Raman spectroscopy for kidney tissue and its neoplasms research

A A Lykina 1, D N Artemyev 1, V I Kukushkin 2, I A Bratchenko 1, N S Aleksandrov 3 and V P Zakharov 1

1Samara National Research University, Moskovskoe Shosse 34, Samara, Russia, 443086
2ISSP RAS, Academician Ossipyan str. 2, Chernogolovka, Russia, 142432
3Sechenov University, Trubetskaya st. 8-2, Moscow, Russia, 119991

e-mail: zuum63@mail.ru, artemyevdn@gmail.com, kukushvi@mail.ru

Abstract. This work is devoted to the study of the spectral properties of intact and tumor kidney tissues. For excitation of Raman scattering and autofluorescence a laser with a wavelength of 785 nm was used. Visually and morphologically unchanged tissues of pig and human kidneys as well as tumor tissue of human kidneys were used as the test samples. The analysis of registered signals was carried out using the method of projection on latent structures to isolate the spectral features between tissue types. The study showed a difference between the registered signals of the clear cell adenocarcinoma and chromophobe types of human kidney cancer, the most informative bands of the Raman spectrum for the determination of the clear cell human kidney cancer were 330-500 cm⁻¹, 870-980 cm⁻¹, 980-1130 cm⁻¹, 1230-1270 cm⁻¹, 1360 -1420 cm⁻¹.

1. Introduction
At the present time, kidney cancer is one of the leading problems in the modern oncology, associated with high increase of sickness rate and difficulty of its diagnosis [1]. Histological research is utilized to investigate pathological processes in biological tissues. Analysis is based on the study of structure, vital activity and development of living tissues at the cellular level. Spectral methods have become widely used for the tissue analysis. They provide an opportunity to measure the optical properties of substances. One of the most promising methods is Raman spectroscopy [2-4]. The purpose of this work is the use of Raman spectroscopy to detect pathological changes in the kidney tissues. Pig kidney tissues were studied as a model medium, which includes the internal substance of the kidney (medulla) and external layer (cortex). The interaction effect of formalin and pig kidney tissues on the spectral characteristics was investigated. Results of model experiments showed an insignificant contribution of formalin to the detected signal of tissue, therefore, spectral properties of intact and tumor tissues of the human kidney were studied. Raman spectroscopy was used as the research method, which allowed for highlighting changes in chemical compounds of the examined samples.

2. Material and methods

2.1 Experimental setup
Raman spectra were collected by setup including thermally stabilized diode laser LML-785.0RB-04 (785 nm, 200 mW), commercial Raman probes (Inphotonics RPB785), and spectrograph Shamrock SR-500i-D1-R with deeply cooled digital camera Andor iDus DU416A-LDC-DD (air-cooled up to...
-70 °C). The optical scheme of the experimental setup for Raman scattering registration is presented in Fig. 1. Detailed information about the experimental setup presented in paper [5].

![Optical scheme of the experimental setup](image)

Figure 1. Optical scheme of the experimental setup (LPF- long-pass filter, BPF - band-pass filter, DM- dichroic mirror, M- deviating mirror, L₁⁻³ - lens).

All spectra were registered in 780-950 nm spectral range, the exposure time was 60 seconds. The three spectra for each studied sample were registered sequentially.

2.2. Tested samples
Samples of the pig kidney tissues were studied as a model medium. Pig kidney tissue was examined immediately after excision (unfixed state), then the sample was placed in formalin (fixed in formalin state). The standardized collection of human tissues (11 samples) with pathological neoplasms was performed. 1 sample corresponded to safe kidney tissue, 9 samples are clear cell types of the kidney cell cancer, and 1 sample is chromophobe subtype of the renal cell cancer. The tested samples were fixed in formalin. Tissue of human samples were obtained from the Sechenov University and its type (intact or tumor) were determined by histological and immunohistochemical methods.

