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Differentiation of Human, Animal and Synthetic Hair by ATR FTIR Spectroscopy

Jeremy Manheim
University at Albany, State University of New York

Differentiation of human, animal and synthetic hair by ATR FTIR Spectroscopy

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Jeremy Manheim

Research Mentor: Kyle Doty, B.S. and Greg McLaughlin, Ph.D.
Research Advisor: Igor K. Lednev, Ph.D.

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Abstract

Hair fibers are ubiquitous to every environment and are the most commonly found form of trace evidence at crime scenes. The primary difficulty forensic examiners face after retrieving a hair sample is determining who it came from. Currently, the methodology of microscopic examination of potential hair evidence is absent of statistical probability and is inherently subjective. Another method, involving DNA analysis, takes months to conduct and the majority of times is unsuccessful due to its degradation and absence from the hair. Here, Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) Spectroscopy coupled with advanced statistics was used to identify a hair sample within a specific confidence solely from its spectrum.

Ten spectra were collected for each of ten human, cat, and dog donors and a single synthetic fiber for 310 total spectra. A spectrum is collected by simply placing a single strand or patch of hair, without preparation, directly across the crystal (500μm) of the instrument. Two Partial Least Squares-Discriminant Analysis (PLS-DA) models were constructed: one to differentiate natural hair fibers from synthetic fibers and the second discriminating human hair from dog and cat hair. Both internal models were successful in separating the desired class from another; synthetic hair was completely separated from actual hair in the binary approach and all human samples were predicted as human in the species specific model.

The species specific training model was tested by loading spectra from ten external donors (three human, two cat and five dog) and examined the model's ability to correctly assign these spectra. The external validation confirmed our model's ability to correctly classify a sample as human as well as properly predict spectra that are not human. It also showed that a breed of dog not accounted for in the training data set was entirely misclassified as cat, but more importantly led to the possibility that different breeds of dog can be separated based on their hair spectra. This preliminary investigation sheds light on the next step of the discrimination process to identify the gender and race of a human hair, as well as the identification of different hair dyes. Overall, the method is able to quantitatively identify a sample of hair as human with a high degree of confidence and is of ample importance to the field of forensic science. The method can be conducted without the need of a specialist, is non-destructive, is extremely quick and requires no sample preparation.

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1. Introduction

Hair fibers are ubiquitous to every environment and are a common form of trace evidence found at crime scenes. The primary difficulty forensic examiners face after retrieving a hair sample is determining its origin; if it came from a human or an animal and, if human, what is the race, gender and type of body hair (e.g. head, pubic, underarm, etc.). Light microscopy is the most commonly employed method for the investigation of hairs in forensic laboratories[1]. Transmitted light and polarized light microscopes are traditionally used to analyze and identify the morphology of a natural fiber[2]. A comparison microscope is used when comparing unknown natural hairs, or fibers, recovered from a crime scene to those of a known origin.[3] Hair classification is dependent on the expertise of the forensic examiner, the quality of the hair sample and the instrumentation used[1]. DNA analysis is another common method employed for the identification of an unknown hair sample. DNA testing is an extensive and costly procedure that requires sophisticated techniques, time and resources[4]. Since hair is so abundant, crime scene investigators collect many unknown fibers for analysis that could have come from a human, an animal or even a wig. The ability to quickly identify a hair fiber as human, animal or synthetic, with statistical support, would be of tremendous assistance to forensic investigations.

Based upon the probability theory, evidence including fingerprints, body fluids, and hair are considered as circumstantial[5]. Fingerprints and body fluids have established probability standards recognized by the criminal justice system that account for points of comparison between known and unknown samples of evidence[5]. The issue preventing the same type of standards for hair analysis is that the method is unable to directly associate the number of different properties between two hairs and the probability that the samples did or did not come from the same individual[6]. Additionally, two examiners who analyze the same hairs may describe the hairs in slightly different ways, placing varying emphasis on certain characteristics,

and often use different descriptive words in their findings[7]. Furthermore, hair comparisons may contain prejudice or bias, on the forensic expert's part, due to interactions with criminal justice personnel[5]. In particular, police and attorneys may have preconceived beliefs on a suspect's guilt, and if these attitudes are expressed to the examiner, it can greatly affect their conclusions when analyzing hair evidence.

Hair is important to the investigation process because it may contain DNA and, in some cases, it is the only evidence available linking a criminal to the crime scene. In the 2009 report, "Strengthening Forensic Science in the United States: A Path Forward," it was concluded that there are no accepted statistics about the frequency with which certain hair characteristics are distributed within a population and that hair comparisons for individualization have no scientific support without nuclear DNA[8]. In early 2013, the F.B.I. began a review of over 2000 convictions based on hair evidence[9]. Of the first 310 cases, DNA analysis revealed that 72 of the convictions were grounded on faulty hair evidence[9]. One case involved a man named Claude Jones who was executed in 2000 after being convicted of killing the owner of a bar. His conviction stemmed from the belief that a hair recovered from the crime scene was his. As part of the F.B.I.'s review, DNA from the hair proved to not have come from Claude Jones[10]. Although this was only one case, there are many more examples where innocent people were wrongly convicted based on improper conclusions drawn by examiners, which reinforces the need for new methods to accurately analyze hair evidence.

Despite its increasing popularity, the process of extracting DNA from a hair fiber is an extensive procedure that does not always generate usable results[11-14]. The majority of the genetic material in hair is located in its root which is generally absent from the hair shaft (i.e. the portion of hair that grows out of the skin)[4]. However, collected hairs absent of the root or follicle material may undergo exhaustive and laborious mitochondrial DNA analysis, even

though success is not guaranteed[4]. DNA analysis is extremely costly and time consuming, not to mention that most laboratories are currently backlogged. A method for determining the identity of an unknown fiber quickly, with a high degree of certainty, and eliminating examiner bias would be extremely useful and cost-effective for the field of forensic science.

