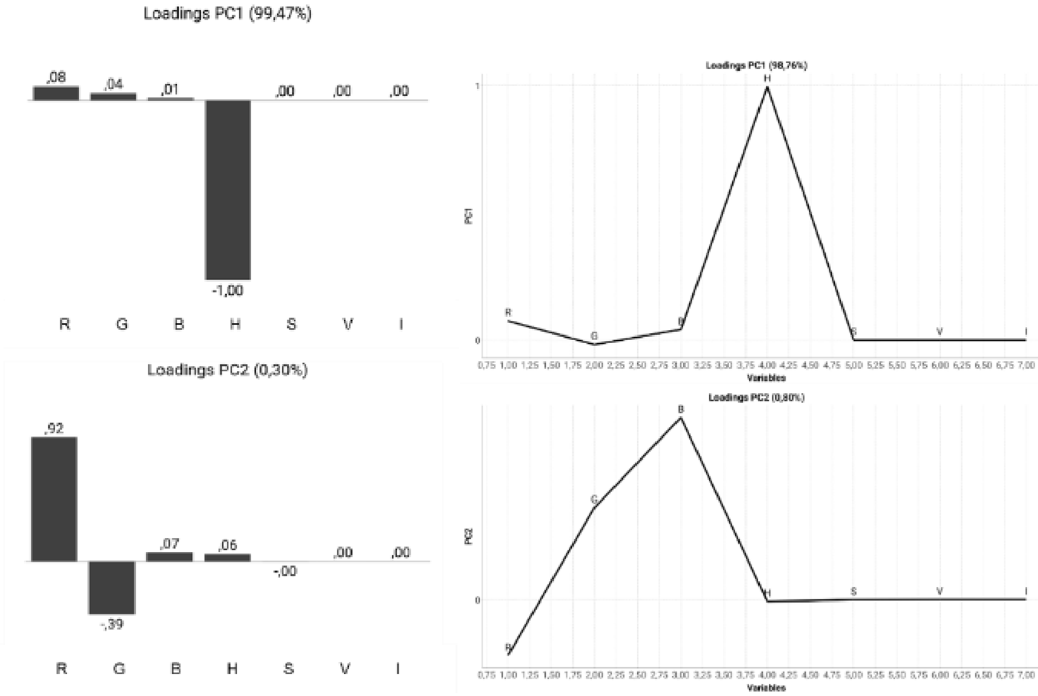
(a)



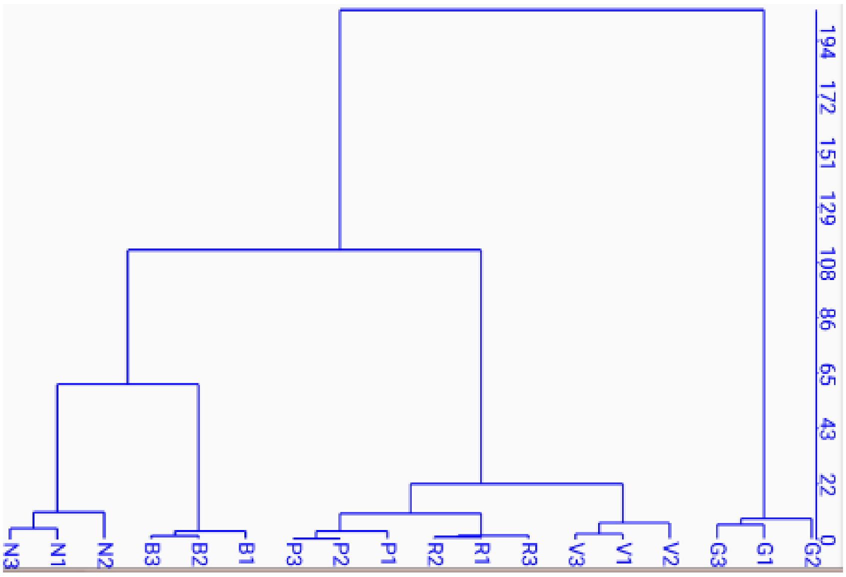
(b)


Scores PC1 (98,76%) x PC2 (0,80%)
PC2
PC1
BW3
BW1
NW1
NW2
NW3
RW3
PW2
PW1
RW3
RW1
RW2
VW1
VW2
GW1
GW2
GW3

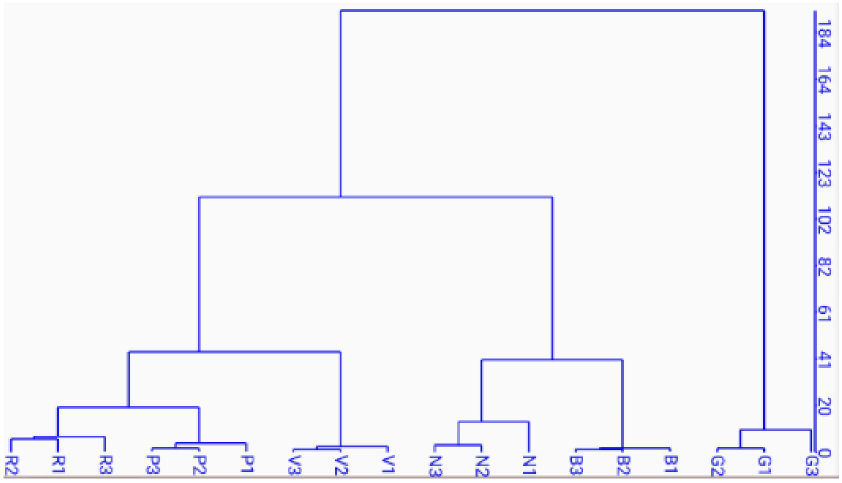
(c)


Loadings PC1 (99,47%)
,08
,04
,01
-1,00
,00
,00
,00
R G B H S V I
Loadings PC2 (0,30%)
,92
-,39
,07
,06
-,00
,00
,00
R G B H S V I
Loadings PC1 (98,76%)
Variables
Loadings PC2 (0,80%)
Variables

(d)


N3 N1 N2 B3 B2 B1 P3 P2 P1 R2 R1 R3 V3 V1 V2 G3 G1 G2
194 172 151 129 108 86 65 43 22 0

(e)



