



# Fourier Transform Infrared Radiation (FT-IR) Spectroscopic Analysis of Oral Squamous Cell Carcinoma - A Preliminary Evaluative Study

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## KEYWORDS

Oral squamous cell carcinoma, FT-IR, non invasive screening, chair side diagnosis

## ABSTRACT:

**Introduction:** Fourier transform infrared spectroscopy is gaining traction in the field of cancer research. The molecular changes that underpin the pathophysiology of an illness are used to diagnose it, not the appearance of the tissue. The aim of this study is to use Fourier transform infrared radiation (FT-IR), an automated technique that more accurately represents morphology, to identify molecular differences between normal and Oral cancer tissue.

**Objectives:** To analyze and compare the molecular composition of normal and cancerous oral tissues using Fourier Transform Infrared (FT-IR) spectroscopy, and to identify specific spectral differences that may aid in the molecular diagnosis of oral cancer.

**Methods:** Fresh tissue samples were obtained from Oral Cancer patients undergoing surgery. The mercury cadmium telluride (MCT) detector on the Nicolet Magna 750 FT spectrometer was used to record the spectra.

**Results:** FT IR spectra showed significant differences in the peaks between the cancerous and normal oral tissue. The stretching vibration of the carbonyl produced the peak at 1645/cm. Compared to normal tissue, the gradient of the protein peak in malignant tissue was greater.

**Conclusions:** Numerous genes regulate the multiple stages of the pathogenesis and development of oral cancer, which are influenced by a wide range of variables. When it comes to protein, nuclear acid, glucose, and adipose in cells, as well as the molecular structure of the molecule, FT IR can offer information that allows for an in-depth understanding of the molecular diagnosis of cancer and future treatment strategies.

## 1. Introduction

Squamous cell carcinomas account for over 90% of these malignancies. Oral cavity squamous cell carcinoma (OSCC) incidence varies noticeably by geography, with developing nations accounting for about two thirds of occurrences[1]. Three well-established risk factors for OSCC include alcohol use, betel quid use, and tobacco use in all its forms[1]. The incidence of the disease in nonsmokers has increased recently, indicating that additional factors might be involved. More research is needed to pinpoint these risk factors, though OSCC patients arrive with advanced illness, with significantly poor overall survival rate[1][2].

A Fourier-transform(FT) spectrometer uses interference to modulate radiation in the time domain, which is then Fourier transformed to provide the spectrum of its source radiation. An outline of its overall functionality is provided here[3][4]. During FT-IR spectroscopy, three primary measuring methods can be employed: transmission, attenuated total reflectance(ATR), and reflection. Even though all methods rely on the fundamental idea of FT-IR spectroscopy, they each analyze the material using a slightly different procedure and have various applications.[5].The technique of Fourier transform infrared (FTIR) spectroscopy holds potential for identifying the molecular fingerprints of biological samples[6]. The basis of this technique is the way the



chemical bonds in the samples vibrate in response to infrared light. FTIR is a label-free, non-invasive technique that plays a crucial role in the early detection of cancer since it can spot early bimolecular changes connected to a tumor before any outward signs of abnormality show up[6,7],[8].

As a result, more research is being done to determine how infrared spectroscopy might be used for illness screening and diagnosis[9]. The hunt for innovative techniques has been made necessary by the dearth of extremely sensitive and focused techniques for the early diagnosis of malignancy. Fourier transform infrared spectroscopy has advantages over existing screening, diagnosis, management, and monitoring methods, is easier to use, faster, more accurate, less expensive, non-destructive, and amenable to automation[10]. Clinical decision-making and patient outcomes may be improved by detecting molecular alterations in the metabolic states of affected patients[1]. A non-invasive technique that is becoming more and more popular is the extracellular vesicle-based method, in addition to the often examined blood and tissue samples[10,11]. In order to detect and prevent oral carcinoma as early as possible, a cutting-edge diagnostic instrument that offers precise OSCC grading for improved oral cancer care together with a quick, unbiased, and accurate diagnosis of OPMDs is clearly needed.[1,7].

