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Visible and near-infrared chemical imaging methods for the analysis of selected forensic samples

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Abstract

This study investigated various chemical imaging methods for the forensic analysis of paints, tapes and adhesives, inks and firearm propellants (absorption and photoluminescence in the UV–vis–NIR regions). Results obtained using chemical imaging technology were compared with those obtained using traditional techniques. The results show that chemical imaging offers significant advantages in the forensic context, for example the ability to display visual and spectral results side by side and to reduce sample preparation, hence minimizing the risk of contamination. Chemical imaging produced a greater discriminating power than traditional techniques for most evidence types. Chemical imaging also eliminated different brands of ammunition based on the fluorescence characteristics of the propellant grains preserving the evidence for further analysis. It is expected that this technology will find broader forensic applications in the future.

Keywords: Forensic analysis; Paint; Ink; Tapes; Propellant; Spectrometry; Chemical imaging

1. Introduction

New technology is constantly being sought for more rapid and more accurate examination of evidence materials in forensic laboratories as well as for field use. In particular, it is becoming increasingly necessary to find improved discriminatory and non-destructive methods for the examination and interpretation of forensic evidence. Chemical imaging is one of the most promising technologies that have been proposed to date. Chemical imaging essentially combines molecular spectroscopy and digital imaging. Forensic science has been identified as a possible application of chemical imaging technology [1,2]. The advantages chemical imaging presents to forensic investigations have been addressed [1]. In particular, applications to fingerprint detection have been reported elsewhere [1,3,4]. The foremost benefits that this emerging technology provides are that it is a non-destructive technique, utilising well-established and peer-reviewed spectroscopic methods. In this study chemical imaging is investigated as an analytical technique for several common types of forensic evidence.

1.1. Chemical imaging

The theory of chemical imaging has been previously described in detail [1,5,6]. In summary, spatial and spectral information is simultaneously recorded using chemical imaging techniques [7]. Light intensity is recorded as a function of pixel location and as a function of wavelength. The resulting data set is a three dimensional image cube (i.e., hyperspectral

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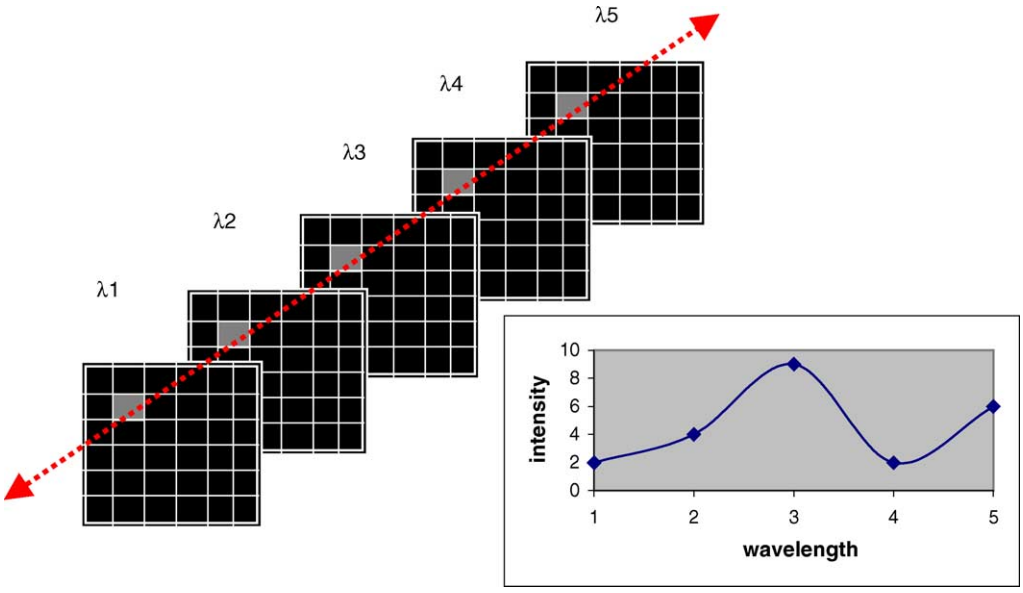

Fig. 1. Different images at selected wavelengths and the spectrum of the highlighted pixel.

image) where there is a full image at each individual wavelength and a full spectrum at each individual pixel [1]. This concept is illustrated by Fig. 1 [8].

Chemical imaging can be used in conjunction with a variety of well-established and accepted spectroscopic techniques. The spectroscopic techniques examined in this study are micro and macro UV–vis–NIR absorption and photoluminescent emission (fluorescence). A number of processing tools in specialised software are used to extract and present relevant information from the large chemical imaging data sets [1,3].

This study investigated and evaluated micro and macro chemical imaging techniques for the forensic examination of paints, tapes and adhesives, inks and firearm propellants (absorption and photoluminescence in the UV–vis–NIR regions). Results obtained using chemical imaging technology were compared with those obtained using traditional techniques. Particular attention was paid to the ease of analysis and discriminatory abilities applicable in a forensic context.

2. Materials and methods

2.1. Chemical imaging

Several different chemical imaging instruments and software versions were used in this study, all from ChemImage Corp (Pittsburgh, USA).

2.1.1. Visible chemical imaging macroscope

The VIS Condor Macroscopic Chemical Imaging System™ used has been described elsewhere [1]. Essentially this macroscope is able to scan from 400 nm to 720 nm in less than 1 nm increments through the utilisation of a liquid crystal imaging spectrometer (LCTF), a CCD camera and macro optics.

ChemImage software (ChemImage Corp.; version 5.0) was used to process the large data sets that resulted from the analysis. The software contains a variety of functions such as pre-processing tools and chemometric functions such as principal components analysis (PCA).

