Supplementary Information for "Analysis of tear fluid protein fractions using surface-enhanced Raman spectroscopy (SERS), followed by high-performance liquid chromatography-light emitting diode-induced fluorescence (HPLC-LED-IF) method".

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Subject Information

All subjects underwent the Schirmer test to assess tear secretion. MDE patients demonstrated reduced tear volume (5–10 mm), whereas C and POAG subjects exhibited normal tear secretion (>15 mm). No additional tear film parameters were measured. The tear break-up time (TBUT) was >10s, <10s for MDE and 9-10s for POAG.

Detailed glaucoma medications for POAG patients are summarized below: Among the 20 POAG subjects, 4 were on a single β-blocker, 5 on a single prostaglandin analogue, 3 on combination therapy of α-agonist, β-blocker, and prostaglandin analogue, 2 on a combination of carbonic anhydrase inhibitor, β-blocker, and prostaglandin analogue, 2 on a single carbonic anhydrase inhibitor, 2 on α-agonist and β-blocker combination therapy, and 2 on other single or dual medications (including β_1-adrenergic blocker and α_2-adrenergic receptor agonist with prostaglandin analogue). All medications were used for one month or longer, as prescribed by ophthalmologists.

MDE subjects were on long-term tear supplements and cyclosporine eye drops for at least three months prior to sampling.

Systemic diseases, contact lens use, and systemic medications were not specifically adressed in this study, as the primary objective was to establish whether the HPLC-LED-IF followed by SERS technique could effectively detect differences in the vibrational characteristics of tear protein fractions. Inclusion and exclusion criteria ensured that participants did not have any major ocular or systemic conditions known to significantly alter tear composition. Samples were collected during daytime hours following a standardized collection protocol however, exact sampling times were not recorded.

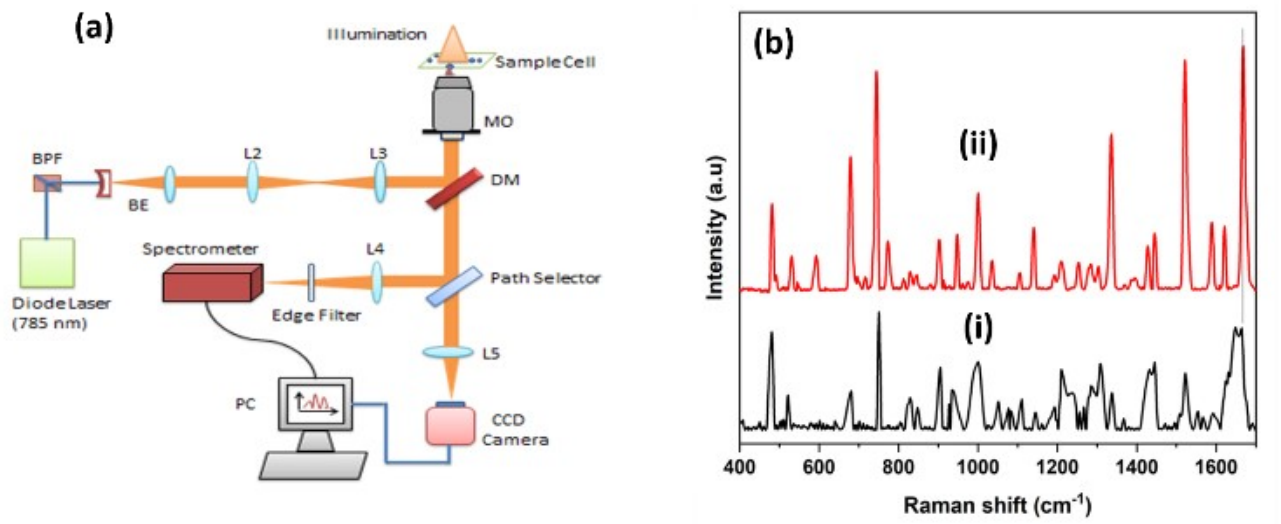

Fig. S1: (a) Schematic Diagram of the micro- Raman setup: 785 nm Diode Laser, BPF-Band Pass Filter, BE-Beam Expander, L2, L3, L4 and L5-Focussing Lenses, DM-Dichroic Mirror, Path Selector, Edge Filter, Spectrometer with CCD, CCD-Camera, PC-Personal Computer, MO-Microscope Objective and Sample Cell, (b) Typical Raman Spectra of (i) Lyz and SERS of Lyz (ii).

