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**Abstract.** The detection of kidney cancers at an early stage is critical for diagnosis and therapy. Surface-enhanced Raman scattering (SERS) is investigated for early detection of cancer cases from biopsy samples. The colloidal silver nanoparticles as the SERS-active nanostructures are directly mixed with homogenized tissue samples. The SERS spectra from the normal and abnormal tissue samples collected from 40 cancer patients, 28 of them at T1 stage and 12 of them at T2–T3 stages, are analyzed using principal component analysis combined linear discriminant analysis with leave-one-out cross-validation method. It is found that the diagnosis sensitivity, specificity, and total accuracy of the approach can be as high as 100%. The results suggest that SERS can be used as a potential technique for the identification of the different tumor stages.  [DOI: 10.1117/1.JBO.20.4.047002]

Keywords: kidney tumor; renal cell carcinoma; surface-enhanced Raman scattering; principal component analysis; linear discriminant analysis.

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## 1 Introduction

Kidney cancer is almost 2% of all cancers worldwide, with its most common types being renal cell carcinoma (RCC) and transitional cell carcinoma (TCC). As the mortality rate due to kidney cancers increases by 2%–3% per decade, about 210,000 new cases are reported each year and over 100,000 patients die due to the disease.[1] Kidney cancer is diagnosed based on the information obtained from imaging techniques, biopsy examinations, and blood and urine testing. The imaging techniques include ultrasound, intravenous pyelogram, computed tomography (CT or CAT) scan, cystoscopy/nephro-ureteroscopy, and magnetic resonance imaging.

The commonly used treatment approach for kidney cancer is surgery including radical nephrectomy, partial nephrectomy (PN), laparoscopic nephrectomy, and robotic-assisted laparoscopic nephrectomy. According to the American Joint Committee on Cancer (AJCC), the most common staging system for kidney cancer is the association between tumor stage and tumor size as T1a $\leq$ 4 cm, T1b $>$ 4 cm but $\leq$7 cm, T2a $>$ 7 cm but $\leq$ 10 cm, T2b $>$ 10 cm.[2] Tumor size is related to the recurrence rate, the survival rate, and the choice of clinical treatment method.[3,4] Several studies have shown that subdividing the T1 tumor stage into T1a and T1b stages is beneficial for a better estimation of the survival rate in patients with tumors in the size of 4 cm or less.[5,6] The lower recurrence rate after the PN process for the tumors <4 cm was reported.[7] In addition, the treatment of tumor sizes less than 4 cm showed no recurrence in the renal cell carcinoma patients followed for 61 months.[8] It was reported that a four-fold increase in the local recurrence was observed with every 1-cm increase in the tumor size.[9] The success rate of the cryoablation and radiofrequency ablation methods to treat kidney tumors is related to tumor sizes that are less than 4 cm.[10–13] The recent reports have shown that the 5-year survival rate is high in patients with RCC in the T1 early stage, and this rate is decreased in RCC patients as the cancer changes from T1 to T4 stages. The survival rate for patients is 94.9% in T1a, 92.6% in T1b, 85.4% in T2a, 70% in T2b, 64.7% in T3a, 54.7% in T3b, 17.9% in T3c, and 27.1% in T4 stage.[2] Hence, early detection and the prediction of the tumor stages increase the survival rate of kidney cancer patients with the prognostic morphologic parameters based on the microscopic morphology of a neoplasm with hematoxylin and eosin staining of cancer cell.[14]

Several spectroscopic techniques have been investigated to differentiate healthy and malignant tissues such as reflectance spectroscopy, fluorescence spectroscopy, light (elastic) scattering spectroscopy, infrared, and Raman spectroscopy.[15–23] Among these techniques, Raman spectroscopy has some advantages, such as narrow spectral bandwidth, minimal interference from water, almost no photobleaching, and very rich spectral information to determine molecular changes in a sample compared to other spectroscopic techniques.

Surface-enhanced Raman scattering (SERS), a mode of Raman spectroscopy, has been investigated for its use in clinical applications to overcome the disadvantages of inherent inefficiency of Raman scattering and strong fluorescence background of biological samples. In an SERS experiment, a nanostructured noble metal substrate such as gold and silver is used to enhance the Raman scattering.[24] In addition to the advantageous features of Raman scattering such as fingerprinting property, narrow bandwidth and minimum sample preparation, a sensitivity increase in the Raman scattering down to a single molecule can be achieved.[25] Since the Raman spectrum from a molecule or molecular structure is its fingerprint, it can be used for label-free detection and identification of a molecule or molecular structure. In our previous studies, we demonstrated

