Fluorescence spectroscopy for the detection of potentially malignant disorders and squamous cell carcinoma of the oral cavity

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Summary Oral cancer is a public health problem with relevant incidence in the world population. The affected patient usually presents advanced stage disease and the consequence of this delay is a reduction in survival rates. Given this, it is essential to detect oral cancer at early stages. Fluorescence spectroscopy is a non-invasive diagnostic tool that can improve cancer detection in real time. It is a fast and accurate technique, relatively simple, which evaluates the biochemical composition and structure using the tissue fluorescence spectrum as interrogation data. Several studies have positive data regarding the tools for differentiating between normal mucosa and cancer, but the difference between cancer and potentially malignant disorders is not clear.

The aim of this study was to evaluate the efficacy of fluorescence spectroscopy in the discrimination of normal oral mucosa, oral cancer, and potentially malignant disorders.

KEYWORDS

Squamous cell carcinoma; Fluorescence spectroscopy; Early diagnosis; Potentially malignant disorders; Oral cavity

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The fluorescence spectroscopy was evaluated in 115 individuals, of whom 55 patients presented oral squamous cell carcinoma, 30 volunteers showing normal oral mucosa, and 30 patients having potentially malignant disorders. The spectra were classified and compared to histopathology to evaluate the efficiency in diagnostic discrimination employing fluorescence. In order to classify the spectra, a decision tree algorithm ([4.5]) was applied. Despite of the high variance observed in spectral data, the specificity and sensitivity obtained were 93.8% and 88.5%, respectively at 406 nm excitation. These results point to the potential use of fluorescence spectroscopy as an important tool for oral cancer diagnosis and potentially malignant disorders.

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Introduction

Annually, about 10 million malignant tumors are diagnosed worldwide, and cancer of the oral cavity represents 6% of all cases [1,2]. Different tumors may affect the oral cavity, and oral squamous cell carcinoma (OSCC) is the most frequent one, representing 90% of all cases [2–5]. Unfortunately, in most cases, the patients present for treatment with advanced stage disease, with consequent decrease in survival rates [1,6]. The treatment of these patients is usually aggressive and may be mutilating [7]. Despite the recent advances in the treatment of OSCC, there has been only a small increase in the survival rate [8].

Disease local and regional control and rehabilitation of patients with advanced oral carcinoma are extremely difficult. On the other hand, initial lesions are more easily removed with less patient mutilation and better prognosis [9,10].

Some OSCC lesions come from pre-existing potentially malignant disorders [6,11] with the annual malignant transformation rate of approximately 1% [11,12]. Patients with previous history of oral cancer, especially those that had been heavy smokers, present an altered oral mucosa (cancerization field). Although oral lesions are common, to predict the biological behavior of them remains a challenge. The standard methodology for the diagnosis of these lesions is clinical examination and histopathological analysis. Most oral potentially malignant disorders (PMD) are clinically present as leukoplakia or erythroplakia, but histologically they can show a wide variety of phenotypes, such as hyperkeratosis, dysplasia or even carcinoma [13].

Currently, there is great interest in optical techniques that use fluorescence to evaluate lesions using a non-invasive procedure. Based on the tissue optical properties and diagnostic sensitivity to capture the fluorescence emitted by native fluorophores, these optical techniques have been indicated to differentiate endogenous tissue variations [14–16]. The fluorescence spectroscopy has become an important tool in detecting potentially malignant and malignant lesions in the oral mucosa [17,18]. The biochemical composition and tissue architecture can be assessed by the emitted fluorescence spectrum, which will be modified in the presence of tissue alterations [19]. Mathematical algorithms can then be developed and optimized to classify the respective tissues in histological categories based on their spectral characteristics [20–23].

The aim of this study was to determine the ability of fluorescence spectroscopy to discriminate normal and abnormal oral mucosa, potentially malignant and/or malignant oral lesions.

