# Micro-Raman spectroscopy of single leukemic cells

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The Raman spectra from leukemic cell line (HL60) and normal human peripheral blood mononuclear cells (PBMCs) are obtained by confocal micro-Raman spectroscopy using near-infrared laser (785 nm) excitation. The scanning range is from 500 to 2000 $cm^{-1}$. The two average Raman spectra of normal PBMCs and carcinoma cells have clear differences because their structure and amount of nucleic acid, protein, and other major molecules are changed. The spectra are also compared and analyzed by principal component analysis (PCA) to demonstrate the two distinct clusters of normal and transformed cells. The sensitivity of this technique for identifying transformed cells is 100%.

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Raman spectroscopy technique can be used to detect substance changes of structure and composition (DNA, protein, nucleic acid, and lipid)[1] at the molecular level, and can differentiate between samples on the basis of the detectable changes in spectral shape or intensity[2,3]. Recently, this technique has emerged as a potential non-destructive diagnostic tool for many kinds of cancer detection, such as breast cancer, colon cancer, esophageal carcinoma, prostate cancer, and stomach cancer[4–6]. However, leukemia, a cancer of blood or bone marrow, has seldom been studied on this aspect up to now. In this study, we demonstrate the use of confocal Raman micro-spectroscopy to obtain the Raman spectra of leukemic cell line (HL60) and normal human peripheral blood mononuclear cells (PBMCs) using near-infrared laser (785 nm) excitation, and analyze the characteristic spectral line from two types of cells with principal component analysis (PCA). Compared to histopathological examination of conventional diagnosis requiring blood or bone marrow tests, confocal Raman micro-spectroscopy is expected to become a new diagnosis method which is direct, rapid, and noninvasive.

It has been reported that for the rat pulmonary epithelial cells at 785-nm laser excitation, no damage was induced to the cells even after 40 min irradiation at 115-mW power[7,8]. Hence, we choose a 785-nm laser with 20-mW output power and 30-s irradiation time for measuring the cells. An entire Raman spectrum can be obtained from an individual living cell with 4-min integration time using a 50× objective. The scanning range was from 500 to 2000 $cm^{-1}$ and the resolution was 2 $cm^{-1}$. Peak frequency calibration and rapid checking of instrumental performance were performed with the silicon phonon line at 520 $cm^{-1}$. Figure 1 shows the average Raman spectra of two cell types, normal PBMCs which were isolated from a volunteer's blood by Ficoll-Hypaque density gradient of heparinized venous blood, and transformed HL60 cells. Each spectrum represents an average of measurements from 12 normal PBMCs, 20 Raman spectra, and 20 HL60 cells, 30 Raman spectra. Prior to Raman spectra analysis, the cells were washed and suspended in phosphate buffered saline. Each Raman spectrum was background-subtracted by polynomial fitting and normalized to the area underneath the curve to enable direct comparison of the data using Origin software. The two spectra exhibit similar peak structures. The peaks at 781, 831, and 1092 $cm^{-1}$ are vested in the mode of the symmetric $PO_2$ stretching vibration of the DNA, and some peaks are attributed to protein vibrations, such as the phenylalanine mode at 1003 $cm^{-1}$. The amide III region overlapping with $CH_2$ deformation mode is at $1302-1337$ $cm^{-1}$, similarly, the amide I band is at 1658 $cm^{-1}$ and the $CH_2$ deformation mode is at 1449 $cm^{-1}$, because the main components of cells are DNA and protein.

We need to confirm that the differences of any spectra between normal PBMCs and HL60 are not attributed to the different regions in which the signals were acquired. Different locations (all near the nucleolus) within a single cell are probed. Figure 2 shows the three reproducible Raman spectra from different locations. As the Raman spectra indicated, from different regions in the same cell, the primary peaks such as 1657, 1449, $1302-1337$, and 1003 $cm^{-1}$ had no major variations in the spectral shape and intensity. But there was some minute difference in some details, which is owing to a little difference of the structure and the amount of some components from different regions.

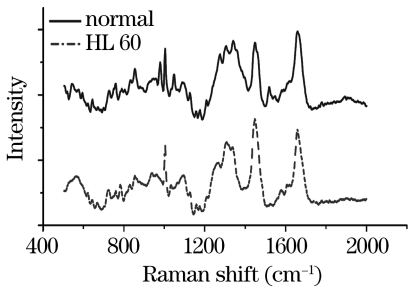


Fig. 1. Average Raman spectra of individual leukemic cell line (HL60) and normal human mononuclear cells.

 

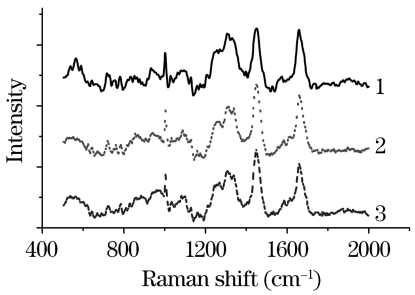


Fig. 2. Raman spectra obtained from the different locations within the HL60 cell.