ATR FTIR spectroscopy is a technique rising in popularity for analytical and biological purposes. It has been employed for the analysis of biomedical samples[15], paint[16, 17], fingerprints[18] and ink[19]. The attributes of ATR FTIR spectroscopy are very attractive for forensics because of its rapid and non-destructive nature, its ease-of-use and minimal to no sample preparation. An infrared spectrum displays the vibrational characteristics of a sample based on the different absorption frequencies of the individual functional groups[20]. The ATR attachment allows for analysis of solid samples, often with no sample preparation[21]. The advantage of combining ATR FTIR spectroscopy with chemometrics is its ability to enhance the selectivity of the instrument and create classification models[16, 22, 23].

Two published studies demonstrate the use of FTIR and chemometrics to differentiate the spectra from different types of hair. Espinoza et al. applied infrared spectroscopy and advanced statistics to the forensic identification of elephant and giraffe hair[24]. They visually observed a difference in the elephant and giraffe hair spectra at a very prominent peak (1032 cm^{-1}), which is due to surface cystine oxides and the presence of cysteic acid. Through the discriminant analysis of their spectral data they demonstrated a performance index of 91.8%, which specifies how well their algorithm can differentiate between elephant and giraffe hair. Another group combined FTIR microscopy and chemometrics to differentiate Asian hair samples and black Caucasian hairs[25]. Using Principle Component Analysis (PCA), they were able to separate the three female Asian hair samples from the three female Caucasian hair samples demonstrating their ability to discriminate between hair from two different races.

Our lab has used Raman spectroscopy, in conjunction with advanced statistics, for differentiation purposes when spectra are visually similar. Some of these studies include body fluid identification[26], distinguishing between species' blood[27], species' bones[28], and mixtures of semen and blood[29]. However, Raman spectroscopy is not an advantageous method to use for hair analysis due to the significant fluorescence interference, as shown in the literature[30, 31]. For this reason our approach was to use ATR FTIR to analyze hair samples. Similar work has been done as part of two theses projects, "Vibrational spectroscopy of keratin fibres: A forensic approach" by Helen Panayiotou[32] and "A forensic investigation of single human hair fibres using FTIR-ATR spectroscopy and chemometrics" by Paul Barton[33], at Queensland University of Technology in Australia. Our study is an expansion upon their work, primarily Panayiotou's 2004 thesis, in a few different ways. First, they treat their hair samples by flattening with a roller[32] prior to analysis whereas we have analyzed all hairs without any sample preparation. Second, our data analysis was performed using a different statistical algorithm better suited for class separation, PLS-DA, and we used ATR FTIR spectroscopy for data collection, rather than Panayiotou's approach of using FTIR micro-spectroscopy in the transflection mode. With ATR FTIR, there is no need for sample preparation and allows for the potential opportunity of on-field analysis due to the availability of portable instruments[34]. Finally, our sample size for species differentiation is over fourteen times larger, focusing on humans, dogs and cats.

Our analysis for the present study is bimodal where the first model discriminates natural hair from synthetic and the second discriminates human hair from other common natural hair sources (i.e. dog and cat hairs). Hair samples were collected from a synthetic wig and a diverse population of humans, dogs, and cats. The spectra were differentiated using Partial Least Squares-Discriminant Analysis (PLS-DA) classification models which were built from a training

dataset of human, dog, and cat spectra. An external validation step was also carried out to test the model's ability to accurately predict a sample to its actual class.

2. Methods and materials

2.1 ATR FTIR spectrometer and hair samples

A PerkinElmer Spectrum 100 FTIR spectrometer with an attenuated total reflectance (ATR) attachment was used for data collection for all experiments. Spectra were collected over a range of 650-4000 cm^{-1} with 10 scans per sample. For each donor, ten averaged spectra were collected. The chemical composition of hair, primarily its proteins, is subject to change after being exposed to various chemical reactions such as bleaching, waving, straightening and extensive sunlight exposure[30, 35-37]. Of the many variables that can influence the chemical make-up of hair only chemically treated (i.e. dye, bleaching, etc.) hairs were excluded from this study. A single hair was placed over the diamond/ZnSe crystal of the instrument in order to obtain a spectrum with optimal signal. For animal donors consisting of only fur hairs, multiple hairs were required because they are fine and shorter compared to that of an animal's guard (outer) hair[38]. For each donor, ten spectra were acquired at various points along several hair fibers, and each spectrum was treated as its own sample. In the case where multiple fur hairs were placed over the crystal, spectra were obtained over different patches of the fur hair.

Spectra from ten different human, dog and cat hair samples were collected as well as from one polyester synthetic hair fiber. The race, gender, and age of the human donors, as well as the breed of dog and cat, were taken into consideration for sample collection. These individual characteristics can be seen in Table 1.

Table 1: The background information of the thirty human, dog and cat donors used in the training data set for all PLS-DA models.

