31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Preliminary Study on the Quick Detection of Acquired Immure Deficiency Syndrome by Saliva Analysis using Surface Enhanced Raman Spectroscopic technique

Wang Yan, Hua Lin, Liu Jinghua, Qu Dian, Chen Anyu, Jiao Yi, Guo Xun, Liu Chunwei, Huang Wen and Wang Hong

Abstract—45 saliva samples of AIDS patients and 55 saliva samples in normal population were detected by the Surface-enhanced Raman spectroscopy (SERS) system. And the spectrum data were analyzed using the Support Vector Machine (SVM) algorithm, one of the data mining technologies. Statistical analysis showed that two groups were distinguished effectively. This study provided a new research direction of the quick non-invasive testing for AIDS.

I. Introduction

Acquired Immure Deficiency Syndrome (AIDS) is a serious, even fatal, disease which damages to the human immune system caused by the human immuno deficiency virus (HIV). Worldwide, 40.3 millions people had been infected by HIV and died 3.1 millions at the end of the year 2005 [1]. In China, the data is increasing too recently and it is emergent to detect the HIV taking easily and quick and prevent the AIDS spread. The AIDS has become the community focus of the whole world for the commonality sanitation.

Nowadays the general detection method of AIDS is to exam the vein blood to find if there is the HIV antibody in it. But it is too inconvenient to collect the vein blood and need more time to get the result. So the research focus is to find a convenient and quick detection method of AIDS instead of using the vein blood.

Human saliva contains abundant proteins and metabolites. Many diseases can be diagnosed by analysis of saliva [2]. There are many advantages of using saliva analysis: the sample collection is easy and noninvasive; the entire procedure of sample collecting can be more conveniently monitored, compared to urine sample collection. As a newly available test method, the saliva test will become an effective supplement, even a replacement to other test methods, such as blood or urine test [3].

Manuscript received June 19, 2009. This work was supported by OptoTrace (Beijing) Technologies, Inc.
Wang Yan, Chen Anyu, Hua Lin, Liu Jinghua and Qu Dian are from Biomedical Engineering College of Capital University of Medical Sciences, Beijing, China 100069.
Chen Anyu is the corresponding author. Adds: Biomedical Engineering College of Capital University of Medical Sciences, Beijing, China 100069 Tel.: +86-010-83911557; Fax: +86-010-83911560
E-Mail: chenay@ccmu.edu.cn
Jiao Yi, Guo Xun, Liu Chunwei, Huang Wen and Wang Hong are from OptoTrace (Beijing) Technologies, Inc., Beijing, China 100088

Raman spectrum is used for investigation of the internal structural property of solids, liquids and gases. It is an effective method to identify unknown chemical materials [4, 5]. It is used to identify the molecular functional cluster to achieve the chemical material fingerprint identification. The Raman spectroscopy technology has many advantages, such as not requiring complicated sample preparation, free of interference from water molecules, fast, noninvasive, portable equipment, etc. [6]. Due to the technology development in the field of optics, electronics, semiconductor and computer software [7], Raman spectroscopy has demonstrated its technical advantages in chemical detection. It is now possible for Raman spectroscopy to enter many new fields of application. In recent years, the development of nanotechnology provides a new life for Raman spectroscopy. Experiments showed that the detection sensitivity of Raman spectroscopy increases with the decrease of dimension of the particles on a sensing substrate surface. The sensitivity is enhanced significantly when the dimension of the particles on the substrate surface is less than 100 nanometers. The Surface-Enhanced-Raman-Spectroscopy (SERS) is a technology using special processed nano sensing chip as substrate to increase the detection sensitivity. Study showed that the sensitivity of SERS can reach the level of part per trillion (ppt) [8]. SERS has demonstrated great potential not only in the field of material molecular structure investigation, but also trace chemical detection. It is an important advantage that SERS detection technology can perform noninvasive and fast test on saliva samples.

In this study, saliva samples of 45 AIDS patients were tested by a portable SERS system from OptoTrace Technologies, Inc., and comparison analysis has been done against the saliva samples of 55 health persons. This study demonstrated the feasibility and effectiveness of a new method of fast detection of the AIDS patient. It is an important advantage that SERS detection technology can perform noninvasive and fast test on saliva samples.

II. Materials and Methods

2.1 Materials

The Raman spectrometer and nano sensing chips are provided by OptoTrace Technologies, Inc.. The Raman spectrometer is RamTracer^{TM}-200 Raman Spectrometer,

978-1-4244-3296-7/09/$25.00 ©2009 IEEE

which main technical specifications: 0-300 mW, 785 nm, frequency stabilized Laser; 2048 pixel CCD; Spectral resolution< 6 cm^{-1}; Spectral range: 250 to 2400 cm^{-1}. The nano sensing chip is as Fig. 1 shown.

