

Raman Spectroscopic Signatures of Prostate Cancer Progression: Correlation with Gleason Score

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Abstract

Prostate cancer (CaP) is a heterogeneous malignancy, and its grading via Gleason scoring is essential for prognosis and therapeutic decisions. However, traditional histopathological methods are limited in detecting subtle biochemical variations. This study applies Raman spectroscopy (RS) to formalin-fixed paraffin-embedded (FFPE) CaP tissues of varying Gleason scores to uncover biochemical signatures associated with tumor aggressiveness. Raman spectral analysis reveals distinct molecular alterations, particularly in lipid, protein, and nucleic acid content, that correlate with Gleason grade progression. Raman data Singular Value Decomposition (SVD) analysis helps differentiate between benign and malignant prostate tissues and further stratify cancerous lesions according to Gleason patterns. This is particularly valuable in distinguishing intermediate-grade tumors (Gleason score 7) from high-grade tumors (Gleason scores 8–10), a distinction that carries critical therapeutic implications. Our findings support the potential of Raman spectroscopy as a label-free, non-destructive diagnostic adjunct in CaP grading and stratification, offering further improved classification accuracy to conventional histopathology.

Highlights

- Raman spectroscopy (RS) revealed progressive biochemical alterations in CaP tissues across Gleason scores 7–10, reflecting metabolic reprogramming, structural remodeling, and genomic activation.
- Lipid-associated bands (987, 1090, 1437, 1462 cm^{-1}) showed reduced intensity in high-grade tumors, indicating disrupted lipid metabolism and membrane remodeling.
- Carbohydrate-related bands (1106, 1122, 1140 cm^{-1}) progressively increased with Gleason score, consistent with elevated glucose uptake and glycosylation activity.
- Protein-associated amide bands (1205, 1237, 1637–1687 cm^{-1}) exhibited spectral shifts, highlighting enhanced protein synthesis, denaturation, and secondary structure changes in aggressive CaP.
- Nucleic acid-associated bands (1044, 1337, 1584 cm^{-1}) intensified with tumor grade, reflecting elevated genomic activity and DNA replication.
- RS combined with multivariate statistical analysis achieved accurate classification of CaP grades, supporting its integration into histopathological workflows for non-invasive, label-free tumor grading.

INTRODUCTION

Prostate cancer (CaP) is the second most common malignancy in men worldwide, exhibiting a spectrum of clinical behaviors that complicate disease management [1–4]. The Gleason score, derived from histopathological patterns of glandular differentiation, is the primary grading system for prostate adenocarcinoma and a robust predictor of tumor aggressiveness, metastatic potential, and cancer-specific survival [5–7]. Serum prostate-specific antigen (PSA) levels complement Gleason grading, and

the strength and consistency of this correlation across discrete Gleason grade groups, as well as their predictive accuracy over an extended follow-up period, remain incompletely defined [8–10]. Despite its clinical utility, Gleason grading may not capture the full biochemical spectrum of tumor progression [11]. While histopathology remains the gold standard for diagnosing human CaP, its inherently subjective nature often results in significant variability among pathologists. Inter-observer disagreement can reach up to 40% and distinguishing between Gleason score 7 (intermediate grade) and Gleason scores 8–10 (high grade) CaP can be nuanced and clinically significant [10–11]. High-grade CaP typically requires prompt treatment, whereas low-intermediate grade CaP may be managed through active surveillance due to its indolent progression. Distinguishing between intermediate-grade (Gleason score 7) and high-grade (Gleason scores 8–10) CaP presents a clinically significant challenge due to overlapping histopathological features and variable biological behavior. Gleason score 7 encompasses two distinct subgroups ($3 + 4 = 7$: more favorable, lower risk of progression) and ($4 + 3 = 7$: higher proportion of aggressive cells, closer to high-grade behavior) with differing prognostic implications; the former is associated with more favorable outcomes, while the latter demonstrates more aggressive characteristics akin to high-grade disease [12–15]. In contrast, Gleason scores 8–10 reflect poorly differentiated or undifferentiated tumor architecture and are consistently linked to higher risks of progression, metastasis, and mortality [16]. The heterogeneity within Gleason 7 tumors, particularly those with a predominant pattern 4, complicates risk stratification and treatment planning, often necessitating adjunctive tools such as genomic classifiers and multiparametric imaging [15–16]. Accurate grading is critical, as misclassification may lead to under- or over-treatment, underscoring the importance of expert pathological review and comprehensive clinical assessment in guiding therapeutic decisions [17–18]. Accurately differentiating between high-grade CaP represents an urgent and essential clinical challenge. Emerging optical imaging technologies, such as Raman spectroscopy (RS) and other genomic classifiers, offer promise for refining risk stratification but require systematic integration with established histological grades [19–21]. RS has become indispensable in biomedical research. This vibrational spectroscopic technique provides label-free biochemical insights at the molecular level. Label-free optical modalities enable direct interrogation of biological specimens without the need for exogenous probes, ensuring that measurements remain nonperturbative and that the structural, functional, molecular, and physiological integrity of cells or tissues is fully preserved [20].

Alterations in molecular signatures underlie cancer invasion and metastasis. Because most biomolecules are Raman-active, each generates a distinct spectroscopic fingerprint, enabling RS to detect even subtle biochemical and molecular shifts in cells and tissues. This sensitivity confers powerful diagnostic potential, allowing RS to reveal chemical changes associated with both disease onset and tumor progression, and it has demonstrated promising accuracy across multiple cancer types [19–22]. To interpret complex datasets, multivariate calibration and classification strategies such as Singular Value Decomposition (SVD) and Linear Discriminate analysis are applied to full-spectrum Raman measurements, elucidating the principal factors driving spectral variability within heterogeneous biological specimens [23–25].

Using RS, we recently showed that the Gal-3 inhibitor induces significant alterations in major biochemical constituents, such as lipids, proteins, and nucleic acids, which may lead to structural and molecular changes in the cancerous prostate tissue, and this highlighted the therapeutic potential of the Gal-3 inhibitor in the prevention of CaP progression and metastases [26]. RS generates a unique molecular fingerprint that reveals the distinct chemical composition of complex biological samples, allowing unambiguous detection of biomolecular features even in heterogeneous tissue environments. This could revolutionize CaP diagnostics by enhancing precision in Gleason scoring, especially in ambiguous cases like Gleason 7 (3 + 4 vs. 4 + 3), where treatment decisions hinge on subtle histological differences. While still in the research phase, RS offers a compelling pathway toward more objective, reproducible, and efficient CaP grading. When applied to FFPE tissue, RS enables retrospective analysis using archival samples, making it a powerful tool for cancer diagnostics and biomarker discovery. In this study, we explore the capability of RS to differentiate CaP tissues across a range of Gleason scores from archival FFPE CaP tissue samples and identify spectral markers indicative of tumor grade. Our data shows that RS has promising potential for non-invasive, rapid assessment of CaP aggressiveness, including correlation with Gleason scores.

