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Noncontact blood species identification method based on spatially resolved near-infrared transmission spectroscopy

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HIGHLIGHTS

- Blood species identification is very important to protect national specially protected wildlife.
- Spatially resolved spectroscopy method performed far better than single-point spectra method.
- The noncontact nature make this approach well-suited for the forensic species identification.

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ABSTRACT

The inspection and identification of whole blood are crucially significant for import-export ports and inspection and quarantine departments. In our previous research, we proved Near-Infrared diffuse transmitted spectroscopy method was potential for noninvasively identifying three blood species, including macaque, human and mouse, with samples measured in the cuvettes. However, in open sampling cases, inspectors may be endangered by virulence factors in blood samples. In this paper, we explored the noncontact measurement for classification, with blood samples measured in the vacuum blood vessels. Spatially resolved near-infrared spectroscopy was used to improve the prediction accuracy. Results showed that the prediction accuracy of the model built with nine detection points was more than 90% in identification between all five species, including chicken, goat, macaque, pig and rat, far better than the performance of the model built with single-point spectra. The results fully supported the idea that spatially resolved near-infrared spectroscopy method can improve the prediction ability, and demonstrated the feasibility of this method for noncontact blood species identification in practical applications.

1. Introduction

The inspection and identification of whole blood are crucially significant for import-export ports and inspection and quarantine departments [1]. On one hand, the export of whole blood products that the national specially protected wildlife were prohibited in our national law. Because the export will result in the loss of national resource information of species. On the other hand, the import of whole blood products must be tested and controlled, in order to avoid the destruction of the ecological environment caused by the invasion of unknown alien species. In import-export inspection and quarantine departments, the general methods for the detection of whole blood products were conventional flow cytometry, which was based on open detection method. The method has two drawbacks, the contamination of whole blood samples brought by contact sampling, and the risk of personnel infection caused by pathogenic factors, that may be carried by whole blood products.

Interspecies blood analysis is also an important part in analytical chemistry and biochemistry. Identifying the species of a blood stain is of great significance to the fields of forensic casework [2,3], wildlife preservation [4]. Currently, species identification can be realized by many analytical techniques [5,6]. Several high-performance liquid chromatography (HPLC) [7] methods can simultaneously identify a substance as blood and determine its species of origin. Mass spectrometry (MS) method [8] was also reported to be effective for species identification. Most of these

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methods are destructive to the sample. So it is in urgent need of noncontact blood identification methods.

Compared to these methods, vibrational spectroscopy methods are simple, quick, and non-destructive [9–13]. Our group has proved that visible diffuse reflectance spectroscopy combined with PLS-DA method can successfully discriminate human blood and nonhuman blood [14,15]. And near-infrared diffuse transmission spectra has been confirmed to be effective for identifying three blood species—macaque, human and mouse [16]. Fig. 1 showed the schematic diagram of different measurement. The measurement of Refs. [14,15] was shown in Fig. 1(A). The detection fiber was under the surface of blood samples, which was in the circular sample dish. The measurement of Ref. [16] was shown in Fig. 1(B). Blood samples were measured in cuvettes, and were radially scanned by the detection fiber. However, all these experiments were performed with samples open. And open sampling may pollute the blood samples. Virulence factors in blood samples can also endanger inspectors. In this paper, measurements were implemented with the blood samples in their original container—vacuum blood vessel, as shown in Fig. 1(C). And blood samples were vertically scanned by the detection fiber to obtain spatially resolved spectra.

In case of blood is a kind of turbid media, which was more complicated than pure absorbing substances, spatially resolved near-infrared spectroscopy method [17,18] was used to improve the performance of identification. Our group has proved its advantage by predicting the concentration of the Intralipid diluted solutions. Many researches have also testified spatially resoled diffuse reflectance spectroscopy and spatial frequency domain reflectance technique were powerful for composition analysis. Erkinbaev's research [19] proved hyperspectral scatter imaging method, which was based on spatially resolved diffuse reflectance technique, has the potential for noncontact and non-destructive application in food industry domain. Sung [20] developed a movable diffuse reflectance spectroscopy system, successfully realized extracting the reduced scattering coefficient of both the top layer and the bottom layer, the thickness and the hemoglobin concentration of the phantoms.

