

L.7: Off-confocal Raman spectroscopy (OCRS) for depth-sensitive measurements

Recently, there has been an increasing interest in investigating Raman spectroscopy based techniques for non-invasive measurement of characteristic Raman signatures of sub-surface layers in highly turbid layered samples. We at LBAS have developed, for the first time to our knowledge, off-confocal Raman spectroscopy (OCRS), a novel technique of depth-sensitive Raman spectroscopy for non-destructive sub-surface interrogation of layered turbid samples. The technique is based on the concept of varying Raman collection zones while keeping the point of illumination fixed on the surface of the target sample.

The system developed employing the principle of OCRS adopts the experimental configuration of a confocal Raman, but employs off-confocal Raman detection for non-destructive sub-surface interrogation at depths beyond the reach of the conventional confocal Raman. It allows subsurface interrogation by moving the tip of the Raman detection fiber (acting as the pinhole aperture) from the focus of the Raman collection objective either by taking the point of detection away from the objective (positive confocal offset) or bringing it closer to the objective (negative confocal offset). The off-confocal separation of the detection point leads to micrometer-scale spatial offset (much like the spatially offset Raman spectroscopy) between the collection and the fixed-point illumination on the surface of the target sample thereby enabling to probe subsurface depths inside the layered sample.

The developed system utilizes a single mode diode laser (central wavelength - 785 nm) as an excitation source. The collimated and filtered laser beam is steered towards the sample by using a dichroic filter and is focused onto its surface through an objective lens of NA of 0.4. The Raman detection is carried out through an optical fiber coupled to an imaging spectrograph equipped with a thermoelectrically cooled, back-illuminated, deep-depletion CCD camera. The collection zone at the sample end is varied by varying at the detection end the distance between the detection fiber and the collection objective (NA – 0.25). While the separation between the two larger than the focal length of the collection objective corresponds to a positive confocal-offset, a separation less than the focal length corresponds to a negative offset. The ability of the system to recover Raman spectra of the subsurface layers was demonstrated using a two-layered (paraffin deposited over acetaminophen substrate) non-biological phantom and a biological tissue sample (chicken tibia).

The Raman spectra measured from the two-layered non-biological phantom is shown for zero-offset (i.e. confocal) as well as positive and negative confocal-offsets (Fig. L.7.1).

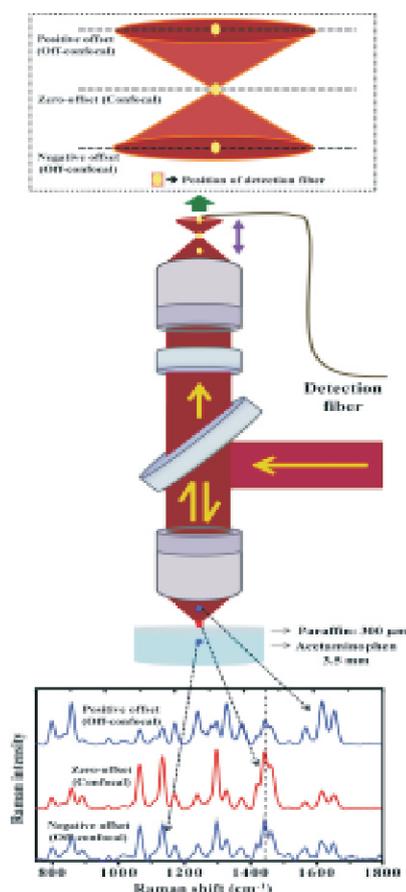


Fig. L.7.1: Experimental setup for off-confocal Raman spectroscopy. Raman spectra measured from a non-biological phantom at confocal and off-confocal positions.

It is clear that the Raman spectrum corresponding to the confocal position is dominated by the Raman bands associated with the top paraffin layer of the phantom. For both the off-confocal spectra (measured by displacing the fiber tip equidistant from the confocal position in two opposite directions along the axis of the microscope objective), the intensities of the Raman bands (e.g. ~ 855 , 1328 cm^{-1} and the triplet peaks between $\sim 1550\text{--}1650\text{ cm}^{-1}$) of the bottom acetaminophen layer are increased relative to the intensities of the Raman bands (e.g. ~ 1295 and 1440 cm^{-1}) of the top paraffin layer (Fig. L.7.1). Further, the Raman spectrum measured at positive confocal-offset is seen to contain a considerably higher contribution of Raman signatures characteristic of acetaminophen compared to the spectrum measured with negative confocal-offset. For detailed description of the study and its results, please refer to *Journal of Optics* 18, 095301, 2016 (doi:10.1088/2040-8978/18/9/095301).

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