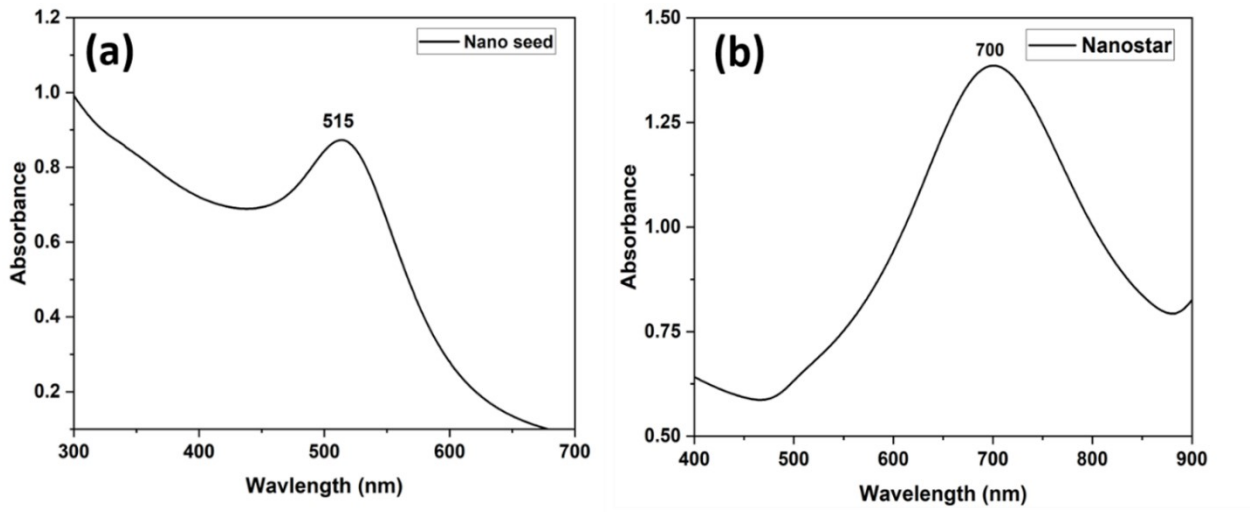

Fig. S2: UV-Absorption spectra of (a) Gold nanoseed colloidal solution and (b) UV-Absorption spectra of gold nanostar colloidal solution.

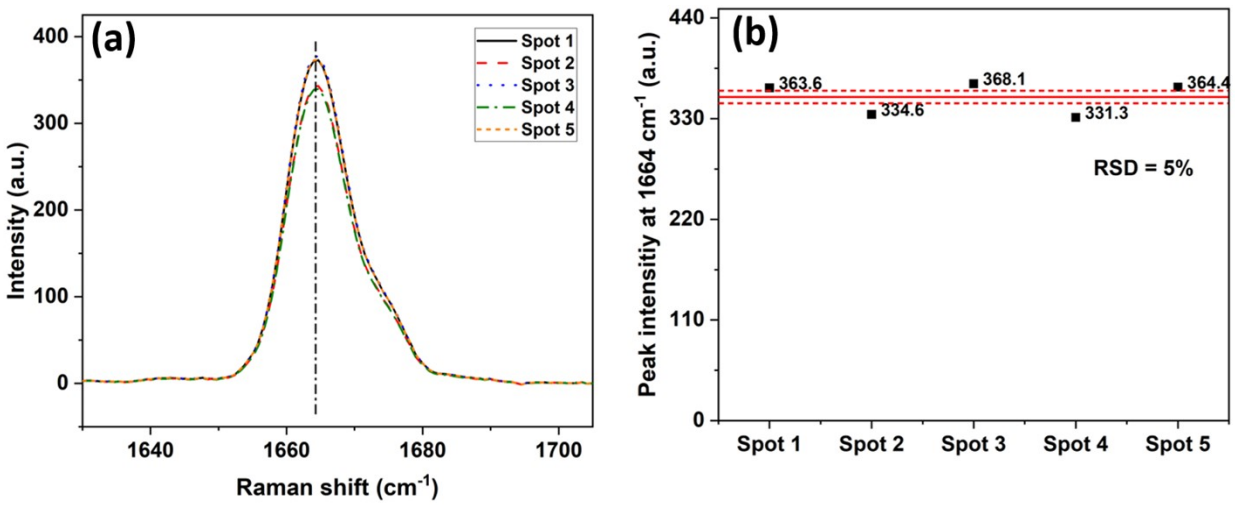

Fig. S3: (a) SERS spectra of Lyz (100 nM) recorded at five different spots on the substrate (laser power: 6.2 mW, acquisition time: 10 s), showing the reproducible peak at 1664 cm^{-1} and (b) Peak intensities at 1664 cm^{-1} from the five spots, with mean value and standard deviation.