MATERIALS AND METHODS

Sample Collection and Preparation

CaP biopsy samples were obtained from the archived tissue bank of the Department of Urology at Western New York Urology Associates, Cheektowaga, NY-14225. Patient tissue samples obtained after informed consent was obtained from patients, as per protocols and approval by our Institutional Review Board (IRB). Tissue sampling was performed using spring-loaded or coaxial needles, typically 18-gauge, under transrectal ultrasound (TRUS) guidance. In some cases, 16-gauge needles were employed to yield larger cores, though cancer detection rates have been reported as comparable. The 18G needles produced cores approximately 0.8 mm in diameter and 15 mm in length. Sections (4 μ m thick) were mounted on calcium fluoride (CaF₂) slides and stored at – 80°C until analysis. To preserve native molecular profiles, no staining, labeling, or chemical treatment was applied. None of the patients received preoperative therapy. All protocols were approved by the Ethics Committee of the Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA. The biopsies represented the following range of Gleason scores: Gleason 7 (3 + 4/4 + 3) (**G7**) – intermediate grade; Gleason 8– 10 (**G8; G9; G10**) high grade. Representative hematoxylin and eosin (H&E) stained sections of CaP tissues used in this study (Fig. 1), which show CaP tissue grades by a pathologist based on Gleason scores ranging from 7 to 10, demonstrate a progressive loss of glandular architecture and increasing cellular atypia compared to normal prostate tissue controls. Normal prostate glands exhibit well-organized acini lined by a uniform layer of epithelial cells with basally located nuclei and intact fibromuscular stroma. In contrast, Gleason score 7 tissues show a mixture of well-formed and poorly formed glands, while Gleason 8 sections reveal predominantly fused or cribriform glands with marked nuclear enlargement. Gleason scores 9 and 10 are characterized by a complete absence of glandular structures, with tumor cells

arranged in solid sheets or single-cell infiltrates, accompanied by prominent nucleoli and areas of necrosis. These histological changes reflect increasing tumor aggressiveness and loss of differentiation with higher Gleason grades.

Raman Spectra Acquisition of CaP Tissue

Paraffin-embedded sections (4 μm) of the CaP tissue samples ($n = 4$) obtained from patient groups: Gleason 7, Gleason 8, Gleason 9, Gleason 10, and controls (non-cancerous prostate biopsies identified by a pathologist through microscopic examination, confirming the absence of malignant cells) were used for the Raman spectral analysis. The paraffin peaks were eliminated by software-guided subtraction of the Raman spectra of standard paraffin from the acquired spectral data of samples. To further minimize paraffin contributions, we identified the singular vector corresponding to paraffin-like spectral features (by comparison with a pure paraffin spectrum) and excluded its contribution prior to downstream analysis. The resulting spectra preserved tissue-specific biochemical signatures while reducing wax-related variance. No staining or chemical treatment was applied to enable preservation of the native biochemical composition of the tissue for Raman analysis. Scattered Raman light from a laser beam focused on a tissue section provided detailed information about the molecular composition of the tissue at the microscopic level. Multiple spectra were obtained per tissue section to account for heterogeneity.

Raman spectra were acquired by a commercial Raman micro-spectroscope (HORIBA XploRA PLUS) equipped with a 1024×256 TE air-cooled CCD chip (pixel size 26 μm , temperature -60°C). Spectra were acquired using a 532 nm laser operating at a power of 0.065 W, with an 1800 grooves/mm grating, a slit width of 100 μm , and a pinhole diameter of 100 μm . Each spectrum was recorded with an acquisition time of 30 seconds and three accumulations to enhance signal quality. A 40x objective was employed for focusing, and a total of 40 spectra per sample were collected for analysis. Spectra were acquired over 950–1800 cm^{-1} (fingerprint region).

Raman Data Processing and Analysis

HORIBA LabSpec6 software was used for the initial data processing: smoothing, baseline removal (polynomial), and normalization (unit vector), necessary to enable subsequent quantitative analysis (23–25). SVD analysis of the spectra was done using Python code to obtain the critical spectral features differentiating between the tissue samples. All Raman spectra from each imaging dataset were aggregated to form an input matrix for the SVD algorithm. In particular, the Raman spectra acquired from individual points within each sample were organized to generate a matrix of size $m \times n$, where m denotes the number of data points per spectrum, and n indicates the total number of spectra within a specific sample [26–29]. In our study, these dimensions were 584 X 80. Subsequently, we applied the SVD function in Python to decompose the input matrix into matrices U , Σ , and V^T . The matrix V was utilized to create SVD scatter plots, whereas the individual SVD components were stored in the matrix U [30–31].

Each scatter plot was based on the leading SVD components and contained two data sets, either a Gleason score (G7, G8, G9, G10) vs. control, or two Gleason scores (G7 vs. G8, G7 vs. G9, G7 vs. G10, G8 vs. G9, G8 vs. G10, G9 vs. G10), where each spectrum was represented as a single point. A separating line was constructed using Linear Discriminant Analysis (LDA), a supervised classification technique that identifies the linear combination of features which maximizes the separation between classes by maximizing the distance between their means while minimizing the variance within each class [32]. A corresponding confusion matrix, summarizing the classification performance for each sample, was also generated. Additionally, the SVD components employed in the scatter plots, each of which contained the spectral features responsible for distinguishing the datasets, were plotted.

Statistical Analysis

Statistical analysis was done using GraphPad Prism (v8; GraphPad Software, Boston, MA). The comparison between the specific Gleason scores (G7, G8, G9, G10) vs. control, and between Gleason scores (G7 vs. G8, G7 vs. G9, G7 vs G10, G8 vs. G9, G8 vs. G10, G9 vs. G10) was done using a one-way ANOVA followed by the Tukey multiple comparison test. Statistical significance was set at $P < 0.05$. Singular Value Decomposition (SVD) was used solely for visualization purposes, not for classification or model fitting. This approach inherently avoids overfitting, as SVD was not part of the predictive pipeline. We selected the first six components based on their cumulative explained variance, which captured the most informative structure in the data while minimizing noise. Linear Discriminant Analysis (LDA) was applied for classification, and we ensured robustness by performing cross-validation during model evaluation. This helped assess generalizability and mitigate overfitting risks. The following flowchart represents our steps:

RESULTS

Raman Spectral Analysis:

Although digital dewaxing substantially reduces paraffin contributions, residual overlap with wax bands cannot be completely excluded in FFPE tissues. We therefore performed an additional SVD-based removal of the paraffin-associated component and confirmed that the main Gleason-dependent trends in nucleic acid and protein bands remained unchanged, supporting the biological origin of the reported spectral differences. Figure 2 presents a comprehensive Raman spectral analysis of CaP tissues across Gleason scores 7 to 10, alongside normal prostate controls. The schematic in panel (a) outlines the experimental setup used for RS, highlighting the excitation source, sample positioning, and signal detection pathway. Panel (b) displays baseline-subtracted and normalized average Raman spectra for each Gleason group and control, with shaded regions indicating 95% confidence intervals. Notably, panel (c) zooms into the $1400\text{--}1500\text{ cm}^{-1}$ region, revealing distinct spectral peaks associated with biochemical alterations in cancer progression. Panel (d) shows a three-dimensional SVD scatter plot, demonstrating clear spectral separation between normal and cancerous tissues, with increasing divergence correlating with higher Gleason scores. Finally, panel (e) presents a heatmap of Raman band

intensities, illustrating progressive shifts in molecular signatures across the Gleason spectrum, underscoring the diagnostic potential of Raman spectroscopy in CaP grading.

