Electronic Supplementary Material (ESI) for Analyst.

Sensitivity of Amide I band to matrix manipulation: a Raman micro-spectroscopy and Spatially Offset Raman Spectroscopy study

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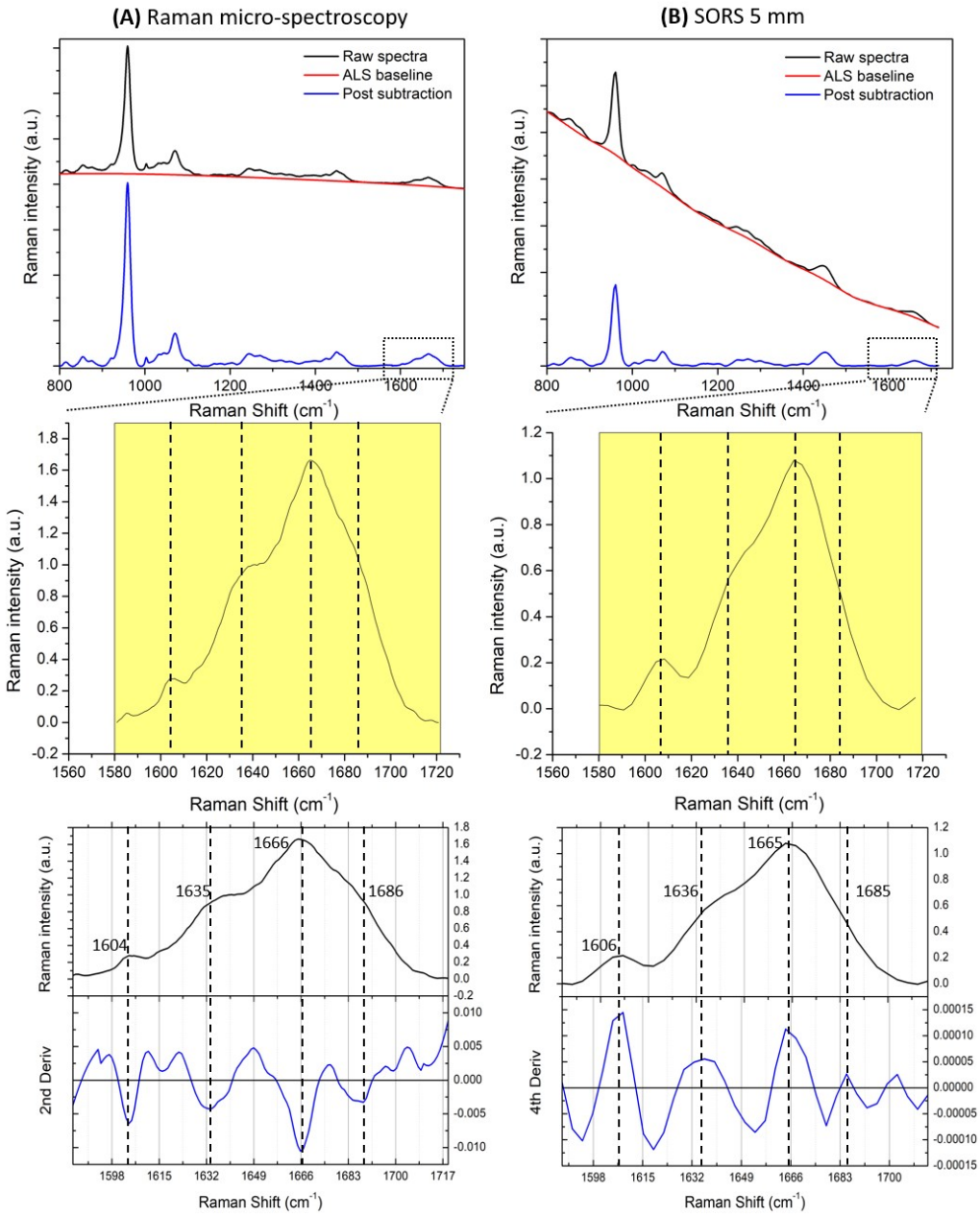

Fig. S1. Representative Raman spectral processing. Data acquired using conventional Raman micro-spectroscopy (A) and 5 mm offset SORS (B) are processed for background subtraction using asymmetric least squares (ALS) method. ALS parameters "λ" and "p" were optimized for μRS (λ=100000, p=0.001) and SORS (λ=4, p=1e^{-4}). λ fits the baseline to the data and p defines the asymmetry of positive versus negative residuals. Amide I band in the region 1580-1720 cm^{-1} is selected (zoomed down) to apply the 2nd derivative (A) and 4th derivative (B) to find the hidden peaks within the band.

