



# An overview of conventional and fluorescence spectroscopy tools in oral cancer diagnosis

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## Abstract

**Background** Oral cancer is a major public health challenge, along with other common diseases worldwide. Risk factors like tobacco usage and alcohol consumption have contributed towards the rise in the development of oral cancer cases. The low survival rate of oral cancer creates a necessity for early detection. At present, clinical examination followed by biopsy and histopathological-based assessment is considered the gold standard in the early diagnosis of oral cancer. However, it also holds drawbacks like invasiveness and time consumption in confirming a diagnosis, which have raised the need for other non-invasive diagnostic techniques. Spectroscopic techniques used as diagnostic tools are to collect the spectral signatures generated by endogenous fluorophore molecules in abnormal and healthy tissues upon excitation with a suitable wavelength.

**Objective** The present review aimed to highlight the potential application of non-invasive spectroscopic tools such as fluorescence spectroscopy in oral cancer detection and diagnosis and with a brief standpoint of the conventional techniques used in oral cancer diagnosis.

**Conclusion** The review emphasizes the potentiality of spectroscopic techniques to replace conventional diagnostic procedures, given their minimally invasive or non-invasive approach, cost-effective nature, and ability to provide a diagnosis in real time.

**Keywords** Oral cancer · Fluorescence · Non-invasive · Spectroscopy

## Introduction

Cancer is one of the major causes of mortality among adults worldwide. A rise in cancer-related deaths is expected, primarily because of an increase in the risk factors for cancer [1]. Oral cancer is a significant health concern, especially in Asian countries, as it shows a great deal of variation worldwide in terms of its incidence rate owing to racial and ethnic differences among the people as well as associated lifestyles [2]. Countries in South and South-East Asia present the most worrisome rate of

incidence, according to the International Agency for Research on Cancer, which includes Sri Lanka, India, and Pakistan, among others [3]. These countries, which are said to be developing, lower-income countries, are often more affected by this burden compared with developed countries and are responsible for two-thirds of the oral malignancies worldwide. The Asian population is more prone to oral cancer compared with other communities due to the habit of chewing tobacco and areca nut in betel quid being rampant in these groups of people. The incidence of oral cancer found to occur more commonly in males than in females [4, 5]. With the increase in age, the risk of developing oral cancer increases. Further, the 5-year incidence and mortality rate as per GLOBOCAN (2018) report indicate oral cancer incidence and mortality are higher in males compared with females [6].

The majority of oral cancer incidence is found to occur in people age 50 years and above. In high-risk countries, oral cancer cases among people below the age of 40 have also been reported. A study conducted by Dikshit R et al. has highlighted oral cancer as one of the fatal diseases in Indian men belonging to the age group of 30–69 years, accounting for 22.9% of the cases, which included cancers of the lip, oral cavity, and

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pharynx, while in women belonging to the same age group, oral cancer accounted for 9.8% of the cancer cases after cervical (17.1%), stomach (14.1%), and breast (10.2%) [1].

Oral cancer could be defined as a non-homogenous group of diseases of the oral cavity, involving the lips, tongue, labial mucosa, buccal mucosa, palate, the floor of the mouth, gingiva, and other anatomical sites. However, literature does not adopt a clear cut definition for the same. Oral cancer has a multifactorial etiology, with tobacco considered a significant risk factor. Apart from smoking or chewing any form of tobacco, along with or without betel quid, excess consumption of alcohol, infection by human papilloma virus (HPV), poor oral hygiene, and poor dentition and even genetic susceptibility are associated with risk factors [7–9]. A considerable minority of the cases have also reported that oral cancer can be caused by factors other than the traditional risk factors. Lower economic status and lack of awareness about oral health are also related to increased risk of developing the disease [10]. The level of education is associated with knowledge about oral cancer, and its risk factors were looked into by Elango JK et al. in a survey conducted among a high-risk population. They reported that higher education level, more significant, was the awareness for both oral cancer and its risk factors ( $p < 0.001$ ), while oral cancer-causing risk habits, which included smoking, chewing pan with/without tobacco, and alcohol, were significantly less prevalent among those with a better level of education [11].

Oral cancers show a low survival rate (less than 50% in most countries) since half of the diagnosed cases would have progressed to an advanced stage. The above progression of the diseases to advanced stage attributed due to the patient's ignorance or reluctance in obtaining proper medical care and poor acceptance of the treatment [10]. Also, the lack of access to cancer treatment centers may further delay and also result from misdiagnosis and inappropriate treatment. On the contrary, in diagnosis at the stage where the primary tumor is localized, the survival rate is better, suggesting that early diagnosis could lead to better chances of survival. Therefore, detection of oral cancer at an early stage or a precancerous lesion before it becomes malignant is the key to ensure effective treatment and reduced morbidity [12].

In the present review, we have attempted to highlight the potentiality of fluorescence-based spectroscopic tools in the early oral cancer detection/diagnosis, while we also tried to summarize some of the conventional techniques used in oral cancer diagnosis with their limitations, which is routinely used in oral oncology screening.

