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Vibrational Spectroscopy Using Infrared Raman Microscope for Cytoscreening

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Cytoscreening of normal and cancer cells was performed using an infrared Raman spectroscopy together with data analysis software. The samples used were WFB (normal cell), W31 (cancer cell) and W14 (attenuated virulence cancer cell). Differential Raman spectra of cell samples were analyzed by using principal component analysis and discriminant analysis. Comparing the characteristic results, the increase of protein amide I, the significant decrease of lipid and the decrease of nucleic acid were observed. The accuracy of cell discrimination was around 93% for WFB, W31 and W14.

Keywords H-ras oncogene-transfected fibroblast; Raman spectroscopy; rat fibroblast; vibrational spectroscopy

1. Introduction

The cytoscreening of cancer has been developed widely among pathologists in recent days. In this technique the endoscopic diagnosis is used as a periodic diagnosis for the early stage cancer detection and the targeted location is enucleated partially from the experience and biopsy of the specialists (pathologists). However it takes several days in general for the diagnostic ascertains, because the pathologist have to make histological stain (hematoxylin-eosin staining) and examine specimen with microscope [1]. Unfortunately the numbers of pathologist in Japan is only 2100 so far, and a lot of experience and knowledge are needed when the pathologist does cytoscreening. This leads to a possibility that the diagnosis is different by different pathologist. Therefore it is desired to do cytoscreening not only from biological viewpoint but also from chemical viewpoint with IR Raman spectra. For this reason, the optical technologies using fluorescence microscope and confocal microscope have been developed recently [2].

Raman effect was discovered by Raman *et al.* in 1928. The molecule absorbs incident photons under photoexcitation and re-emits Raman photons. In this process, the transition of energy level occurs and then the frequency shift of emitted photons is observed. This frequency shift is called 'Raman shift'. Since the Raman shift shows a specific spectrum even the excitation wavelength is different, then

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the detection of scattering light leads to the Raman spectroscopy. When the detector is a single channel, the measuring time is very long for Raman scattering detection. Therefore the multichannel detector is desirable for shortening the measuring time.

In the present study, we use a near IR micro Raman system to discriminate specific Raman scattering of normal and cancer cells of WFB, W31 and W14 (see below). Because the sample preparation of these cells the complicated processes such as a cell immobilization are not necessary and the measuring technique is simple, our approach will bring a lot of improvement to the usual biopsy.

2. Materials and Methods

2.1. *Cell Culture*

Cells used were WKA rat normal fibroblast (WFB) and H-ras oncogene-transfected rat fibroblast (W31, W14) in this study. WFB is a normal cell, whereas W31 is a cancer cell and W14 is an attenuated virulence cancer cell [3]. WFB and W31 were cultured in the Dulbecco's modified Eagle's medium (D-MEM, SIGMA-ALD RICH Corporation, UK) supplemented with 10% inactivated fetal bovine serum (FBS), and W14 was cultured in the modified Eagle's medium (MEM, SIGMA-ALD RICH Corporation, UK) in a dish of $\varphi = 100\,\text{mm}$ for 3 days. Each cell was cultured in the atmosphere of CO_2 concentration 5%, humidity 100% and at 37°C. And then WFB and W31 were cultured 1 day in D-MEM and W14 was cultured 1 day in MEM. Cells were scaled with cell scraper and set into centrifuging tube. After centrifugation, cells were rinsed with phosphate buffer solution (PBS). After re-centrifugation cells were scraped up with spatula. Scraped up cells were put on CaF_2 glass of low infrared absorption.

2.2. *Raman Spectra Measurement*

The sample was set on the stage and excited by Nd:YAG laser whose wavelength is 1064 nm. The Raman scattering was detected with a spectroscope (PJ320, HORIBA, Japan) and the measured Raman shift range was $400\,\text{cm}^{-1} \sim 2000\,\text{cm}^{-1}$ in wave number. Photodetector was a multichannel analyzer (OMA V 1024-1.7, Roper Scientific, Germany) using InGaAs (element number is 1024). The measuring time was 4 minutes for each, which is much shorter than the usual measurement. The 30 samples of WFB, W31 and W14 cells for each were measured.