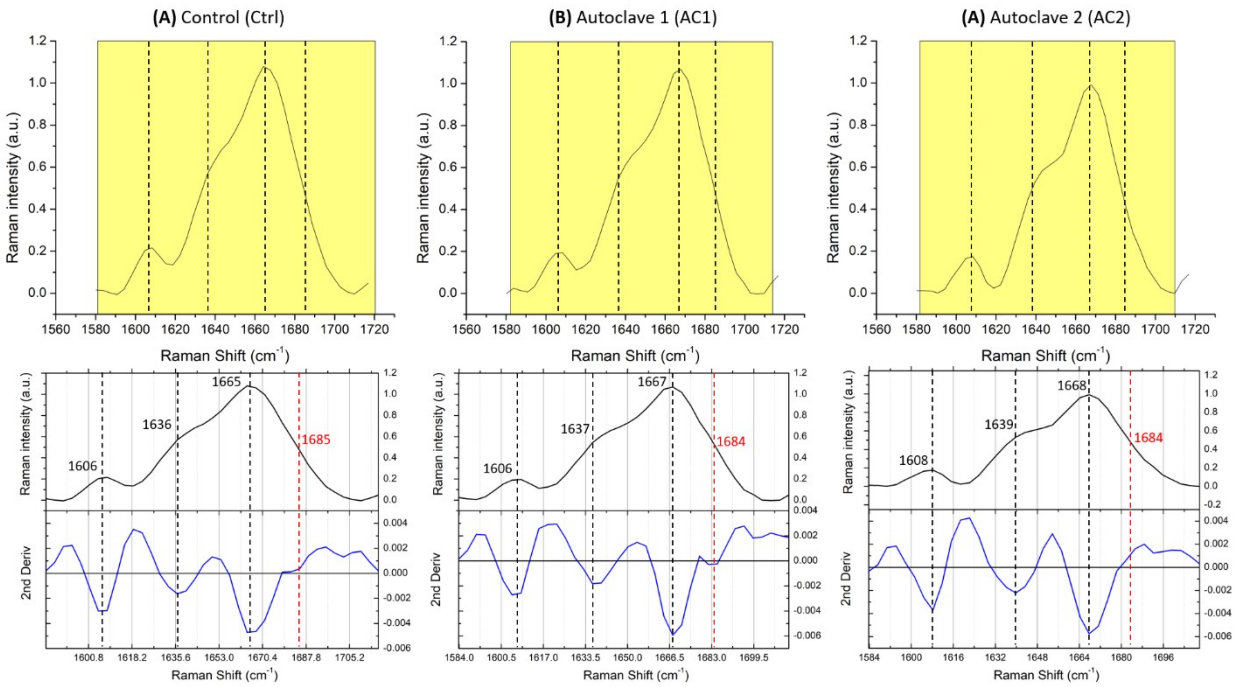

Fig. S2. Amide I sub-peaks using 2nd derivative for SORS acquired data. Sub-peaks identified using 2nd derivative approach for the SORS acquired data (5 mm offset is represented here) failed to identify the 4th sub-peak regardless of the sample condition i.e., control (A) or autoclaved AC1 (B) and AC2 (C).

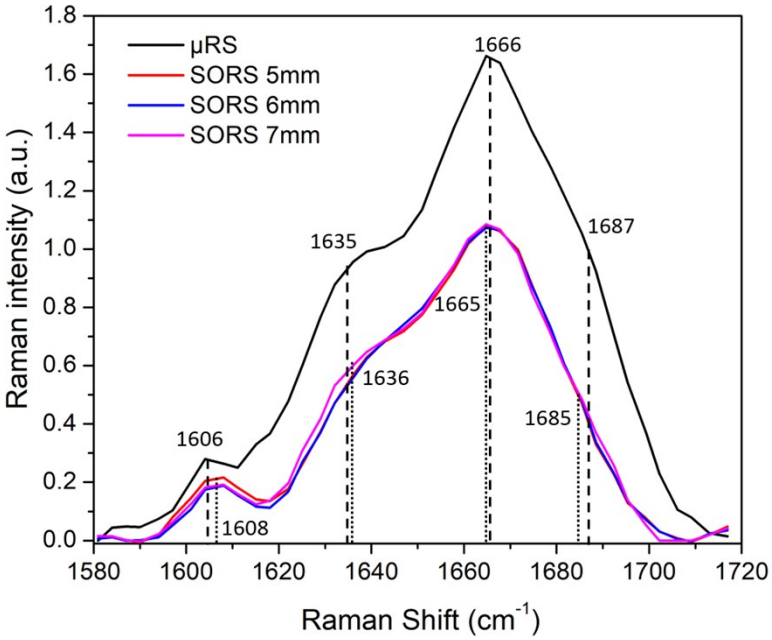

Fig. S3. Amide I band comparison (μRS vs. SORS). Raman spectra were pre-processed for background subtraction and Amide I band is separately plotted here for comparing the intensity and band shape when acquired using Raman micro-spectroscopy and SORS. Intensity of RS is higher compared to SORS irrespective of offset rings. The slight differences between peak positions are labelled. Average peak positions are used for SORS.