Table 1 presents the key spectral changes observed in CaP tissue, emphasizing distinct Raman bands associated with Gleason scores G7 through G10 in comparison to non-cancerous control samples. These spectral shifts reflect molecular changes that correlate with disease severity and progression. Notably, specific Raman bands demonstrate consistent variation across increasing Gleason stages, suggesting their potential utility as biomarkers for tumor aggressiveness. The biological significance of each spectral feature, including its association with lipid metabolism, protein synthesis, and structural remodeling, is elaborated in the subsequent discussion.

Table 1 Comparative Raman band analysis in prostate cancer with respectto normal prostate control		
Raman band (cm-1)	Intensity	Biological relevance
987	Decrease	Hydrogen atoms rocking vibrations in small peptides
1044 & 1090	Decrease	-O-P-O- (symmetric) of nucleic acids and C-C in phospholipids (with pH change)
1106	Decrease	Carbohydrates such as α-D-Glucose
1122, 1319 & 1584	Decrease	Cytochrome C characteristics band and Cytochrome C shoulder band; ν(C-C) skeletal vibration of the acyl backbone in lipid (trans conformation)
1122 & 1140	Decrease	C-N stretch, C-O/C-C stretch in proteins and carbohydrates
1167	Decrease	C-H in-plane bending for aromatic amino acids in proteins
1205	Decrease	ν(c-C ₆ H ₅), phenylalanine, tryptophan in proteins; Adenosine, Thymine (ring breathing modes of the DNA/RNA bases) in nucleic acids; amide III (protein)
1237	Decrease	Amide III band in proteins; CH ₂ deformations in lipids
1337	Increase	C-N signals from deoxynucleotides
1418 & 1462	Increase	C-H asymmetrical bending mode of CH ₂ /CH ₃ in lipids and proteins
1437, 1637 & 1687	Decrease	Characteristic band for L-Glutamate
1662	Decrease	ν(C = C stretch) in lipids; amide I proteins; ring breathing ν(pyrimidine) of nucleoside base
1437 & 1672	Increase	Specifically for nucleoside base (Thymine)

In the RS analysis of CaP tissue, a prominent peak observed at 987 cm⁻¹ is indicative of molecular alterations associated with disease progression. This spectral feature is commonly linked to vibrational modes of cholesterol and cholesterol esters, which are known to accumulate in CaP cells, particularly in

androgen receptor-negative subtypes [33–34]. The presence of this peak reflects a shift in lipid metabolism, a hallmark of cancerous transformation. Additionally, the 987 cm^{-1} region may capture changes in protein structure and synthesis, as cancer cells often exhibit elevated protein turnover and remodeling.

Significant spectral changes were observed in CaP tissue across Gleason scores 7 to 10, with notable shifts in Raman bands at 1044 cm^{-1} and 1090 cm^{-1} . The 1044 cm^{-1} band, attributed to phosphate vibrations from nucleic acids and phospholipids, showed increased intensity in higher Gleason grades, reflecting enhanced genomic activity and membrane remodeling associated with tumor progression [35–36]. Similarly, the 1090 cm^{-1} band, linked to C–C and C–O stretching in lipids and carbohydrates, exhibited a progressive increase from Gleason 7 to 10, indicating metabolic reprogramming and lipid accumulation characteristic of aggressive CaP phenotypes [37].

The observed increase in Raman intensity at 1106 cm^{-1} across CaP tissues with Gleason scores 7 to 10 suggests a significant shift in cellular carbohydrate metabolism [38]. This band is closely associated with C–O and C–C stretching vibrations found in α -glucose and other carbohydrate structures. Its enhancement in higher-grade tumors may reflect elevated glucose uptake and utilization, consistent with the metabolic reprogramming characteristic of malignant transformation. The correlation between this spectral feature and α -glucose implies a potential link to the Warburg effect, wherein cancer cells preferentially metabolize glucose through glycolysis even under aerobic conditions. These findings support the role of the 1106 cm^{-1} band as a biomarker for altered glucose dynamics and underscore its relevance in distinguishing aggressive CaP phenotypes [39–41].

Distinct spectral features at 1122 cm^{-1} , 1319 cm^{-1} , and 1584 cm^{-1} were observed in CaP tissues and demonstrated increasing intensity with higher Gleason scores (G7–G10). The 1122 cm^{-1} band is attributed to C–O stretching vibrations in carbohydrates and phospholipids, and its enhancement suggests altered membrane composition and glycosylation patterns associated with malignant transformation. The 1319 cm^{-1} band corresponds to CH_2/CH_3 bending modes in lipids and proteins, indicating increased lipid turnover and structural remodeling in more aggressive tumors. The 1584 cm^{-1} band is linked to C = C stretching in aromatic amino acids and nucleic acid bases, reflecting elevated protein synthesis and genomic activity in high-grade cancer [42–43].

A notable increase in the Raman band at 1140 cm^{-1} was observed in CaP tissues with escalating Gleason scores from 7 to 10. This band is primarily attributed to C–C and C–N stretching vibrations, commonly found in proteins, lipids, and amino acid side chains [41–44]. The progressive enhancement of this spectral feature suggests a shift in the biochemical composition of the tumor microenvironment, particularly reflecting increased protein synthesis and lipid remodeling associated with malignant transformation [44–45].

A progressive increase in the Raman band at 1167 cm^{-1} was observed in CaP tissues with advancing Gleason scores from 7 to 10. This band is primarily attributed to C–H in-plane bending and C–C stretching vibrations, commonly found in lipid chains and protein side groups [45–49]. The enhanced

signal intensity at this frequency suggests elevated lipid turnover and protein conformational changes, both of which are characteristic of malignant transformation and tumor progression.

A progressive enhancement of the Raman band at 1205 cm^{-1} was observed in CaP tissues with increasing Gleason scores from 7 to 10. This band is primarily attributed to C–C and C–N stretching vibrations, which are characteristic of protein backbones and lipid-associated structures [48–49]. The elevated intensity at this frequency suggests increased protein synthesis, amino acid turnover, and membrane remodeling, all of which are hallmarks of malignant progression [50–53]. These biochemical changes support the metabolic demands of rapidly proliferating cancer cells and reflect the structural adaptations required for invasion and metastasis. In CaP, especially across Gleason scores 7 to 10, Amide III shifts may indicate structural remodeling of proteins- these shifts arise from N–H bending and C–N stretching in the protein backbone. In cancerous tissues, changes in this band may reflect protein conformational shifts and altered structural integrity [54].