2.1.2. Visible-near infrared chemical imaging macroscope

The VIS/NIR Condor Macroscopic Chemical Imaging System™ is an upgrade of the VIS Condor version. This instrument is able to scan from 400 nm to 1100 nm in less than 1 nm increments. This is made possible through the use of multiple LCTFs. The VIS/NIR Condor uses a back-illuminated charged-coupled device (CCD) 1024 × 1024 pixels and imaging optics (16:1 visible macro zoom lens). The resulting data was viewed and processed using ChemAnalyze software (ChemImage Corp., version 7.0).

2.1.3. Visible chemical Imaging microscope

The CI-Trace™ chemical imaging microscope used in some of the experiments is equipped with a LCTF capable of scanning 400 nm to 720 nm for the colour and fluorescence analysis of samples. Magnification was up to 100x with samples in this study being examined under 10× magnification. The software used to process data from the microscope was ChemImage (ChemImage Corp., version 5.0).

Table 1
Identities and origins of pressure sensitive adhesive tape samples analysed

No.	Brand	Adhesive	Bought	Made	Width (mm)
P081	Reno	Acrylic	Cyprus	–	48
P071	Scotch	Acrylic	Australia	Taiwan	48
P020	Tristar	Acrylic	Australia	Taiwan	36
P087	Tesa	Acrylic	Finland	–	36
P052	Syrom 90 S.P.A	Rubber	Italy	–	48
P041	Huskytape	Rubber	Australia	Italy	36
P065	Johnson Tape	Acrylic	Pakistan	Taiwan	48
P056	Scotch	Acrylic	Australia	China	24
P073	Tartan 3690	Rubber	Australia	USA	48
P092	Sellotape	Rubber	Australia	Australia	24
P072	Scotch	Acrylic	Australia	Taiwan	48
P062	Fender	Acrylic	Thailand	–	48
P006	Bullseye	Rubber	Australia	Korea	48
P054	Scotch	Acrylic	Italy	–	48
P060	Staples	Acrylic	USA	Taiwan	48
P012	Cling	Acrylic	Australia	–	48
P093	Sellotape	Rubber	Australia	Australia	48
P019	Tristar	Acrylic	Australia	Taiwan	38
P066	Johnson Tape	Acrylic	Pakistan	Taiwan	48

2.1.4. Light sources and liquid tunable filters specifications

The light source for the experiments using infrared excitation was a standard desk lamp. All other excitations were achieved using a Xenon lamp (e.g. a Polilight model PL500-500 W; Rofin, Australia-or equivalent).

The full width at half maximum (FWHM) of the liquid tunable filters (LCTF) used was in the range of 5–50 nm, depending on the wavelength.

2.2. Paint analysis

Two multi layered and multi coloured paint chips were used for the comparison of traditional techniques and chemical imaging. These paint chips were obtained from insurance car yards as part of a previous survey of automotive paints and mounted on glass slides.

2.2.1. Chemical imaging

Chemical imaging analysis was carried out using the visible microscope on cross sections of the paint chips. Colour analysis was conducted from 400 nm to 720 nm using white light excitation in the reflectance mode. Fluorescence analysis was carried out from 440 nm to 720 nm using a xenon excitation source and a fluorescence cube equipped with a 410 nm excitation filter, dichroic mirror and 420 nm barrier filter.

2.2.2. Traditional techniques

A stereomacroscope was used to observe the optical characteristics of the paint chips.

Reflectance spectrometry of the paint chip cross-sections was performed using a SEE 2100 UV–vis–NIR microspectrophotometer. Three spectra for each layer of the two samples were collected from different locations along the paint chip to account for any variation within the sample. Each spectrum comprised of fifty scans. The spectra of each sample were then averaged to obtain a single merged spectrum. White layers in the paint chip were analysed in the UV range (220 nm to 430 nm) and all other layers were analysed in the UV–vis–NIR range (430 nm–1000 nm).

2.3. Tape and adhesive analysis

A variety of clear and brown tapes were used for this study. The tapes ranged in brand, size, adhesive type, country of manufacture and country of purchase. Samples of each tape were mounted on glass slides (Table 1).

2.3.1. Chemical imaging

The VIS Condor system was used for the chemical imaging analysis of the tape backing and adhesive. Small sections of the tapes were cut and placed on a single glass slide allowing for the simultaneous analysis of all tapes in a single field of view. The adhesive side was analysed by placing the tape backing side down on top of double sided tape attached to the glass slide. The tapes and adhesives were both analysed from 400 nm to 720 nm using white light and 350 nm excitation.

The visible chemical imaging microscope was also utilised for the tape and adhesive analysis. Colour analysis was conducted from 400 nm to 720 nm and fluorescence analysis was carried out from 440 nm to 720 nm using 350 nm bandpass excitation.

2.3.2. Traditional techniques

A stereomacroscope was used to observe the optical characteristics of the different adhesive tapes.

The Poliview system (Rofin, Australia) was used to examine the presence or absence of fluorescence of the tape backing using excitation at 415 nm, 450 nm and

555 nm and observation at 495 nm, 570 nm and 620 nm, respectively.

Reflectance analysis of the tape backing was performed using the SEE 2100 microspectrophotometer in the UV–vis–NIR range. The backing of each tape sample was analysed by scanning 50 times at five different places along the sample and an average representative spectrum was obtained for each sample.

The backing of the tape samples were also analysed by the Nicolet Nic-Plan IR microscope with a zinc selenide crystal ATR accessory. The infrared detector was a cooled Mercury Cadmium Telluride (MCT) detector with a spectral range of 650 cm^{-1} to 4000 cm^{-1}. Two spectra from different places along the sample were collected to ensure reproducibility with each spectrum comprising of 128 scans and a resolution of 4 cm^{-1}.

