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Multi-configuration Raman spectrometer for Early Stage Diagnosis of Oral Cancer

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ABSTRACT

Oral Squamous Cell carcinoma (OSCC) is one of the most common and aggressive oral malignancies. Despite all significant advances in medicine, five-year survival rate is still 40%-60%. Diagnosis in early stages is critical as it can improve the survival rate and the quality of life after treatment. This study aims to develop a strategy for diagnosing oral cancer non-invasively in the early stages and to provide better surgical guidance by differentiating healthy and tumor tissues by using Raman spectroscopy. For this purpose, a multimodal Raman system is developed to detect oral cancer biomarkers in patient's saliva specimen and to study different tissue types in oral cavity with Raman spectroscopy. The developed system is quite compact, easy to use and portable. It can be easily modified for *in vivo* and *ex vivo* analysis and can work in both reflection and transmission mode in case of *ex vivo* measurements. This paper compares the surface enhancement and background spectra from different plasmonic nanoparticles. Lastly, bovine serum albumin (BSA) and uric acid were used as model analytes to at physiologically relevant concentrations to test the performance of the system.

Keywords: oral cancer, Raman spectroscopy, multimodal spectroscopy, SERS, saliva

1. INTRODUCTION

Oral Squamous Cell carcinoma (OSCC) is one of the most common and aggressive oral malignancies. Despite all significant advances in medicine, five-year survival rate is still 40%-60%. It includes a variety of tumors occurring in oral cavity at various anatomical positions such as tongue, buccal mucosa, mouth floor, gums and palate. Most common causing agents of oral cancer are betel quid and areca nut usage, smoking tobacco, consumption of processed meat and use of alcohol or alcohol containing mouth wash. It is one of the three most common cancers in some of the Asian-pacific countries [3]. According to an estimate of World health organization (WHO) global incidence rate of oral cancer is 4 cases per 100,000 people. However it varies from region to region across the globe [3]. Late-stage diagnosis and lack of access to treatment is common in low- and middle-income countries. When diagnosed at early stage, response to treatment is more effective and less expensive. As a result, probability of survival could be more than 80% and quality of life after treatment can be improved. Which is 30-50% in advanced stage - III or IV stage cancers.

Currently, biopsy and histopathology are considered the gold standard procedure for oral cancer diagnosis albeit being invasive, and thus not suitable for regular sampling. To this end, there is a dire need to develop a painless, non-invasive, rapid and inexpensive diagnostic procedure for OC that can detect the malignancy at early stages with high accuracy. Diagnosis in early stages is critical but it can improve the five year survival rate dramatically.

Optical spectroscopy can be an invaluable tool in this regard. Such a technique has the potential to diagnose oral dysplasia and early stage cancer non-invasively, by optically detecting subtle biochemical changes, that occur before any change in tissue morphology. Additionally, optical spectroscopy offers many advantages over traditional approaches including cost, speed, objectivity, sensitivity, painless, easy to perform real time diagnosis in clinical environment. In

this study, different modes of Raman spectroscopy are described to analyze biochemical markers non-invasively. It is a powerful tool to measure vibrational fingerprints of the molecule and from that information structural, physical and chemical properties of that particular sample can be determined. It requires minimal sample preparation and uses visible light to probe vibrational states of the molecules. Contrary to IR spectroscopy, Raman spectroscopy is more suited for the analysis of aqueous samples because water is a weak Raman scatterer. Additionally, the relatively narrow Raman peaks – hence richer molecular information, along with the ease of sample preparation make Raman spectroscopy a potential tool for early detection of oral cancer.

In this study we report the development of a multimodal Raman system for oral cancer screening and accurate tumor margin detection during surgery. This developed system is easy to use, compact and portable. It can work in both reflection and transmission mode and can be used for in vivo and ex vivo analysis. To compensate for the weak Raman signals, plasmonic enhancements were used in this study. This study also examines the SERS enhancement and background from different commercially available and home-made nanoparticles. Lastly, for the purpose of proof-of-concept demonstration, bovine serum albumin (BSA) and uric acid were used as model at the similar concentration range as present in human saliva to test the performance of the system.