Donor #	Human (age)	Dog	Cat
1	Asian female (18)	Barbet	Maine Coon
2	Caucasian female (20)	Maltese	Ragdoll
3	Caucasian male (20) A	Cocker Spaniel	Domestic Short Hair (Grey A)
4	Caucasian male (20) B	Dachmund Mini	Domestic Short Hair (Black A)
5	Caucasian female (40)	Pug	Domestic Short Hair (black-and-white A)
6	Hispanic female (20)	Golden Retriever	Domestic Short Hair (White)
7	Hispanic male (20)	Unknown Dog	Domestic Short Hair (Brown)
8	African American female (21)	Yorkshire Terrier	Domestic Short Hair (Black B)
9	Egyptian male (20)	Briard	Domestic Short Hair (Grey B)
10	Ecuadorian male (20)	Beagle	Domestic Short Hair (black-and-white B)

2.2 Data preparation and statistical treatment

All data preparation and statistical models were performed with the PLS Toolbox 7.0.3 (Eigenvector Research, Inc.) operating in MATLAB version R2010b. The model for differentiating natural hair from synthetic hair was built using the full spectrum collected (650-4000 cm^{-1}). All 310 spectra were imported into a dataset; the dataset was preprocessed using transmittance log, second-order derivative, normalization by total area and finally mean centering. The model created for discriminating human hair from animal hair (species specific) was built using spectra truncated to the data range of 650-1827 cm^{-1}. The 300 total spectra (excluding the ten synthetic fiber spectra) were imported into a data matrix and preprocessed the same way as the binary model. All models were cross-validated using the venetian blinds method.

2.3 External validation

The training model was tested by loading external donors (three human, two cat and five dog) into the model to test its ability to correctly predict the identity (class) of an untrained sample. All external samples were preprocessed in the same manner as the training data but not included as part of the training dataset used to build the models.

3. Results

The main objectives of this study were to discriminate natural hair from a synthetic fiber and differentiate human hair from animal hair using chemometric modeling of ATR FTIR spectroscopic data. Preliminary experimentation determined the model selection and data processing steps. PLS-DA models were chosen to build simple classification models using the infrared spectra of a synthetic fiber and human, dog, and cat hair. The number of latent variables for each model was selected by choosing a local minimum of total data variance captured using a scree plot (not shown). The PLS-DA models were constructed in two fashions, first by classifying each spectrum as either natural or synthetic and secondly, focusing on the individual species, to determine if a more specific assignment could be made. The second model was used to make class predictions of 10 external natural hair donors that were not accounted for in the training dataset.

3.1: Natural hair v. synthetic hair (binary)

The prominent features of an infrared spectrum of natural hair correspond to specific vibrational modes of the amino acids and lipids present[39]. The averaged raw spectra for human, dog, cat and synthetic hair, as shown in Figure 1, reveal visual differences between natural hair and synthetic hair. These differences include the absence of the Amide A peak at 3300 cm^{-1} and the more intense CH_3/CH_2 (alkane stretching) peak at 2950 cm^{-1} in the averaged synthetic hair fiber spectrum. Additionally, various spectral inconsistences exist between the two hair types in the fingerprint region (650-1827cm^{-1}) including peaks at ~1400 and ~1450 cm^{-1} for synthetic hair and peaks at ~1520 and ~1620 cm^{-1} only present in natural hair spectra. These peaks most likely correspond to C=N and C=O respectively[32]. Due to these spectral differences, the polyester synthetic hair spectrum can be visually differentiated from a spectrum of natural hair quite easily.

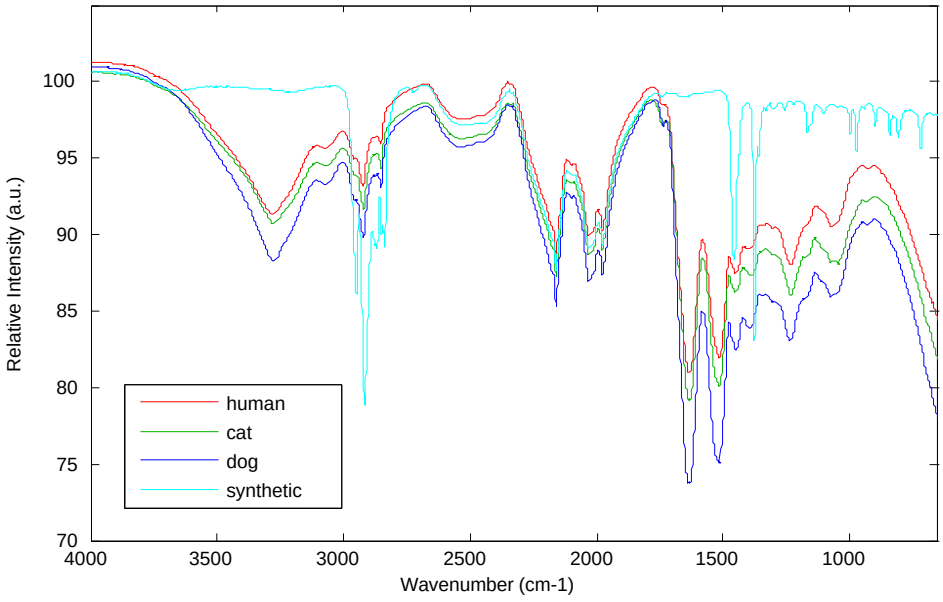

Figure 1: The raw mean spectra of human, cat, dog, and synthetic hair samples.

For statistical analysis, all 310 spectra from the 31 donors were used to build a Partial Least Squares-Discriminant Analysis (PLS-DA) model using four latent variables. Before the model was built, all spectra were preprocessed as described in Section 2.2. Initially, the human, cat, and dog samples were grouped together as one class (natural hair) and compared to synthetic hair in a binary approach. Under cross-validation (CV) all of the synthetic hair samples were correctly classified as seen in Figure 2. A cross validation model works by treating all of the trained spectra as unknowns, and tries to properly predict them. The results of perfect separation between the synthetic hair and natural hair were not surprising since the averaged natural hair spectra looked visually different from the averaged synthetic hair spectrum. These results demonstrate that our model can efficiently discriminate samples of natural hair from synthetic hair with 100% accuracy.