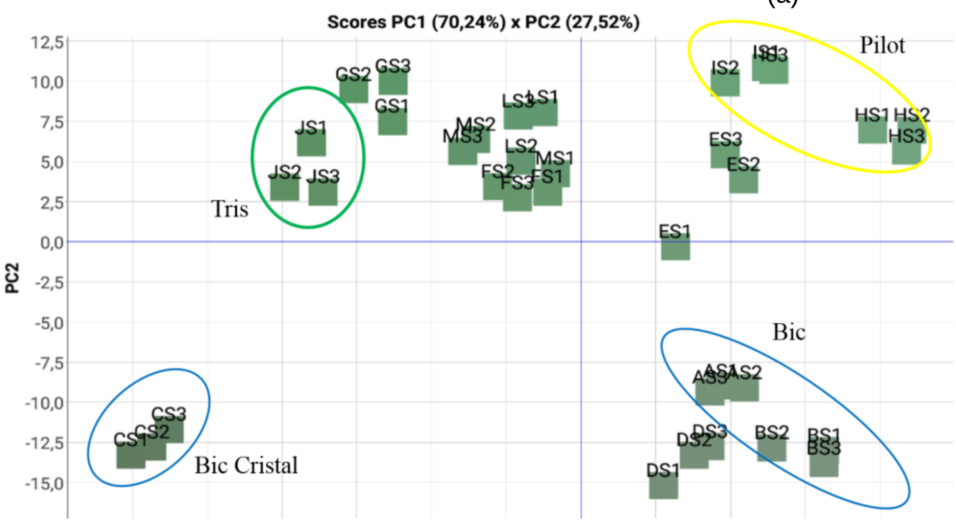
(f)

Figure 4. PCA and HCA results for triplicate images of the same region of part of a graphism, for colorful ballpoint pens. A) Selected region for the graphism; b) Scores Graph for PC1xPC2 using Motorola® mobile device; c) Scores Graph for PC1xPC2 using Samsung® mobile device; d) Loading Graphs for PC1 and PC2, for Motorola® (left) and Samsung® (right) devices; e) HCA dendrogram using Motorola® mobile device; and f) HCA dendrogram using Samsung® mobile device.

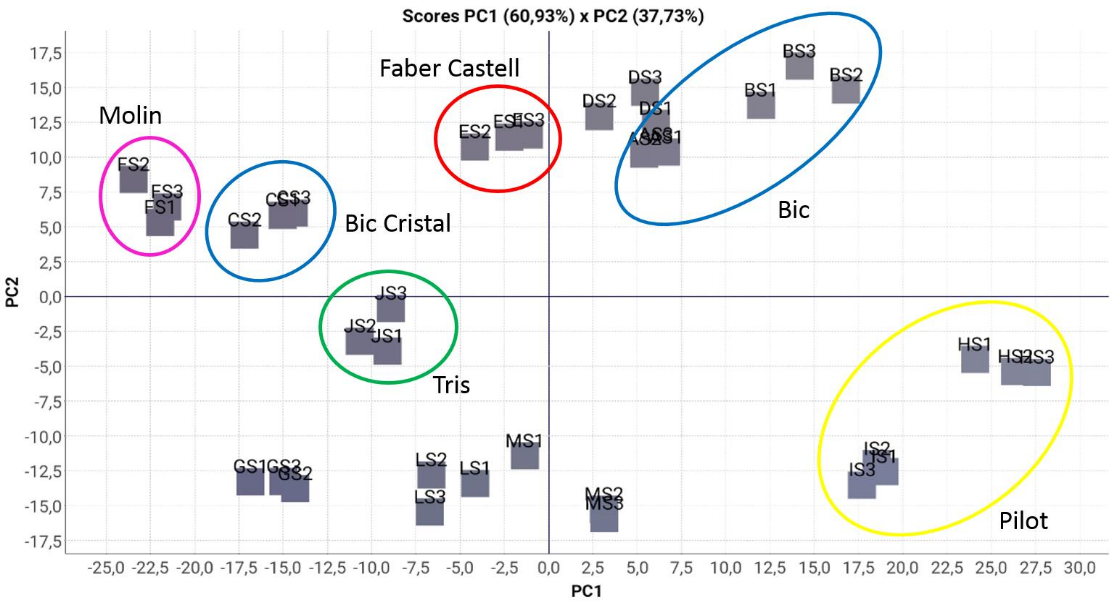
Subtitles:

Pen Brand	Representation
Bic 1	A
Bic 2	B
Bic Cristal 1.6mm	C
Compactor 07	D
Faber Castell Trilux 032 Medium	E
Molin Trion 1.0	F
Paper Mate Kilometrica 100 1.0M	G
Pilot BP-S	H
Pilot Super Grip 1.6	I
Tris Click 1.0M	J
Uni Laknock 1.0	L
Unisa-S Fine	M

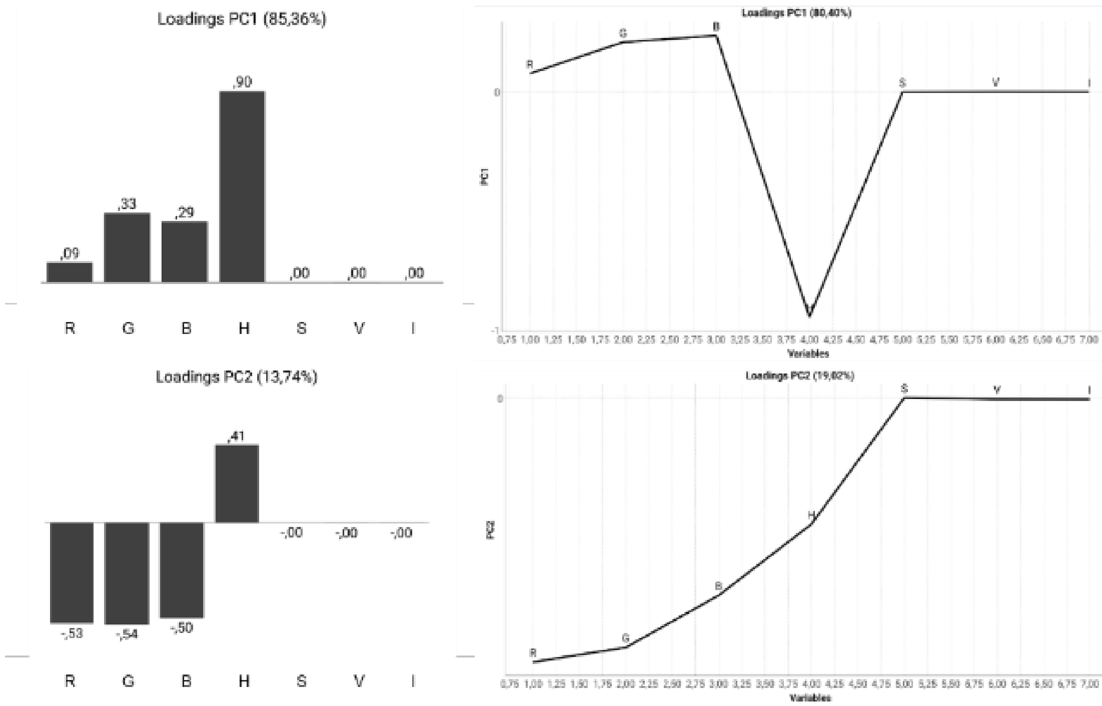
(a)



