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Forensic Applications of Infrared Chemical Imaging: Multi-Layered Paint Chips

ABSTRACT: This paper examines the potential of infrared chemical (hyperspectral) imaging as a technique for the forensic analysis of automotive paint chips in particular, and multicomponent (e.g., layered) samples in general. Improved sample preparation procedures for the infrared analysis of paint chips are detailed, with the recommendation that where mounting resins are chemically incompatible with the sample, it is better to mount and section the sample in a soft wax from which the sections can be removed and pressed into a KBr disk for transmission analysis. Infrared chemical images of multilayered paint chips have been successfully obtained, with the chief advantage over conventional infrared analysis being that thousands of infrared spectra are collected in a few minutes across the whole sample, at a spatial resolution of around 5 μm. As with conventional infrared spectroscopy, chemical species can be identified from their spectra, but the wealth of information available can be also extracted in a number of different ways that make multicomponent spectral (and hence chemical) comparisons between two samples easy to visualize and understand. In one approach, the infrared chemical images of two paint chips being compared side-by-side can be viewed as a "movie," in which each frame is an intensity map of the two samples at a given wavenumber (frequency) value. In another approach, the spectra (pixels) in the image files are classified into chemically similar groups, resulting in a "cluster" image that makes it possible to simultaneously compare all of the layers in two paint chips. These methods are applicable to other multicomponent samples, and also to other chemical imaging techniques.

KEYWORDS: forensic science, chemical imaging, paint, infrared, FTIR, hyperspectral imaging, multicomponent samples, chemometrics

Chemical Imaging in Forensic Science

Generally speaking, chemical or hyperspectral imaging is the ability to map the spatial distribution of chemical species across a sample surface. It allows for the visual enhancement of the sample and the chemical identification of its components. Since spectroscopy is one of the most important tools for identifying and quantifying chemical species, a chemical (hyperspectral) image is usually constructed by collecting multiple spectra in the frequency (ν) or wavelength (λ) range of interest in an array across the sample. The resulting dataset consists of intensity values at each position in an $x \times y \times \lambda$ datacube (Fig. 1). This data can be represented and analyzed in a number of different ways. It can be visualized as a spectrum at each pixel in the image, or as series of $x \times y$ images, one for each wavelength value collected in the spectrum. The latter visualization represents the simplest form of chemical imaging, since a particular wavelength may correspond to a spectral band of a specific molecule, and thus an image at this wavelength will selectively map the distribution of that molecule across the sample.

The very large three-dimensional datasets collected by most chemical imaging instruments contain a wealth of information that is not accessible using the simple visualizations mentioned above. For this reason, multivariate statistical techniques are employed to reduce the data and/or classify the spectra, and thus form more informative images than the raw data.

The potential of chemical imaging in forensic science has been apparent since instrumentation became available in the late 1990s. Techniques currently available include UV-visible/fluorescence imaging (1), near infrared (NIR) imaging (2), Raman mapping and imaging (3), mid-infrared mapping and imaging (4), and compositional mapping using electron microprobe analysis (5,6). All of these techniques can be applied in forensic situations. Chemical imaging in the visible and NIR spectral regions is currently being investigated to gain improved contrast and discrimination in the imaging of fingerprints, textile fibers and questioned documents (1,7). Infrared and Raman mapping (as distinct from imaging) are now the poor relations of chemical imaging in that they require the point by point acquisition of spectra across the sample, an extremely time-consuming process. However, technological improvements in the areas of array detectors, liquid crystal tunable filters, infrared interferometers and computing power in recent years have meant that true mid-infrared and Raman chemical imaging systems are becoming available to the forensic scientist (8).

Infrared Chemical Imaging

Infrared chemical imaging and Raman chemical imaging have enormous potential in forensic science because of their greater chemical specificity compared with UV-visible chemical imaging techniques. This specificity comes from the sheer abundance of narrow, highly resolved bands in the vibrational spectrum of even relatively simple molecules, and the fact that these bands correspond to particular functional groups of the molecules. As a simple example, the UV-visible spectrum of a colorless polymer may contain as few as one or two broad UV absorptions that may do little to distinguish the polymer from similar or even quite dissimilar materials. The addition of a colored dye to the polymer may introduce one or two broad absorptions into the visible region of the spectrum, but the ability to spectroscopically distinguish this sample

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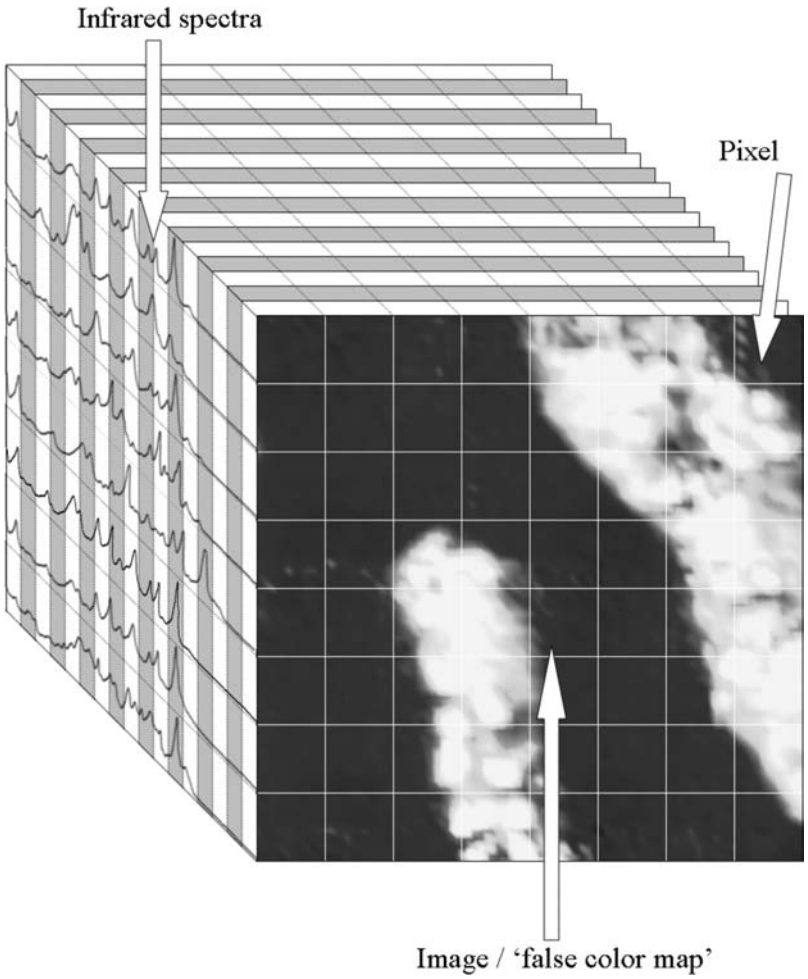