Patients and methods

This prospective study was performed at the Department of Head and Neck Surgery and Otorhinolaryngology, A.C. Camargo Cancer Center and LELO-USP (Special Laboratory of Laser Dentistry), São Paulo, Brazil. The clinical research protocol was approved by the Ethical Committee of the participating institutions, and all recruited patients that agreed to participate as volunteers signed an informed consent form.

The fluorescence spectroscopy system is composed of an excitation light source, an interrogation probe, a portable spectrophotometer USB 2000 (Ocean Optics, USA) and a laptop. Two solid state lasers, a diode emitting at 406 nm and a doubled-frequency neodymium: YAG at 532 nm, were used as excitation light sources. The interrogation probe is a V-type fiberoptic probe, where one tip is connected to the spectrophotometer and the other one to the excitation laser. Two optical fibers of 600 μm in diameter are positioned side by side. One conducts the excitation light and the other collects the re-emitted light by the tissue. The outer diameter of the interrogation tip is 1.6 mm and the total diameter of the area that is placed in contact with the tissue surface is 3.2 mm. The spectroscopic data was acquired using the software OOIBase32 (Ocean Optics, USA).

All subjects were interviewed and the data filled in a standardized interview form containing information regarding the habits (including tobacco and alcohol consumption), family history, marital status, education, occupation, and other factors that were considered as a risk factor for OSCC development. Clinical examination was performed and examiner clinical impression was recorded. In patients with cancer (C) and PMD, clinical characteristics, including lesion dimensions, color, and site were evaluated. In vivo fluorescence spectroscopy measurements were performed under both excitation wavelengths, collecting as many points needed to cover the whole lesion surface, at least one hour after the last meal. In the case of identification of superficial clinical heterogeneities, as leukoplakia or erythroplakia, fluorescence spectra were taken and identified to correlate with distinct clinical patterns. Tissue research was not taken from area of necrosis, usually present at the center of ulcerated lesions. In each chosen area, five spectroscopic measurements, one at each wavelength were taken. The patient evaluation was performed in the surgical theater at A.C. Camargo Hospital, after induction of general anesthesia, and at outpatient clinics at LELO, before the biopsy procedure or surgical resection of the lesions.
A 3 mm punch biopsy was taken at patients with cancer at the time of surgery. Biopsy sites were matching with specific spectroscopic measurement sites, and were performed according to heterogeneity of the lesion defined by the physician based on clinical judgment. Tissue specimens were stored in plastic vials containing 10% formalin solution for histology processing.

In volunteers with normal mucosa, and no history of cancer, fluorescence measurements were taken from the border of the tongue (BOT), dorsum of the tongue (DOT), floor of the mouth (FOM), lower labial mucosa (LB), buccal mucosa (BM), gingiva or alveolar ridge, hard palate and lip, with at least 5 spectroscopy measurements for each excitation wavelength. From each area, an exfoliative cytology was performed.

The spectra were analyzed according to the different anatomical sites and amended by tissue injury compared to normal tissue. The results were correlated with the histopathological diagnosis. Different types of computational processing were conducted to evaluate the discrimination among normal mucosa (NM), PMD, and cancer (C).

Initially, all spectra were analyzed based on classification and independent of anatomical site. In a second analysis, principal component analysis (PCA) was performed, classifying the spectra also according to the anatomical site. PCA was applied using three types of spectrum preprocessing: normalization by maximum intensity, standardization and analysis of the first derivative and the second derivative.

Classification using spectral indices (SI) based on qualitative analysis of the spectra was also performed. The SI was defined comparing emission intensities at wavelengths modified in the PMD and OSCC groups, when compared to the NM. The index used to classify the spectra can be expressed by the following equation:

\[ R = \frac{\int_{623.1}^{643.2} I(\lambda) d\lambda}{\int_{485.4}^{506.6} I(\lambda) d\lambda} \]

where \( I \) is emission intensity, in arbitrary units, and \( \lambda \) is the emission wavelength.