The tape adhesive was examined by diffuse reflectance infrared spectroscopy (DRIFTS) using the Nicolet 760 Magna IR Spectrometer with DTGS detector. Adhesive was removed from the tape backing using a metal probe and dissolved in 1 mL of chloroform. Finely ground potassium bromide was used to prepare sample cups for DRIFTS analysis. Two drops of the chloroform solution were placed on the potassium bromide and then heated for 15 min at 100 °C to remove the solvent. Sample cups were then placed in the Baseline DRIFTS accessory in the spectrometer and spectra collected. Each adhesive was sampled twice and one spectrum was collected for each of the two samples. The spectral range was 400 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Each spectrum comprised of 256 scans.

2.4. Ink analysis

Ink was placed on a white paper support in the form of lines and letters. Nineteen inks were used in the initial study, and the brand, type and colour of these is listed in Table 2. A second study used nine blue ballpoint inks, as listed in Table 3.

2.4.1. Chemical imaging

The first part of the ink analysis focussed on a variety of blue and black ball point pens and the VIS Condor. The Condor was used to examine the inks from 400 nm to 720 nm using white light and 350 nm to 380 nm bandpass excitation. The chemical imaging microscope was used to examine the samples from 400 nm to 720 nm using white light and 410 nm excitation.

Subsequently, blue ballpoint pen ink was analysed using the VIS/NIR Condor. Data was collected from 400 nm to 1100 nm using white and infrared light as excitation.

Table 2
Ink brands and types used in initial chemical imaging study

Brand	Type	Black	Blue
BIC	Cristal Ball Pens	√	√
Papermate	Flexigrip Ultra	×	√
Papermate	Kilometrico	√	√
The Price Brand	Ballpoint Pen	√	√
Staedtler	Stick 430M	√	√
Sanford	Saga	√	√
Pilot	Supergrip Fine	√	√
Artline		√	√
Uni	SA-S	√	√
Pentel	Star V	√	√

Table 3
Ink brands and types used in subsequent chemical imaging study

No.	Brand	Type
Pen #0001	Office Works	Ballpoint Pen Medium Point
Pen #0003	Office Works	Retractable Ballpoint Pen Medium Point
Pen #0005	BIC	Classic Ballpoint Fine Point
Pen #0007	BIC	Cristal Ballpoint Medium Point
Pen #0009	Staedtler	Stick 430 M
Pen #0011	Penline	Four Colour Ballpoint Medium Point
Pen #0013	Penline	B.P. Clear Medium Ballpoint
Pen #0015	Pilot	BPS-GP-M
Pen #0017	Pilot	BPGP-10R-M Super Grip

2.4.2. Traditional techniques

Samples were analysed using the high resolution Video Spectral Comparator (VSC 2000), SEE 2100 microspectrophotometer and thin layer chromatography (TLC). The VSC 2000 was utilised for fluorescence (spot filters from 400 nm to 750 nm), UV and infrared transmission analysis. The SEE 2100 was utilised for UV–vis reflectance analysis. Two spectra were obtained for each sample and these two spectra were averaged to obtain a representative spectrum with a reduced amount of noise. Ink samples were analysed by thin-layer chromatography (TLC). Pyridine was used as a solvent and the plates were developed with ethyl acetate, absolute ethanol and distilled water. Results were examined under visible and UV light.

2.5. Firearm propellant analysis

In this study, two different brands of firearm ammunition were examined to see if chemical imaging could distinguish them. The ammunition examined was Winchester Power-Point. 22 rimfire and PMC Zapper. 22 rimfire. One of the ammunition samples was fired into a black cotton target at close range (PMC Zapper). Raw propellant was also removed from an unfired cartridge for each brand.

2.5.1. Chemical imaging

The target and raw propellant were then examined using the VIS/NIR Condor. Data was collected from 400 nm to 720 nm using 450 nm excitation.

2.6. Calculation of discriminating power

The ability of chemical imaging to discriminate samples of interest was assessed by calculating the discriminating power (DP). The DP is the probability that two items selected at random from a relevant population do not match using a

given protocol. In this case, DP = number of differentiated pairs/number of all possible pairs.

3. Results and discussion

3.1. Paint analysis

Chemical imaging allows visual and spectral data to be collected simultaneously. Spectral data of all layers can be collected in one experiment, compared with a single experiment for each layer of paint when using microspectrophotometry. The same analysis by microspectrophotometry takes a longer amount of time due to the number of spectra required to obtain an average spectrum, in contrast, chemical imaging is a more efficient time saving technique for this type of analysis.

Fluorescence chemical imaging is able to be performed on the same instrument without the removal of the sample. The risk of contamination is greatly reduced because of the reduced sample preparation. Fluorescence chemical imaging provides strong images for paint cross sections, visually highlighting the differences between the layers as an alternative to a comparison of spectra. For example, Fig. 2 shows a cross section of a paint chip viewed under 4× magnification. Fig. 3 shows the same paint chip viewed at 20× magnification using fluorescence excitation at 350 nm and extracted at 535 nm. Fig. 4 shows differences in the same paint cross section when extracted at 595 nm. By exploiting the characteristics visible at each wavelength, the different constituents in the paint chip can be recognized.

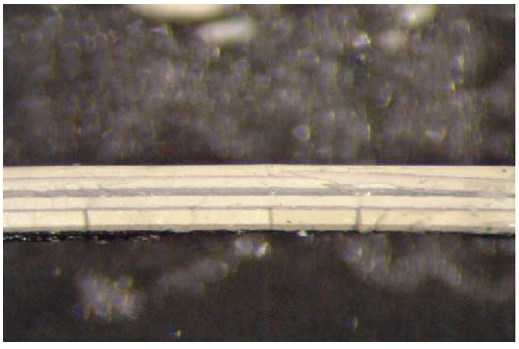

Fig. 2. White paint chip consisting of 10 layers.