FIG. 1—*Diagrammatic representation of "datacube" generated by focal plane array (FPA) detector (reproduced with permission from Ref. 8).*

from others with similar coloration (especially those identically dyed) is limited.

The vibrational (mid-infrared or Raman) spectra of the polymer will, however, usually contain at least several (but often dozens of) sharp bands corresponding to specific structural components of the molecule that enable it to be differentiated even from closely related structures. Infrared (or Raman) spectra can be used to precisely identify materials in a heterogeneous sample; when collected in the form of an image, the spectra can be used to map the spatial distribution of the different chemical components of the sample to a resolution of around 5 μm (for mid-infrared imaging) or better (Raman imaging).

There have been numerous applications reported for infrared chemical imaging covering a broad range of areas including polymer analysis (9), cancer detection (10), lipid analysis in obesity research (11), and bone analysis (12) among others. Further information can be found on infrared chemical imaging theory and other applications in (13).

Infrared Microscopy in Forensic Science: Paint Chips

Paint evidence is frequently encountered by forensic laboratories, particularly in cases involving road accident investigations such as hit-and-runs, where paint may be transferred from the automobile onto the victim's clothing. Paint samples may be received as smears or intact paint fragments, possibly consisting of multiple layers.

With optical microscopy, infrared spectroscopy is one of the most commonly used techniques in the analysis of paint, yielding information on the identity and relative concentrations of major binder, pigment and resin components (14,15). This information can be used in two ways: it may provide an investigative lead by identifying the make and model of the automobile by comparison with database spectra; secondly, if there is reference material available from a suspect car, then the chemical information can be analyzed and compared to determine whether the paint materials could have a common origin (16).

Numerous sampling techniques have been reported in the literature for the infrared analysis of paint chips, including transmission (17), reflection (18,19), attenuated total reflection (ATR) (16), transflection (20), and diffuse reflectance (DRIFTS) (21). Transmission experiments often involve the use of microsampling accessories such as the diamond anvil cell (22). In general, transmission analysis is preferred over reflection analysis, even if it does require more sample preparation time. Drawbacks with reflection analysis include poor signal-to-noise ratio, spectral distortion (e.g., derivative-shaped peaks due to anomalous dispersion effect) and problems encountered with reproducibility (14). Band shifts of up to 30 cm^{-1} have also been reported for reflection spectra relative to the corresponding transmission spectra (19). Transmission spectra are therefore more easily compared with reference databases, which are generally compiled using transmission data. Reflectance spectra can be useful in certain circumstances, however, such as for the

analysis of trace smears of paint on tools or paint embedded in fabric, which may be difficult to remove and manipulate (16,18). High quality infrared spectra of individual paint layers can be collected using an attenuated total reflectance (ATR) microscope objective, but the ATR analysis of multi-layered samples is still tedious and difficult as it requires the separation of the individual paint layers.

The development of infrared microscopy has enabled the analysis of the very small paint samples that are often received by forensic laboratories, and has meant that the separation of each individual layer prior to analysis is no longer necessary because apertures can be used to limit the analysis area to one layer at a time (14). For transmission analysis, this requires that a thin cross-section of the paint layers be prepared, generally by embedding the paint chip in a suitable resin and using a microtome to cut sections 1–10 μm in thickness (17).

Numerous different embedding media have been suggested for paint chips prior to infrared analysis, including wax (23), gelatin (water-soluble) (17), polyesters (24), and cyanoacrylates (25). The ideal properties of a resin medium were originally outlined by Derrick et al., and modified for forensic analysis by Chang et al. to include: (i) curing at room temperature without shrinking; (ii) not reacting with or infiltrating the sample; and (iii) being easy to microtome (23,26). In a recent study, seven different embedding methods were evaluated for their use on forensic automotive paint evidence. Overall, the best results were obtained using a fast-epoxy resin, but infiltration problems were minimized with the use of wax (26).

A method that avoids the use of any resin material was proposed by Van der Weerd (27). This involves doubly polishing a paint chip embedded in a KBr pressed pellet. Drawbacks include the potentially lengthy trial and error nature of the method, the inability to determine the final thickness, and the destruction of the majority of the paint evidence. An advantage is that no specialized equipment, such as a microtome, is required.

Whether the paint layers are separated prior to analysis, or embedded and microtomed, very thin paint layers, such as thin base coats (12–20 μm), can cause difficulties. The manual separation of thin layers is obviously problematic; small sample sizes and/or incompletely separated layers may result. The sequential analysis of layers in a transverse-sectioned paint chip usually requires the use of an aperture, which allows the isolation of a small area of the sample. Aperturing has the disadvantages of reduced signal to noise (necessitating longer collection times) and the limiting of the spectral range to wavelengths smaller than the size of the aperture (16). With both methods of analysis, it may also be possible to overlook a layer, if two adjacent layers are similar in appearance. If each layer of a multilayered paint chip is analyzed individually, this will be a fairly time-consuming process, particularly for chips from older cars that have been repainted and repaired numerous times (28). In one actual case study reported by Zieba-Palus, an automobile paint sample was reported to have nineteen layers (29).