2.3. Data processing methods
Projection to latent structures (PLS) method was used for the experimental data analysis [6]. The objective with PLS is to select a model with the appropriate number of components that has good predictive ability.

The registered signal includes the autofluorescence (AF) and Raman scattering, so a raw spectrum preprocessing was performed for the autofluorescence background removal. The Savitzky–Golay smoothing filter was used to reduce the noise effect on the registered signals [7]. The background signal was eliminated by baseline correction with asymmetric least squares smoothing [8]. The method uses a smoothed signal as the baseline for raw signal estimation. The method includes the parameters responsible for the signal smoothing procedure, the baseline position for raw signal and number of iterations. The data were normalized [6] using the standard deviation of a random variable with normal distribution. Preprocessing methods were implemented in the cloud service TPT cloud (https://tptcloud.com/).

Spectral informative bands of the regression model were determined from the analysis of the variable importance in projection (VIP) [9]. The higher VIP-score of an individual variable corresponds to the more significant values in the regression model. Variables with a low VIP-score are less important, and may be regarded as candidates for exclusion from the model.
3. Results and discussion

3.1. Study of porcine kidney biological tissues

At the first stage, the experimental setup parameters were selected to eliminate thermal damage of the examined samples. Then model experiments of Raman scattering registration for kidney tissues were performed. The interaction effect of formalin and kidney tissues on the spectral characteristics was analysed. Pig kidney tissues (cortex and medulla) were used as a model samples. Each registered signal was pre-processed by methods of AF background removal, signal smoothing, and data normalization for pure Raman spectra analysis. Raman spectra of pig kidney tissues include the peaks of proteins and lipids: 876 cm\(^{-1}\) (hydroxyproline C\(_5\)H\(_9\)N\(_3\) - collagen), 1031 cm\(^{-1}\) (C-H in-plane bending mode of phenylalanine - lipids and proteins), 1450 cm\(^{-1}\) (CH\(_2\) deformation - lipids and proteins) and 1542 cm\(^{-1}\) (Amide II - proteins) [2, 3]. Raman spectra of formalin include peaks corresponded to: 1040 cm\(^{-1}\) (anti-symmetric mode due to the C–O–C groups within the oligomer chains), 1258 cm\(^{-1}\) (CH\(_2\) deformation), 1410 cm\(^{-1}\) (In-plane bending modes connected with the OH group) and 1555 cm\(^{-1}\) (CH\(_2\) deformation) [10, 11]. Raw signals and Raman spectra of the renal cortex and medulla substances of pig kidney in two states are presented in Fig. 2 and Fig. 3.

![Figure 2. Normalized raw signal of pig kidney cortex in two states (a) and their pure Raman spectra (b).](image-url)

Raw signals and Raman spectra of medulla and cortex substances of the pig kidney in Fig. 2 and 3 coincide with each other in form and intensity of the Raman bands. It could be noticed that intensity of raw signals and the Raman spectra of the cortical and cerebral substances of the porcine kidney are different due to the formalin effect on the spectral characteristics. The intensity of raw signal of cortex fixed in formalin decreased by 7% relative to registered signal of cortex in the unfixed state. Raw signals intensity of medulla substance in unfixed state and fixed in formalin remain unchanged. Raman spectra of formalin (Fig. 2.b and Fig. 3.b) have the most intensive peak at 1040 cm\(^{-1}\). The contribution of this band to Raman spectra of pig kidney tissue is negligible relative to bands at 1200-1300 cm\(^{-1}\), 1370-1470 cm\(^{-1}\) and 1500-1590 cm\(^{-1}\). The intensity of raw signals of cortex substance differs to fixed/unfixed states, it can be assumed that the density of internal and external substance is
different. The absence of formalin bands in the Raman spectrum allows for studying spectral features of human kidney tissues fixed in formalin.

