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**Research Article**

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# Detection and analysis for abnormal liver using Raman spectroscopy of serum

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**ABSTRACT**

*In this paper, Raman spectroscopy of serum was detected and analyzed by multivariate analysis and classification methods to identify its diagnosis potential for liver diseases. Serum taken from 81 volunteers (31 healthy, 27 liver cancer and 23 liver cirrhosis) was measured. Principal component analysis (PCA) was utilized on the first order derivative spectra of the original Raman spectroscopy. Linear discriminant analysis (LDA) and artificial neural networks (ANN) were then used separately on the selected PCs obtained from PCA. The diagnosing accuracy of PCA-LDA is 85%, and is 80% for PCA-ANN. In conclusion, this preliminary study demonstrated that Raman spectroscopy of serum can be used in the screening of liver cancer.*

**Keywords:** liver cancer, Raman spectroscopy, serum

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## INTRODUCTION

Liver cancer has about 740,000 new diagnosed cases annually worldwide, and the number of deaths is approximately 690,000 each year[1]. Though the best way of reducing death rate of liver cancer was early diagnosis and consequent expedient treatment, no effective screening method is available till now. Because water which is abundant in biological liquids is Raman inactive, Raman spectroscopy are commonly used for the detection of bio-fluids[2–4]. Serum contains a great quantity of biomarkers, and its composition changes can reflect the corresponding variation of body status. Therefore, it is feasible to use serum as a predictor for liver diseases[5,6].

Because of the low scattering cross section and consequent weak signal, it is rather difficult for the detection of low concentration serum. So, data reduction method of principle component analysis (PCA) and classification methods of linear discriminant analysis (LDA) and artificial neural network (ANN) were applied in our data analysis to analyze the Raman spectroscopy of serum. PCA is a multivariate statistical technique that has been successfully deployed in the data reduction of spectroscopy. It can simplify the large amount of datasets into several principal components (PCs) which can represent the whole range of the original Raman spectroscopy[7]. LDA and ANN are two effective discriminating techniques, they have shown high performance in classifying spectroscopy on the spectroscopic diagnosis of cancers[8,9].

The purpose of this study is to identify whether Raman spectroscopy of serum coupled with chemometrics data analysis techniques could efficiently distinguish between liver cancer, liver cirrhosis patients and normal controls. Two classifying methods (LDA and ANN) were utilized separately on the PCs of PCA to get the algorithm for liver diseases diagnosis.

## EXPERIMENTAL SECTION

Blood samples were taken from 31 healthy people, 27 liver cancer patients and 23 liver cirrhosis patients provided by Liaoning Cancer Hospital & Institute. All samples were phlebotomized before breakfast in the morning to avoid the interference of food. Approximate 2mL venous blood was collected, and no anticoagulant was added. Within half an hour, each blood sample was centrifuged at a speed of 3000 rpm for 10 min. Serum samples were measured Raman spectroscopy as soon as possible, to avoid component changes of serum. When measuring, about 1 mL serum sample was injected into a clean dry sample tube, and all the spectral measurements were performed at room temperature in dim light.

The excitation source was 514.5 nm wavelength laser produced by an Argon ion laser (The 772 factory, Nanjing, China). Scanning spectral range was from 520 nm to 640 nm with the laser power of 3.5 mW. About 1 mL of serum sample was put into a transparent tube between the chopper and the double spectrometer when the spectra were measured.

Every sample of all the three groups was tested three times and an average spectroscopy was recorded. Before the PCA analysis, Savitzky-Golay filter determined by an unweighted linear least squares method was used to smooth the spectra. This processing is provided by the smooth function in the Curve Fitting Toolbox of Matlab.

