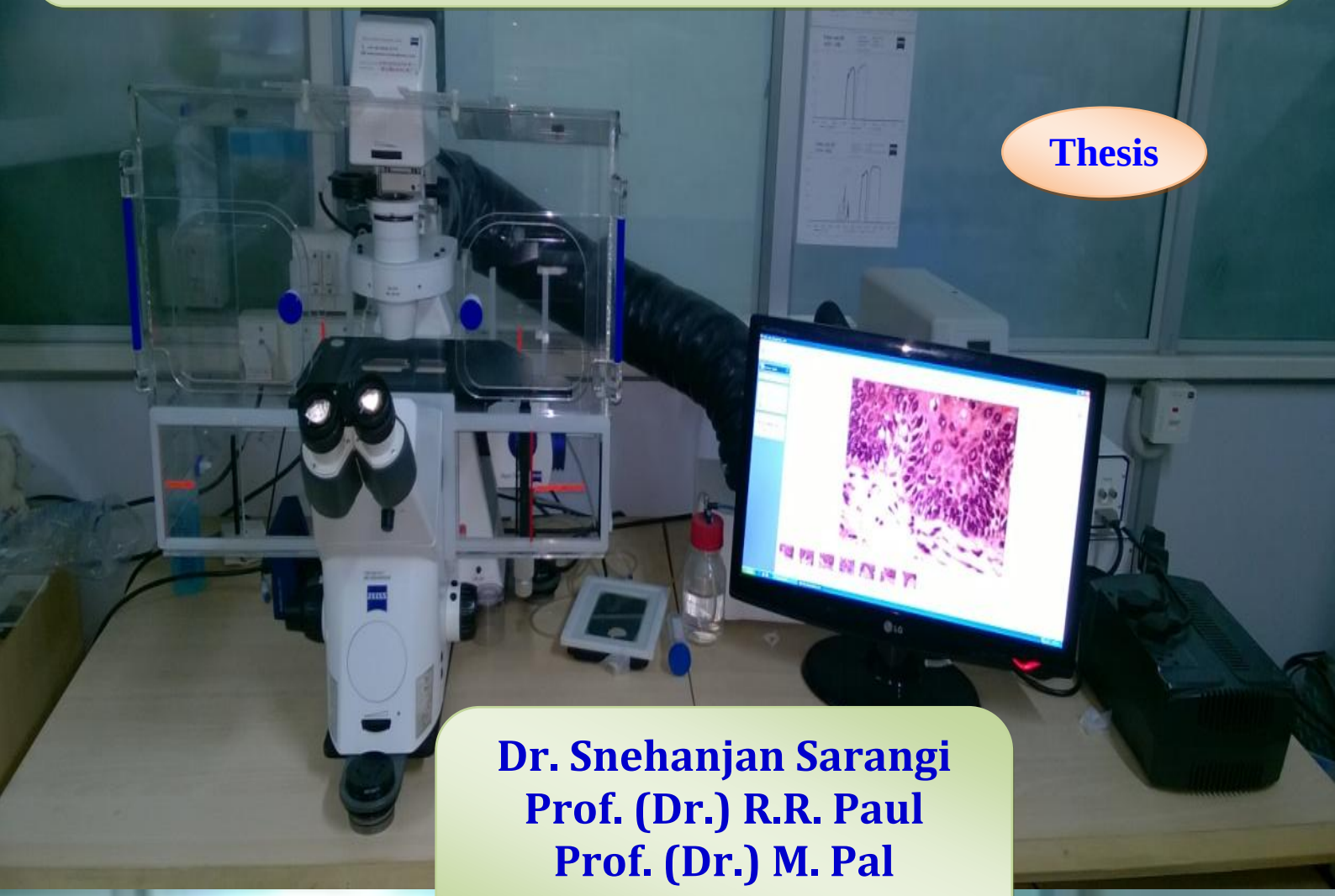
CHARACTERIZATION OF OPTICAL BIOPSY (OCT IMAGES) OF ORAL SQUAMOUS CELL CARCINOMA (OSCC) AND ITS CORRELATION WITH HISTOPATHOLOGICAL ATTRIBUTES

Thesis



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PREFACE

The present study had been conducted on a total number of six subjects in Guru Nanak Institute of Dental Sciences and Research and SMST, IIT, Kharagpur during the time period of December 2014- August 2015 for characterization of Optical biopsy (OCT images) of Oral squamous cell carcinoma (OSCC) and its correlation with histopathological attributes, to establish the efficacy of Optical coherence tomography(OCT) as an adjunctive non invasive diagnostic imaging modality, for an efficient and painless diagnosis Oral squamous cell carcinoma.

The study subjects were thoroughly interrogated, examined, assessed and all the analysed relevant observations were recorded carefully. Then real time *in vivo* OCT imaging from the representative areas of the lesion with prior informed consent was performed. Thereafter, incisional biopsies were performed from the exact site and the biopsied specimens were subjected to an *ex vivo* OCT imaging protocol after a time lapse of six hours, which was the time required for transportation of the tissues to SMST. Afterwards, the biopsied samples were histopathologically evaluated, for the presence of corroborative architectural analytical parameters, with the OCT images.

OCT images appear as varying areas of hyper/hypo lucid zones depending upon the area through which the light is backscattered or transmitted. Hyper lucid zones appear as brighter areas and hypo lucid zone appears as darker areas.
Optical coherence tomography is an evolving imaging technology and a non invasive imaging technique for evaluating oral structures. It can be used for the diagnosis of oral premalignant and malignant changes in the oral mucosa. Because of its higher resolution and penetration depth it can be used for imaging the normal and abnormal changes in the oral mucosa ,but a lot of research is required still, to establish OCT as a non invasive diagnostic imaging

modality which might be a replacement for gold standard histopathology. As it is a new and emerging technology in oral health sciences, several ongoing studies can provide relevant information regarding the use of OCT, which might be of routine diagnostic value. It can be said that the performed study is a step in the right direction in this regard, as it might do value additions to this exciting and innovative bio-engineering research arena and enrich future researchers to pursue further studies. However, the SS –OCT cannot discern cellular details. So, a higher resolution OCT, such as the HD-OCT can be useful in identifying cellular details. Hence, studies involving increased number of samples would be quite helpful in establishing the credentials of OCT as a non invasive alternate imaging modality for screening and diagnostic purposes. Further studies may improve the resolution of the system and the probe capabilities which shall provide valuable information on the development and progression oral precancers and cancers.

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THESIS

SUBMITTED TO

THE WEST BENGAL UNIVERSITY OF HEALTH SCIENCES,

KOLKATA

As partial fulfilment of requirement for **Master of Dental Surgery (M.D.S)**

Degree in the speciality of Oral and Maxillofacial Pathology.

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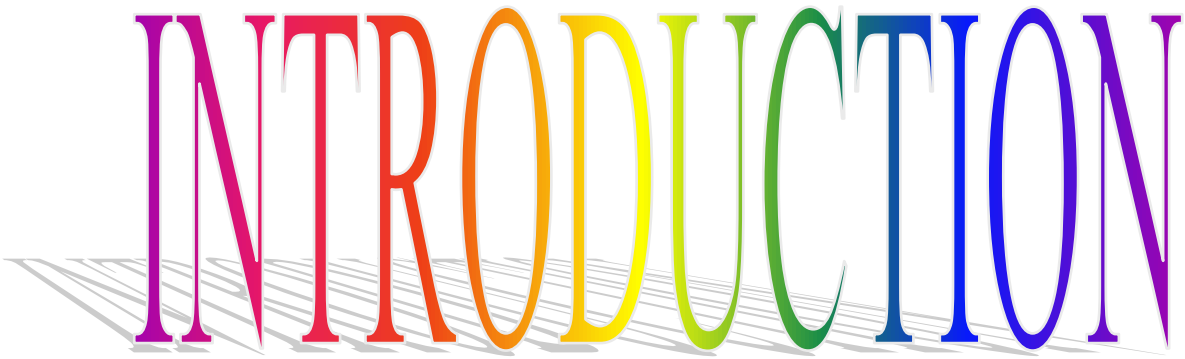
LIST OF ABBREVIATIONS	
BMZ	BASEMENT MEMBRANE ZONE
OSCC	ORAL SQUAMOUS CELL CARCINOMA
CCD	CHARGED COUPLED DEVICE
CT	COMPUTED TOMOGRAPHY
FD-OCT	FOURIER DOMAIN OPTICAL COHERENCE TOMOGRAPHY
HD-OCT	HIGH DEFINITION OPTICAL COHERENCE TOMOGRAPHY
H/P	HISTOPATHOLOGY
LP	LAMINA PROPIA
MRI	MAGNETIC RESONANCE IMAGING
OCT	OPTICAL COHERENCE TOMOGRAPHY
PET	POSITRON EMISSION TOMOGRAPHY
SD-OCT	SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY
SS-OCT	SWEPT SOURCE OPTICAL COHERENCE TOMOGRAPHY
ITF	INVASIVE TUMOR FRONT
VCSEL	VERTICAL CAVITY SURFACE EMITTING LASER
MEMS	MICRO ELECTRO MECHANICAL SYSTEM
IHC	IMMUNOHISTOCHEMISTRY
OPL	OPTICAL PATH LENGTH
OPD	OPTICAL PATH DIFFERENCE
RPE	RETINAL PIGMENT EPITHELIUM

FIGURES AND TABLES


INTRODUCTION

1. **INTRODUCTION**

According to World Health Organization, Cancer is designated as a complex disease in genes that encodes protein that controls cell cycle, cell motility, cell survival and angiogenesis. Therefore, it is an end product of an unregulated proliferation of cells resulting from the accumulation of sequential genetic alterations (mutations) in a precursor cell. The resultant "cancer" is a population of cells that continue to mutate and that secrete self-perpetuating growth factors and angiogenic factors. [1]

About 2% of all malignancies occuring in the body arise in the oral cavity. In some areas of the world this percentage is even higher. The majority of malignancies (94%) consist of squamous cell carcinomas of the covering oral mucosa. [2]

The incidence of oral squamous cell carcinoma (OSCC) differs widely in various parts of the world with the highest incidences found in South East Asia such as Sri Lanka, India, Pakistan and Bangladesh. The etiological factors can be chiefly attributed to usage of tobacco (chewable, non chewable tobacco) and allied products etc. [3]

Mostly, the male-female ratio is approximately 2:1 for oral carcinomas, [4] except for carcinomas of the vermilion border of the lower lip. Most of the cases occur after the fourth decade of life. The commonest site of involvement is the tongue, usually the posterior lateral and the ventral surfaces followed by the floor of mouth, soft palate, gingiva, buccal mucosa, labial mucosa & hard palate. [5]

OSCC often presents as: Exophytic (mass-forming; fungating, papillary, verruciform), Endophytic (Invasive, burrowing, ulcerated), Leukoplakic (white patch), Erythroplakic (red patch), Erythro leukoplakic (Combined red-and-white

patch). [5] The lesions often are part of a diffuse area of clinical leukoplakia-erythroplakia .They are usually asymptomatic, and may produce signs of induration of the affected areas or limited jaw opening if it infiltrates into the regional muscle bed.[6]

There may be a varied histopathologic appearance of oral squamous cell carcinoma. The commonality is that the basement membrane of the surface epithelium has been violated and that the neoplastic epithelium infiltrates the connective tissue. Inter observer variations determine the degree of differentiation, based on how much it resembles to the parent cells of the normal squamous epithelium: well-very similar; moderate-so so; and poorly differentiated-that is barely discernible. The undifferentiated category is usually a diagnosis made with the help of immune histochemical phenotyping of the tissue. The degree of keratinisation may also correlate with the degree of differentiation, and may in some cases correlate with prognosis. [7]

The predictive/prognostic potentiality of Oral squamous cell carcinoma is presently assessed through biopsy followed by histopathological evaluation. Biopsies being an invasive surgical procedure, many a times, patients are reluctant to undergo such modality, specially when it is regarding early diagnosis & screening for assessment of progression of the disease process to various grades of OSCC. Moreover, this invasive procedure is also contraindicated in highly medically compromised patients. Because of the disadvantages, the introduction of any non invasive procedure is the need of the hour, especially for periodic evaluation and screening of oral malignancies. [8]

Optical coherence tomography(OCT) is an optical signal acquisition &processing method which captures micrometer resolution, three dimensional images from within an optical scattering media(e.g. biological tissue).Recently,

this novel technique, first introduced by Fujimoto et. al in 1991 [9], a non-ionising, near interferometric, non-invasive, subsurface imaging modality, is being advocated with a view to assess the micro features of oral mucosal tissue; in terms of imaging and diagnosing epithelial and sub epithelial dysplastic changes within the oral mucosal tissues involving oral [10] premalignant disorders as wells as in the detection of early oral squamous cell carcinoma. OCT uses near-infrared light to produce cross-sectional tissue images with lateral resolution. [10]

OCT can assess oral mucosal tissues at cellular and sub cellular levels at depths of 1-2mm below the surface in biological tissue. This[11] optical modality, provides high resolution (10—20 μm) cross sectional images of tissue *in situ*, up to 10 times higher than conventional ultrasound, magnetic resonance imaging (MRI), or computed tomography and can provide "**OPTICAL BIOPSY**" without the need for excision and processing of specimen as in conventional biopsy and histopathology. [12]

However, the limitation of these studies is that the correlation of the OCT images with their histopathology counterparts has not yet been successfully corroborated.[13]Keeping these factors in mind, the present study had been designed and carried out with the following aims and objectives.