Alternation in vibrational modes of the indole ring in tryptophan can signal oxidative stress, protein folding anomalies, or metabolic changes in tumor cells, and the symmetric ring breathing of the benzyl side chain in phenylalanine alters the reflects changes in protein expression, folding, and oxidative stress responses. Phenylalanine peak typically serves as a stable internal marker due to its sharp and consistent peak its increased intensity may reflect elevated protein synthesis or aromatic amino acid enrichment in cancerous tissues [55–56].

A progressive increase in the Raman band at 1237 cm^{-1} was observed in CaP tissues with advancing Gleason scores from 7 to 10. This band corresponds to the Amide III region, primarily arising from N–H bending and C–N stretching vibrations in the protein backbone. The enhanced signal at this frequency indicates alterations in protein secondary structure, such as shifts between α -helical and β -sheet conformations, which are commonly associated with malignant transformation [57–60]. These structural changes reflect the increased protein synthesis and turnover required to support rapid cellular proliferation and invasion in high-grade tumors.

The Raman band at 1337 cm^{-1} is typically attributed to nucleic acid and protein vibrations, particularly related to adenine and CH deformation modes. In the context of CaP, higher Gleason scores often correlate with increased intensity or shifts in the 1337 cm^{-1} band, due to changes in cellular composition. The 1337 cm^{-1} band may reflect elevated nucleic acid content or altered protein structures, which are common in more aggressive tumors [61–63].

Raman Band 1418 cm^{-1} is attributed to CH_2 bending vibrations in lipids and proteins.

And in the context of CaP, a shift in this band may contribute to Changes in lipid composition and protein structure, which are common in malignant transformation. A decrease in lipid-associated bands like 1418 cm^{-1} may indicate higher Gleason scores, reflecting more aggressive cancer. Lower intensity at this band may suggest loss of normal glandular architecture and increased cellular atypia. Raman Band 1462 cm^{-1} is associated with CH_3 deformation modes, primarily from proteins and lipids, and this band has

been observed to shift or change in intensity with increasing Gleason grade and potentially reflects altered protein synthesis or membrane composition in cancerous cells [63–64].

The 1437 cm^{-1} Raman band is associated with CH_2 bending vibrations in lipids, and exhibited reduced intensity in higher-grade tumors, suggesting a depletion of lipid content consistent with malignant transformation. The 1637 cm^{-1} band, attributed to amide I vibrations from protein secondary structures, showed spectral shifts and intensity changes indicative of altered protein conformation and synthesis in aggressive cancer phenotypes. Similarly, the 1687 cm^{-1} band, also linked to amide I vibrations, demonstrated elevated signals in high Gleason score samples, reflecting increased cellular turnover and protein denaturation [62–63]. The 1437 cm^{-1} band is associated with CH_2 bending in lipids, which may be altered due to increased glutamine-driven lipid synthesis in cancer cells. Glutamine contributes to fatty acid biosynthesis, and changes in this band may reflect that metabolic shift. While the 1637 cm^{-1} and 1687 cm^{-1} bands correspond to amide I vibrations, linked to protein secondary structures. Elevated glutamine metabolism supports protein synthesis and turnover, especially in rapidly proliferating cancer cells; therefore, shifts or intensity changes in these bands may indicate increased protein content or altered folding, consistent with glutamine-driven growth.

The 1662 cm^{-1} Raman band is attributed to amide I vibrations, particularly from $\text{C} = \text{O}$ stretching in protein backbones. CaP cells often exhibit increased protein synthesis and turnover, leading to detectable changes in this band. The 1662 cm^{-1} band intensity and position can vary with Gleason grade and, at lower Gleason scores (G7), tend to show more organized protein structures, with a stable amide I signal, while at higher Gleason scores (G8–G10) often exhibit increased or shifted 1662 cm^{-1} signals, reflecting disordered protein structures and higher metabolic activity. In CaP, nucleoside metabolism is dysregulated, including increased synthesis and turnover of DNA and RNA, and while pyrimidine ring breathing itself is not captured in these bands, Raman shifts in the 1400–1700 cm^{-1} range may reflect secondary biochemical effects of nucleic acid dysregulation such as altered protein expression, chromatin remodeling, and lipid biosynthesis [61–64].

The 1437 cm^{-1} band is attributed to CH_2 scissoring vibrations in lipid acyl chains and in CaP, where lipid metabolism is often disrupted, a decrease in intensity at 1437 cm^{-1} has been observed in higher-grade tumors, reflecting reduced lipid content and altered membrane composition. Lower signal intensity at this band may indicate higher Gleason scores, as aggressive tumors tend to have diminished lipid signatures due to increased cellular proliferation and membrane remodeling. The 1672 cm^{-1} Raman band falls within the amide I region, primarily associated with $\text{C} = \text{O}$ stretching vibrations in protein backbones, and potential shifts or increased intensity at 1672 cm^{-1} suggest changes in protein secondary structure, such as increased β -sheet content or protein denaturation, which are common in malignant tissues. Additionally, elevated or shifted signals at this band have been linked to higher Gleason grades, reflecting greater cellular atypia and metabolic activity. The 1672 cm^{-1} band may reflect thymine's $\text{C} = \text{O}$ stretching, while 1437 cm^{-1} is not directly linked to thymine but may indicate broader biochemical changes in CaP [61–65].

We should note that the artifacts around 1060 and 1140 cm^{-1} are due to the digital paraffin removal procedure, and the broad feature observed near 1100 cm^{-1} is likely influenced by residual paraffin contributions. This wavenumber region contains overlapping bands originating from both prostate tissue and paraffin, leading to partial spectral interference. The sharp peak near 1300 cm^{-1} also corresponds to paraffin-related vibrational modes. The elevated baseline between 1050–1150 cm^{-1} likely reflects a combination of tissue autofluorescence and residual paraffin contributions. Although baseline correction and digital dewaxing were applied, complete removal of wax-related background is challenging in FFPE sections and represents an inherent limitation of this sample type.

Figure 3 illustrates the effectiveness of Raman spectral analysis in differentiating CaP tissues across Gleason grades by applying a Linear Discriminate Analysis (LDA), indicated by a dashed line, to the SVD scatter plots. The scatter plots reveal distinct clustering of spectral data corresponding to normal controls and Gleason scores 7 through 10, indicating clear biochemical separation among tissue types. Spectral clusters for higher Gleason grades (G9–G10) show greater divergence from normal tissue, reflecting increased molecular heterogeneity and malignancy. Accompanying confusion matrices quantify the LDA classification performance for each Gleason group, demonstrating high accuracy in spectral differentiation. The matrices highlight robust sensitivity and specificity, particularly for distinguishing high-grade CaP from lower-grade and normal tissues, underscoring the diagnostic potential of RS combined with multivariate analysis.