![Figure 3](image1.png)

**Figure 3.** Normalized raw signal of pig kidney medulla in two states (a) and their pure Raman spectra (b).

3.2. Research of biological tissues of human kidneys

The next stage of research was to register signals of kidney tumor tissues morphologically correspond to the clear cell and chromophobe types of cancer. Signals were compared with registered signal of safe human kidney tissue. Typical raw signals of human kidney tissues are shown in Fig. 4.

![Figure 4](image2.png)

**Figure 4.** Normalized raw signals of human kidney tissue.

Registered signals of safe tissue and chromophobe renal cell kidney cancer of human coincided in shape and didn’t have Raman peaks on the entire spectral range. Raw signal of clear cell adenocarcinoma kidney cancer has only one strong Raman peak at 1335 cm$^{-1}$, which corresponds to CH$_2$ wagging vibrations in collagen and nucleic acid [2, 4].

Raw spectra of tumor and safe human kidney tissues were processed by methods of AF background removal, signal smoothing, and data normalization to obtain pure Raman spectra, which presented in
Fig. 5. Informative bands of Raman spectra of human kidney tissues were determined by VIP-scores. VIP-variables were used to isolate the clear cell adenocarcinoma among other samples. Figure 5 shows all Raman spectra and VIP-variables of the regression model for our dataset.

Figure 5. VIP-variables of PLS model for clear cell adenocarcinoma discrimination from other kidney tissue types (Phe- phenylalanine, Trp-tryptophan).

Informative Raman peaks of human kidney tissues corresponded to bonds of protein and lipids: 876 cm\(^{-1}\) (hydroxyproline C\(5\)H\(9\)NO\(3\) - collagen), 975 cm\(^{-1}\) (CH stretching bonds of phenylalanine - protein), 1002 cm\(^{-1}\) (phenylalanine - protein), 1031 cm\(^{-1}\) (C-H in-plane bending mode of phenylalanine - lipids and proteins), 1260 cm\(^{-1}\) (Amide III - lipid), 1335 cm\(^{-1}\) (CH\(_2\) wagging - collagen), 1450 cm\(^{-1}\) (CH\(_2\) deformation - lipids and proteins) and 1630 cm\(^{-1}\) (Amide I - lipids and proteins). It was found that the intensities of bands on the spectral range 300-500 cm\(^{-1}\) of chromophobe cancer and adenocarcinoma human kidney cancer have higher AF intensity, than AF intensity of the safe tissue. Raman spectra of intact kidney tissue after background removal acquired Raman bands at 1031 cm\(^{-1}\), 1260 cm\(^{-1}\) and 1335 cm\(^{-1}\) with respect to raw signals, which allowing for distinguishing it from pathological formations. In the Raman spectra of intact tissue and cancer tissues we observed the bands at 1335 cm\(^{-1}\), 1260 cm\(^{-1}\), 1630 cm\(^{-1}\), which are corresponding to components of human kidney [2, 4]. Informative Raman peaks of the clear-cell human kidney cancer are 876 cm\(^{-1}\), 975 cm\(^{-1}\), 1002 cm\(^{-1}\), 1335 cm\(^{-1}\) and 1450 cm\(^{-1}\), which belong to proteins and lipids. The selected Raman bands have a wide spectral range, and VIP variables highlight the most informative part of the band for discrimination clear-cell cancer tissues. VIP-variables with maximal intensities (most informative bands) located at 1335 cm\(^{-1}\), 1002 cm\(^{-1}\) and 330-500 cm\(^{-1}\) (AF). It could be noted that Raman bands at 1630 cm\(^{-1}\) and 1450 cm\(^{-1}\) are’t informative for PLS model because VIP scores at these positions are close to zero.