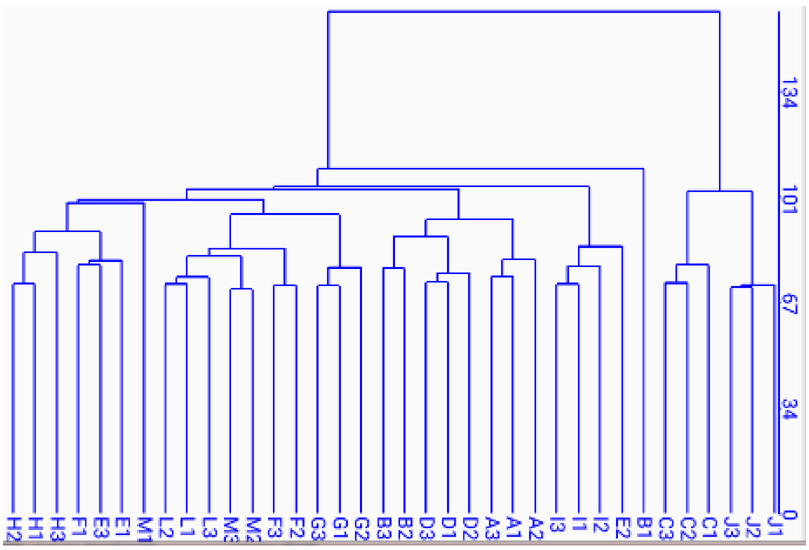
(b)


Scores PC1 (60,93%) x PC2 (37,73%)
Faber Castell
Molin
Bic Cristal
Bic
Tris
Pilot
PC1
PC2

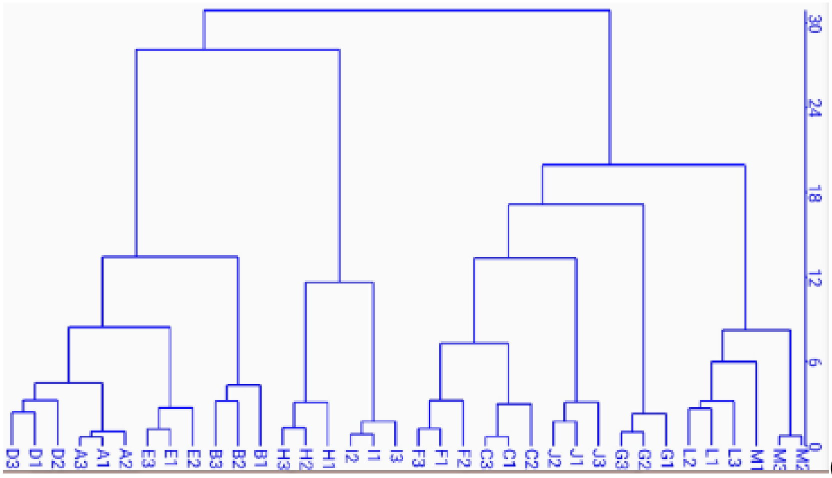
(c)


Loadings PC1 (85,36%)
Loadings PC2 (13,74%)
R
G
B
H
S
V
I

(d)



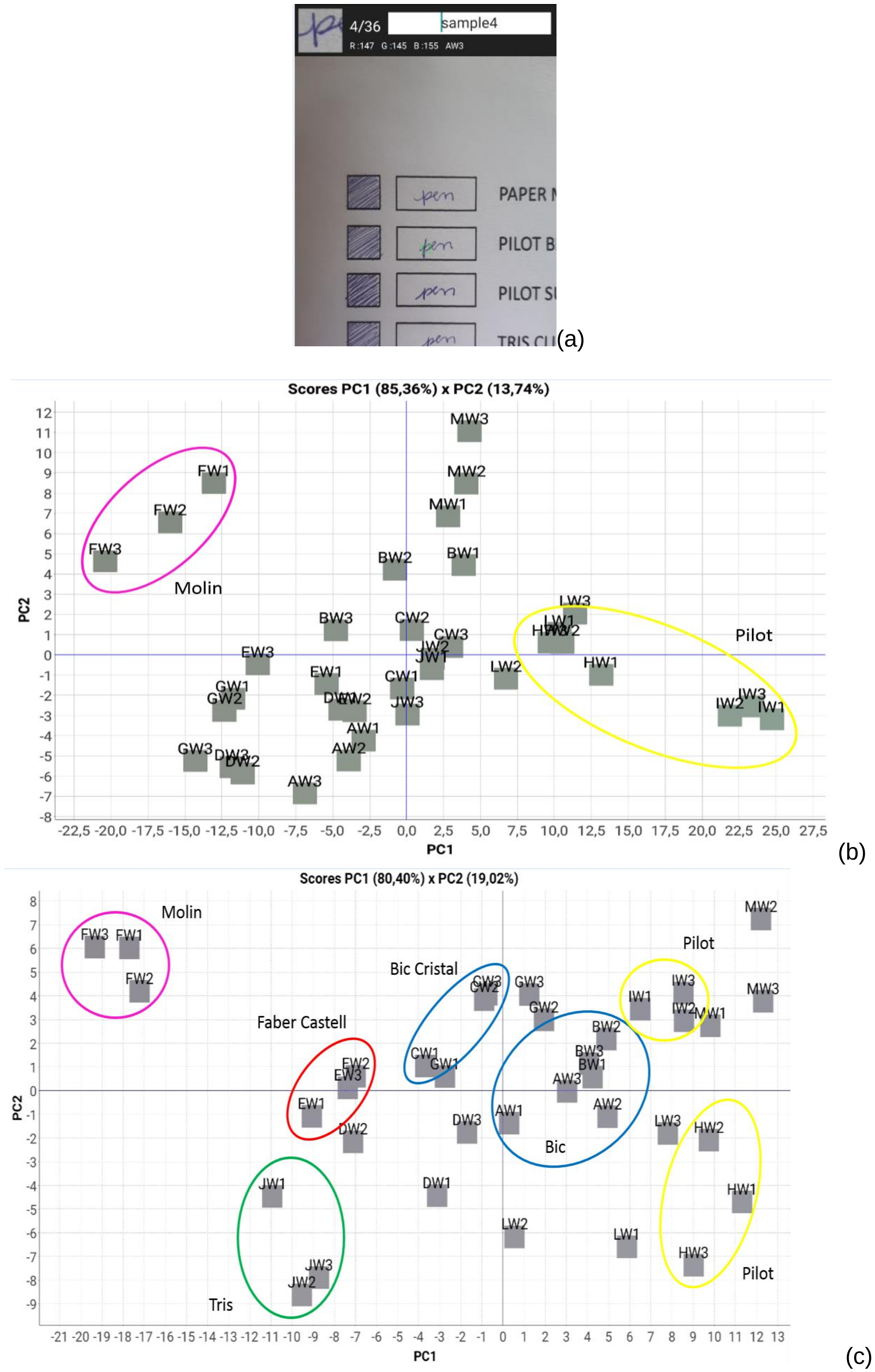
(e)

