Scientific report

Project title: Raman Spectroscopy for Ultra-Sensitive Salivary Diagnosis and Radiotherapy Treatment Monitoring of Oral CancerPerioada de raportare: 01.01.2019 – 31.12.2019

Phase 2: Establish the biomolecular composition of salivary samples (continuation E1). Identification of spectral salivary biomarkers specific to oral cancer. Assessing the capacity of a portable Raman spectrometer for the analysis of salivary samples

Summary:
The objective of the first stage was to establish the biomolecular composition of healthy salivary samples and to identify spectral salivary biomarkers specific to oral cancer. During this stage, for the current reporting period, we have envisioned five activities that lead to the achievement of the objective associated with this stage. Therefore, the first step was to establish a protocol for preparing salivary samples for Raman spectroscopic measurements and / or surface enhanced Raman (SERS). The salivary samples were prepared and the spectroscopic signals were used to analyze the salivary biomolecular composition and to differentiate between healthy and cancerous samples by identifying specific biomarkers for oral cancer. The verifiable results of the project at this stage are presented in Table 1.

Table 1: Stage results of the RAMSES-PD 145/2019 project

Phase Activities	Results	Verfiable results
Phase 2: Establish the biomolecular composition of salivary samples (continuation E1). Identification of spectral salivary biomarkers specific to oral cancer. Assessing the capacity of a portable Raman spectrometer for the analysis of salivary samples		
Activity2.1. Establish a protocol for preparing the salivary samples for Raman and SERS measurements	• Protocol for preparing salivary samples for Raman spectroscopic investigations	Experimental method reported at INCDTIM – Annex 1
Activity 2.2. Identifiy the biomolecular composition of control salivary samples	• Build a spectral data abse (continuare E1). • Analyse the biomoleculare composition of control salivary samples by assigning the Raman and/or SERS bands	Scientific paper sent to Analytical Letters on 30th Oct. (ID: LANL-2019-1000)
Activity 2.3. Analyse the biomolecular composition of oral cancer saliva samples	• Colecting salivary samples from oral cancer suffering patients and preparing them for spectroscopic measurements • Acquire Raman and/or SERS spectra • Examine the biochemical salivary changes charactersitic to the oral cancer compared to the control group.	Scientific paper sent to Lasers in Medical Science on 17th Sept. (ID: LIMS-D-19-00430R1)
Activity 2.4. Identify the salivary biomarkers specific to oral cancer	• Determine the biomarkers specific to oral cancer diagnosis using mutivariate analisys • Corelate the spectral data with the medical diagnosis	Method for oral cancer salivary diagnosis based on Raman and/or SERS investigations
Activity 2.5. Validate the capacity of a portable Raman system for saliva samples analysis	• Identify the salivary biomarkers specific to the control and oral cancer using a portable raman system.	Validate the spectra acquired with the portable Raman system (on-going)

	• Corelate the spectral data with the ones obtained in the laboratory.	Report on the capacity of the portable Raman system (on-going)

Activity 2.1.
Establishing a protocol for the preparation of salivary samples and the acquisition of Raman and / or SERS spectra

To achieve the results of this research activity, salivary samples were collected from healthy volunteers and from patients with confirmed oral cancer. In order to keep track of the collected samples and the necessary details about each sample, a database was prepared with information about patients, such as diagnosis, tumor location, medical treatment, age, smoking status, and the method of collecting the sample (time, date, quantity harvested, food intake). In order to establish an adequate protocol for the preparation of salivary samples for spectroscopic measurements, different methods were tried and the spectroscopic response was acquired in each case, in order to analyze its intensity and reproducibility, as well as the signal-to-noise ratio. Following these investigations, an adequate method of preparation of salivary samples was established, which was then applied on several samples to meet the following goals set out in the activity plan of this research project. The need to establish a standardized protocol for the preparation of saliva samples is indicated by the very different spectroscopic signal (both Raman and surface-enhanced Raman - SERS) obtained from salivary samples and reported so far in the literature [1-3]. The diversity of the experimental conditions, the stages of sample preparation (speed of centrifugation, time, drying or not, the substrate involved, etc.) and the inter- and intra-individual diversity can lead to differences between the spectra reported by different research laboratories.

One of the main benefits of saliva as a diagnostic tool is the easy, non-invasive, safe collection that can be performed by personnel who do not require special training, as compared to other body fluids that involve discomfort problems, as is the case, for example, of urine collection [4]. However, some difficulties are encountered due to the fact that the salivary constituents vary depending on the method of collection, the degree of salivary flow and other factors, such as smoking status or individual circadian rhythm. Therefore, standardizing the methods of collecting, processing, and preserving saliva is a mandatory condition for salivary diagnosis. The composition of the saliva depends on the salivary glands that produce it and the methods of collection, which can be classified according to the use or not of stimuli. The stimulated methods will use chewing gum or lemon to increase the salivary flow, however, these affect the pH of the saliva, while the unstimulated methods, the flow is affected by the degree of hydration. Regardless of the method of collection, subjects should be instructed to rinse their mouths with water before collecting saliva to avoid contaminants. The preparation steps usually include centrifuging the samples at different speeds and for different periods of time. Centrifugation is used to separate cells, subcellular organs, large molecules (eg. proteins) and food debris, in order to obtain samples of pure saliva. A recent study followed the effect of centrifugation at different speeds on the salivary protein profile and observed that increasing the speed of centrifugation decreased the frequency of occurrence of salivary proteins with molecular masses ≥30 kDa [5]. Therefore, our protocol for preparing the salivary samples included, with the modification of some parameters or the omission of some stages, the following:

1. Centrifuging the sample at different speeds
2. Collecting the supernatant
3. Lyophilization of the sample

4. Obtaining a mixture of supernatant and colloidal gold nanoparticles (for SERS measurements)
5. Deposition of the sample on different substrates for spectrum acquisition.