2. SYSTEM

Raman spectroscopy is a nondestructive optical approach for probing the vibrational modes of molecules. These vibrational modes serve as chemical fingerprints that allows molecules to be identified or distinguished. Owing to its chemical sensitivity, Raman spectroscopy thus has the potential to identify tumor in oral cavity at early stages by differentiating tumor and healthy tissue on the basis of their overall biochemical composition. A multimodal Raman system developed in our lab is shown in figure 1 for the complete screening of oral cancer, starting from the detection of oral cancer biomarkers in saliva specimen of the patient. However, the concentration (amount) of these biomarkers in the saliva is low and requires amplification of inherently weak Raman signals for sensitive detection. For this purpose, surface enhanced Raman spectroscopy is used which involves mixing the salivary samples with plasmonically-active nanoparticles. By virtue of intense localized surface plasmon fields on the nanoparticles, Raman signals from analytes adsorbed in the vicinity of or absorbed on the nanoparticles will thus experience tremendous enhancements (between 10^4 – 10^6). As a result, the detection sensitivity can be increased while maintaining the FWHM of sharp Raman peaks. Recently, surface enhancement is observed in photonics crystal fibers. PCFs are specially designed optical fibers that possess holes allowing incorporation of liquid or gas analyte inside them. PCF fiber gives light a chance to interact with a large number of analyte molecules and metallic nanoparticles along the length of the PCF in a relatively small volume (20 - 100nL range) resulting in improve sensitivity. As the light can interact with overall large number of analyte molecules and nanoparticles it limits the effect of nanoparticles variability by providing averaged signals, which leads to better repeatability and reproducibility [7].

