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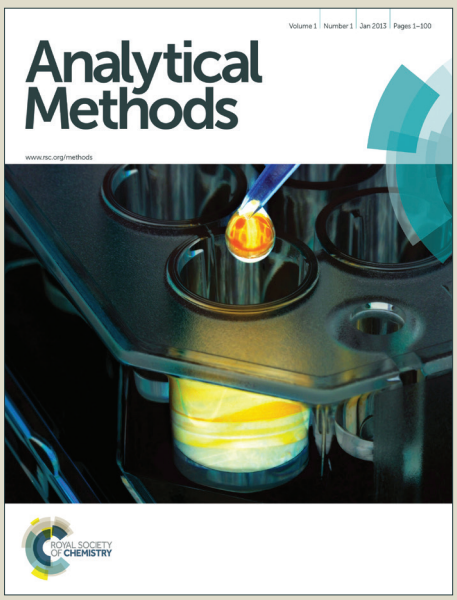

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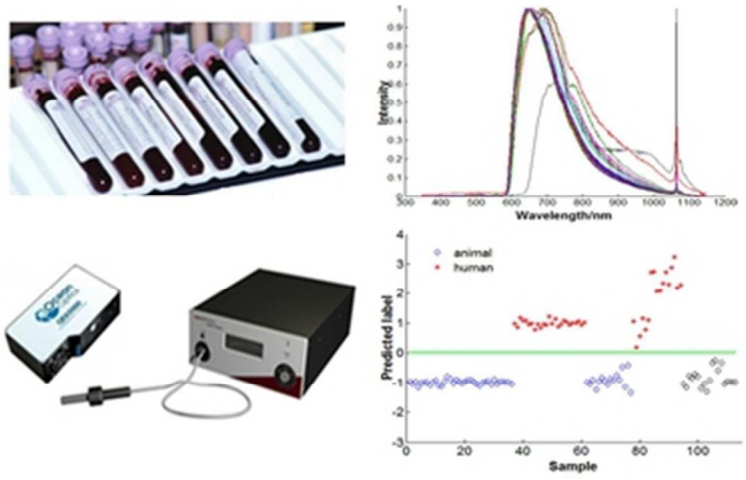

Discrimination of human blood
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Analytical Methods

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ARTICLE

Discrimination of human and nonhuman blood using visible diffuse reflectance spectroscopy

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Customs inspections are very important to protect our national property. Human blood is an important part of national property. So, discrimination of human and nonhuman blood is very important. In this paper, visible diffuse reflectance spectra and Partial Least Square Discrimination Analysis (PLS-DA) were used to discriminate human and nonhuman blood. A blind test and an external validation test were used to assess the PLS-DA model. The model demonstrated 100% accuracy in its differentiation between human and nonhuman blood. The results further demonstrate a great potential of visible diffuse reflectance spectroscopy for blood inspection.

1. Introduction

The identification of blood is very important in forensic investigation [1], the customs import and export sample detection and many other fields. There are a variety of presumptive tests for identifying blood [2, 3]. A common confirmatory blood test is a microcrystal assay (e.g. Teichmann or Takayama assays). Then the Ouchterlony or similar immunochromatographic assays can be used to determine if the blood is nonhuman [2]. DNA analysis is always used in identifications [4]. Both presumptive and confirmatory tests are destructive, so that they consume a portion of the sample. To minimize this consuming, a modified testing scheme is employed. Typically, if a stain has been presumptively identified as blood, further characterization cannot be carried out. If a DNA profile is not extracted from the sample, then the suspected blood would be supposed as animal origin. This is problematic, because there is a lack of evidence that the sample is of human origin and in fact blood. This approach could also be detrimental for crime labs since time and money would be wasted on nonhuman or non-blood samples. Therefore, a non-destructive and efficient screening technique for discrimination of human blood would be highly valuable. Compared to these methods, spectral method can be quick, stable, accurate and environmental [5-9]. Spectral method is promising to apply on screening blood samples for customs supervision demands.

