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Validation of Optical Coherence Tomography in the assessment of  
oral potentially malignant disorders and oral cancer

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## Abstract

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Currently, the “gold standard” for the approach to the diagnosis of potentially malignant disorders and oral carcinoma involves three steps: i) a visual recognition of macroscopic features, ii) selection of the most representative site for biopsy, and iii) histopathology evaluation of hematoxylin and eosin (H&E). However, each of the different steps can be characterized by some limitations.

To obviate these problems, it would be advantageous to introduce a non-invasive imaging tool for *in vivo* and real-time microscopic evaluation of the lesion, both in the diagnostic step and in the selection of the site to be biopsied.

The term "optical biopsy" includes a wide range of dental and medical screening systems. To date, only fluorescence based systems are used in clinical practice.

OCT is a diagnostic imaging method, first applied in 1991 by Huang et al.<sup>9</sup> in ophthalmology: it is a non-invasive imaging technique analogous to ultrasound, that measures the amplitude of backscattered light generated from a light source as a function of depth. OCT uses lowcoherence interferometry to produce microscopic cross-sectional images of the tissue architecture similar to histology

The aim of this *in vivo* study is to generate a bank of pathological OCT data of the oral tissues to allow identification of cellular structures of pathological processes and compare it to the “gold standard” histopathology with the aim to create a diagnostic algorithm which can be used in the early detection of oral disorders.

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## Summary

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In our study we included consecutive patients referred to the Oral Medicine Sector "Valerio Margiotta" of the University of Palermo, during the period of June 2015 to September 2017, who presented the following suspected oral lesions: oral carcinoma, leukoplakia (idiopathic, from smoking), erythroplakia, oral lichen planus, lichen-like lesions (e.g. lichenoid reactions, cGVHD).

The same protocol was followed for all patients:

- 1) Clinical examination
- 2) Photo report
- 3) Examination through the use of in vivo OCT
- 4) Biopsy and histological examination

For each selected OCT image the same characteristics were analyzed: basal membrane status, basal membrane quality (excellent, good, adequate, poor), changes in the lamina propria (no change, no obvious change and obvious change), changes in the epithelial layer (increased, decreased, no change), changes in the keratin cell layer (increased, decreased, no change).

Furthermore, changes in the thickness of the epithelial layer and the keratin cell layer were described, considering the normal values reported in the literature.

In view of the characteristics of the OCT images, another suspicion diagnosis was formulated.

During the period of June 2015 to September 2017, 80 patients were involved in the study.

Basic histological layers were identified on most of the images.

OCT could identify diseased area but could not provide a diagnosis or differentiate between oral potentially malignant disorders while OCT sensitivity and specificity for invasive carcinoma were 94%.

This study confirms the feasibility of using OCT to identify architectural changes in malignant lesions, but, to date, with the use of this method it is not possible, however, to distinguish between cellular and sub-cellular changes, for staging of potentially malignant disorders.

## Background Rationale and Objectives

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### 1.1 Background

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Oral squamous cell carcinoma (OSCC) is the most common oral and maxillofacial malignancy<sup>1</sup>.

If detected at an early stage, survival from oral cancer is better than 90% at 5 years, whereas survival of patients presenting with late stage disease is only 30%. The 5- year survival rate for oral cancer has remained less than 50% over the last 50 years<sup>2</sup>.

OSCC can be preceded by oral “potentially malignant disorders”; in 2005, the World Health Organization recommended the use of the term oral “potentially malignant disorders” (PMDs) instead of precancerous lesions/disorders. However, a definition of oral PMDs had not been proposed at that time. Sarode et al.<sup>3</sup> attempted to propose a definition for oral PMDs; namely, it is a group of disorders of varying etiologies, usually tobacco, characterized by mutagen associated, spontaneous or hereditary alterations or mutations in the genetic material of oral epithelial cells with or without clinical and histomorphological alterations that may lead to oral squamous cell carcinoma transformation. Currently identified oral PMDs include leukoplakia, erythroplakia, palatal

lesions associated with reverse smoking, oral lichen planus, oral submucous fibrosis, actinic keratosis and discoid lupus erythematosus<sup>4</sup>.

These lesions have greater potential for malignant transformation than other oral lesions. The early detection and diagnosis of oral PMDs allow clinicians to monitor and treat oral carcinogenesis at intraepithelial stages, including mild, moderate, and severe dysplasia, as well as carcinoma in situ. This is crucial for improving the survival rate and reducing morbidity, disfigurement, function loss, treatment duration, and hospital costs of OSCC patients.

Currently, the “gold standard” for the approach to the diagnosis of potentially malignant disorders and oral carcinoma involves three steps:

- a visual recognition of macroscopic features,
- selection of the most representative site for biopsy, and
- histopathology evaluation of hematoxylin and eosin (H&E)

However, each of the different steps can be characterized by some limitations.

Conventional oral examinations, including visual inspection and palpation, are the routine methods for the screening of oral lesions. However, subtle lesions may pass undetected, and it is difficult to make a distinction among benign, premalignant, and malignant lesions. It has been reported that dysplasia or micro-invasive carcinoma can occur in clinically normal-appearing mucosa<sup>5</sup>.

Eighty-five% of potentially malignant oral disorders and / or malignant tumors may present as white lesions (leukoplakia), with variable cellular changes. Other less common presentations include red lesions (erythroplakia) and mixed lesions (leuko-erythroplakia). In addition, many cases of oral carcinomas are reported in the literature in patients with oral Lichen Planus, from Graft versus Host Disease and from actinic cheilitis. The evolution of all these lesions is not predictable, but the location, size, margins, color and morphology can help the clinician to recognize the lesions that could undergo malignant transformation<sup>6</sup>.

Clinical diagnosis of the potentially malignant disorders is not simple, however, since the macroscopic aspect is not conclusive to obtain a certain diagnosis; for example, frictional keratosis can clinically simulate a leukoplakia, and inflammatory lesions may simulate an erythroplakia. Several authors have reported that generic dentists are extremely sensitive

but poorly specific to identify these conditions, while general practitioners (with less training in the identification of oral diseases) are less sensitive and more specific. Furthermore, it must be considered that even for an oral medicine specialist it is difficult to make a differential diagnosis between lesions clinically similar but which may have very different histocytological characteristics<sup>6</sup>.