2.3. *Data Analysis*

In this study we used two analysis methods, Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), for the analysis of Raman spectra [4,5]. Analysis software used is 'R'. PCA package used is 'stats' (statistics) and the function is 'prcomp' (principal component analysis). LDA package used is 'MASS' (modern analysis statistics with S) and the function is 'lda' (linear discriminate analysis).

3. Results and Discussion

3.1. *Raman Spectra of the Cells*

Figure 1 shows the Raman spectra of three cells, WFB, W31 and W14, which is averaged for 30 different samples for each cell. The Raman characteristic peaks of amide

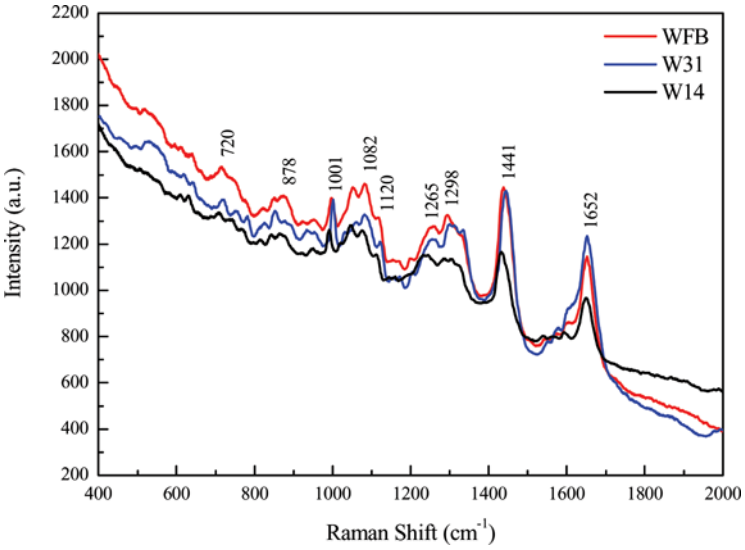

Figure 1. Raman spectra of the cells: WFB (normal cell), W31 and W14 (cancer cells).

I(1652 cm^{-1}) and amide III (1265 cm^{-1}) were detected. The other peaks observed were as follows: tyrosine at 825 and 853 cm^{-1}, phenylalanine at 1001 and 1032 cm^{-1}, and aromatic amino acid at 1173, 1207 and 1612 cm^{-1}, vibration peaks of CH_2 for lipid at 1298 and 1441 cm^{-1}, symmetric expansion and contraction vibrations of phosphoester bond that composes a part of the frame of DNA at 825 and 1082 cm^{-1}, vibration of the frame of DNA including deoxyribose at 878 cm^{-1}.

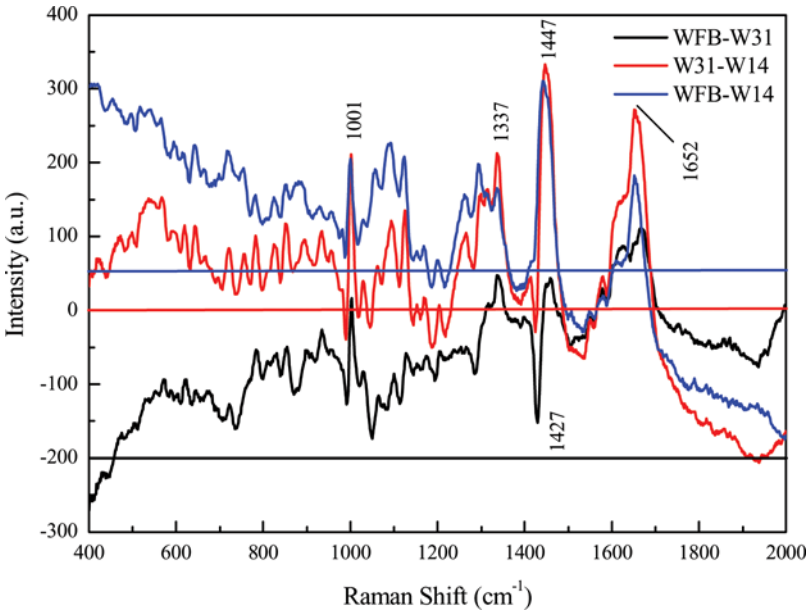

Figure 2. Differential Raman spectra of the cells.

It should be noted that DNA bases have the peaks of adenine (720 cm^{-1}), thymine (753 cm^{-1}), thymine and cytosine (782 cm^{-1}), adenine and guanine (1580 cm^{-1}).