De Wael et al. applied vibrational spectroscopy to the problem of species identification of blood samples [10]. Their work illustrates the inability to differentiate between blood particles originating from human, cat and dog samples through their infrared and Raman spectra. However, Raman spectroscopy has been proved a technique that has the potential both as a non-destructive confirmatory identification for blood [11, 12] and as a species of origin assay. Virkler's work [7] has proved the possibility of identifying human, feline and canine blood by combining Near-infrared Raman spectroscopy and Principle Component Analysis (PCA) method. McLaughlin [13] has realized the discrimination of human and animal blood traces via Raman spectroscopy and Partial Least Square Discrimination Analysis (PLS-DA) model. Their model demonstrated 100% accuracy to differentiate between human and nonhuman blood.

Diffuse reflectance spectroscopy can be used for composition analysis [14-17]. The diffuse reflectance spectra contain quantitative information about structure and composition of samples. Compared to Raman technology, DRS maybe more portable and affordable. So far there is no report diffuse reflectance spectroscopy can do the discrimination of human blood and nonhuman blood. In this paper, we used visible diffuse reflectance spectroscopy combining PLS-DA method to discriminate human and nonhuman blood.

2. Materials and Methods

2.1 Blood samples

Sixty blood samples of macaque, rat, chicken, pig and guinea pigs were formally delivered by Institute Zoology, the Chinese Academy of Sciences. Fifteen human blood samples were formally delivered by Tianjin people hospital. All experiments performed were in compliance with relevant laws, as well as the guidelines of Institute Zoology, the Chinese Academy of Sciences, Tianjin people hospital and State Key Laboratory of Precision Measurement Technology and Instruments, Tianjin University. All the institutes mentioned above had approved the experiments. The volunteers had given their consents for the experiments. The anticoagulants were added to the blood

samples after the blood samples acquired immediately. The spectra were obtained within 48 hours (after 24 hours) after the samples acquired. Before the measurement the samples are prepared with ice. Each sample was prepared by placing about 1 mL on a circular sample dish. Each sample was measured twenty times, with integration time of ten milliseconds.

2.2 Measurement system

A supercontinuum white light laser source (Superke Compact, NKT, Denmark), a visible spectrometer (QE65000, Ocean Optics, USA) and a fiber probe were used to get the diffuse reflectance spectra. The source and the spectrometer used are shown in Fig.1. The laser provided by the supercontinuum white light laser source was 100 mW. The supercontinuum white light laser source has a spectral range of 600-2500 nm. The spectrometer has a spectral range of 350–1150 nm. The fiber probe used was shown as Fig. 2. The white central region contains the illumination fibers with a diameter of 0.6 mm, and the surrounding gray region contains the collection fibers with an outer diameter of 5.8 mm. The visible diffuse reflectance spectra were collected with the optical fiber under the surface of the blood sample surface.

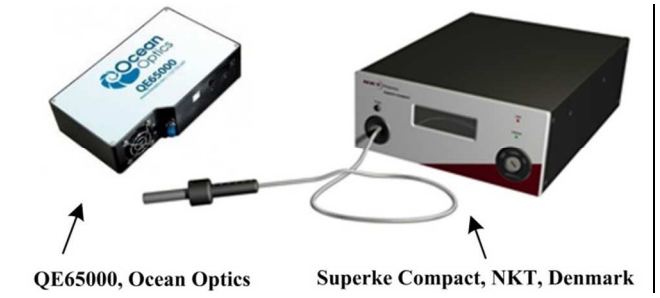

Fig.1 Devices used in the experiments

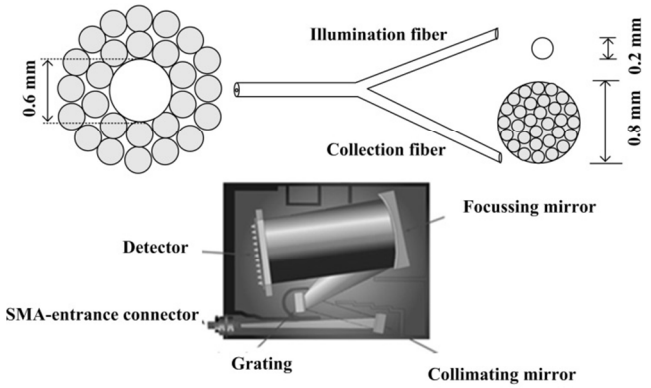

Fig.2. Schematic of the probe geometry used in this study, with the white central region containing the illumination fibers and the surrounding gray region containing the collection fibers.

