L.5: Depth-sensitive Raman Spectroscopy (RS) combined with Optical Coherence Spectroscopy (OCT) for layered tissue analysis

There is an increasing current interest in the development of dual-modal instruments combining the molecular specificity of Raman Spectroscopy (RS) with the sensitivity and rapid screening capabilities of high resolution microscopic optical imaging like Optical Coherence Tomography (OCT). However, a major limitation of the systems reported thus far is that there is no direct correlation between the OCT information at a given depth to the Raman signal of that particular depth. We, at LBAID, have developed a combined RS-OCT system capable of obtaining Raman signal and the OCT image from the same depth of a layered tissue sample.

The depth-sensitive RS-OCT system was developed by combining a Mach-Zehnder interferometer based real-time, time-domain (TD) OCT system with a Raman spectroscopy system with confocal configuration. The sample arm of the TD-OCT system was modified to allow for co-alignment of the OCT probe beam with the Raman excitation beam along with filtered detection of the Raman scattered light. Figure L.5.1 shows the schematic of the combined RS-OCT system.

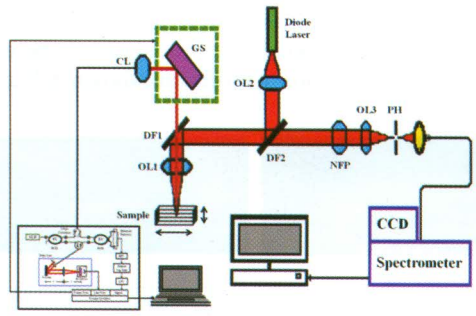

Fig.L.5.1: Experimental setup for combined depth-sensitive Raman spectroscopy and optical coherence tomography

The TD-OCT system utilizes a broadband superluminescent diode, with a centre wavelength of 1310nm. The depth-sensitive Raman part utilizes a single mode 785nm diode laser (DL) as the excitation source. The co-aligned beams with the help of a dichroic filter (DF1) are focused through a microscope objective (NA-0.40) onto the sample kept on a micrometer-controlled stage. For depth-sensitive Raman measurements, the stage is moved in steps of 10 μm and the Raman beam backscattered from the sample is focused (after filtering) onto a 100 μm core diameter optical fiber coupled to the entrance port of an imaging spectrograph equipped with a thermoelectrically cooled, back-illuminated, deep-depletion CCD camera. The combination of the microscope objective and the pinhole aperture rejects the out-of-focus Raman scattered light thereby allowing for depth separation with a depth resolution of ~220 μm.

The combined depth-sensitive RS-OCT device was validated using two samples, one prepared by depositing a thin layer of paraffin over acetaminophen and the other was a goat mucous membrane having an epithelial layer on top of a relatively thicker stromal layer. Figure L.5.2 shows the OCT images and the corresponding Raman spectra for the samples. For both the samples clear distinction is observed between the Raman spectra measured from the two layers as seen in the corresponding OCT images. For example, the Raman bands around 1295 and 1440 cm^{-1}, characteristics of paraffin, are seen to be significantly enhanced in the measured Raman spectrum corresponding to the upper layer. Similarly, the Raman peaks at ~855, 1235, 1328 cm^{-1} and the triplet peaks over 1565-1653 cm^{-1}, characteristics of acetaminophen, are observed to be considerably intense in the measured spectrum corresponding to the lower layer. Similarly, one can observe significant differences in the spectra of the two tissue layers. For example, the intensities of the amide-I band located at ~1670 cm^{-1} the amide-III Raman bands at ~1246, 1269 and 1447 cm^{-1}, characteristics of proteins, are observed to be considerably higher in the Raman spectrum of the stromal layer as compared to the upper epithelial layer. In contrast, the Raman bands at ~1002, 1035, 1209 and 1534 cm^{-1}, believed to be associated with various aromatic amino acids, are observed to be stronger in the Raman spectrum of the epithelial layer as compared to the stromal layer.

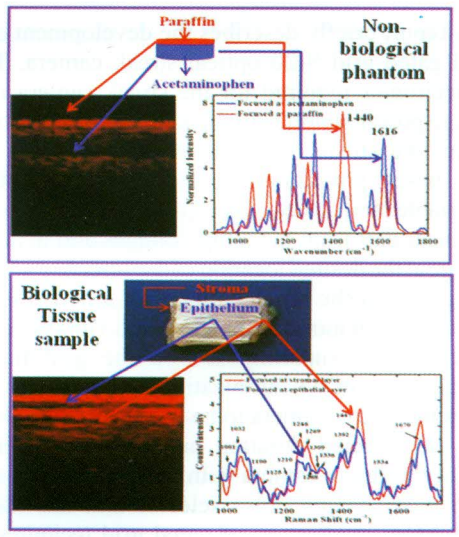

Fig. L.5.2: Raman spectra and OCT images of (a) paraffin-acetaminophen phantom and (b) goat mucous membrane.

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