2. AIMS AND OBJECTIVES

1. To record the cardinal features of the light microscopic histopathological images of Oral squamous cell carcinoma (OSCC).

2. To capture and characterize the Optical Coherence Tomographic (OCT) images of OSCC.

3. To compare and corroborate the light microscopic histopathological images to that of images obtained through OCT with a view to validate OCT as an alternative non-invasive diagnostic modality of Oral squamous cell carcinoma.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

A thorough review of English literature was done regarding histopathology and allied OCT attributes of Oral cancer.

3.1 EPIDEMIOLOGICAL DATA

According to Global cancer facts and figures, American Cancer Society, oral squamous cell carcinoma (OSCC) is the most prevalent malignant neoplasm of the oral cavity.[14] Because of this dominance, the term ‘‘oral cancer’’ is used synonymously with oral SCC. [15]

According to Moradzadeh, Halimi et al, the most common type of cancer affecting the oral cavity and oropharynx is squamous cell carcinoma (SCC), which is estimated to constitute approximately 94% of all oral malignancies.[20]

The incidence of squamous cell carcinomas of the oral cavity differs widely in various parts of the world and ranges from approximately 2 to 10 per 100,000 populations per year. Oral and pharyngeal cancers are the sixth most common cancers internationally. [16] In the United States, there are about 30,000 new cases of oral and pharyngeal cancers diagnosed each year. In India, the incidence rate is 20 per 100,000 population and accounts for over 30% of all cancers in the country. [17]

3.2 ETIO-PATHOGENESIS

Epidemiologic studies have established associations between a number of environmental factors and the incidence of cancer. Some of these associations are so strong as to represent cause and effect relationship. The main etiological factor of OSCC is the use of different forms of tobacco(smoking, chewing and smokeless tobacco).[18] Apart from that alcohol, several microbes, fungi, viruses (particularly human papilloma viruses 16, 18, and 32), irradiation, ultraviolet light, naturally occurring genetic defects found in some syndromes, and random

spontaneous genetic mutations are also responsible. Cancer etiology is very complex and is related to the type of carcinogen- its dose, host associated factors, duration, frequency, and application. [18]

The normal precursor cells in the basal layer are often exposed to the carcinogen (benzopyrene, benzanthracene) of cigarette smoking and perhaps alcohol (nitrosamine) at early age .The carcinogens damage the DNA of these cells by forming electrophilic intermediates. [19]

3.3 CLINICAL FEATURES

As per Wahi et al, the lesion is mostly seen in tongue-posterior lateral and ventral aspect [26] and also on floor of mouth, soft palate, gingiva, buccal mucosa, labial mucosa, and hard palate. The lesion manifests itself as Exophytic (mass-forming; fungating, papillary, verruciform), Endophytic (Invasive, burrowing, ulcerated), Leukoplakic (white patch), Erythroplakic (red patch) and Erythro leukoplakic (Combined red-and-white patch). Induration is mostly present which is sometimes associated with pain due to secondary infection. [27]

3.4 HISTOPATHOLOGICAL APPEARANCE OF ORAL SQUAMOUS CELL CARCINOMA (OSCC)

There may be considerable range in the histopathologic appearance of oral squamous cell carcinoma. The commonality is that the basement membrane of the surface epithelium has been violated leading to the infiltration of the neoplastic epithelium into the connective tissue. The extent of the infiltration may range from a few small islands to strands, broad sheets, and large islands that may obliterate the supporting tissue. [21]

The appearance of the invading epithelium is variable. In case of the well differentiated tumors, the cellular atypia and mitotic activity may be minimal. [22] At the other end of the spectrum are the poorly differentiated lesions in which keratin formation is not seen and maturation of tumor cells cannot be appreciated-the tumor cells are quite immature and indiscernible in relation to their parent precursors. In these instances, mitoses are usually more prevalent and cellular atypia is more marked. Poorly differentiated carcinomas are usually diagnosed by the use of IHC to identify cytokeratins within the tumor cells. [23]

Dysplastic changes in OSCC can broadly be classified under architectural changes and cellular changes. Architectural changes include ***tear drop shaped appearance of rete pegs, loss of polarity (i.e. lack of progressive maturity of the cells), and loss of adhesion between cells, formation of squamous keratin pearls, basilar hyperplasia and hypertrophy.*** Cellular changes include ***hyperchromatic nucleus, prominent nucleolus, altered nuclearcytoplasmicratio, poikilokarynosis, increased and abnormal mitosis, nuclear pleomorphism, dyskeratosis.***[24]

The stroma typically contains an inflammatory infiltrate of lymphocytes, plasma cells, and macrophages. Particularly when the neoplasm infiltrates within skeletal muscle, large numbers of eosinophils may be seen. Sometimes liberation of keratin will induce a giant cell response. The stromal inflammation appears to be a host
response to the tumor and infiltrating islands will often be surrounded by inflammatory cells. Many carcinomas have dysplastic epithelium at their margins, while a considerable number show a normal adjacent epithelium. [25]

Wahi and Evans et al, are of the view, that lack of differentiation is considered to spell for poor prognosis while closeness of structural characteristic of tumour to parent tissue is considered to be favourable. [26]According to them, squamous cell carcinoma is characterized histopathologically by Invasive islands and cords of malignant squamous epith**e**lial cells. [27]

3.5 HISTOPATHOLOGICAL GRADING SYSTEMS OF OSCC

Very many grading systems have been put forward by several competent pathologists through the years. A few significant ones are discussed here.

3.5.1 **Broder's(1927)** suggested a system of grading tumors based on the degree of differentiation along with amounts of keratin production- in which a grade I lesion was highly differentiated (its cell were producing much keratin) while grade IV was poorly differentiated (the cells were highly anaplastic and showed practically no keratin formation. [28] .His classification has been used for many years in squamous cell carcinoma and is based on proportion of neoplasm resembling normal squamous epithelium.

3.5.2 Pindborg and others (1973) claimed that OSCCs are classified microscopically based on a method which takes into account a subjective assessment of the degree of keratinisation, cellular and nuclear pleomorphism and mitotic activity. The grades are well differentiated (grade 1), moderately differentiated (grade 2) and poorly differentiated (grade 3). Well and moderately differentiated tumours can be grouped together as low grade and poorly differentiated and undifferentiated tumours as high grade. [29]

3.5.3 The Jacobson(1973) system not only includes the morphologic parameters "structure", "tendency of keratinization", "nuclear aberrations", and

"number of mitosis", but also an evaluation of tumor-host relationship as estimated by parameters such as "mode," "stage of invasion", "vascular invasion" and "degree of lymphoplasmocytic infiltration".[30] **Willen et al (1975)** also improvised upon system of Jacobson et al. They consisted of the deletion of two morphological parameter "structure" and "vascular invasion". The results showed no definitive correlation between the clinical stage and histopathologic grading of malignancy. [31]

3.5.4 <u>Anneroth, Batsakis and Luna (1987)</u> opined that the histopathology grade of a tumor is related somewhat to its biologic behaviour. They also use Jakobsson et al. system for application to squamous cell carcinoma in the tongue and floor of mouth. One of the parameters, "vascular invasion" was omitted. In other words a tumour that is mature enough to closely resemble its tissue of origin seems to grow at a slightly slower pace and to metastasize later in its course. Such a tumour is called *low-grade/grade* I or *well-differentiated* squamous cell carcinoma. A tumour that often enlarges rapidly, and metastasizes early in its course is termed *high-grade*, *Grade* III/IV *poorly differentiated.* A tumour with a **microscopic appearance somewhere between these two** extremes is labelled a "moderately differentiated" carcinoma. [32]

3.5.5 <u>Bryne's (1989, 1992) (ITF)</u> Invasive Tumor Front Grading System - Bryne M. (1998) presented a hypothesis suggesting that molecular and morphological characteristics at the invasive front area of various squamous cell carcinomas may reflect tumor prognosis better than other parts of the tumor. He further stated that several molecular events of importance for tumor spread, like gains and losses of adhesion molecules, secretion of proteolytic enzymes, increased cell
proliferation and initiation of angiogenesis were observed. [33, 34]

3.6 OPTICAL DIAGNOSTIC MODALITIES

Over the past few years, there have been very successful attempts in the diagnosis of tissue pathology using **optical biopsy or optical diagnostics**. In theory, a beam of light fired into tissue should provide an optical signature of that tissue highlighting the precise, micrometer resolution three dimension images. [35]

Several studies have sought to investigate the diagnostic utility of Optical coherence tomography to detect and diagnose oral and oropharyngeal malignancy.[36]

Optical coherence tomography (OCT) is an optical signal acquisition and processing method which captures micrometer resolution, three dimensional images from within optical scattering media (e.g. biological tissue). [37]

3.6.1 Introduction and history-

There have been three basic approaches to optical tomography since the early 1980s: diffraction tomography, diffuse optical tomography and optical coherence tomography (OCT). Optical techniques are of particular importance in the medical field, because these techniques promise to be safe and cheap and, in addition, offer a therapeutic potential. Advances in OCT technology have made it possible to apply OCT in a wide variety of applications but medical applications are still dominating. [38]

Tomographic techniques generate slice images of three-dimensional objects. Optical tomographic techniques are of particular importance in the medical field, because these techniques can provide non-invasive diagnostic images. There is a fundamental difference between optical tomography techniques and x-ray and magnetic resonance techniques. Since optical techniques are dominated by diffraction the Fourier slice theorem cannot be used.

3.6.2 <u>There are two fundamental optical tomography techniques</u>: Diffuse optical tomography (DOT), and optical diffraction tomography (ODT). Optical coherence tomography (OCT) is physically founded on ODT. The vast majority of applications of these techniques are in the biomedical field. [39]

DOT uses diffusely propagating photons. Spatially and/or temporally modulated light is launched into the tissue and multiple scattered. Back-projection methods, perturbation methods, and nonlinear optimization methods are used to derive tomographic images from the transmitted light (Arridge and Schweiger 1997, Depeursinge 2002). [40] It uses single scattered light and derives tomography images by the Fourier diffraction projection theorem (Born and Wolf 1999). Recently, it has been shown, that standard diffraction tomographic methods can also be used for imaging with diffuse-photon density waves (Li *et al* 1997). [41]

3.6.3 <u>OCT uses ballistic and near-ballistic photons</u>- Laterally adjacent depth-scans (similar to the more familiar A-scans of ultrasound imaging technology) are used to obtain a two dimensional map of reflection sites in a sample. Initially, OCT techniques were based on low time-coherence interferometry (LCI) depth-scans performed in the time domain. In a first approach towards tomography imaging a cross-sectional topographic image of the retinal pigment epithelium (RPE) of a human eye obtained *in vivo* by the dual beam LCI technique was presented at the ICO-15 SAT conference by Fercher (1990) and published by Hitzenberger (1991). [42] OCT using fiber optic Michelson LCI was pioneered by Fujimoto and co-workers (Huang *et al* 1991). First *in vivo* tomograms of the human retina were published by Fercher *et al* (1993a) and Swanson *et al* (1993) .[43]

Later Chinn *et al* (1997) used wavelength tuning interferometry (WTI) to synthesize OCT images, whereas Hausler and Lindner (1998) generated OCT images using spectral interferometry. For a review of early work in LCI and OCT key papers published by Masters are quite relevant (2001). [44]

3.6.4 **Specific advantages of OCT** are its high depth and transversal resolution, the fact, that its depth resolution is decoupled from transverse resolution, high probing depth in scattering media, contact-free and non-invasive operation, and the
possibility to create various function dependant image contrasting methods.[45]