2.3 Data preparation and statistical treatment

All data preparation and construction of statistical models were performed with MATLAB 8.2.0. For each blood sample, twenty spectra ware collected. These twenty spectra were averaged to form a single spectrum representing one sample. Data standardization was used before constructing the model. PLS-DA method was used to discriminate human and nonhuman blood samples.

3. Results and discussion

3.1 Spectral analysis of training dataset

The training dataset consisted of ten human blood samples and thirty animal blood samples—twenty macaque, ten guinea pig blood samples. The preprocessed training dataset spectra are shown in Fig. 3A. A comparison of the animal and human class mean spectra is shown in Fig. 3B. These spectra are the total averaged data for their respective classes after preprocessing.

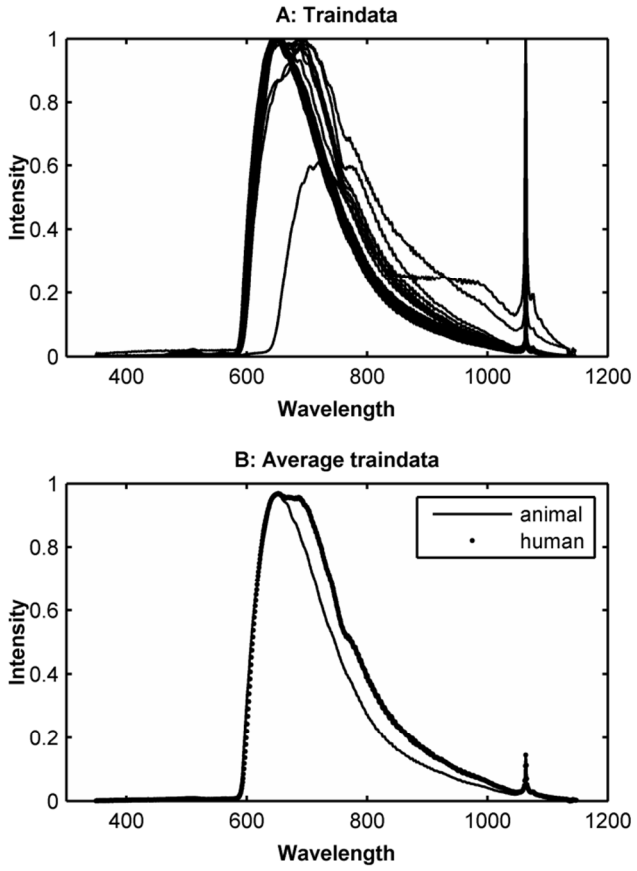

Fig.3. (A) Comparison of the preprocessed visible diffuse reflectance spectra and (B) Average spectrum of all animal (n=30) spectra and all human (n=10) spectra.

3.2 Construction of a binary model

The leave-one-out cross-validation method was used to choose the best number of factors to use. Root-Mean-Square Error of Prediction (RMSEP) value versus the number of PLS factors was shown in Fig.4. When the number of factors was eighteen, RMSEP value can be the smallest. So, eighteen factors were used to build the PLS-DA model.

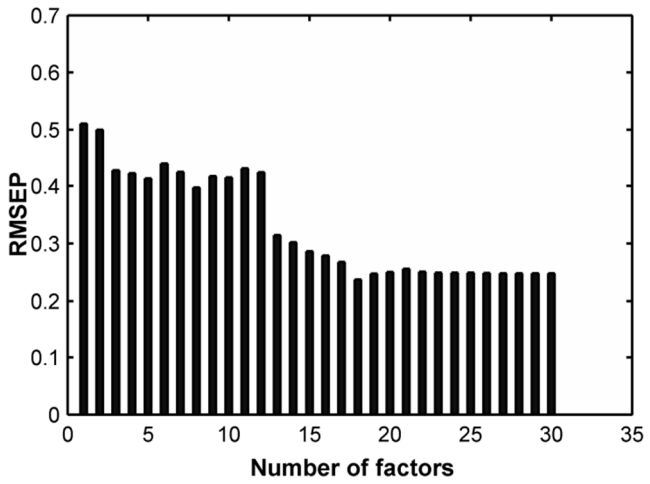

Fig.4. RMSEP for the calibration set versus different PLS factors.

A PLS-DA model was constructed with training dataset classified as either human or animal. The model was built with eighteen factors. Prediction scores using the PLS-DA model of training dataset were shown in Table 1 and Fig.5. This illustrates the classification ability of the model for every spectrum in the training dataset.