## Objectives

The gingivobuccal sulcus of the jaw, the tongue, and the floor of the mouth are the most frequently occurring locations of oral squamous cell carcinoma[12]. It is important to recognize that carcinoma in India is not the same as carcinoma in other nations. The most common reasons are using pan masala, a mixture of tobacco, betel nut, and unknown substances added for flavor and color, and chewing tobacco (smokeless tobacco)[13]. An established analytical method with a wide range of uses is FT IR spectroscopy[7]. Biomolecular fingerprinting, a term used to describe the biochemical profile of proteins, nucleic acids, lipids, and carbohydrates in biological samples, is provided by FT IR spectroscopy[6]. Patient survival rates can be raised and medical costs can be decreased by early identification of oral cancer[8]. A sensitive and reliable molecular-based diagnostic tool is desperately needed for the early identification of oral

malignancy[14]. Because of its capacity to yield both qualitative and quantitative data regarding the biochemical composition and molecular-level structural alterations in intricate biological systems, Fourier transform infrared spectroscopy is gaining traction in the field of cancer research. The molecular changes that underpin the pathophysiology of an illness are used to diagnose it, not the appearance of the tissue[15]. This technique is adaptable and can be used to bodily fluids, tissues, or cells. The aim of this study was to use Fourier transform infrared radiation (FT-IR), an automated technique that more accurately represents morphology, to identify significant differences between normal and oral malignant tissue.

## 2. Methods

The study was conducted in accordance with SRB/SDC/OPATH-2202/23/218 ethical guidelines after being approved by the institutional scientific review board. Following the standards of the 2013 amendment of the 1964 Declaration of Helsinki, this study was conducted. Case samples were collected from patients histologically diagnosed with oral squamous cell carcinoma during intraoperative frozen section. Control tissue samples(healthy tissue) were obtained from tissues attached to the apical area of the tooth after orthodontic extraction. All participants provided written informed consent. Confidentiality was maintained consistently.

Spectroscopy with Fourier transform infrared was used to study both normal and oral malignant tissues, and the unique spectra features were examined. A mercury cadmium telluride (MCT) detector was used to record the spectra on a Nicolet Magna 750 Fourier transform spectrometer. The near infrared (NIR) at 0.76-2.5 microm (12500-4000 cm<sup>-1</sup>) is one of the three infrared spectrum areas. the 2.5-25 microm (4000-400 cm<sup>-1</sup>) range of the mid-infrared (MIR). 400-10 cm<sup>-1</sup> is the far-infrared range in 25-1000 micrometers. Mid-infrared, which includes a fingerprint region between 1800 and 900 cm<sup>-1</sup> for protein, lipid, carbs, and nucleic acid, is the most often used region for biological applications. This differentiation is based on three categories that are created by the infrared wavelength. The electromagnetic spectrum is near infrared, which has the shortest wavelength of the three, just after visible red light. Situated between near and far infrared, mid infrared has



a longer wavelength. On the electromagnetic spectrum, far infrared has the longest wavelength and is therefore closest to radio waves and farthest from visible light.

In our study, the case (tumour) and normal tissues were collected from patients histologically diagnosed with oral squamous cell carcinoma during intraoperative frozen section. Infrared (NIR) wavelength range: 0.76 - 2.5 micron (12500 - 4000  $\text{cm}^{-1}$ ). The range of 2.5 to 25 micron (4000-400  $\text{cm}^{-1}$ ) is mid-infrared (MID). Far-infrared (FIR) wavelengths: 400–10  $\text{cm}^{-1}$  at 25–1000 microns. The MIR region, which has a fingerprint of 1800–900  $\text{cm}^{-1}$  for proteins, lipids, carbohydrates, and nucleic acids, is the area that biological applications most frequently used.