On the other hand, when it is decided to carry out a diagnostic biopsy, the choice of the site to be examined is crucial, as the histological characteristics can be considerably variable in clinically non-uniform lesions. If only histologically characterized areas of less severe cellular changes are chosen, the less severe cellular pattern observed may be interpreted as representative of the lesion as a whole (especially lesions presenting other areas associated with cellular atypia) and adequate management may not be performed. Tumour detection is further complicated by a tendency towards field cancerisation, leading to multicentric lesions, all of which may not be clinically visible.

In addition, biopsy is a real surgery procedure, with all its possible consequences for the patient and the medical report can require several days because it involves the necessary processing of the tissue, fixation and examination<sup>7</sup>.

Moreover, subjectivity and observer variability (intra- and interobserver variability) are very common in the histologic diagnosis of oral PMDs and oral cancer<sup>7</sup>.

Early intervention (i.e. medical and / or surgical) can reduce morbidity and to date biopsy is the most sensitive and specific means to confirm the suspicion of an oral potentially malignant disorder or oral cancer.

## 1.2 Rationale

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To obviate these problems, it would be advantageous to introduce a non-invasive imaging tool for *in vivo* and real-time microscopic evaluation of the lesion, both in the diagnostic step and in the selection of the site to be biopsied. These methods should be objective, proposing a quantitative and reproducible analysis.

The term "optical biopsy" includes a wide range of dental and medical screening systems. To date, only fluorescence based systems are used in clinical practice. Many other systems are still in the clinical trials phase, including elastic scattering spectroscopy (ESS), path length differential spectroscopy (DPS), infrared spectroscopy, Raman



spectroscopy, confocal imaging, microendoscopy, optical coherence tomography (OCT) and molecular imaging<sup>8</sup>.

OCT is a diagnostic imaging method, first applied in 1991 by Huang et al.<sup>9</sup> in ophthalmology: it is a non-invasive imaging technique analogous to ultrasound, that measures the amplitude of backscattered light generated from a light source as a function of depth. OCT uses lowcoherence interferometry to produce microscopic cross-sectional images of the tissue architecture similar to histology. The heart of this system is the fiber optic Michelson interferometry : light in a system is broken into two arms: a reference arm (a mirror) and a sample arm (used to scan the sample of tissue). By combining the light from both arms an interference pattern is produced, which allows the depth of the reflection in the sample to be calculated. This pattern is picked up by the detector and converted into a representative image pixel. In this way with OCT it is possible to obtain high resolution images (3-5 $\mu$ m of axial resolution against the 0.3 mm of the ultrasounds and 20  $\mu$ m against 1mm for the transversal resolution), permitting visualization up to a tissue depth of 1–2 mm. Therefore, the invasive and extensive malignant lesions in the deeper layers of the tissue are out of the OCT images. Images can be obtained in vivo using a flexible fiber optic probe.

### **1.3 Objectives**

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The aim of this in vivo study is to generate a bank of pathological OCT data of the oral tissues to allow identification of cellular structures of pathological processes and compare it to the “gold standard” histopathology with the aim to create a diagnostic algorithm which can be used in the early detection of oral disorders.

## **Patients and Methods**

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### **2.1 Ethics**

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All the procedures reported were approved by the Internal Ethical Committee of the University Hospital A.O. U. P “Paolo Giaccone” of Palermo (Internal registry: 11/2016).

### **2.2 Patients**

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In our study we included consecutive patients referred to the Oral Medicine Sector "Valerio Margiotta" of the University of Palermo, during the period of June 2015 to September 2017, who presented the following suspected oral lesions: oral carcinoma, leukoplakia (idiopathic, from smoking), erythroplakia, oral lichen planus, lichen-like lesions (e.g. lichenoid reactions, cGVHD). Informed consent was obtained from each patient explaining the nature of the study. Exclusion criteria involved patients under 18 years of age and those with previous history of cancer of the oral cavity and the oropharyngeal/laryngeal regions.

## 2.3 Methods

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The same protocol was followed for all patients:

- 1) Clinical examination
- 2) Photo report
- 3) Examination through the use of *in vivo* OCT
- 4) Biopsy and histological examination

All data: demographic, habits (smoking and alcohol) and clinical features of the lesions (site, clinical features, lesion morphology and color) were collected in a dataset. For each patient a clinical diagnosis formulated during the first visit was also recorded.

The photographic report was performed in order to record not only the clinical features of the lesion, but also for the purpose of recording the site in which the survey was performed through OCT and biopsy sampling, especially in cases of patients presenting widespread disorders in different sites of the oral cavity (e.g. in patients with oral Lichen planus, or proliferative verrucous leukoplakia.)

For this study we used *in vivo* VivoSight OCT (Michelson Diagnosis). We decided to use the dermatological probe because independently conducted clinical research have demonstrated the effectiveness of this new medical technology. Namely, more than 50 papers have been published on the use of the VivoSight® Optical Coherence Tomography in dermatology, demonstrating both its clinical value and potential applications. The light source used had an imaging wavelength of  $1305 \pm 15$  nm 1310 nm, axial optical resolution of  $<10$   $\mu$ m, and lateral optical resolution of  $<10$   $\mu$ m, with a maximum image width of 6 mm and a focal depth of 2 mm.

No preliminary preparation of the site is required to perform an OCT examination.

The greatest limitation of the probe used for this study is represented by its size, as it is suitable for dermatological use and not in the oral cavity. For this reason, the patients were also selected in consideration of the site of manifestation of the pathology: namely, we selected patients who showed gingival lesions in the II and V vestibular sextant, lesions on the back (excluding the posterior 1/3 portion), lingual margin or belly, lesions

of the mucous membrane genes (1/3 anterior portion), lesions on the mucosa upper and lower labial, lesions on upper and lower lip.