## Conventional diagnostic techniques

The fact is that the oral cavity is accessible for the visual and tactile examination and can be viewed to detect oral cancer or

even for its precancerous change [9]. Presently, the assessment of the oral cavity is carried out either by oral examination, invasive approach (oral biopsy), and vital staining techniques (toluidine blue, Lugol's iodine), or by light-based optical tools such as chemiluminescence techniques.

## Oral examination

Conventional oral examination (COE) is carried out with the help of an incandescent light, which is used routinely in the screening of oral cancer. Visual examination to screen patients is advantageous as it is non-invasive and causes no pain and is also well accepted among the clinicians socially. However, studies have highlighted that the method is a bit obscure for screening premalignant or early lesions. On the contrary, few studies report that the COE method has better sensitivity and specificity. Presently there is hardly any test that can reveal an accurate detection of oral lesions when it comes to the screening program; the only method used for screening in the oral malignant or lesion is a conventional oral examination. Some of the earlier studies have reported the COE-based screening for the detection of potential oral malignant lesions or cancers performed by health experts [13, 14].

A hospital-based study was carried out by Chang et al. where a total of 13,878 participants were included in the study. The study aimed to demonstrate the effectiveness of oral visual screening in determining the potential risk of oral cavity cancer in chronic smokers, alcohol consumption, and betel quid chewers. The results clearly show that oral screening is effective and should be used regularly as a routine screening procedure to detect oral cancer at the earliest [15]. In another study, Walsh et al. have also highlighted the diagnostic methods, such as COE, vital stain rinsing, optical tools, and biomarker applications in oral cancer detection. The review mainly includes the sensitivity and specificity of the methods. The study shows that COE has better sensitivity, which was higher than 70%, and the specificity was more than 90% compared with other techniques used in oral cancer screening [16]. Table 1 below highlights the results of a few studies where COE is used as a screening tool in oral cancer.

Thus, based on earlier studies, it has been reported that COE could be used to screen the oral lesions and cancers. However, it is not useful in differentiating between the lesions that could progress to cancer and which would be in a benign state. Also, chromosomal aberrations may exist in areas of the mouth appearing to be normal, which could later lead to the development of cancer [22]. Also, certain precancerous lesions get hidden beneath normal-appearing mucosa and which is difficult to detect during COE and could lead to oral malignancies. Therefore, all suspicious lesions should be evaluated

**Table 1** Studies mentioning sensitivity and specificity for the detection of oral abnormalities by conventional oral examination

Authors	Objective of the study	Subject	Type of lesion	Conclusion	Sens (%)	Spec (%)
Monterio et al. [17]	To determine the usefulness of oral cancer screening for the detection of oral cancer and oral potentially malignant disorders (OPMDs) in the population of Oporto City, Portugal.	727	OPMD and oral cancer	The study suggests the new cases of cancer and potential malignant disorders can be detected by oral screening and can help in early detection and raise awareness of cancer in Portuguese population.	96	98
Scott SE et al. [18]	The study aims to determine the potentiality of oral screening examination as a tool to detect the potential malignant oral lesions in oral cancer.	53	Oral screening	The study summarizes that the leaflet-based self-identification is not effective in detection of potential malignant oral lesion.	33	54
Mathew B et al. [19]	A randomized intervention-based study carried out in order to evaluate the effectiveness of oral visual screening in prevention of oral cancer screened by trained health workers.	2069	Premalignant or malignant	The study outcomes show that experienced trained health care persons can perform oral cancer screening as effectively as doctors.	94.3	99.3
Julleian et al. [20]	To establish the sensitivity and specificity of oral examination in detection of oral cancer and precancer.	24	Premalignant or malignant	The study was able to show an effective way by which oral lesions can be screened.	74	99
Downer MC et al. [21]	To determine and establish the sensitivity and specificity of oral screening test in detection of oral cancer and precancerous, and further to evaluate a pilot screening program in the work environment.	292	Oral lesion	The study summarizes that the pilot screening program shows oral screening program is effective in screening in oral cancer, and the program can be made more feasible and cost-effective with inclusion of more experienced oral examiner.	71	99

histologically to determine their potential in turning into a malignant condition.

## Oral biopsy

Oral biopsy is considered an essential clinical tool in the diagnosis of oral lesions ranging from simple lesions such as precancerous lesions to cancerous lesions. The biopsy procedure helps the clinicians to determine the proper diagnosis of the diseases, which eventually will help patients in the timely treatment of the diseases. The suspicious oral lesions are evaluated either by oral brush cytology or by surgical biopsy.

The scalpel or punch biopsy, followed by its histopathological assessment, is considered the gold standard for the diagnosis of oral lesions [23]. However, it is not conceivable to perform a biopsy in all cases, as some of the patients might not give consent as their lesion may be asymptomatic. In some cases, the patients are critically ill due to which the procedure could not be possible to carry out. An alternative to the above, the clinicians go for an oral cytology approach, where they monitor changes in cytology of oral cells by performing a procedure such as brush cytology. The brush cytological study is minimally invasive and well accepted among the patients and gives consent to undergo the procedure, due to which it has been considered an alternative diagnostic option to a more

invasive and painful biopsy approach [24]. The technique holds the potential in determining the pathological changes in the oral lesion. The technique mainly involves the collection of oral cells by rinsing the oral cavity after scraping the affected oral cavity area using an oral brush.