Infrared chemical imaging readily suggests itself as a powerful method for discrimination within evidence types such as paint chips, because it simultaneously delivers chemical and spatial information about the sample. For example, infrared chemical imaging has been applied to paint cross-sections in order to investigate art restoration work conducted on one of Rembrandt's paintings (30). However, this analysis was carried out in reflection mode, which led to poor signal-to-noise and the introduction of spectral artifacts caused by specular reflection, necessitating the subsequent use of the Kramers-Kronig transform, a mathematical correction algorithm. For the purposes of identifying various materials used to restore the art work, this sampling method was satisfactory; however, transmission analysis is preferred in forensic work, as previously noted.

At the time of writing this article, no publication concerning the forensic analysis of paint chips using infrared chemical imaging could be found. In this paper, we investigate the potential of infrared chemical imaging, with and without associated multivariate analysis techniques, in forensic identification and classification studies, using automobile paint chips as an example. In addition, for the specific case of paint chip analysis, we propose a new method for the preparation of samples prior to analysis by infrared microscopy/imaging.

Materials and Methods

Six multi-layered and multi-colored paint chips of varying quality were obtained from a previous study on automotive paint (31). These paint chips were collected from insurance car yards and had 4–8 layers each. The samples were set in a relatively soft medium such as paraffin (Paraplast®, Oxford Labware) or Fimo (014 Transparent, Eberhard Faber) prior to transverse sectioning using a Leica RM2165 motorized microtome. Paint chip sections of 5–10 μm thickness from a given sample were allowed to separate or fall out of the embedding medium, and were then carefully laid onto a bed of spectroscopic grade potassium bromide (KBr, Merck) powder in a dye. The KBr powder and paint chip sections were then pressed into a conventional 13 mm diameter disk, using a press. Individual paint chips were selected for imaging if they remained flat (unfolded) and parallel to the surface of the disk after pressing.

A variety of other resins were tested including Serifix (Struers), cyanoacrylate (Selleys 'Fix'N'Go' Supaglue), Spurrs embedding resin (soft formulation, ProSciTech), and a UV-curable resin (Loctite). It was necessary to reduce the amount of hardener by half in the Serifix recipe to obtain a resin of suitable hardness for microtoming. Paint sections of 5–10 μm thickness were microtomed and placed on a KBr plate for imaging.

Infrared chemical imaging of the paint chips in the KBr medium was carried out using a Digilab Stingray system, comprised of an FTS7000 FTIR spectrometer, coupled to a UMA600 infrared microscope fitted with a Lancer 64 × 64 focal plane array (FPA) detector. Images and spectra were collected and processed in the first instance with Digilab Win IR Pro software. All samples were imaged in transmission mode using either the normal field of view, in which each individual image tile is approximately 350 × 350 μm in size, or the expanded field of view (EFOV) setting, which produces an image tile of approximately 700 × 700 μm in size. Within each image datacube, absorbance spectra were collected at 8 cm^{-1} resolution, using 64 or 256 co-added scans. The spectral range collected was 900-4000 cm^{-1}, with the lower value being determined by the limit of the FPA detector. Background image files were collected from vacant areas of the KBr disks/plates. Some samples were also imaged in reflectance mode, with most parameters similar to those for the transmission experiments, except that (i) background images were collected from infrared-reflective metal oxide-coated glass slides (Kevley Technologies), and (ii) a minimum of 1024 co-added scans were often required to obtain a reasonable signal-to-noise ratio.

Classification of infrared chemical images was carried out in specialist chemical imaging software packages, ENVI 4.0 (Research Systems Inc.) and Cytospec 1.05.10 (www.cytospec.com), which were also used to preprocess the spectra. Hierarchical cluster analysis (HCA) was carried out in Cytospec using the D-values distance method and Ward's algorithm for clustering. Prior to this analysis, spectra were truncated to a spectral range of 1000–2000 cm^{-1}

(leaving just the fingerprint region which contains most of the discriminating power of an infrared spectrum) and then converted to their second derivatives and normalized in the spectral domain. Supervised classification of images was carried out in ENVI using the Mahalanobis distance method to classify spectra as members of user-defined "regions of interest." Spectra were preprocessed as for HCA, except that spectra were further truncated to meet the requirement that they contain fewer variables than pixels were defined in each region of interest.

Results and Discussion

Sample Preparation Considerations

As mentioned earlier, the preparation of multi-layered paint samples for infrared microscopic analysis is not straightforward. In order to access all of the advantages that chemical imaging offers in the analysis of this type of evidence, cross sections must be prepared. For reflected light analysis, whether visible or infrared, the cross sections can be prepared simply by cutting a mounted or unmounted sample with a scalpel or a microtome. However, since in most cases the quality of (non-ATR) infrared reflection spectra will not be adequate for identification or discrimination purposes, the sample must be prepared as microtomed sections thin enough for transmission analysis. The choice of mounting resin is critical, and the chief conclusion that can be drawn from the literature surveyed in the introduction is that no single resin material is ideal for all infrared microscopy applications. In fact, our own investigations in this area lead us to speculate that few of the embedding resins or waxes commonly used for optical and electron microscopy are suitable for infrared microscopy; many epoxy and polyester resins are designed to infiltrate the sample, so are immediately undesirable here, while paraffin waxes do not adhere sufficiently to the sample, allowing it to fall out after sectioning. Some epoxy resins require curing at elevated temperatures, and actually dissolve paint samples. It was found in our studies that the Spurrs resin (epoxy-based) and cyanoacrylate either partially or completely dissolved the paint samples, therefore making these resins unsuitable for embedding forensic paint samples.

In order to avoid interference with sample spectra, the ideal resin for embedding samples prior to sectioning for infrared analysis would have either no infrared spectrum, or a very simple spectrum with a very small number of absorption peaks. Few carbon or silicon-based polymers with appropriate physical properties meet this criterion; the paraffin waxes do seem ideal from this point of view, but have other drawbacks as mentioned above. The traditional medium for embedding samples prior to macroscopic infrared analysis has been the halide salts, such as sodium chloride (NaCl) and potassium bromide (KBr). These are transparent to infrared radiation above characteristic low wavenumber frequencies, and are usually mixed with powdered sample and pressed in a dye into a pellet or disk. The efforts of Van der Weerd (27) to capture paint chips in KBr sought to take advantage of the non-interfering nature of KBr, but seem unnecessarily laborious and difficult.