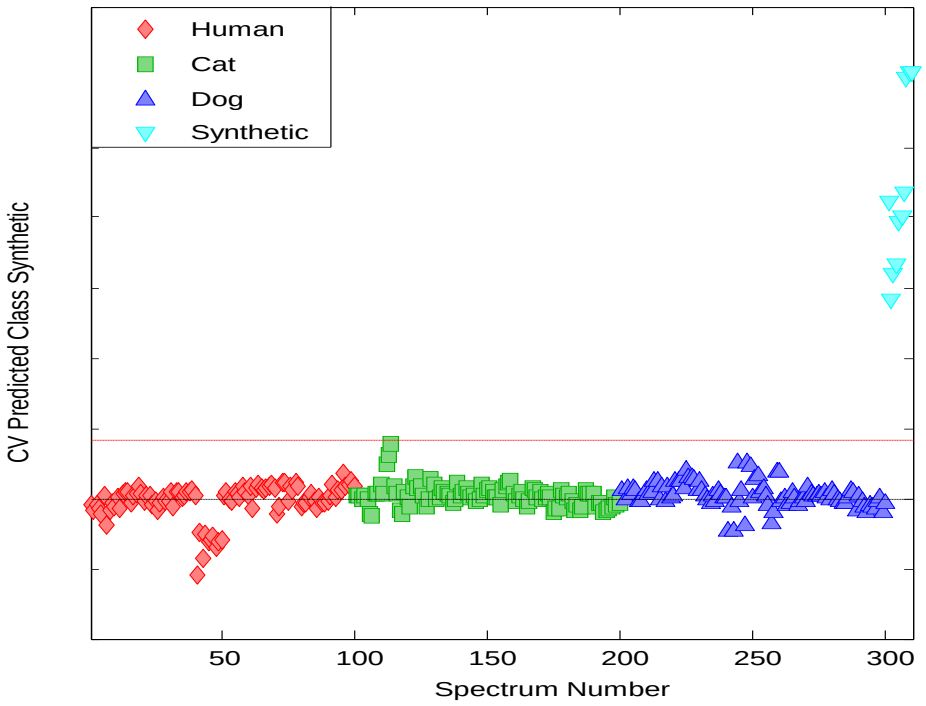

Figure 2: Cross-validated synthetic hair class predictions for all 310 spectra analyzed in the binary model (natural v. synthetic). All spectra above the red threshold line are predicted to the synthetic class and all below are predicted as not synthetic.

3.2: Human, cat, and dog hair (species specific)

The 300 spectra of the human, cat, and dog hairs were truncated to 650-1827 cm^{-1}. An artifact around 2350 cm^{-1}, consistent with atmospheric CO_2[40], is not a vibrational mode of hair and to ensure that the air did not influence our results we only analyzed the specified region. Although not shown here, the full hair spectrum was also analyzed and the results were very similar, informing us that the air artifact would not significantly alter our results. From visual inspection, all natural hair spectra shown below in Figure 3 appear to be identical in terms of the number of spectral features and their location. For this reason we utilized classification statistical analysis in an attempt to extract any differences which could not be visualized.

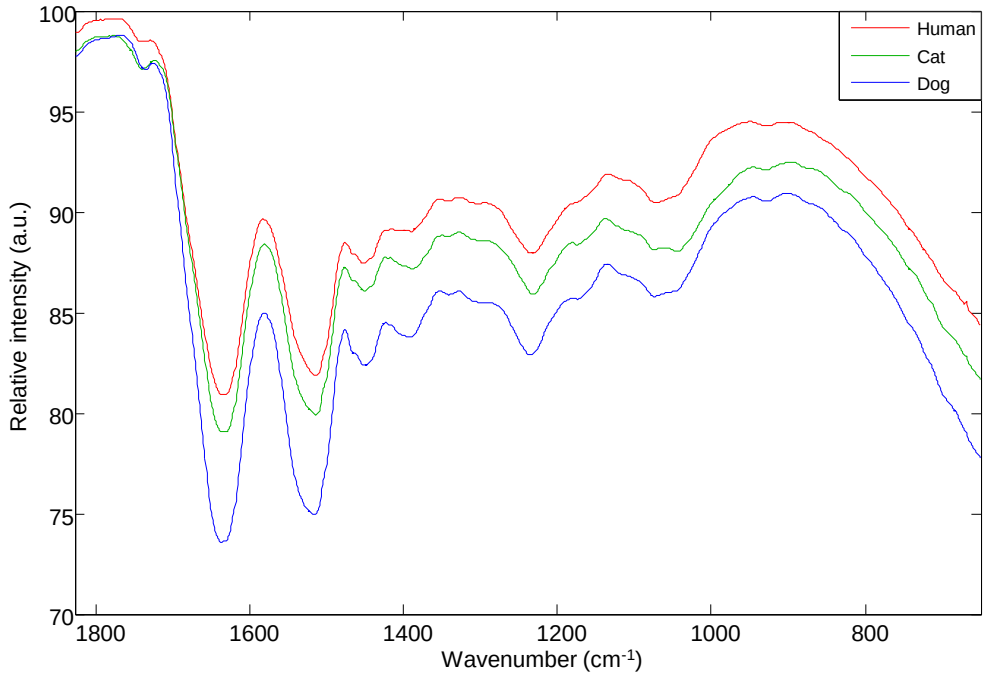

Figure 3: The truncated, raw mean spectra of human, cat, and dog hair samples.

A second PLS-DA model was built to analyze the training dataset classified by their species of origin: human, cat or dog. The spectra were preprocessed in the same way as in the binary approach and ten latent variables were selected to build the model. According to the strict

class predictions, which assigns a sample to its nearest class and samples with a large uncertainty are unassigned, all of the human and cat spectra in the training dataset were correctly assigned to their proper class. Only one dog (Cocker Spaniel) spectrum was predicted incorrectly, as unassigned. Using this approach both the human and cat classes showed 100% correct classification while the dog class showed a marginally lower rate at 99%. Although these strict predictions are informative, cross-validated analysis provides more reliable classification results.