Finally, computational classifiers were also used to achieve a more robust system for spectra classification. In one analysis, the classifiers were applied to the database without any spectral normalization and a second analysis with the spectra normalized by its maximum emission. This approach could lead to a better comprehension about the role of spectrum intensity in the quality of classification. Three different classifiers were used: Naive Bayes, k-nearest neighbor (kNN) and a decision tree C4.5 (J48), all of them implemented on open source software WEKA [44]. All classifiers were tested for both excitation wavelengths in the exact same conditions. Moreover, the excess of variables (wavelengths) may contain unnecessary and redundant information, which can compromise the classifier performance. Thus, variables selection methods were applied prior to computational classifiers in order to keep only the relevant ones. The selection methods used were ReliefF and CFS (correlation-feature selection), both present in WEKA software.

Results

One hundred and fifteen subjects over 18 years old, both genders, smokers and nonsmokers were investigated in this study. The subjects were enrolled in the study as 55 OSCC patients, 30 patients with PMD, and 30 volunteers with normal oral mucosa, without history of malignancy in airway digestive tract. Of the total of 115 subjects, 54 (47%) were women and 61 (53%) men. The mean age was 58.59 years old, while among women was 54.5 years and for men was 62.69. Each biopsy was analyzed by a certified oral pathologist blind to the fluorescence results, describing the diagnosis and histopathological characteristics (Figs. 1 and 2). Five patients presented more than one lesion, resulting in 99 interrogated lesions. Classification was based on histopathological diagnosis as gold standard. Fluorescence patterns were determined, identifying spectroscopic features for each type of mucosa evaluated. The sample distribution based on the histopathological analysis and site location is presented at Table 1.

Among 83 individuals with lesions in the oral mucosa, 56 were OSCC patients and underwent surgery for resection of the lesion with tridimensional surgical margins. The most affected anatomical site was the BOT (25 lesions, 44.5%), followed by the BM (11 lesions, 19.5%), FOM and LM, 5

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lesions each, and alveolar ridge (retromolar region) with 4 lesions.

The qualitative comparison between the effectiveness of violet and green light excitation for fluorescence in the detection of oral cancer was performed. The oral mucosa showed distinct fluorescence features in distinct interrogated sites.

Variation at fluorescence intensity was observed depending on oral site, and inter- and intra-patient measurements. Due to the fact that these differences may result from probe positioning, and pressure, the intensity emission raw values were not used for diagnostic. As overall analysis of the fluorescence spectral behavior, the higher emission intensities were present at palate and alveolar ridge, for 406 nm excitation. The tongue and the FOM are highly vascularized tissues, and hemoglobin is the main biological absorber resulting in the low intensity of the spectra collected classification (Figs. 3 and 4). We observed that the center of the cancer lesion emits very low fluorescence signal, while the borders emit distinct fluorescence emissions depending on the heterogeneity of the lesion and site location.

The BOT site showed the major spectral differences in the shape and intensity of the fluorescence emission. This site is a transition region of dorsal and ventral tongue. The dorsum of the tongue shows a unique mucosa with the presence of large papillae and high bacteria colonization that produces porphyrin compounds, which emits a high intensity red fluorescence. Analyzing the fluorescence from erythropapillae lesions, a lower intensity is observed when compared to the NM emission intensity, due to higher hemoglobin content, which is the main endogenous absorber. Excitation at 406 nm provided the spectra with best fluorescence discrimination in all analysis performed. Although, excitation at 532 nm reach a deeper region in the tissue, the endogenous fluorophores involved in OSCC are better excited at the ultraviolet region and the initial changes of epithelial tissue start at the most superficial layers.

Spectral results from NM at normal volunteers and NM at PMD patients presented distinct patterns.