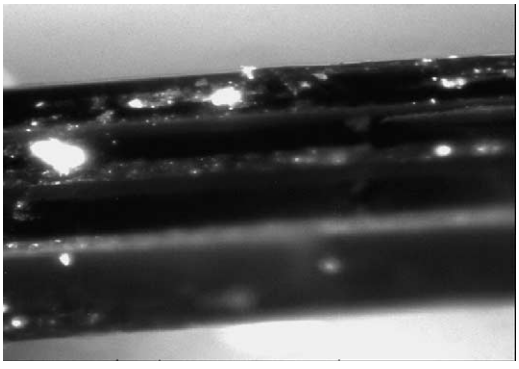

Fig. 3. Fluorescence chemical imaging of white paint chip at 535 nm with 350 nm excitation.

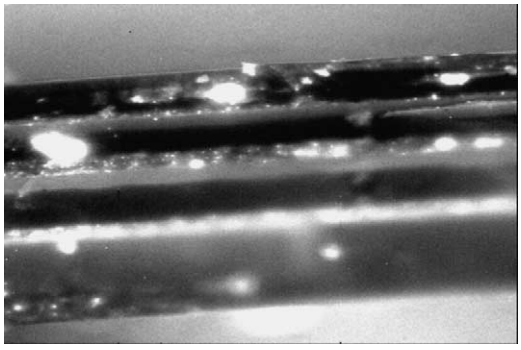

Fig. 4. Fluorescence chemical imaging of white paint chip at 595 nm with 350 nm excitation.

This is a powerful way of presenting data when comparing a known and an unknown paint sample. Using chemical imaging software, images can be placed side by side and a 'video' of the images of the experimental range can be played. This easily conveys the similarities and differences of the paint cross-sections to the layperson, such as a jury member when presenting evidence in court.

Alternatively, the microspectrophotometer is able to scan in the UV range as well as the visible and near-infrared region of the sample, which may be significant for some samples. The chemical imaging system is not able to do this. It can only scan in the region of the liquid crystal imaging spectrometer that is installed. However, the electronically tunable filter does replace the need for barrier filters in fluorescence analysis, of which only a few are available for the microspectrophotometer.

3.2. Tape and adhesive analysis

Macroscopic colour chemical imaging was able to discriminate and classify many of the different brown tape and adhesive samples giving a discriminating power of 0.92 for the tape backings and 0.72 for the tape adhesives. The colour analysis for clear tapes was obviously non-discriminatory.

The analysis of the tape backings by ATR infrared spectroscopy identified all of the tape backings as polypropylene.

The fluorescence analysis of the tape backings under the Polilight at three different wavelengths offered discrimination for the brown tapes only with a discriminating power of 0.56. Fluorescence chemical imaging on the other hand, gave a discriminating power of 0.61 for the adhesives and a slightly better discriminating power of 0.64 for the tape backings. The reason that the chemical imaging system picked up fluorescence of the clear tape backings and the Polilight did not may be due to the increased sensitivity of the chemical imaging system and the processing techniques

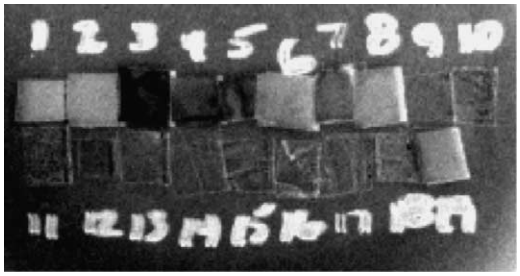

Fig. 5. Macroscopic colour chemical imaging of tape backings (extracted at 400 nm).

Table 4
Discriminating powers of techniques used for tape analysis

Examination	Tape	Discriminating power
Optical	Brown (backing)	0.95
Fluorescence polilight	Brown (backing)	0.56
DRIFTS	Brown (adhesive)	0.38
DRIFTS	Clear (adhesive)	0.75
VIS Condor (colour)	Brown (adhesive)	0.72
VIS Condor (colour)	Brown (backing)	0.92
VIS Condor (fluorescence)	Brown (adhesive)	0.61
VIS Condor (fluorescence)	Brown (backing)	0.64

Macroscopic chemical imaging appears to be an adept screening tool for the discrimination of similar coloured tape samples with the ability to analyse a large number of samples at once (Fig. 5).

The microscope system on the other hand is more suitable for the comparison of a limited number of samples, with more sensitive spectral data being available (Table 4).

3.3. Ink analysis

Microspectrophotometry provided a good degree of discrimination for the blue and black ballpoint pen inks, with discrimination powers of 0.87 and 0.92, respectively. TLC provided a slightly lower degree of discrimination with a discriminating power of 0.82 and 0.81, respectively for the blue and black inks.

Macroscopic colour chemical imaging of both black and blue inks proved successful with a slightly higher degree of discrimination than was achieved with microspectrophotometry. Discrimination powers of 0.96 for blue inks and 0.94 for black inks were obtained (Table 5).

Chemical imaging is an adept method of analysis for the differentiation of inks, especially since a large number of samples can be analysed at once, saving a great deal of time. Macroscopic chemical imaging would be a proficient tool for the analysis of altered documents as well as for the comparison of ink samples. Although spectral data was not utilised, the characteristics of the inks over the visible region provided means for discrimination and it is likely that with further investigation spectral data could be utilised. Although microscopic chemical imaging was able to differentiate all ink samples, it is best suited for the comparison of a limited number of samples (typically one on one comparison) as the benefit of being able to analyse large amounts of samples at once is lost. However, it may provide additional information for the analysis of microscopic alterations, line crossings and forgeries.