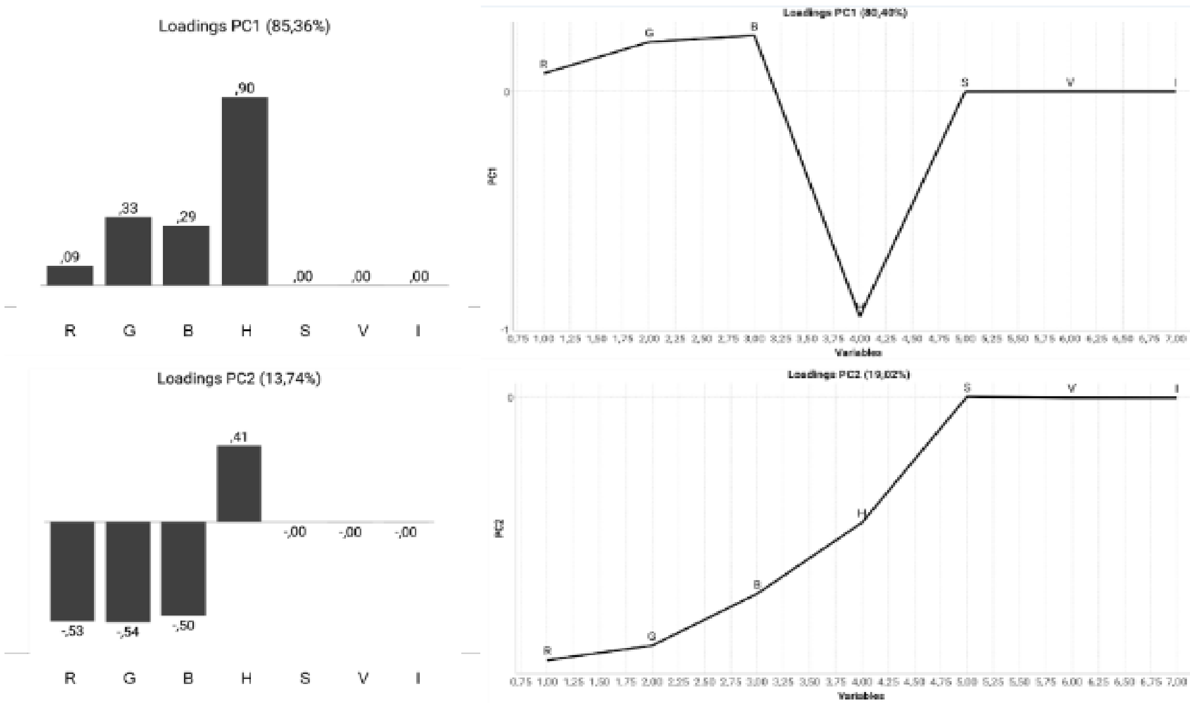


(f)

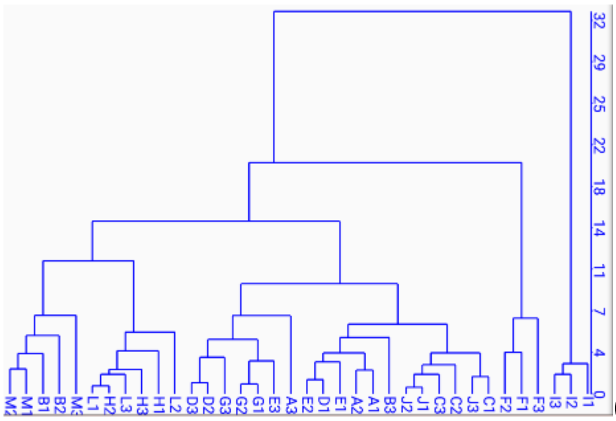
Figure 5. PCA and HCA results for triplicate images of the same region of each square for blue ballpoint pens. a) Pen codes for the score graphs; b) Scores Graph for PC1xPC2 using Motorola® mobile device; c) Scores Graph for PC1xPC2 using Samsung® mobile device; d) Loading Graphs for PC1 and PC2, for Motorola® (left) and Samsung® (right) devices; e) HCA dendrogram using Motorola® mobile device; and f) HCA dendrogram using Samsung® mobile device.

Figure 6 shows the results for image collection from the graphism “pen”, using three images from the same region represented in Figure 6a, for all blue ballpoint pens. PCA (6b, 6c and 6d) and HCA (6e and 6f) are demonstrated, for both mobile devices. Using Motorola® device, the differentiation between pens became harder in this experiment, but it is possible to differentiate some brands, like Molin® from Pilot® brand (as shown in Figure 6b), for example. Also, analyzing different pairs of triplicates, it is possible to differentiate some other brands too, like Paper Mate® (G) from Unisa-S® (M). When analyzing graphisms, the pressure between each written word is different even when performed by the same writing fist, which could be influencing the amount of ink deposited on paper and, consequently, the color captured by the mobile device camera. However, when the Samsung® device was used, a better differentiation was observed. Figure 6c shows good differentiation for Molin®, Faber Castell®, Tris®, Bic® and Pilot® brands. This result is the same as the one obtained from the square’s images (Figure 5b). Comparing Motorola® and Samsung® results, Samsung® showed improved repeatability and consistency. Loadings graphs also shows RGB and hue (H) impact (PC1 and PC2) for both devices (Figure 6d). For Motorola® device, HCA properly separates Pilot® and Molin® brands, but it shows the others pen clusters containing different pens amongst the triplicates (Figure 6e); using Samsung® device, pen clusters are better distributed and distinguished from each other (Figure 6f).

