Current Optics and Photonics
Vol. 3, No. 2, April 2019, pp. 150-153

ISSN: 2508-7266(Print) / ISSN: 2508-7274(Online)
DOI: https://doi.org/10.3807/COPP.2019.3.2.150

Evaluation of Larynx Cancer via Chemometrics Assisted Raman Spectroscopy

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(Received October 14, 2018 : revised January 28, 2019 : accepted February 17, 2019)

Larynx cancer is a potentially terminal and severe type of neck and head cancer in which malignant cells start to grow and spread upwards in the larynx, or voice box. Smoking tobacco, drinking hot beverages and drinking alcohol are the main risk factors for these tumors. In this study, we aimed to develop a precise, accurate and rapid chemometrics assisted Raman spectroscopy method for diagnosis of larynx cancer in deparaffinized tissue samples. In the proposed method, samples were deparaffinized and 20 microns of each tissue were located on a coverslip. Both healthy (n = 13) and cancerous tissues (n = 13) were exposed to a Raman laser (785 nm) and excitations were recorded between wavenumbers of 50~1500 $cm^{-1.}$ An Orthogonal Partial Least Square algorithm was applied to evaluate the Raman spectrum obtained. Sensitivity and specificity of the proposed method is high enough with the aid of Principal Component Analysis (PCA) to test the whole model. Healthy and cancerous tissues were accurately and precisely clustered. A rapid, easy and precise diagnosis algorithm was developed for larynx cancer. By this method, some useful data about differences in biomolecules of each group (phospholipids, amides, tyrosine, phenylalanine collagen etc.) was also obtained from the spectra. It is claimed that the optimized method has a great potential for clustering and separating tumor tissues from healthy ones. This novel, rapid, precise and objective diagnosis method may be an alternative for the conventional methods in literature for diagnosis of larynx cancer.

Keywords : Larynx cancer, Raman spectroscopy, Chemometrics, Diagnosis
OCIS codes : (170.0170) Medical optics and biotechnology; (170.6510) Spectroscopy, tissue diagnostics; (170.1790) Confocal microscopy; (170.5660) Raman spectroscopy; (170.1610) Clinical applications

I. INTRODUCTION

Larynx cancer is one of the most prevalent head and neck squamous cell cancers (SCC) [1]. A rapid increase is being observed in incidence of head and neck cancer, accounting for over 550,000 cases annually worldwide, and almost 30% are new larynx cancer cases [2]. Incidence of larynx tumours may vary from region to region due to different prevalence of risk factors, mostly tobacco, alcohol and consumption of hot beverages, and for that reason this type of cancer is mostly observed in men because of their greater exposure to those effects [3-5]. The three main locations for the tumor are the glottis, which is the most common, the supraglottis and the subglottis [6]. Several symptoms are reported for larynx cancer, including hoarseness, pain, sore throat, and persistent cough, depending on the stage and dimension of the tumor [7-9]. According to the data, 13,000 new cases are expected in a year in the USA with a 70% survival rate [10]. Early diagnosis is a crucial step to increase survival [11, 12]. As a result of this, different methods have been developed for diagnosis of larynx cancer. Several methods were routinely applied including subjective and objective examination, and laboratory examination. Laryngoscopy is one of the most used methods

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to determine tumor formation. Microlaryngoscopy is another method to get substantial data about the localization and difference in neoplasmatic cells. These methods give a chance to apply histhopathological measurements by taking samples. Radiological approaches in larynx cancer diagnosis have also been considered via laryngotomography that may provide evaluation of subglottic region, ventrical and recesus piriformis. Today, histological examination is still accepted as the most trustworthy method in the surgery of larynx cancer. Other alternatives for the diagnosis of tumors in the larynx are ultrasonographic examinations, computer tomography and magnetic tomography of nuclear resonance. Currently, photonics strategies are also taken into account with the aid of chemometrics for the diagnosis of tumors. These invasive methods have a great potential either to get information from the tissue or to give an objective diagnosis of cancer, positive or negative. Photonics methods are rapid, simple sample preparation and they have objective numeric results. For these reasons, these methods are currently very popular [13]. Raman spectroscopy is one such method used in diagnosis. In this study it is aimed to compare larynx tumor and healthy tissues via Raman spectroscopy.