Oral computer-assisted brush cytology (OralCDx) is the most standard and routinely brush cytology procedure for assessing the oral lesions [25]. Several studies have reported encouraging results with oral brush cytology in evaluating cancerous lesions. Table 2 illustrates the utilization of oral brush cytology methods highlighting the sensitivity and specificity of the technique.

At present, a biopsy followed by a histopathological assessment of a tissue sample suspected of malignancy is considered the gold standard for the diagnosis of oral cancer. Histopathological evaluation of tissues involves a visual analysis of tissues at the cellular level, which enables the clinicians to make decisions on whether the tissue section under evaluation is malignant or not, based on the features like shape and distribution of the cells and the nuclei [31, 32]. Further, during the procedure, the site of the mouth from where the tissue specimen obtained during biopsy should be such that it should be able to represent the most severe changes in the oral cavity. Therefore, the accuracy of diagnosis depends on the clinician's choice of an appropriate biopsy site and the pathologist's interpretation based on the histopathological findings

**Table 2** Studies mentioning sensitivity and specificity for the detection of oral abnormalities by oral brush cytology

Authors	Objective of the study	Subject	Type of lesion	Conclusion	Sens (%)	Spec (%)
Goodson et al. [26]	The study was performed to determine the adjuvant use of oral brush biopsy techniques in the management of potentially malignant disorder in UK hospital population.	310	Oral potentially malignant disorder	The study reports that oral brush biopsy technique provides reliable diagnosis along with conventional histopathology and can be used in long-term use in monitoring of patients.	60	99
Kaur et al. [27]	The study was to evaluate the efficiency of brush biopsy and along with DNA image cytometry as adjunctive role in diagnosis of oral cancer.	112	Oral cell carcinoma	The study reports that DNA image cytometry along with brush biopsy cytology has better sensitivity in detection of oral cancer.	92	100
Gupta et al. [28]	To evaluate and compare the applicability of exfoliate cytology and brush biopsy in detection of oral premalignant and oral cancer.	117	Oral premalignant and malignant lesions	The study concludes that modified oral brush cytology has higher sensitivity and specificity than exfoliate cytology.	81.6	68.4
Reddy SG et al. [29]	To evaluate the performance diagnostic ability of oral brush biopsy compared with histopathology group of potential malignancy.	60	Malignant lesions	The study reports oral brush biopsy can be used as diagnostic tool if coupled with molecular markers like tenascin and keratins.	43.5	81.25
Delavarian et al. [30]	To determine the diagnostic value of modified liquid-based brush biopsy technique using OralCDx brush in detection of oral potential malignant lesions and oral cancer.	26	Oral premalignant and malignant lesions	The study reports that modified liquid-based cytology could be used in clinical uses and can overcome conventional cytology approach.	88.8	100

[33]. Due to the above problem with visually identifying sites for biopsy, which represents the most malignant transformation, it is subjective and depends entirely on the examiner and, this might lead to selecting an inappropriate lesion which in turn often requires redoing the biopsy, adding to the patient's distress [34].

Further, the interpretation of microscopic sections includes the presence of artifacts that are common in biopsy specimens of the oral mucosa, which can result in a change in morphology and cytology, which will render the specimen useless and their presence depends on the type of biopsy technique used.

## Staining techniques

The conventional vital staining techniques such as toluidine blue, Lugol's iodine staining, and Rose Bengal staining are commonly used in oral cancer diagnosis [35]. The toluidine blue technique is the most accepted staining technique and coupled with other modalities to visualize the oral lesions. The staining technique helps in the diagnosis of oral cancer and has been used to detect both premalignant and malignant conditions. Toluidine blue is a vital stain used to visualize changes in the mucosa of the oral cavity as it is capable of staining nucleic acids. The dye binds DNA and RNA, which is seen to be higher side during the onset of malignancy [36], while Lugol's iodine binds to the glycogen content present in

the epithelium cells. The stain has been used in discriminating against the inflammatory or cancerous oral lesion from normal epithelium as they contain lower glycogen contents [37].

Various studies have reported the application of these stains in oral cancer detection; some of these studies are highlighted in Table 3 above. A study conducted by Chainani W et al. has reported the sensitivity and specificity of toluidine blue to the detection of high-risk lesions (carcinoma in situ or OSCCA). Of the 100 lesions evaluated from 43 patients, 77 lesions were biopsied and included for analysis. The results of the toluidine blue technique showed a sensitivity of 94% in the case of high-risk lesions, while that for OSCCA was 100%. However, the authors pointed out the limitations in the study where they found the specificity of 45% and 39% for the above cases, respectively [43]. To increase the sensitivity and specificity and overcome the limitations of the staining techniques, some authors have combined two different staining techniques to detect abnormal oral lesions. A study was carried out by Nagaraju K et al. where the authors determined the diagnostic applicability and reliability of toluidine blue with Lugol's staining. The results show the combined staining technique can assess and discriminate between the premalignant and malignant oral lesions with a sensitivity of 92.7% and specificity of 60%, respectively [41]. The staining of the oral lesion by toluidine blue is shown in Fig. 1.