Figure 4 highlights the discriminative power of Raman spectroscopy in differentiating CaP tissues across Gleason scores and from normal controls. Singular vector component plots reveal distinct spectral variations, with clear differences between cancerous and non-cancerous tissues and among Gleason grades G7 to G10. These spectral distinctions reflect underlying biochemical changes associated with tumor progression and aggressiveness. Complementing these findings, receiver operating characteristic (ROC) curve analyses for each comparison dataset demonstrate high classification performance, with area under the curve (AUC) values indicating strong sensitivity and specificity. Together, these results underscore the feasibility of Raman spectroscopy as a non-invasive diagnostic tool for CaP grading and detection.

DISCUSSION

The RS analysis of CaP tissues across Gleason scores 7 to 10 revealed a series of biochemical alterations that reflect the progressive nature of malignancy. These spectral changes provide molecular insights into the metabolic reprogramming, structural remodeling, and genomic activity characteristic of aggressive CaP phenotypes.

A prominent peak at 987 cm^{-1} , indicative of lipid metabolism shifts and protein remodeling, underscores the early biochemical hallmarks of cancerous transformation. This band's presence across Gleason grades suggests its potential utility as a marker for disease progression. Complementary changes in bands at 1044 cm^{-1} and 1090 cm^{-1} further support this, with increased intensity reflecting enhanced

phosphate vibrations from nucleic acids and phospholipids, and C–C/C–O stretching in lipids and carbohydrates, respectively. These findings align with known patterns of membrane remodeling and genomic activation in high-grade tumors.

Carbohydrate metabolism also emerged as a key differentiator, with progressive increases in the 1106 cm^{-1} band, which is associated with α -glucose structures, suggesting elevated glucose uptake and utilization. This is consistent with the Warburg effect and other metabolic shifts observed in malignancy. Additional carbohydrate- and lipid-associated bands at 1122 cm^{-1} , 1140 cm^{-1} , and 1167 cm^{-1} demonstrated similar trends, reinforcing the role of glycosylation and membrane composition changes in tumor progression.

Protein-related bands showed a strong correlation with Gleason scores. The 1205 cm^{-1} and 1237 cm^{-1} bands, linked to C–C/C–N stretching and Amide III vibrations, respectively, increased with tumor grade, reflecting elevated protein synthesis and backbone remodeling. The 1337 cm^{-1} band, associated with nucleic acid and protein vibrations, particularly adenine and CH deformation, also intensified in higher-grade tumors, suggesting increased genomic activity and altered protein structure.

Lipid-associated bands such as 1319 cm^{-1} , 1418 cm^{-1} , and 1437 cm^{-1} exhibited reduced intensity in high Gleason scores, consistent with lipid depletion and membrane disruption. These changes may be driven by glutamine-dependent lipid synthesis pathways, which are upregulated in aggressive CaP. The 1462 cm^{-1} band, linked to CH_3 deformation in proteins and lipids, showed shifts in intensity, further supporting altered membrane dynamics and protein turnover. Lipid-dominated bands at 1418, 1437, and 1462 cm^{-1} showed reduced intensity in higher Gleason scores. While these modes partially overlap with paraffin vibrations in FFPE sections, their persistence and Gleason-dependent modulation after additional paraffin-component removal suggest a substantial contribution from tissue lipids and membrane-associated CH deformations.

The Amide I region bands, namely 1637 cm^{-1} , 1662 cm^{-1} , 1672 cm^{-1} , and 1687 cm^{-1} , demonstrated significant spectral shifts and intensity changes with increasing Gleason scores. These bands reflect changes in protein secondary structure, including increased β -sheet content and denaturation, indicative of elevated cellular turnover and metabolic stress. Notably, the 1672 cm^{-1} band may also capture C = O stretching in thymine, suggesting a link to nucleic acid metabolism and DNA replication activity in high-grade tumors.

The RS findings presented in this study offer clinically significant insights into the biochemical landscape of CaP progression and its correlation with Gleason scores. The lipid-associated bands 987 cm^{-1} , 1090 cm^{-1} , 1437 cm^{-1} , and 1462 cm^{-1} demonstrated reduced intensity in higher-grade tumors, reflecting disrupted lipid metabolism and membrane remodeling, which are hallmarks of aggressive cancer phenotypes. These changes suggest potential utility in identifying tumors with high proliferative capacity and altered energy storage pathways. Carbohydrate-related bands at 1106 cm^{-1} , 1122 cm^{-1} , and 1140 cm^{-1} showed progressive enhancement with increasing Gleason scores, indicating elevated glucose uptake and glycosylation activity, which are biochemical features consistent with the metabolic

reprogramming observed in malignancy. Protein structure and turnover were captured through amide-related bands at 1205 cm^{-1} , 1237 cm^{-1} , 1637 cm^{-1} , 1662 cm^{-1} , 1672 cm^{-1} , and 1687 cm^{-1} , which exhibited spectral shifts and intensity changes in high-grade tumors, reflecting increased protein synthesis, denaturation, and secondary structure alterations. These features are clinically relevant for assessing tumor aggressiveness and may inform therapeutic strategies targeting protein homeostasis. Nucleic acid-associated bands, including 1044 cm^{-1} , 1337 cm^{-1} , and 1584 cm^{-1} , showed increased intensity in higher Gleason scores, suggesting elevated genomic activity and DNA replication, which could aid in identifying tumors with high mutational burden or proliferative potential. Collectively, these spectral markers provide a molecular fingerprint of CaP progression and offer a non-invasive, objective complement to traditional histopathological Gleason scoring. Their integration into diagnostic workflows could enhance grading accuracy, reduce inter-observer variability, and support personalized treatment planning based on tumor biochemistry.

This study demonstrates the diagnostic utility of RS in grading CaP and distinguishing malignant from normal tissue. Figures 2, 3, and 4 reveal progressive biochemical alterations across Gleason scores, captured through distinct spectral features. Figure 2 shows average Raman spectra and SVD-based separation, highlighting key peaks (1400–1500 cm^{-1}) linked to tumor-associated molecular changes. Figure 3 presents SVD scatter plots with LDA clustering and confusion matrices, confirming accurate spectral classification, especially for high-grade CaP, and Fig. 4 further supports this conclusion with singular vector plots and ROC analyses, showing high sensitivity and specificity. Collectively, these findings validate RS as a robust, non-invasive tool for CaP assessment and support the integration of RS with multivariate statistical techniques for non-invasive, label-free CaP detection and grading. The ability to differentiate between Gleason scores and identify high-grade malignancies offers promising clinical utility, particularly in guiding biopsy decisions, monitoring disease progression, and personalizing treatment strategies.