Table 1 Summary of model predictions from validation spectra.

Spectrum	Binary	Actual identity
Sample 1-Sample 20	Animal	Macaque
Sample 21-Sample 30	Animal	Guinea pig
Sample 31-Sample 40	Human	Human

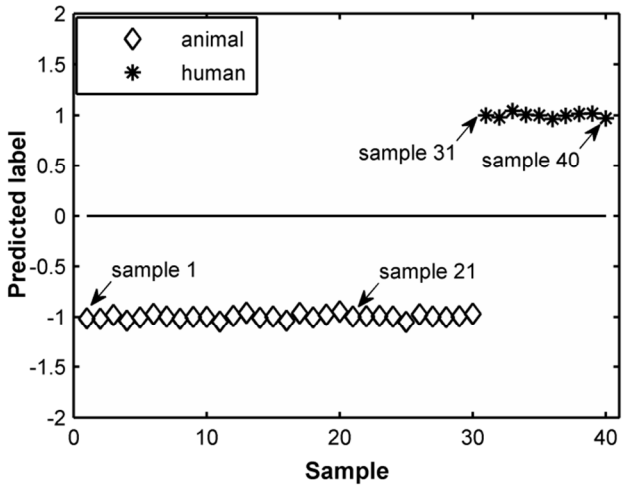

Fig.5.Prediction scores using the PLS-DA model of training dataset.

3.3 Blind test and external validation

To confirm the performance of the model built with eighteen factors, predictions were calculated for thirty-five unknown spectra–nine macaque, seven guinea pig, five human samples (the species within the model) and twelve rat, one chicken, one pig samples (the species out of the model) (Section 2.3). The prediction results for these 35 spectra for the model are displayed in Table 2 and Fig. 6.

Table 2 Summary of model predictions from blind and external validation test spectra.

Spectrum	Binary	Actual identity
Unknown 1-Unknown 9	Animal	Macaque
Unknown 10-Unknown 16	Animal	Guinea pig
Unknown 17-Unknown 21	Human	Human
Unknown 22-Unknown 33	Animal	Rat
Unknown 34	Animal	Chicken
Unknown 35	Animal	Pig

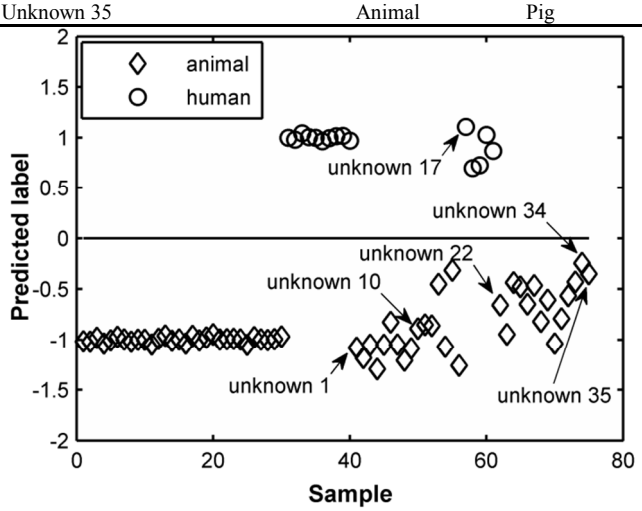

Fig.6. Prediction scores using the PLS-DA model of training dataset and unknown samples. The line represents the default classification threshold.

These results illustrate the classification ability of the method to identify human and nonhuman blood samples. The remarkable classification performance and the non-destructive nature of visible spectroscopy make this approach well-suited for human blood discrimination. This is especially important in cases where the sample is limited to nondestructive occasions. Furthermore, visible spectral analysis supports the idea that rapid species identification is feasible.

4. Discussion

The human blood samples used in the first model are all measured 24 hours after blood samples obtained. And the model described above can only predict blood samples after 24 hours well, can't predict blood samples within 24 hours well. When we take the preservation time into consideration, adding six guinea pig blood samples and 15 human blood samples measured within 24 hours to the training dataset, the model can predict guinea pig blood samples and human blood samples measured within 24 hours very well. The training model is shown in Table 3 and Fig.7. And the prediction results are shown in Table 4 and Fig. 8. So we can get the conclusion that samples measured with different days should be added to the training model to improve the predicting performance of the model.