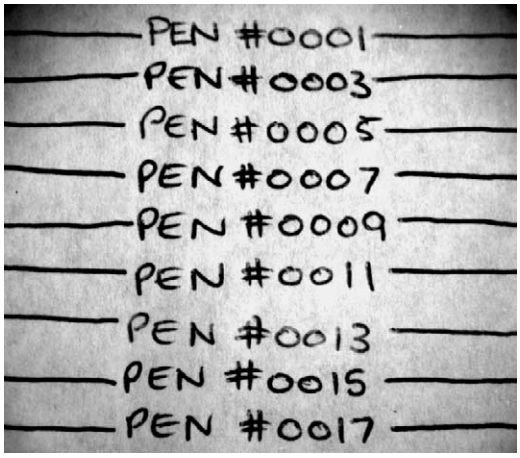

Fig. 6. Ink data set collected using white light. Frame extracted at 600 nm.

Fig. 7. Ink data set after processing. Third principal component extracted.

Table 5
Discriminating powers of ink analysis experiments

Examination	Discriminating power (blue inks)	Discriminating power (black inks)
Microspectrophotometry	0.87	0.92
Thin layer chromatography	0.82	0.81
VIS Condor (colour)	0.96	0.94
VIS microscope chemical imaging (colour)	1.00	1.00
VIS microscope chemical imaging (fluorescence)	1.00	1.00

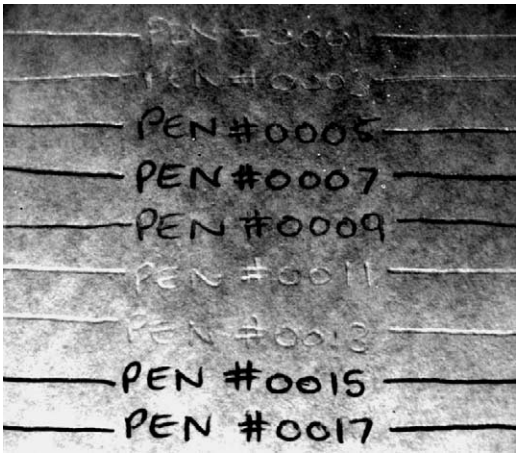

Fig. 8. Ink data set after processing. Fourth principal component extracted.

The current techniques for the analysis of ink samples are TLC and microspectrophotometry. TLC is a valuable method for the differentiation of ink samples, but it is destructive and microspectrophotometry offers a slightly lower degree of discrimination than macroscopic chemical imaging (Table 5).

Chemical imaging data sets obtained in the NIR region were processed using a number of tools contained in the ChemAnalyze software. Pre-processing tools, such as background division, baseline correction, zero offset and normalisation, which can all be used to minimise spectral artefacts due to the instrument (and unrelated to the sample), were used on the ink data sets. The data was then treated with a multivariate classification technique, principal components analysis (PCA). Basic processing tools such as brightness and contrast control were also employed to improve the quality of the resulting image.

Fig. 6 is an extract of the data set before any processing was conducted. Figs. 7–11 are extracted images that represent the principal components containing useful information for the comparison of the blue inks. Using these images, all

Fig. 9. Ink data set after processing. Seventh principal component extracted.

Fig. 10. Ink data set after processing. Tenth principal component extracted.

Fig. 11. Ink data set after processing. Twelfth principal component extracted.

of the pens can be distinguished with the exception of two pairs, which are similar pens from the same brand: 11 and 13 (Penline brand) and 15 and 17 (Pilot brand).

Fig. 12 shows comparison data that was collected with the VSC using green excitation light and a 655 nm longpass observation filter. These conditions reveal three groups of

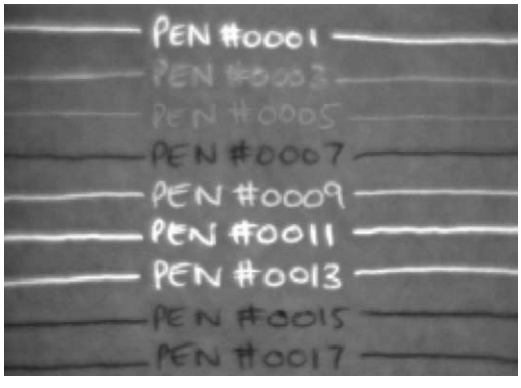

Fig. 12. Comparison data of blue ballpoint inks using the VSC 2000.

