L.2 : Raman spectroscopy for *in vivo* diagnosis of oral neoplasia

It is now well recognised that optical spectroscopy can play a very important role in early non-invasive diagnosis of the cancer of oral cavity. While fluorescence spectroscopy, diffuse reflectance spectroscopy, or a combination of these, has provided very encouraging results, the use of Raman spectroscopy for clinical diagnosis was limited by the necessity of large data collection time owing to the weakness of the Raman signal. With continued improvements in detectors and spectroscopic systems, it has now become possible to acquire reasonable Raman spectrum of human tissue with clinically acceptable data collection time. Laser Bio-medical Applications and Instrumentation Division of RRCAT has assembled a near-infrared Raman spectroscopy setup for studies on human tissue and evaluation of its use for the diagnosis of the cancer of oral cavity. These studies are expected to compliment and advance the ongoing studies on the use of fluorescence spectroscopy for the same purpose.

A schematic of the Raman system is shown in Fig.L.2.1. The portable system utilizes a 785 nm diode laser coupled to a fiber-optic Raman probe that delivers the laser light onto the tissue. The Raman scattered light collected by the same probe is filtered through an in-line notch filter and is then fed into a spectrograph where it is dispersed onto a thermoelectrically cooled, back-illuminated, deep-depletion CCD camera controlled by a laptop computer for recording the signal. After detailed characterization of the system by recording Raman spectra from various known Raman standards like naphthalene, acetaminophen, glucose etc., it was used for acquiring spectra from *ex-vivo* tissue samples. Integration time of 2-5 seconds, considered clinically acceptable, was found to generate good quality tissue Raman spectra.

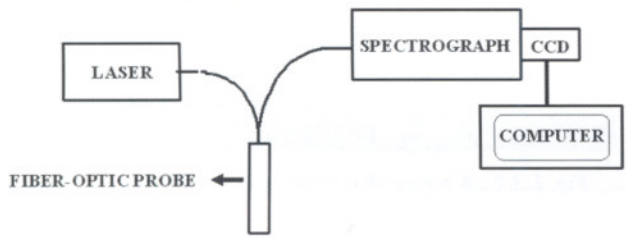

Fig. L.2.1: Schematic of the experimental set-up for measuring in vivo tissue Raman spectra.

NIR Raman spectra were collected from 36 normal volunteers and 20 patients undergoing routine examination of oral cavity at the Out Patient Department of Modern Dental College, Indore and Government Cancer Hospital, Indore. For each patient, spectral measurements were taken from multiple sites of abnormal as well as apparently uninvolved contralateral regions of the oral cavity. The different tissue sites investigated belonged to either of the three histopathologic categories: 1) squamous cell carcinoma, 2) sub-mucosal fibrosis, and 3) normal squamous tissue. A probability based multi-class diagnostic algorithm, was developed to analyse and quantify the diagnostic content of the Raman spectra corresponding to these different oral tissue sites. It was found that using the algorithm it was possible to distinguish neoplastic from normal oral tissue sites, based on their Raman spectra, with a predictive accuracy of more than 90% with respect to histology as the gold standard. Fig. L.2.2 shows the predicted posterior probability of belonging to a tissue category for all the oral tissue sites investigated. These probability values were computed by the multi-class diagnostic algorithm in a leave-one-out cross validation mode and were based on the NIR Raman spectra acquired from these tissue sites whose histopathologic class labels were already known.

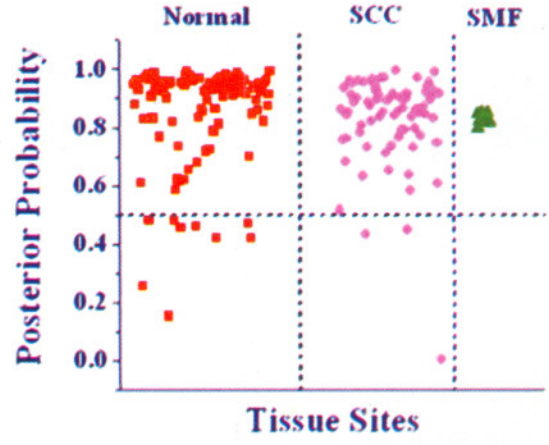

Fig. L.2.2: Predicted posterior probability of being classified to normal, submucosal fibrosis and squamous cell carcinoma for all the investigated oral tissue sites. The probability values were based on the NIR Raman spectra acquired from these tissue sites whose histopathologic class labels were known.

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