We therefore believe that the method outlined in the experimental section for embedding paint chip sections in KBr has potential for defeating most of the problems identified here for other methods. The use of a low melting point paraffin (or similar material such as Fimo) in which to embed the paint chip for sectioning obviates problems of infiltration, dissolution and spectral interference. The paraffin sets in minutes, after which time the sample can be readily microtomed. The tendency of the thin sections of paint to fall out of the microtomed paraffin slices is now treated as an advantage, because a clean transfer can be made to the KBr prior to pressing. Although it might be considered that the "liberated" paint section could be used "as is" for infrared microscopy, it was often found to be too delicate to handle and/or not sufficiently flat for imaging analysis. This overall method has been found to work well for multilayered paint chips of reasonable quality, but is not recommended, for obvious reasons, for samples with a tendency to separate or crumble easily. Care must also be taken to avoid smearing of paraffin across the paint surface when microtoming.

Other resins found to be satisfactory for embedding paint samples include the Serifix and UV-curable resin (which must be used with a high powered UV source to allow for complete and rapid curing). However, it was found that for older, more porous paint samples, the resins were found to have infiltrated the samples, as spectral bands due to the resins could be seen in spectra of the paint layers. The older samples were also found to fall apart and crumble when microtomed in these resins.

Infrared Chemical Images

Infrared chemical imaging was found to be a powerful technique for analyzing multi-layered paint samples. There are a number of advantages of infrared imaging over conventional infrared microscopy as applied to the analysis of multilayered samples such as paint chips. These include: (i) the simultaneous collection of spectra from all layers at once, so that only a single experiment is required in order to (potentially) identify all of a sample's components; (ii) potentially hundreds of spectra are collected from each layer of the chip, a feature that greatly increases confidence in any conclusions drawn, and lends itself to multivariate classification techniques; and (iii) any (potentially characteristic) inhomogeneities present in the layers can be revealed.

As mentioned earlier, infrared chemical imaging data can be visualized in a number of ways. In one mode, single-point spectra can be extracted from any pixel in any layer in the paint sample and then classified or submitted for a spectral library search as would be done for conventional infrared spectral data. In a more powerful representation of the data, it can be viewed as a series of images produced by mapping the spectral intensity, at any given wavenumber value, across the field of view. These images can be based on the simple spectral intensity (e.g., absorbance) at a single wavenumber value, or they can be formed using the integrated intensity under a spectral peak that represents a chemical component of the sample. In either case, the spatial distribution of a chosen chemical component can be displayed, using either a grayscale or a "false" color scale (e.g., red for high through to blue for low) to indicate intensity.

Figures 2(*a*) and 2(*b*) show, respectively, the visible light image and an infrared chemical image of a five-layer automotive paint chip (Ford Telstar 1982, chip #36). Figures 2(*c*) to 2(*g*) show characteristic infrared spectra from each of the five layers. Using the flowcharts, tables and spectra in references 14 and 16, the paint types in the layers can be identified as follows: (*c*) epoxy enamel with clay extender; (*d*) and (*e*) polyester-melamines; (*f*) acrylic urethane with clay pigment extender; and (*g*) acrylic urethane. The infrared image was formed using integrated spectral intensity under a peak centered near $1606\,cm^{-1}$, which corresponds to a C=C stretch of the aromatic bisphenol ring in the epoxy resin (c, top layer). This choice permits all five layers to be seen together, because of the contrast yielded by the different intensities at that frequency in the corresponding layers (blue indicating low

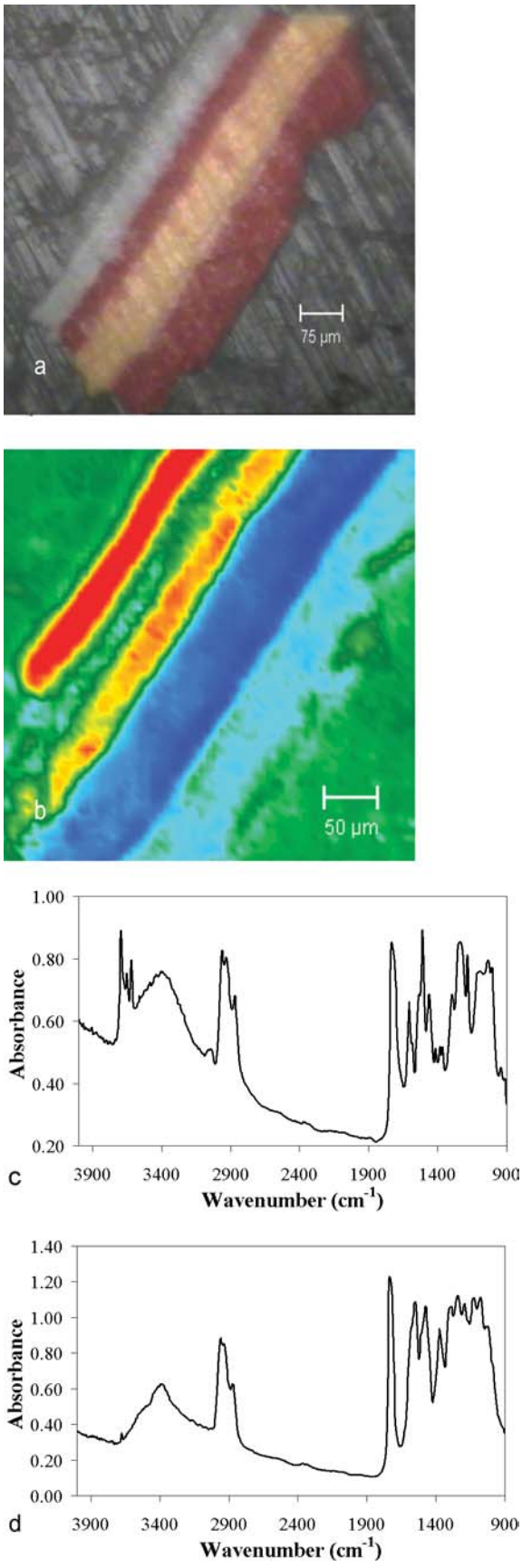

FIG. 2—*a) Visible light image of paint chip #36; b) FTIR chemical image of paint chip #36 at 1606 cm^{-1}; c–g) Infrared spectra of paint layers 1–5.*