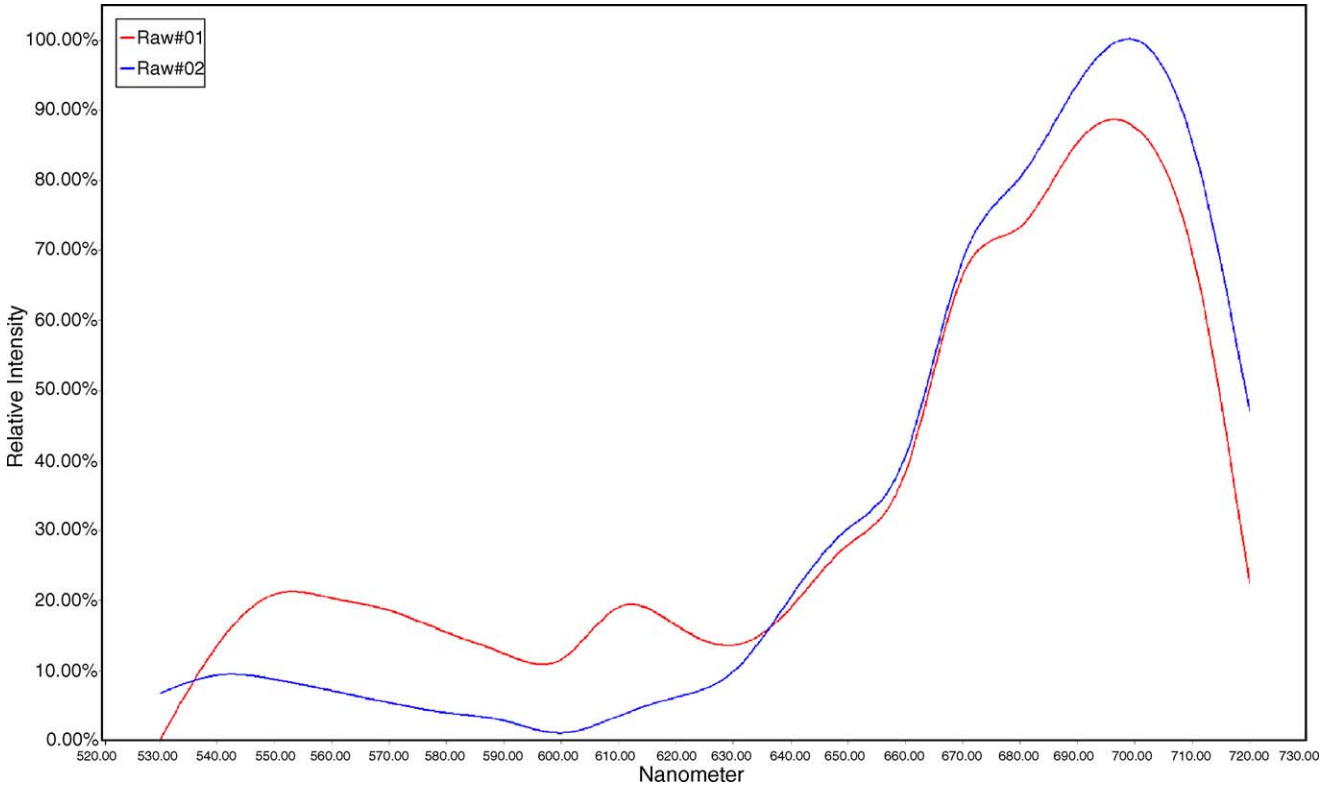

Fig. 13. Spectral (fluorescence) comparison of the two brands of raw unfired propellant.

inks. Further analysis with the VSC enabled four groups of inks to be distinguished.

The three dimensional nature of the chemical image data means that it contains enough information to allow more inks to be distinguished through the use of multivariate statistical techniques such as PCA. When discriminating powers are calculated, the VIS/NIR Condor scores well with a discriminating power of 0.94 when compared to that of the VSC (0.81).

Compared with traditional methods, chemical imaging methods provided the most discrimination with the added benefit of both visual and spectral information if needed. This reinforces the value of chemical imaging for forensic ink analysis and supports the suggestion that chemical imaging could be used to replace optical microscopy and microspectrophotometry for this type of application. TLC can be used as a confirmatory test if required subsequently. Chemical imaging has also been used for increased dis-

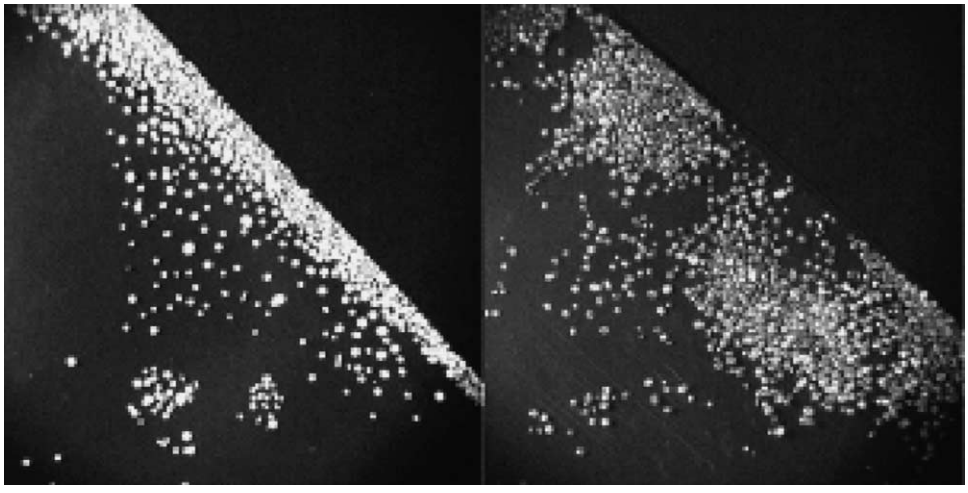

Fig. 14. Five hundred and thirty nanometers extract of propellant data set. Left raw #1 and right raw #2.