CONCLUSION

CaP diagnosis and treatment strategies are based on the architecture of tissue structures, such as the Gleason score and the blood PSA levels. While biopsies and PSA levels are reliable technologies for oncologists due to their clinical utility, other alternatives that can capture the biochemical landscape of CaP progression are warranted. These involve identifying molecular changes, biomarkers, and proteomic shifts that signal disease advancement and recurrence. Collectively, our findings highlight the utility of RS in capturing the biochemical landscape of CaP progression. The observed spectral trends across Gleason scores support the integration of Raman-based diagnostics into histopathological workflows, offering a non-invasive, molecularly informed approach to tumor grading. The clinical relevance of these RS findings lies in their potential to enhance CaP diagnosis, grading, and treatment planning through non-invasive biochemical profiling. Future studies should explore the integration of these spectral markers with machine learning models to enhance the accuracy and reproducibility of Gleason scoring.

Declarations

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CONFLICT OF INTEREST STATEMENT:

The authors confirm that there are no relevant financial or non-financial interests to report.

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Figures

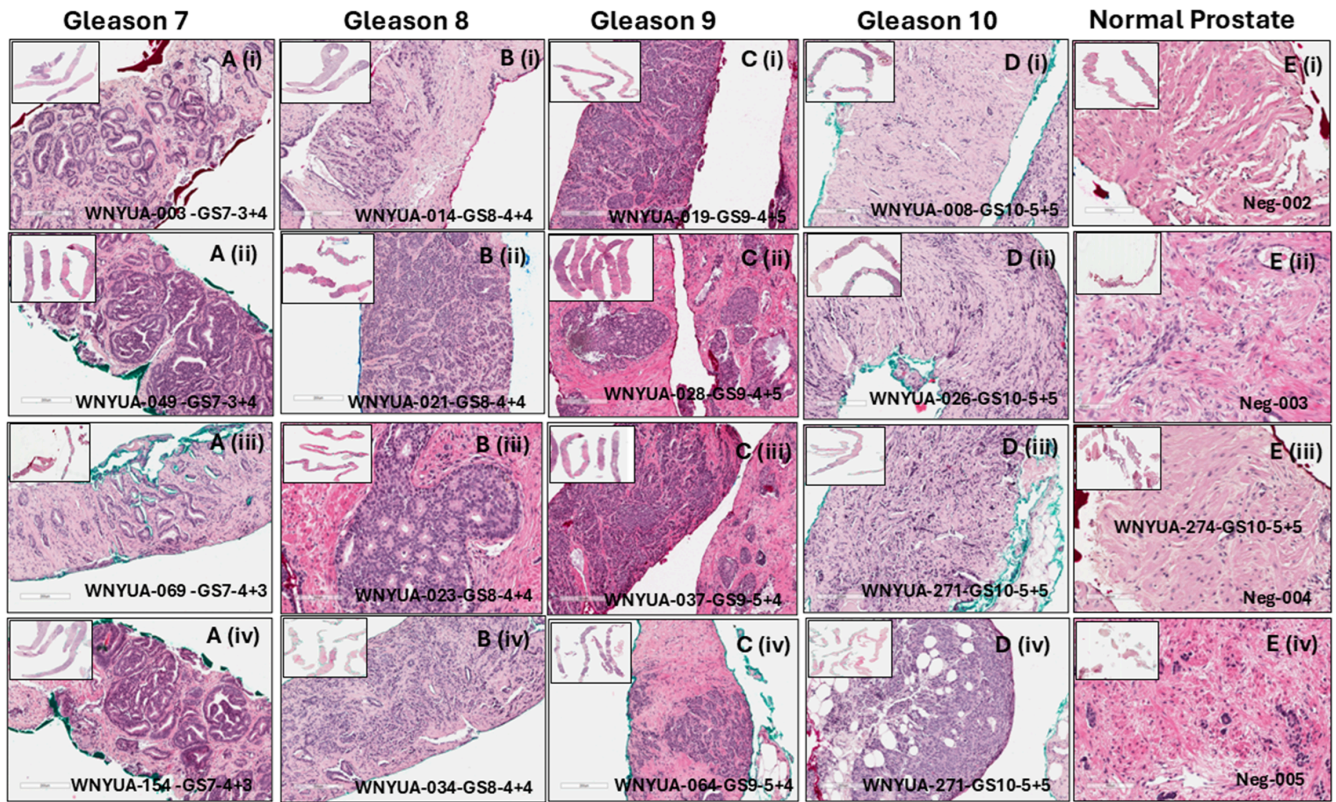


Figure 1

Representative H&E-stained sections of prostate tissue from all patient samples analyzed in the study (n=4) in each category (A i-iv), Gleason score 7; CaP tissue with mixed well-formed and poorly formed glands. (B i-iv) Gleason score 8; CaP tissue displaying fused and cribriform glands with nuclear atypia. (C i-iv) Gleason score 9 CaP tissue showing solid sheets of tumor cells and loss of glandular structure. (D i-iv) Gleason score 10 CaP tissue with undifferentiated tumor cells in single-cell infiltrates and extensive necrosis. (E i-iv) Normal prostate tissue showing well-organized glandular architecture and uniform epithelial cells. Scale bars: 100 μ m for each section, and Inlet image scale = 3 μ m