Figure 4 shows the cross-validation prediction plot which illustrates the probability that a given spectrum will be classified as human. All of the human hair spectra lie above the classification threshold (red dotted) line, signifying a 100% correct class prediction rate. However, one cat (Ragdoll) and one dog (Barbet) spectrum are above the threshold line and are therefore false positive predictions. This means that 90% of the spectra from the Ragdoll and Barbet donors were correctly classified as opposed to 100% classification rate for all other donors' spectra. The single misclassified spectra could be due to any sudden instrument movements or background contaminants since nine out of ten spectra along the same hair fiber were properly predicted. Overall this represents a correct classification rate of 99% for both the cat and dog classes as not human under cross-validation. These results conclude that our model has no false negative assignments and is capable of predicting a sample of human hair as human with 100% accuracy.

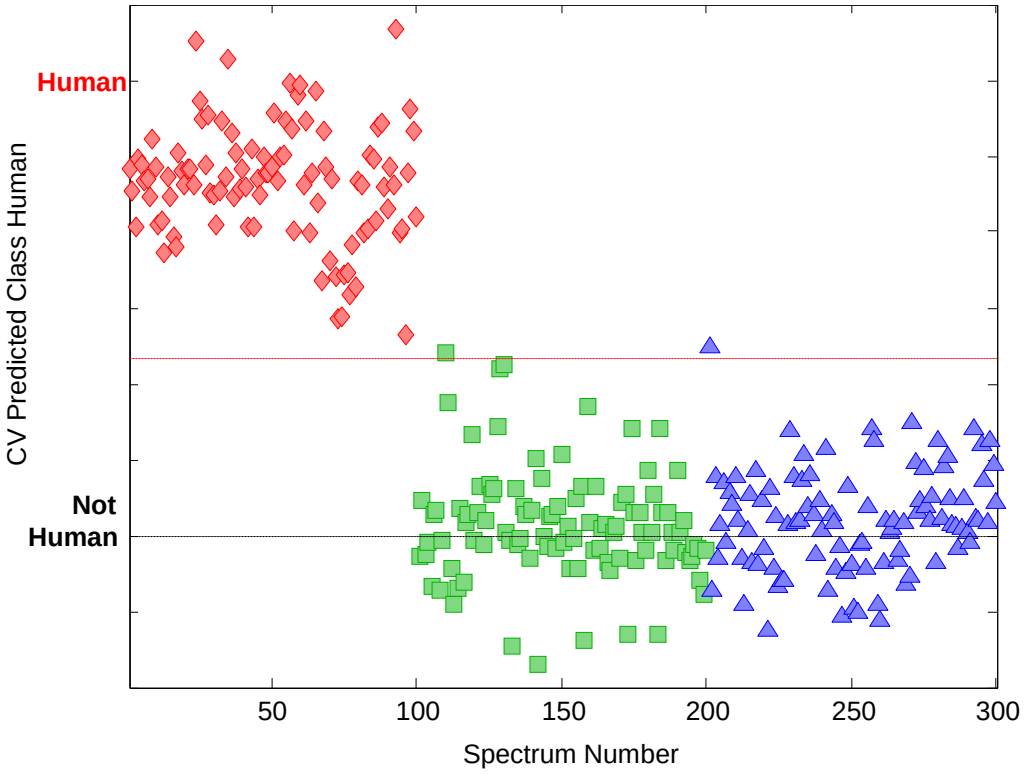

Figure 4: The cross-validated model predictions for human hair. The red threshold line represents the default classification threshold where all spectra above are predicted as human and all below are predicted as not human.

3.3: External validation

To test the reliability of the model, an external validation was conducted for ten new donors: three humans, two cats, and five dogs. The spectra for the new donors were collected and preprocessed following the same procedure detailed in Section 2. The gender, race and age of the human donors and the breed of dog and cat for the untrained donors are listed in Table 2. The 100 untrained spectra (ten per donor) were loaded into the binary and species specific models, separately, and predictions were made.

External Donor (Class)	Description
Human	Hispanic Female, age 24
Human	Caucasian Female, age 22
Human	Caucasian Male, age 30
Cat	Calico
Cat	Domestic Short Hair (Grey C)
Dog	Maltese B
Dog	Maltese C
Dog	Maltese D
Dog	Golden Retriever B
Dog	Pomeranian

Table 2: The new donors collected for the external validation

Examining the prediction plot for the species specific model demonstrates how well the model correctly predicted for the human class. Figure 5 shows complete separation between the classes and all 130 human spectra (100 in the training set and 30 external) lie above the threshold (red dotted) line, with one external sample lying close to it. In addition, all of the cat and dog spectra (training and external) are well below the threshold line signifying zero false positive predictions for the human class. Therefore, the accuracy of the model for predicting a hair sample as human or nonhuman under strict class prediction conditions was 100%.

