L.13: Use of Raman optical tweezers for cell cycle analysis

Flow cytometry (FCM) has become an indispensable tool for understanding the complex cellular processes and is widely used for the analysis of cell cycle, cell-drug interactions, toxicology and cell sorting. However, FCM requires labelling of cells with suitable fluorescent labels which can affect their functionality. Therefore, development of Raman flow cytometers where cells can be analysed in label free manner based on their Raman finger prints and would remain functional after analysis is a topic of much current interest. Further, combining optical tweezers with Raman spectroscopy provides the advantage that a cell can be trapped and Raman interrogated in a flowing suspension and translated into a desired sorting channel after a decision has been made on the basis of its Raman signatures. One important application of a cytometer is the analysis of the distribution of cells across the cell cycle. This can be done by Raman spectroscopic measurement of the DNA content in single cells. Previous attempts to measure DNA content using Raman spectroscopy have suggested the need of physical isolation or spectral demarcation of nucleus from the cytoplasm. As the nucleus, being the optically densest part, gets localized in the trap and hence dominates the Raman spectrum in Raman tweezers therefore at Laser Biomedical Applications & Instrumentation Division, we have analysed the use of Raman optical tweezers for cell cycle analysis.

For these experiments, human colon carcinoma (colo-205) cells were synchronized in G0/G1 and G2/M phases using standard protocols. The single beam (785 nm) Raman tweezers was used to trap the cells and record their Raman spectrum. A fraction of these cells was stained with nucleus labelling dye DAPI and was also subjected to fluorescence microscopy. This was done to determine the synchronization level of the cell populations using conventional fluorescence measurement as well as to investigate the correlation, if any, between the Raman and fluorescence measurements on single cell basis. The Raman spectra of the cells from synchronized cell populations are shown in Fig.L.13.1(A). It can be seen that the DNA Raman band at 783 cm^{-1} gets enhanced in case of G2/M phase cells when compared to the G0/G1 phase cells. Figure L.13.1(B) shows the histograms for the distribution of intensity of band at 783 cm^{-1} for the cell populations in G0/G1 and G2/M phase. It can be seen that the histogram for cells in G2/M phase cells peaks at approximately double value of I_{783cm}^{-1} as compared to the histogram for the cells in G0/G1 phase. Further, it may be noted that the other prominent nucleic acids bands at 1095 and 1575 cm^{-1} also gets enhanced but overlaps with nearby Raman bands from lipids and proteins peaks making it difficult to correctly estimate the contribution of DNA to its intensity. Therefore, the band at 783 cm^{-1} appears to be a better choice for the determination of the DNA content in the cell focal volume.

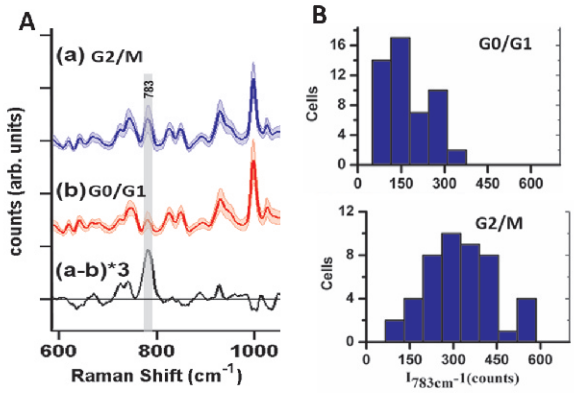

Fig. L.13.1: (A)) The mean Raman spectra of 45 cells each from cell populations synchronized in G0/G1 and G2/M phases, acquired using Raman optical tweezers. Thick lines show the mean spectra and the shaded area represents the standard deviations. (B) Histograms of the intensity of DNA band at 783 cm^{-1} for these cells.

To check the correlation between the Raman and the fluorescence measurement on single cell basis, the Pearson correlation coefficient between the intensity of the Raman band at 783 cm^{-1} and the DAPI fluorescence integrated over the cell nuclei was calculated and found to have a value of ~ 0.62. It may be noted that the Raman probe beam focal volume was ~ 1x1x3 μm^3 and thus the I_{783cm}^{-1} represented the DNA content in this fractional volume of cell nucleus probed by the Raman excitation beam. In contrast, the integrated fluorescence represents the entire volume of the nucleus. The lower value of the correlation coefficient may therefore be because of the fact that not only the amount of DNA but also its arrangement in the nucleus undergoes change during different phases of the cell cycle. An improved correlation is to be expected if the Raman beam focal volume is large enough to overlap with the entire volume of the cell nucleus.

To validate the use of 783 cm^{-1} peak for label free determination of DNA content in cells, Raman spectra was also recorded from live-unstained cells from both the G0/G1 and G2/M phase populations. The results obtained were similar to those shown in Fig. L.13.1. These results confirm the suitability of the technique for cell cycle analysis in a label free manner without the need for isolation of cell nucleus. For more details, please refer to *S. Ahlawat et al., Analyst, **141**, 1339 (2016).*

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