PhD Thesis Title: Exploring the viability of vibrational spectroscopy for diagnosing cancer in human surgical samples

Author: Gourav Kumar Jain
Email: gourav108@gmail.com
Institution: SMS Medical College and Hospital, Jaipur-302004 Ind**ia**
Supervisor: Prof. Arun Chougule
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ABSTRACT:

Cancer is the most frequently diagnosed non-communicable disease resulting in high mortality worldwide. Despite significant scientific advances in cancer detection methodologies, there is a need for non-destructive and rapid cancer detection methods for the population. Label-free bio sensing methods have made enormous progress in recent years due to their ability for rapid and inexpensive bio-detection in small reaction volumes. The basis of cancer detection by Raman spectroscopy (RS) is the theory directly related to molecular vibrations based on the presence of molecular concentration in the biological specimen. RS is a non-destructive, versatile and label free technique and these features are desirable for cancer diagnostics. The present work focuses on identifying the features and parameters of RS for the detection of cancer in human breast surgical samples.

The biological samples were obtained from the modified radical mastectomy (MRM) surgical procedure. Malignant tissue sections were obtained from the gross tumour volume. The normal tissue sections (control samples) were obtained from 2 to 3 cm away from the gross tumour volume with a safe clean margin of the breast tissue resection during the MRM surgical procedure. Confocal spontaneous RS in the reflection mode is performed using an incident excitation laser monochromatic beam of 532 nm. The specimen was focused through a 20x dry objective and a 4.0–2.9 mm operating distance. The laser beam of 532 nm with laser power 5mW was utilized to record the Raman spectra with an integration time of 10x3 s. The notch filter was employed to remove the Rayleigh's scattered light. After conformal focus, the RS was performed three consecutive times for the biological sample and the average spectra were obtained to reduce uncertainty in the measurements.

In conclusion, the average Raman spectra of normal and cancerous tissues demonstrated significant spectral differences, and the intensity ratio of the Raman biomarkers in the Raman fingerprint region range 1000–1637 cm−1 provided pivotal information for the detection of breast cancers. The results of the present work suggest that the tumour vascularity kinetics associated with the tutor proliferation shall be measured with the haemoglobin peak at 1587 cm−1. Moreover, the peak has a major contribution from vibrations in the haemoglobin originating from the blood vessels. It acts as a key and effective Raman marker for the identification of the phosphorylation status in the rapidly dividing nature of cancer cells. The differences observed among Raman profiles of cancerous tissues are more prominent compared with normal breast tissues. Notable spectral differences are present in both the absolute and relative intensities of the Raman peaks in the spectral profile. After collecting and analyzing all the spectroscopic data, this chapter formulates those three ratios of Raman intensities 1587 cm−1/ 1637 cm−1, 1587 cm−1/ 1000 cm−1 and 1587 cm−1/ 1375 cm−1 with statistically reliable differences were reported. Noteworthy, it is found to be sensitive enough to differentiate between normal and cancerous breast tissues. Certainly, RS presents strong evidence for the detection of cancer and potential to enter into clinical practice.

References to author publications that relate specifically to the dissertation:

1. **Jain GK**, Verma R, Chougule A, Singh B. Raman spectroscopy study of healthy and cancerous human breast tissue for cancer detection. ***Explor Anim Med Res***. 2022;12(2):160-166. DOI: 10.52635/eamr/12.2.160-166

2. 1**Jain GK**, Verma R, Chougule A, Singh B. Bioethical education and standardization of sample handling procedures in Raman spectroscopy research studies involving human subjects. ***Indian J Sci Technol***. 2022;15(24):1187-1194. DOI: 10.17485/IJST/v15i24.1016
3. **Jain GK**, Verma R, Chougule A, Singh B. Scientific advances in cancer detection using Raman spectroscopy. ***Asian Pac J Cancer Prev***. 2024;25(11):3977-3986. DOI: 10.31557/APJCP.2024.25.11.3977