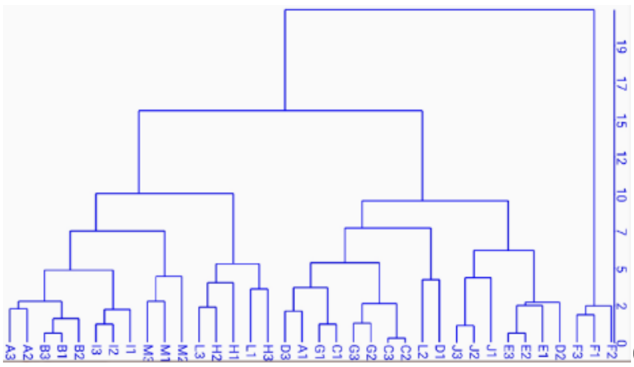

4/36
sample4
R:147 G:145 B:155 AW3
PAPER M
PILOT B
PILOT S
(a)
Scores PC1 (85,36%) x PC2 (13,74%)
Molin
Pilot
PC1
PC2
(b)
Scores PC1 (80,40%) x PC2 (19,02%)
Molin
Faber Castell
Bic Cristal
Bic
Pilot
Tris
PC1
PC2
(c)



(d)



(e)



(f)

Figure 6. PCA and HCA results for triplicate images of the same region of part of a graphism, for blue ballpoint pens. a) Selected region for the graphism; b) Scores Graph for PC1xPC2 using Motorola® mobile device; c) Scores Graph for PC1xPC2 using Samsung® mobile device; d) Loading Graphs for PC1 and PC2, for Motorola® (left) and Samsung® (right) devices; e) HCA dendrogram using Motorola® mobile device; and f) HCA dendrogram using Samsung® mobile device.

To confirm PCA and HCA results, sets of differentiated pens in the score graph were evaluated, in pairs and in a group, with the PLS-DA method. Figure 7shows an example of PLS-DA comparing one Bic® pen and one Tris® pen. Figure 7a shows the calibration model, after the capturing of five images from Bic® pen ink named as “0” (does not belong to the class) and five images from Tris® pen ink named as “1” (belong to the class in question); figure 7b shows the regression line for sampling with known concentrations (validation), which means collecting the images and informing the app their respective codes (“0” and “1”). Lastly, figure 7c shows the blind sampling without informing their codes, to evaluate if the PLS-DA method can actually separate the pens in two different classes; the graph shows Bic® samples closer to “0”, while Tris® samples are closer to “1”, demonstrating that Bic® samples does not belong to the same class as Tris® samples.

Another model maintaining Tris® brand as the class of interest was tested, including three other pen brands – previously separated in the PCA and HCA graphs (Bic®, Pilot®, Molin®) – which were classified together as not belonging to Tris® class. In this model, it were captured five images of Bic® samples, five images from Pilot® samples and five images of Molin® samples, all named as “0”. Then, five images of Tris® samples were collected as “1”. Figure 9 shows the graphs for the calibration model for this experiment (Figure 8a), for the validation model, informing the app about their codes (Figure 8b) and for the blind sampling, without informing any code to the app (Figure 8c), and it is possible to observe that Tris® samples are closer to “1” in the sampling graphs (Figures 8b and 8c), while all the other brands does not belong to this class, being closer to “0” in the graphs.

Figure 9 shows an experiment performed with two Bic® pens, to demonstrate when two pens cannot be differentiated from each other. Figure 9a shows the calibration model, using five images of a Bic® pen named as “0” and five images from another Bic® pen, named as “1”. At figure 9b and 9c it is possible to observe that the app could not distinguish the images from each other, as it can be seen for different pen brands at Figures 7 and 8.

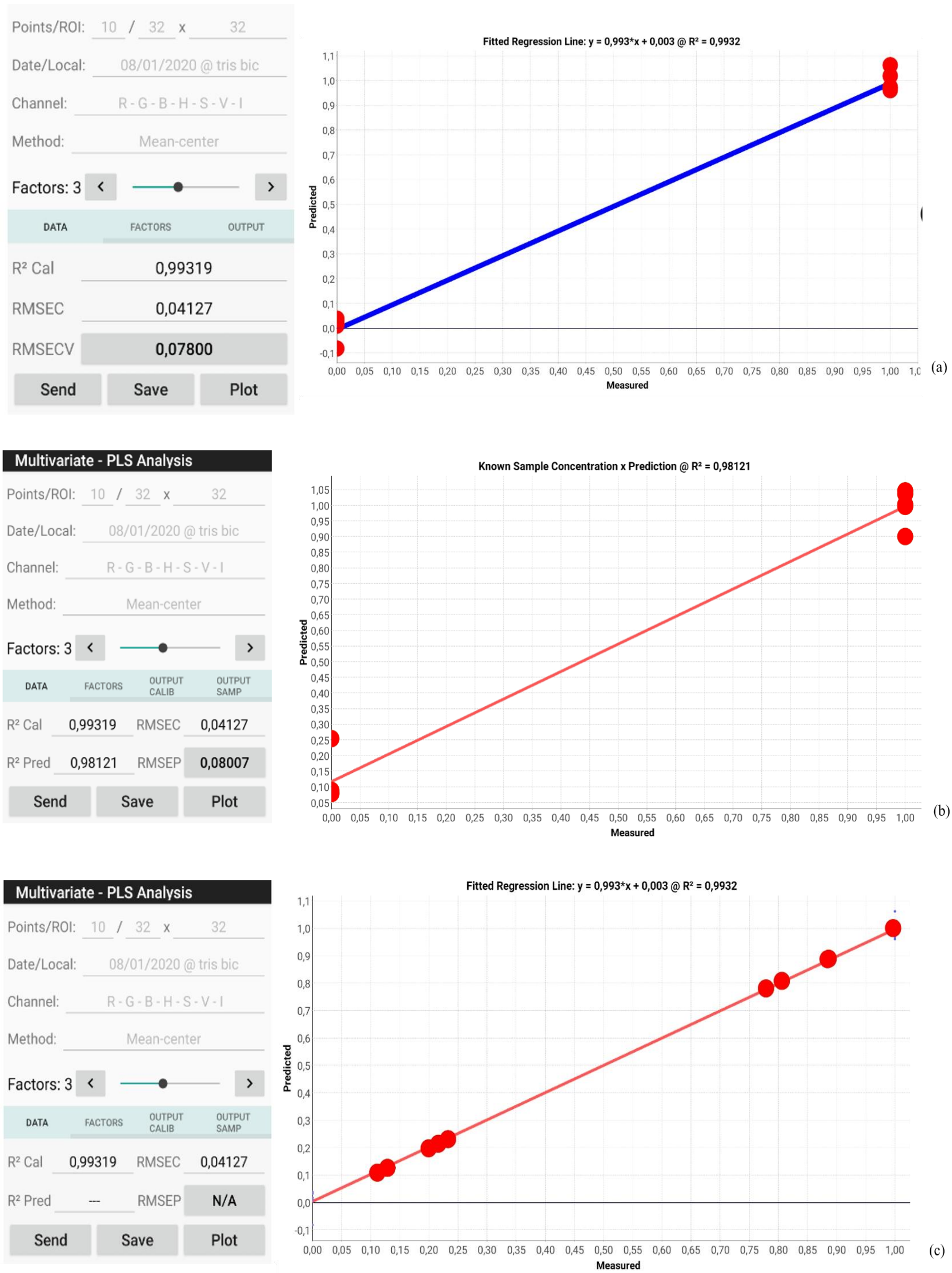


Figure 7. PLS-DA analysis comparing a Bic® pen and a Tris® pen: a) PLS-DA model, using Bic® samples as not belonging to the class ("0") and Tris® as the evaluated class ("1"); b) validation of samples, informing the app each pen code ("0" and "1"); c) blind samples to test the PLS-DA model, without informing any code to the app.