Although the staining techniques are quite a sensitive tool, however, they possess some limitations such as erroneous

**Table 3** Studies mentioning sensitivity and specificity for the detection of oral abnormalities by toluidine staining and Lugol's iodine

Author	Objective	Subjects	Type of lesion	Conclusion	Sens (%)	Spec (%)
Vijayakumar V et al. [38]	To evaluate the efficacy of toluidine blue dye as an adjunct to standard clinical examination to determine cancer lesions of oral cavity.	55	Malignant	The study reports the toluidine blue staining can be potential adjunct in the diagnosis related to oral and oropharyngeal cancers.	92.6	67.9
Chaudhari A et al. [39]	To compare the effectiveness of self-examination, clinical examination, and staining methods using 1% toluidine blue and Lugol's iodine in determining the prevalence of malignant lesions.	82	Premalignant and malignant	The study concludes that a self-examination tool is effective in detection of oral cancer. Further, the use of vital stains is an effective screening tool in detection of oral lesion in a given population.	94.7	83.3
Rodriguez P et al. [40]	The objective of the study was to determine the efficacy of toluidine blue staining as a detection tool for detecting malignant and dysplastic oral lesions.	160	Malignant and dysplastic	The study infers that TB staining can be a useful diagnostic tool in the detection of oral cancer. However, the findings should be correlated with clinical and histopathological findings.	65.5	73.3
Nagaraju K et al. [41]	To determine diagnostic efficiency of toluidine blue with Lugol's iodine in determining the risk of oral cancer lesions developing into cancer.	30	Premalignant and malignant	The combined staining was able to discriminate the inflammatory lesions. The study concludes that combination of toluidine blue with Lugol's iodine can be used to assess the progressiveness of diseases.	92.7	60
Allegra E et al. [42]	To determine the usefulness of toluidine blue as marker in order to differentiate lesions and in early diagnosis of oropharyngeal carcinomas.	32	Premalignant	The study reports the use of toluidine stain is a reliable aid where the clinical examination fails to determine and differentiate the oral lesions of higher risk population.	53	80

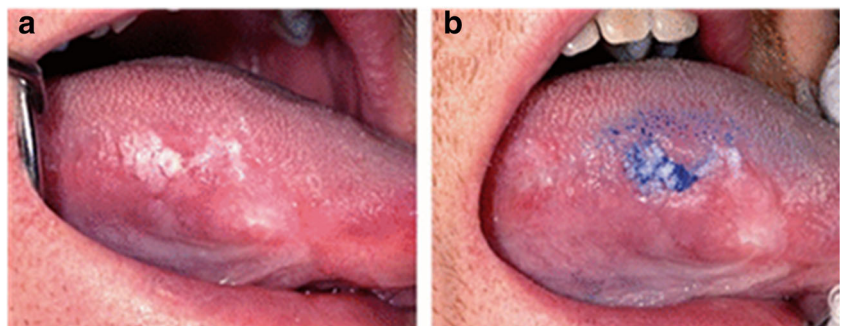
retention of stain in regions that show inflammation and ulcers and inability to detect deeper-lying dysplasia in the epithelium and the hindrance in dye-uptake lesion area caused by the mucous [38].

## Chemiluminescence

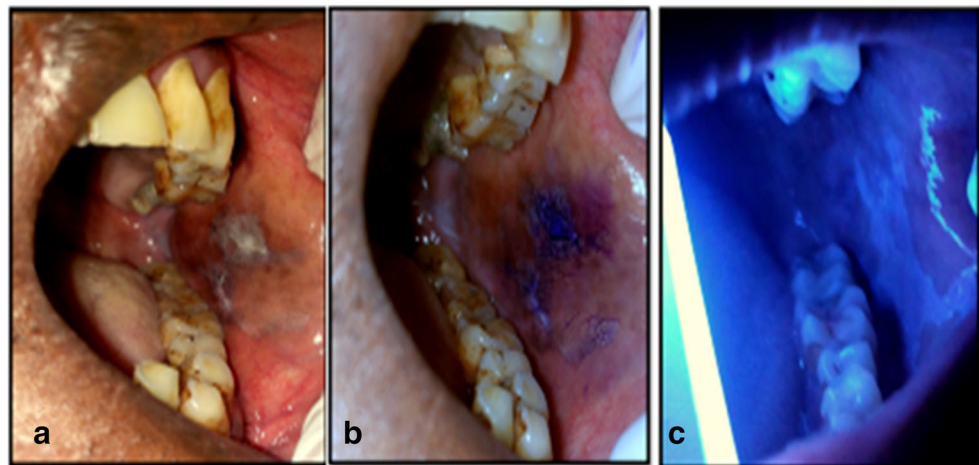
Chemiluminescence is a light-based screening technique used to examine the oral cavity. The method principally depends on alterations in the refractive index of the mucosa corresponding to the alterations in the metabolism and structure occurring due to malignancy. Normal mucosa absorbs the blue-white light, while the cells which are dysplastic and neoplastic reflect it. The