The contact with the mucosa was obtained through the use of hard plastic spacer rings. For lesions larger than 6 mm, OCT images were obtained at the central area of the lesion.

The most significant OCT images among those collected were selected retrospectively; so the main investigator was not influenced by the clinical features of the lesion and by the suspected diagnosis.

For each selected OCT image the same characteristics were analyzed:

- 1) Basal membrane status (intact, breach, difficult to assess)
- 2) Basal membrane quality (excellent, good, adequate, poor)
- 3) Changes in the lamina propria (no change, no obvious change and obvious change)
- 4) Changes in the epithelial layer (increased, decreased, no change)
- 5) Changes in the keratin cell layer (increased, decreased, no change)

Furthermore, changes in the thickness of the epithelial layer and the keratin cell layer were described, considering the normal values reported in the literature.

In view of the characteristics of the OCT images, another suspicion diagnosis was formulated.

After the OCT examination, excisional or incisional biopsy of the lesion was performed. The biopsy specimens were processed routinely in 10% formalin and embedded in paraffin. Representative sections of lesions were selected by a pathologist and photographed under light microscopy.

The images obtained through the two methods were then compared.

## **2.4 Statistical analysis**

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For the quantitative variables, the mean and the standard deviation and the relative range were calculated; for the demographic variables, for the evaluation of the mucosa structure and for the comparison between the OCT diagnosis and the histological diagnosis, the frequency distribution will be reported.

## Results

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During the period of June 2015 to September 2017, 80 patients were involved in the study: 47 females (59%) and 33 males (41%); their age range was 22-90 and 24-89 years, respectively. The mean age was 63 years. All patients presented with clinical features suggestive of potentially malignant disorders and oral cancer.

Demographic information of each patient was collected in a dataset. Smoking habits was also highlighted. (**Table 1**) Detailed clinical examination was performed on each patient to assess the site and the clinical characteristics of the lesion (**Table 2**). All lesions were photographed.

The lesions were mainly identified in the tongue (39%), buccal mucosa (31%), gingiva (24%), and floor of mouth (4%).

The majority of the lesions (n=27) appeared clinically as plaque, 10 were erosive lesions, 10 were papular/reticular, and 10 were atrophic lesions.

Lesions color was variable: 43% were red, 36% were white, and 21% were mixed white / red.

The suspect diagnosis revealed 33 Oral Lichen Planus, 19 oral carcinoma, 7 leukoplakia followed by 3 cases (4%) of suspected candidotic leukoplakia, 3 cases (4%) of suspected proliferative verrucous leukoplakia, 3 cases (4%) of lichen-like lesions, 1 case (1%) of erythroplakia and 1 case (1%) of actinic cheilitis(**Table 3**)

The pathological diagnosis (**Table 4**) identified the presence of n = 38 cases of Oral Lichen planus, n = 18 cases of invasive oral cell squamous cell carcinoma, n = 11 cases

of oral leukoplakia, n = 5 cases of low-grade dysplasia, n = 2 cases of proliferative verrucous leukoplakia, n = 1 case of high-grade dysplasia, n = 1 case of carcinoma in situ, n = 1 case of carcinosarcoma, n = 1 case of hyperplastic candidiasis.

OCT images were then examined and compared with the pathological results.

OCT imaging showed distinct layers: basic histological layers (keratin, epithelium, and lamina propria) were identified in most of the images (**Table 5**). In 48% of cases the quality of the basement membrane was considered good, while in 29% of cases it was considered excellent. In 88% of cases it was possible to evaluate the status of lamina propria. However, in 15 cases (19%) the basement membrane could not be identified, and in 14 cases (18%) it was not possible to identify the lamina propria.

The keratin layer appears as a bright line on the uppermost part of the epithelium. Its thickness was reduced in the OCT images in non-keratinized tissues.

In hyperkeratosis, this layer showed hyper-reflection and moderately increased in thickness. In cases of high-grade dysplasia and oral carcinoma, the layer appears hypo-reflective or absent, especially in cases of ulcerative lesions (**Fig. 1**).

The epithelial layer appeared to be more hypo-reflective compared with the keratin layer. It also appeared increased in thickness in the cases of architectural changes.

The demarcation between the two different signal intensities of the epithelium and of the lamina propria is represented by the basement membrane. The basement membrane appeared to be intact in potentially malignant disorders without dysplasia or with dysplasia, while it was breached in cases of invasive oral carcinoma (**Fig.2**).

The keratin and epithelial layers thickness was variable: the average thickness in images of potentially malignant disorders without dysplasia was  $19 \pm 14 \mu\text{m}$  and  $36.4 \pm 15 \mu\text{m}$  respectively; the average thickness was  $18 \pm 14 \mu\text{m}$  and  $36.5 \pm 13.9 \mu\text{m}$  respectively in cases of dysplasia; in the case of carcinoma in situ the thickness was  $4 \mu\text{m}$  for the keratin layer and  $43 \mu\text{m}$  for the epithelial layer; the keratin and epithelial layers' thickness was respectively  $3 \pm 13 \mu\text{m}$  and  $47.8 \pm 14.8 \mu\text{m}$  in cases of invasive carcinomas. It must also be considered that in n=14 cases (73.6%) it was not possible to identify the keratin layer.

OCT was able to differentiate normal tissue from malignancy tissue; in all cases the OCT images showed a loss of continuity of the basement membrane, a loss of epithelial and lamina propria architecture. Moreover, most of them had a hypo-reflecting keratin layer,

and in almost all cases it was not possible to identify this structure, while the epithelial thickness appeared to be increased.

In the cases of invasive carcinoma, it was possible to identify the presence of epithelial invasion in the chorion, in association with the breach of the basement membrane. **(Fig.3)**.

OCT sensitivity and specificity for invasive carcinoma were at 94%.

With the use of this method it is not possible, however, to distinguish between cellular and sub-cellular changes, which would allow for identifying the different potentially malignant disorders.

Differentiation between normal and pathological tissue was mainly based on the identification of disorganized layers.