II. METHOD

2.1. Instrumentation

Raman measurements were performed in backscattering geometry via a WItech Alpha 300R Confocal Raman Microscope following with a Raman Spectroscopy System UHTS 300 charge coupled device operating at -60°. Grating set at 600 g/mm, (BLZ = 500 nm). Vertical shift speed was 16.25 μs and horizontal shift speed was 0.033 MHz. All measurements were carried out by using a 785 nm laser as excitation source. This wavelength was found to be advantageous for tissue analysis by eliminating strong fluorescence emissions that interfere with desired signals. Raman signals were taken through a confocal pinhole, which is about 100 μm diameter, by using a 0.9 numerical aperture (NA) objective of 100x magnification. Rayleigh scattering, fluorescence emission, humidity and dust interferences were eliminated by the software.

2.2. Sample Preparation

Twenty-six different subjects were kindly provided by Ataturk University, Faculty of Medicine, Department of Pathology. All subjects were Caucasian and male. Histopathological diagnosis test were applied into 26 tissue samples. It is the 'reference method' for Raman spectroscopy analysis. The proposed reference method covers almost three days of run time. According to the reference method 13 malign larynx tumour tissues were diagnosed whereas 13 samples were determined to be healthy. All samples were deparaffinized and cut into 1 cm × 1 cm dimension. The thickness of each aliquot was about 20 microns. After that samples were put onto coverslips and the 785 nm laser was applied to get Raman spectra of each tissue. All spectra were obtained from the software and exported to MATLAB PLS Toolbox 8.0 for chemometrics analysis. Raman mapping was also carried out by utilizing two-laser beams by filtering the Rayleigh scattering.

2.3. Data Processing and Statistical Analysis

In Raman scattering measurements, sample spectra include too much interference in solid samples due to light scattering, differences in spectroscopic path length variation of the sample and homogeneity problems, especially in biological samples. In order to get rid of unwanted systematical variation, there are two different approaches for pre-processing the raw data, differentiation and signal correction. Savitzky-Golay smoothing, Multiple Signal Correction (MSC), Fourier transformation, Orthogonal Signal Correction (OSC) are the most cited and applied algorithms for pre-processing the raw spectra. These signal correction algorithms are based on a mathematical function that filters the results of any spectroscopic data to remove undesired light scattering interference and other undesirable parts of the data. to enhance the sensitivity and selectivity of the method. In this study, we aim to divide healthy and cancerous tissues into two main clusters. In order to do that the most common classification algorithm, Principal Component Analysis (PCA) was applied to raw Raman spectrometry data.

By this mathematical function raw Raman spectra data are filtered and clustered with a higher sensitivity and specificity.

2.4. Raman Mapping

Raman mapping was achieved by using the WItech Alpha 300R Confocal Raman Microscope. Smooth scan features were applied on the data. Both scan width and height were determined to be 20 μm. Pixels for width and height were 1024 × 128, providing an acceptable resolution

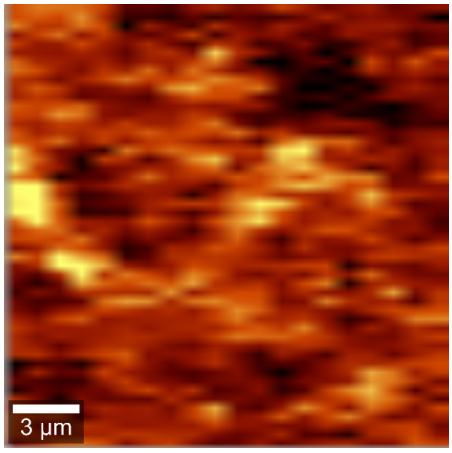

FIG. 1. Raman mapping of healthy larynx tissues.