chemiluminescence-based technique is marketed under the names of Vizilite plus and MicroLux DL. Vizilite Plus is a disposable light packet, while MicroLux DL is reusable. Vizilite Plus tool is a combination of chemiluminescence and toluidine blue (TB). The toluidine blue (TB) stain specifically stains the DNA. The procedure mainly involves 1-min rinsing with a 1% acetic acid solution, followed by the clinician observation of the rinsed lesion sites using the tool which emits light in a wavelength from 430 to 580 nm (as shown in Fig. 2) [44, 45]. The sensitivity of Vizilite was reported by Chaudhry A et al. where the authors claimed Vizilite-based detection showed better sensitivity in identifying leukoplakia [46]. Further, several studies have reported the application of the chemiluminescence tool in screening the oral lesions by clinicians as shown in Table 4 below.

**Fig. 1** TBlue staining of leukoplakia, **a** before staining and **b** retention of TBlue dye by the white patches, reprinted from Awan K. et al. [36] with permission of John Wiley and Sons



**Fig. 2** Oral lesion at buccal mucosa under **a** incandescent light **b** stained with toluidine blue and **c** Aceto-white delineation of oral lesion on Vizilite plus, reprinted from Jain N. et al. [44] with permission of Elsevier



Although the above technique possesses better application compared with other conventional methods, however, they possess some limitations such as interference of saliva in visualizing lesions, inability to identify erythroplakia, and to differentiate non-dysplasia from dysplasia has been reported.

### Non-invasive fluorescence-based tools in oral diagnosis

When an experienced practitioner performs a visual examination, he limits to differentiate between premalignant lesions

**Table 4** Reports on Vizilite-based detection of oral lesions

Study	Objective	Subjects	Type of lesion	Conclusion	Sens (%)	Spec (%)
Vashisht et al. [47]	To detect the dysplastic epithelial changes in potentially malignant epithelial lesions (PMELs) and oral squamous cell carcinoma (OSCC) using chemiluminescence and toluidine blue staining methods and comparing with histopathological examination.	60	Premalignant and malignant	The study concludes chemiluminescence-based optical tools possess higher sensitivity and specificity in screening PMELs compared with toluidine blue. Thus, chemiluminescence-based tools can be used effectively as a diagnostic aid.	95.5	84.6
Rajmohan et al. [48]	To determine the efficacy of the chemiluminescent light (Vizilite) to diagnose premalignant and malignant and compare them with toluidine and exfoliate cytology outcomes.	30	Potential malignant disorder	The results clearly showed the chemiluminescent light showed better discrimination ability between precancerous and cancer group compared with other two techniques.	85	100
Ujaneoy et al. [49]	A cross-sectional study to compare the performance of chemiluminescence, toluidine blue, and histopathology for detection of the high-risk precancerous oral lesion.	44	Potential malignant disorder	The study concludes that chemiluminescent-based assessment is not very sensitive compared with toluidine blue staining which shows higher sensitivity.	59	78
Awan et al. [50]	To accurately determine the OPMD by using commercially available chemiluminescent detection kit-Vizilite.	126	Oral potential malignant disorder	The Vizilite holds the ability to detect OPMD; however, it fails to outline margins accurately in dysplastic lesions. The technique holds potential in the general mucosal examination and may further improve the visualizations of leukoplakias.	87	24
Epstein et al. [51]	To evaluate the effectiveness of improvised visualization (Vizilite) in visualizing the mucosal lesions which could help in early diagnosis.	84	Oral potential malignant disorder (OPMD)	The chemiluminescent light (Vizilite) was effective in detection of red and white lesion showing enhanced brightness.	100	50

from the conditions that are inflammatory and one that can lead to malignancy. Also, in many of the cases, the patient would get diagnosed at a later stage of cancer by the time they reach the clinician, followed by their histopathological assessment, which is very subjective. Impediments such as these, presented by conventional diagnostic methods in early detection of oral cancer, have led to the need for the development of alternative diagnostic modalities. The optical tools based on autofluorescence are effective in discriminating dysplastic lesions from early neoplastic and also provide information regarding changes at the biochemical and molecular levels. Autofluorescence is a phenomenon that takes place when an intrinsic fluorophore molecule gets excited by a light source of a particular wavelength, and emits fluorescence upon relaxation. On absorbing incident light of suitable wavelength, the electrons in the fluorophores get excited from the ground vibrational states to the higher energy states. The electrons then relax to the 1st excited state of the fluorophore through non-radiative relaxations followed by its transition to the ground vibrational levels through radiative relaxations known as fluorescence and thereby releasing the excitation energy of the fluorophores at higher wavelength compared with the excitation wavelength of the fluorophore as shown in Fig. 3 [52, 53].