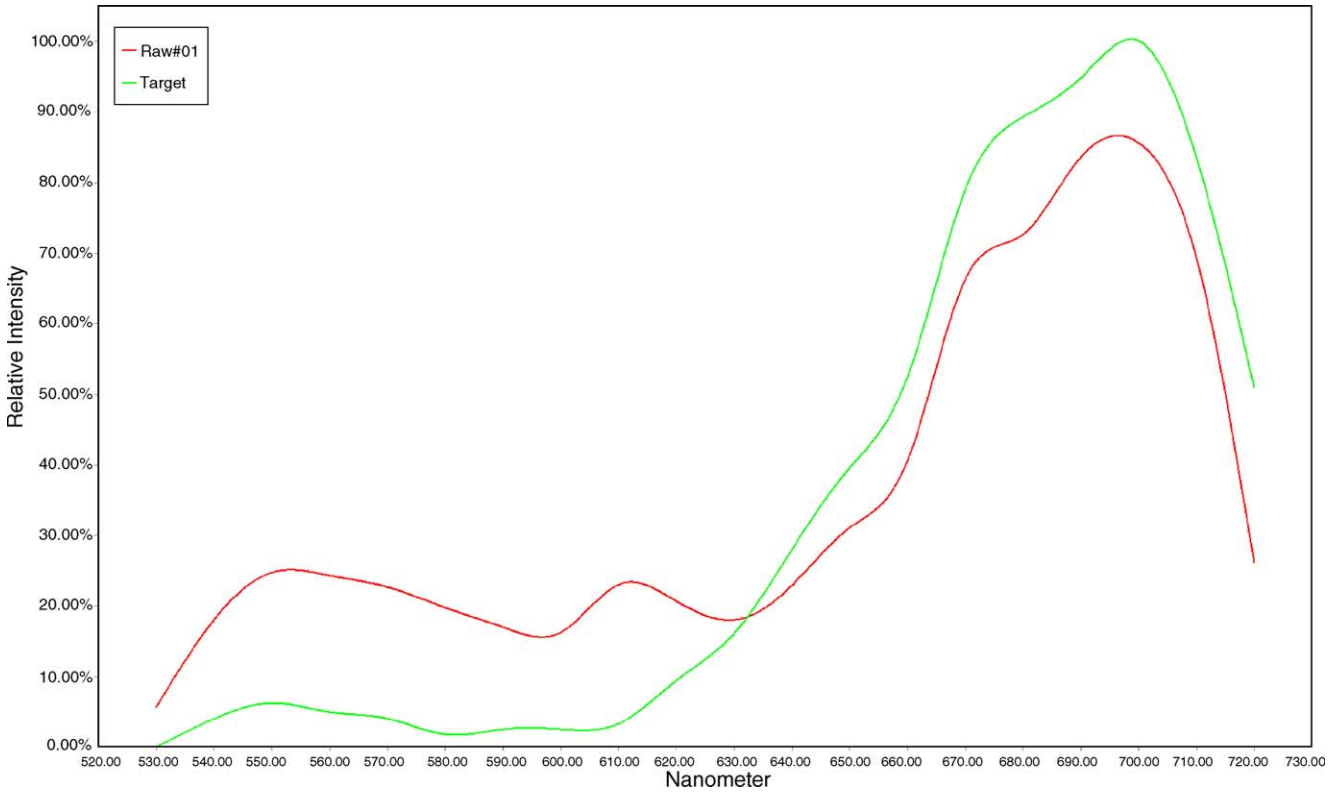

Fig. 15. Spectral (fluorescence) comparison of propellant fired onto target and raw #1.

crimination of developed TLC plates compared to visual inspection.

3.4. Firearm propellant analysis

Only simple pre-processing tools were required to enhance the propellant data sets. All data was treated with background division. When more than one image is being examined the concatenate tool is used to combine the images making analysis and results display straightforward.

Figs. 13–18 represent the comparison of two known unfired propellants, raw #1 (PowerPoint) and raw #2 (Zapper), with an unknown ammunition that was fired into a piece of black cotton fabric. Each spectral line represents the average of five propellant particles.

The comparisons reveal two interesting results. Firstly it shows that the fluorescence spectra of the two propellant

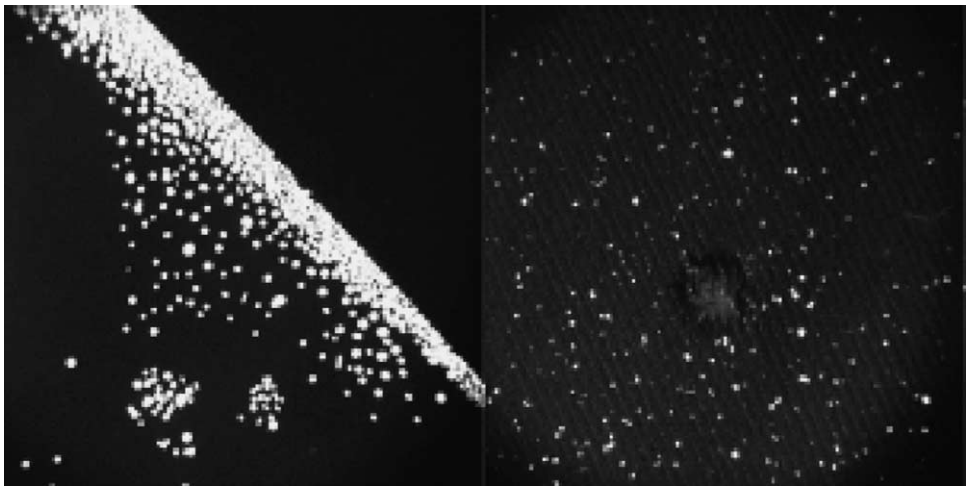

Fig. 16. Seven hundred nanometers extract of propellant data set. Left raw #1 and right target.