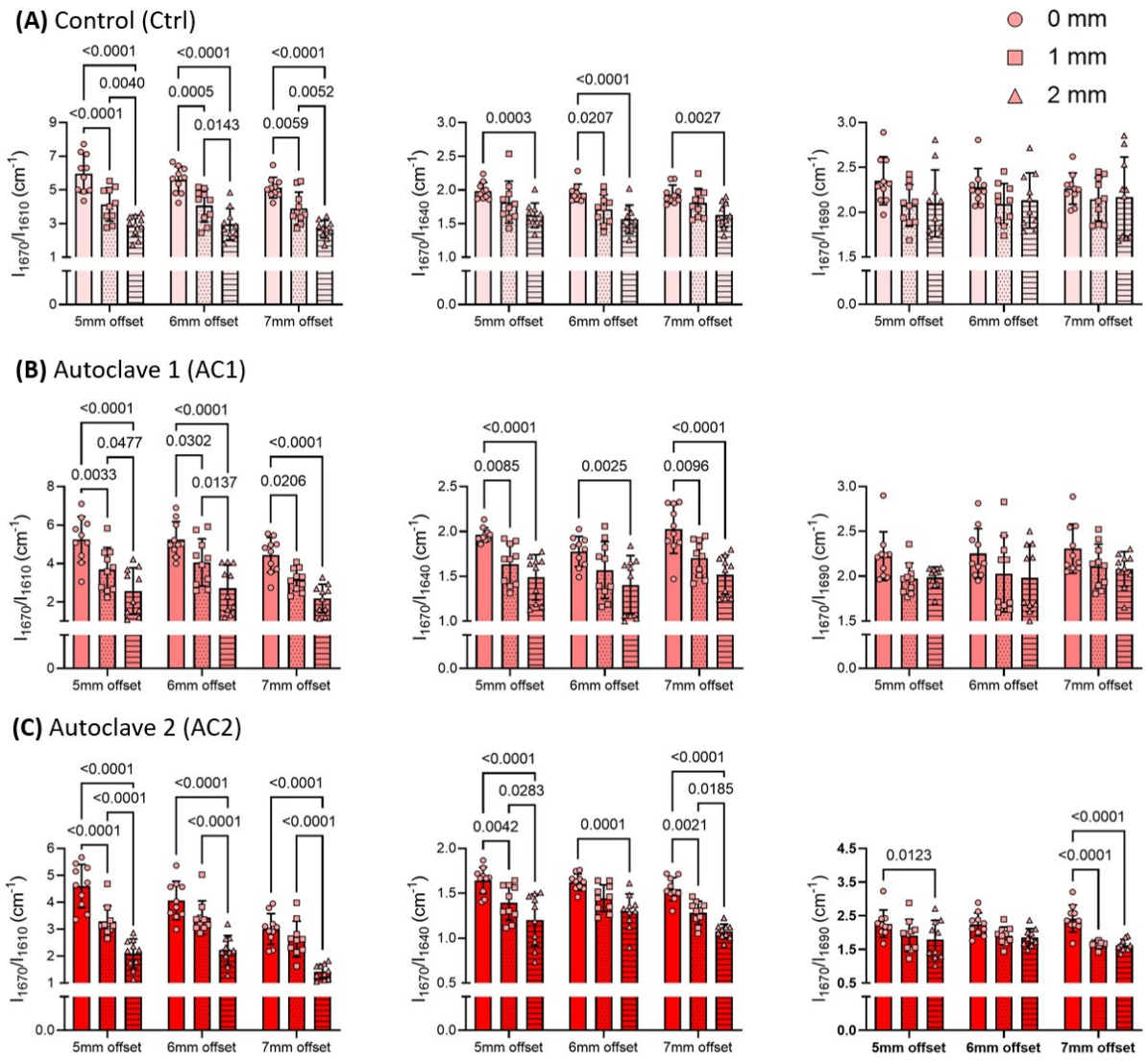

Fig. S4. Raman signal attenuation resulted in dropping Amide I sub-peak ratios through layers. Regardless of the bone condition as Ctrl (A), AC1 (B), AC2 (C), sub-peak ratios significantly dropped when compared between 0 mm thickness (direct bone), 1 mm and 2 mm layers.