The non-invasive nature of this imaging modality coupled with (i) a penetration depth of 2–3 mm, (ii) high resolution (5–15um), real-time image viewing, and (iii) capability for cross-sectional as well as 3D tomographic images, provide excellent prerequisites for in vivo oral screening and diagnosis. [46]

3.6.5 ***Optical coherence tomography has most often been compared with ultrasound imaging***. Both technologies employ back-scattered signals reflected from different layers within the tissue to reconstruct structural images, with the latter measuring sound rather than light. The resulting OCT image is a two-dimensional representation of the optical reflection within a tissue sample. Cross-sectional images of tissues are constructed in real time, at near histologic resolution (approximately 5–15 μm with current technology). These images can be stacked to generate 3D reconstruction of the target tissue. This permits in vivo non-invasive imaging of epithelial and subepithelial structures, including: (i) depth and thickness, (ii) histopathological appearance, and (iii) peripheral margins of the lesions. [47]

3.6.6 <u>Principle</u>: It is an interferometric technique, typically employing near-infrared light. Optical coherence tomography (OCT), first reported by Fujimoto *et al.* in 1991 is a non-invasive, non-radioactive optical diagnostic tool based on interferometers. By using a low-coherence broadband near-infrared light source, it is possible to obtain excellent spatial resolution (~20 μm) and real-time images. OCT was first applied *in vitro* in human retina and in atherosclerotic plaque [58, 59]. It is an optical imaging technique that enables cross-sectional imaging of microstructure of tissue in situ.High-definition OCT is a non-invasive technique for morphological investigation of tissue with cellular resolution filling the imaging gap between reflectance confocal microscopy & conventional optical coherence tomography. With improvement of optical specifications and system capabilities, OCT demonstrates great potentials in research topics and clinical applications to date. [48]

3.6.7 <u>Background of technique</u>--OCT is based on depth resolved detection of elastic light scattering. When light is directed at a tissue sample, it will be partially back scattered. This back-scattered light is measured at different depths at a particular location on the tissue using low-coherence interferometry resulting in a reflection profile in the depth (z−) direction. The magnitude of the OCT signal at each depth is determined by the different cellular structures in the imaged volume (typically in the order of 103μm3 or 1 pl) and as a result it differs per tissue type. [49]

Several adjacent depth profiles can be acquired in the lateral (x−) direction and displayed as a gray-scale image in real time which is known as an OCT B scan. Subsequently, the OCT beam can be scanned across a tissue sample in the other lateral (y−) direction, resulting in a 3D image representation with acquisition speed reported up to several volumes per second. [50]

3.6.8 System setup

The OCT system consists of an interferometer, generally constructed from fiber optic components, illuminated by a broad wavelength–range light source operating in the near infrared (typically 1,250–1,350 nm for non-ophthalmic applications). A small fraction of the light is guided towards a "reference" mirror; the majority is directed to the tissue, using handheld XY-scanning devices or miniaturized endoscopic probes (Figure 1). Both fractions are combined and directed towards a detection unit and subsequent computer processing. [51]

OCT is an interferometer-based system with a low coherence length broadband light source. The lights reflect from the sample and reference arms interference within a Michelson or Mach-Zehnder interferometer. This interference signal is acquired by a photodiode (PD) or charge-coupled device (CCD) that is dependent on the type of OCT. Figure 1 shows the first OCT type, time domain OCT (TD-OCT). TD-OCT acquires various optical path lengths (OPLs) by moving a reference reflector. The light interference occurs when the OPL of the reference and of lights reflected by samples are the same. Furthermore, the constructive interference (bright lines) arises when the optical path difference (OPD) between two lights is an integer multiple of the wavelength. Therefore, a low coherence length light source is usually used for observing only one interference envelope from a selected depth. The relationship between OPL and OPD is described as Equation:

$$OPD = \Delta OPL = \Delta(k \times n \times d)$$

Where *k* is the wave number, *n* is the refractive index of material and *d* is the propagation length in air. [51]

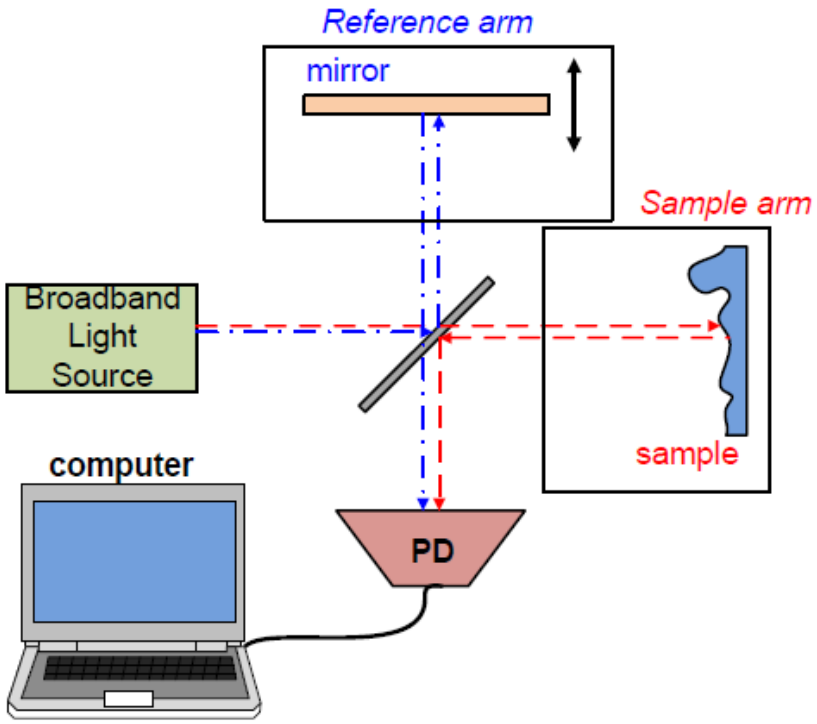


Figure 1-System setup of TD-OCT

Another common type of OCT is the spectral domain OCT (SD-OCT) (Figure 2) or Fourier domain OCT (FD-OCT). Unlike TD-OCT, the OPL in SD-OCT is decided from different wavelengths and no moving reflection mirror is necessary. A SD-OCT system is setup with almost same components as TD-OCT but with an additional grating (for spatial Fourier transform), sensor array (usually CCD array) or spectrometer. A SD-OCT system setup is shown in Figure.

Figure 2. System setup of SD-OCT.

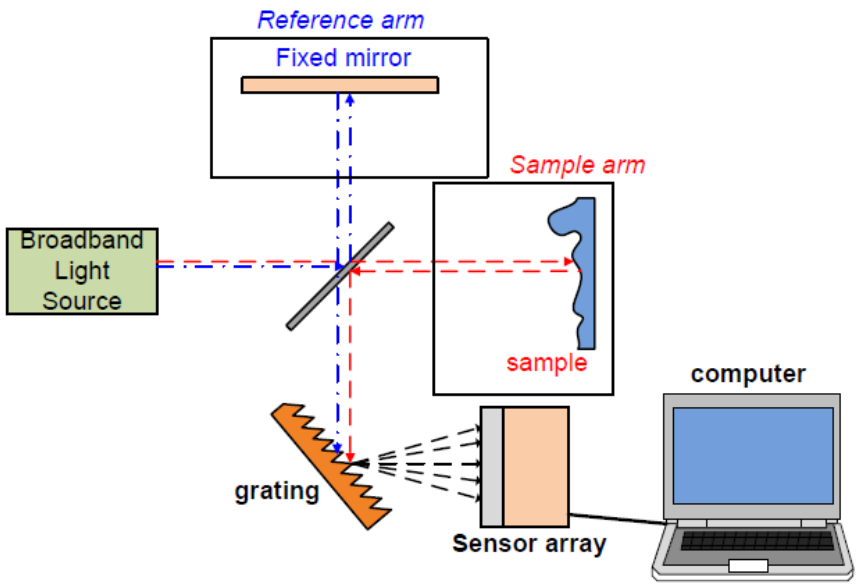


Figure 2- Setup of SD-OCT

The OCT axial resolution is related to the light source coherence length, which is a function of the light source bandwidth (Δλ). The light source coherence length can be described by Equation :[52]

$$l_c = \frac{2c\ ln\ 2}{\pi}\frac{1}{\Delta v} = \frac{2\ ln\ 2}{\pi}\frac{\lambda_0^2}{\Delta\lambda} \approx 0.44\frac{\lambda_0^2}{\Delta\lambda}$$

Where λ0 is the centre wavelength

3.6.9 Different Scanning procedures used in OCT:-

1. **A Scan:** A-scan, also called as axial scan, is obtained by focusing test and recombining the reflected light with the reference. The information thus obtained corresponds to the depth of the tissue which is determined by the optical reflectance of the tissue.

2. **B Scan:** B-scan or longitudinal scan is generated by collecting many single axial scans linearly across the tissue and in subsequent transverse positions. The images thus obtained will have both depth axis and lateral or angular axis.

3. **T Scan:** T-scan or en-face scan is produced by transversally scanning the beam over the target maintaining the reference mirror fixed to generate a reflectivity profile in angle or lateral position.

4. **C Scan:** C-scan, also called as transverse slice scans, are made from many T-scans in the transverse plane. Different transversal slices are collected for different depths either by advancing the optical path difference in steps after each complete transverse scan, or continuously at a much slower speed. [53]

Disposable in vivo OCT probes with a diameter less than 1 mm are developed and already used in several medical settings, enabling OCT to be used in endoscopes or in combination with 16G/18G needles to access internal tissue. Clinical value of OCT images depends on obvious factors such as high resolution, high-imaging speed, and adequate contrast to discriminate between benign and malignant tissues. [54]

3.6.10 Contrast in OCT is caused by spatial differences in refractive index of different tissue constituents, e.g., contrast originates from reflection of different structures. It is known that the refractive index is proportional to the density of the cells and cell structure. Because malignant cells display an increased number, larger and more irregularly shaped nuclei with a higher refractive index

and more active mitochondria, OCT images are expected to be different in malignant tissue compared to normal and benign tissue [55]

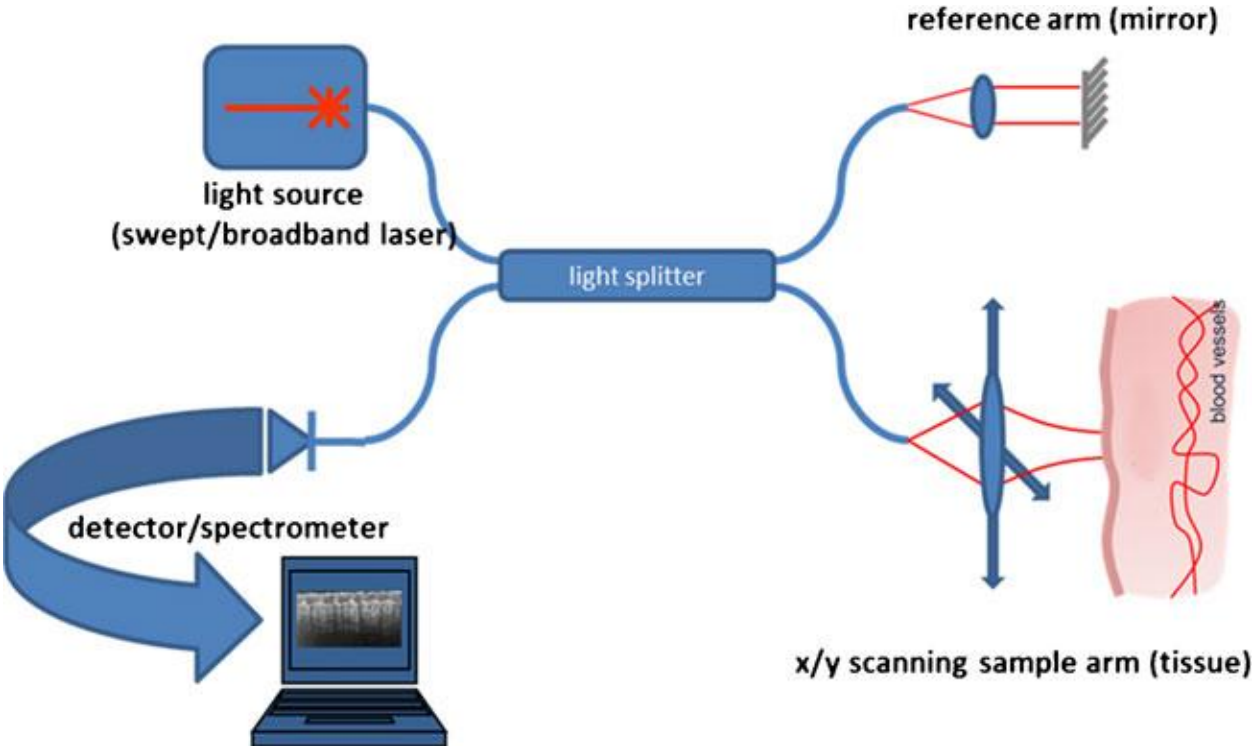


Figure 3-Schematic overview of the OCT system. An optical beam (from the light source) is split into two arms. One arm is directed at the tissue (scanning sample arm), the other at a mirror (the reference arm). The reflected light from these two paths is recombined and the differences between these two paths can be shown in a grayscale image