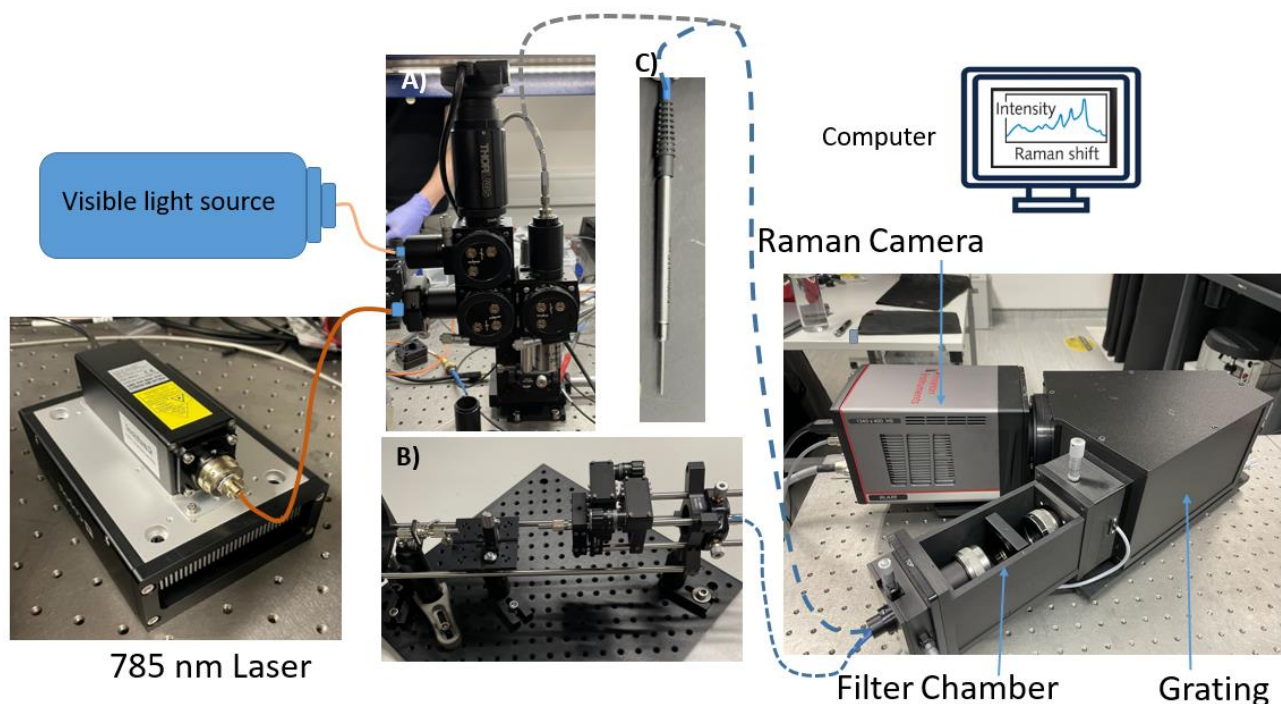


Figure 1. Home built multi-configuration Raman system

The system described here is compact and easy to use. It consists of interchangeable Raman acquisition subunits: an epi microscopy setup (for salivary analysis), a transmission setup (PCF analysis) and an optical-fiber probe (for in-vivo study). Its portability means it can be used for the bedside clinical diagnostics and the two subunits can be easily switched between *in vivo* or *ex vivo* analysis depending on the requirement. When connected with a probe, the system can be used for the *in vivo* analysis of the healthy and malignant tissue in oral cavity. The probe can be replaced with a home-built microscope that enables the collection of Raman photons using an objective lens in upright position (reflection mode) (Figure 1A). The stage in upright position that can work in the reflection mode has an automated z axis adjustment and can accommodate the liquid and solid samples. Saliva specimen can be placed on any 2-dimensional substrate for the analysis or even in the cuvette for the diffused focus. In this work, to collect the salivary Raman signature, plasmonically-active gold nanoparticles are mixed with the salivary samples to observe under the Raman microscope.

To further increase the overall interaction length and to get the enhanced signal the transmission mode will be used with PCFs, arranged in the transmission set-up (Figure 1B). This setup holds the PCF loaded with mixture of nanoparticles and saliva sample. Light will enter from one end of the fiber and the transmitted light will be focused on the collection fiber of the Raman camera through multiple lenses.

Apart from analysis of saliva samples for the screening of oral cancer, the system can be used with a handheld probe for *in vivo* analysis of the oral cavity (Figure 1C). These probe-based measurements can assist the identification of cancer lesions for inform biopsy based on the spectral information.

Figure 2a represents geometry of a typical PCF placed in the new transmission mode stage shown in figure 2b. It is a suspended core PCF (SuC-PCF) with air holes in the cladding as big as possible ($>50\mu\text{m}$) to allow the incorporation of the liquid analyte and nanoparticles easily. Figure 3c represents the PCF fiber placed in the transmission mode stage.

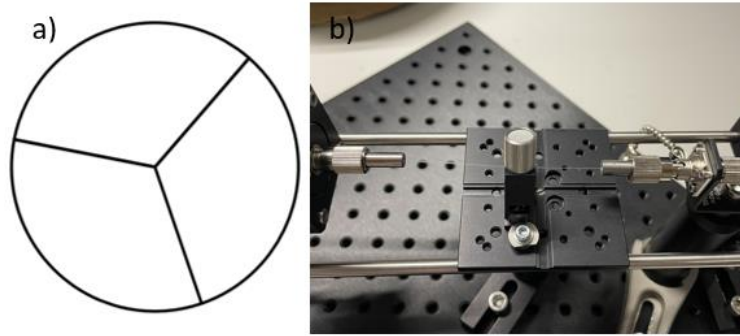


Figure 2. a) Geometry of a typical suspended core photonic crystal fiber (SuC-PCF) b) PCF in Home built transmission mode stage

3. RESULTS

Raman spectroscopy differentiate between the saliva of healthy and tumor patients on the basis of spectral features. In past few decades, hundreds of salivary biomarkers have been reported to diagnose oral cancer in early stage, including circulating tumor cells, genomic or proteomic biomarkers[8]. To analyse most of these biomarkers highly sensitive methods are required because of their low concentration in saliva. Table 1 shows some of the potential biomarkers and their concentration in saliva of healthy volunteers and in people suffering through oral cancer.