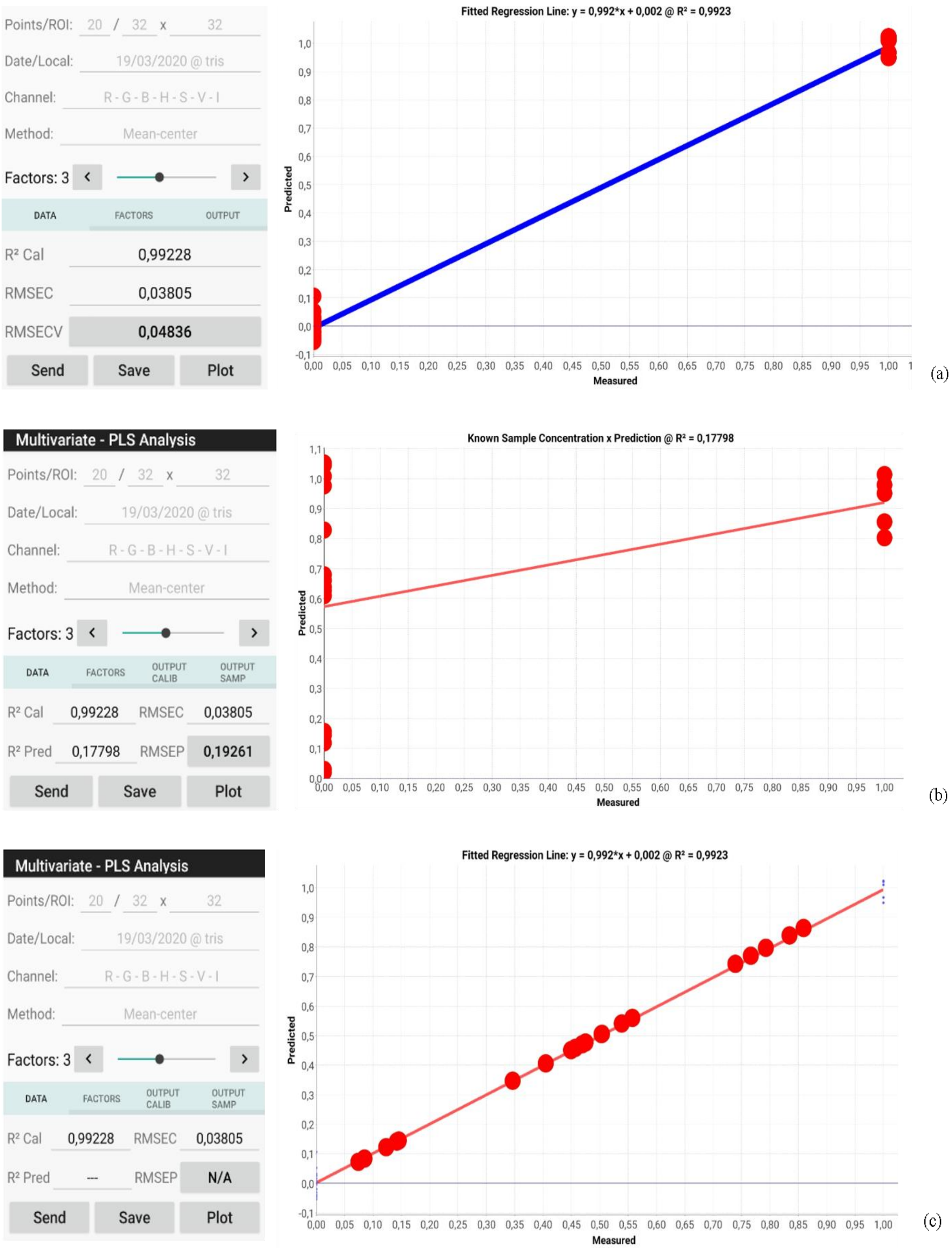


Figure 8. PLS-DA analysis comparing a Tris® pen against Bic®, Pilot® and Molin® pens : a) PLS-DA model, using Bic®, Pilot® and Molin® samples as not belonging to the class (“0”) and Tris® as the evaluated class (“1”); b) validation of samples, informing the app each pen code (“0” and “1”); c) blind samples to test the PLS-DA model, without informing any code to the app.

Figure 9. PLS-DA analysis comparing images from a Bic® pen ("0") and another Bic® pen ("1"). A) PLS-DA model; b) validation of samples, informing each pen code; c) blind samples to test the PLS-DA model.

In Figures 7, 8 and 9, we can observe the estimated errors for the training set (Root Mean Square Error of Calibration – RMSEC) and the test set (Root Mean Square Error of Prediction – RMSEP). Both errors should be as low as possible, demonstrating similarities between the images for the samples of same class. When analyzing two pens of different brands (Bic® and Tris®), RMSEC was 0.04127 and RMSEP was 0.0807, both lower than 0.1 (Figure 8b). Adding more pens as not belonging to Tris® class showed a similar RMSEC value (0,03805), but it increased RMSEP value (0.1926), which it is expected considering that there are three different pens tagged as "0" (Figure 8b). Analyzing the prediction graphs (Figures 7c and 8c), it can be seen that Tris® samples are separated from the others, closer to "1". Comparatively, when analyzing two pens of the same brand (Bic®), RMSEC was 0.02538, while RMSEP was 0.2771, which demonstrates a high error for prediction and indicates that the pen colors could not be differentiated from each other (Figure 9b). Also, all Bic® samples are positioned together in the graph, close to "0" (Figure 9c).

4. Discussion

Given the results we have found when studying nine different pen brands, it is possible to infer that some pen brands can be distinguished from each other using PhotoMetrix PRO® app, but not all of them at the same time. Also, Samsung® mobile device camera has shown better discrimination, when compared to Motorola® device camera, which can be explained by the higher quality of Samsung®'s camera. Furthermore, different mobiles devices should be studied for its capability of differentiate blue ballpoint pens.