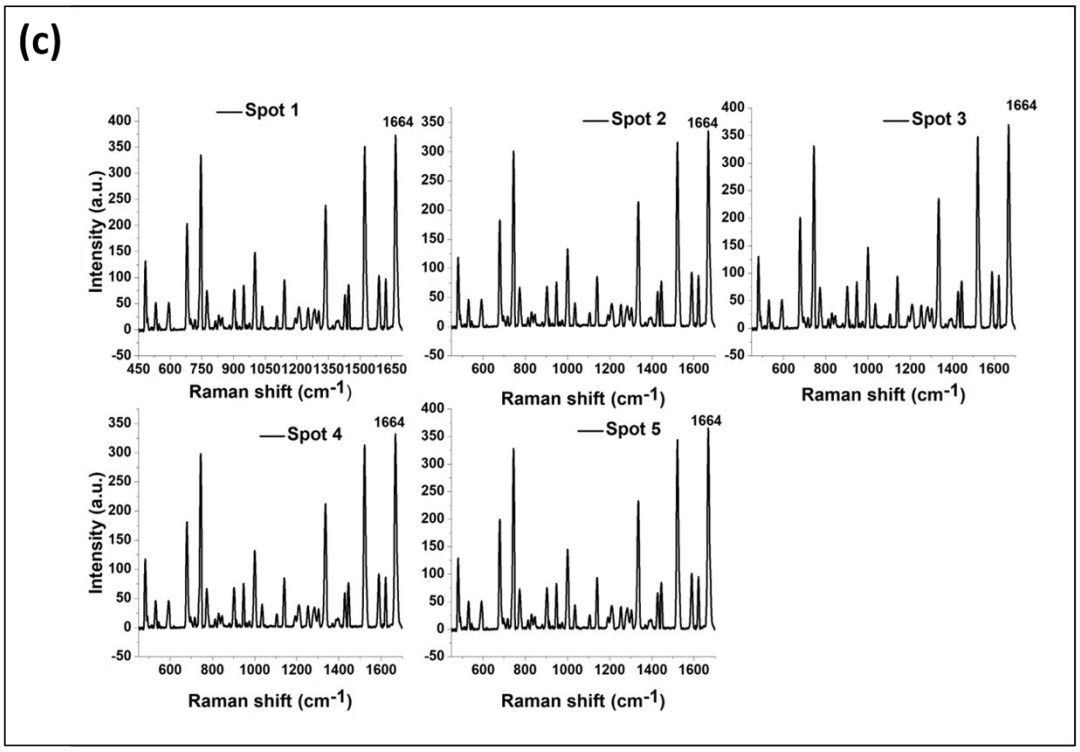

Fig. S3: (c) SERS (average of 25 spectra) of Lyz from five different spots.

Table S1: Multi-confusion matrix for PF1.

Class	Confusion Matrix(TP, Misclassified)	Sensitivity (%)	Specificity (%)	Precision (%)	Accuracy (%)
C	18 (1 as MDE, 1 as POAG)	85	90	81	88
MDE	18 (1 as C, 1 as POAG)	80	87.5	76	85
POAG	17 (3 as MDE, 0 as C)	80	95	89	90

Table S2: Multi-confusion matrix for PF2.

Class	Confusion Matrix(TP, Misclassified)	Sensitivity (%)	Specificity (%)	Precision (%)	Accuracy (%)
C	17 (2 as MDE, 1 as POAG)	85	90	81	88
MDE	16 (3 as C, 1 as POAG)	80	87.5	76	85
POAG	16 (1 as C, 3 as MDE)	80	95	89	90