In OCT images of invasive carcinoma, epithelial thickness was found to be greater when compared to the thickness in cases of potentially malignant disorders and dysplasia, while the thickness of the keratin layer was significantly reduced, and in most cases absent. This is not possible in cases of dysplasia: the epithelial thickness has proved variable and in the absence of the loss of continuity of the basement membrane. The sensitivity and specificity in these cases was found to be 20% and 50%, respectively.

Regarding the potentially malignant disorders in the absence of dysplasia, in 100% of cases it was possible to identify the presence of hyperkeratosis, so a hyper-reflective horny layer. Furthermore, OCT showed a sensitivity and specificity of 27% and 75% respectively for leukoplakia

A greater variability of the characteristics of the OCT images was identified in cases of Lichen Planus Orale: in cases presenting a clinical picture of desquamative gingivitis, it was possible to identify the presence of erosions (associated with the absence of the keratin layer in the OCT image) or the presence of a sub-epithelial blister, associated with an intense hyper-reflective area of the basement membrane **(Fig. 4.)**

In reticular or plaque features a hyper-reflexivity keratin layer was found, whether associated or not with the presence of a hyper-reflective area of the basement membrane.

For oral lichen planus the sensitivity was 45%, while the specificity was 75%.



## Discussion

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The term "optical biopsy" refers to all the non-invasive diagnostic methods that use the properties of the light waves and the ability of the different tissues to absorb and reflect the same, to enable the operator to have a clinical support for real-time diagnosis, for the follow-up and for the selection of the most representative site for biopsy; to date this has been entrusted to the objective examination and therefore to the personal experience of the single operator<sup>8</sup>.

It has been shown that the OCT method is effective for invasive carcinoma, but it is difficult to identify the different pictures of potentially malignant disorders.

Dysplasia is identified through cytological changes (cell nucleus size, number of nuclei, nucleus / cytoplasm ratio and amount of chromatin) and architectural changes<sup>7</sup>; to date it has been impossible to identify the cellular and sub-cellular changes with the use of the OCT tool: we must therefore consider that it is not possible to identify the presence of early cellular atypia in the absence of changes in the tissue architecture; indeed, the OCT has the ability to identify only architectural changes. The system for the assessment of oral dysplasia developed by the World Health Organization<sup>3</sup> is partly applicable in OCT. Many studies<sup>10-12,12-18</sup> have investigated the ability of the OCT methodology to identify changes in images of oral potentially malignant disorders, but no study has identified solid diagnostic criteria.

As showed in this study, it is possible to identify the presence of hyperkeratosis in 100% of cases.

In an in vivo study<sup>14</sup> involving 50 patients with a clinical diagnosis of suspected oral leukoplakia or erythroplakia, the efficacy of the OCT in the early detection of dysplasia was evaluated. OCT images of dysplastic lesions showed increased epithelial thickness and loss of stratification. This criterion, however, was not considered significant for classifying diagnostic criteria for dysplasia.

The ability of the OCT to accurately measure the keratin layer and the thickness of the epithelium has nonetheless been a fundamental factor. A study by Fomina et. al. has shown that the increase of mitotic activity together with the loss of cell cohesion could be responsible for the increase in epithelial thickness.

However, the study by Hamdoon<sup>12</sup> showed that the thickness of the mucosa layers alone is not conclusive for the formulation of a diagnosis.

As also demonstrated in this study, there is a marginal difference between all groups of diseases, so it is difficult to confirm whether the increase in the epithelial layer may or may not be associated with the presence of carcinoma, or only be related to the presence of local inflammatory processes.

Some studies have showed, however, the ability of OCT to differentiate between malignant and non-malignant diseases of the oral mucosa: the basement membrane should be used as a reference layer<sup>13</sup>. The continuity or breach of the basement membrane can help to determine whether the lesion that we are seeing is malignant or not. In all the cases in our study with histological diagnosis of oral squamous cell carcinoma, the observation of OCT images showed a loss of continuity of the basement membrane; moreover, the normal architectural division of the mucosa appeared extremely distorted and the basement membrane could be identified, when possible, only for short sections. Such a structure is extremely breached; furthermore, some cases showed the presence of a structure called the "vertical icicle shaped structure", and identified in the OCT images of cutaneous melanomas. These are hyper-reflective structures generated by the epithelial layer and are inserted into the chorion, interrupting the continuity of the basement membrane. The "vertical icicle shaped structures" have been associated with the presence of dense infiltrates of tumor cells and lymphocytes in histological examination. In our study it was possible to identify very similar structures in 14 cases of invasive cancer.

An interesting finding emerged from this study concerns Oral Lichen Planus: in all cases clinically diagnosed as desquamative gingivitis, it was possible to confirm, through OCT images, the diagnosis of Lichen Planus Orale. One of these cases showed the presence of a sub-epithelial blister, associated with the presence of a hyper-reflective area of the

basement membrane, probably correlated with the presence of a dense inflammatory infiltrate<sup>15</sup>.

The method has also shown its limits: the first is due to the fact that the method is still, to date, dependent on the operator. Therefore, it does not allow for overcoming the limits of clinical examination and evaluation of histological images, which are strongly related to the operator.

A second limitation is the co-registration of OCT images and histological images: the exact control of the histopathological plan may be difficult due to the formalin contraction effect and the lack of vascularisation, as well as of the artifacts of histological processing. Another limitation is represented by the fact that the penetration depth of OCT is strongly dependent on the tissue and is, in any case, limited to a few millimeters (about 2mm); this makes it impossible to evaluate the correct extent of the tumor infiltrate.

Furthermore, we must consider that the sample size prevents us from determining the accuracy for the evaluation of potentially malignant disorders; this is mainly due to the fact that, considering the use of a dermatological probe, the site of the lesion was one of the criteria for inclusion / exclusion of the study. However, it must be considered that the use of the *in vivo* probe allows to obviate the artifacts that could be generated in OCT images, due to the lack of tissue perfusion. Future studies are needed for the validation of a probe for intra-oral use, in order to use OCT *in vivo*.