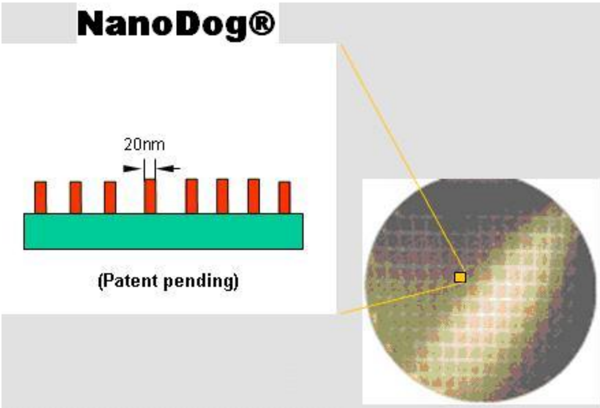

Fig. 1. Testing Nano Sensing Chip

2.2 Sample Collection and Preparation

In this research, the saliva samples of AIDS patients were collected by the clinical laboratory of Beijing YouAn Hospital, the special hospital for the AIDS patients. And the health saliva samples were directly get from the students and teachers of China Capital University of Medical Science, who had the normal health examination just before the experiment. The samples were collected as the same step: mouth was rinse with water 10 minutes before saliva collection, then 1-1.5 ml saliva was collected into a clean container. Saliva samples were centrifuged at 14,000 rpm for about 10 minutes, as the Fig.2 shown

Fig. 2. Saliva Drop on the Chip. The photo is obtained under the microscope.

III. Testing Progress

Only 10 μl centrifuged sample was transferred to the surface of a nano sensing chip using micropipette once a time. Raman test can be carried out right after the saliva sample dried.

The laser output power of the RamTracer™-200 Raman spectrometer was set to 100 to 200mW. The signal to noise ratio is generally proportional to the power of the laser. The higher the power is, the better the signal to noise ratio is. But the sample may be damaged if the power is too high. The surface-enhanced Raman scattering of the sample was received by RamTracer™-200. And the Raman spectrum related to the sample molecular structure was obtained. Fig. 2 shows the Raman spectrum of a saliva sample. Horizontal axis is Raman shift in the unit of cm^{-1}, while the vertical axis is the intensity of Raman scattering.

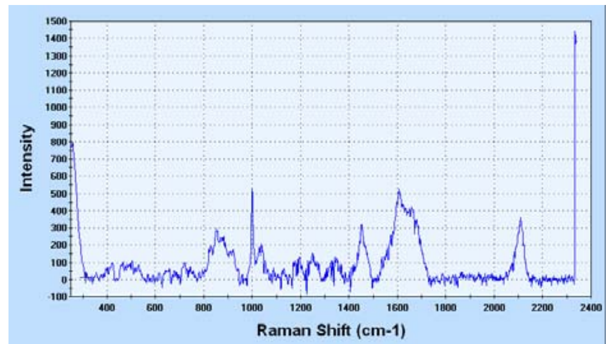

Fig. 3. Raman Spectrum of a Saliva Sample. There are many Raman Spectrum peaks and the typical peaks using to identify the normal and the AIDS are in research now.

The Raman peaks in Fig. 3 represent the characteristic of the molecular structure of the sample being tested. The chemical contents of the sample can be obtained by analyzing the peak position and the relative peak intensity, but it is on researching now. In medical research, by comparing the Raman spectra of samples from patients with certain disease against that from healthy people, we may discover a distinctive Raman peak(s). This distinctive Raman peak can be considered as a specific marker related to a disease; hence it can be used in the diagnosis of this disease.

IV. Results And Discussion

All 45 patients are diagnosed as AIDS by Beijing YouAn Hospital (Beijing, China), compared to 55 normal people are examined by the School Hospital of China Capital University of Medical Science. Because there is almost no HIV antibody in the saliva, the differences between the health and AIDS patients' samples in the Raman spectra are hardly distinguished by naked eyes.

So the Support Vector Machine (SVM) algorithm [9] is used to classify the samples between the health and AIDS by Raman shifts. SVM maps the input data vectors into an n-dimensional space by using Kernel function (linear function, polynomial Kernel function, Gauss Radial Basis Kernel (GRBK) Function, Sigmoid Kernel Function, etc.). The input data is viewed as two sets of vectors in the n-dimensional space. A maximized training is performed by SVM using the data sets, to search the Optimum Separating Hyperplane (OSH) so that the distance from the hyperplane to the nearest positive and negative data point has been maximized. The data classifier being tested is obtained by calculating the relative positions of the projection points of the two vectors on the hyperplane. Thus the classification of the samples is achieved.