3.6.11 **Types of OCT**-There are two main types of OCT. Time domain and spectral domain. In time domain OCT (TDOCT) the path length of the reference arm is scanned in time. Interference (i.e. series of dark and bright fringes) is only achieved when the optical path difference (OPD) lies within the coherence length of the light source. The envelope of this modulation changes as the OPD is varied, where the peak of the envelope corresponds to path-length matching. [56]

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3.6.11.1 Time domain, spectral domain OCT and their essential applications

Several reports deal with this type of OCT. TDOCT has been used for evaluation of indirect dental restorations, apical microleakage after laser – assisted endodontic treatment, monitoring the periodontal ligament changes induced by orthodontic forces and orthodontic interfaces. [57]

In spectral domain OCT (SDOCT), the spectrum at the output of the low coherence interferometer is measured. Due to the Fourier relation (Wiener-Khintchine theorem between the auto correlation and the spectral power density) the depth scan (A-scan) is calculated by a Fourier-transform from the acquired
spectra, without movement of the reference arm. [56]

Because all depths are obtained in one measurement, SDOCT improves imaging speed dramatically. SDOCT has also an improved signal to noise ratio in comparison to TDOCT, the higher the number of separate spectral windows used in the spectrometer, the larger the signal to noise ratio. The width of the Spectral windows limit the axial scanning range, while the full spectral bandwidth sets the axial resolution.

3.6.11.2 Swept source OCT

SDOCT can be also divided into swept source (SS) OCT and camera based, Fourier domain (FD) OCT. In SSOCT, a narrow band optical source is used, whose frequency is tunable in time. Point photo detectors are used. The depth resolution is inverse proportional to the tuning bandwidth while the axial range is limited by the coherence length of the source, the narrower the line width, the longer the axial range. [58, 59]

3.6.11.3 Frequency domain OCT

In FDOCT, a broadband optical source is used and the spectrum is acquired using a dispersive detector, such as a diffraction grating and a linear detector array. The optical source bandwidth determines the depth resolution while the axial range is limited by the spectrometer resolution. Compared with TDOCT, SDOCT has the advantage of increased phase stability for functional imaging.

3.6.11.4 SD OCT Disadvantages

However, the SDOCT has three main disadvantages: decay of sensitivity with OPD, impossibility to move the focus to the depth investigated while scanning and symmetric (ghost) images if the OPD = 0 position .The impossibility of focusing at selected depths renders the technology unsuitable to high transversal resolution microscopy, where TDOCT is favored. If minute details of defects are to be identified in dental constructs, then TDOCT is better. In case large size images are to be generated from soft moving tissue, then SDOCT methods should be used. [60]

Several OCT systems have received FDA approval for clinical use, and OCT is deemed by many as an essential imaging modality in oral premalignancy and malignancy. In vivo image acquisition is facilitated through the use of a flexible fiber optic OCT probe. The probe is simply placed on the surface of the tissue to generate real-time, immediate surface and sub-surface images of tissue microanatomy and cellular structure, while avoiding the discomfort, delay and expense of biopsies.[61]

3.6.12 **OCT Studies involving Gastro intestinal tract, retina, esophagus, larynx lung and pleura** -A substantial amount of research work has been carried out involving the GI tract and retina.

3.6.12.1 **Studies involving GI Tract** Several *in vitro & in vivo* studies demonstrating the feasibility of OCT in the GI tract had been performed. In the in vitro studies the GI tract wall was identified as a multiple layer structure characterized by a sequence of hyper- and hypo-reflective layers (Fig 4), with a variable homogeneity of the back-scattered signal. Neoplastic and normal tissue also showed different light backscattering patterns. [62]

In vivo studies confirmed the possibility of OCT to recognize the multiple-layer structure of the GI wall; the possibility to introduce the OCT probe into a standard transparent catheter for cannulation during an ERCCP procedure permits the epithelial layers of the pancreato-biliary ductal system and sphincter of Oddi to be investigated; thereby increasing diagnostic accuracy. [63]

3.6.12.2 **Studies involving the retina** OCT showed a double-layered structure in the normal sensory retina with a highly reflective layer located in the inner retina and a low reflective layer located in the outer retina. The retinal pigment epithelium (RPE) and choriocapillaris were imaged as a layer with the highest reflection. (Fukuchi et al) [64](Figure 5)

3.6.12.3 **Optical Coherence Tomography of the Esophagus and Proximal Stomach in Health and Disease** Zuccaro J et al carried out a study which revealed a highly detailed view of the GI wall, with clear delineation of a multiple layered structure. They were able to distinguish squamous mucosa, gastric cardia, Barrett's esophagus, and cancer. This technique holds great

potential as an adjunct to the surveillance of patients with Barrett's esophagus, ulcerative pancolitis, and other premalignant conditions. [65]

Optical coherence tomography for the staging of tumor infiltration in superficial esophageal squamous cell carcinoma (SESCC) was carried out in a study by Hatta W et al. In this study, the authors defined the criteria for OCT imaging for staging tumor invasion based on the esophageal cancer treatment guidelines and found that OCT could be a novel technology with a high degree of accuracy for the preoperative staging of SESCCs. [66]

3.6.12.4 <u>Study on laryngeal thickness</u> A study on Laryngeal epithelial thickness was carried out by Kaiser M.L. et al (Figure 6). Calibrated measurements of epithelial thickness at various laryngeal subsites were recorded. [67]

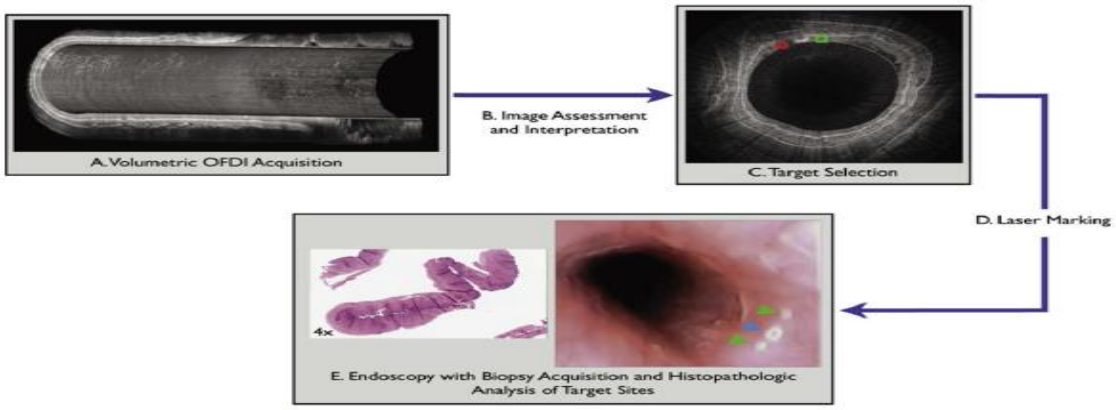

Figure 4-Guided biopsy with the use of laser marking and endoscopic OCT imaging. (A) volumetric OCT imaging of the region of interest in the oesophagus; (B) assessment and interpretation of the volumetric data set; (C) selection of point of interest; (D) laser marking at corresponding sites on the luminal wall; and (E) endoscopic biopsy at the marked sites for histopathologic analysis.

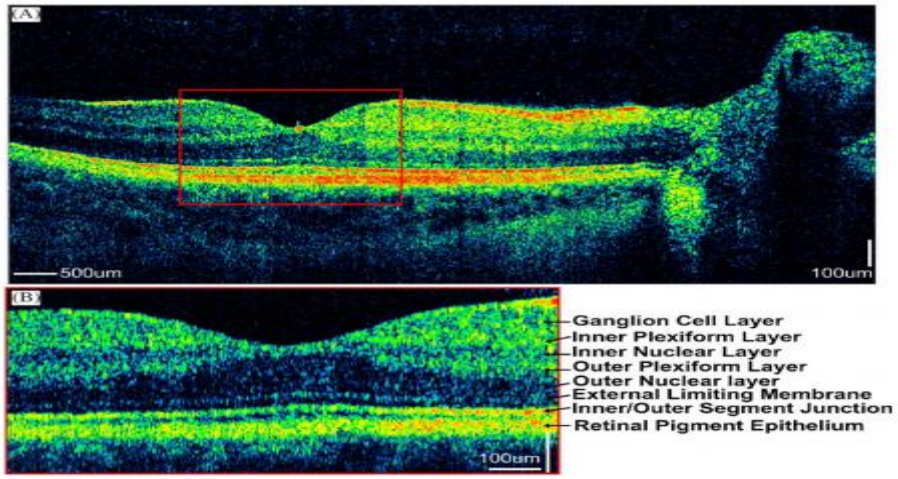

Figure 5-A high quality standard-resolution cross sectional spectral optical coherence tomography retinal imaging showing different layers of the retina

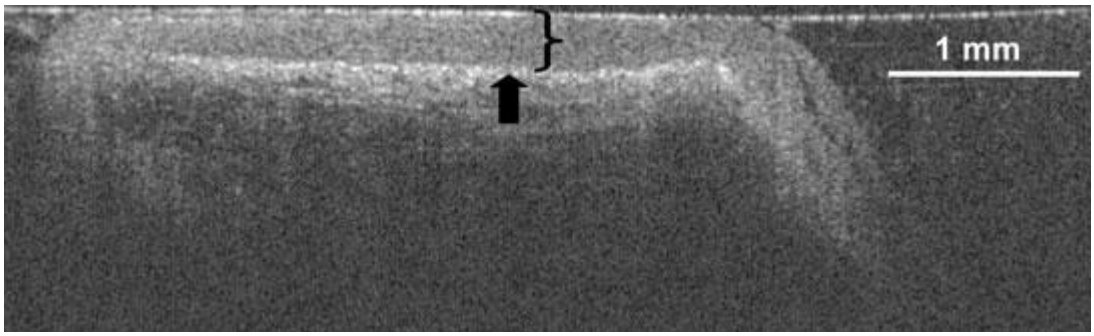

Figure 6-Optical coherence tomography image in the coronal plane of the true vocal cords-The bracket marks the extent of the epithelium, with an arrow indicating the basement membrane.