To conclude, it is clear that OCT is a valid tool in the diagnosis of oral carcinoma, but shows numerous limitations in the early diagnosis of dysplasia.

Moreover, in consideration of the limitation of the method owed to the fact that it is strongly operator-dependent, it would be useful to devise a diagnostic support based on computer analysis systems applied to OCT images of malignant lesions and potentially of oral mucosa, based on a set of algorithms able to process OCT images and to automatically recognize the characteristics typical of the aforementioned lesions, in order to create an automated diagnostic algorithm, thus obviating the close relationship between the method and the experience of the clinician.

**Tables and Figures**

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**5.1 Tables**

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<b>Gender</b>		
	<b>Male</b>	<b>33 (41%)</b>
	<b>Female</b>	<b>47 (59%)</b>

<b>Smoking status</b>		
	<b>Smoker</b>	<b>27 (34%)</b>
	<b>Former smoker</b>	<b>8 (10%)</b>
	<b>Never smoker</b>	<b>45 (56%)</b>

**Table 1:** Demographic information

<b>Clinical feature</b>		
	<b>Bullae</b>	<b>3 (4%)</b>
	<b>Neof ormation</b>	<b>9 (11%)</b>
	<b>Plague</b>	<b>27 (34%)</b>
	<b>Papular/reticular</b>	<b>10 (13%)</b>
	<b>Ulcer</b>	<b>9 (11%)</b>
	<b>Others</b>	<b>22 (27%)</b>

<b>Colour</b>		
	<b>White</b>	<b>29 (36%)</b>
	<b>White/Red</b>	<b>17 (21%)</b>
	<b>Red</b>	<b>34 (43%)</b>

<b>Location</b>		
	<b>Tongue</b>	<b>31 (39%)</b>
	<b>Gengiva</b>	<b>19 (24%)</b>
	<b>Buccal Mucosa</b>	<b>18 (23%)</b>
	<b>Floor of mouth</b>	<b>3 (4%)</b>
	<b>Others</b>	<b>9 (12%)</b>

**Table 2:** Clinical characteristics of the lesion

<b>Clinical diagnosis</b>		
	<b>Oral Cancer</b>	<b>19 (24%)</b>
	<b>Actinic Cheilitis</b>	<b>1 (1%)</b>
	<b>Erythroplakia</b>	<b>1 (1%)</b>
	<b>Gengivite desquamativa</b>	<b>9 (11%)</b>
	<b>Graft vs Host disease</b>	<b>1 (1%)</b>
	<b>Leukoplakia</b>	<b>7 (9%)</b>
	<b>Hyperplastic oral candidiasis</b>	<b>3 (4%)</b>
	<b>Proliferative verrucous leukoplakia</b>	<b>3 (4%)</b>
	<b>Lichen like</b>	<b>3 (4%)</b>
	<b>Oral Lichen Planus</b>	<b>33 (41%)</b>

**Table 3:** Clinical diagnosis

<b>Histologic diagnosis</b>		
	<b>Carcinoma in situ</b>	<b>1 (1%)</b>
	<b>Oral cancer</b>	<b>18 (23%)</b>
	<b>Carcinosarcoma</b>	<b>1 (1%)</b>
	<b>Dysplasia high-grade</b>	<b>1 (1%)</b>
	<b>Dysplasia low-grade</b>	<b>5 (6%)</b>
	<b>Frictonal keratosis</b>	<b>1 (1%)</b>
	<b>Leukoplakia</b>	<b>11 (14%)</b>
	<b>Hyperplastic oral candidiasis</b>	<b>1 (1%)</b>
	<b>Proliferative verrucous leukoplakia</b>	<b>2 (3%)</b>
	<b>Oral Lichen Planus</b>	<b>38 (48%)</b>
	<b>Ulcer</b>	<b>1 (1%)</b>