Initially, principal component analysis (PCA) was performed to compare the raw spectra of PMD versus NM from the PMD patients versus NM from volunteers, without discrimination of the anatomical site. Secondly, the comparison was performed of PMD, OSCC and NM from volunteers. No satisfactory result was achieved for 406 or 532 nm excitation, even after pre-processing data, as spectral normalization tools.

The spectral index analysis was performed for spectra collected from normal volunteer (NM) and normal contralateral mucosa (NC). Fifty patients and 75 sites were selected. However only 36 cases presented contralateral normal mucosa (NC), for inpatient comparison. High variance was observed for the calculated index values of C and NC oral mucosa. The standard deviation for the normal oral mucosa is lower when compared to the cancer at the same

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Total number of lesions</th>
<th>Number of lesions by histological diagnosis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Potentially malignant disorders</td>
</tr>
<tr>
<td>Border of tongue</td>
<td>39</td>
<td>14</td>
</tr>
<tr>
<td>Floor of the mouth</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>Labial mucosa</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Gingiva</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Retromolar</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Palate</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1 Distribution of lesions by anatomic site correlated with the histopathological diagnosis.
site location. This result is correlated to the distinct histology and clinical patterns for the oral carcinoma that are presented. Despite of this, the spectral indexes analysis did not result in clear distinction between the classification groups at a global comparison. In an intra-site analysis the NM, C and PMD groups were separated. However, due to small sampling, statistical analysis could not be evaluated.

The classification using computational classifiers was performed in the whole dataset (NM, PMD, and C). In the proposed analysis with the computational classifiers (P vs. NN), the computational k-nearest neighbor (kNN), was used followed by two other algorithms: the Bayes and J48. The missing values in Table 2, marked with (***), are algorithms that were time consuming and did not show expressive results. The J48 decision tree presented the best result, both for 532 nm and 406 nm excitations. For excitation at 406 nm, algorithm J48 + CFS showed 88.5% of sensitivity and 93.8% of specificity, while for excitation at 532 nm the same method obtained 87% of sensitivity and 91.2% of specificity (both with normalization by the maximum intensity emission) (Table 2). Considering just PMD and NM group, the J48 + CFS shows a sensitivity equals to 92% and a specificity of 96.5%.

Discussion

Changes on tissue architecture and biochemical composition that occur during establishment and progression of the pathology result in modification of the tissue fluorescence emission. The optical path of the photons traveling in the tissue is driven by these microscopic characteristics. The collection geometry of the spectroscopy system may also play an important role on the final collected fluorescence spectrum [24—26]. For oral cancer diagnostics, excitation wavelength presented as more effective is in the 400—450 nm interval [25,27]. Our results also showed that the 406 nm excitation presented more tissue information concerning malignant characteristics, even though 532 nm could potentially interrogate a deeper tissue layer.

The collected fluorescence spectra differ depending on the excitation wavelength, oral site, biochemical composition and tissue architecture [28,29]. Distinct excitation wavelengths present different photon energies, resulting in distinct light-tissue interaction, and may excite different endogenous fluorophores [26,30].
Native tissue fluorescence originates from endogenous fluorophores, such as collagen, elastin, structural components of the tissue, and other biomolecules. The fluorescence associated with the presence of aromatic amino acids and proteins shows information about the cellular and tissue structure [23,30]. In contrast, the concentration of derivatives of pyroxene, NADH and FAD, varies with the metabolic status of tissue [31] and their levels are altered in the presence of pathological conditions. The fluorescence of these molecules is dependent on its concentration and distribution as well as physicochemical properties of the environment. Because the modification of protein and metabolic conditions occurs during malignant transformation, this fluorescence interrogation is a useful tool to differentiate tumor from normal tissue [26].

When considering the collection of the emitted fluorescence at tissue surface, not only the fluorophores distribution contributes for the spectral intensity and shape of fluorescence detected, but also the absorbers and scatterers distributions. Collagen is one of the main contributors for the oral mucosa fluorescence, and the decreased emission is related to the breakdown of the fiber links, resulted from tumor cells invasion. Neoplasia progression also involves angiogenesis, and structural epithelium changes, resulting in modification of tissue absorption and scattering [27,31,32].