Table 1. List of the important salivary biomarkers for oral cancer diagnostics and their concentration in healthy volunteers and oral cancer patients

Some important salivary biomarkers for oral cancer				
Biomarkers	Conc. In healthy volunteer	Conc. In oral cancer patients	Trend	Ref.
<i>Proteins</i>	<i>0.17-0.36 g/l</i>	<i>0.192-0.67 g/l</i>	Increase	[1]
<i>Uric Acid</i>	<i>4.24 – 27.1 µg/ml</i>	<i>7.26 – 30.75 µg/ml</i>	Increase	[1]
<i>Fucose</i>	<i>3.18 mg/dl</i>	<i>11.66 mg/dl</i>	Increase	[2]
<i>Lipids (Linoleic Acid)</i>	<i>339.3 + 267.9 ng/ml</i>	<i>1092.3 ± 1927.8 ng/ml</i>	Increase	[4]
<i>Interlukins (IL - 6)</i>	<i>0 pg/ml</i>	<i>86.5 pg/ml</i>	Increase	[5]
<i>CD 44</i>	<i>1.09 ng/ml</i>	<i>7.85 ng/ml</i>	Increase	[6]

Among all these biomarkers we have used uric acid and albumin protein to use as representative analytes in the concentration range as present in human saliva to test the performance of our system and methodology. Due to their low concentration in saliva and low sensitivity of Raman system, gold nanoparticles (AuNPs) were used for surface enhancement. Different sized commercially available nanoparticles were tested using rhodamine B (Rhd B) as an analyte. And on the basis of enhancement output shown in figure 3, 60 nm AuNPs were selected for the further analysis as 60nm AuNPs provided best enhancement with every concentration of Rhd B.

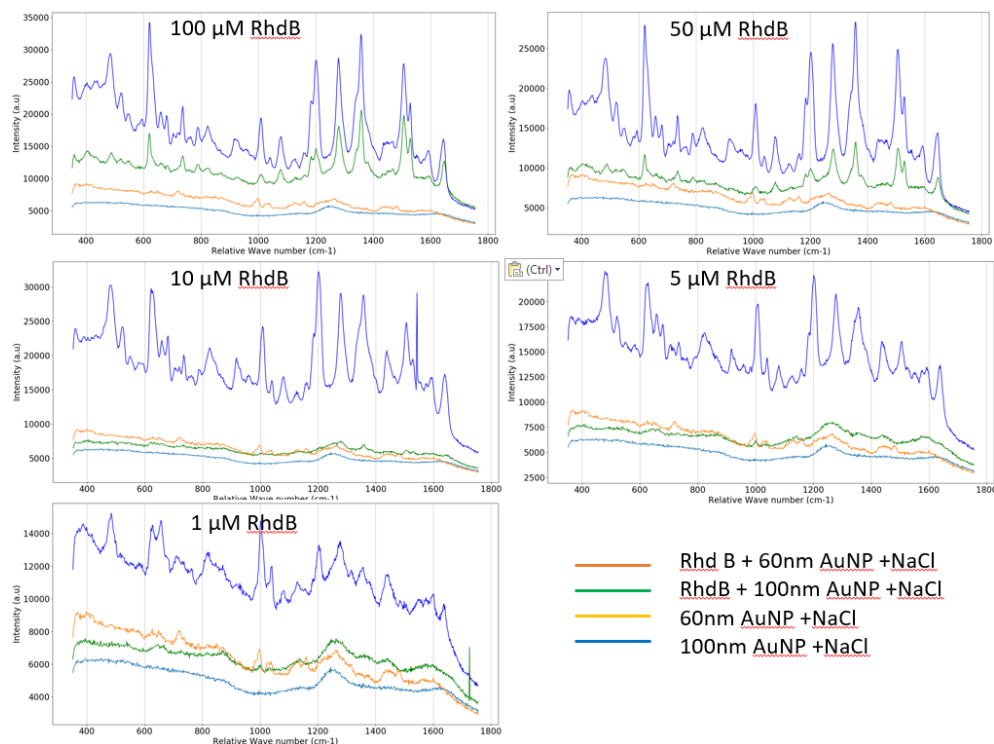


Figure 3. Selection of nanoparticles for SERS using Rhod B

Spectra in figure 4 represents the different concentrations of uric acid in the range that is present in human saliva with 60nm commercially available AuNP. These spectra are slightly different then the spectra of the solid uric acid. Some of the peaks found in solid uric acid were not visible and some shifted slightly in these spectrum this could be attributed to a different molecular orientation and interaction of the uric acid molecules with the nanoparticles [9]. If 635 cm^{-1} peak is considered as the Raman signature of uric acid which is enhanced here. These results depict that uric acid can serve as a potential biomarker for the screening of saliva samples.

