Investigation of Live White Blood Cells using Raman Tweezers spectroscopy

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Introduction

- WBC are classified into Granulocytes (Neutrophils, Basophils, Eosinophils), Lymphocytes (B-cells, T-cells, NK cells) and Monocytes (Macrophages, Dendritic cells).
- Live cell spectroscopy of these cells are performed by using Raman Tweezers technique in which cells are trapped by optical tweezers.
- Optical Tweezers is based on optical-radiation pressure due to different optical forces acting on the cell (Scattering force and Gradient force).
- Using focused laser beams, Arther Ashkin has manipulated particles ranging in size from atoms to cells and their components.
- Raman Tweezers couples optical trapping along with Raman spectroscopy has been explored for studying the spectral features of optically immobilized, single, live white blood cells (WBCs).

Objective

Classification of different types of WBCs using Raman tweezers spectroscopy technique.

Experimental/Methodology

- The separation of WBCs is performed by centrifuging the whole blood (about 2 ml) at 3000 rpm for 5 min at room temperature.
- The buffer solution used for suspending WBCs is isotonic solution (Normal Saline Solution – 0.9%)
- An inverted microscope with a 100X oil immersion microscope objective was used to trap the cell.
- The laser power - 11 mW.
- The exposure time- 60s.
- Number of accumulations- 2
- A CCD camera was used to capture the microscopic images of the cells.