Tissues possess endogenous fluorophores, which include aromatic amino acids, collagen, elastin, porphyrins, and metabolic products such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotides (FAD). During the neoplastic and pre-neoplastic stages, these endogenous fluorophores demonstrate alterations in their metabolism and structures. Thus, a light of a particular wavelength is excited upon these endogenous fluorophores present in the tissues. They display different optical properties (absorbance, reflectance, and fluorescence) which are sensitive to their microenvironment changes, which in turn get reflected in their corresponding wavelength and emission profiles [54]. The observed fluorescence from tissues under pathological conditions truly reflects the change in biochemical and tissue architecture. Abnormal conditions such as dysplasia may lead to disturbance in the fluorophore distribution and quantity, showing corresponding loss of tissue autofluorescence as

compared with the healthy tissues. The NADH and FAD are important fluorophores in the epithelium of the oral mucosa, while in the stroma, it is the collagen that is dominant. Any change in the levels of these fluorophores is indicative of the metabolic status of the cells. Hence, change in spectral intensity from disease tissues could be contributed by a change in the concentrations of fluorophores [55–57].

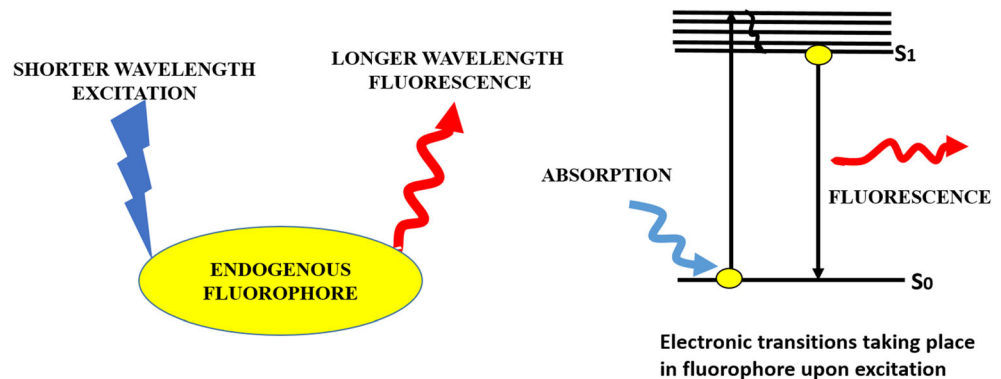
Autofluorescence-based tools are non-invasive, easy to handle, and also minimize unnecessary surgical biopsy interventions. Due to these properties, the techniques can be used as a potential tool to monitor the malignant area in medically compromised patients. The autofluorescence-based diagnostic tools for oral cancer include the following:

- Visual autofluorescence (visually enhanced lesion scope) VELscope
- Autofluorescence spectroscopy

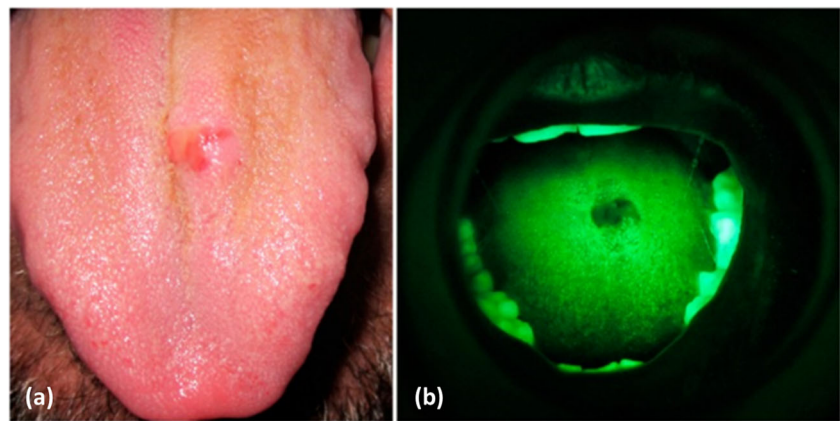
### VELscope

Over the last few decades, tissue fluorescence has been exploited much to detect oral lesions by involving a variety of devices. VELscope is one such device that is based on fluorescence emitted by the natural fluorophores of the oral tissues upon suitable light excitations in the region 400–600 nm. It is a handheld device that consists of an arc lamp as an excitation light source to shine into the oral cavity and a series of reflectors and filters to view the emitted fluorescence through them. The device avoids procedures like rinsing of the oral cavity with chemical agents [57, 58]. The diagnostic ability of VELscope depends on the differences in the fluorescence emitted by the diseased and healthy tissues. Pale green is seen in the case of normal mucosa, while dark coloration is seen in dysplastic tissues, as shown in Fig. 4 [59]. Various studies have reported using VELscope in the diagnosis of oral lesions which are listed in Table 5. Further, some studies have highlighted the limitations of using VELscope alone in the diagnosis of potentially malignant disorders or oral squamous cell carcinoma [65].