Tissues and kidney cells have already been studied using Raman spectroscopy. In work [4] samples of chromophobe cancer types and renal oncocytoma were studied. The most informative bands for sample separation were 1035 cm\(^{-1}\), 1120 cm\(^{-1}\) and 1612 cm\(^{-1}\) peaks. Raman peaks of human kidney tissues [4] have coincidences with our data on wavenumbers 1035 cm\(^{-1}\) and 1336 cm\(^{-1}\). In this paper, spectral differences of human kidney tissues could be caused by the presence of high concentrations of mitochondria in the cytoplasm for renal oncocytoma cells [12]. The study [13] demonstrated SERS method with silver nanoparticles for the differentiation of cancer and healthy kidney cells. The most informative peaks of cancer cells were 667 cm\(^{-1}\) (lipoprotein and glutathion), 800 cm\(^{-1}\) (cytosine, proteins, lactic acid and glutathione), 993 cm\(^{-1}\) (phenylalanine and aspartic acid), 1330 cm\(^{-1}\) (proteins) and 1576 cm\(^{-1}\) (proteins) bands. Raman peak at 993 cm\(^{-1}\) is increased for cancer cells relative to healthy cells. This fact indicates a concentration increase of phospholipids in cancer cells, because of possible increase in production of fatty acids. In cancer cases the cell division is accelerated, so they
need to produce new phospholipids to maintain cell membrane integrity. Positions of Raman peaks of kidney tissues in our work and work [13] have coincidences on wavenumbers of 1002 cm\(^{-1}\) and 1336 cm\(^{-1}\).

4. Conclusion

The conducted research showed the Raman signals of the pig kidney cortex fixed in formalin have an insignificant contribution of formaldehyde due to differences in the density of medulla and cortex substances. The Raman signals of the medulla and the cortex remain unchanged for both (unfixed and fixed in formalin) states.

Spectral differences of human kidney clear-cell adenocarcinoma are probably caused by the presence of mitochondria high concentrations in the cytoplasm of cancer cells associated with intensity differences at 876 cm\(^{-1}\), 975 cm\(^{-1}\) and 1335 cm\(^{-1}\).

5. References

[1] Humphrey P A, Dehner L P and Pfeifer J D 2008 *The Washington Manual of Surgical Pathology* (Philadelphia: Lippincott Williams & Wilkins.)
[2] Huang N, Short M, Zhao J, Wang H, Lui H, Korbelik M and Zeng H 2011 *Optics Express* 19 22892
[3] Lorinz A, Haddad D, Naik V, Naik R, Fung A, Cao A, Manda P, Pandya A, Auner G, Rabah R, Langenburg S E and Klein M D 2004 *J. Pediatr. Surg.* 39 953
[4] Stewart S, Kirshner H, Treado P J, Priore R and Tretiakova M 2014 *J. of Raman Spectroscopy* 45 274
[5] Lykina A A, Artemyev D N and Bratchenko I A 2017 *JBPE* 2 3167
[6] Esbensen K H 2000 *Multivariate Data Analysis* (New Jersey: In Practice 4-th ed.)
[7] Ruffin Ch and King R L 1999 *IEEE International* 2 275
[8] Eilers P H C and Boelens H F M 2005 *Baseline Correction with Asymmetric Least Squares Smoothing* (United States: Leiden University Medical Centre)
[9] Farrés M, Platikanov S, Tsakovski S and Tauler R 2015 *Journal of Chemometrics* 29 528
[10] Huang Z, Mcwilliams A, Lam S, English J, Mclean D I, Lui H and Zeng H 2003 *International J. of oncology* 23 649
[11] Lebrun N, Dhamelincourt P, Focsa C, Chazaillon B, Destombes J L and Prevost D 2003 *J. of Raman Spectroscopy* 34 459
[12] Kalasinsky K S, Hadfield T, Shea A A, Kalasinsky V F, Nelson M P, Neiss J, Drauch A J, Vanni G S and Treado P J 2007 *Anal. Chem.* 79 2658
[13] Mert S and Culha M 2014 *Applied Spectroscopy* 68 617

Acknowledgments

This research was supported by the Ministry of Education and Science of the Russian Federation.