To evaluate the collected data, the proximity of each image in a triplicate indicates how this sample is differentiated from another sample triplicate in the visual space of PCA. As closer as the samples of the same triplicate are in the PCA graphs, it means that the image collection was correctly proceeded; otherwise, the image collection should be re-done. In some cases, it will be easy to delimitate a categorical conclusion; however, in some cases it will be necessary to proceed with another technique for ink analysis, to avoid false positives or negatives results. So, it is important for the analyzer to acquire chemometric knowledge before interpreting the results. Also, our results show that Molin®, Tris®, Faber Castell®, Bic® and Pilot® brands could be differentiated from each other even when capturing images from a graphism, so pens of those brands could be used as a control of differentiation when analyzing unknown pen brands. This means that the analysis of unknown ink brands should include similar graphisms performed with known pen ink brands that can be differentiated from each other in the PCA score graph.

As a qualitative analysis, PhotoMetrix PRO® may be useful to discard or to confirm visual ink analysis. In many situations, Forensic Experts must answer to questions regarding different pen inks in a document, but they do not have access to expensive instruments, such as a Video Spectral Comparator®, a FTIR or a Raman Spectrometer. When visually comparing color tonalities, the subjective perception from one person might be different from other one; in those cases, the use of PhotoMetrix PRO® app might be very helpful in obtaining a conclusion.

Previously, the same pen brands were studied by our research group, either for ink differentiation[8] or ink dating[30], using multivariate statistics. For pen inks differentiation, a Q-Exactive® Orbitrap analyzer was used to collect spectrometric data from colorants and additives patterns to distinguish pens from each other. In this study, PCA and HCA grouped pens of same brand, approximated pens of similar composition and differentiated pens with distinct formulations, and all of the pens studied for PhotoMetrix PRO® analysis could be differentiated from each other using its chemical data[8]. Regarding ink dating analysis, infrared spectra of pens of different brands/models and ages was collected, and PCA and HCA analysis showed discrimination between pens of different ages[30]. Asri *et al*.[14,16] also discusses the importance of chemometric techniques in ink analysis. Using both FTIR and Raman Spectroscopy chemical data, they have showed that PCA is a powerful tool to recognize patterns of similarities and differences between inks of different pen brands.

Similarly to our work, Valderrama & Valderrama (2016)[31] published a study with digital images, using an IOS smartphone to capture images from different ballpoint, gel, rollerball and felp-tip pens. Using PLS-DA to compare the inks images, they have found proper results to differentiate those pens. In this study, all inks were applied into small squares, uniformly. However, when considering Forensic Practice, usually pen ink differentiation has a demand on signatures and distinct writings, which may have been written at different times, trying to imitate the original ink color and writing. Thus, it is easier to differentiate distinct pen kinds, such as ballpoints, gel, rollerball and felp-tip pens, than pens of same kind but different brands, such as different ballpoint pen brands. Therefore, our study tried to distinguish blue ballpoint pens from each other, and also to apply the method in writings, in an attempt to reproduce real cases.

Innovatively, our study showed that the differentiation between blue ballpoint pens, using MIA of digital images captured with PhotoMetrix PRO®, is possible between some pen brands. It showed some limitations, but it also opened a space for future research. It is important for those results to be replicated by different analyzers, also including a larger number of pen brands. As the pressure for graphisms had an influence on the images capture, even for a same person writing it, a bigger study involving different writers and graphisms should be performed, considering that the amount of ink applied on paper could be an interference. It is important to highlight the importance of testing those results with different mobiles phones. Thus, considering the long time between a document forgery and its analysis by a forensic expert, a study involving ink`s age should also be considered.

5. Conclusion

PhotoMetrix PRO® is a free app for download in different phones devices, and it is an easy tool to manipulate, which allows multivariate analysis in several areas. By applying this method for colorful ballpoint pens, our results showed a great differentiation from each other, as expected. Considering blue ballpoint pens, it is possible to differentiate between some of the studied brands, while there is some limitation to distinguish between others pen brands. For blue pens of different brands, but visually similar color, it was not possible to discern it from each other using this technique. However, PhotoMetrix PRO® use is very interesting to obtain validated results in specific cases. Besides its limitations, this technique could be further studied, with different pens and mobile devices, so it can be possibly applied in some specific cases in Forensic Documentoscopy. Also, this method could be studied in other forensic areas.

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References

1. Ezcurra M; Góngora, JMG; Maguregui, I; Alonso, R. Analytical methods for dating modern writing instrument inks on paper. Forensic Sci Int, 2010; 197: 1-20. https://doi.org/10.1016/j.forsciint.2009.11.013
2. Câmara & Silva, E.S.; Feuerharmel, S. Documentoscopia: Aspectos Científicos, Técnicos e Jurídicos. 1 Ed. Millenium Editora, Campinas/SP, 2014. 734p.
3. Bugler J; Hans B, Anton D. Age Determination of Ballpoint Pen Ink by Thermal Desorption and Gas Chromatography–Mass Spectrometry. J Forensic Sci, 2008; 53(4): 982-988. https://doi.org/10.1111/j.1556-4029.2008.00745.x
4. Andrasko, J. HPLC analysis of ballpoint pen inks stored at different light conditions. J Forensic Sci, 2001; 46(1): 21-30. https://doi.org/10.1520/JFS14907J
5. Gorziza RP; Carvalho CMB; Gonzalez M; Leal LB; Korndorfer T; Ortiz RS; Trejos T; Limberger RP. Blue and Black Ballpoint Pen Inks: a Systematic Review for Ink Characterization and Dating Analysis. Braz J Forensic Sci, Med Law & Bioethics, 2019; 8(3):113-138. https://doi.org/10.17063/bjfs8(3)y2019113
6. Saini K, Rathore R. Differentiation of Red and Black Ballpoint Pen Inks using High Performance Thin Layer Chromatography and Gas Chromatography-Mass Spectrometry. Arab J Forensic Sci & Forensic Med, 2018; 1 (7), 829-841. https://doi.org/10.26735/16586794.2018.011
7. Sharma V, Kumar R. Fourier transform infrared spectroscopy and high performance thin layer chromatography for characterization and multivariate discrimination of blue ballpoint pen ink for forensic applications. Vib Spec, 2017; 92: 96–104. https://doi.org/10.1016/j.vibspec.2017.05.006
8. Bello de Carvalho CM, Ortiz RS; Reis M, Zamboni A, Limberger RP, Ferrão MF, Vaz Boniek G. Characterization and Differentiation of Ballpoint Pen Ink Strokes on Paper Using Orbitrap Mass Spectrometry and Multivariate Statistic. Forensic Sci Add Res., 2018; 2(2): 1-8. https://doi.org/10.31031/FSAR.2018.02.000537
9. Sun Q, Luo Y, Yang X, Xiang P, Shen M. Detection and identification of dyes in blue writing inks by LC-DAD-orbitrap MS. Forensic Sci Int, 2016; 261: 71–81. https://doi.org/10.1016/j.forsciint.2016.01.038
10. Kumar R, Sharma V. A novel combined approach of diffuse reflectance UV–Vis-NIR spectroscopy and multivariate analysis for non-destructive examination of blue ballpoint pen inks in forensic application. Spectrochim Acta A Mol Biomol Spectrosc, 2017; 175: 67–75. doi: 10.1016/j.saa.2016.12.008. https://doi.org/10.1016/j.saa.2016.12.008
11. Lee LC, Shandu KTS, Razi NSM, Ishak AA, Osman K. Forensic analysis of blue ballpoint pen inks using ultraviolet-visible spectrometer and ultra-performance liquid chromatograph. Malays J Anal Sci, 2015;19 (2): 397 – 401.