Next, we will conduct a brief overview of the steps followed, presenting variations of some stages and the resulting conclusions. The Raman or SERS spectra acquired in each case were compared in order to determine which preparation method presents reproducible, intense Raman signal, with well-defined bands, and with the best signal-to-noise ratio. Thus, the centrifugation of the salivary samples was performed at a speed selected based on the scientific literature in the field, but also on the basis of the Raman spectroscopic signal obtained after the centrifugation of the samples. Both non-centrifuged samples and samples centrifuged at different speeds were probed and it was observed that centrifugation removes impurities from saliva samples that otherwise impede the acquisition of Raman spectra. The collected supernatant was subsequently lyophilized overnight at -50^{o}C and it was observed that freezing the sample and its dehydration lead to an intense, reproducible signal, with well-defined bands and low background. Figure 1 shows Raman spectra acquired from the same salivary sample divided into 3 batches prepared in different ways. Of these, only the first one was centrifuged at 9000 g for 10 minutes, at -10^{o}C, while bacthes 2 and 3 were not centrifuged. The supernatant obtained in the first case was collected and lyophilized together with sample 2. The third sample was deposited directly on a MgF_2 substrates, as were the other two samples and subjected to Raman spectroscopic measurements. In Fig. 1 are also presented the optical images that show the location from where the spectra were obtained.

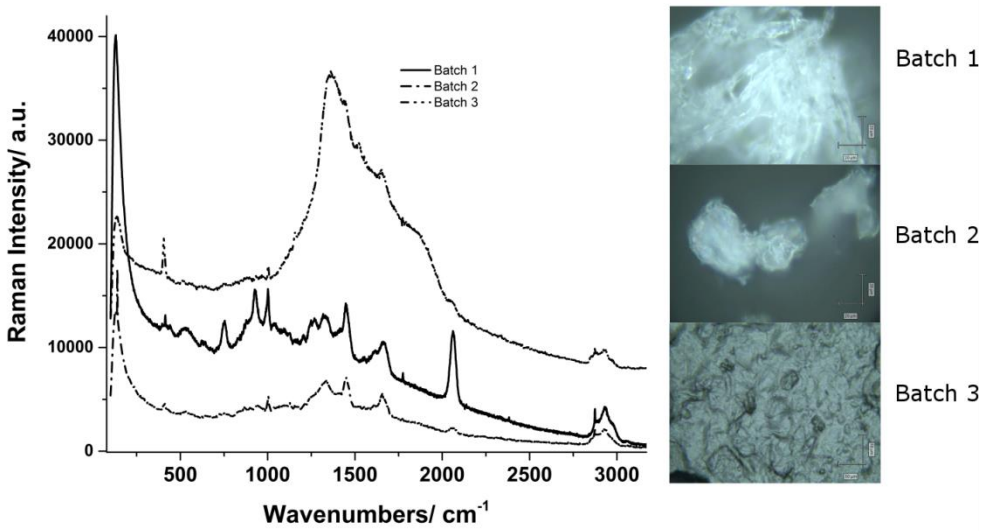

Figure 1. Raman spectra obtained from the three saliva batches prepared in different ways. A laser diode emitting at 785 nm was used to excite the spectra.

The first batch showed a high signal-to-noise ratio, with well-defined Raman bands. The second batch presented a well-defined Raman spectrum, however, the relative intensity of the Raman bands was lower compared to the spectrum collected from the first batch, despite the lower background signal. The spectrum collected from the last batch showed a high background that prevented the identification of Raman bands in the fingerprint region. The lower quality of this spectrum could be attributed to the impurities and residues found in the sample of non-centrifuged saliva, the signal of which overlaps with the Raman signal of interest. On the other hand, the lyophilization procedure seems to reduce the amount of residues in the sample, which leads to the acquisition of a better resolved Raman spectrum. As can be seen in Fig. 1, however, centrifugation at 9000 g followed by lyophilization allowed to obtain a high quality Raman spectrum. Therefore, the protocol used to prepare this saliva sample was further applied to all investigated samples.

For SERS spectroscopic measurements, the supernatant collected after centrifugation was mixed in a 1:1 ratio with colloidal gold nanoparticles of 50 nm diameter, purchased from Sigma Aldrich. A few microliters of the supernatant mixture with Au colloid was deposited on a microscope glass and allowed to dry. Although the obtained SERS spectra show well-amplified Raman bands, there was an intermittent effect of on and off signal, showing SERS bands at a position and moment of acquisition which disappeared at the next acquisition, making the SERS characterization more difficult. Figure 2 presents a SERS spectral map. The map was obtained by plotting the scores of the first principal component (PC) obtained following principal component analysis (PCA) at each position where a SERS spectrum was acquired.