PF1

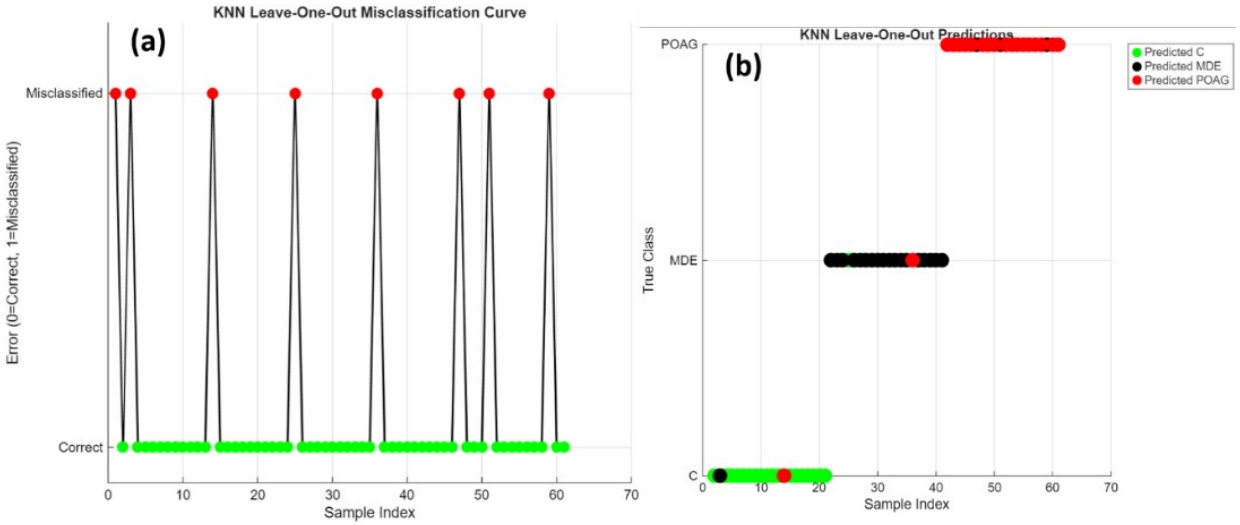

Fig. S4: (a) Results of leave-one-out cross-validation for the KNN classifier. The misclassification curve displays correct predictions (green at baseline) and errors (red above baseline), showing that only a small number of scattered misclassifications occurred and (b) the prediction plot compares true classes with predicted outputs, illustrating that classes C and POAG were classified with high accuracy.

PF2

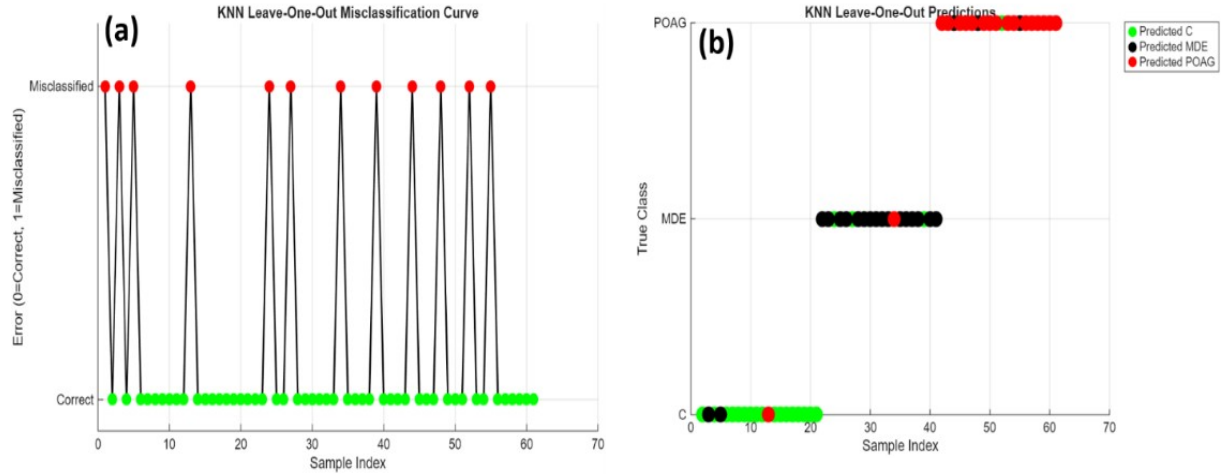

Fig. S5: (a) Results of leave-one-out cross-validation for the KNN classifier. The misclassification curve displays correct predictions (green at baseline) and errors (red above baseline), showing that only a small number of scattered misclassifications occurred and (b) the prediction plot compares true classes with predicted outputs, illustrating that classes C and POAG were classified with high accuracy.