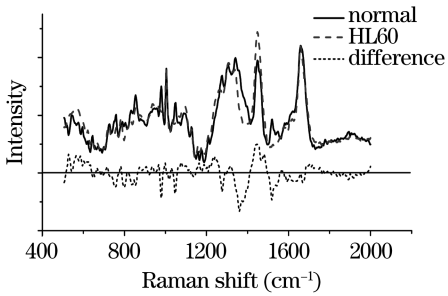


Fig. 3. Average Raman spectra obtained from normal and HL60 cells. The difference spectrum is also given.

As shown in Fig. 3, overall the two average spectra exhibit similar peak structures and locations, but the intensities of the peak at 980 $cm^{-1}$ assigned to deoxyribose of DNA and the peak at 1520 $cm^{-1}$ considered as A of DNA become significantly weaker in transformed cells. The intensities of 781 and 1092 $cm^{-1}$ modes of the symmetric $PO_2$ stretching vibration of the DNA backbone are significantly stronger in transformed cells. It is owing to the changes of the structure and the amount of nucleic acid, protein, and other major molecules of DNA in transformed cells. The amide I of protein at 1660 $cm^{-1}$ shifts slightly to lower wave numbers (1657 $cm^{-1}$) in the transformed cells. Comparing the intensity of the amide III of protein at 1305 $cm^{-1}$ with the C–H deformation vibration at 1337 $cm^{-1}$, we found that the one in the transformed cells is stronger. But the 1274 $cm^{-1}$ peak also assigned to amide III and the 758 $cm^{-1}$ peak to tryptophan become weaker in the transformed cells. It is not entirely clear what causes these changes in the transformed cells, but it appears plausible that this could be due to a somewhat different protein composition for the majority of proteins in transformed cells. The detailed spectral assignments are shown in Table 1.

**Table 1. Peak Positions and Assignment of Raman Spectra**[9−12]

| Peak Positions ($cm^{-1}$) | Assignment |
|---|---|
| 642 | P: C-S Stretch; Tyrosine: C–C Twist |
| 725 | A,P: C–S |
| 758 | T,P: Tryptophan |
| 781 | DNA: O–P–O |
| 831 | O–P–O Asymmetric Stretch, Tyrosine |
| 854 | Tyrosine, Ring Breath |
| 938 | P: C–C Stretch Backbone |
| 980 | DNA: Deoxyribose |
| 1003/1004 | Symmetric Ring Breathing Mode of Phe |
| 1032 | Phe, P: C–N Stretch |
| 1048 | Glycogen: C–O, C–C |
| 1092 | DNA: O–P–O, P: C–N |
| 1127 | P: C–N Stretch |
| 1156 | P: C–C, C–N Stretch |
| 1174 | Tyr, Phe, P: C–H Bend |
| 1274 | P: Amide III |
| 1305 | P: Amide III |
| 1337 | A, G, P: C–H Def. |
| 1449 | P: Deformation Mode |
| 1520 | A |
| 1660/1657 | P: Amide |

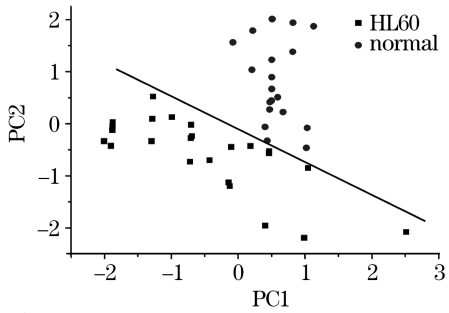


Fig. 4. PCA of all individual spectra of normal PBMCs and leukemic cell line (HL60).

PCA was used to reduce the large amount of spectral information contained in the Raman spectra into $2-3$ important parameters (principal components). A scatter plot generated from this data shows clusters of points representing different cell groups. We compare the normal and HL60 cells using only the first and the second principal component values, as shown in Fig. 4. It can be seen that the spectra could be clustered into normal and tumor groups using a solid line that represents potential diagnostic algorithms. The sensitivity of this technique for identifying transformed cells is calculated to be 100%.

In conclusion, Raman spectroscopy is a noninvasive method for analyzing molecular composition in biological samples. In this work, normal human mononuclear cells and transformed cells are well identified and classified by Raman spectroscopy technique and PCA. The results show that normal and transformed cells are separated into distinct clusters. The sensitivity of this technique for identifying transformed cells is calculated to be 100%. Hence, Raman spectroscopy as a potential clinical diagnosis of leukemia is perspective in rapidity and accuracy based on their inner biochemical signatures.

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