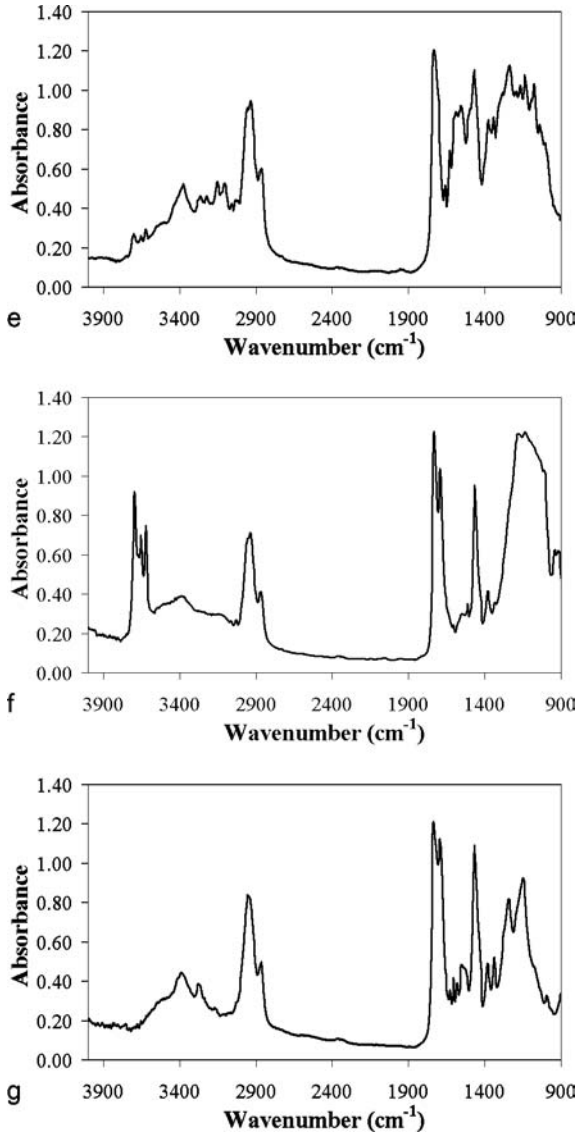

FIG. 2—*Continued.*

intensity, red indicating high), even though only one layer contains that precise functional group. By itself, this image could be more informative than the visible light image (if it revealed a previously undetected layer); in any case, it represents only a tiny fraction of the information available in the entire infrared image datacube. Multivariate techniques for better accessing the entirety of this information are discussed in the next section, but one simple way to visualize more of the information is to view a "movie" constructed using the full sequence of chemical images across the spectral range. As the movie plays, different chemical components of a heterogeneous sample, such as a paint chip, are highlighted sequentially. This capability can be used to make very powerful comparisons between two samples, such as a paint chip from a hit-and-run scene and one from a suspect vehicle. The two infrared images, collected under identical conditions, can be joined side-by-side. If the two paint chips are from the same source, each frame of the resulting movie will show the corresponding layers of the two chips behaving identically to each other (in terms of intensity patterns) at every wavenumber value across the spectrum. Figure 3 shows four frames of such a movie. With a color intensity scale, this is a particularly powerful way of highlighting the similarities and

