L.10: Extraction of tissue Raman spectra from the raw fluorescence background

Raman spectroscopy due to its high sensitivity to subtle biochemical changes as well as amenability to non-invasive applications has tremendous potential for differential tissue diagnosis. However, successful use of Raman spectroscopy for all tissue applications requires various data-processing steps to be followed to extract rather weak tissue Raman signals from the measured raw spectra. These include system calibration, fluorescence background subtraction, and noise smoothing. Perhaps the most challenging of these is the problem of orders of magnitude stronger background fluorescence, the primary reason why most researchers in the field have moved to the near-infrared (NIR) wavelengths for excitation.

Over the years a variety of methods of varying rigor have been proposed for extraction of Raman signal by minimizing the fluorescence background. Though each of these methods has been shown to be useful in certain situations, polynomial fitting, particularly modified iterative polynomial fitting, is considered superior to the rest of the methods due to its ability to preserve to a large extent the spectral contours and intensities of the input Raman spectra. Although the method has been shown to be successful for effective background reduction and is extensively used by the Raman community dealing with tissue diagnostic applications, it has one major shortcoming. The method is sensitive to the choice of the spectral region to be used in the fit thus leading to Raman spectra of significantly different lineshapes and intensities for different start and stop wavenumber settings. This effect is the most prominent if the chosen spectral region happens to be close to the cut-on of the Rayleigh filter or contains significant dip or hump caused by several factors like the spectral response of the system. At Laser Biomedical Applications and Instrumentation Division (LBAID) of RRCAT, a method has been developed for fluorescence background removal that overcomes the shortcomings of the modified polynomial method and results in faithful representation of Raman spectrum.

The method is based on iterative smoothing of the measured Raman spectrum. The central idea of this approach is to iteratively smooth the raw Raman spectrum, by using moving average of the spectral data, such that Raman peaks are automatically eliminated, leaving only the baseline fluorescence, to be subtracted from the raw spectrum. The scheme allows retrieval of all Raman peaks and shows good range independence.

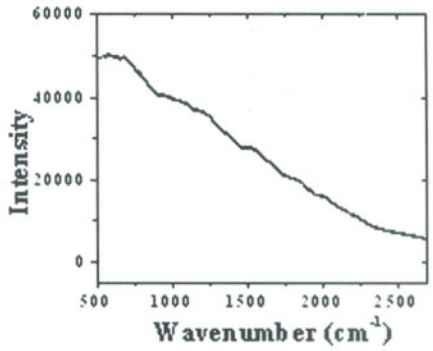

Fig.L.10.1 Raw NIR Raman spectrum of normal squamous tissue of human oral cavity.

Fig.L.10.1 shows a raw tissue Raman spectrum measured with 785nm excitation from a normal squamous tissue site of human oral cavity. Fig.L.10.2(a) demonstrates the Raman signatures, retrieved using the iterative smoothing method, from the raw Raman spectrum shown in Fig.L.10.1. Two different spectral ranges were chosen over which the Raman signal was extracted. Range-1 corresponds to 900-2700 cm^{-1} and Range-2 corresponds to 500-2400cm^{-1} respectively. For comparison sake, the same raw Raman spectrum was also subjected to the modified polynomial-based background removal method. The extracted spectra are shown in Fig.L.10.2(b). It's clear from the figures that when the modified polynomial fit method was used to extract Raman spectra by fitting over different spectral ranges, it led to significant changes in the spectral contours and peak intensities. In contrast, our iterative smoothing method when applied on the same raw spectrum of human oral tissue over the two different spectral ranges, led to Raman spectra (Fig.L.10.2(a)) of identical spectral contours and intensities.

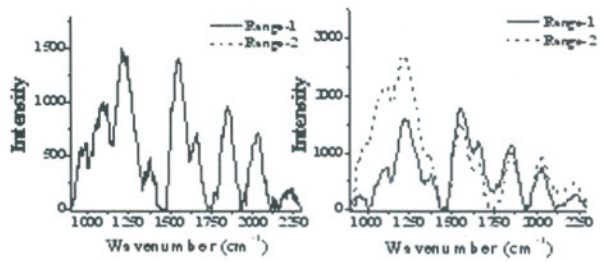

Fig.L.10.2 Raman spectra extracted from the raw spectrum in Fig. L.10.1 by (a) iterative smoothing method and (b) modified polynomial fit method applied on two different spectral ranges. Range-1 corresponds to 900-2700 cm^{-1} and Range-2 corresponds to 500-2400 cm^{-1}.

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