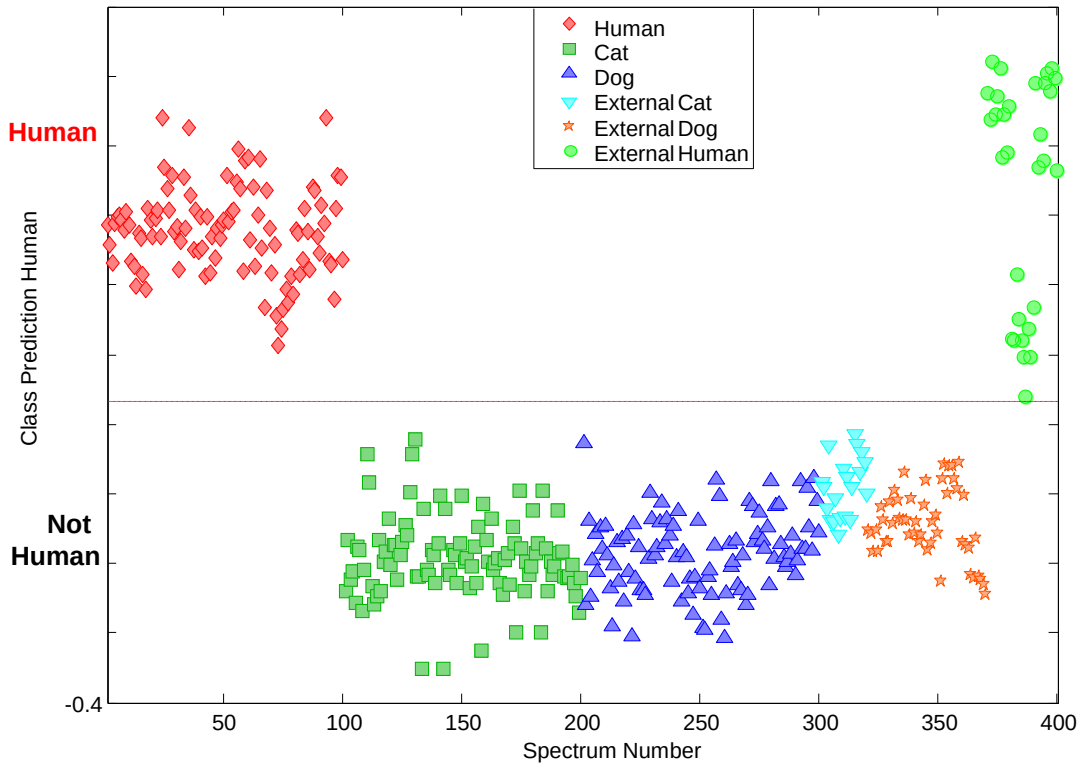

Figure 5: Human class predictions for all 400 samples analyzed, including untrained donors, in the species specific PLS-DA model. Red line represents the default classification threshold where all spectra above are predicted to the human class and all spectra below are predicted as not human.

For the species specific model, under strict class predictions (Figure 6), twenty-nine of the thirty external human spectra were correctly predicted as human; the other spectrum was unassigned. All twenty external cat hair samples were correctly classified as cat. In addition, all of the external Golden Retriever (dog) donors were classified correctly as dog. However, of the three external Maltese donors (B, C, and D), three samples from donor C were misclassified (two of which were unassigned and one predicted as cat) and eight samples from donor D were misclassified (four were unassigned and four were misclassified as cat). Lastly, all ten spectra from the internal Pomeranian donor were misclassified as cat.

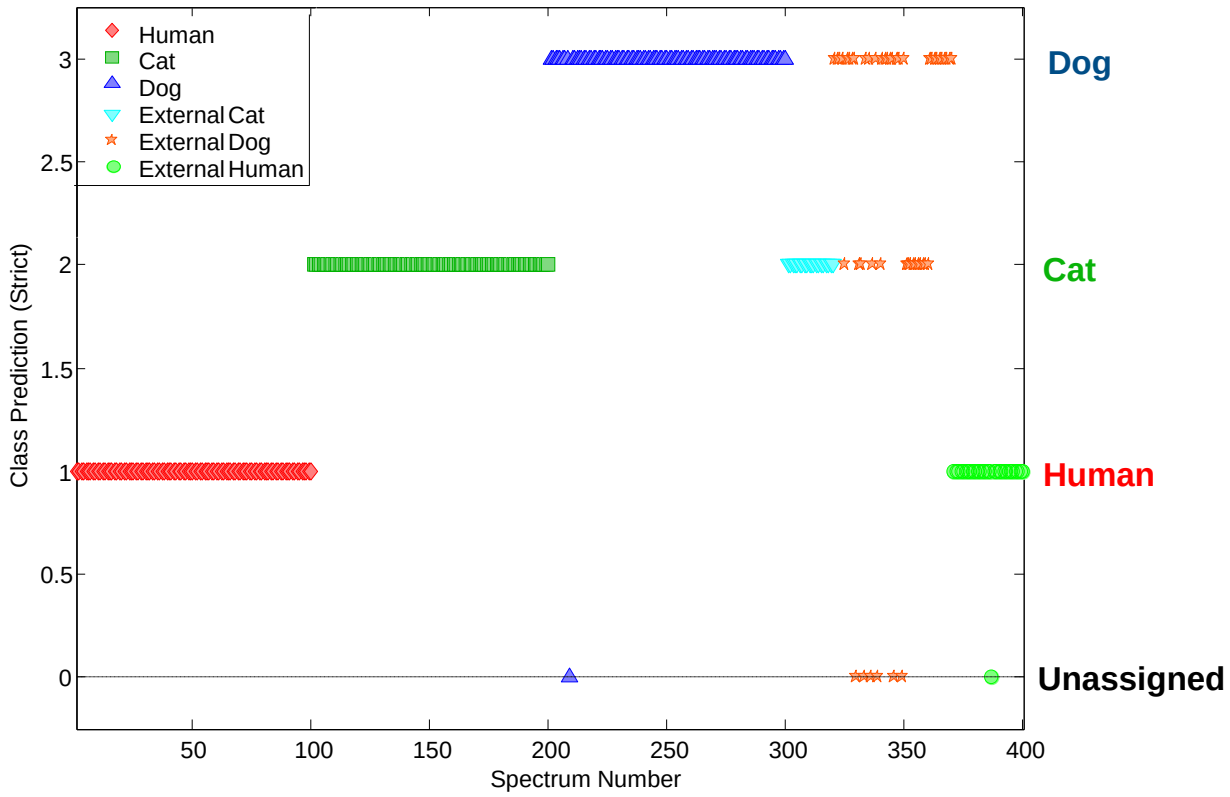

Figure 6: Strict class predictions for the external validation samples loaded into the species specific PLS-DA model. Deviations from each class' horizontal line represent a misclassification.