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the feasibility of using SERS for tissue differentiation.[26,27] The label-free detection and minimal sample preparation to obtain molecular information from a sample improves the efficiency in early detection of cancer.[28–33] Recently, SERS-based diagnosis of cancer has been emerged as a powerful approach to detect many cancer types such as esophageal, nasopharyngeal, gastric, breast, ovarian, thyroid, bladder, lung, colorectal, and renal cancer.[29,30,33–40]

In this study, SERS is evaluated for the identification and the classification of 40 normal and 40 abnormal pathologically evaluated tissue samples obtained from kidney cancer patients at different stages. The pattern recognition algorithm, a principal component analysis (PCA) combined with linear discriminant analysis (LDA), was used for the evaluation of the tissue samples. The accuracy of the classification results was predicted using leave-one-out cross-validation (LOO-CV) method. The results show that the classification of T1a, T1b, T2a, T2b, and T3a stage of renal cell carcinoma, and T3 stage of transitional cell carcinoma is possible with the SERS spectra obtained from tissue samples. This study demonstrates that SERS-based detection of early stage kidney cancer from tissue samples is a promising approach in clinical diagnosis.

## 2 Materials and Methods

### 2.1 *Synthesis of Silver Nanoparticles*

The Lee and Meisel method for preparation of colloidal silver nanoparticles (AgNPs) was used.[41] Eighteen mg of $AgNO_3$ was dissolved in 100 mL of distilled water by stirring. Then the solution was heated to boiling, and 2 mL of 1% (w/v) trisodium citrate solution was dropped into this boiling solution. Finally, the solution was kept boiling until it was only half of the initial volume. The solution was concentrated by centrifugation at 5500 rpm for 30 min, and one-third of the supernatant was decanted to increase the final concentration of the AgNPs colloidal suspension to four times, which was called 4×. The maximum of the UV/visible spectrum from the resulting AgNP suspension was at 420 nm [Fig. 1(a)] indicating the average size of the AgNPs as 50 nm. The final density of the AgNPs in the 4× concentrated suspension was calculated as $2.08 \times 10^{11}$ particles/mL.[42]

### 2.2 *Kidney Cancer Tissue Sample*

The biopsy samples of kidney tissues were obtained from the cancer patients with consent of the ethical approval from the Department of Urology at Okmeydani Education and Research Hospital. The tissue samples obtained during surgery were separated into two parts. One portion was saved for the histopathological examination, while the other half was stored at −80°C in plastic tubes until the sample preparation for the SERS measurements. The histopathological tumor staging was performed by a panel of pathologists. Table 1 shows the kidney tumors stages, the age, and the gender of the biopsied patients. For the SERS measurements, the sample preparation was performed using our previously reported method.[26] Briefly, a piece of tissue approximately in the size of $2 \times 2 \times 3$ $mm^3$ was cut from sample tissue and tumor samples and placed in a mortar with 5 mL of liquid nitrogen to be crushed with a pestle. This crushed and liquefied tissue was mixed with 200 $\mu$L of the AgNP suspension. Then a 5 $\mu$L volume of this mixture was placed onto a $CaF_2$ slide, and each of the $CaF_2$ slides was dried in an inverted position under sterile conditions.[43] The average number of spectra collected on each tissue sample was 10 with three repeated Raman measurements. A total of 800 SERS spectra were acquired from 40 normal and 40 abnormal tissues, in which 280 SERS spectra were from T1 stage tumor tissues, 120 SERS spectra were from T2–T3 stage tumor tissues, and 400 SERS spectra were from the normal tissues.

### 2.3 *Raman System and Surface-Enhanced Raman Scattering Measurements*

A Renishaw InVia Reflex Raman microscopy system (Renishaw Plc., New Mills, Wotton-under-Edge, UK) equipped with an 830-nm diode was calibrated by using the silicon phonon mode at 520 $cm^{-1}$. The incident laser power was 3 mW on the sample, and the spectral data acquisition time was 10 s. The SERS spectra were acquired over a spectral range of $400 - 1800$ $cm^{-1}$ with a 50× microscope objective (NA: 0.50). The SERS spectra were collected from randomly selected points on the sample using the "map image acquisition method" function in WIRE 2.0 software, and the WIRE 2.0 software carried out the spectral analyses.