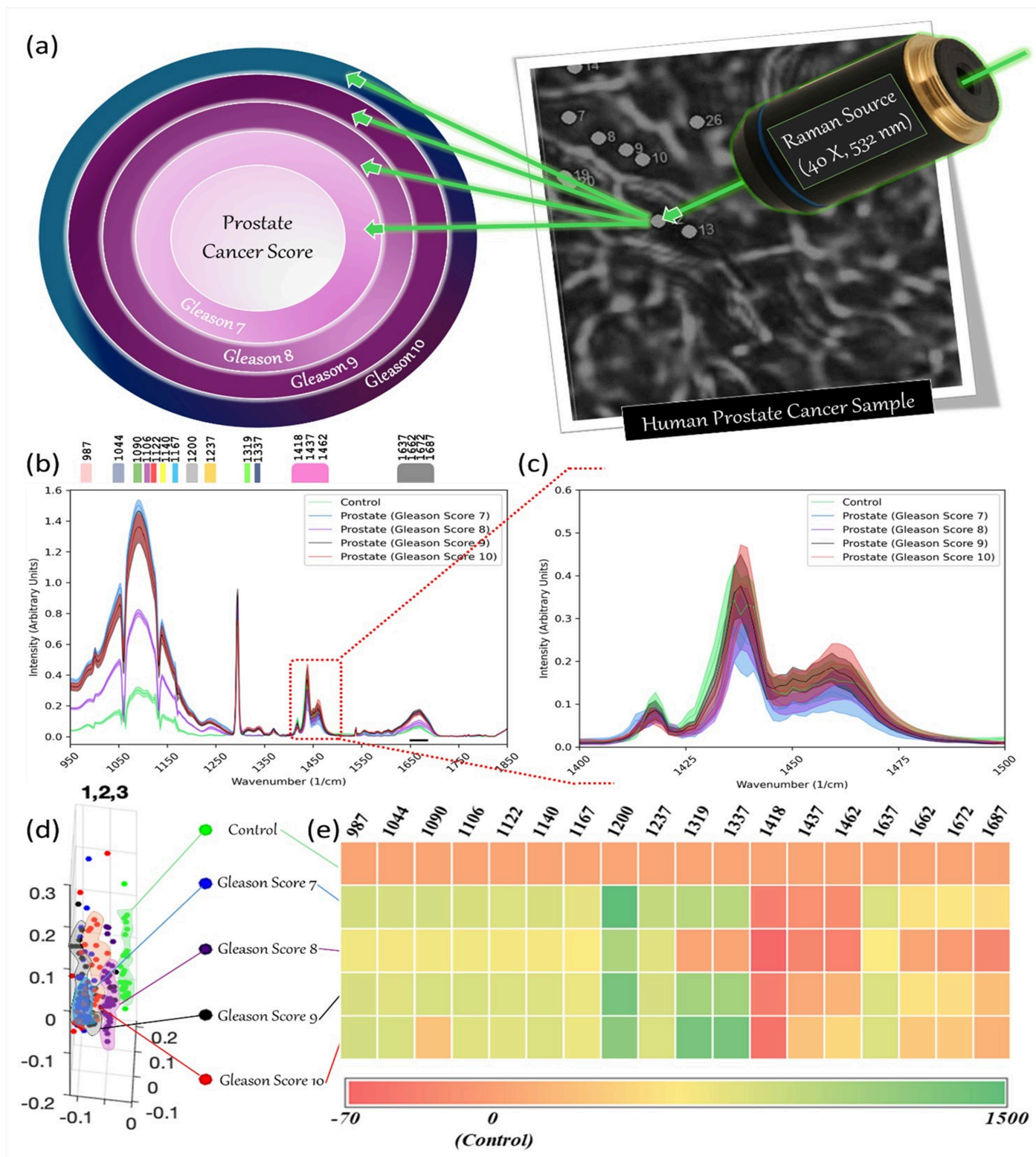


Figure 2

Representative Raman spectral analysis of CaP tissues with Gleason scores G7–G10 and normal controls. (a) Schematic overview of the Raman spectroscopy experimental setup. (b) Baseline-subtracted and normalized average Raman spectra for each Gleason score group and control, shown with 95% confidence intervals. (c) Inset highlights prominent spectral peaks within the 1400–1500 cm⁻¹ region. (d) Three-dimensional singular value decomposition (SVD) scatter plot illustrating spectral

separation between control and Gleason score groups. (e) Heatmap depicting intensity variations of key Raman bands across Gleason scores.

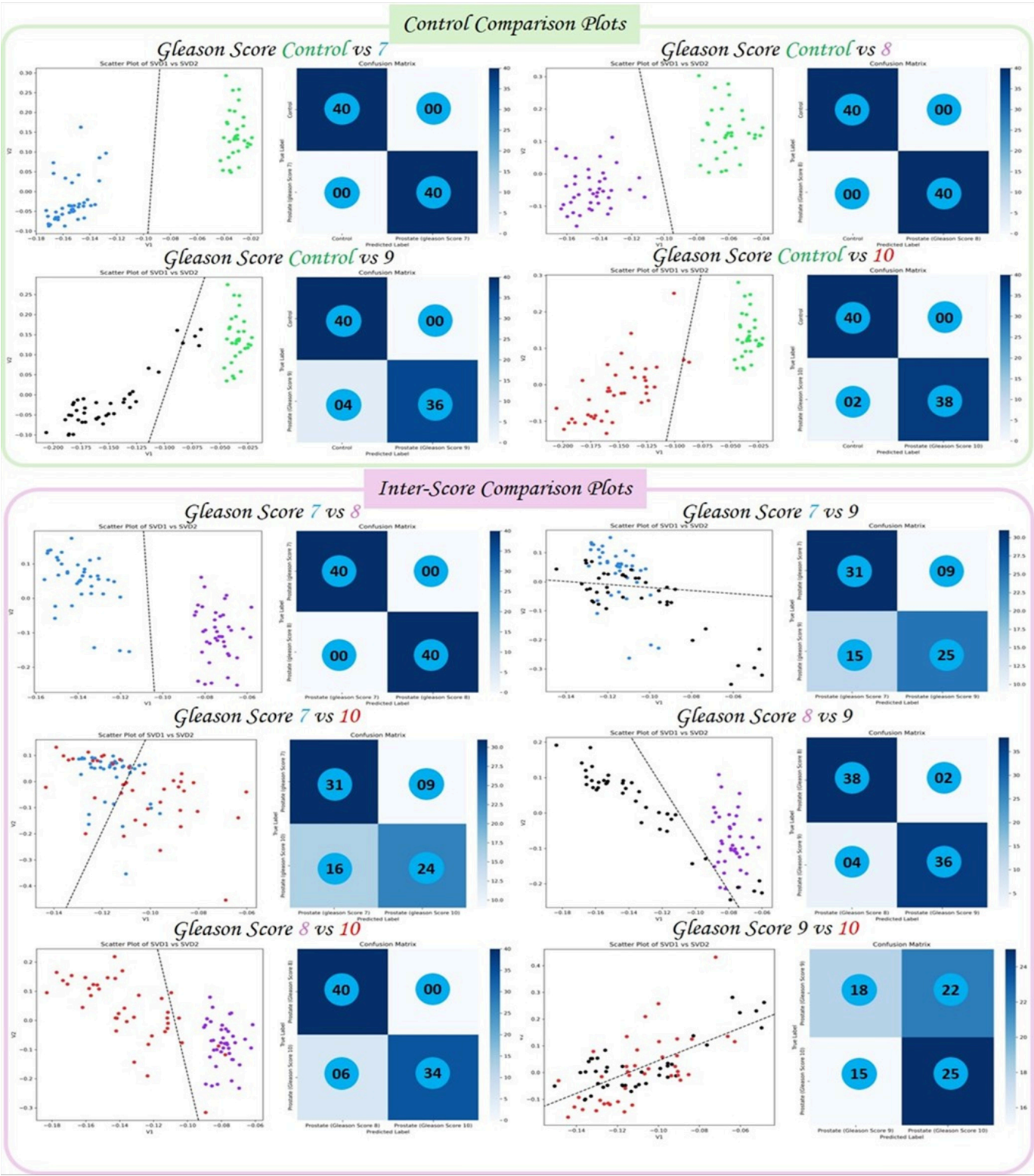


Figure 3

Singular value decomposition plots demonstrating clear separation of Raman spectral clusters corresponding to different Gleason grades of CaP. The accompanying confusion matrices illustrate

classification performance for each Gleason group, highlighting the accuracy of spectral differentiation across cancer grades.