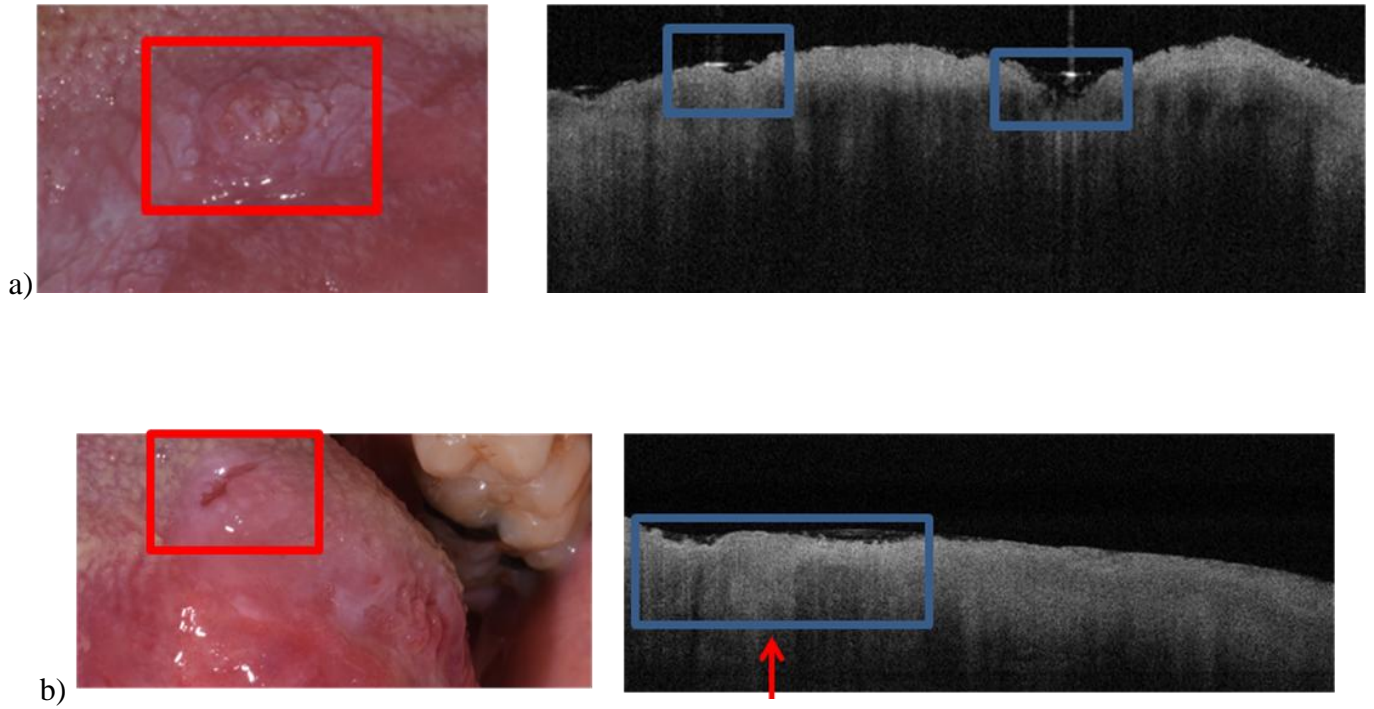
**Table 4:** Histologic diagnosis

<b>BM status</b>	
<b>Breach</b>	<b>20 (25%)</b>
<b>Difficult to assest</b>	<b>14 (18%)</b>
<b>Intact</b>	<b>46 (57%)</b>
<b>BM quality</b>	
<b>Adequate</b>	<b>4 (5%)</b>
<b>Excellent</b>	<b>23 (29%)</b>
<b>Good</b>	<b>38 (48%)</b>
<b>Poor</b>	<b>15 (19%)</b>
<b>LP layer</b>	
<b>No changes</b>	<b>48 (60%)</b>
<b>No clear changes</b>	<b>14 (18%)</b>
<b>Obvious changes</b>	<b>18 (22%)</b>
<b>Ephitelial layer</b>	
<b>Decreased</b>	<b>4 (5%)</b>
<b>Increased</b>	<b>56 (70%)</b>
<b>No changes</b>	<b>20 (25%)</b>
<b>Keratin layer</b>	
<b>Decreased</b>	<b>20 (25%)</b>
<b>Increased</b>	<b>16 (20%)</b>
<b>No changes</b>	<b>44 (55%)</b>

**Table 5:** OCT assessment of the epithelial structure

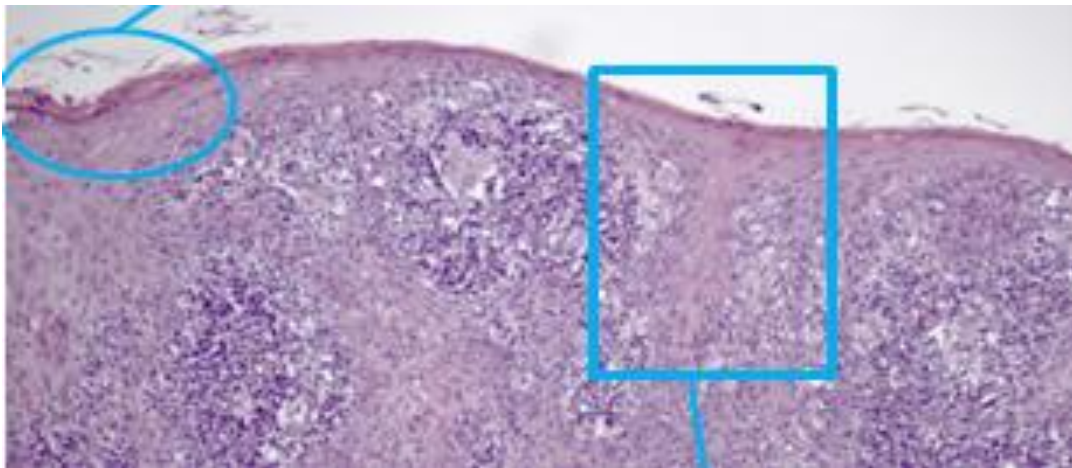
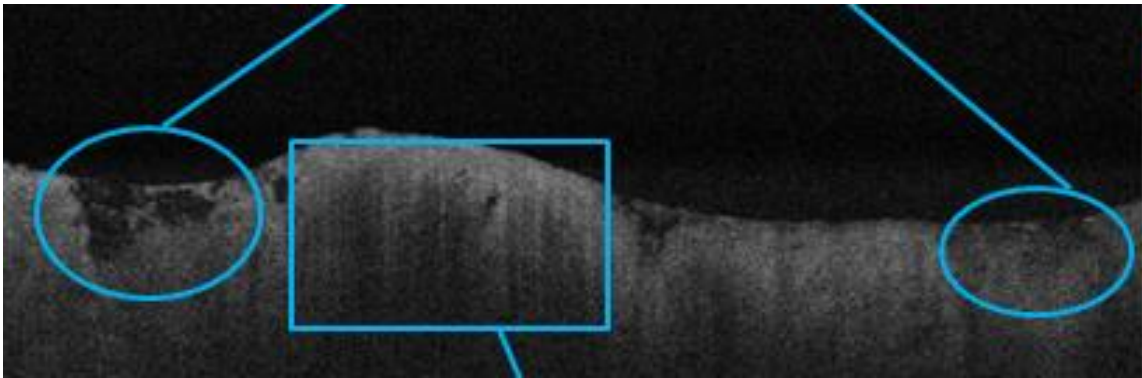
## 5.2 Figures