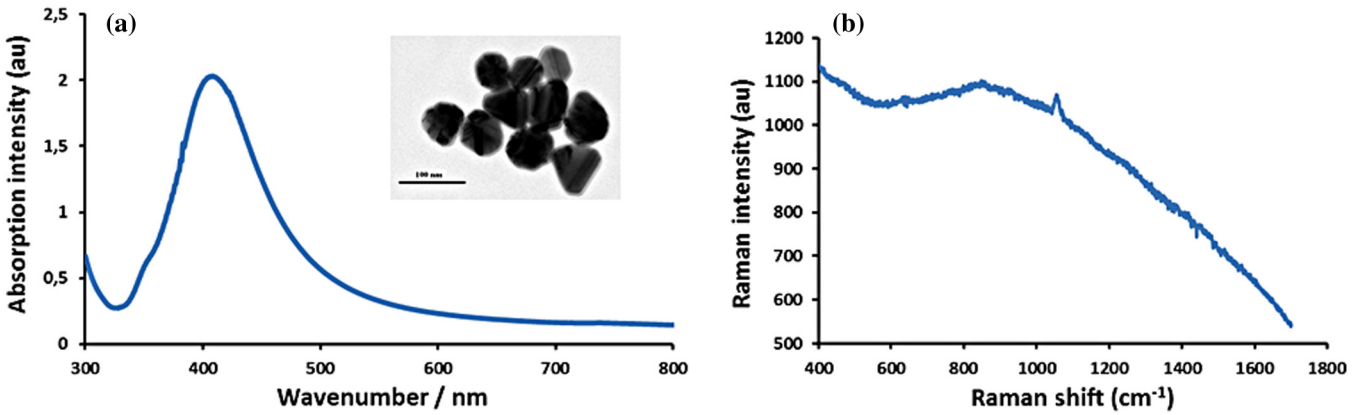


**Fig. 1** (a) UV/visible absorption spectra of silver nanoparticle (AgNP) colloidal suspension. (b) Surface-enhanced Raman scattering (SERS) spectrum of colloidal AgNPs. The insert shows the transmission electron microscopy micrograph of AgNPs.

**Table 1** Histopathological tumor staging, age and gender of patients that tissues are biopsied.

| SN | CT | TS | G | A |
|---|---|---|---|---|
| S1 | RCC | T1b | F | 48 |
| S2 | RCC | T1a | M | 56 |
| S3 | RCC | T2b | M | 67 |
| S4 | RCC | T1a | F | 69 |
| S5 | TCC | T3 | M | 74 |
| S6 | RCC | T1b | M | 64 |
| S7 | RCC | T2a | F | 51 |
| S8 | RCC | T1b | F | 65 |
| S9 | RCC | T1a | M | 49 |
| S10 | RCC | T1a | M | 71 |
| S11 | RCC | T1b | F | 63 |
| S12 | RCC | T1b | M | 48 |
| S13 | RCC | T1b | M | 56 |
| S14 | RCC | T1a | M | 61 |
| S15 | RCC | T1a | F | 49 |
| S16 | RCC | T2a | M | 65 |
| S17 | RCC | T1b | F | 59 |
| S18 | RCC | T1b | F | 62 |
| S19 | RCC | T2a | M | 39 |
| S20 | TCC | T3 | M | 61 |
| S21 | RCC | T3a | M | 71 |
| S22 | RCC | T1a | M | 45 |
| S23 | RCC | T1a | M | 58 |
| S24 | RCC | T1b | F | 44 |
| S25 | RCC | T1b | M | 62 |
| S26 | RCC | T3a | M | 55 |
| S27 | RCC | T1b | M | 67 |
| S28 | RCC | T1b | F | 68 |
| S29 | RCC | T3a | M | 71 |
| S30 | RCC | T3a | M | 58 |
| S31 | RCC | T3a | M | 61 |
| S32 | RCC | T1b | F | 46 |
| S33 | RCC | T3a | M | 60 |
| S34 | RCC | T1a | F | 62 |
| S35 | RCC | T1b | M | 59 |
| S36 | RCC | T1a | F | 48 |
| S37 | RCC | T1a | M | 64 |
| S38 | RCC | T1b | M | 73 |
| S39 | RCC | T1b | F | 56 |
| S40 | RCC | T1a | M | 51 |

Note: SN: Sample number; CT: Cell type; TS: Tumor stage; G: Gender; A: Age.

### 2.4 *Spectral Data Processing and Analysis*

Each of randomly selected 10 SERS spectra obtained from each tissue sample was normalized to reduce the variations in the Raman intensity and to permit comparison of the spectral shapes. The spectral dataset was processed with the statistical package for the social science (SPSS) package (SPSS Inc., Chicago, Illinois), which contains PCA and LDA algorithms for statistical analysis to clarify the significant spectral characteristics of each tissue type and differentiate tissue types from each other for tumor stage identification. PCA was applied before LDA to reduce the number of dimensions ($d = 2009$) in the original high-dimensional dataset. To apply PCA, the spectral data observations having 2009 predictors were placed into a data matrix using 10 SERS spectra for each tissue sample. The eigenvalue decomposition was performed to the covariance matrix of the spectral data matrix. The resultant eigenvectors were obtained, and the original spectral dataset was projected into the new coordinate system defined by the principal directions of variance, called the principal components (PCs), which are the linear combination of the original data variables.[44] One way analysis of variance (ANOVA)[45,46] is used to determine the most significant PCs ($p < 0.05$), then PCs were used in LDA to generate diagnostic algorithms for the classification of the tumors with different stages. LOO-CV[47] methodology was applied to demonstrate the accuracy of the classification.[48,49] The significant differences in the Raman peak intensities for each tissue class were obtained by applying one-way ANOVA with a Tukey *post-hoc* test (significance level $p < 0.05$).[45,50,51]