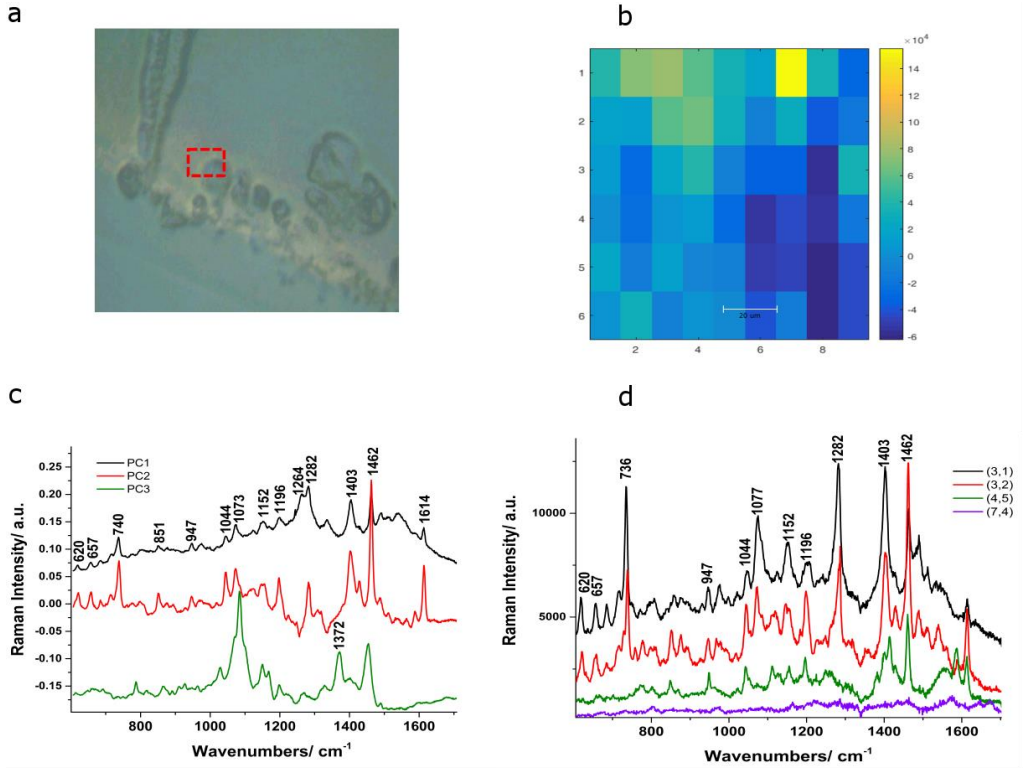

Figure 2. (a) Optical image showing the location of an 18 x 12 µm area on a saliva sample collected from a healthy volunteer, from which the SERS map (red border) was acquired. (b) The SERS map reproduced by the PC1 score representation. The highest intensity is colored yellow and the lowest intensity is dark blue. (c) The first 3 principal components (PC1 90.5%, PC 5%, PC3 2%). (d) SERS spectra collected from random points on the map, as indicated in the legend with coordinates (x, y).

PCA was applied to all the spectra collected from the saliva sample from an area of 18 x 12 µm, from which SERS spectra were acquired with a 2 µm step, resulting in a total of 54 spectra. Fig. 2d shows SERS spectra collected from different locations on the map, as indicated by the positions (x, y) in the figure legend. As can be seen from the distribution of colors associated with the intensity of the SERS spectra represented in Fig. 2b, the spectra with the highest intensity were grouped in certain areas of the map. The spectra collected from these points (eg. position (3,1) and (3,2)) showed high intensity reproducible SERS signals with well resolved bands. Going further away from this area to a place where the SERS map intensity is lower, a low intensity SERS spectrum is obtained showing a different signal from the previous ones. Moving further from the initial position to location (7.4), a SERS spectrum with low signal-to-noise ratio was obtained, which shows unresolved SERS bands. Given this distribution of the signal, we can assume that the gold nanoparticles were clustered during the air drying of the sample, which led to fluctuations of the signal intensities and frequencies. Therefore, the amplification of the characteristic signals of the biomolecules present in the saliva supernatant is observed mainly in the places where the Au nanoparticles accumulate forming "hot spots", which leads to strong improvements of the Raman signal scattering. However,

the SERS spectra showed variability even at these locations, which could indicate that the amplification of Raman modes depends on the number of nanoparticles and biomolecules present in the focal point of the laser, how the biomolecules interact with the nanoparticles and various orientations of molecular species relative to the surface of nanoparticles [6].

Activities 2.2.-2.3:

Identification of the biomolecular composition of the control salivary samples (continuation E1).

Analysis of the biomolecular composition of salivary samples obtained from patients with oral cancer.

In the following, we will present the results obtained in activities 2.2-2.3 together, due to the similar investigation approach addressed for both salivary samples collected from healthy volunteers and from patients suffering from oral cancer. Thus, the approved protocol for sample preparation was used on a larger batch of salivary samples for both Raman and SERS measurements, the purpose of which was to identify and analyze the biomolecular composition of salivary samples and to perform a differentiation of the spectroscopic signals collected from the two types of pathologies.