A high quality standard-resolution cross sectional spectral optical coherence tomography retinal imaging was performed by Costa R. et al that showed different layers of the retina which were very much evident in the OCT image. [68]

3.6.12.5 <u>Study on airway, lung and pleura</u> Two-dimensional and 3-dimensional optical coherence tomographic imaging of the airway, lung, and pleura was carried out by Hanna N et al. This study confirmed the feasibility of high-resolution OCT imaging of the airway and pleura for evaluating distal airway, lung, and pleural pathology to obtain optical images at near-histologic levels in vivo. Differences in tissue layers of the airway and pleura were clearly distinguishable and corresponded closely to standard H&E images subsequently obtained from the excised tissues.[69] (Fig 7)

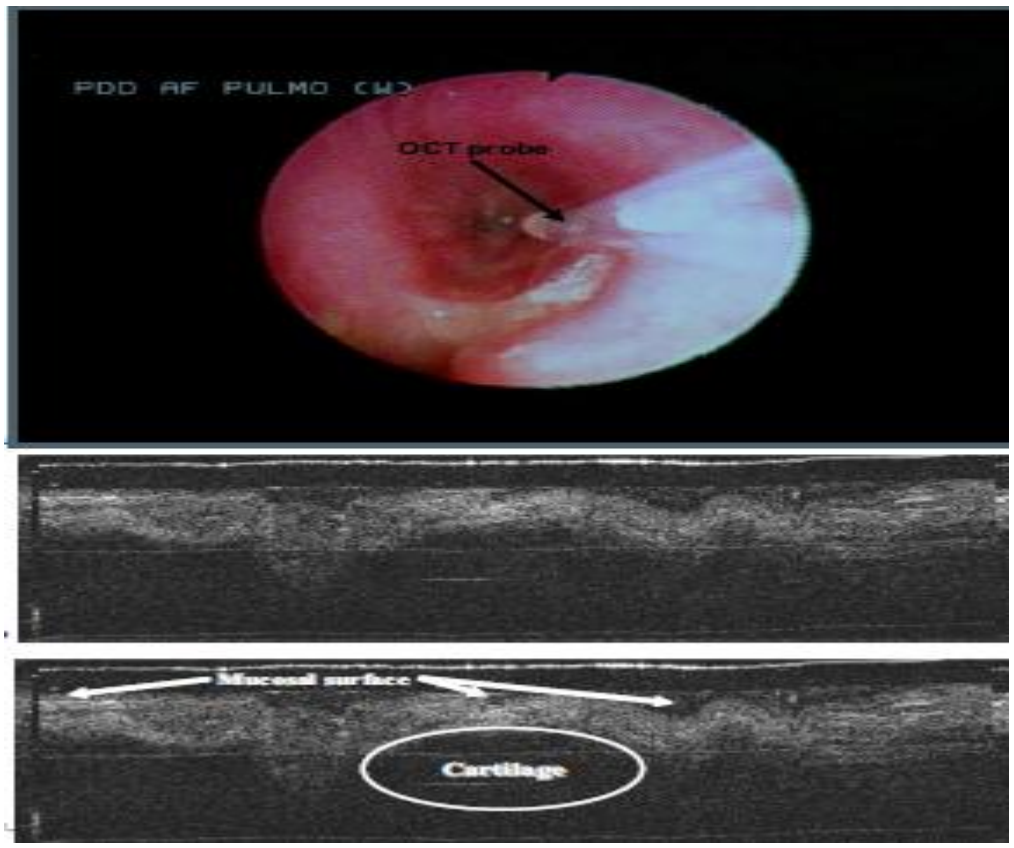

Figure 7-Image (a) of an OCT probe (O) within a human airway (right upper lobe bronchus) obtained through a flexible fiber bronchoscope. In vivo OCT image of an inflamed right upper lobe bronchus without (b) and with (c) labels.

3.6.13 <u>OCT imaging showed distinct zones of normal and altered architectural changes involving the oral mucosal tissues in a study</u> by Hamdoon Z et al. Basic histological layers(keratin cell layer,epithelium,lamina

propria) and micro anatomical structures(blood vessels, tongue papilla & glandular ducts) were identified in most of the cases. The basement membrane was clearly identified in many cases.[70]

- Structural identification and validation with histopathology was variable. Correlation of OCT vis a vis histopathology was 97% in assessing the basement membrane, 93.5% in terms of the epithelium and its changes and 94% in assessing keratin layer and its changes.Rete ridges were correlated in 89% of the OCT specimens in the author's study . Correlation was less achievable in case of blood vessels (77%) and salivary ducts (60%) [70]

- According to a study carried out by Prestin S et al, in normal keratinized mucosa, the keratin layer appears as thin bright line on the most upper layer of the epithelium. It is absent in non-keratinized epithelium. Dysplasia cases (mild, moderate) mainly demonstrate hyper-signal; however severe dysplasia and carcinoma-in-situ have hypo reflective layer due to disorganized tissue differentiation. Invasive carcinoma can either have hypo reflective kearatin layer or no layer at all due to structural damage from ulceration.[71]

- Description of epithelial layer- As per Jerjes WK et al, in normal mucosa the epithelium has relatively lower signal intensity than keratin layer or lamina propria-with a homogenous structure and having hard distinction between spinous and granular cell layer. In dysplastic cases, slight to moderate increase in this layer thickness is evident and is usually associated with architectural changes in

severe dysplasia and carcinoma in situ.In case of invasive carcinoma,epitheleial layer shows significant increase in thickness in the areas of focal invasion.[72]

- Lamina propria-Irregular and unclear architecture in the lower lamina propria is noted in case of cancers.[70]

- Description of basement membrane- As per Jerjes WK et al, the demarcation between the two different signal intensities of epithelium and lamina propria represents the basement membrane- it may appear as a linear or undulated structure due to tissue formalin shrinkage effect. Intact basement membrane is seen in cases of dysplasia; while complete or partial loss or breach of the basement membrane is usually evident in the cases of invasive carcinoma.

- Other micro anatomical structures-prominent blood vessels might appear as lines with hyperechoic signal and associated hypo echoic shadow. [73]

- Agreement between the clinician and the histopathologist were achieved in cases of oral cancer-100% in terms of identifying the basement membrane status and 100% in cases of thickened epithelial layer. [73]

- Hence, some significant diagnostic criterions were laid down regarding OCT evaluation of the tissues and their correlation with histopathological attributes- involving invasive carcinoma by Jerjes

WK et al. These include breakdown of the basement membrane, irregular and unclear structure in the lower lamina propria, and hypo reflective signal for keratin layer along with increase in epithelial thickness in the majority of the invasive lesions examined. [73]

- However, this study had a few disadvantages, namely accurate co-registration of OCT and histopathology images, a relatively small sample size and lack of optimum tissue perfusion.[73]

In one study, performed by Wilder Smith et al, involving 50 patients with suspicious lesions including oral leukoplakia or erythroplakia, the effectiveness of OCT was evaluated for detecting oral premalignant disorders and malignancy. OCT images of the dysplastic lesions revealed visible epithelial thickening, loss of epithelial stratification and epithelial down growth. This criterion is not adamant enough to draw firm conclusion and to grade oral premalignant disorders. [74]

Criteria for oral cancer diagnosis was established using OCT images by the absence or disruption of the basement membrane and the epithelial layer that was highly variable in thickness, with areas of erosion and extensive epithelial down growth and invasion into the sub epithelial layers.

In another study, by Tsai MT et al, 97 patients were subjected to OCT imaging to detect neoplasia in the oral cavity. The results revealed that the main diagnostic criterion for high-grade dysplasia and carcinoma in situ was the lack of a layered structural pattern. Diagnosis based on this criterion for dysplastic⁄malignant vs. benign⁄reactive conditions was achieved. [75]

In a similar study, performed by Upile T et al, OCT images of suspicious oral lesions were obtained in ex vivo form. It was found that OCT was able to

distinguish various layers of oral mucosa. In addition segregating benign from malignant lesion was easily feasible. [76]

In another study, done by Rotzchild et al, in a total number of 143 healthy test persons, epithelial thickness of oral mucosa was determined at 7 different sites with help of OCT.Special attention was directed to those sites having the highest incidence for development of dysplasia and carcinomas. The highest values were found in buccal mucosa (294 μm), hard palate (239 μm), and the thinnest in epithelium at floor of mouth (99 μm) respectively. [77]

MATERIALS AND METHODS

4. MATERIALS AND METHODS

The explorative study was conducted in the Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research **(GNIDSR)**, Kolkata and School of Medical Science & Technology **(SMST)**, IIT, Kharagpur, West Bengal, during the period of December 2014-August 2015.

4.1 SELECTION OF STUDY SUBJECTS

For selection of the study subjects, the patients attending the outpatient department of GNIDSR,Kolkata, with their age ranging from 35-60 years, were screened clinically with a view to detect the presence of Oral squamous cell carcinoma as per the clinical criterion laid down by Neville and Marx & Stern et al.[5,6] Initially twenty five patients were selected for the study based on the presence of the relevant clinical signs and symptoms of OSCC.After the primary selection, all of these patients were subjected to routine medical and haematological investigations and informed consent was taken. Five of the patients had severe medical/systemic complications, and therefore excluded from the study. Five of the patients denied extending their consent for further diagnosis and treatment procedure.

4.2 RECORDING OF CLINICAL DATA

Special care was taken to record the clinicopathological data of the fifteen patients suffering from OSCC with a view to facilitate the grabbing of OCT images. Among these fifteen patients, only ten patients having involvement of buccal mucosa adjacent to commissural region were included in the study. These selected study subjects were then individually interviewed using a questionnaire having information on their occupation, economic status, alcohol consumption, type of tobacco habit, daily tobacco use frequency, duration of the

habits along with details of subjective and objective features of them were recorded properly in the specially prepared clinical case record sheets as per guidelines mentioned by Neville and Marx & Stern et al.[5,6] Clinical photographs were taken from appropriate sites.

4.3 GRABBING OF *in vivo* OCT IMAGES

All these selected OSCC individuals were subjected to the grabbing of *in vivo* OCT images with the help of a THORLABS endoscopic swept source OCT machine OCS1310V1 (SS-OCT)(Figure-9,10) involving the diseased site. Then all the real time images were categorized and stored in a computer. The system is based on a patented Micro-Electro-Mechanical (MEMS)-tuneable Vertical Cavity Surface Emitting Laser (VCSEL) that is specially designed for optimal performance in OCT applications. Very quick(line, roster, zigzag) Four point averaging B scans were performed in a 3mm X axis and 3mm Z axis with the clearance between the object and OCT source being approximately 2.5 cm in case of both *in vivo and ex vivo OCT* images. The axial resolution was <16 μm and the lateral resolution 25 μm at focus and the centre wavelength was 1300 nm. (Figure 8)

4.4 INCISIONAL BIOPSY & COLLECTION OF TISSUE SAMPLE

Following this incision biopsy was performed over the same representative site of the same patient after few days. Among these remaining ten OSCC patients, four did not turn up for the biopsy. Hence, they were excluded from the study. After achieving local anaesthesia using lignocaine hydrochloride with adrenaline (1 in 100000), incision biopsies from the representative sites were performed using 5mm punch. The specimens were taken with sufficient depth up to sub mucosa without any laceration in any form. Following the surgical procedure, complete haemostasis was achieved with placement of sutures. Proper post-operative medications, post-operative care and follow ups were

performed in each and every patient. The patients were called after seven days for suture removal.

4.5 PRESERVATIONS OF TISSUE SAMPLES All the biopsied specimens were fixed in 10% buffered formalin. The collected specimens were carefully labelled.

4.6 GRABBING OF *ex vivo* OCT IMAGES The *ex vivo* OCT images of the specimens were grabbed by a THORLABS endoscopic swept source OCT machine OCS1310V1. All the information were categorized and stored in a computer.[78] A time gap of five hours was present between performing the *ex vivo* OCT imaging and the biopsy procedure as the specimens were transported to IIT, Kharagpur.

4.7 PROCESSING, SECTIONING AND STAINING OF TISSUE SAMPLES The tissue, fixed for 24 hours in 10% buffer formalin was first dehydrated in ascending grades of alcohol, replacing the fixative and water with the dehydrating fluid. It was then cleared in xylene. Adequate care was taken to avoid entrapment of air bubble. The samples were sectioned by maintaining the following sequential steps. The cleared tissues were then embedded in molten paraffin and blocks were prepared at 64ºC. Sections of 5µm thickness were cut down using Rotary Microtome [LEICA RM 2125 RT].

Following this, the sections were then stained with Haematoxylin & Eosin in accordance to the standard protocol laid by Bancroft & Gamble et al[78]:

The sections were dewaxed, hydrated through graded alcohol to water.

Stained in Alum haematoxylin (Harris's haematoxylin) for 05-15 minutes (regressive staining)

Thoroughly washed in running tap water until blue (05 m

Differentiated in 01% acid alcohol [1% HCL in 70% Alcohol] for 05 seconds]

↓

Thorough washing in tap water was done for another 05 mins

↓

Stained in Eosin (01%) for 15 seconds

↓

Washed in running tap water for 10 mins

↓

Dehydrated through alcohol, cleared and mounted.

4.8 EVALAUATION OF H&E STAINED SECTIONS AND GRABBING OF LIGHT MICROSCOPIC IMAGES

All the aforesaid stained sections were viewed under light microscope [Olympus CH20i, Objective 10x and 40x] and the clinical diagnosis of Oral squamous cell carcinoma were confirmed and findings were recorded according to the criteria laid by Neville and Marx & Stern et al.[5, 6] Photomicrographs of selected sites from the sections were also taken by an inverted Bright field transmission microscope, the Observer.Z1, Zeiss Germany and Axiocam MRc imaging modality with the help of 5x objective.(Figure 11)

4.9 COMPARISON AND CORRELATION OF OCT IMAGES WITH LIGHT MICROSCPOIC IMAGES

Thereafter, all the variables were studied on both *in vivo* as well as *ex vivo* OCT images to identify the architectural changes caused by the pathological aspects of OSCC. This included the identification and characterization of-

EPITHELIAL PARAMETERS

- **Thickness of the epithelium**
- **Surface zone of epithelium**
- **Subsurface zone of epithelium**
- **Epithelio-mesenchymal junctional zone**
- **Configuration of the rete pegs**

CONNECTIVE TISSUE PARAMETERS

- **Lamina propria zone**
- **Blood vessels**

All the OCT as well as microscopic images were carefully recorded and evaluated by several experts including several competent Oral Pathologists and Researchers and the observations were carefully analysed, recorded and stored in a computer for further study .