Table 3 Summary of model predictions from (discussion) validation spectra.

Spectrum	Binary	Actual identity
Sample 1-Sample 20	Animal	Macaque
Sample 21-Sample 30	Animal	Guinea pig (>24 h,<48 h)
Sample 31-Sample 36	Animal	Guinea pig (<24 h)
Sample 37-Sample 46	Human	Human (>24 h,<48 h)
Sample 47-Sample 61	Human	Huamn (<24 h)

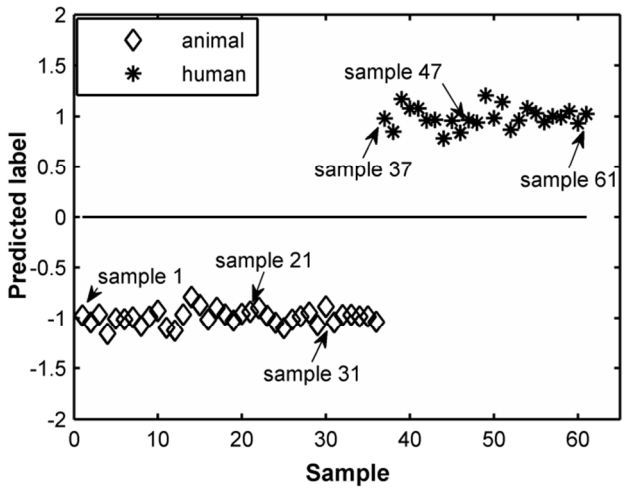

Fig.7.Prediction scores using the PLS-DA model of training dataset.

Table 4 Summary of model predictions from (discussion) blind and external validation test spectra.

Spectrum	Binary	Actual identity
Unknown 1-Unknown 9	Animal	Macaque
Unknown 10-Unknown 16	Animal	Guinea pig (>24 h,<48 h)
Unknown 17-Unknown 22	Human	Human (<24 h)
Unknown 23-Unknown 33	Human	Human (>24 h,<48 h)
Unknown 34-Unknown 45	Animal	Rat
Unknown 46	Animal	Chicken
Unknown 47	Animal	Pig
Unknown 48-Unknown 52	Animal	Guinea pig (<24 h)

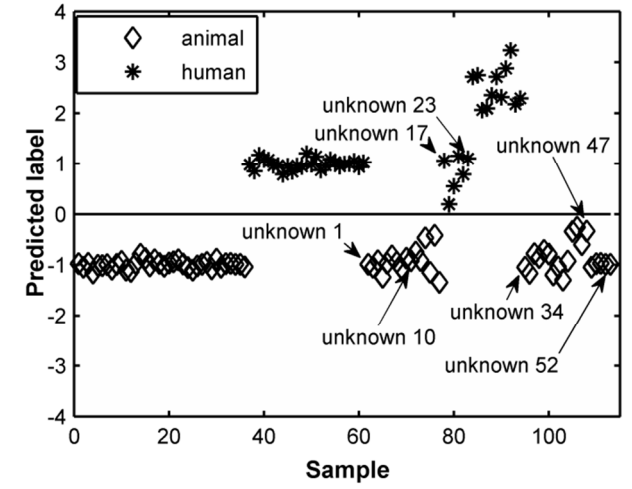

Fig.8. Prediction scores using the PLS-DA model of training dataset and unknown samples. The line represents the default classification threshold.

5. Conclusions

The combination of visible diffuse reflectance spectroscopy and PLS-DA method was demonstrated to be a powerful tool toward the human identification of a blood sample. A binary PLS-DA model was constructed using a training dataset formed from visible spectral data collected from human blood and two animal species' blood (macaque and guinea pig). The constructed model demonstrated very good internal classification ability with zero occurrences of false negative assignments of the human class. Furthermore, the model performed well under two performance measures – a blind test of internal samples and an external validation test of animal spectra (rat, chicken and pig) which was excluded from the training dataset. In order to improve the predicting performance of the model, blood samples measured in different days should be added to the training dataset. The model will be further strengthened by adding more species and more samples. This method has good potential to apply on screening blood samples for customs supervision demands, since it is advantageous over the current methodology, primarily due to the non-destructive nature of analysis.

Acknowledgements

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Notes and References

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