**Fig. 3** Block diagram depicting the generation of fluorescence along with its underlying phenomenon



**Fig. 4** **a** Clinical oral examination of the tongue and **b** loss of fluorescence at the region of oral lesion observed under VELscope, reprinted from Cicciù M. et al. [59] with creative copyright Licensee MDPI, Basel, Switzerland



## Autofluorescence spectroscopy

Fluorescence spectroscopy is a very sensitive technique that can detect minor metabolic or pathological changes taking place in the cells and tissues by illuminating the concerned fluorophores with appropriate monochromatic sources of light [66]. The fluorescence properties can differentiate abnormal

tissues from the normal with higher sensitivity and specificity and thus providing an opportunity to use in diagnostic decision-making, including the detection of premalignant conditions and thereby bringing down the chances of repeated biopsies [67]. There are several reports of using fluorescence spectroscopy in the past to distinguish malignant tissues of different organs from their normal healthy counterparts in

**Table 5** Reports on VELscope-based detection of oral lesion

Authors	Objective	Subjects	Type of lesion	Conclusion	Sens (%)	Spec (%)
Ganga et al. [60]	To determine the efficacy of VELscope in the detection of dysplastic or neoplastic changes in oral lesions as compared with COE.	200	Oral lesion	The study reports that VELscope itself cannot be used as a definitive method in diagnosis. Further, the results should be correlated with other existing gold standard methods such as conventional oral examination, surgical biopsy, and histopathological evaluation.	76	66.3
Huang et al. [61]	The purpose of the study is to develop a novel quantitative analysis approach of autofluorescence-based imaging for oral cancer screening.	140	OPMD and OSCC	The study reports novel quantitative analysis of autofluorescence-based images along with QDA classifier can be used in differentiation of oral cancer and precancerous lesions from normal oral mucosa.	98.3	77.6
Hanken et al. [62]	The aim of the study was to determine the ability of autofluorescence to differentiate the benign, premalignant, and malignant oral lesions and further to compare the outcomes with histopathological findings.	60	OPMD	The study reports that VELscope can be used by experienced physician to find oral malignant lesion in the area of altered mucosa. However, the results obtained should be interpreted carefully due to frequent false positive results. Further, the device should be handled by experienced clinicians.	97	33
Rana et al. [63]	The purpose of the study was to determine the applicability of the device in oral cancer prevention by using as adjunct to standard clinical examination in the clinical settings.	123	OPMD	The study reports the potential application of VELscope as an adjunct along with clinical oral examination in the prevention of oral cancer.	100	74
Scheer et al. [64]	To determine the fluorescence loss with VELscope device and to determine its ability to differentiate different oral lesions before its histological confirmation.	64	OSCC	The study reports that autofluorescence imaging using VELscope can be able to differentiate malignant and potential malignant oral lesion from normal mucosa in high-risk patients. However, the technique was not able to discriminate benign lesion from malignant or premalignant.	100	80



vitro/in vivo. The technique has shown its ability to detect even benign tissues from normal along with malignant tissues [68, 69]. The application of this technique in oral cancer detection is very well documented, and some studies are listed in Table 6. The fluorescence spectroscopy, when used along with suitable statistical analyses, has shown very high sensitivity and specificity in the discrimination of pathological conditions. The study by Jayanthi J L et al. applied linear discriminate analysis on the autofluorescence spectra recorded from 15 healthy people and 34 oral cancer patients. The results demonstrated clear differentiation of premalignant dysplasia from squamous cell carcinoma, as well as benign hyperplasia and hyperplasia from normal with a sensitivity of 86%, 78%, and 92%, and specificities of 90%, 100%, and 100%, respectively [75]. Similarly, in another study carried out by Sudha K D et al. the authors performed MATLAB-based PCA and k-means nearest neighbor (k-NN) analysis on 142 fluorescence spectra obtained from pathologically clinical samples recorded at 325-nm excitation using laser-induced fluorescence as a tool. The various features such as mean, median, maximum intensity, energy, spectral residuals, and standard deviation extracted from the fluorescence spectra of the normal, premalignant, and malignant oral tissue samples. In the study, the

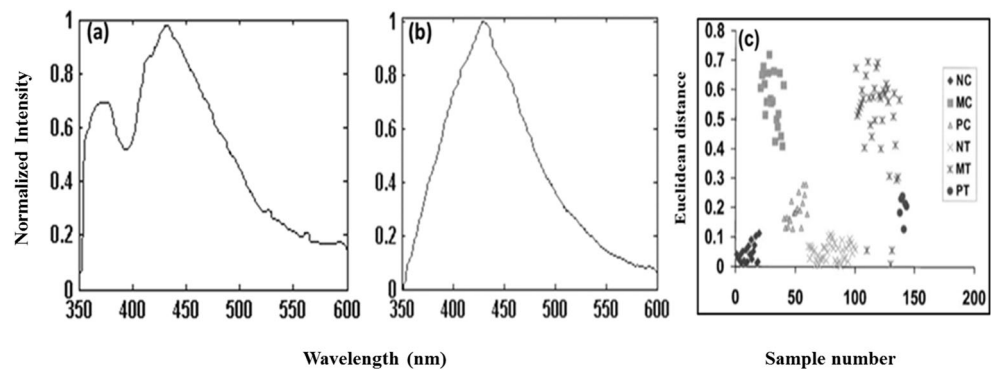
authors were able to show a robust application of PCA and k-NN analysis by which it can discriminate and also able to classify the unknown sample into a diagnostic group, which helps in reducing the misclassification as shown in Fig. 5. The authors were able to achieve sensitivity, specificity, and accuracy of 100, 94.5, and 96.17%, respectively. Based on the above study, the authors have tried to show that the laser-induced fluorescence technique can effectively be used to discriminate between normal, premalignant, and malignant conditions. Further, the time required to acquire and analyze the data is very less, and thus it holds very potentially as an alternative tool in the diagnosis of diseases [76].