Our aim was to analyze the Raman / SERS signal characteristic of salivary samples, as well as to detect the biomolecular changes induced by the disease in the saliva of patients suffering from oral cancer and to differentiate between the spectroscopic signal corresponding to the healthy salivary samples from the cancerous ones. For this, we acquired Raman spectra from a healthy sample and a cancerous saliva sample, respectively, and their average Raman spectra were calculated and displayed in Fig. 3a. The average spectra are displayed together with the standard deviation, which is higher for the cancer spectra, pointing out the molecular heterogeneity of the cancer sample. Saliva contains electrolytes, mucus, proteins, enzymes, such as lipase, amylase, antibacterial compounds (thiocyanate), lysozyme, and epithelial cells from which DNA can be extracted. Their contribution to the Raman spectra is evident through the bands observed, and the signals obtained are similar to the previous reports in the specialized literature in the field [7]. The contribution of lysozyme to the Raman spectrum of saliva is observed through the bands located at 755, 929 cm^{-1}, as well as by the tryptophan bands at 1342, 1451 cm^{-1}, and the amide vibrations at 1662 cm^{-1}. Phenylalanine is contained in both amylase and lipase [7] and results in a strong Raman band located at 1002 cm^{-1}. The 2061 cm^{-1} band is most likely caused by thiocyanate, which is found in the saliva of smokers as well as of non-smokers as an antibacterial agent [8,9].

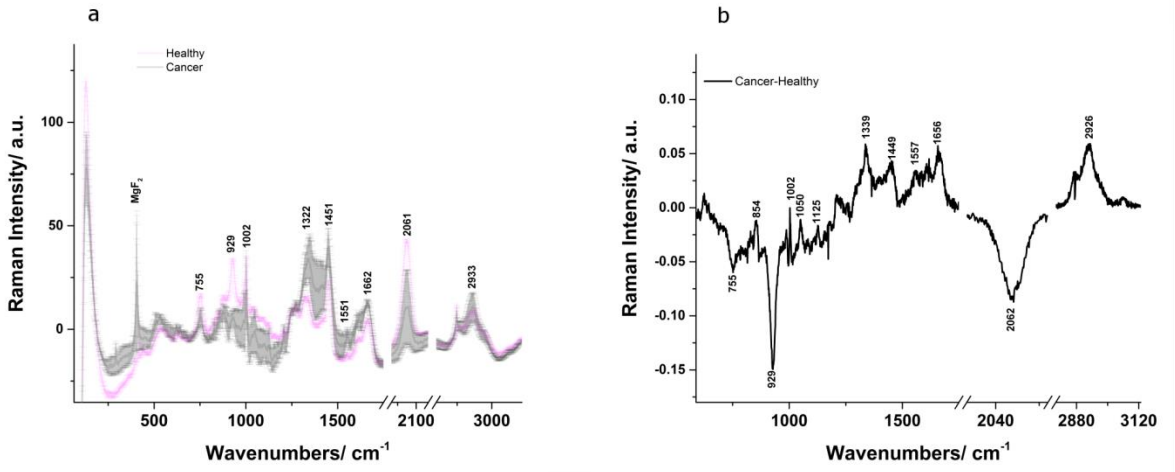

Figure 3. (a) Raman spectra mediated characteristic to a healthy, respectively a cancerous saliva sample. The vertical lines represent the standard deviation calculated for each wave number. (b) Difference spectrum calculated by subtracting the average spectrum of healthy saliva from the average spectrum of cancerous saliva.

To identify the spectral differences between the healthy and cancerous saliva samples, we calculated the Raman difference spectrum (average spectrum characteristic of the cancerous saliva sample minus the average spectrum characteristic of healthy saliva), which is shown in Fig. 3b. The

main spectral differences can be observed in the fingerprint regions of 700-150 cm $^{-1}$ and 1280-1700 cm $^{-1}$, as well as for the band 2061 cm $^{-1}$ and the area of 2920 cm $^{-1}$ representative for CH_2, CH_3 stretching vibrations. The difference spectrum showed an increased contribution of the Raman bands located at 854, 1002, 1050, 1125, 1339, 1449, 1557, 1656 and 2926 cm $^{-1}$ in the cancerous spectra, mainly allocated to amino acids (tyrosine, tryptophan), proteins (phenylalanine, collagen), glucose (glycogen), but also nucleic acids (guanine) and lipids. On the other hand, a higher intensity of the bands 755, 929 and 2062 cm $^{-1}$ was observed in the healthy saliva spectra. The higher contribution of the 2062 cm $^{-1}$ band attributed to thiocyanate may indicate the smoking status of the healthy volunteer.