System Specifications[a]	
Center Wavelength	1300 nm
A-Scan Line Rate	100 kHz[b]
Imaging Range	12 mm
Axial Resolution	<16 µm
Lateral Resolution (with LSM03 Scan Lens)	25 µm at Focus

Figure 8-Specifications of THORLABS endoscopic SS-OCT OCS1310V1 machine

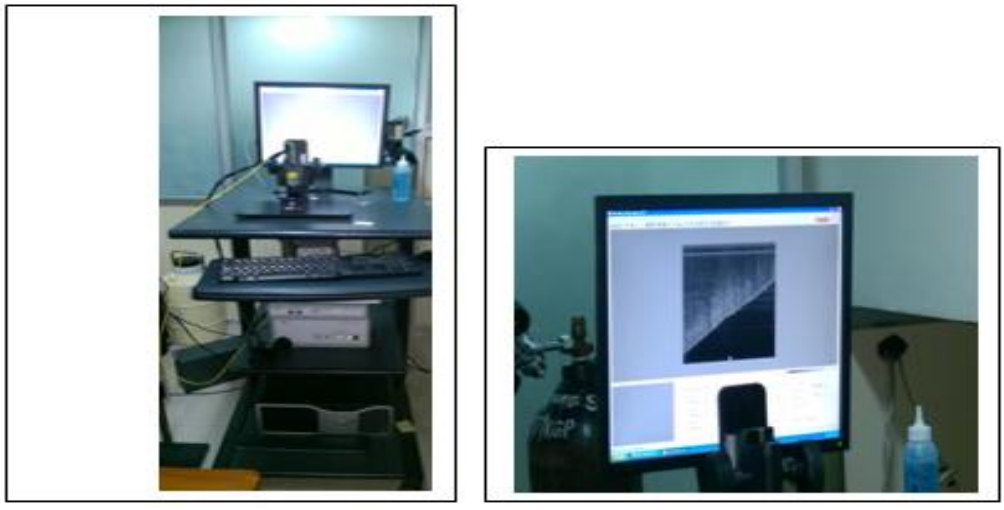

Figure 9- THORLABS endoscopic OCS1310V1 SS-OCT System

Figure 10- OCT image grabbing in progress

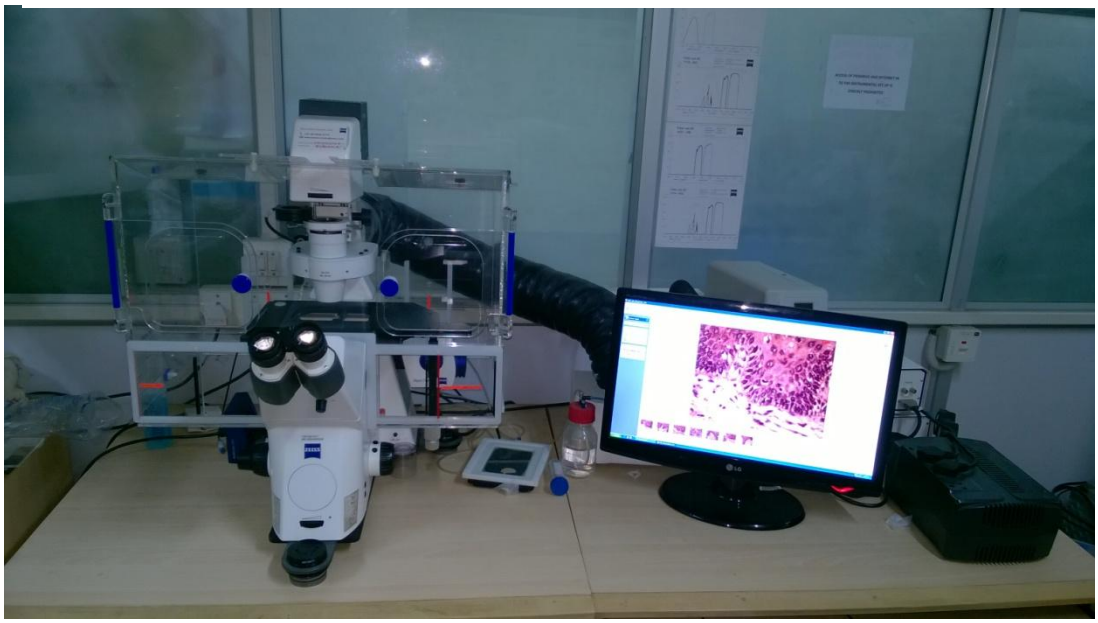

Figure11- Carl Zeiss OBSERVER.Z1 bright field transmission optical microscope with Axiocam MRc imaging modality

RESULTS

5. **RESULTS**

The study was conducted on a number of six study subjects, of both genders, with their age ranging from 35-60 years. The representative site was chosen as the buccal mucosa adjacent to commissural region and the images were grabbed from the same site. (Figs 12a, 12b) The study was conducted in the department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research (GNIDSR) and School of Medical Science and Technology (SMST), IIT, Kharagpur during the time period of December 2014-August 2015.

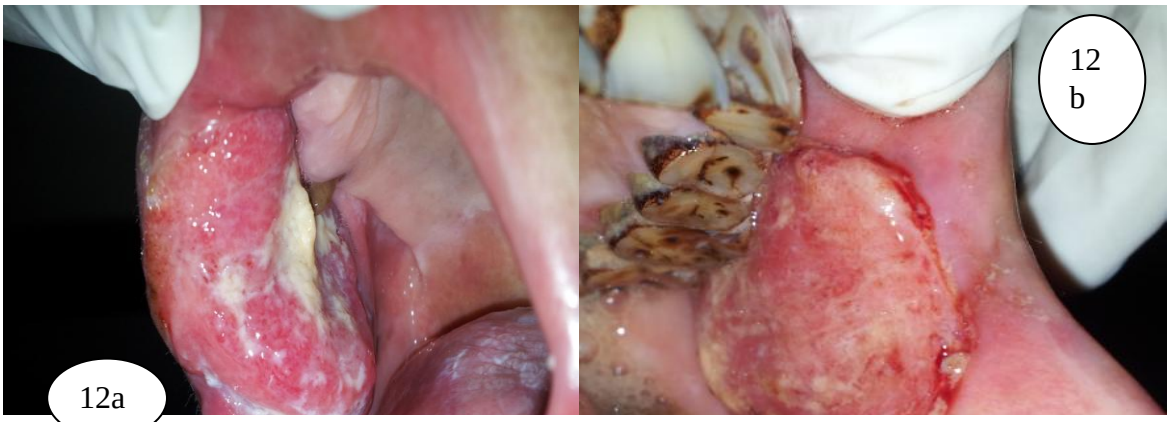

Fig 12a depicts presence of an exophytic ulceroprolifeartive relatively large, diffuse mass involving the right commissural region and extending to the right buccal vestibule and buccal mucosa -evident with marked surface nodularity and granularity and Fig 12b depicts an ulcerated exophytic mass involving left buccal mucosa adjacent to commissural region.

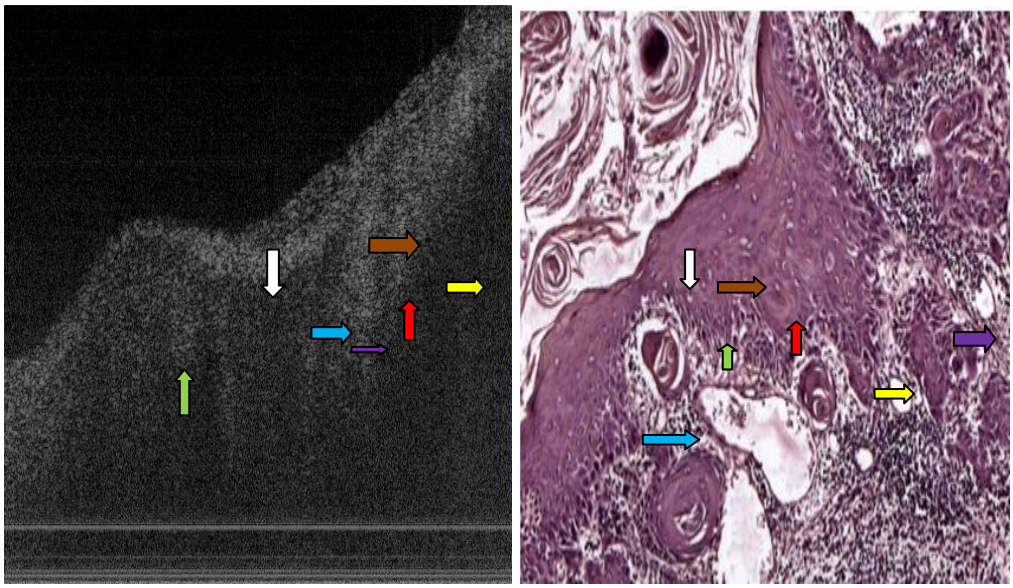

Figure 13a depicts in vivo OCT image of OSCC and 13b shows a photomicrograph of histopathological image of same site of OSCC in 5x

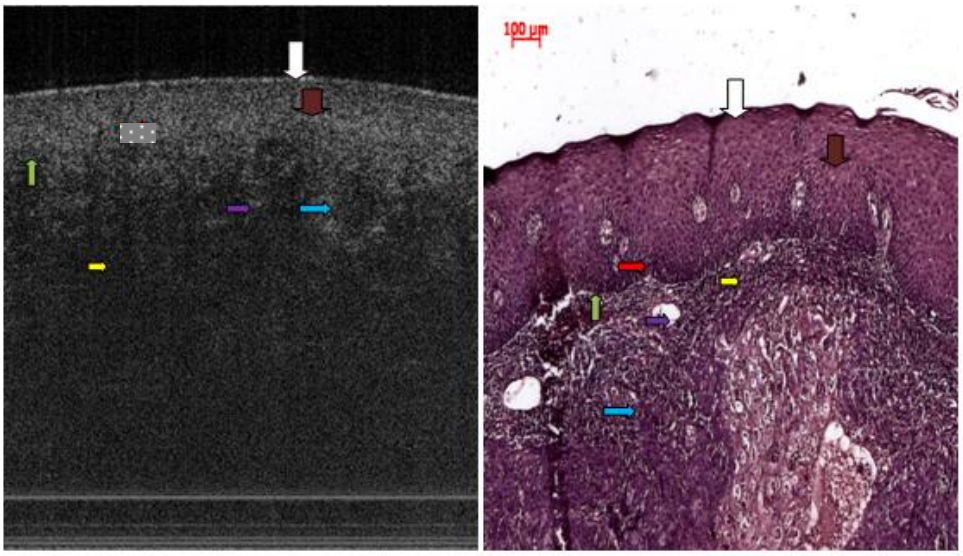

Figure 14a depicts in vivo OCT image of OSCC and 14b shows a photomicrograph of histopathological image of same site of OSCC in 5x magnification

⇨ SUPERFICIAL ZONE OF EPITHELIUM, ➡ REST OF THE EPITHELIUM, ➡ BASEMENT MEBRANE ZONE,
➡ LAMINA PROPRIA, ➡ INFILTRATING NEOPLASTIC EPITHELIAL ISLANDS, ➡ BLOOD VESSEL,

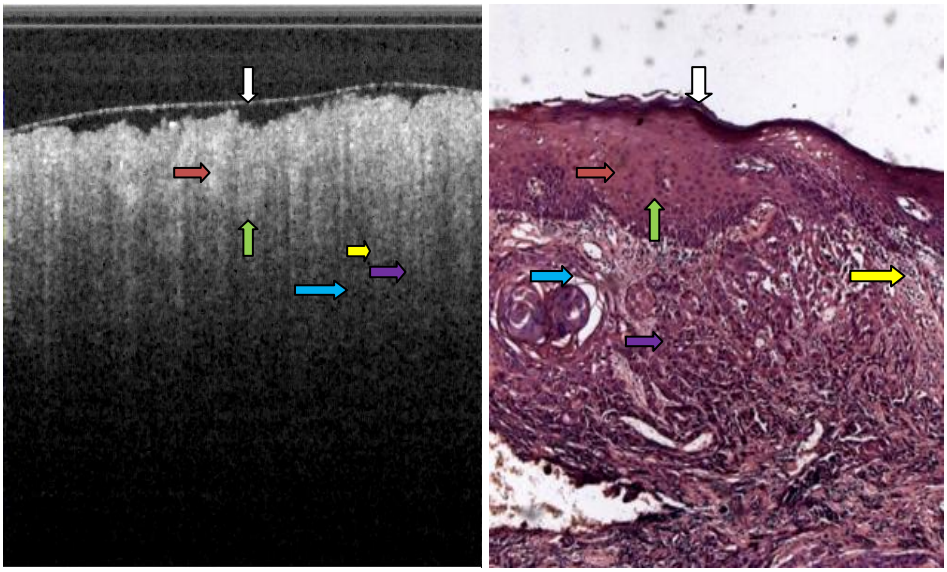

Figure 15a depicts in vivo OCT image of OSCC and 15b shows a photomicrograph of histopathological image of same site of OSCC in 5x magnification