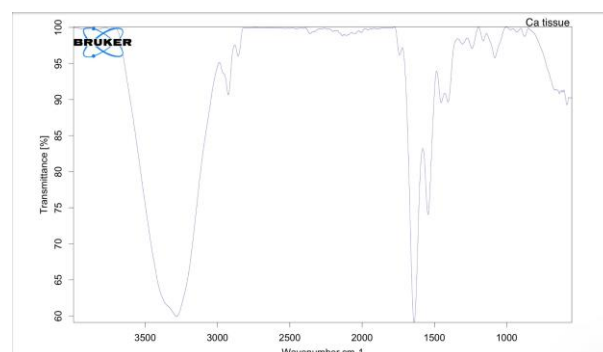


**Figure 1:** Nicolet magna 750 Fourier transform spectrometer with a mercury cadmium telluride (MCT) detector.

### 3. Results

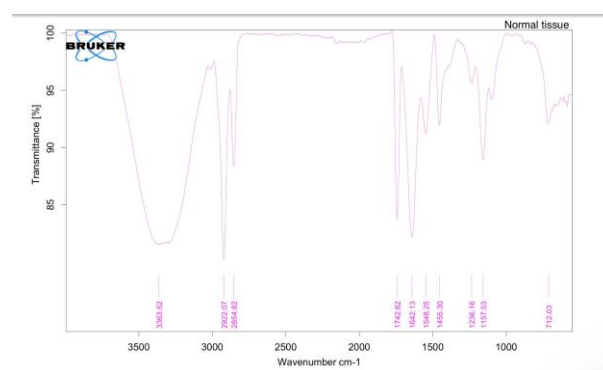
Significant differences were found between normal and cancerous oral tissue in the FT-IR spectra. The diagnosis of tumour cells may be established at the molecular level owing to the information about molecular structure that FT IR could provide. Help to identify or be able to recognize unfamiliar materials, It can ascertain a sample's uniformity or quality. It can ascertain how many ingredients are present in a mixture. This information might indicate changes in protein, nuclear acid, glucose, and fatty tissue in the cells along with the structure array of the molecule.

It is important to assess and document the link between wavelength and transmittance %. distinct wavelengths correspond to distinct protein types (Amide I and Amide II), lipids, lipid DNA/RNA, and glycogen DNA/RNA. The most crucial peak level that needs to be taken into account is the mid and far infrared radiation.



**Figure 2:** The spectra of oral squamous cell carcinoma tissue at wavelength of 3500-1000  $\text{cm}^{-1}$ .

The peak wavelength at 1640.08  $\text{cm}^{-1}$  shows expression of protein (amide I) in cancerous tissue, meanwhile the adjacent peak at 1544  $\text{cm}^{-1}$  shows evidence of protein (amide II) level in malignant tissue. The peak at wavelength of 1452  $\text{cm}^{-1}$  represents lipid level in malignant cells and the neighboring peaks show glycogen DNA/RNA, lipid DNA/RNA.



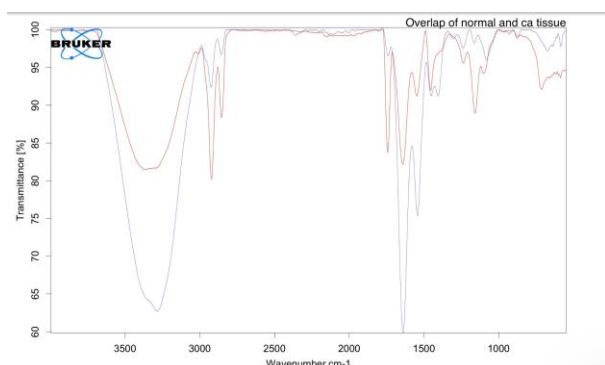
**Figure 3:** The spectra of normal tissue at wavelength of 3500-1000  $\text{cm}^{-1}$ .

### 4. Discussion

Oral squamous cell carcinoma (OSCC) accounts for the majority of malignant neoplasms in the mouth, accounting for around 80–90% of cases. The mouth cavity is recognized to be between the sixth and the ninth most common anatomical place for cancer, despite the fact that oral cancer incidence varies greatly around the globe [1,2]. This is primarily dependent on the patient's gender and the country (or even a specific region in certain cases). Notwithstanding this average incidence, in certain places, particularly in Southeast Asia, it may serve as the most frequent site for cancer [16]. The main



risk factors and etiologies of oral squamous cell carcinoma (OSCC) are UV radiation and smoking/drinking behaviors[17]. Through the use of FT-IR, molecular structural information may be collected, reflecting changes in nuclear acid, sugar, fat, protein, and cell structure as well as changes in the structure of the molecule's space array, allowing for the molecular level identification of cancer cells[3].



**Figure 4:** The spectra showing overlap of normal and cancerous tissue at wavelength of 3500-1000 cm-1.

The Fourier transform infrared spectroscopy(FT-IR) in which after the computer does the transformation, the user is presented with the desired spectral information for analysis. This includes samples of either fresh or frozen biological tissues kept at the area where the infrared beam strikes the target. Within a few minutes, the spectroscopy will analyze and provide results. To avoid obscuring the biological ratios associated with the material, we procured new tissue samples.