Another objective of this study was to differentiate the saliva collected from healthy individuals from saliva corresponding to patients with oral cancer, based on SERS spectral characteristics. The average SERS spectra characteristic for each group were calculated and are shown in Fig. 4a. The shaded region represents the standard deviations from the average spectra. The thickness of this area suggested a low reproduction of SERS spectra. This result indicated that, based on the spectral differences between healthy saliva and oral cancer, SERS diagnosis is possible. The difference spectrum obtained by subtracting the average SERS spectrum of healthy saliva from that of the cancer group further confirmed the discrepancy of saliva composition between oral cancer and healthy samples (see Fig. 4b). The differences between SERS data characteristic of oral cancer and healthy group are given by several main bands located at 2126, 1659, 1585, 1510, 1469, 1390, 1274, 1189, 1093, 820, 631, 483 and 284 cm^{-1}. Qiu *et al.* previously identified several of these SERS bands in a study that involved non-invasive detection of nasopharyngeal cancer and attributed them based on the literature in the field to glycogen at 496 cm^{-1}, tyrosine and lactose at 635 cm^{-1}, phenylalanine. at 1003 cm^{-1}, unsaturated fatty acids at 1270 cm^{-1}, collagen and phospholipids at 1448 cm^{-1} and nucleic acids at 1662 cm^{-1} [3]. Li and co-workers identified among other bands the 620 cm^{-1} and assigned it to proteins or adenine, the 820 and 1280 cm^{-1} and assigned them to phospholipids, amide III, proteins and / or lipids and a band at 1390 cm^{-1} [10]. Moreover, the emergence of a 2126 cm^{-1} thiocyanate band in the difference spectrum indicated that thiocyanate could be used as a biomarker for differentiating between healthy and cancerous saliva.

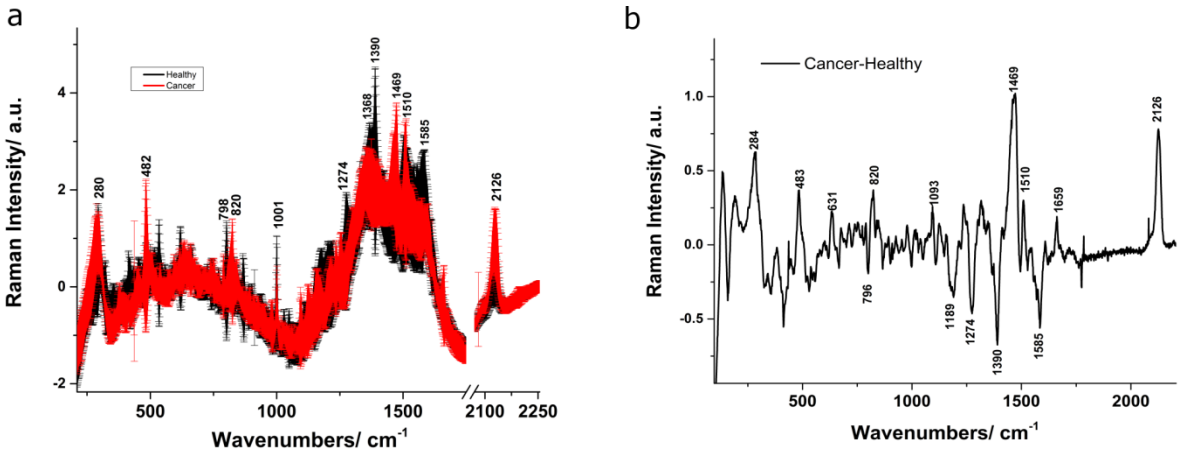

Figure 4. (a) The average SERS spectra characteristic to oral cancer and healthy saliva, respectively. Shaded areas represent the standard deviations. (b) The difference spectrum obtained by subtracting the healthy saliva characteristic spectrum from the oral cancer group.

Activity 2.4. Identification of salivary biomarkers specific to oral cancer.

In order to discriminate spectrally between the two groups of saliva, a multivariate investigation based on principal component analysis (PCA) was applied, a statistical method of reducing the size of the initial space to several main components. Each component can be assigned to the original spectrum by a score indicating the weight of the respective component in the initial spectrum. The spectra corresponding to the first three main components, which captured 97.8% of the spectral

variation, are shown in Fig. 5. PC1 and PC2 consisted mainly of characteristics that can be related to salivary constituents and can be identified in the difference spectrum, while PC3 captured a slight variation in noise between the two groups. Fig. 5b shows a three-dimensional scatter diagram showing the distribution of scores corresponding to the first three PCs for both healthy and cancerous saliva spectra. A clear distinction can be observed between the two groups of saliva. The separation of the spectra was mainly due to the band from 929 cm^{-1} attributed to lysozyme and proline, but the thiocyanate band from 2061 cm^{-1} also had an important contribution, as well as the band from 2933 cm^{-1} representing CH_2 and CH_3 stretching vibrations.

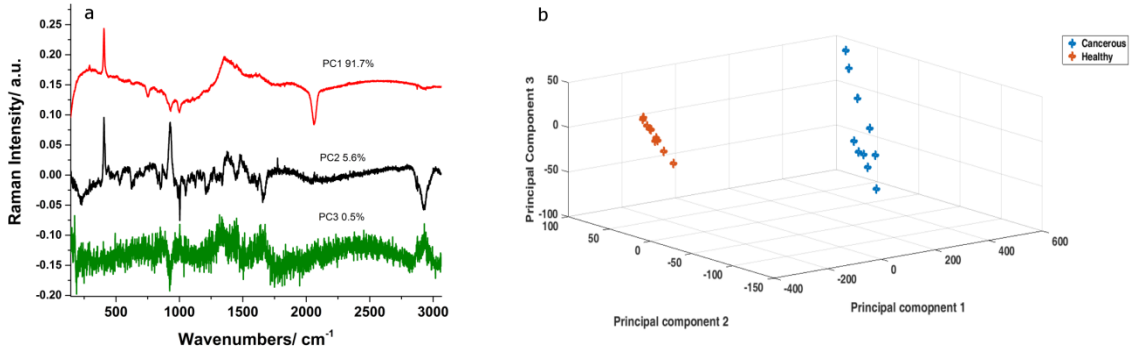

Figure 5. (a) The first 3 PCs used to differentiate the Raman spectra. (b) Separation of scores of the first 3 PCs characteristic of healthy saliva (red pentagrams), respectively cancerous saliva (blue pentagrams).