## 3 Results and Discussion

### 3.1 *Spectral Characteristics of Normal and Cancerous Kidney Tissues*

SERS spectra were recorded from each type of normal and cancerous tissues. The mapping method of WIRE 2.0 software was applied to collect at least 10 spectra from randomly selected points from the dried sample composed of homogenized tissue and the colloidal AgNPs. The histopathologic stages of the cancer tissues according to TNM (tumor, node, and metastasis) classification were T1a, T1b, T2a, T2b, and T3a for RCC and T3 for TCC. The mean spectra of RCC tissue from the different stages T1a (120), T1b (160), T2a (30), T2b (10), and T3a (60) with the mean spectra of TCC tissue from the T3 (20) stage tumor and the mean spectra of normal tissues (400)

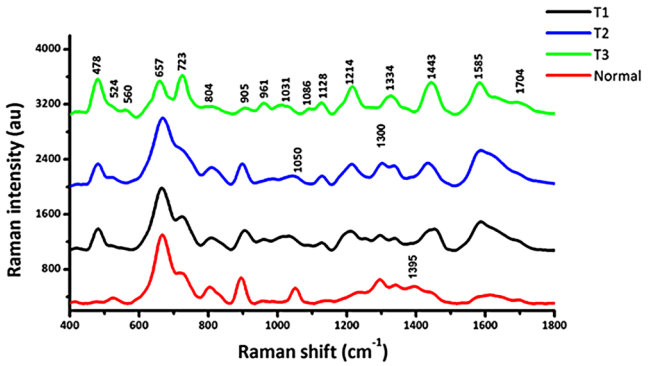


**Fig. 2** Mean SERS spectra obtained from normal tissues and cancerous tissues at T1 (T1a-T1b), T2 (T2a-T2b), T3 (T3a) stages.

were normalized to the integrated area under the curve in the range of $400-1800\ \mathrm{cm}^{-1}$ to enable a better comparison of the spectral shapes and the relative peak intensities among on the spectra obtained from the different tissue samples. Figure 2 shows the comparison of the normalized average SERS spectra acquired from the normal tissues and cancerous tissues at T1 (T1a–T1b), T2 (T2a–T2b), and T3 (T3a) stages. The major SERS peaks, which can be attributed to biochemical constituents such as nucleic acids (478, 560, 723, 1086, and 1334 $\mathrm{cm}^{-1}$), proteins (524, 657, 804, 1031, 1050, 1214, 1300, 1395, 1443, 1585, and 1704 $\mathrm{cm}^{-1}$), carbohydrates (905 $\mathrm{cm}^{-1}$), and lipids (961, 1128, and 1443 $\mathrm{cm}^{-1}$), were obtained from normal and abnormal tissue subjects. The tentative assignments for the observed SERS bands are listed in Table 2 in order to understand the possible molecular basis of the changes in the tissue samples based on previously reported studies in the literature.[40,52–63] Figure 3 shows that the normalized intensities of SERS peaks at 478, 560, 723, 961, 1031, 1086, 1128, 1214, 1334, 1443, 1585, and 1704 $\mathrm{cm}^{-1}$ are higher for the tumor tissues than those of the normal tissues, while the intensities of the SERS bands at 657, 1050, and 1395 $\mathrm{cm}^{-1}$ are higher on the spectra obtained from the normal tissues. The statistical significance of differences in the peak intensities between the different pathology groups and normal group was identified using one-way ANOVA with a Tukey *post-hoc* test (significance level $p < 0.05$).