12. Calcerrada M, González-Herráez M, Garcia-Ruiz C. A microdestructive capillary electrophoresis method for the analysis of blue-pen-ink strokes on office paper. J Chromatogr A, 2015; 1400: 140–148. https://doi.org/10.1016/j.chroma.2015.04.036
13. Gallidabino M, Weyermann C, Marquis R. Differentiation of blue ballpoint pen inks by positive and negative mode LDI-MS. Forensic Sci Int, 2011, 204 (1-3): 169–178. https://doi.org/10.1016/j.forsciint.2010.05.027
14. Asri MNM, Hashim NH, Desa WNSM, Ismail D. Pearson Product Moment Correlation (PPMC) and Principal Component Analysis (PCA) for objective comparison and source determination of unbranded black ballpoint pen inks. Aust J Forensic Sci, 2016; 1: 1-18. https://doi.org/10.1080/00450618.2016.1236292
15. Silva CS; Borba FSL, Pimentel MF, Pontes MJC, Honorato RS, Pasquini C. Classification of blue pen ink using infrared spectroscopy and linear discriminant analysis. Microchem J, 2013; 109: 122-127. https://doi.org/10.1016/j.microc.2012.03.025
16. Asri MNM, Desa WNSM, Ismail D. Raman spectroscopy of ballpoint-pen inks using chemometric techniques. Aust J Forensic Sci, 2016; 1: 1-11.
17. Borba FSL, Honorato RS, Juan A. Use of Raman spectroscopy and chemometrics to distinguish blue ballpoint pen inks. Forensic Sci Int, 2015; 249: 73–82. https://doi.org/10.1016/j.forsciint.2015.01.027
18. Lennard C, El-Deftar MM, Robertson J. Forensic application of laser-induced breakdown spectroscopy for the discrimination of questioned documents. Forensic Sci Int, 2015; 254: 68–79. https://doi.org/10.1016/j.forsciint.2015.07.003
19. Kula A, Wietecha-Posłuszny R, Pasionek K, Król M, Woźniakiewicz M, Kościelniak M. Application of laser induced breakdown spectroscopy to examination of writing inks for forensic purposes. SciJustice, 2014; 54: 118–125. https://doi.org/10.1016/j.scijus.2013.09.008
20. Denman JA, Skinner WM, Kirkbride KP, Kempson IM. Organic and inorganic discrimination of ballpoint pen inks by ToF-SIMS and multivariate statistics. Appl Surf Sci, 2010; 256: 2155–2163. https://doi.org/10.1016/j.apsusc.2009.09.066
21. Ferreira PS, Abreu e Silva DF, Augusti R, Piccin E. Forensic analysis of ballpoint pen inks using paper spray mass spectrometry. Analyst, 2015, 140: 811–819. https://doi.org/10.1039/C4AN01617C
22. Silva VAG, Talhavini M, Peixoto ICF, Zacca JJ, Maldaner AO, Braga JWB. Non-destructive identification of different types and brands of blue pen inks in cursive handwriting by visible spectroscopy and PLS-DA for forensic analysis. Microchem J, 2014; 116: 235–243. https://doi.org/10.1016/j.microc.2014.05.013
23. Hoffman E, Stroobant V. Mass spectrometry: principles and applications,3rd ed. Chichester: John Wiley and Sons Ltd., 2007.

24. Brereton RG.Chemometrics for Pattern Recognition, 1st ed. Chichester: Wiley, 2009. https://doi.org/10.1002/9780470746462
25. Helfer GA, Magnus VS, Böck FC, Teichmann A, Ferrão MF, Costa AB. PhotoMetrix: An Application for Univariate Calibration and Principal Components Analysis Using Colorimetry on Mobile Devices. JBraz Chem Soc, 2017; 28 (2): 328-335. doi: 10.5935/0103-5053.20160182. https://doi.org/10.5935/0103-5053.20160182
26. Grasel FS, Ferrão MF, Helfer GA, Costa AB. Principal Component Analysis of Commercial Tannin Extracts Using Digital Images on Mobile Devices. J Braz Chem Soc, 2016; 27(12): 2372-2377. https://doi.org/10.5935/0103-5053.20160135
27. Lumbaque EC, Silva BA, Bock FC, Helfer GA, Ferrao MF, Sirtori C. Total dissolved iron and hydrogen peroxide determination using the PhotoMetrix PRO application: A portable colorimetric analysis tool for controlling important conditions in the solar photo-Fenton process. J Hazard Mat, 2019; 378:120740. https://doi.org/10.1016/j.jhazmat.2019.06.017
28. Böck FC, Helfer GA, Costa AB, Dessuy MB, Ferrão MF. Rapid Determination of Ethanol in Sugarcane Spirit Using Partial Least Squares Regression Embedded in Smartphone. Food AnalMethod, 2018; 11:1951-1957. https://doi.org/10.1007/s12161-018-1167-4
29. Helfer GA, Tischer B, Filoda, PF, Parckert, AB, Santos, RB, Vinciguerra LL, et al. A New Tool for Interpretation of Thermal Stability of Raw Milk by Means of the Alizarol Test Using a PLS Model on a Mobile Device. Food Anal Method, 2018; 11: 2022-2028. https://doi.org/10.1007/s12161-018-1190-5
30. Carvalho CMB, Reis M, Ortiz RS, Ferrão MF, Limberger RP. Application of Multivariate Statistics (PCA and HCA) on ballpoint pen ink Infrared spectra for dating of forensic relevant documents. Journal of the American Society of Questioned Document Examiners Inc., 2019; 22(1): 19-35.
31. Valderrama L, Valderrama P. Nondestructive identification of blue pen inks for documentoscopy purpose using iPhone and digital image analysis including an approach for interval confidence estimation in PLS-DA models validation. Chemometr Intell Lab, 2016; 156: 188–195. https://doi.org/10.1016/j.chemolab.2016.06.009