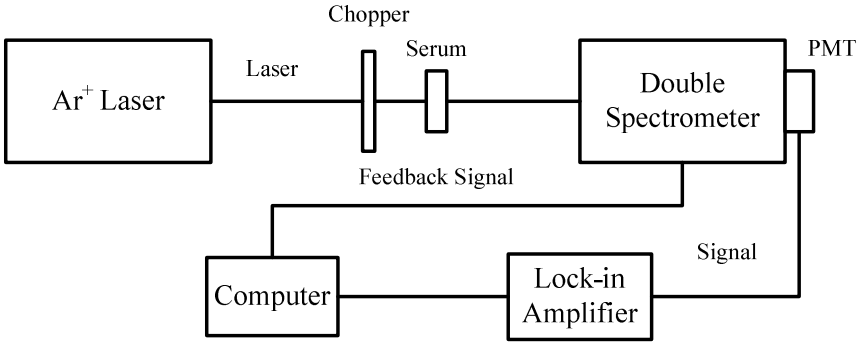


**Fig. 1. Schematic drawing of the Raman spectroscopy system**

PCA is an orthogonalizational data transition method. It can reduce the data dimension of Raman spectra to a much lesser amount of new variables while retaining the most diagnostically significant information of the original spectra[10]. By doing PCA, only a small proportion of the spectroscopy with diagnostic information was retained. After this data reduction, linear discrimination analysis (LDA) and artificial neural network (ANN) were used on the PCs of the PCA to discriminate different groups of samples. Then the diagnostic evaluation of the PCA-LDA and PCA-ANN was calculated to see the diagnostic ability of the two algorithms. All the data analyses stated above were performed using the Matlab software (The MathWorks, Inc. U.S.A).

## RESULTS AND DISCUSSION

### Raman spectroscopy

Three Raman peaks are observed in all the three groups of samples (liver cancer, liver cirrhosis and controls), they are located at 1020 $cm^{-1}$, 1158 $cm^{-1}$ and 1515 $cm^{-1}$, separately (Fig 2(a)). The band at 1515 $cm^{-1}$ and 1158 $cm^{-1}$ were both assigned to beta carotene[11], and the intensity of these two peaks decrease with the deterioration of liver diseases (the intensity of liver cancer has the lowest intensity, while the normal people have the highest). Beta carotene existed in human bodies as a cancer resistant substance as it can suppress the production of oxygen radicals. For the Raman peak at 1020 $cm^{-1}$(aromatic ring breathing of phenylalanine[12]), the peak height for liver cirrhosis and the normal are similar, but were higher than that of liver cancer. This indicates that serum from liver cirrhosis patients and normal people have similar chemical components. These results are consistent with the pathological explanation that liver cirrhosis is less severe than liver cancer.

### Statistical Analysis

First order derivative spectroscopy of the average Raman spectra were obtained and used for the PCA to eliminate the interference of background fluorescence (Fig 2(b)). Then all the first order derivative spectroscopy was input into PCA to do the K-L transformation of Matlab.

The contribution rate of the first two PCs (PC1 and PC2) to the total variation of spectra is over 97%, so the data of the spectrum can be represented by the first two PCs. Thus, only the first two PCs were used for further classification analysis.