**Table 2** Peak positions and tentative assignment of Raman bands.[40,52–63]

| Peak positions ($\mathrm{cm}^{-1}$) | | | | |
|---|---|---|---|---|
| Normal | T1 stage | T2 stage | T3 stage | Major assignment |
| — | 478 | 478 | 478 | DNA/ RNA |
| 524 | 524 | 523 | 524 | S—S disulfide stretching in proteins |
| — | — | — | 560 | G/ T/ U |
| 666 | 662 | 664 | 657 | C—S stretching mode of Cys |
| 726 | 724 | 722 | 723 | A (ring breathing mode of DNA/RNA bases) |
| 804 | 807 | 805 | 804 | Asn |
| 901 | 902 | 904 | 905 | Pro/Val/Glycogen |
| 962 | 960 | 963 | 961 | Cholesterol |
| 1031 | 1030 | 1033 | 1031 | C—H in-plane bending mode of Phe |
| 1050 | 1048 | 1048 | 1050 | C—O stretching, C—N stretching in proteins |
| — | — | — | 1086 | Phosphodiester groups in nucleic acids |
| 1129 | 1128 | 1129 | 1128 | C—C stretching mode of lipids |
| 1216 | 1214 | 1214 | 1214 | C—$C_6$—$H_5$ stretching mode in Tyr and Phe |
| 1298 | 1298 | 1300 | 1300 | Ser/Glu/Lipids |
| 1335 | 1337 | 1335 | 1334 | DNA—purine bases/ Collagen |
| 1395 | — | — | — | Protein |
| 1445 | 1445 | 1443 | 1443 | Collagen and phospholipids |
| 1586 | 1585 | 1584 | 1585 | C=C bending mode of Phe |
| 1704 | 1704 | 1704 | 1704 | Phe |

Note: A-Adenine; Asn-Asparaginase; Cys-Cysteine; Glu-Glutamate; G-Guanine; Ser-Serine; T-Thymine; Tyr- Tyrosine; Phe-Phenylalanine; Pro-Proline; U-Uracil; Val-Valine.