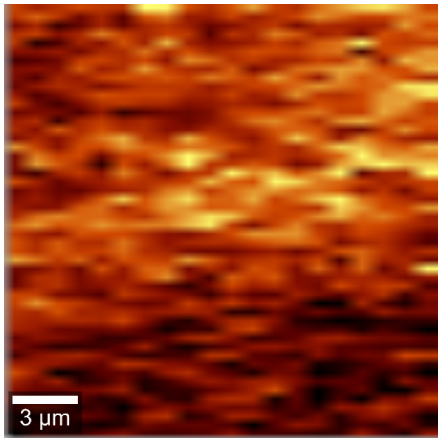

FIG. 2. Raman mapping of larynx cancer tissues.

for both and healthy tissues. In Figs. 1 and 2, Raman mappings were monitored for wavenumber 1341 cm^{-1} to monitor collagen formation in tissues. Alteration in intensity of this band exhibits the differences between cancer and healthy tissue. This wavelength is selected due to its importance. Collagen alteration could be one of the strong evidences for cancerous cells and we aim to underline the change in collagen level of cancerous cells related to healthy tissues.

III. RESULTS

A sample for healthy and cancerous tissue Raman spectra is shown in Fig. 3. According to the spectra, some important biomolecules were identified. At 854 cm^{-1} tyrosine

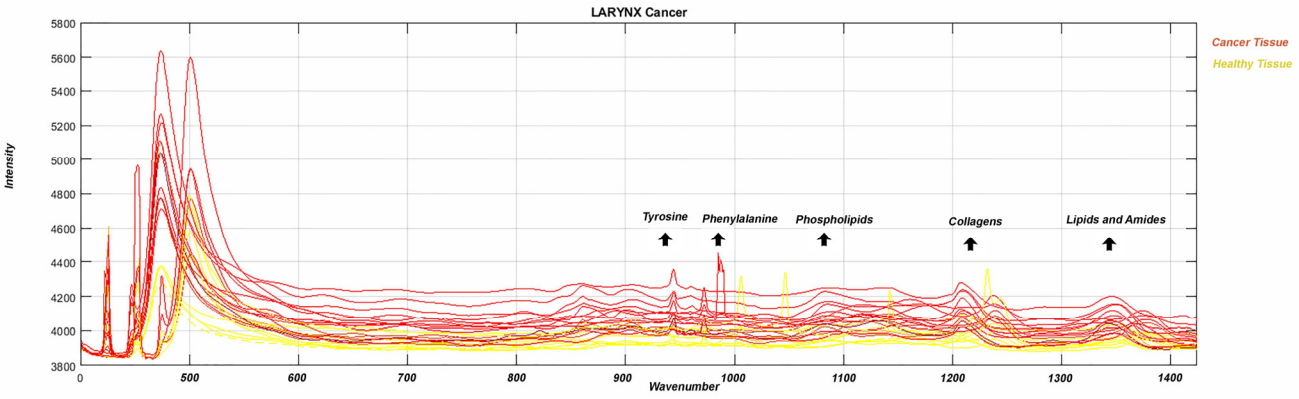

FIG. 3. Raman spectra of healthy and cancerous larynx tissues.