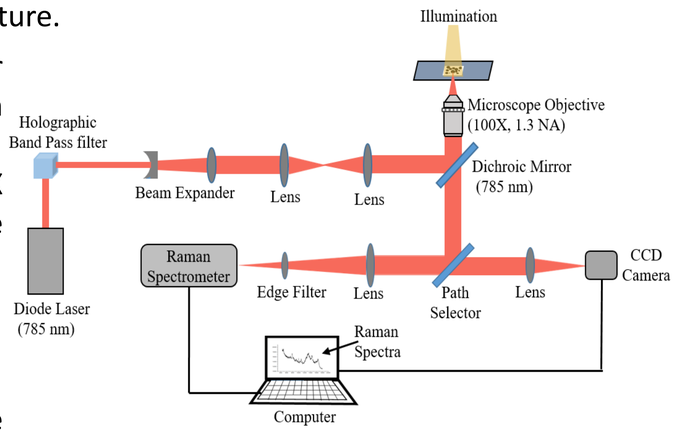

Fig 1. Schematic of Raman Tweezers experimental setup

Results and Discussion

Fig.2 : Microscopic Image of Neutrophil

Fig..3 : Microscopic Image of Lymphocyte

Fig.4: Microscopic Image of Monocyte

Fig.5 : Raman spectrum of an optically trapped Neutrophil

Fig.6 : Raman spectrum of an optically trapped Lymphocyte

Fig.7 : Raman spectrum of an optically trapped Monocyte

Results and Discussion

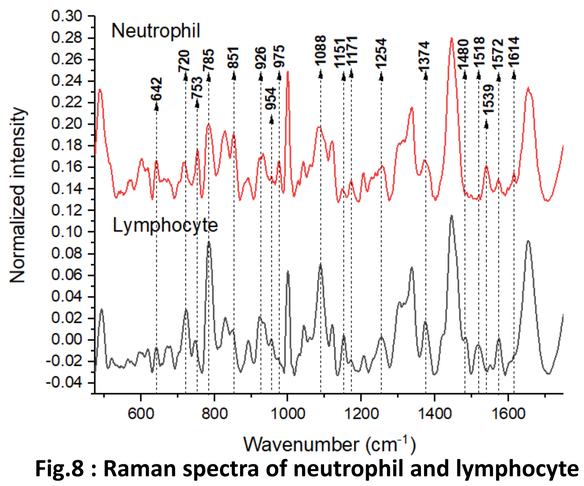

Fig.8 : Raman spectra of neutrophil and lymphocyte showing the peak differences

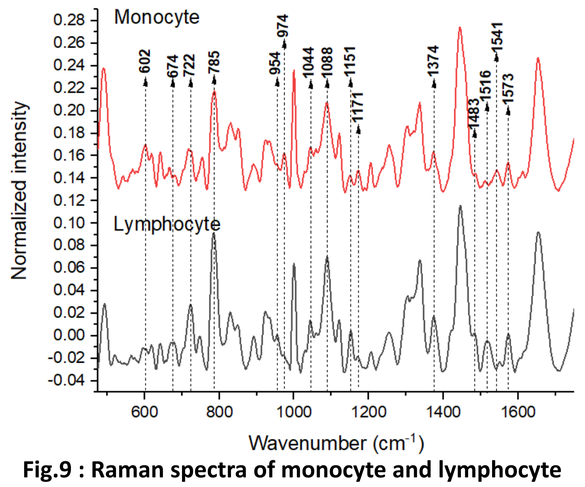

Fig.9 : Raman spectra of monocyte and lymphocyte with Raman signature showing cell differences.

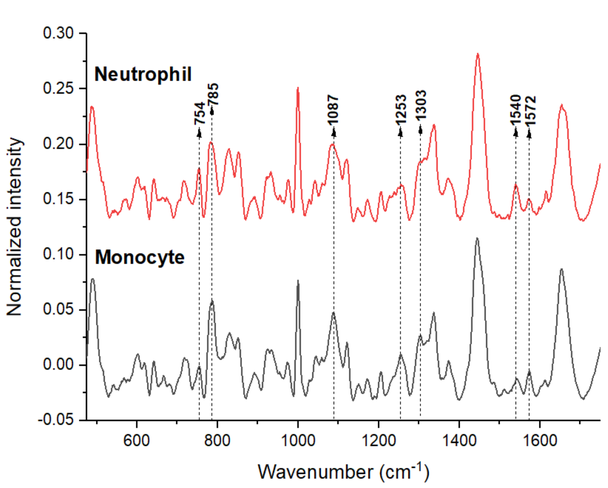

Fig.10 : Raman spectral differences of Neutrophil and Monocyte

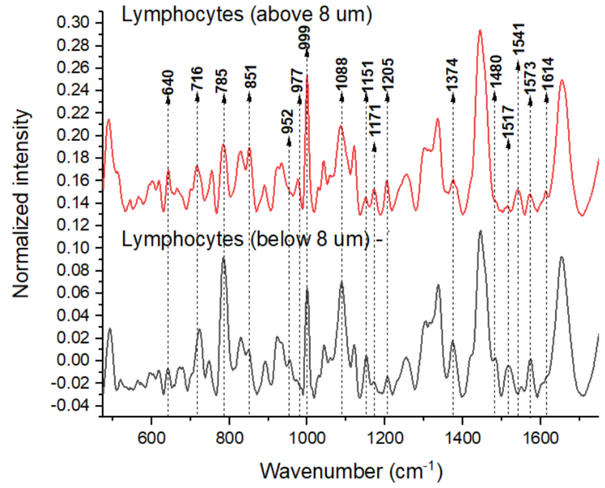

Fig.11 : Raman spectra of lymphocyte (above 8 µm) and lymphocyte (below 8 µm) with Raman peaks

Conclusions

- The average diameter of Monocyte is found to be 9.73 µm, while that of Lymphocyte (below 8 um) is 6.95 µm and Lymphocyte (above 8 um) is 9.24 µm. Neutrophil has the average diameter of 9.97 µm.
- Significant variations were evident in spectral features of nucleic acids and proteins amongst three different classes of white blood cells – Lymphocytes, Monocytes and Granulocytes.
- An enhancement in the Raman band at 974 cm^{-1} resulting from deoxyribose was evident for Granulocytes in comparison with Lymphocytes.
- Raman peaks corresponding to nucleic acid are more intense in Lymphocyte spectrum, whereas in neutrophil, protein peaks are more intense.
- Peak at 1483 cm^{-1} due to Adenine, Guanine and CH deformation, has more intensity in Lymphocytes followed by Monocyte and with almost negligible peak in Neutrophil.

References

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