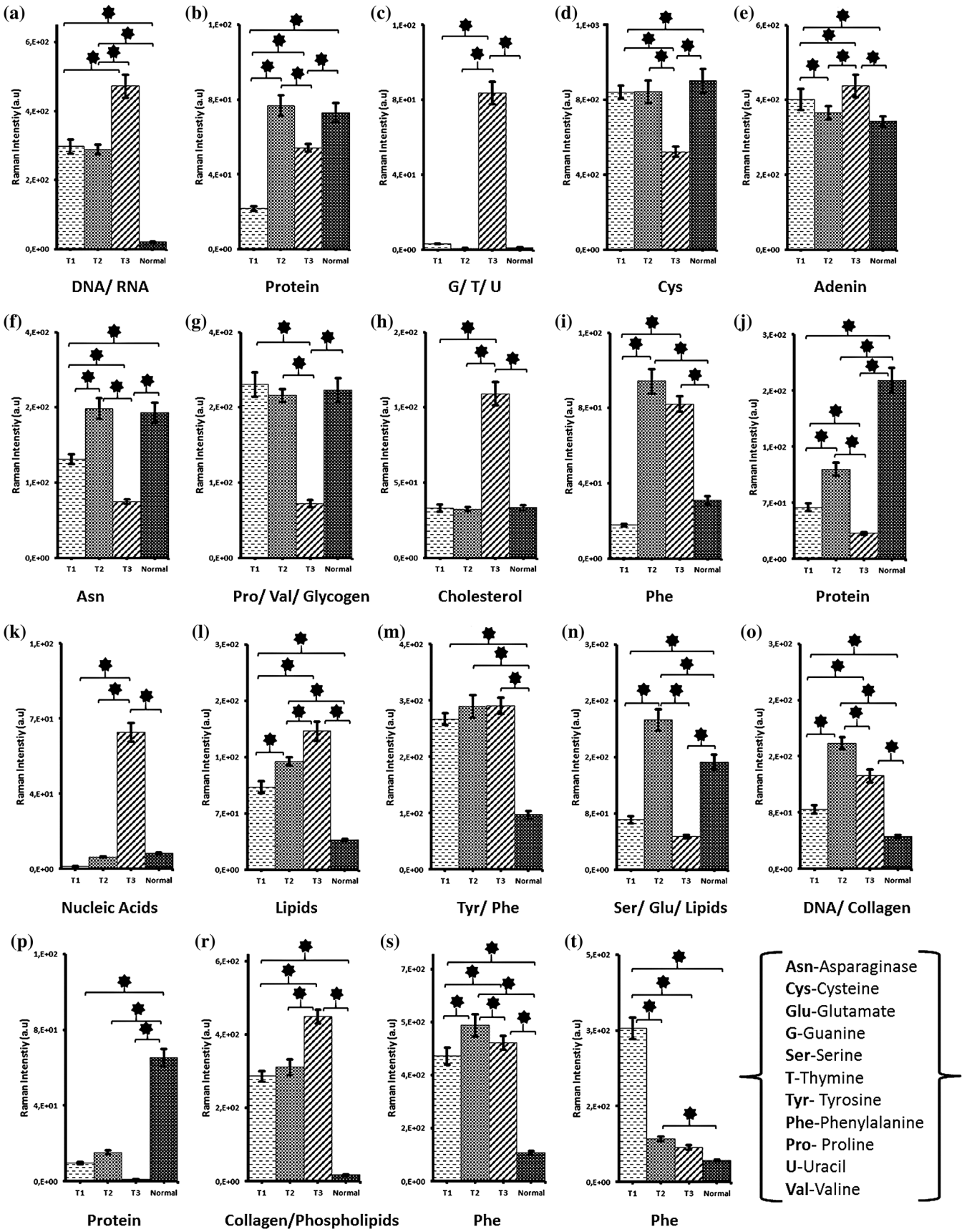


**Fig. 3** Column plots of 19 significant SERS peak intensities for the four tissue types (normal, T1, T2, and T3 stage cancers) (a) 478, (b) 524, (c) 560, (d) 657, (e) 723, (f) 804, (g) 905, (h) 961, (i) 1031, (j) 1050, (k) 1086, (l) 1128, (m) 1214, (n) 1300, (o) 1334, (p) 1395, (r) 1443, (s) 1585, (t) 1704 $cm^{-1}$. Error bars represent the standard deviation for SERS spectra obtained from each type of tissue. *Significantly different from each other [$P < 0.05$, one-way analysis of variance (ANOVA) with Tukey's HSD *post-hoc* test].

The selected spectral intensities, where the standard deviations do not overlap, were displayed in Fig. 3, and the differences are thus significant and reproducible. The significant decrease and increase for biomolecules is relative to the total Raman active components in the different tissue groups. These spectral intensity differences for the different pathological tissues and normal tissues could be evaluated for a better understanding of molecular changes between malignant and normal tissue types. The peak intensities at 478, 723, and 1334 $cm^{-1}$, primarily related to nucleic acids, were found to be increased in the cancer groups, indicating the uncontrolled fast replication of DNA in cancer cells. This is associated with the increased nucleic acid content in cancer cells. The band intensities at 560 and 1086 $cm^{-1}$ were higher on the spectra obtained from tissues at the T3 stage than the normal and T1–T2 stages.[64] The band at 961 $cm^{-1}$, attributed to cholesterol, is more intense on the spectra obtained from the tumors, especially at the T3 stage. This increased intensity may be attributed to an increased cholesterol synthesis in cancer tissues.[65,66] Phenylalanine-related bands at 1031, 1214, 1585, and 1704 $cm^{-1}$ in cancer groups are associated with increased phenylalanine contents relative to the total Raman-active components in cancer tissues. [58,63] In a study, it was found that the uptake rate of amino acids in cancer was higher.[67] The increased band intensity at 1128 $cm^{-1}$ in the cancer groups may be attributed to the increased lipid concentration in the tumors. The studies comprising the high levels of fatty acid synthesis related to the tumor aggressiveness are consistent with our study results.[68–70] The band at 1443 $cm^{-1}$, which is probably characteristic of collagen and phospholipids, shows a higher intense signal in malignant tissues and indicates that the collagen synthesis significantly increased in the cancerous tissues.[71] The bands at 657, 1050, and 1395 $cm^{-1}$, more intense in the normal tissue, are assigned to proteins, and the bands at 524, 804, and 1300 $cm^{-1}$, probably originating from proteins or lipids, were more intense in the normal tissues than the tumors at the T1 and T3 stages, even though it was the most intense on the spectrum from the cancerous tissue at the T2 stage. Note that there is a possibility that more than one band's vibrations might contribute to the band observed on the spectra. In addition, the intensity of the band at 905 $cm^{-1}$, associated with protein or glycogen, was higher on the spectra obtained from the normal tissues than that of the tumor at the T3 stage. However, the highest band intensity at 905 $cm^{-1}$ was obtained from tumors at the T1 and T2 stages.