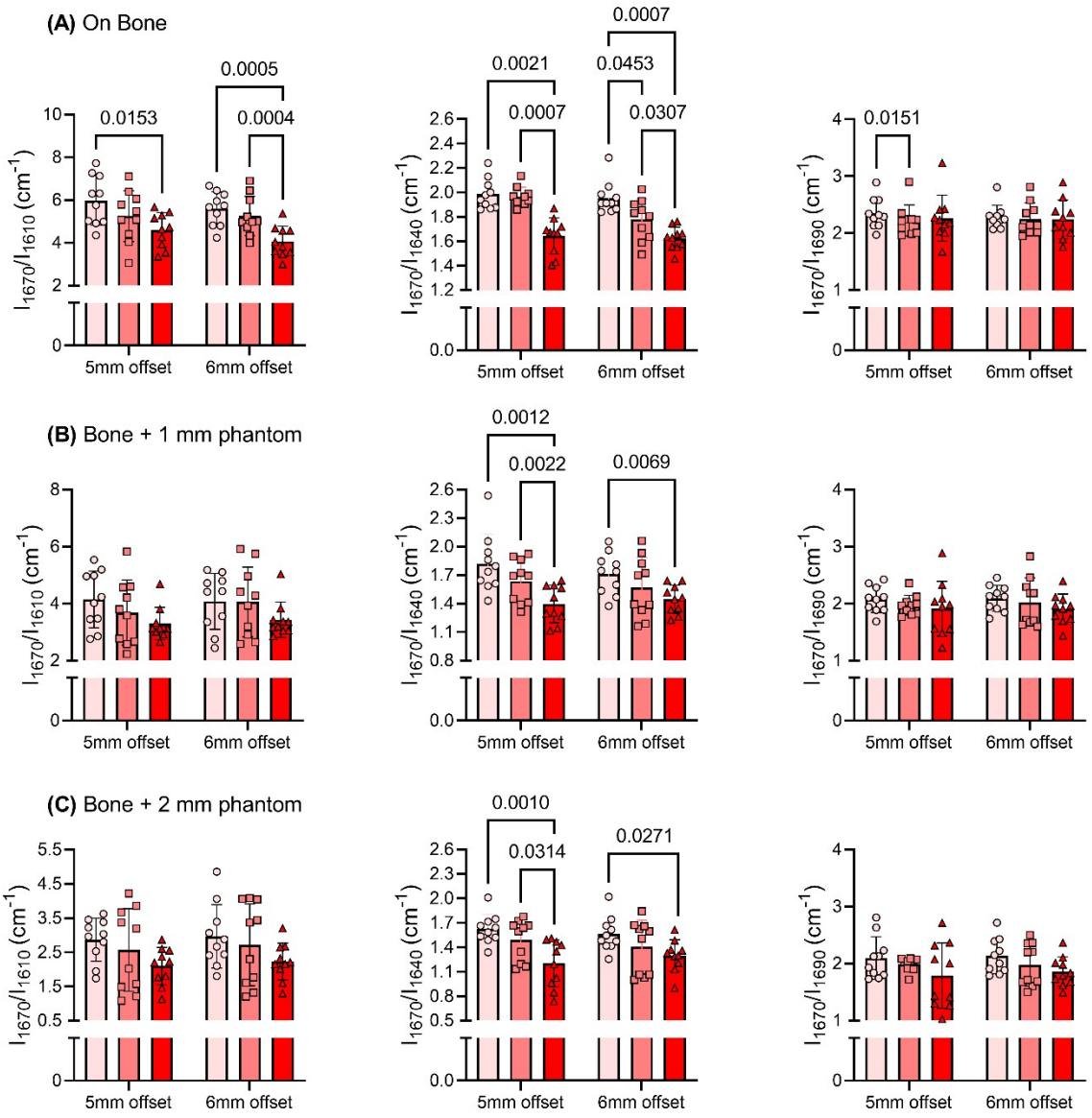

Fig. S5. Sensitivity of SORS to autoclaving when spectra were acquired through tissue phantom layers with a thickness of 2 mm. 4 sub-peak ratios detected through 4th derivative test are plotted against autoclave treatment for 5 mm and 6 mm offset. ~I_{1670}/I_{1640} ratio was decreased irrespective of the layers and offsets. For other ratios (~I_{1670}/I_{1610}, ~I_{1670}/I_{1690}) both 5 mm and 6 mm offset lost the sensitivity with layer thickness of 1 mm and 2 mm.