In order to understand why some dog spectra were misclassified as cat, differences in hair between individual dogs of various breeds was investigated. The results for this test revealed that all of the individual dogs (ten spectra from each dog) of ten different breeds, with the exception of three spectra, were correctly assigned to their correct class (Figure 7). All of the breeds of dog appear to be differentiating from one another, which still does not explain why the Pomeranian breed in particular was the only breed being misclassified within our model. However, since only one donor from each breed of dog was analyzed, the differences observed could be an individual difference or breed differentiation. More dog donors from each breed would need to be collected in order to make any definite conclusions from these results.

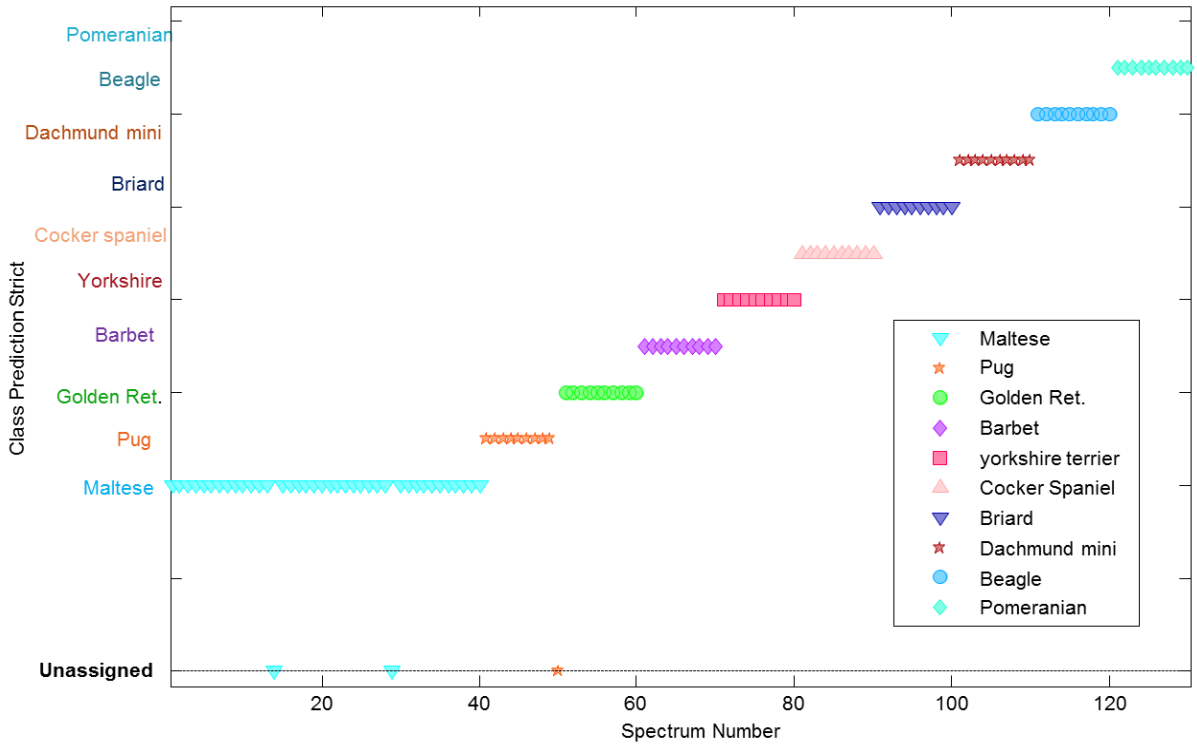

Figure 7: Strict class predictions for the individual dog donors. Deviations from each class' horizontal line represent a misclassification.

To further investigate the Pomeranian dog breed misclassification, we also created a second binary PLS-DA to differentiate dog hairs from cat hairs and included the Pomeranian dog donor in the dog class. An influence plot was analyzed, which groups spectra similar to each other within a 95% confidence interval and all spectra plotted outside the interval are considered extremely different. Analysis of the model revealed all ten Pomeranian spectra had higher Hotelling T^2 values, and were grouped together, separate from all other hair spectra. Hotelling T^2 values are directly related to the amount of variation in each sample. So, higher Hotelling T^2 values suggest that those spectra are somehow inherently different than the other spectra, yet similar within themselves because of their close grouping. Although the Pomeranian dog was shown to be different from the other dog and cat spectra, it still does not explain its misclassification as cat, but rather illuminates the unique characteristics in the chemical spectra for the Pomeranian dog donor.