### 3.2 *PCA-LDA as Diagnostic Algorithms*

Renal cell carcinoma staging has been used as a significant prognostic factor for kidney cancer patients because the survival rate of patients with renal cancer is reduced from the early stage to the late stage. The PCA-LDA model was built to predict the classification of tissue types and to improve the diagnostic utility of kidney cancer patients. The SERS spectra acquired from normal and abnormal tissues were processed in SPSS software package (SPSS Inc., Chicago, Illinois) for PCA-LDA analysis after the intensity of the spectra was scaled within a similar range using the min–max normalization method to compare the relative peak intensities among the normal tissue and tumor stages in a more precise manner. The significant PCs obtained using one-way ANOVA comparison test ($p < 0.05$) was used in LDA to generate a diagnostic assay. The scatter plot of the posterior probabilities based on the linear discriminant scores of the normal and the cancerous tissues using the PCA-LDA diagnostic algorithm is provided in Fig. 4. Each dot on the plot is associated with the SERS spectra acquired from each type of tissue. The LDA scatter plot of the classification model developed to differentiate the cancerous and the normal tissue samples shows a good discrimination among the normal and abnormal tissues at the different tumor stages. The discrimination results based on SERS spectra using a leave one out-cross validation method to evaluate the performance of the PCA-LDA models for the classification of different tumor stages in terms of sensitivity, specificity and 95% confidence interval of accuracy was displayed in Fig. 5. The RCC tumors at T1a stage related to PCs, which were subtracted from SERS spectra, were well differentiated from RCC tumors at T1b, T2a, T2b, and T3a stages, TCC tumors at T3 stage and the normal tissues with the diagnostic sensitivities of 100%, 100%, 65%, 100%, 89%, and 65%, the specificities of 100% from all types of tumor stages and 70% from the normal subjects, and the accuracy of 100%, 100%, 88%, 93%, 100%, and 69%, respectively. The discrimination results of the diagnostic combinations of RCC tumors at T1b stage versus T2a, T2b, and T3a stages, TCC tumors at T3 stage and normal tissues, and RCC tumors at T2a stage versus RCC tumors at T2b, T3a stages, TCC tumors at T3 stage and the normal tissues were achieved with a sensitivity, specificity, and accuracy of 100% while the posterior probabilities of RCC tumors at T2b stage versus RCC tumors at T3a stage, TCC tumors at T3 stage and normal tissues were obtained with sensitivities of 88%, 100%, and 80%, specificities of 43%, 100%, and 84%, and accuracies of 57%, 100%, and 83%, respectively. The RCC tumors at the T3a stage versus normal tissues were diagnosed with a sensitivity, specificity, and accuracy of 100%, and TCC tumors at the T3 stage were classified with a sensitivity of 100% and 70%, a specificity of 98% and 60%, and an accuracy of 98% and 62%, respectively.

The tumors at the T1, T2, and T3 stages with normal tissues were also differentiated using PCA-LDA models of the spectral

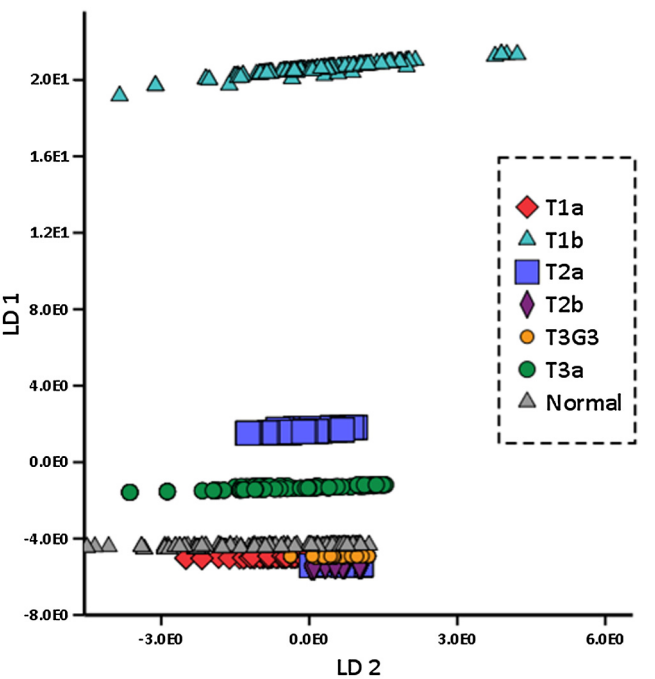


**Fig. 4** Scatter plot of posterior probabilities for classification of normal tissues and tumors.

| | T1b | T2a | T2b | T3 | T3a | Normal |
|---|---|---|---|---|---|---|
| **T1a tumor stage (RCC)** | 100 | 100 | 65 | 89 | 100 | 65 |
| | 100 | 100 | 100 | 100 | 100 | 70 |
| | 100 | 100 | 88 | 93 | 100 | 69 |
| **T1b tumor stage (RCC)** | 100 | 100 | 100 | 100 | 100 | 100 |
| | 100 | 100 | 100 | 100 | 100 | 100 |
| | 100 | 100 | 100 | 100 | 100 | 100 |
| **T2a tumor stage (RCC)** | 100 | 100 | 100 | 100 | 100 | 100 |
| | 100 | 100 | 100 | 100 | 100 | 100 |
| | 100 | 100 | 100 | 100 | 100 | 100 |
| **T2b tumor stage (RCC)** | 88 | 100 | 80 | | | |
| | 43 | 100 | 84 | | | |
| | 57 | 100 | 83 | | | |
| **T3 tumor stage (TCC)** | 100 | 70 | | | | |
| | 98 | 60 | | | | |
| | 98 | 62 | | | | |
| **T3a tumor stage (RCC)** | 100 | | | | | |
| | 100 | | | | | |
| | 100 | | | | | |
| **Sensitivity %** | | | | | | |
| **Specificity %** | | | | | | |
| **Accuracy %** | | | | | | |