CHARACTERIZATION OF EX VIVO OCT IMAGES AND THEIR CORRELATION WITH HISTOPATHOLOGY

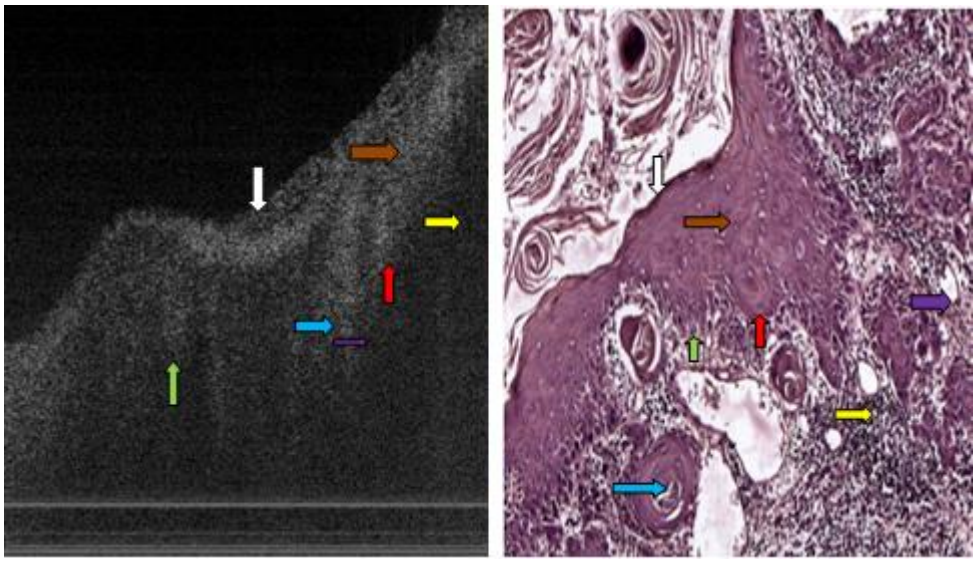

Figure 16a depicts ex vivo OCT image of OSCC and 16b shows a photomicrograph of histopathological image of same site of OSCC in 5x

⇨ SUPERFICIAL ZONE OF EPITHELIUM, ➡ REST OF THE EPITHELIUM, ➡ BASEMENT MEBRANE ZONE,
➡ LAMINA PROPRIA, ➡ INFILTRATING NEOPLASTIC EPITHELIAL ISLANDS, ➡ BLOOD VESSEL,

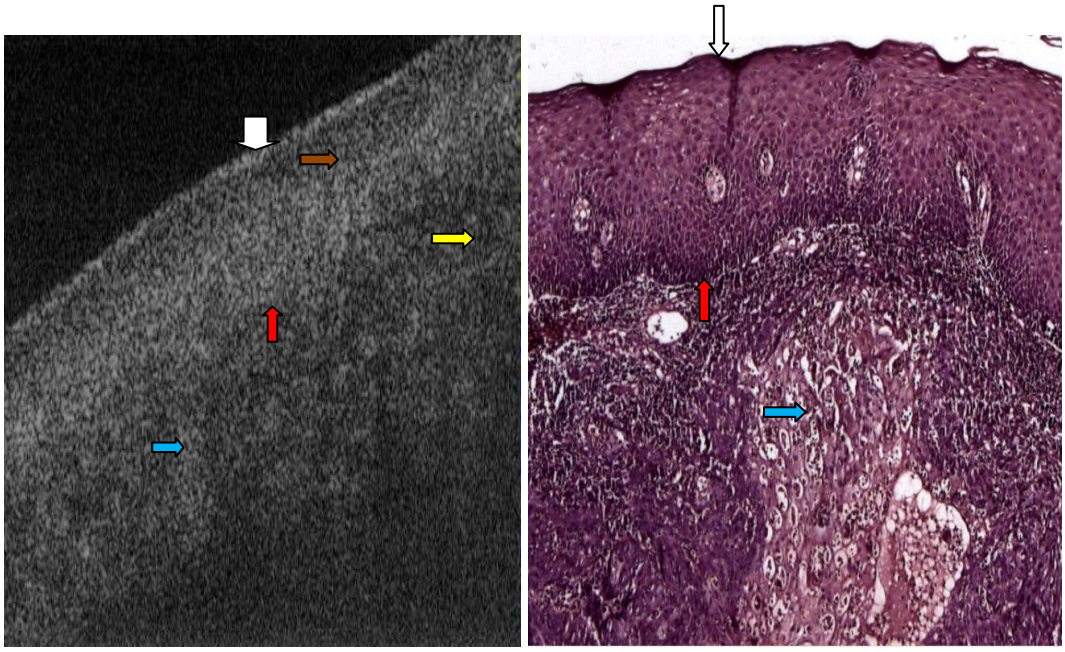

Figure 17a depicts ex vivo OCT image of OSCC and 17b shows a photomicrograph of histopathological image of the same site of OSCC in 5x magnification

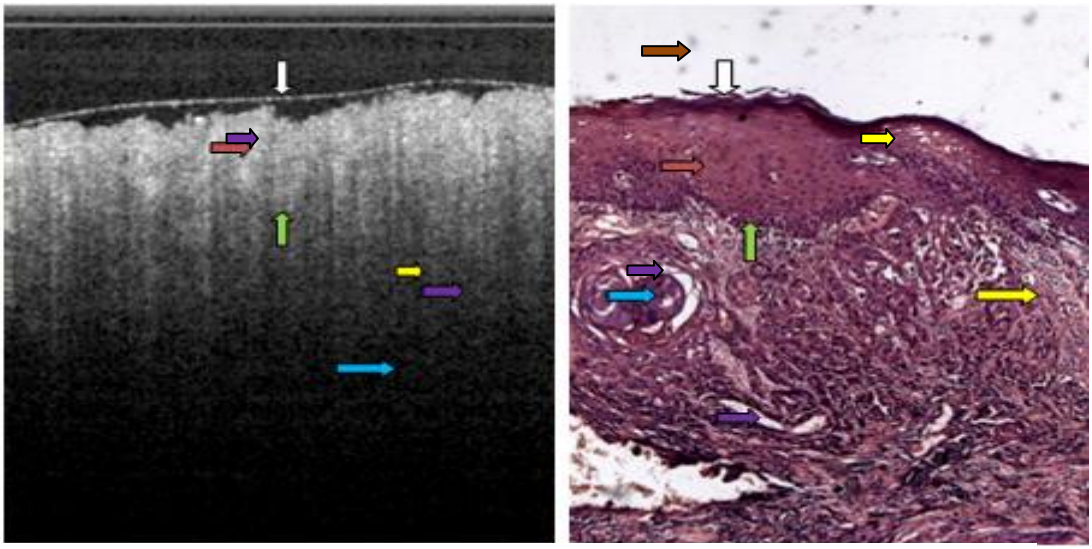

Figure 18a depicts ex vivo OCT image of OSCC and 18b shows a photomicrograph of histopathological image of same site of OSCC in 5x magnification

SUPERFICIAL ZONE OF EPITHELIUM, REST OF THE EPITHELIUM, BASEMENT MEBRANE ZONE,
LAMINA PROPRIA, INFILTRATING NEOPLASTIC EPITHELIAL ISLANDS, BLOOD VESSEL,

The grabbed photomicrographs of H&E stained sections were compared and correlated with their corresponding in vivo and ex vivo OCT counterparts regarding the presence of features which could be corroborative to the OCT images, and were thoroughly analysed and evaluated by several competent Oral Pathologists and Researchers in the field of biomedical optics. The following observations were noted.

5.1 THICKNESS OF SURFACE EPITHELIUM

5.1.1 The light microscopic images Revealed an increase in the thickness. (Hyperplastic) (Fig 13b-18b, Table 1)

5.1.2 The *in vivo* as well as *ex vivo* OCT images Revealed a less hyperlucid zone. (Figs 13a-18a, Table 1).

5.2 THE SURFACE ZONE OF THE EPITHELIUM

5.2.1 The light microscopic images Exhibit hyperkeratinisation in all the subjects (hyperparakeratinization in 100% of the cases). (Fig 13b-18b, Table 1)

5.2.2 The *in vivo* OCT images Represented as a hyper lucid bright band in 83.3 %(Figs 14a,15a) and hypolucid zone similar to deeper layers in 16.7% (Fig 13a,Table 1)

5.2.3 The *ex vivo* OCT images Revealed a hyper lucid bright band in 83.3 %(Figs 17a, 18a) and hypolucid zone similar to deeper layers in 16.7% (Fig 16a,Table 1)

5.3 SUBSURFACE ZONES OF EPITHELIUM

5.3.1 The light microscopic images Showed an increase in the thickness of the strata of the epithelium (basal, spinous, granular and keratin layer) (Figs 13b-18b, Table 1).

5.3.2 The *in-vivo* OCT Demonstrated a relatively less hyperlucid zone with reduced brightness in 83.3 %(Figs 13a, 14a) and enhanced brightness in 16.7%. (Fig 15a,Table 1).

5.3.3 *Ex vivo* OCT Revealed a relatively less hyperlucid zone with reduced brightness in 83.3 %(Figs 16a, 17a) and enhanced brightness in 16.7%. (Fig 18a,Table 1).

5.4 EPITHELIO-MESENCHYMAL JUNCTIONAL ZONE

5.4.1 The light microscopic images Showed a wavy, ill demarcated, undulating zone with breach in continuity. (Figs 13b-18b,Table 1)

5.4.2 In the *in vivo* and *ex vivo* OCT images (Figures 13a-18a) Revealed a wavy,dark,ill demarcated undulating hypolucid zone .(Table 1)

5.5 CONFIGURATION OF THE RETE PEGS

5.5.1 The light microscopic images Exhibited irregular, bulbous or wavy elongated projections. (Figs 13b-18b)

5.5.2 *In vivo* OCT images Showed torn, relocated structures (66%) (Fig 13a, 15a) or wavy, undulating structures (33%) (Fig 14a, Table 1).

5.5.3 *Ex vivo* OCT images Revealed torn, relocated structures (66%) (Fig 16a, 18a) or wavy, undulating structures (33%) (Fig 17a, Table 1).

5.6 THE LAMINA PROPRIA

5.6.1 The light microscopic image Showed irregular, complex, homogenous, fibro vascular connective tissue with its components.(Fig 13b-18b, Table 1)

5.6.2 In case of both *in vivo* and *ex vivo* OCT images The lamina propria appeared as an irregular patchy hyperlucid zone interspersed with hypolucid areas. (Figs 13a-18a, Table 1)

5.7 BLOOD VESSELS

5.7.1 The light microscopic images Showed well delineated endothelial lined lumen. (Fig 13b-18b, Table 1)

5.7.2 In cases of both *in vivo* and *ex vivo* OCT images A dark hypolucid lumina surrounded by hyperlucid linear bands was noted. (Figs 13a-18a, Table 1).