An additional differentiation of the two groups was obtained by hierarchical cluster analysis (HCA). To apply the HCA, we calculated the Euclidean distances between all spectra and checked the cluster tree using the correlation coefficient, which represents the fidelity of the differences given by the tree among the spectral observations. The correlation coefficient obtained was 0.97, indicating a high quality solution. The resulting dendrogram is shown in Fig. 6 which presents the 20 spectra used in this analysis and their connections. The spectra can be clustered into three groups: healthy saliva (spectra numbered 1-10), subgroup 1 of cancerous saliva (spectra 16-20) and subgroup 2 of cancerous saliva (spectra 11-15). A similar classification was obtained following the PCA discrimination based only on the first two PCs (which are not presented here). The cancer saliva spectra were grouped into two subgroups because of background differences observed between the spectra collected from the two different positions in the sample, as well as slight differences in position and relative intensity of the observed Raman bands. This investigation is in accordance with the results shown in Fig. 3, which revealed by analyzing the standard deviation, a greater variability of the cancer spectra. The subgrouping of these spectra indicates the heterogeneity of the cancer sample. Further research aimed to identify the spectral differences that contribute most to this specific grouping.

The Raman spectra characteristic of each of the three groups are shown in Fig. 6b and present contributions from the main salivary components, as well as the Raman bands identified in the calculated difference spectrum. The main differences were observed in the spectral region 1240-1340 cm^{-1}. This fingerprint area comprises major vibrations of proteins, lipids and nucleic acids. The amide III vibration around 1270 cm^{-1} is usually observed in the Raman spectra of biological samples and this vibrational mode is a complex one involving several chemical bonds. The characteristic spectra of both cancer subgroups showed a sharp decrease of the bands 1270 and 1244 cm^{-1} of amide III and an increase of a band centered around 1345 cm^{-1} in the case of the first cancer subgroup, respectively of the band from 1338 cm^{-1} for the second cancer subgroup. These two bands are mainly attributed to CH_2, CH_3 collagen vibrations, nucleic acids and lipids [11].

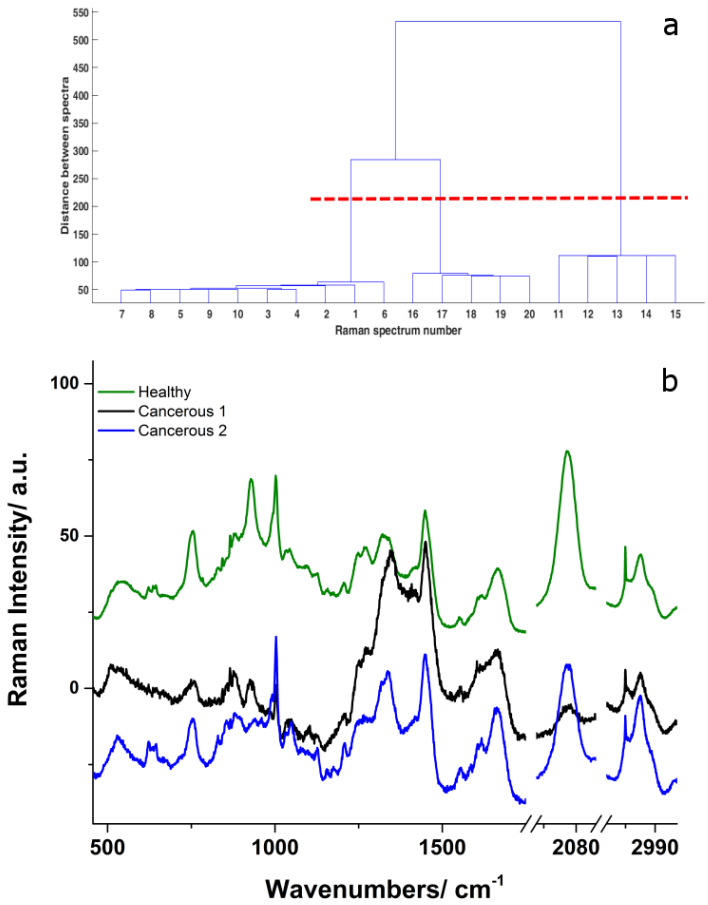

Figure 6. (a) Dendrogram showing the separation of healthy Raman spectra from cancerous ones. The red horizontal line indicates a possible division of the dendrogram tree into three clusters. (b) Raman spectra characteristic of each of the three groups: healthy, cancerous 1, and cancerous 2.