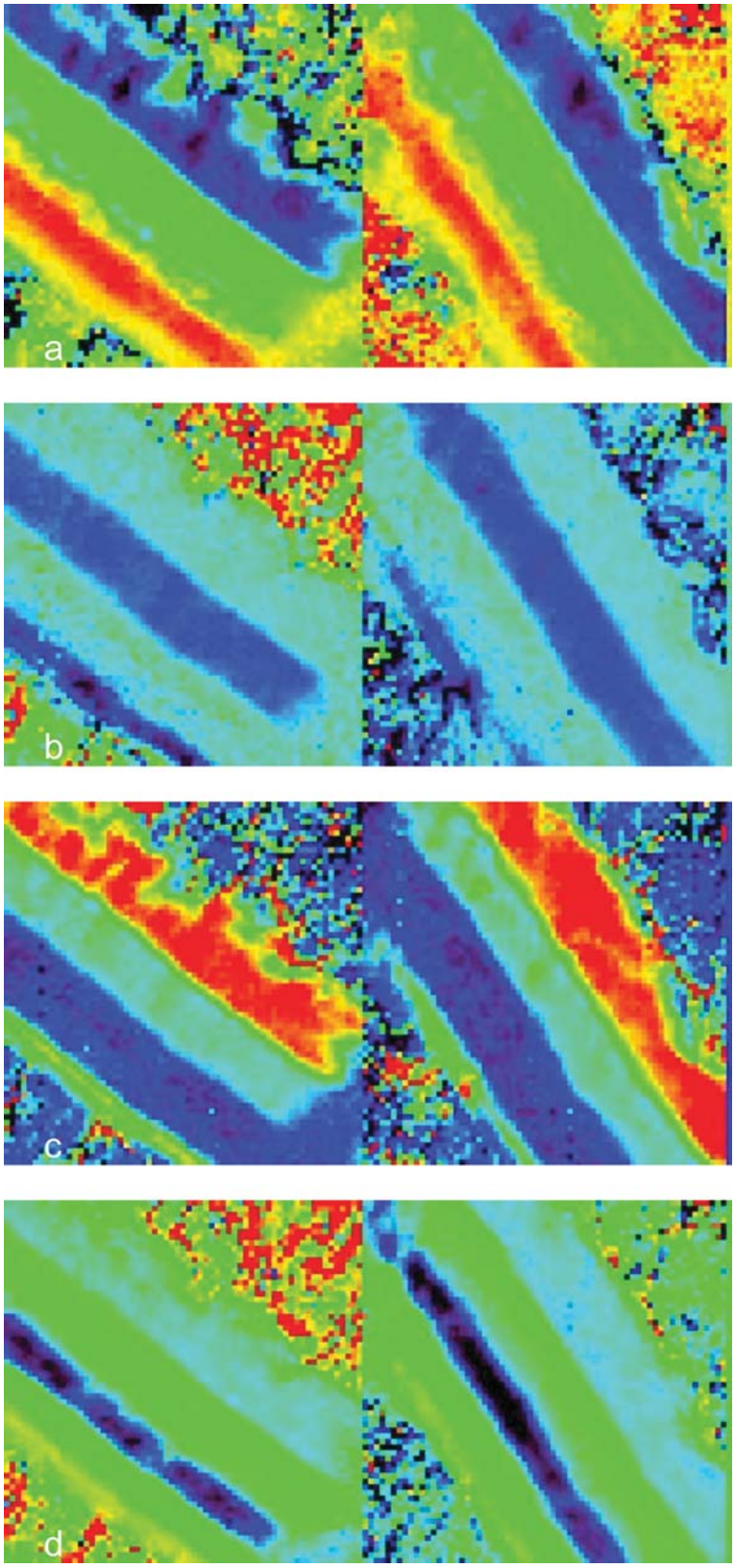

FIG. 3—*a–d) Four image frames captured at various wavelengths from movie comparing two paint chips from same source (#36).*

differences between two paint chips. This representation is more easily understood by a layperson such as a jury member than a comparison of individual infrared spectra, a significant advantage for forensic evidence.

Most of the potential drawbacks of infrared chemical imaging correspond with those of conventional infrared spectroscopy, including the sample preparation issues discussed earlier in this article, and problems encountered when collecting data in reflectance

mode. For certain samples, obtaining an acceptable signal-to-noise ratio in the spectra means longer acquisition times with the FPA detector compared to a conventional MCT infrared microscope detector (5–6 min for 256 scans, compared with less than 1 min), but the extra time is small on an absolute scale, and the quantity of information collected in a chemical image is far greater, since it consists of 4096 spectra from across the whole sample. One imaging-specific disadvantage of infrared imaging systems as they are currently manufactured is that the spectral range at the low end does not extend below about 900 cm^{-1} (for the Digilab system used in our laboratory; 700 cm^{-1} for the Perkin Elmer Spectrum Spotlight), compared with about 600 cm^{-1} for an MCT detector on a conventional infrared microscope. This means, for example, that some vibrations of inorganic components of a sample, such as some paint pigments, will not be observable.

Cluster Analysis: Comparison of Chemical Images

Modern multivariate image analysis techniques are currently being used successfully in the classification of pixels in hyperspectral (chemical) image data in the disparate fields of remote sensing and cancer histopathology (10,32). In remote sensing applications, spectral information (often consisting of just four visible or near infrared wavelengths) is used to accurately classify pixels in satellite and aerial images according to vegetation type, land use and mineral/soil types. A lot of published research has also been devoted to the classification of pixels in the infrared images of thin tissue sections. This type of work aims to use the very subtle differences in the infrared spectra of abnormal tissue to map the location of cancerous or potentially cancerous cells as an alternative to traditional staining techniques. Although the differences between the spectra of the different tissue types are very slight, the very large number (hundreds or thousands) of spectra involved mean that multivariate statistical techniques can be used to draw valid conclusions from the data. The application of these techniques to forensic samples such as paint chips actually poses less of a challenge because the differences between the infrared spectra of different paint layers are generally far greater than (say) the differences between normal and abnormal tissue from a human lymph node.

The statistical techniques that are used in the classification of hyperspectral or chemical images can be divided into two broad categories, supervised and unsupervised classification. Supervised methods rely upon information input by the operator when classifying pixels in an image, while unsupervised methods do not. Detailed descriptions of chemometric classification techniques can be found elsewhere (33), but here we will confine ourselves to brief summaries and an example, using infrared chemical images of paint chips, of how supervised and unsupervised classification techniques might be used in a forensic setting. While in the case of paint chips these approaches might seem unnecessary for, say, a comparison of two paint chips with the same sequence of ten brightly colored layers, their value will be more apparent for more visually ambiguous evidence. Because the clustering techniques can assign a "false" color to each chemically distinct region (cluster) in a chemical image, the resulting image represents a powerful way to make multiple chemical comparisons easy to understand for a layperson such as a juror.

When a forensic laboratory uses infrared spectroscopy to analyze a paint chip, the objectives are usually to identify the component paints as accurately as possible and/or to compare a paint chip from an unknown source (e.g., crime scene) with another from a known source (e.g., suspect vehicle). The former objective can best be accomplished by comparing spectra with those in infrared libraries. If a visible light comparison is inconclusive, a comparison of infrared spectra from corresponding layers of the two paint chips will provide information as to the chemical composition of the different layers. As mentioned earlier, one of the advantages of infrared chemical imaging over conventional infrared analysis in this situation is that potentially hundreds of spectra from each layer of a paint chip can be collected in the same time it would take a conventional infrared microscope to collect one spectrum. Cluster analysis of the resulting spectra/image can identify and clearly display the location of all spectra that are classified as being "the same" (within certain limits) in the image of a paint chip, and, more powerfully, in the image of two paint chips that are being compared.

An example of an unsupervised cluster analysis technique is hierarchical cluster analysis (HCA) (33,34). In this technique, the "distance" in multivariate space (a measure of similarity/dissimilarity) between each pair of spectra in an image is first calculated. Next, the two most similar spectra in the dataset are linked and placed into a group, or cluster, together. The distances between this group and each of the other spectra in the image are then calculated, and the linkage process is repeated, resulting in another spectrum being placed in a group, but at a lower level of similarity. This overall procedure is repeated until all of the spectra are in one group. The result of this sequential grouping process is a *dendrogram*, a tree-like structure that shows the stage at which each spectrum was grouped with other spectra (Fig. 4).