**Fig. 5** Leave-one-out cross-validation (LOO-CV) classification results of normal tissues and tumor tissues (T1a, T1b, T2a, T2b, and T3a stages of RCC; T3 stage of TCC).

data obtained from the normal and the abnormal tissue samples. As compared to the classification results in Fig. 5, Fig. 6 shows a better discrimination among the different tissue classes with a diagnostic sensitivity of 89%, 96%, 94%, 70%, 97%, and 98%, specificity of 100%, 100%, 96%, 83%, 94%, and 77%, and accuracy of 99%, 97%, 95%, 81%, 95%, and 85%, respectively, for the classification between T1 and T2 stage cancers; T1 and T3 stage cancers; T1 stage cancer and normal tissues; T2 and T3 stage cancers; T2 stage cancer and normal groups; T3 stage cancer and normal tissue, respectively. The classification of tumors at the advanced stages (T2–T3), at the early stage (T1), and for normal tissues was obtained with sensitivities of 93% and 100%, specificities of 98% and 86%, and accuracies of 91%, and 90%, respectively, indicating that the classification of normal tissues, early stage tumors, and advanced stage tumors is possible with a high diagnostic efficacy.

The three-dimensional scatter plot of the diagnostic probabilities of LD1, LD2, and LD3 discriminants were shown in Fig. 7, illustrating a good classification among the different tumor stages and the normal tissue groups. The diagnostic performance of PCA-LDA models on the classification of tissue types has been acquired with an improved accuracy by the selection of significant PCs and different Raman bands ($p < 0.05$).

## 4 Conclusions

The differences in the biochemical components of normal and cancer tissues are reflected on the SERS spectra, which can be used in PCA-LDA multivariate statistical models for the tissue differentiation. The differentiation of the tumors at different stages and the normal tissues with a sensitivity of 89%, 96%, 94%, 70%, 97%, and 98%, a specificity of 100%, 100%, 96%, 83%, 94%, and 77%, and an accuracy of 99%, 97%, 95%, 81%,

| | T1 (T1a-T1b) | T2 (T2a-T2b) | T3 (T3a) | T2+T3 (Advanced stage) |
|---|---|---|---|---|
| **Normal** | 94 | 97 | 98 | 98 |
| | 96 | 94 | 77 | 86 |
| | 95 | 95 | 85 | 90 |
| | **T1 (T1a-T1b)** | 89 | 96 | 93 |
| | | 100 | 100 | 100 |
| | | 85 | 97 | 91 |
| | | **T2 (T2a-T2b)** | 70 | **Sensitivity %** |
| | | | 83 | **Specificity %** |
| | | | 81 | **Accuracy %** |

**Fig. 6** LOO-CV classification results of normal tissues and tumors at T1, T2, and T3 stages.

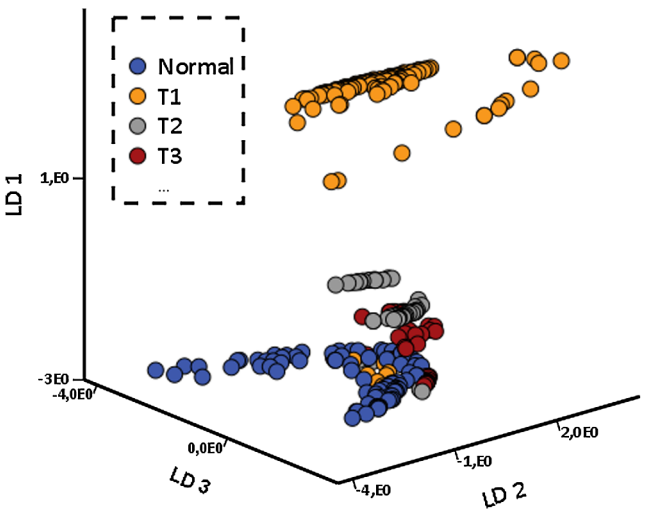


**Fig. 7** Three-dimensional (3-D) scatter plot of diagnostic probabilities of normal tissues and tumors at T1, T2, and T3 stages.

95%, and 85% between T1 and T2 stages; T1 and T3 stages; T1 stages and normal; T2 and T3 stages; T2 stages and normal; and T3 stages and normal tissue, respectively, has been successfully demonstrated using PCA-LDA. The diagnostic discrimination of tumors at advanced (T2–T3) stages against the early stage (T1) and normal tissues resulted in sensitivities of 93% and 100%, specificities of 98% and 86%, and accuracies of 91% and 90%, respectively.

In conclusion, the SERS has the ability to differentiate the early stage kidney cancers, T1, and advanced stage kidney cancer, T2–T3, and normal tissues, using PCA-LDA diagnostic classification algorithms. The results suggest that the SERS spectra from tissue samples can be used to reach a clinical decision for the stage determination of kidney cancers.

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