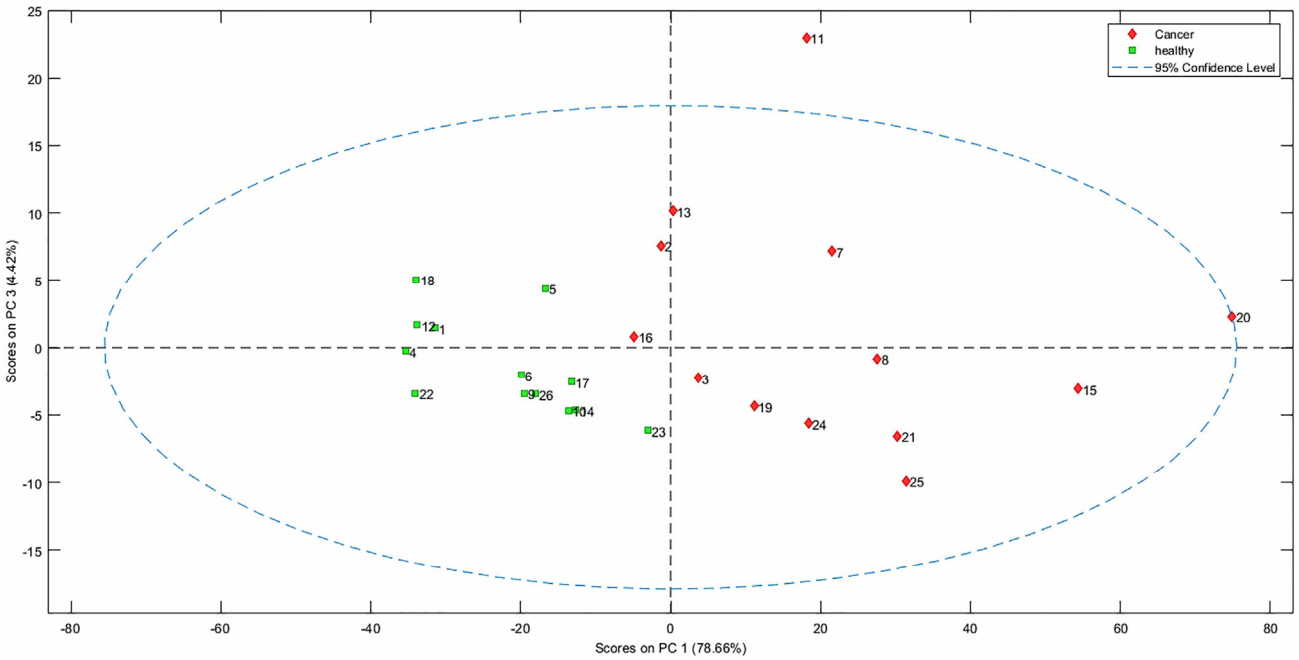

FIG. 4. Graph of scores on PC1 vs PC3.

molecules have been detected in ring breathing mode. The 1004~1033 cm^{-1} band expressed C-C vibrations of phenylalanine. Phospholipids were mostly seen at 1130 cm^{-1} [14]. The tyrosine band is identified at 1209 cm^{-1}. Collagen, lipids and amide formations were also detected between 1360~1400 cm^{-1}. Collagen levels of cancerous and healthy tissue were also monitored by Raman mapping to show deformation due to the malignant cells in Figs. 1 and 2 [15].

This Raman map shows the differences of collagen level in both tissue samples. Differences are evident between cancer and healthy tissue. Data were also evaluated via the chemometrics algorithm. In this study, PCA were selected to classify both groups. All Raman data were exported into MATLAB 2017 software. PCA suggested four principal components to explain the method. PC1 vs PC2 score plot was monitored in Fig. 4. This figure exhibits the successs of the proposed method. All samples were divided into two groups via the chemometrics algorithm. Cross validation of the proposed method was carried out as venetian blinds w/10 splits and 1 sample per split. Root Mean Square Error of Cross Validation (RMSECV) values were 0.2335 while %Variance captured was 94.38. This data confirms the goodness of fit between the proposed model and the obtained spectrum of larynx cancer and healthy tissue samples.

IV. CONCLUSION

Chemometric analysis and Raman mapping of collagen bands brought about a novel study for diagnosis of larynx cancer. Healthy and cancerous tissues were successfully discriminated by this model in a short time without using chemicals. The method offers several advantages that include short analysis times, absence of chemicals, high accuracy, and good sensitivity. This proposed method not only give researchers important information for variation in several metabolites to illuminate the mechanism of cancerous tissue but also reveal a new, rapid, accurate and sensitive diagnosis algorithm that is an alternative for the routine histopathological analysis. This study also monitored for the first time the collagen variations between healthy and cancer tissue via a Raman imaging system.

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