While comparing the normal and cancerous tissue the peaks show higher protein levels in the cancerous tissue while in normal tissue it shows limited % of transmittance.lipid level shows decreased wavelength while comparing to protein peaks. Other molecules like glycogen DNA/RNA, lipid DNA/RNA level in cancerous tissues show lower peaks than normal tissue. The following factors may be associated with the decreased lipid content in malignant tissues. First of all, because cancer grows quickly and requires more food and energy, it may use fat as a source of these things, preventing fat from building up in cancerous tissues and cells. The second possible explanation is because the tumor developing malignant components mechanically exclude normal cells and tissues, including fat cells[18].

DNA/RNA level in malignant tissue indicates the proliferative nature of the tumor, increased in this peak represents the growth and extension of cancer tissues. According to recent developments, near infrared spectroscopy is a very adaptable analytical tool that may be used in a wide variety of studies. In dentistry, hand-held infrared (IR) tools are used as an early diagnostic tool to examine cancer cells before more invasive treatments like biopsies. New breakthroughs in infrared source, waveguide, chip integrations, detectors and software development are driving the rapid, low-cost, accurate, and automated diagnostic tool that is FTIR in the clinical setting.

### CONCLUSION:

The findings suggest that clinical diagnostics may benefit from the application of Fourier infrared spectroscopy. The ability to distinguish between diseased and normal oral instances with high sensitivity, specificity, and accuracy has been proven by FTIR in combination with chemometric data processing.

### References

1. Rabin M: Oral Squamous Cell Carcinoma: From Diagnosis to Treatment. Nova Science Publishers; 2021.
2. Oral Squamous Cell Carcinoma: New Insights for the Healthcare Professional: 2013 Edition. ScholarlyEditions; 2013.
3. Tudini A, Ti S-S: Analysis of Emission Data from a Perkin-Elmer 1710 FTIR Spectrometer. 1990.
4. Mattson JS: Infrared, Correlation, and Fourier Transform Spectroscopy.
5. Pachler KGR, Matlok F, Gremlich H-U: Merck FT-IR Atlas: eine Sammlung von FT-IR Spektren. Vch Pub; 1988.
6. Mantsch HH, Chapman D: Infrared Spectroscopy of Biomolecules. Wiley-Liss; 1996.
7. IOS Press (Firm): Vibrational Spectroscopy in Diagnosis and Screening. IOS Press; 2012.
8. Stuart BH: Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons; 2004.



9. Mackenzie MW: *Advances in Applied Fourier Transform Infrared Spectroscopy*. John Wiley & Sons; 1988. Chennai: A Retrospective Study. *Cureus*. 2024, 16:e52774.
10. Salih S: *Fourier Transform: Applications*. BoD – Books on Demand; 2012.
11. Smith BC: *Fundamentals of Fourier Transform Infrared Spectroscopy*. CRC Press; 2011.
12. Website. Accessed: Muthusamy, M., Ramani, P., Arumugam, P. et al. Assessment of various etiological factors for oral squamous cell carcinoma in non-habit patients- a cross sectional case control study. *BMC Oral Health* 25, 62 (2025). <https://doi.org/10.1186/s12903-024-05406-z>.
13. Esakki A, Poothakulath Krishnan R, Pandiar D, Ramani P, Jayaraman S: The Evaluation of Tumor Budding in Oral Squamous Cell Carcinoma Arising in the Background of Oral Submucous Fibrosis. *Cureus*. 2024, 16:e69830.
14. Krishnan RP, Pandiar D, Ramani P, Jayaraman S: Molecular profiling of oral epithelial dysplasia and oral squamous cell carcinoma using next generation sequencing. *J Stomatol Oral Maxillofac Surg*. 2024, 126:102120.
15. R P Jr, Yuwanati M, Sekaran S, M S: miRNA Associated With Glucose Transporters in Oral Squamous Cell Carcinoma: A Systematic Review. *Cureus*. 2023, 15:e46057.
16. Asarkar AA, Chang BA, de Bree R, et al.: Primary Management of Operable Locally Advanced Oral Cavity Squamous Cell Carcinoma: Current Concepts and Strategies. *Adv Ther*. Published Online First: 20 April 2024. 10.1007/s12325-024-02861-6
17. Zou J, Dai Y, Xu G, Kai Y, Lan L, Zhang J, Wang Y: Identification of two distinct head and neck squamous cell carcinoma subtypes based on fatty acid metabolism-related signatures: Implications for immunotherapy and chemotherapy. *Medicine* . 2024, 103:e37824.
18. Reichal P, Ramani P, Kizhakkoottu S: Association of Site and Recurrence in Oral Squamous Cell Carcinoma Patients Visiting Private Hospital in