3.2. *Differential Raman Spectra of the Cells*

The differential Raman spectra of the cells were determined by the subtraction of spectra observed for each cell as shown in Figure 2. The substantial change of different cell appeared at 1441 cm^{-1} and 1665 cm^{-1}. The peak at 1441 cm^{-1} is the vibration of CH_2 and CH_3. The peak of 1441 cm^{-1} showed slight shift to the higher frequency side and the lower frequency side [6]. It is suggested that the former appearance is due to the vibration of protein and the latter due to the vibration of lipid. As for the difference between WFB and W14, it could be considered as a decrease of lipid peak. The other lipid peaks observed at 1047 cm^{-1} and 1285 cm^{-1} were also found being decreased.

The peak at 1665 cm^{-1} is assigned to the vibration of amide I of protein, and the differential spectra showed a slight shift to the lower frequency side. In addition, the

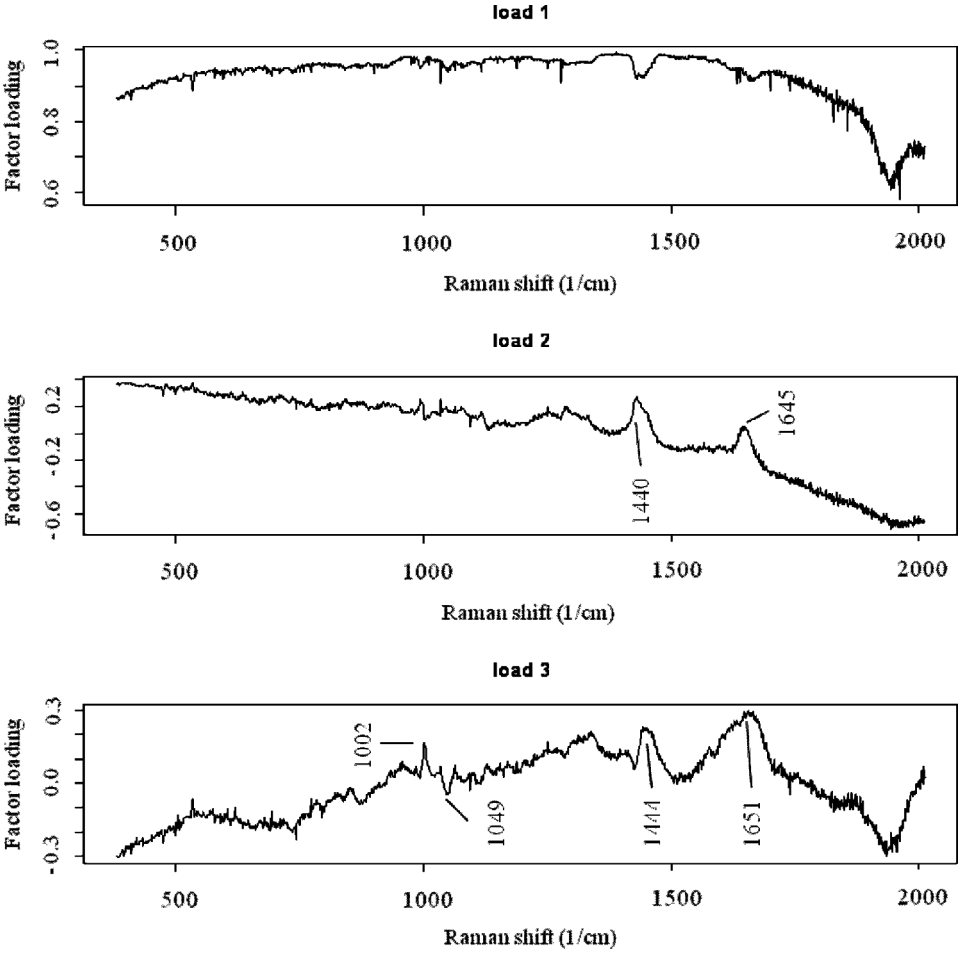

Figure 3. Loading curves of WFB-W31.

peaks of protein at 935 cm^{-1}, 1001 cm^{-1} and 1337 cm^{-1} increased. The decrease of vibrational peaks of framework DNA at 878 $cm^{-1}$and 1082 cm^{-1} for WFB-W31 were observed, whereas the case of WFB-W14 this tendency was not found. Wave numbers of DNA bases (700 cm^{-1} range) showed little change for W31 and W14, that is, the differential spectra is almost unchanged.