R software (a language and software platform for statistical computing and graphics) was used to implement SVM; the Kernel function used is GRBK Function.

The software of the OptoTrace Technologies, Inc. provided can give 2090 Raman shifts from 250 to 2339 cm^{-1} in one sample. We get the whole 100 samples both the health and the AIDS patients together to build a sample data base. In this study, total about 2090 Raman shifts are analyzed from 100 samples, and statistically it is a small-sample multi-index analysis. The ten times cross validated algorithm is adopted. The all 100 samples are divided into 10 parts: the nine parts of ten are used as the training set and one part is used as the testing set. The TABLE I shows the result.

Using statistic analysis of 45 samples of AIDS patients and 55 samples of the health described above, we can effectively distinct the AIDS patient from the health people. The analysis shows that the sensitivity is 95.6%; the specificity is above 100%.

TABLE I
SENSITIVITY AND SPECIFICITY OF SERS CLASSIFICATION OF THE HEALTH AND AIDS

		SERS Classification Result	
		HIV	Health
Saliva Sample	HIV	43	2
	Health	0	55

Sensitivity = Test Positive/(Test Positive + False Negative)
= 43/(43+2) = 95.6%
Specificity = Test Negative/(Test Negative + False Positive)
= 55/(55+0) = 100%

It is pointed out that many pattern recognition methods such as SVM, can involve in the overfitting problems inevitably. In order to validate our algorithm, we implement the same process to another data set which includes 44 drugging individuals and 52 normal controls. We apply the optimum separating hyperplane acquired by SVM model to classify 11 new rehabilitation samples and the results show that the diagnostic accuracy is 90.9%. In fact, sample size is an important factor for training the reasonable model. Accordingly, more available samples will be collected in our future works, which will make our model more exactly in classifying prospective blinded samples.

V. CONCLUSION

Based on the above analysis, the Raman spectrum of the AIDS patient's saliva is sensitive to that of the health people using the SERS technology. The study using SERS technology to quickly detect the AIDS is seldom in research both in China and other countries. Our study is preliminary and the samples need to increase and to built an accurate model to confirm the testing results. The biochemical content(s) of the AIDS patients' saliva need to be further identified and understood.

SERS test on saliva samples is fast, noninvasive, with very easy sample collection, and cost effective. The entire process of sample collecting can be conveniently monitored without intrusion of privacy. SERS test on saliva samples is promising to be used as a new test technology for health screening and disease diagnosis.

REFERENCES

[1] http://data.unaids.org/Publications/IRC-pub06/epi_update2005_en.pdf (URL)

[2] Fitzmaurice, M; Haka. A. A et al. Raman Spectroscopy: development of clinical applications for breast cancer diagnosis. SPIE, 2005, 1, 5862

[3] Tatiana, A.; Anatoly, G. S.; Vladimir, G. Y.; et al. Positive and negative, drying drops of biological liquids: dynamics of the optical and mechanical properties. Application in rapid medical diagnostics, SPIE, 2005, 188, 5692.

[4] Moskovits, M. Surface-enhanced Raman spectroscopy. Rev. Mod. Phys.1985, 57, 783-826.

[5] Otto, A; Mrozek I; Grabhorn H; Akemann, W. Surface-enhanced Raman scattering. J. Phys. (Condensed Matter) 1992, 4, 1143-1212.

[6] Corset, J; Aubard, J. Surface-enhanced Raman scattering: new trends and applications. J. Raman Spectrosc. (Special Issue) 1999, 29, 22.

[7] Yao, J. L; Xu, X; Wu, DY; Yong X.; Bin R.; Zhong-Qun T.; Gu-Ping P.; Dong-Mei S. and Kuan-Hong X. Electronic properties of metal nanorods probed by surface-enhanced Raman spectroscopy. Chem. Commun. 2000, 22, 1627.

[8] Wang, H H; Liu, C Y; Wu, S B et al. Highly Raman-enhancing substrates based on silver nano particle arrays with tunable sub-10 gaps. Adv. Mater. 2006, 18, 491-495.

[9] Gong, B; Guo, Z; Li, X; et al. A Genetic Algorithm-Support Vector Machine Hybrid for predicting Affection Status of a Sib Pair Based on Genome-wide SNP IBD Profiles. Genet. Anal. Workshop 14 Conf. Trans. 2004, 9, 20-24.