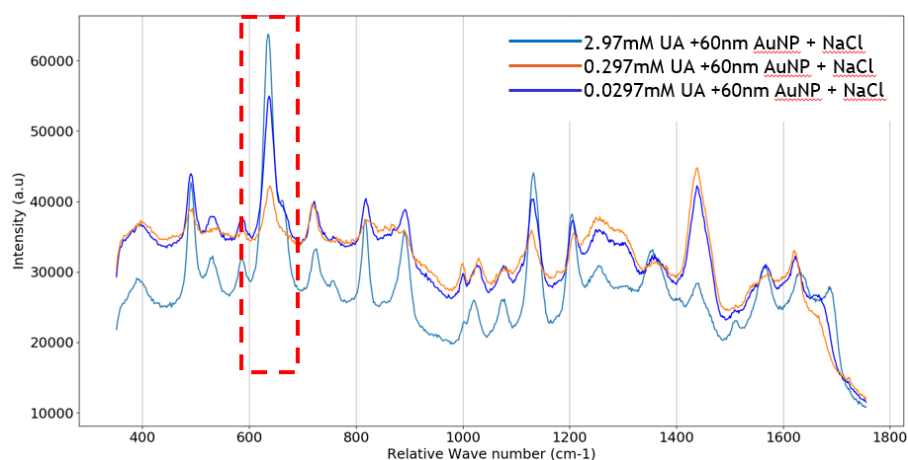


Figure 4. Uric acid Raman spectrum with 60nm commercially available gold nanoparticles with the new Raman system

Experiment with RhdB and uric acid confirms the presence of some background signal that can be associated with the used commercially available nanoparticles. That is why homemade nanoparticles were synthesized and tested with BSA.

Figure 5 represents the background spectrum from 100nm and 60nm commercially available nanoparticles in comparison with our home made nanoparticles. Throughout the experiment NaCl has been used for conjugating nanoparticles.

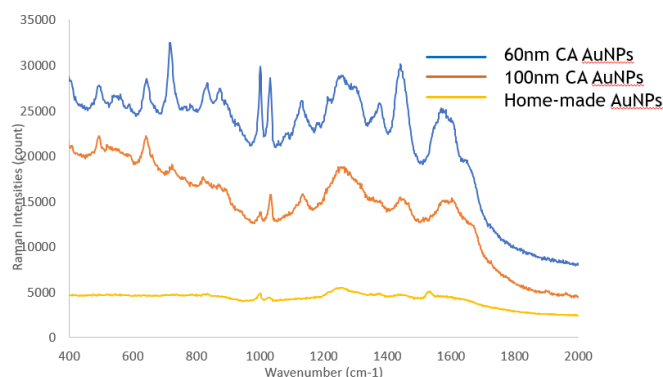


Figure 5. Comparison of SERS background spectra of home-made AuNP and commercially available (CA)-AuNPs

Figure 6 represents the spectra of BSA with home-made nanoparticles. This spectra is in good agreement with the published literature [10].



Figure 6. SERS spectra of BSA with home-made AuNP.

4. CONCLUSION

Development of a multimodal Raman system is reported in this study. This novel system is compact and has a flexible design. It is suitable for both in vivo and ex vivo analysis. The system is easy to use and alterations between probe and microscopic design is quite easy. The ex vivo mode offers two kinds of stage to work in reflection and transmission mode. Performance of the system was tested for the future application by using uric acid and BSA as model analytes. Background comparison of commercially available nanoparticle and home-made nanoparticles was also given.

5. FUTURE PERSPECTIVE

In future, the developed system will be used for the detection of oral cancer and pre-malignant oral diseases by planning a complete screening program. It will involve the detection of salivary biomarkers of oral cancer and *in vivo* tissue differentiation.

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