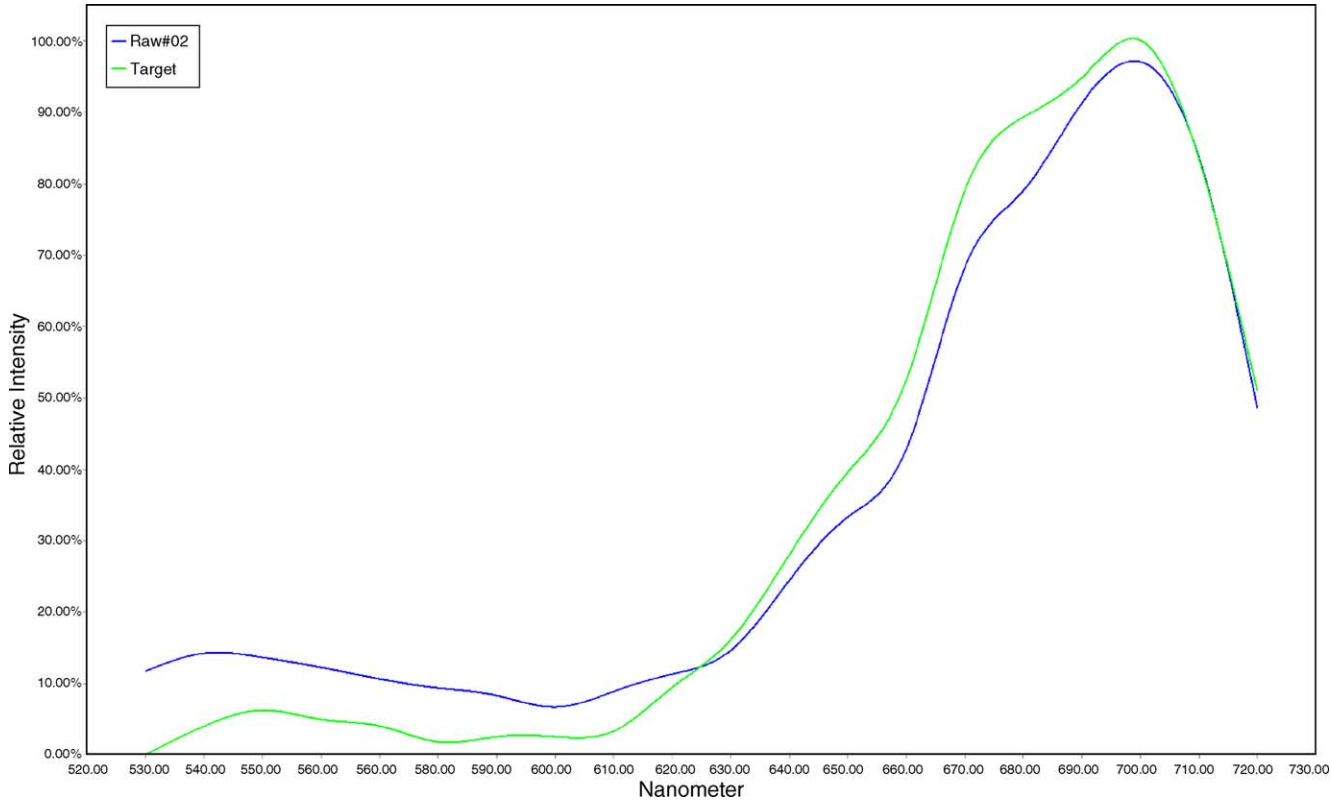

Fig. 17. Spectral (fluorescence) comparison of propellant fired onto target and raw #2.

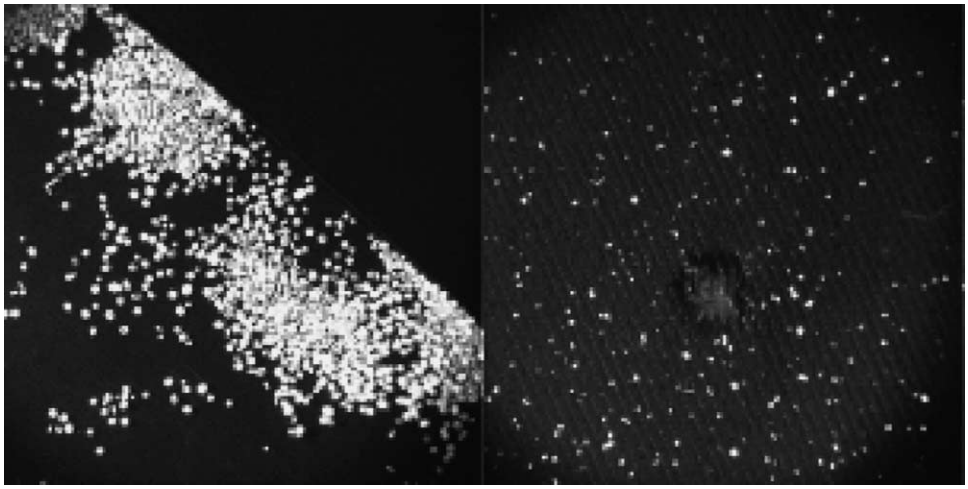

Fig. 18. Seven hundred nanometers extract of propellant data set. Left raw #2 and right target.

brands used do not change significantly after firing. Secondly the results show that it is possible to narrow down the brand of ammunition used in a shooting based on the fluorescence spectra.

The comparison in this study reveals that the ammunition that was fired into the black cotton fabric is indistinguishable from raw #2 (Zapper) (Figs. 17 and 18). This result indicates that chemical imaging can be used to examine the fluorescence properties of firearm propellants.

4. Conclusions

Results of this study show that chemical imaging has great potential in the forensic analysis of materials, including paints, tapes and adhesives, inks and firearm propellants.

The analysis of paint chips exploited some of the main advantages that chemical imaging technology provides, the ability to display visual and spectral results side by side and the lack of sample preparation required. Also, because spec-

tral data from the entire field of view is collected at one time, analysis time is greatly reduced compared with traditional techniques that require multiple spectra to obtain an average profile of the sample.

The results of the tape and adhesive study show that chemical imaging was able to produce a greater discriminating power (ability to discriminate two samples randomly selected from a chosen relevant population) than most traditional techniques. The macroscopic Condor also added the advantage of being able to analyse many samples at once, making comparisons easier.

Ink analysis on questioned documents was shown to benefit greatly from chemical imaging analysis. Although traditional techniques produce good discriminating powers, chemical imaging was able to discriminate all brands of inks. Chemical imaging also has the benefit of being a non-destructive technique unlike TLC.

Currently there is no widely used technique for the fluorescence analysis of firearm propellants. The initial results of this study demonstrate the possibility of using chemical imaging technology to eliminate different brands of ammunition based on the fluorescence characteristics of the propellant grains without destroying the evidence before further analysis can be conducted.

The recent upgrade of the VIS Condor at the Australian Federal Police to a VIS/NIR Condor and the promising results obtained has prompted research to continue, including emphasis on the analysis of questioned documents and firearm propellants so that the technology can be fully validated for these applications.

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