The healthy saliva spectrum, on the other hand, while showing an increase in the bands 1270 and 1248 cm^{-1} compared to the cancerous spectra, showed a band increased of the 1320 cm^{-1}, which is attributed to guanine and amide III. The decrease of the relative intensity of the amide III modes together with the increase of the nucleic acid intensity and of the characteristic modes of collagen or lipids in cancerous saliva suggest a change in the molecular structures of the proteins and a higher percentage of nucleic acids content in association with the tumor transformations. In the lower spectral region of the wavenumbers, some differences were observed for the relative intensity of the band 929 cm^{-1}, which was higher in the characteristic spectra of healthy saliva, the appearance of the unidentified shoulder from 990 cm^{-1} in the spectra of healthy saliva and of the subgroup 2 of cancer and the disappearance of the bands from 620 and 640 cm^{-1} from the spectrum characteristic of the first subgroup of cancer. In addition, the relative intensity of the thiocyanate band at 2063 cm^{-1} is higher in the spectra characteristic of healthy samples and subgroup 2 of cancer. The chemometric analysis presented here showed that the acquired Raman spectra can be used to differentiate healthy saliva from cancerous one. However, this study is a preliminary one and further investigations involving more saliva samples collected from both healthy and cancer patients are needed to confirm the identified spectral differences. Currently, we are working on the database of Raman spectra collected from healthy and cancerous salivary samples and we intend to apply the same analysis as presented here on a batch of at least 30 salivary samples.

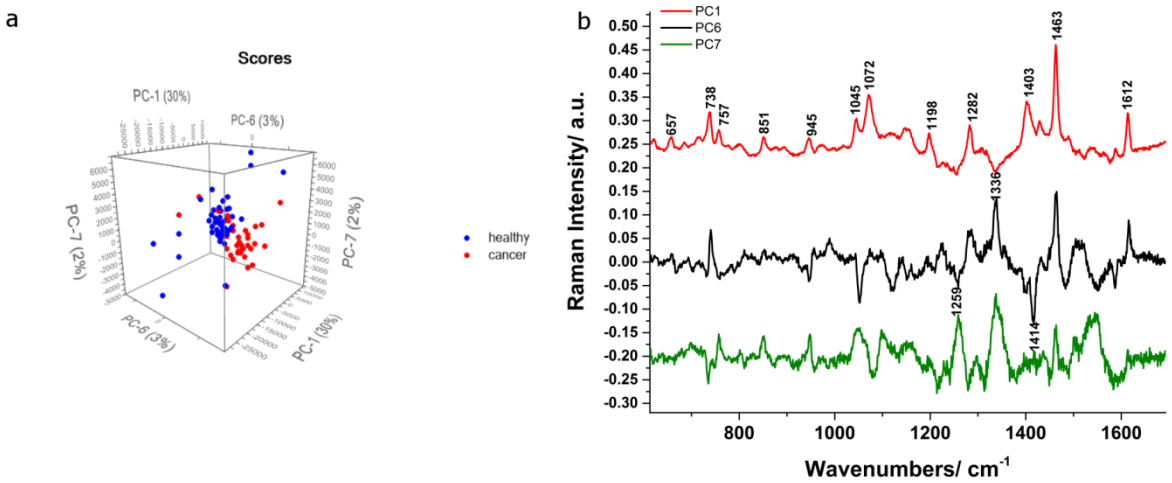

Figure 7. (a) Three-dimensional scatter plot of PCA result (PC1 vs PC6 vs PC7 scores) for saliva groups with oral cancer (red points) and healthy saliva (blue points), respectively. (b) The main PCs used for spectrum separation, indicating the SERS bands that contribute most to the separation of oral cancer saliva from the healthy saliva group.

The differentiation of healthy salivary samples from cancerous ones was also tested for SERS spectra acquired from the two groups. For this, two SERS maps containing a total of 90 spectra were used. PCA was applied in order to obtain new variables and principal scores of the components that best explain the differences between the spectra. PCA can classify or organize Raman and SERS data that cannot be visually examined and has significantly contributed to cancer detection, proving to be an invaluable tool for comparing complex biological samples. To illustrate the use of PC scores for classification of diagnosis, a comparison between normal and cancer groups is shown in Fig. 7, as a 3D scatter plot of PC1, PC6 and PC7 on the three axes. For this analysis, all spectra in the spectral range of 600-1720 cm^{-1} were used. The characteristics of the PCs correspond to the SERS spectra, presenting bands at positions similar to those of the spectra. The total variation summed by the first seven PCs was 82%. PC6 and 7 were used in the 3D graph, together with PC1, as these PCs showed the best separation between the SERS spectra obtained from the two groups. In some cases, there is no clear distinction between groups using only the first components, as they may not contain any ability to discriminate. This does not necessarily mean that there is no distinction between groups, but only that the largest source of variation is similar in both. Therefore, the examination of superior PCs can lead to the expected discrimination. Figure 7a shows that scores PC1, PC6, and PC7 for the healthy saliva and oral cancer group, respectively, were distributed in separate axes, forming separate clusters. This shows that we were able to differentiate between SERS spectra of oral cancer and healthy saliva. The separation of the spectra was mainly due to the 1463 cm^{-1} band attributed to the CH_2 bending mode of lipids and proteins or sucrose. The other SERS marker bands that contributed to the spectra discrimination are shown in Fig. 7b and are located at 1612, 1414, 1403, 1336, 1259, 1072 and 738 cm^{-1}. These bands are attributed to lipids, fatty acids, nucleic acids, amino acids, such as tyrosine, tryptophan and proteins, as well as thiocyanate (738 cm^{-1}) [11].