According to Schwarz et al. [18], during carcinogenesis in the oral cavity, structural and biochemical changes in the epithelium and stroma may alter the optical properties of dysplastic and cancerous tissues. Increased nuclear size and cytoplasm/nucleus ratio, microvascular changes, degradation of collagen fibers, increased of hemoglobin, and changes in mitochondrial concentration of NADH and FAD, leading to changes in the optical scattering, absorption and intrinsic characteristics of tissue autofluorescence.

The fluorescence spectroscopy using a fiberoptic probe interrogation provides the evaluation of a small tissue volume, especially when considering the excitation wavelength at the violet spectrum. Poh et al. [27] using wide field fluorescence imaging showed the effectiveness of a simple handheld fluorescence system (excitation at 400–460 nm) to delineate the full extent of a tumor which, in turn, can be useful in guiding the complete tumor resection in the operating room. Optical changes, specifically the loss of fluorescence in the mucosa lesion and surrounding tissues were used to map the field cancerization. Correlation of histopathological characteristics and specific genetic alterations indicate that fluorescence visualization is improved lesion delineation (size) and field cancerization detection compared to clinical judgment [16,27,32,33]. The fluorescence spectra collected in the same tissue show different ways depending on the excitation used. This variation occurs because the penetration depth and the excited fluorophores in each wavelength. The excitation at 532 nm has a greater penetration depth compared to excitation at 406 nm [25]. Moreover, the characteristics of the tissue in which the endogenous fluorophores are present have higher influence. The hemoglobin, which is present in the vascular spaces and in the stromal layer absorbs a portion of the emitted fluorescence. This finding is particularly visible in the excitation at 406 nm. Epithelial fluorophores such as NADH also play a role in the features of the spectra of shallow depths and stromal fluorophores, such as collagen, contribute to the measured signal from deeper regions [8,18,19].

Schwarz et al. [34] suggest that short wavelengths may be more sensitive than the profound changes in the uptake of optical scattering properties, where occur the initial changes detected in epithelium, such as nuclear size and nucleus/cytoplasm ratio, and in the stromal surface region. In non-keratinized tissue diagnostic performance was achieved using only optical spectra short and medium depths. The discrimination spectra normal and abnormal sites is better when used excitations smaller ranges, which is heavily skewed to the epithelial layer and minimizes the effects of the absorption of hemoglobin [19,35]. Our optical system do not have discrimination of the information from distinct tissue layers, the final response is the contribution of the total volume. This result in a less precise information of each fluorophore contribution to the final spectrum, but

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Representation of the mathematical processing performed for analysis, with normalization by maximum, system fluorescence spectroscopy to discrimination between normal and abnormal mucosa.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>VE Sensibility</td>
</tr>
<tr>
<td>Bayes</td>
<td>0.513</td>
</tr>
<tr>
<td>J48</td>
<td>0.829</td>
</tr>
<tr>
<td>kNN k = 3</td>
<td>0.647</td>
</tr>
<tr>
<td>kNN k = 7</td>
<td>0.641</td>
</tr>
<tr>
<td>kNN k = 11</td>
<td>0.638</td>
</tr>
<tr>
<td>kNN k = 15</td>
<td>0.641</td>
</tr>
<tr>
<td>kNN k = 19</td>
<td>0.636</td>
</tr>
<tr>
<td>Bayes + RelieFF (10%)</td>
<td>0.515</td>
</tr>
<tr>
<td>J48 + RelieFF (10%)</td>
<td>0.834</td>
</tr>
<tr>
<td>Best kNN + RelieFF (10%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Bayes + CFS</td>
<td>0.567</td>
</tr>
<tr>
<td>J48 + CFS</td>
<td>0.87</td>
</tr>
<tr>
<td>kNN + CFS</td>
<td>0.672</td>
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<tr>
<td>VI Sensibility</td>
<td>0.618</td>
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<tr>
<td>Specificity</td>
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<td>Accuracy</td>
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<td>FP rate</td>
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<tr>
<td>0.768</td>
<td>0.859</td>
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<tr>
<td>0.75</td>
<td>0.845</td>
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<tr>
<td>0.748</td>
<td>0.843</td>
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our results showed that even the superficial information can achieve satisfactory diagnostic performance.