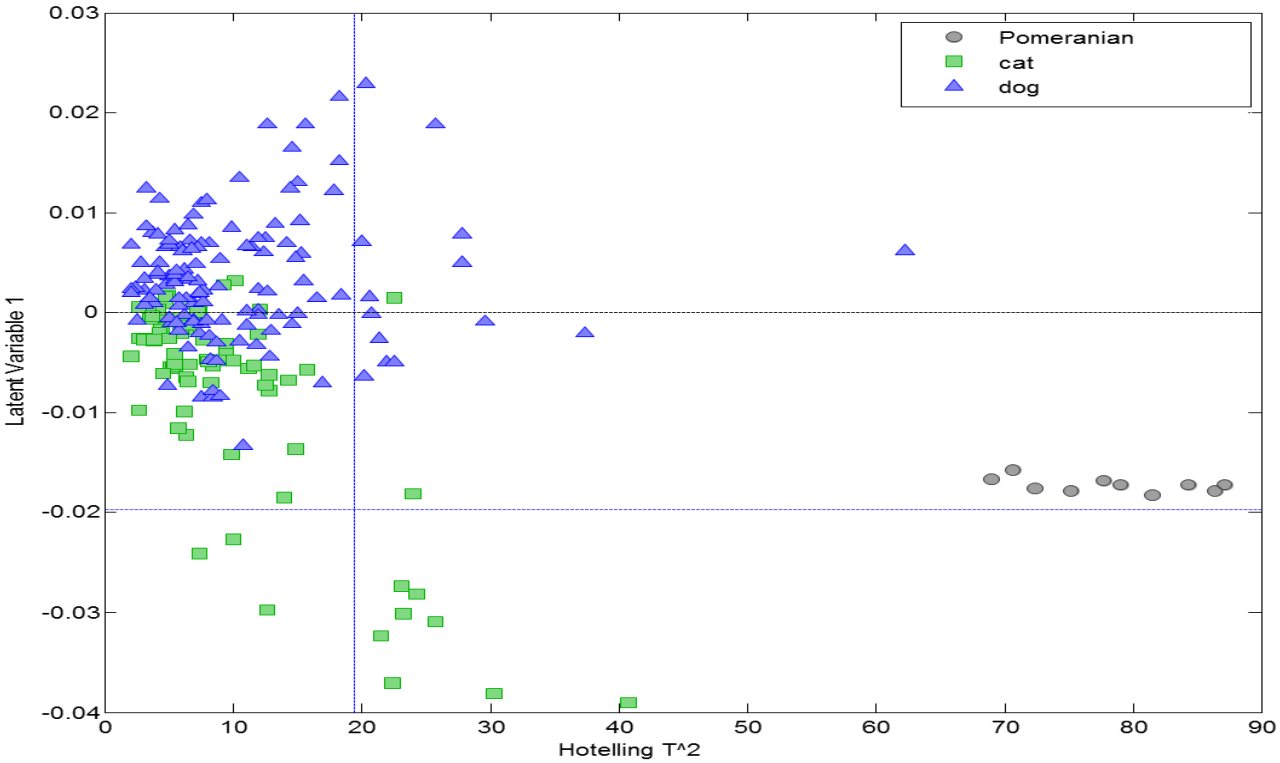

Figure 8: The influence plot for the dog v. cat binary model. All spectra to the left of the vertical blue line are within a 95% confidence interval.

4. Discussion

The differentiation of human hair from cat and dog hair is difficult to observe from their raw spectra, unlike for synthetic hair from natural hair. The species specific PLS-DA model was able to successfully make this differentiation based on the shape of each component latent variable (Figure 9). These variables represent the prominent discriminating factors between spectra and are denoted by different peaks in the fingerprint region. This observation implies that there is more than one characteristic peak which differentiates the individual classes of hair from one another; latent variables one, two and three as shown in Figure 9 depict where these characteristic peaks are. The dominant features are in the regions 1739-1742 cm^{-1} (C=O stretch), 1467-1477 cm^{-1} (CH_2 bend) and 1230 cm^{-1}(amide III). One possible explanation for the model's

discriminatory power is based on the different combinations of amino acids that form keratin, the structural protein from which hair is constructed. Hair is chemically composed of 65-95% proteins and the content of the different proteins vary among different donors[41]. The 2004 study conducted by Panayiotou at Queensland University of Technology determined differences between the relative intensity areas of various peaks for seven different samples: human, cat, dog, horse, cow, feather, and wool[32]. As it pertains to our project, that study found that human and dog hairs have lower Amide I (α-helix) content than cats and that humans have lower Amide II (α-helix) content than cats and dogs. Based off this research and the complex nature of keratin, it can help explain the subtle differences identified by the species specific model. Here, we show that spectra collected from multiple points along a donor's single hair fiber can still be predicted as its correct species (class).

The most important results are that a sample of human hair can be quickly and non-destructively analyzed, and subsequently identified with a high degree of confidence. Our rapid analysis and superb probability prediction results have been accomplished without human bias, and could potentially be of great use for the forensic investigative process. The non-destructive nature of using ATR FTIR spectroscopy makes this method ideal for the forensic identification of an unknown hair sample. The developed methodology has the potential to differentiate gender, race, other animal species, and even hair dyes. Furthermore, the presence of portable FTIR instrumentation supports the idea that on-field analysis of a hair fiber is feasible.

Conclusions

The combination of ATR FTIR spectroscopy and chemometrics was demonstrated to be a powerful tool toward the differentiation of hair samples from three species. Two PLS-DA models were constructed: one focusing on the differentiation of natural hair fibers from synthetic

fibers and the second discriminating human hair from animal hair. Both models were successful in separating the desired class from another; synthetic hair was completely separated from natural hair in the binary approach and all human samples were predicted as human in the species specific model. The external validation step confirmed our model's ability to correctly predict a sample as human with zero false positives. A larger sample size for the dog class would help account for the misclassified Pomeranian donor, but this is beyond the scope of this project.

Of the many variables that can alter one's hair chemistry, only chemically treated hairs (i.e. dye, bleaching, etc.) were excluded. All other potential external interferences (e.g. sun damage, type of shampoo, physical treatment, etc.) were not taken into account for this study and did not preclude a high differentiation efficiency of the method. Overall, this demonstrates the significance of the model's unique ability to quantitatively identify a sample of hair as human with a high degree of confidence. But, most importantly, the method can be conducted without the need of a trained expert, is non-destructive, requires no sample preparation, with rapid identification, making it of ample importance to the field of forensic science.

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