Activity 2.5. Validation of the capacity of the portable Raman spectrometer for the acquisition of conclusive spectra from salivary samples.

The idea from which this research activity started is based on the possibility of including the micro-Raman technique in the medical offices or hospitals. Thus, software could be developed to help diagnose diseases based on the Raman signal and a previously built database. Such an implementation could be used by personnel who do not need knowledge in the field of Raman spectroscopy. The first steps to develop this idea are to validate the capacity of the portable Raman spectrometer for the acquisition of the Raman spectra from salivary samples. Salivary samples should be subjected to a preparation protocol as simple, fast, and inexpensive as possible. That is

why we are currently trying to obtain reproducible Raman signal with the lowest signal-to-noise ratio from unprocessed salivary samples, using a portable Raman spectrometer (BWTeK).

Conclusions:

The objectives of the stage were fully achieved, obtaining the following results:

- Scientific report
- Protocol for the preparation of salivary samples for Raman and / or SERS spectroscopic measurements
- 2 manuscripts sent for publication in ISI journals

Achievement indicators met during this stage:

- Participation in the specialized course entitled "VIII Ciamician Photochemistry School", which took place in Bologna, Italy, from 10-14.06.2019
- 1 participation at the international conference Processes in Isotopes and Molecules, PIM 2019 held in Cluj-Napoca, Romania, between 25-27.09.2019 with poster: A. Falamas, M. Hedesiu, "Surface Enhanced Raman Spectroscopy (SERS) Investigations of Saliva for Oral Cancer Diagnosis "
- Manuscript entitled "Raman spectroscopic characterization of saliva for the discrimination of oral squamous cell carcinoma", A. Falamas, C.I Faur, M. Baciut, H. Rotaru, M. Chirila, S. Cinta Pinzaru, M. Hedesiu, sent for publication in the journal Analytical Letters
- Manuscript entitled "Surface Enhanced Raman Spectroscopy (SERS) Investigations of Saliva for Oral Cancer Diagnosis", A. Falamas, H. Rotaru, M. Hedesiu, sent for publication in the journal Lasers in Medical Science
- Method of preparaing the salivary samples for Raman and / or SERS spectroscopic measurements related to INCDTIM

Bibliography:

[1] Connolly J M, Davies K, Kazakeviciute A, Wheatley A M, Dockery P, Keogh I and Olivo M 2016 Non-invasive and label-free detection of oral squamous cell carcinoma using saliva surface-enhanced Raman spectroscopy and multivariate analysis *Nanomedicine Nanotechnol. Biol. Med.* **12** 1593–601

[2] Feng S, Huang S, Lin D, Chen G, Xu Y, Li Y, Huang Z, Pan J, Chen R and Zeng H 2015 Surface-enhanced Raman spectroscopy of saliva proteins for the noninvasive differentiation of benign and malignant breast tumors *Int. J. Nanomedicine* **10** 537–47

[3] Qiu S, Xu Y, Huang L, Zheng W, Huang C, Huang S, Lin J, Lin D, Feng S, Chen R and Pan J 2016 Non-invasive detection of nasopharyngeal carcinoma using saliva surface-enhanced Raman spectroscopy *Oncol. Lett.* **11** 884–90

[4] Ziauddin Medical University, Anjum A and Hosein M 2019 Diagnostic Importance of Saliva – An Overview *J. Pak. Dent. Assoc.* **28** 129–35

[5] Baskoro B D, Nugraha R A, Puspitawati R and Redjeki S 2017 Effect of centrifugation at 7,000 g, 8,000 g, and 9,000 g on the salivary protein profile ≥30 kDa *J. Phys. Conf. Ser.* **884** 012013

[6] Tsuge K, Kataoka M and Seto Y 2000 Cyanide and Thiocyanate Levels in Blood and Saliva of Healthy Adult Volunteers. *J. Health Sci.* **46** 343–50

[7] Virkler K and Lednev I K 2008 Raman spectroscopy offers great potential for the nondestructive confirmatory identification of body fluids *Forensic Sci. Int.* **181** e1–5

[8] Rodrigues L M, Magrini T D, Lima C F, Scholz J, da Silva Martinho H and Almeida J D 2017 Effect of smoking cessation in saliva compounds by FTIR spectroscopy *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **174** 124–9

[9] Schultz C P, Ahmed M K, Dawes C and Mantsch H H 1996 Thiocyanate Levels in Human Saliva: Quantitation by Fourier Transform Infrared Spectroscopy *Anal. Biochem.* **240** 7–12

[10] Li X 2012 Spectral analysis of human saliva for detection of lung cancer using surface-enhanced Raman spectroscopy *J. Biomed. Opt.* **17** 037003

[11] Movasaghi Z, Rehman S and Rehman I U 2007 Raman Spectroscopy of Biological Tissues *Appl. Spectrosc. Rev.* **42** 493–541

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