In this paper, we used spatially resolved Near-Infrared spectroscopy method for blood species identification. We compared the performance of the model built with multi-point spectra and the model built with single-point spectra. And we explored the optimal points with the best effect for identifying the five species.

2. Materials and methods

2.1. Blood samples

Two hundred and thirty blood samples of chicken, goat, macaque, pig and rat were formally delivered by Institute Zoology, the Chinese Academy of Sciences. Each sample was provided about 5 mL by an individual donor. Animal blood was collected from both genders to ensure donor diversity. All experiments performed were in compliance with relevant laws, as well as the guidelines of relevant institutes. All the institutes mentioned above had approved the experiments. The anticoagulants were added to the blood. The blood samples were measured within 48 h. Each sample was prepared in its original collected container—vacuum blood vessels. Each point was measured with integration time of 50 ms.

2.2. Measurement system

The measurement system was shown in Fig. 2, consisted of a white light laser source (Supercontinuum Laser, SC-5-FC, China), a Near-Infrared spectrometer (AvaSpec-NIR256-2.5-HSC, Avantes, Holland), an optical fiber probe, an electric control translation stage and a laptop, to get the diffuse transmitted spectra. The power provided by the supercontinuum white light laser source was 800 mW, the power was adjusted by 30%, namely 240 mW. The spectrometer has a spectral range of 900–1750 nm. The electronically controlled translation stage (NFP-1462CZ, Zolix, China) with an orientation precision of 1 μm, was used to carry out the scanning on the surface of the vacuum blood vessels by the detection fiber along the vertical direction. The near-infrared diffuse transmitted spectra were collected beginning with the collection fiber and the laser center to center. The scanning range of the probe was from the center probe to 4.5 mm away at intervals of 0.5 mm. There were a total of 10 points for each sample in the vertical direction.

2.3. Data preparation and blind test

All data processing was performed with MATLAB 8.2.0. Wavelet decomposition and reconstruction method was used to remove the noise. The signal was two-layer decomposed with sym10, the high frequency coefficients all set to zero, getting the de-noising signal by reconstruction of the treated wavelet coefficients. Then the spectra were standardized. PLSDA methods were used in this research for the identification. The training dataset consisted of near-infrared diffuse transmitted spectra of thirty chicken blood samples, thirty goat blood samples, thirty macaque blood samples, thirty pig blood samples and thirty rat blood samples, for a total of one hundred and fifty spectra. The training data was chosen randomly. To evaluate the performance of the classification model, a set of eighty unknown samples were prepared for the blind test, from the available blood samples, including ten chicken blood samples, twenty goat blood samples, twenty macaque blood samples, twenty pig blood samples and ten rat blood samples. To convert three-dimensional spectra data to two-dimensional spectra data, for each blood sample, the spectra collected from point 1 to

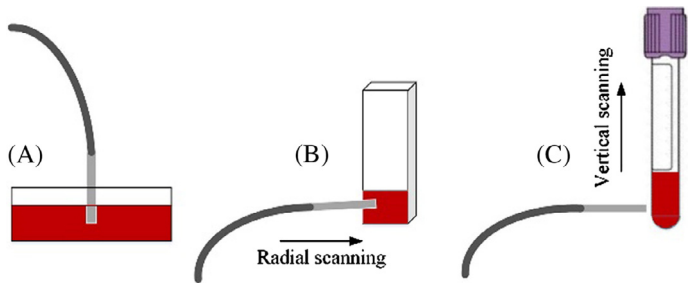

Fig. 1. Comparison of different measurement method. (A) Measurement in Refs. [14,15]: the detection fiber being under the surface of blood samples in the circular sample dish. (B) Measurement in Ref. [16]: blood samples being measured in cuvettes, and radially scanned by the detection fiber. (C) Measurement in this research: blood samples being measured in vacuum blood vessel, and vertically scanned by the detection fiber.