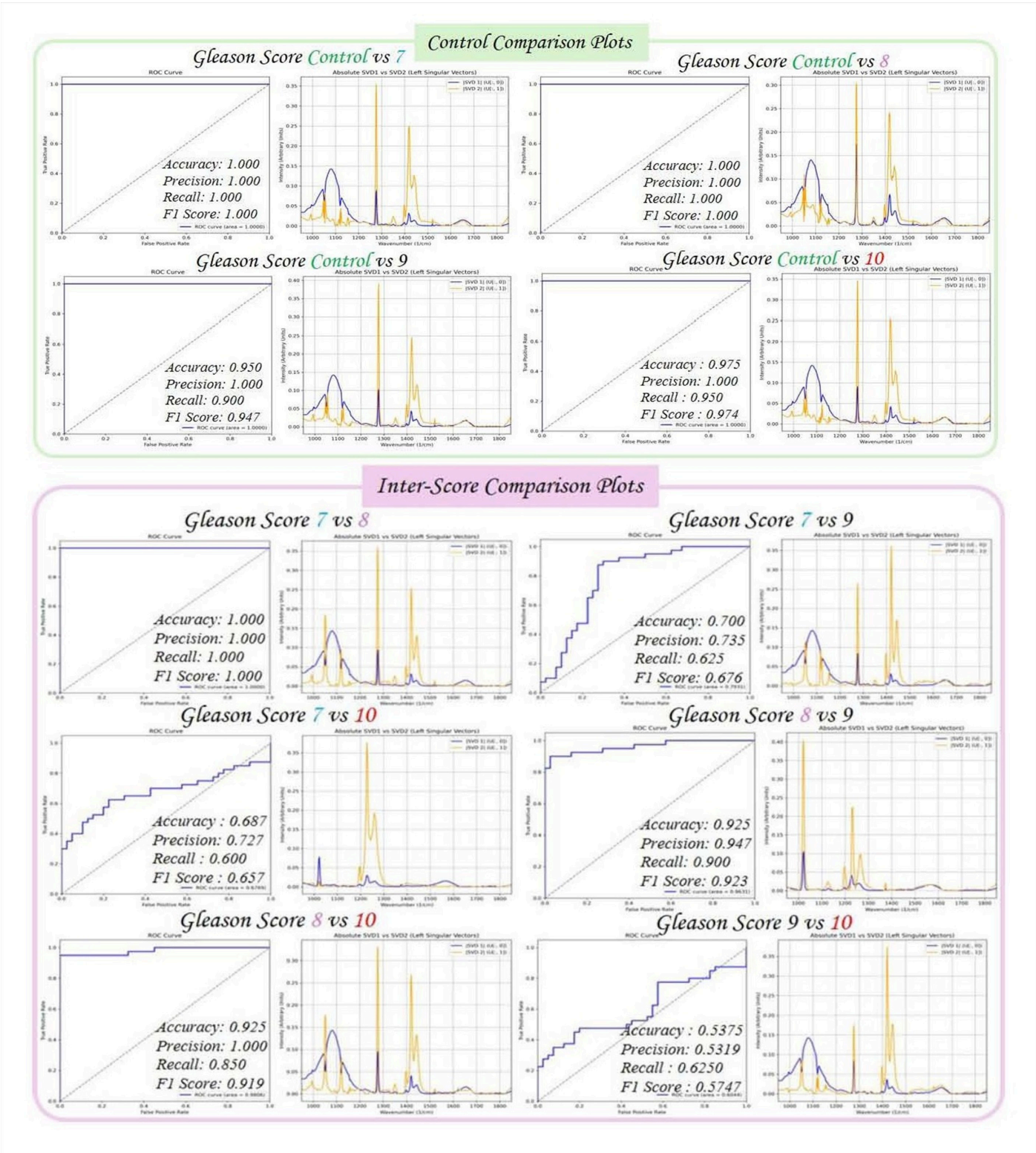


Figure 4

Singular vector component plots illustrate distinct spectral variations between CaP tissues of Gleason scores G7 to G10 and normal controls, alongside ROC curve analyses for each comparison dataset. The

distinct features observed in the plots underscore the potential of Raman spectroscopy to differentiate between CaP grades and between cancerous and non-cancerous tissue, supporting its feasibility as a diagnostic tool.

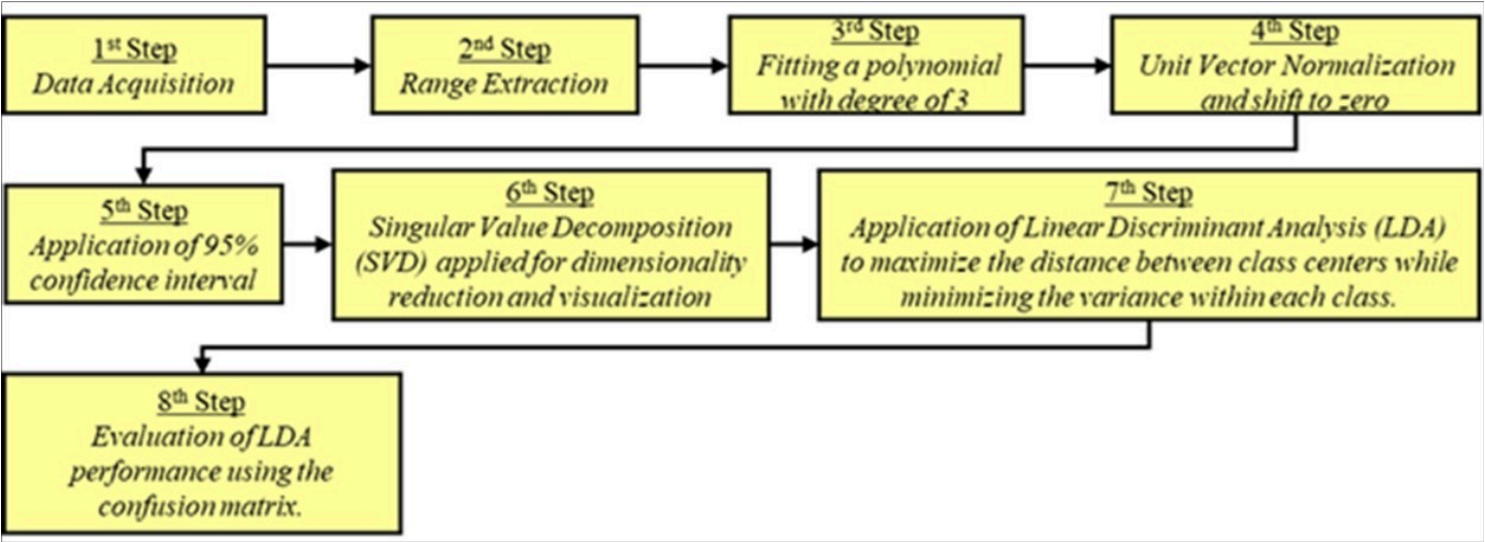


Figure 5

Unnumbered image in the Materials And Methods section.