3.3. *Principal Component Loadings*

Figures 3–5 show the three loading curves for W31-WFB, W31-W14 and W14-WFB, respectively. It is clearly observed that the peak of aromatic amino acid became smooth, and the vibration of the base of DNA was observed. As for the first principal component, the characteristic peaks of protein amid I, lipid, and amino acid were observed. Since the aromatic amino acid was observable in the second principal component, the peak of lipid is also observed at 1337 cm^{-1}. It seems that the structure of the protein is changed judging from the shift of amid I peak [7]. As for the third principal component, the vibration of base units of DNA was mainly observed.

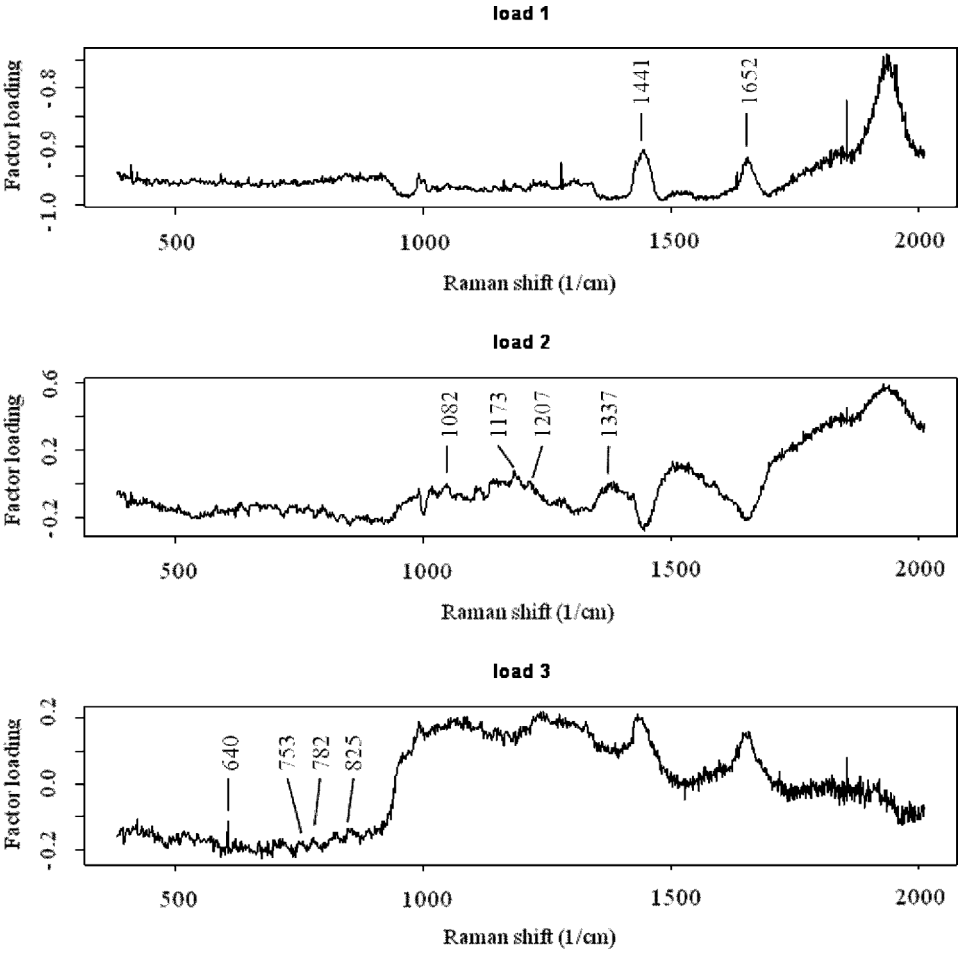

Figure 4. Loading curves of W31-W14.

