RAMAN SPECTROSCOPY OF MAMMALIAN CELLS

ABSTRACT

A procedure for distinguishing tumor cells from corresponding normal cells by virtue of their Raman spectra is being investigated. There are many problems associated with Raman spectroscopy of cells suspended in a liquid medium. Among these problems are fluorescence of the medium, large Rayleigh scattered light, and accompanying time dependent intensity fluctuations of the scattered light due to cell settling. These effects tend to mask the Raman scattered light from the cells. Fluorescence can be avoided by using a simple transparent medium, and cell movement can be eliminated by suspension of the cells in agarose. A relatively simple filter for reducing the Rayleigh scattered light can be made from sodium vapor when using a dye laser as the exciting source.

Results of experiments performed on various biological samples including normal and transformed mammalian cells will be presented.

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SUMMARY

A number of investigators have reported[1-3] that significant differences exist between the Raman spectra of normal and tumor cells. Such differences may provide a new method for early detection of various types of cancer. Little or no detail has been given in the literature relating to the experimental conditions for the reported results. This paper presents problems associated with Raman scattering from biological materials and results of continuing experiments pertaining to the development of a consistent method to obtain Raman spectra of mammalian cells.

Experimental Method

Figure 1 shows schematically the experimental setup. The

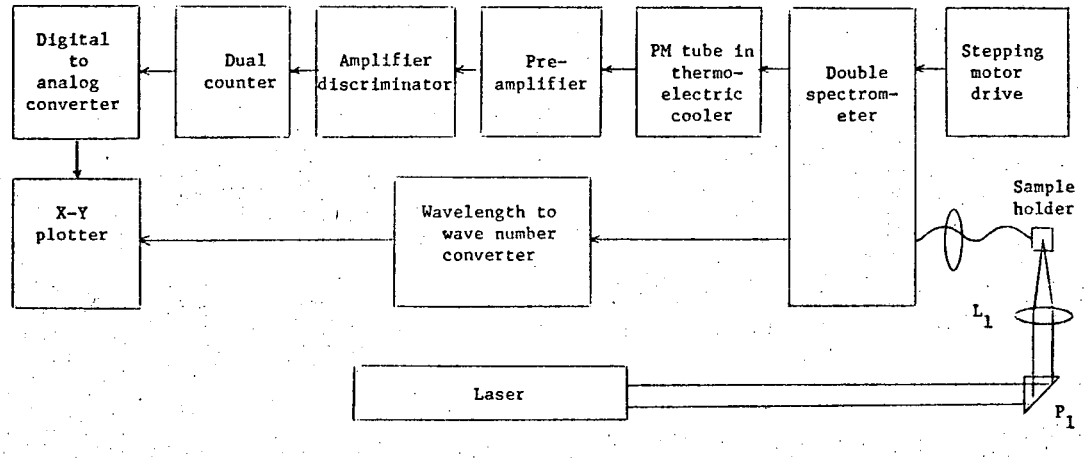

Fig. 1. Diagram of the experimental setup for Raman spectroscopy of cells.

exciting laser can be an argon ion (Spectra Physics 171), krypton ion (Spectra Physics 165), or a continuously tunable dye laser (Coherent Radiation 490), depending on the frequency of interest. A prism P_1 together with a lens L_1 of focal length 37 mm were used to focus the beam on the center of the sample holder (quartz cuvettes of base dimensions 1 cm x 1 cm and height of 2 cm). Lens L_2 (a Nikor 52 mm focal length f 1.2 lens) forms an image of the

scattered light on the entrance slit of a double spectrometer (Spex 1402). A photomultiplier tube (RCA C31034-02) housed in a thermoelectric cooler at -30°C to reduce dark count and photon counting electronic processing equipment (Ortec models, preamplifier 9301, amplifier discriminator 9302, photon counter model 9315, digital-to-analog counter 9325) measured the scattered photon density. The spectra were recorded by using an X-Y plotter.

Results and Discussion

Raman spectra obtained with the previously described system using water, benzyne, egg white, and ovalbumin agree with published data[4,5] and serve as a check for the system. Reproducible spectra were also achieved from experiments performed on samples of RNA and DNA. The anslysis was then extended to spectroscopy of BHK cells suspended in liquid media.

The necessary control experiments were carried out using the suspension media. A frequency scan of a growth medium, Eagle's minimal essential medium (MEM), was carried out. It was observed that MEM fluoresces in a broad frequency range. Other biological media were also studied to find a nonfluorescent nutritious medium. Spectra taken of phosphate buffered saline solution (PBS) showed no indication of fluorescence and therefore proved to be suitable for Raman spectroscopy. Another problem associated with spectroscopy of cells suspended in liquid media was the variation in intensity of the scattered light due to settling of the cells. The results of time scan and frequency scanning experiments that we have done on BHK cells suspended in PBS have shown that the time dependent intensity variations are so significant that they can produce the appearance of false lines. To overcome this problem we have experimented with cells suspended in agarose (a common polysaccharide solidifying agent). Since cells are effectively immobilized, there is no significant time dependent intensity variation. Furthermore, agarose shows no fluorescence in the region of interest, and we have been able to obtain consistent spectra. It therefore appears that by suspending cells in agarose, it should be possible to obtain reproducible Raman spectra of the cells.

In Raman spectroscopy of cells, it is essential[1-3] to keep the cells healthy and viable during the complete period the spectra are being recorded. It is an objective of this research to develop a medium that supports a long-time survival of cells with continuing metabolic activities.

The Rayleigh scattered light is particularly troublesome at frequencies closer to laser line. There are several ways that can be reduced. A system similar to that developed by Kirby and Duffey[6] is being incorporated. This system uses a filter consisting of sodium vapor between the lens L_2 and the spectrometer. The exciting dye laser is tuned to one of the sodium D lines and a simple etalon is used to reduce the number of cavity modes. The sodium vapor will

absorb the elastically scattered light, with a corresponding reduction in the Rayleigh scattered light. Preliminary spectra that we have obtained from scans of BHK cells suspended in agarose indicate presence of possible Raman lines in the low frequency range (lower than 200 cm^{-1}). It is necessary to filter the Rayleigh background to enhance the lines. After reproducible methods for cell spectroscopy have been obtained, Raman spectra of normal cells and corresponding tumor cells will be run, and these results together with our spectra of egg white, ovalbumin, RNA, and DNA will be presented at the meeting.

References

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