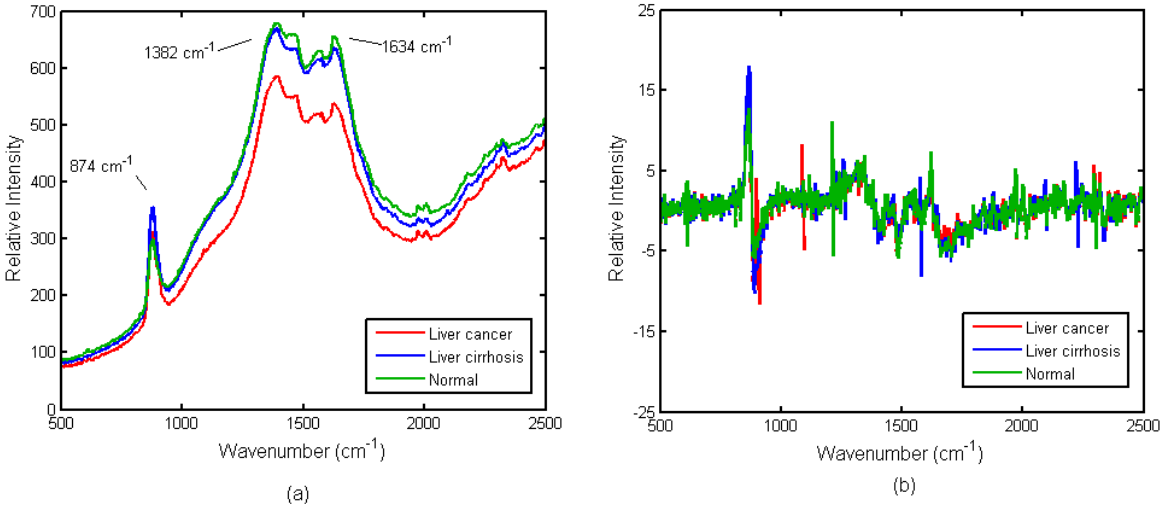


**Fig. 2. (a) Averaged Raman serum spectra of each group; (b) Averaged first order differentiated serum spectra**

To build the discrimination model of LDA, the first two PCs were selected as the input variables. Fisher's linear discriminant function was used as the discriminant function. We got three linear discriminant functions for classifying the three groups (Fig 3). The broken lines are diagnostic lines got from Fisher's discriminant function of LDA: 0.45 × PC1 + 0.18 × PC2 = - 3.41 (between liver cancer & liver cirrhosis); 0.89 × PC1 + 0.46 × PC2 = - 0.57 (between liver cancer & normal, not shown,); 0.44 × PC1 + 0.28 × PC2 = 2.85 (between liver cirrhosis & normal). This PCA-LDA has a diagnostic accuracy of 85% and a specificity of 90%. The sensitivity for liver cancer is 81% and 83% for liver cirrhosis (Tab 1).

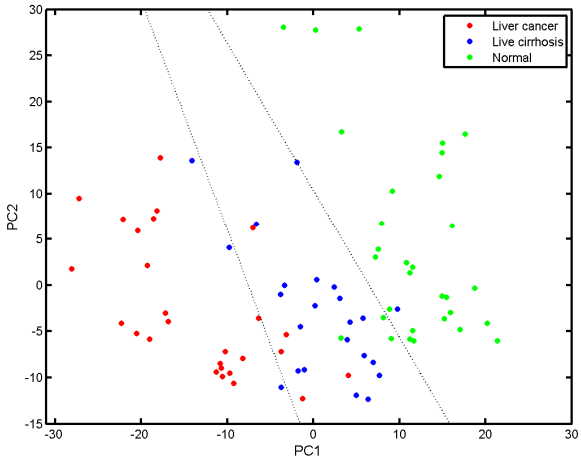


**Fig. 3. Linear discriminant analysis (LDA) on the first 2 PCs of principal component analysis (PCA)**

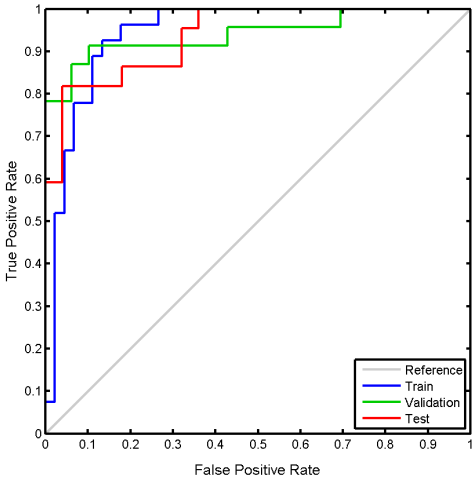


**Fig. 4. ROC graph of the ANN process**

Neural network architectures with nodes from 2 to 20 were tested for the classification of the PCs. The network was set with 2 hidden layers, with 20 neurons in each layer. Then the PCs of PCA were input to this ANN system. The samples were separated into training set (49 samples), validation set (16 samples), and test set (16 samples) when being processed in the neural network. The prediction accuracy of the ANN system is 80%, the sensitivity is 93% for liver cancer and 78% for liver cirrhosis, and the specificity is 71% (Tab 1).

**Table 1 Diagnostic evaluation of the PCA-LDA and PCA-ANN technique**

| Method | Group | n | | | Accuracy (%) | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|---|
| | | cancer | cirrhosis | healthy | | | |
| PCA-LDA | Cancer | 22 | 3 | 0 | | 81 | |
| | Cirrhosis | 5 | 19 | 3 | 85 | 83 | 90 |
| | Healthy | 0 | 1 | 28 | | N/A | |
| PCA-ANN | Cancer | 25 | 3 | 6 | | 93 | |
| | Cirrhosis | 1 | 18 | 3 | 80 | 78 | 71 |
| | Healthy | 1 | 2 | 22 | | N/A | |

## CONCLUSION

In summary, we investigated the applicability of using Raman spectroscopy of serum combined with PCA-LDA/ANN for the discrimination of liver cancer patients, liver cirrhosis patients and healthy people. Small differences at the three Raman peaks indicate a composition change in serum for different groups of samples. By using PCA-LDA/ANN, features of the original Raman spectroscopy were reserved and classified. We got the diagnosing accuracy of 85% for PCA-LDA, and 80% for PCA-ANN. Our work showed the feasibility of the clinical use of serum Raman spectroscopy for the diagnosis of liver diseases.

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