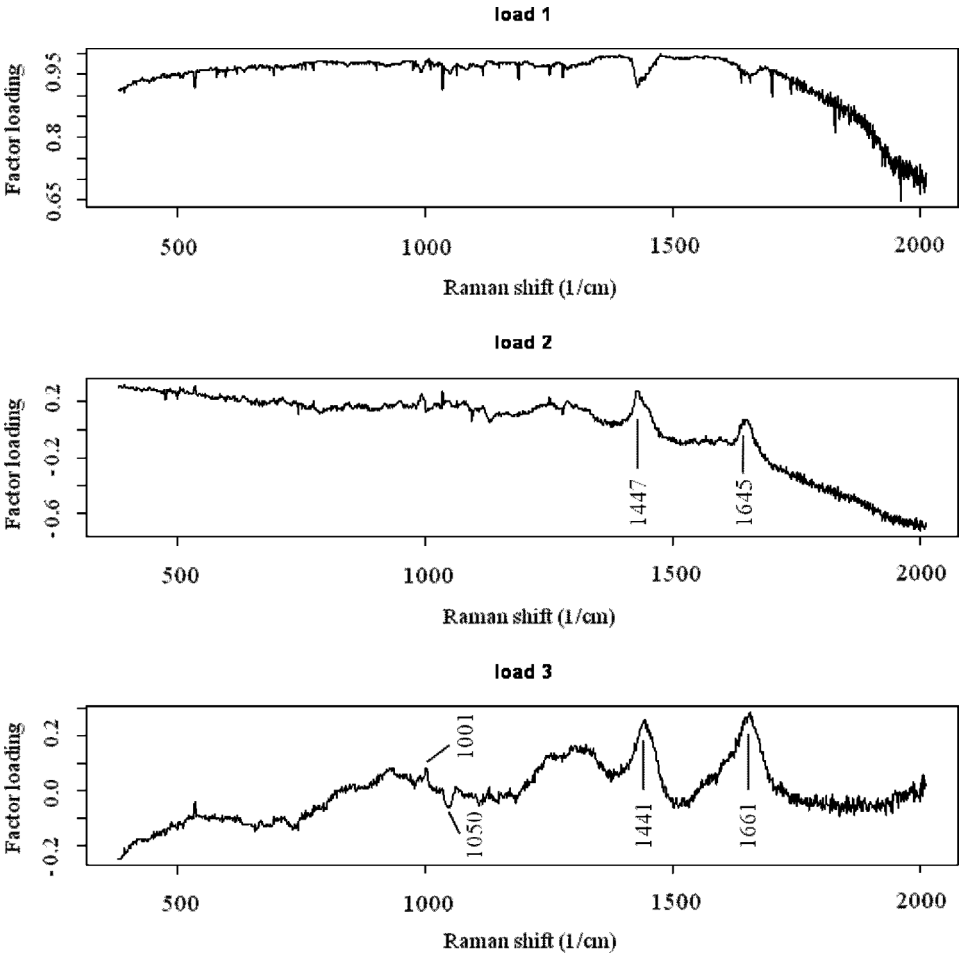

Figure 5. Loading curves of WFB-W14.

3.4. *Discrimination of Cells by PCA and LDA Analysis*

From the PCA and LDA analysis of differential Raman spectra for cells we could determine the type of cell (WFB, W31 or W14) as shown in Table 1. In W31 and WFB group, the 28 of 30 W31 cell which confirmed pathologically were assigned as W31 (sensitivity 93%) and 28 of 30 WFB cell as WFB (specificity 93%). In W31 and W14 group, the 29 of 30 W31 cell confirmed pathologically were assigned as W31 (sensitivity 97%) and the 30 of 30 W14 cell as W14 (specificity 100%). In W14 and WFB group, the 28 of 30 W14 cell which confirmed pathologically were assigned

Table 1. Discrimination of cells

	WFB	W31		W31	W14		WFB	W14
Positive	28	28	Positive	29	30	Positive	30	28
Negative	2	2	Negative	1	0	Negative	0	2

as W14 (sensitivity 93%) and the 30 of 30 WFB cell as WFB (specificity 100%). These results suggest that our analytical method using PCA and LDA is quite useful to discriminate normal cell WFB and cancer cells W31 and W14 clearly.

4. Conclusion

We have demonstrated the discrimination of normal and cancer cells using the infrared Raman spectroscopy together with the analytical software of PCA and LDA. A certain difference was successfully confirmed from the differential Raman spectra of WFB, W31 and W14 in the wave number region of protein and lipid. Even though there remains some extent of ambiguity for the discrimination either normal or cancer cells, we believe this method is simple and available for cytoscreening of cancer cells in future.

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