When followed in the reverse sense, the dendrogram can be used to identify ever-increasing numbers of clusters (with ever-increasing intra-cluster similarity) into which the spectra can be divided. Starting with all of the spectra grouped into one cluster (at the bottom of Fig. 4), the resulting image would be completely uniform. At the next step in similarity (moving up the dendrogram), the image can be divided into two clusters; this shows all of the spectra in the image placed into either of two groups based on gross similarities/dissimilarities. For an object with a finite number of chemically distinct regions, the image can be divided into greater and greater numbers of clusters until such time as the resulting image has no further physical meaning.

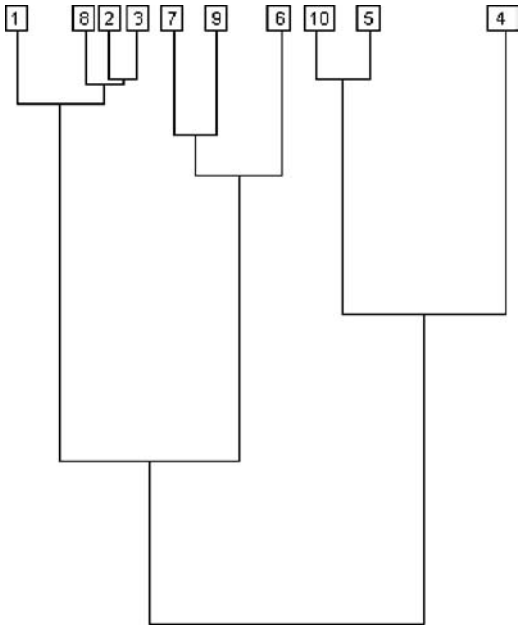

FIG. 4—*Dendrogram result from a hypothetical hierarchical cluster analysis.*

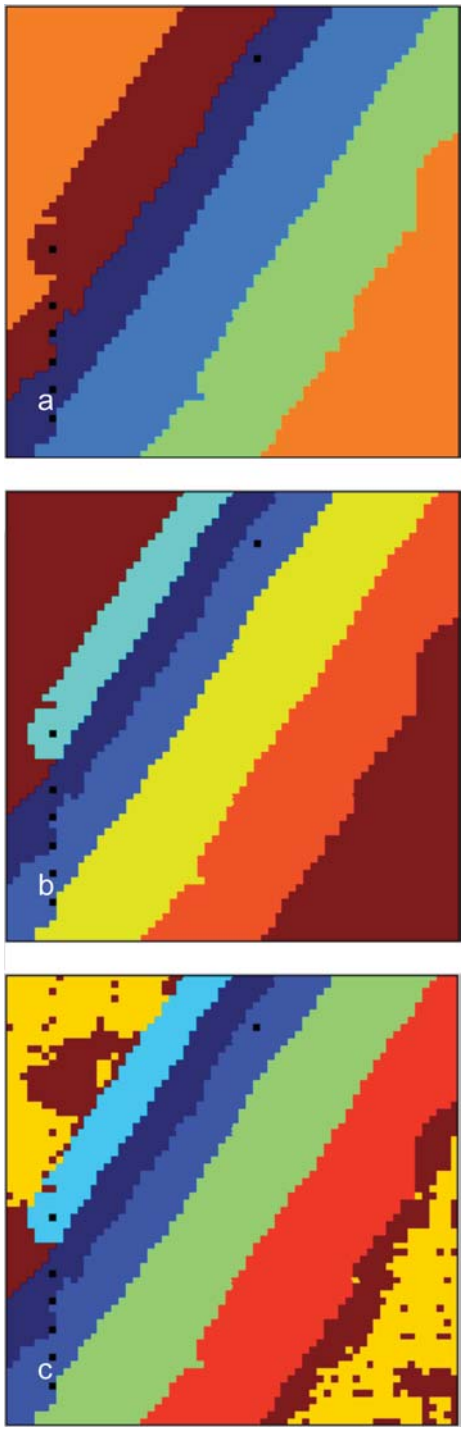

FIG. 5—*Cluster images resulting from hierarchical cluster analysis (HCA) of paint chip #36 with a) five clusters b) six clusters and c) seven clusters.*

Figure 5 shows the results of HCA on the five-layer paint chip discussed earlier. In Fig. 5(*a*), the first five clusters have been used to form the image, while in Fig. 5(*b*), the first six clusters are used. Since the visible light and raw infrared image analyses indicate that the chip has five layers, we would expect a maximum of six che mically distinct regions in the image (the five layers, assuming that they are all different from each other, plus the mounting material). Indeed, the six clusters shown in Fig. 5(*b*) do correspond to the five known paint layers and the mounting medium.

If there were more chemically distinct layers that had not been apparent in the visible or raw infrared images, an image based on seven or more clusters would show the location of such layers. On the other hand, if in reality there are no more chemically distinct regions in the sample, the addition of more clusters to the image will result in the subdivision of existing clusters into random or incoherently located pixels. This is illustrated in Fig. 5(*c*) where seven clusters have been used to form the image. The only change from Fig. 5(*b*) is that the areas corresponding to the KBr mounting material around the paint chip have been split into two clusters. Although some of these lie together near the edge of the chip, reference to the spectra at these pixels and to the visible image clearly shows that they lie outside of the physical boundaries of the chip. Images based on larger numbers (>7) of clusters serve only to strengthen the conclusion that Fig. 5(*b*) is the most accurate representation of the location of the chemically distinct regions in the image, and that there are only six of these. Note that images based on several clusters above the "expected" number should always be examined, because a layer that has only subtle chemical differences from another may not be separated before other clusters (particularly those containing poor quality or low intensity spectra, such as those from pure KBr) start to break down into regions of different signal-to-noise (but with no chemical difference) or into physically meaningless shapes.

Supervised image classification procedures implemented in the ENVI package require the user to indicate sets of pixels in an image (regions of interest, ROIs) that are known to represent chemically distinct areas of the image. The software is then able to classify the remainder of the pixels (spectra) in the image according to their similarity to spectra in these regions. This is done by calculating the distance (in multivariate space) between the average spectrum in each ROI and each of the unclassified spectra in the rest of the image. Spectra that lie within a specified maximum distance (threshold) of the average spectrum of a region of interest are classified as belonging to that region (cluster). In ENVI, the distance method that yielded the best results in this study for the classification of images containing paint chips was the Mahalanobis distance method (32).