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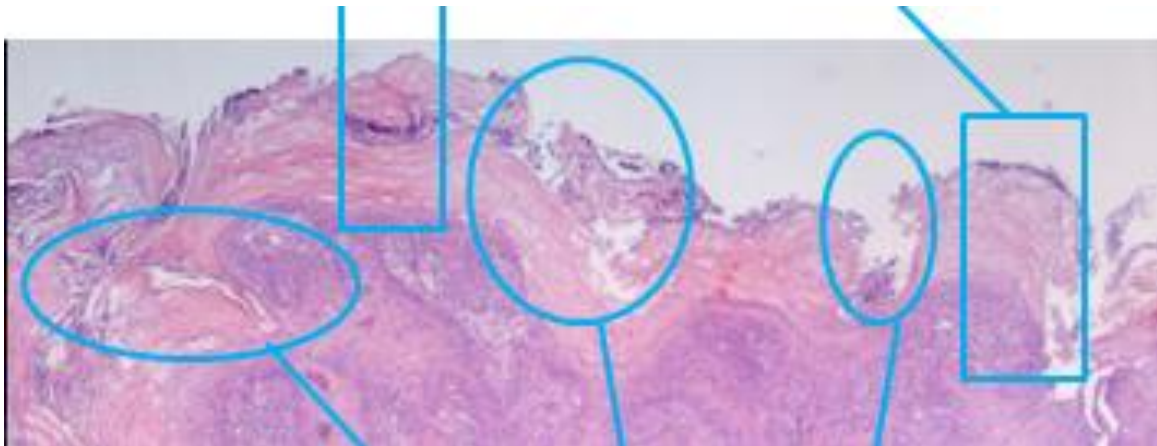
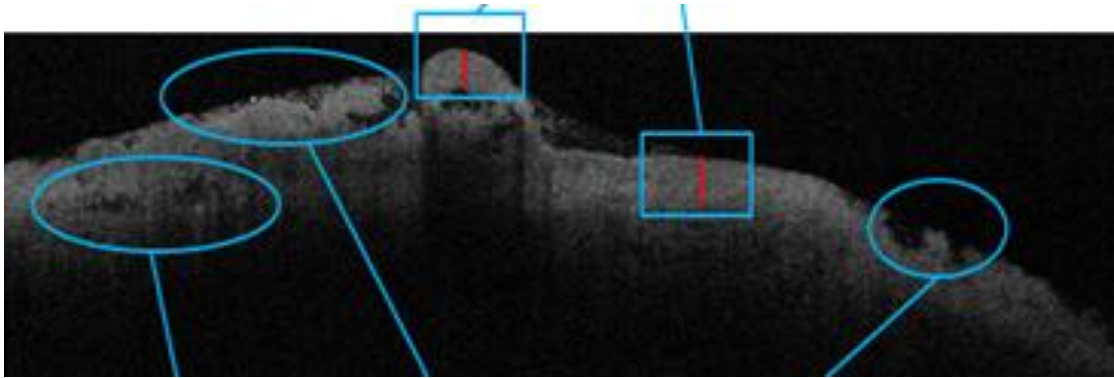


**Fig. 1:** a) high-grade dysplasia and b) carcinoma in situ: the keratin layer appears hypo-reflective or absent.

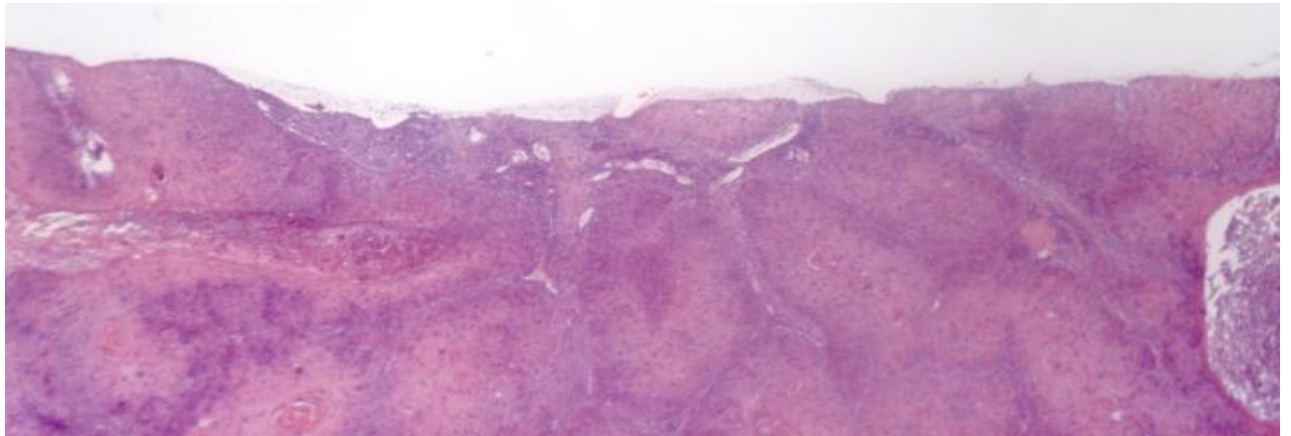
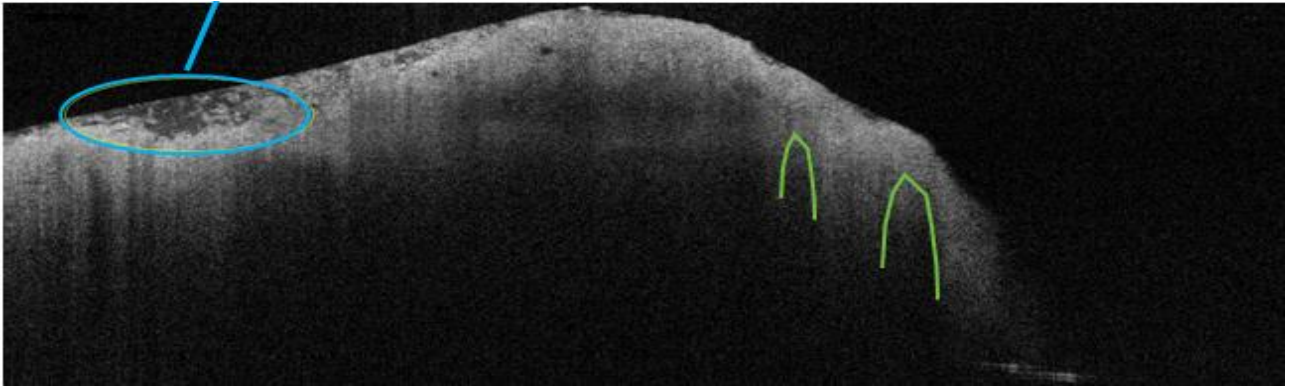