While a study carried out by Yuvaraj M et al. monitored the spectral variation of porphyrin fluorophore in saliva samples collected using autofluorescence spectroscopy from oral squamous cell carcinoma (OSCC) patients and healthy individuals, the results demonstrate predominant emission patterns in the case of cancer patients as compared with the normal at 405-nm excitations. They observed additional spectral peaks at 625 and 687 nm in the cancer samples as compared with control due to the porphyrins. However, both of these peaks were absent in normal saliva samples [77]. The above observations have

**Table 6** Studies mentioning sensitivity and specificity for the detection of oral abnormalities by fluorescence spectroscopy

Study	Objective of the study	Subject	Type of lesion	Conclusion	Sens (%)	Spec (%)
Besty J et al. [70]	To evaluate the applicability of laser-induced fluorescence in detection and differentiation of different grades of dental plaque based on fluorescence intensity ratio in 300 patients upon 404-nm diode excitation.	300	Oral screening	The diagnostic algorithm-based spectral intensity ratio (F510/F630) could help in detecting precisely the minute changes in the oral cavity.	100	100
Francisco A L N et al. [71]	To determine the ability of fluorescence spectroscopy in discrimination of normal and malignant mucosa at 408 and 532-nm excitations.	30	Oral lesion	The results summarize the potential ability of fluorescence spectroscopy to evaluate objectively the oral lesions based on the fluorescence properties of tissues at different wavelengths.	87.4	77.6
Mazumder S K et al. [72]	To evaluate the potential of autofluorescence spectroscopy to classify the oral tissue spectra into different categories of oral cancer.	170	Oral lesion	The study reports that the oral cancer can be classified based on the in vivo autofluorescence properties of oral tissues.	98	100
Cristina K et al. [73]	To determine the efficacy of fluorescence spectroscopy to detect squamous cell carcinoma in animal model at different laser excitations of 442 and 532 nm and to evaluate the sensitivity and specificity by using KNN analysis.	72	Oral lesion	The study concludes the diagnostic potentiality of laser-induced fluorescence spectroscopy to discriminate normal and malignant conditions with higher sensitivity and specificity in 442-nm excitations as compared with 532 nm.	95.7	91.6
Mazumder SK et al. [74]	To analyze the fluorescence spectra of normal and malignant sites in vivo using algorithm based on nonlinear maximum representation and discrimination feature (MRDF) method and compare with linear PCA-based algorithm.	29	Oral screening	The nonlinear MRDF algorithm-based assessment of fluorescence spectra provides better diagnostic performance compared with linear PCA-based algorithms due to its better ability to discriminate oral cancer tissues compared with healthy individual.	95	96

**Fig. 5** The normalized fluorescence spectra of oral tissue. **a** Normal and **b** malignant and **c** scattered plot of 143 spectra from normal, premalignant, and malignant samples (NC, normal calibration; MC, malignant calibration; PC, premalignant calibration; NT, normal test; MT, malignant test; and PT, premalignant test), reprinted from Sudha KD. et al. [76] with permission of SPIE



clearly demonstrated the potential of identifying porphyrins in saliva samples as a biomarker for oral cancer diagnosis using autofluorescence non-invasively. The above study reflects the potential use of fluorescence spectroscopy in early detection of premalignant lesions, even before when there are no visible clinical symptoms. The technique records fluorescence spectra qualitatively and is relatively inexpensive, portable, and easy to operate even by a technician with minimum training. These impeccable qualities make the fluorescence spectroscopy a promising tool to screen people with minimal complexity. They can provide a powerful and robust diagnostic platform for disease screening and subsequent treatment opportunities.

## Conclusion

Oral cancer is preventable, and yet the present-day incidence and mortality rates remain to be high. Despite the existence of numerous conventional diagnostic techniques, many of which are invasive, and most of the cases are detected after they would have progressed to an advanced stage. The prevention entails in averting the progression of a premalignant disorder into full-blown cancer, by focusing on early detection strategies by using fluorescence-based spectroscopic tools, highlighted in this article. The fluorescence technique, explicitly fluorescence spectroscopy, holds the potential to identify oral precancerous and cancerous lesions with high sensitivity at an early stage, even when external morphological changes may not be visible. Besides, their cost-effectiveness, speed, and ability to provide real-time analysis of diagnosis objectively make them ideal diagnostic tools. Further, the technique holds immense potential in providing the molecular information generated from a fluorophore upon excitation. Therefore, it could increase the chances of survival, decrease morbidity, reduce the mortality rates, and ensure effective treatment, post-treatment, and a better quality of life for the patient.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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