Pavlova et al. [35] suggest that oral epithelium can be divided into three layers with different optical properties. The oral epithelium is composed of a superficial layer of keratin, which varies in thickness depending upon the anatomic location. The main fluorophore of the surface epithelium is keratin, in which there is a layer of non-keratinized epithelium and is occupied by intermediate and basal cells metabolically active and with less dispersion. The fluorescence from the non-keratinized epithelium is associated with metabolic indicators NADH and FAD, which increase in samples of oral dysplasia.

The presence of inflammation may be an additional complicating factor for spectroscopic diagnosis of oral lesions; autofluorescence due to reduced inflammation can be difficult to distinguish from the reduced autofluorescence due to neoplasia. As inflammation primarily affects the stroma while the dysplastic epithelium changes occur, the depth sensitive spectral data, particularly obtained from superficial layers may provide more useful information for discriminating benign lesions from malignant or dysplastic lesions [18].

Erythematous lesions have a lower fluorescence intensity compared to leukoplakic lesions, probably the largest presence of hemoglobin. Leukoplakic lesions behave differently in re-emission of light because they are rich in keratin, which has a prominent role in the phenomena of scattering and absorption and fluorescence intensity re-emitted [36–38]. Erythropalakic mucous membranes were already diagnosed with a more advanced degree of atypia and frequently with an in situ or microinvasive cancer, these lesions produced more altered fluorescence spectra compared to normal and there was a tendency to have lower re-emission of light. These results corroborate the study Ebihara et al. [14].

Pavlova et al. [35], using a Monte Carlo model, found that 43–66% of the total light detected with a depth selective probe, ranging from 280 nm to 420 nm, originated from the thickness of the non keratinized epithelium. The identification of bacterial contamination is important because some bacteria produce porphyrins, and this may change the result of the intrinsic fluorescence of the tissue. Leaving the place of contamination by oral microorganisms optical discrimination has been primarily associated with a decrease in the intensity of fluorescence emitted in the tumor region as compared to the healthy regions. This fact has been correlated with decreased crosslinking of collagen fibers and matrix components [8,36].

Similar results have been previously reported in other clinical studies on detection of oral cancer. de Veld et al. [42] observed that dysplastic sites and tumors are accompanied by a progressive drop in fluorescence intensity, whereas Lane et al. [31] reported a loss of signal in wide field fluorescence images from potentially malignant and malignant oral lesions compared to normal mucosa around the lesion. The reduced fluorescence associated with neoplasia was seen in a wide range of excitation wavelengths of 330–470 nm in the study of Schwarz et al. [34].

Pavlova et al. [26] studied the UV excitation from four different locations in the tongue of a single patient. The lesion diagnosed with severe inflammation showed a large drop in both fluorescence as epithelial and stroma. Moreover, while the fluorescence of the stroma of normal tissue stemmed from collagen fibers, the stromal inflamed fluorescence originated mainly inflammatory cells. The oral lesion diagnosed with dysplasia was characterized by increased thickness fluorescent epithelial cells throughout the epithelium and a decrease in fluorescence stromal surface.

McGee et al. [40] found that discrimination of dysplastic lesions versus cancer was more successful when algorithms are designed for individual sites. Similar results were obtained in this study. The combination of sites with the same spectral properties (floor of mouth and border of the tongue) produced a more accurate and consistent discrimination performance than algorithms developed for all sites.