**Fig 2a:** Oral Cancer. The images were compared to the gold standard histopathology.

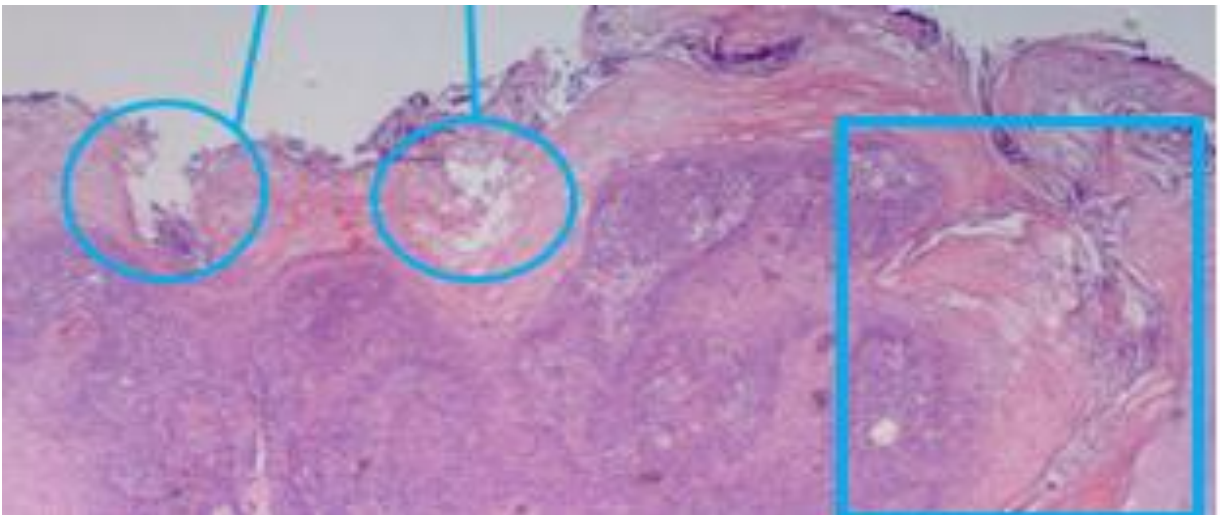
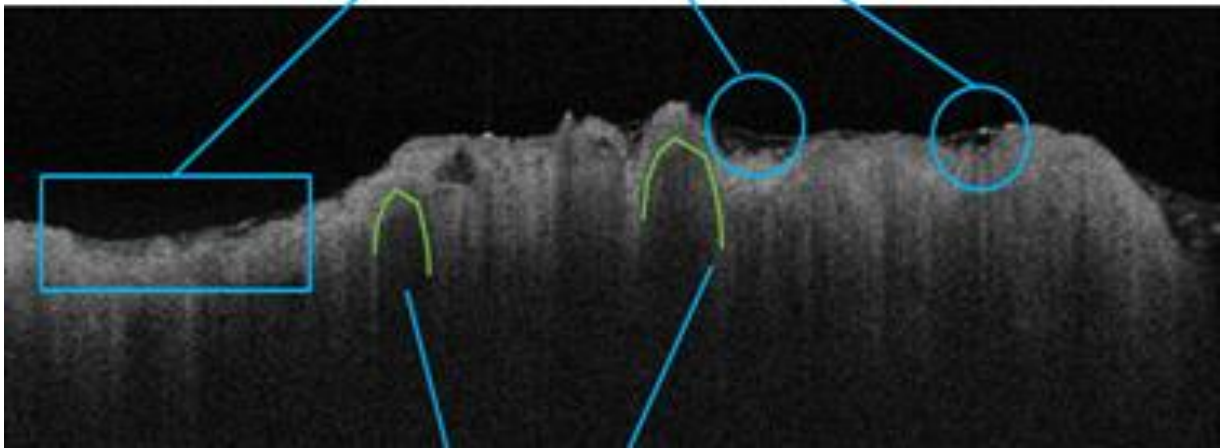
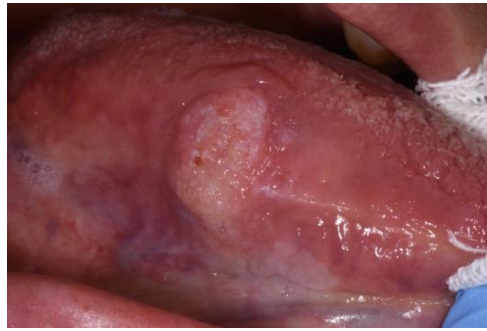


**Fig 2b:** Oral Cancer. The images were compared to the gold standard histopathology.

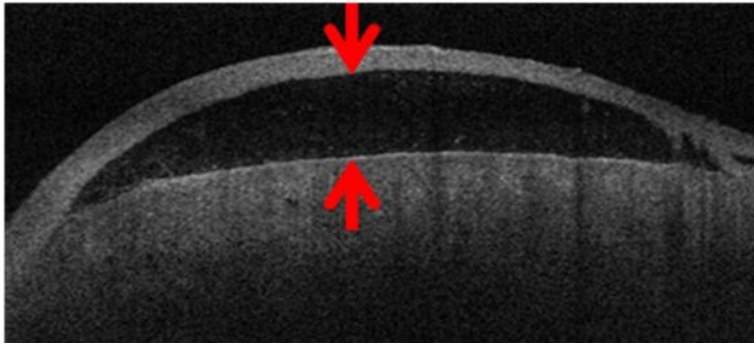


**Fig 3a:** Invasive carcinoma: it was possible to identify the presence of epithelial invasion in the chorion, in association with the breach of the basement membrane. The images were compared to the gold standard histopathology.





**Fig 3b:** Invasive carcinoma: it was possible to identify the presence of epithelial invasion in the chorion, in association with the breach of the basement membrane. The images were compared to the gold standard histopathology.



**Fig 4:** Subepithelial bullae, associated with an intense hyper-reflective area of the basement membrane

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