This method is illustrated in Fig. 6, in which the two paint chips (BMW 320i, #264) are classified together using the ENVI supervised Mahalanobis distance classification routine. Figure 6(*a*) shows the chemical image of the chips at 1492 cm^{-1} with pixels selected by the user in each of the six regions of interest in the left hand image only (the five layers apparent in visible light and infrared images, plus the surrounding KBr mounting material). Figure 6(*b*) is the resulting image when the software is allowed to classify all of the pixels in the combined image of the two paint chips. As with the HCA results, the fully classified image allows multiple chemical comparisons to be made rapidly at one glance, in a way that is very easy to understand. Of the two methods, HCA (as the unsupervised method) involves no subjective input from the operator, and so may therefore be preferred in forensic applications. However, the supervised classification procedure described here can be performed with a prescribed maximum spectral distance threshold that would specify mathematically the greatest variation allowed between two spectra that are classified as representing the same chemical substance. This would confine the input from the operator to the selection of the regions of interest in the sample.

The first key advantage of using chemical imaging data in combination with multivariate techniques (or even simply displaying it as "movies") in the comparison of heterogeneous samples for forensic purposes is that thousands of spectra are being compared

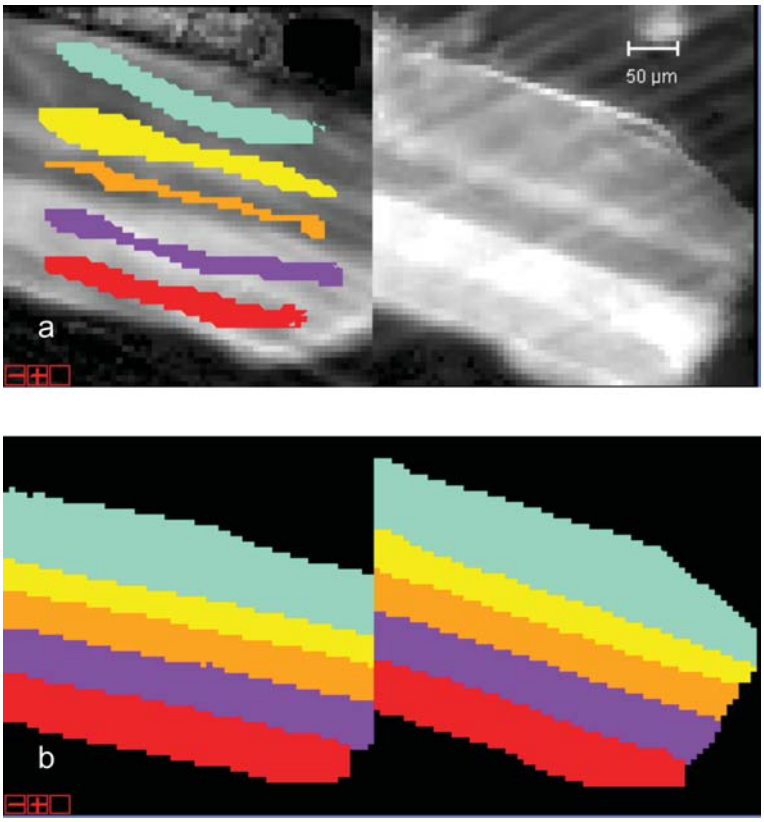

FIG. 6—*a) Chemical image (1492 cm^{-1}) of two paint chips from the same source (#264) with regions of interest (ROI) selected using ENVI. b) Resulting cluster image of paint chip using Mahalanobis distance classification method.*

with thousands of spectra, rather than one spectrum with one spectrum. In the case of paint chips, the *whole* of one sample can be compared with the whole of another in terms of chemical composition and the physical ordering of the chemical components. This dramatically reduces the chances of error when conclusions are being drawn from such comparisons. The second key advantage is that chemical imaging data (and the results of multivariate image classification) can be displayed in ways that make chemical differences and similarities between heterogeneous samples easy to visualize and understand. Importantly, both of these advantages can be exploited using any type of chemical imaging data, not just infrared chemical images.

Conclusions

Infrared chemical imaging has a number of important advantages over conventional "single-point" infrared spectroscopy with respect to the analysis of multi-layered paint chips. These advantages stem from the simultaneous collection of thousands of infrared spectra across the sample in a matter of minutes. Most importantly, the data can be used to visualize the spatial distribution of the different chemical components of a sample, an important advantage in comparison studies, since this spatial distribution (e.g., the ordering of layers) can itself be an important characteristic of a sample. Several different visualization/comparison techniques are available: simple chemical imaging at fixed wavenumber frequencies, chemical image "movies" that allow two samples to be visually compared across the entire infrared spectrum, and cluster images, in which multivariate statistical techniques are used to group regions of the chemical image by similarity. These visualization options make comparisons using chemical imaging data easier for the layperson (such as a juror) to understand than a comparison of infrared spectra. Because the multivariate image analysis techniques compare hundreds or thousands of spectra in each sample, they also greatly reduce the chance of erroneous conclusions being drawn.

The main disadvantages of infrared chemical imaging are chiefly those of conventional infrared analysis, particularly those concerning sample preparation, although we have sought to improve aspects of sample preparation in the work described in this article. The infrared spectral range is somewhat restricted using a focal plane array detector (compared with conventional infrared detectors), and there is the requirement that the sample be flat. We have discussed aspects of the availability of infrared chemical imaging instrumentation elsewhere (8), but, in general, this should improve as the technology becomes less expensive.

Another conclusion from this work is that, although we have examined only one evidence type in this particular study, it seems obvious that infrared chemical imaging should be investigated as a tool for the forensic analysis of other multi-component samples, as many of the same advantages should apply. Equally, the multivariate image classification (clustering) techniques described in this article should be applicable to the analysis of analogous data collected from other parts of the electromagnetic spectrum, such as chemical images from the UV-visible and near-infrared regions.

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