The similarity between the shapes of the spectra of normal and cancerous tissues significantly reduces the reliability of this technique for cancer detection. Several methods have been investigated to solve this problem by a statistical analysis of spectral data to reduce the ‘noise’ or the employment of excitement in multiple lengths. Multivariate analysis based on principal components analyses (PCA) and support vector machines have been introduced to identify spectral features which can be correlated with the tissue pathology condition, but there are various types of mathematical procedures that may be performed for optimal discrimination (classification) of the samples [16].

In the evaluation of biopsy specimens, Fryen et al. [41] observed that autofluorescence becomes heterogeneous in areas with neoplastic cell pleomorphism. The different degrees of keratinization according differentiation of injuries cause more distinct viewing of the change, unlike normal epithelium. Even small precancerous lesions such as dysplasia and carcinoma in situ show different degrees of autofluorescence.

Heintzelman et al. [8] found that the wavelengths of 350 nm excitation, 380 nm and 400 may be suitable for oral cancer detection. By using the excitation wavelengths of 350 nm and 400 nm in the oral cavity, obtained a fluorescence emission at 472 nm, with 90% sensitivity and 88% specificity. In validation tests of the experiment, the authors achieved 100% sensitivity and 98% specificity, using the same parameters of wavelength. According to the authors, the oral mucosa showed a higher emission intensity of autofluorescence (450 nm) at a wavelength of 380 nm excitation relative to neoplastic mucosa.

de Veld et al. [39] evaluated by fluorescence spectroscopy 97 volunteers with normal oral mucosa using seven different wavelengths of excitation in 13 anatomical locations. The intensity of autofluorescence showed its maximum between 500 and 510 nm. The lower fluorescence intensities were observed at the border of lip semimucosa and border of the tongue, and the largest in palate and buccal mucosa. The authors found that there are small spectroscopic differences between different anatomical locations. The total intensity of fluorescence cannot be used as a reference to compare with a database, since there are different intensities of autofluorescence in different anatomical sites, and the variance between patients. Palmer et al. [43] observed that the largest differences between normal and dysplastic tissue were found in the excitation wavelength of 410 nm. Dysplastic and malignant samples had increased fluorescence above 600 nm, when
compared with normal samples indicating that dysplastic lesions exhibit a fluorescent characteristic which is not present in normal mucosa. The method used by the authors showed sensitivity, specificity and accuracy of fluorescence to differentiate histologically normal tissue from dysplastic and neoplastic 90%, 91% and 91%, respectively.

The high level of sensitivity and specificity obtained with a noninvasive procedure demonstrates the power of fluorescence spectroscopy as a diagnostic tool for detection of carcinoma, especially considering that the carcinoma group showed great variability. A diagnostic technique with fast response can provide important information for the clinician, aid in the classification of the lesion, scanning large areas, the delimitation of the lesion margin, and also in the choice of the biopsy site [25].

The clinical characteristics were observed in various different behaviors of fluorescence spectra. The excitation under violet light showed higher levels of sensitivity and specificity for discrimination of normal versus carcinoma compared with excitation at 532 nm. The analysis result showed better results with the algorithm J48 of violet excitation, compared to 532 nm.

Our results demonstrate the potential of diagnostic fluorescence spectroscopy in an objective and non-invasive, dysplastic and cancerous sites distinguishing normal oral mucosa. Furthermore, these results support the use of a system for optical spectroscopy depth susceptible to improve performance in diagnosis.

The fluorescence spectroscopy has significant sensitivity and specificity for diagnosing neoplastic tissue, both in excitation at 406 nm and at 532 nm. Excitation at 406 nm was more efficient and can be used as an auxiliary tool in clinical diagnostic discrimination of mucosa normal versus potentially malignant disorder versus oral cancer.

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Detection of potentially malignant disorders