Table 1

SERIAL NO	TARGETED ZONES	HISTOPATHOLOGICAL FEATURES		IN-VIVO OCT FEATURES		EX-VIVO OCT FEATURES		REMARKS	
		NORMAL ORAL MUCOSA(NOM)	OSCC	NOM	OSCC	NOM	OSCC	IN VIVO OCT IMAGE	EX VIVO OCT IMAGE
1.	SUPERFICIAL ZONE OF EPITHELIUM (KERATIN LAYER)	KERATINIZED	HYPERKERATINIZED	HYPERLUCID	HYPERLUCID	HYPERLUCID	HYPERLUCID	5/6 CORRELATION	5/6 CORRELATION
2.	REST OF THE EPITHELIUM	NORMAL THICKNESS	INCREASED THICKNESS(HYPERPLASTIC)	LESS HYPERLUCID	LESS HYPERLUCID	LESS HYPERLUCID	LESS HYPERLUCID	6/6 CORRELATION	6/6 CORRELATION
3.	EPITHELIO-MESENCHYMAL JUNCTIONAL ZONE	PROMINENT	ILL DEMARCATED	ISO LUCID	HYPOLUCID	ISO LUCID	HYPOLUCID	4/6 CORRELATION	4/6 CORRELATION
4.	CONFIGURATION OF RETE PEGS	NICELY CONTOURED	TORN AND RELOCATED, BULBOUS OR WAVY ELLONGATED PROJECTIONS	NICELY CONTOURED	ILL DEFINED OR IRREGULAR	NICELY CONTOURED	ILL DEFINED OR IRREGULAR	4/6 CORRELATION	4/6 CORRELATION
5.	LAMINA PROPRIA	CAN BE DISCERNED JUXTAEPITHELIALLY	IRREGULAR COMPLEX HOMOGENOUS STRUCYURE	COMPLEX(MIX OF HYPER AND HYPOLUCIDITY)	COMPLEX(MIXTURE OF HYPER AND HYPOLUCIDITY)	COMPLEX(MIXTURE OF HYPER AND HYPOLUCIDITY)	COMPLEX(MIXTURE OF HYPER AND HYPOLUCIDITY)	2/6 CORRELATION	2/6 CORRELATION
6.	MICROANATOMICAL STRUCTURES(BLOOD VESSELS)	SPACE LINED BY ENDOTHELIAL CELLS	SPACE LINED BY ENDOTHELIAL CELLS	HYPOLUCID LUMINA SURROUNDED BY TWO HYPERLUCID	HYPOLUCID LUMINA SURROUNDED BY HYPERLUCID LINEAR ZONES	HYPOLUCID LUMINA SURROUNDED BY HYPERLUCID	HYPOLUCID LUMINA SURROUNDED BY HYPERLUCID LINEAR ZONES	5/6 CORRELATION	5/6 CORRELATION


DISCUSSION

6. DISCUSSION

The diagnosis of OSCC might pose a challenge to clinical pathologists and oncopathologists. From this viewpoint, OCT provides an easy to perform non-invasive, alternate diagnostic imaging modality to aid in the early screening and diagnosis of malignancies as an adjunct to gold standard histopathology.OCT might therefore help to bridge the gap between clinico-pathological and diagnostic aspects. The present study provides some useful information in this regard. Some significant parameters of OCT images of OSCC and its correlation with histopathological attributes have provided us with vital details, based on which, we can go a step forward to establish OCT as a non invasive diagnostic imaging modality.[80]

In the present study, the in vivo and ex vivo OCT images were analyzed, characterized and corroborated with their histopathology counterparts and some significant analyses were made.[81]

- ***The Thickness of the epithelium*** In the present study, *both in vivo and ex vivo* OCT images revealed a less hyperlucid hyperplastic zone. (Figs 13a -18a,Table 1) Prestin S et al. could also delineate this hyperlucid zone, using OCT. [71] Histopathologically, an increase in the thickness of epithelium was noted and Marx R et al [6] also recorded similar observations (Figs 13b-18b, Table 1).
- Therefore, OCT might be a helpful tool in delineating the domains of the epithelium.

- ***The surface zone of epithelium*** In the present study, the in vivo and ex vivo OCT images appeared as a hyper lucid bright band in 83.3% cases (Figs 14a,15a,17a,18a, Table 1) and a less hyperlucid zone (Figure 13a,16a,Table 1) similar to deeper layers in 16.7% of the present cases. Hamdoon Z et al noted the surface zone, as a thin bright hyperlucid line

on the uppermost layer of epithelium.[70] In the present study, hyperkeratinisation was demonstrated. (Figs13b-18b, Table1) in all the instances (hyperparakeratinization). This hyperkeratinisation (ortho or parakeratinization) was also observed by Neville BW[5] and Odell W et al.[25]

- The hyperlucid appearance might be due to the fact that the keratin layer contains very less water content and thick keratin-a protein of high molecular weight .Therefore it appears as denser and produces a greater degree of backscattered light demonstrating the hyper lucid appearance. In one of the in-vivo OCT images, the surface zone appeared as hypo lucid which might be due to production of a low degree of backscattered light. Thus SS-OCT can be used to identify the surface keratinisation of OSCC mucosal tissues.

- ***The subsurface zones of the epithelium*** In the present study, both *in vivo and ex vivo* OCT images, exhibited a relatively less hyperlucid zone with reduced brightness in 83.3 %(Figs13a, 14a, 16a, 17a, Table 1) and enhanced brightness in 16.7 %(Figs 15a, 18a, Table 1) cases. OCT studies by Wilder S et al revealed a relatively less hyperlucid zone compared to the surface zone of epithelium[74] The histopathological findings showed an increased thickness of the epithelial strata (i.e. basal, spinous, keratin and granular layer) (Fig 13b-18b,Table 1) Sapp J et al noted a visible thickening of the subsurface epithelial zones in a study .[24]
- The less hyperlucid appearance could be due to the reason, that the cells, in this region are not that tightly packed, and hence the structures allow the transmission or passage of more light thereby creating a reduced brightness.[82]The enhanced brightness in 16.7% of the cases might be due

to tissue fixation, processing and formalin shrinkage effects, which could lead to loss of relevant optical information. This is especially true for *the ex vivo OCT* images. So, the optical characteristics of the tissue changes dramatically. Hence, SS-OCT can demarcate the subsurface zones of the epithelium.

- ***The epithelio-mesenchymal junctional zone*** In the present study, both *in vivo and ex vivo OCT images,* this zone appeared as wavy, dark ill-demarcated, undulating, hypolucid zone .(Figs 13a-18a, Table 1) [82] The lucidity appears to be intermediate between the superficial epithelium and underlying lamina propria. Jerjes WK et al observed this junctional zone, as a zone having intermediate lucidity between upper epithelium and underlying connective tissue. The histopathology counterparts show a wavy, ill demarcated, undulating zone (Figs 13b-18b, Table 1) and Rajendran R et al also noted a wavy, ill demarcated, undulating zone in his study.[27]
- The junctional zone shares the characteristics of both overlying epithelium and underlying connective tissue .A demarcation between the two might be appreciated in the SS-OCT images in terms of varying lucidity parameters.[73]

- ***Infiltration of the neoplastic epithelial islands within the fibro vascular connective tissue stroma*** It is a hallmark of OSCC. In the OCT images, these might appear as round to ovoid hyperlucid areas with their lucidity mimicking that of the epithelium (Figs 13a-18a). A thorough review of the English literature revealed that studies with OCT findings similar to the present study have yet not been performed. Histopathologically, this zone is represented by the infiltration of neoplastic epithelial islands

within the fibrovascular connective tissue stroma (Figs 13b-18b).Similar histopathological observations have been reported by Marx R et al.[6]

- The hyperlucid appearance might be due to the neoplastic epithelial cells, which are pleomorphic and possess a much higher synthetic and proliferative activity as well as increased mitotic figures-hence these are optically much denser than their normal counterpart. Thus SS-OCT images might be helpful in representing the infiltrating neoplastic epithelial islands in cases of OSCC.

- ***The configuration of the rete pegs*** The present study revealed a torn and relocated appearance (66.6%)(Figs13a,15a,16a,18a,Table 1) or bulbous, wavy elongated projections (33.3%) (Figs 14a, 17a, Table 1) involving the *in vivo and ex vivo OCT images*.OCT studies by Tsai MT et al demonstrated the lack of a layered structural pattern involving the rete pegs.[75] The histopathological findings revealed irregular, bulbous or wavy elongated projections. (Table 1). Such an observation was also made by Lund C et al.[22]

- Hence, the OCT images might be useful in delineating the varying contours of the rete pegs.

- ***The lamina propria*** In the present study, both *in vivo and ex vivo OCT* images revealed an irregular patchy hyper lucid zone interspersed with hypolucid areas (Figs 13a-18a, Table 1) Hamdoon Z et al also noted an irregular and unclear architecture of the lamina propria with varying degrees of lucidity.[70]. The histopathological images revealed irregular complex homogemous fibrovascular connective tissue with its

constituents (Figs 13b-18b, Table 1) and an irregular,dense,fibrovascular connective tissue was demonstrated by Akhtar M et al.[23]

- The different signal intensities are apparent, which might be due to different optical properties of the substances present within the lamina propria; some of which are optically dense such as the collagen fibres thereby allowing little light to pass through them and reflects most of it; whilst some are optically less dense, and therefore appeared as hypolucid. [83] Therefore SS-OCT can be a useful tool in demonstrating the lamina propria of OSCC mucosal tissues.

- ***The blood vessels*** The present study demonstrated dark hypolucid lumina surrounded by hyperlucid linear bands in the OCT images (*both ex and in vivo)* (Figs 13a-18a, Table 1). OCT findings alike our study were reported by Hamdoon Z et al.[70] Histopathologically, a well delineated endothelial lined lumen was found.(Figs 13b-18b, Table 1) .Similar observations were noted by Marx R et al.[6]

- The inner lumen of the vessel appears hypolucid as blood is not that dense and hence allows the passage of light more easily whereas the walls of the vessel is much denser in comparison and as a result there is a greater degree of reflection and backscattering of the near infrared light.[82] OCT images, therefore, might be a helpful tool in pointing out blood vessels within the connective tissue.

Both the *in vivo and ex vivo OCT images* yielded a lot of relevant information, based on which, we could go a step further in establishing OCT images as a safe, non-invasive, adjunctive imaging modality. But in the *ex vivo images,* the essential parameters were clearly not as easily discernible as their in vivo

counterparts.[83] Also, the *ex vivo* OCT was performed post biopsy, which belies the very purpose of being a non invasive procedure.

So it seems that the *in vivo* OCT images are superior to the *ex vivo OCT* ones in terms of their correlation with the histopathology counterparts.[84] The *in vivo* OCT imaging of living tissues may represent a truer biologic picture than any image produced by other high resolution modalities. With the capability to perform a tomography study, one could easily monitor disease, document progression, and target tissue biopsy specimens to regions of greater histopathologic yield.[85, 86 87]

Still the use of this novel, non-invasive, imaging technique provides morphological imaging with sufficient resolution and penetration depth, resulting in quasi-histological images. However, the cytomorphological characteristics of the various layers of cells were not clearly discernible. So, the present study might benefit greatly from an increased number of study subjects and improved imaging tools.

SUMMARY AND CONCLUSION

7. SUMMARY AND CONCLUSION

The present study had been conducted on a total number of six subjects in Guru Nanak Institute of Dental Sciences and Research and SMST, IIT, Kharagpur during the time period of December 2014- August 2015 for characterization of Optical biopsy (OCT images) of Oral squamous cell carcinoma (OSCC) and its correlation with histopathological attributes, to establish the efficacy of Optical coherence tomography(OCT) as an adjunctive non invasive diagnostic imaging modality, for an efficient and painless diagnosis Oral squamous cell carcinoma.

The study subjects were thoroughly interrogated, examined, assessed and all the analysed relevant observations were recorded carefully. Then real time *in vivo* OCT imaging from the representative areas of the lesion with prior informed consent was performed. Thereafter, incisional biopsies were performed from the exact site and the biopsied specimens were subjected to an *ex vivo* OCT imaging protocol after a time lapse of six hours, which was the time required for transportation of the tissues to SMST. Afterwards, the biopsied samples were histopathologically evaluated, for the presence of corroborative architectural analytical parameters, with the OCT images.

OCT images appear as varying areas of hyper/hypo lucid zones depending upon the area through which the light is backscattered or transmitted. Hyper lucid zones appear as brighter areas and hypo lucid zone appears as darker areas.

Optical coherence tomography is an evolving imaging technology and a non invasive imaging technique for evaluating oral structures. It can be used for the diagnosis of oral premalignant and malignant changes in the oral mucosa. Because of its higher resolution and penetration depth it can be used for imaging the normal and abnormal changes in the oral mucosa ,but a lot of

research is required still, to establish OCT as a non invasive diagnostic imaging modality which might be a replacement for gold standard histopathology. As it is a new and emerging technology in oral health sciences, several ongoing studies can provide relevant information regarding the use of OCT, which might be of routine diagnostic value. It can be said that the performed study is a step in the right direction in this regard, as it might do value additions to this exciting and innovative bio-engineering research arena and enrich future researchers to pursue further studies.However, the SS –OCT cannot discern cellular details. So, a higher resolution OCT, such as the HD-OCT can be useful in identifying cellular details. Hence, studies involving increased number of samples would be quite helpful in establishing the credentials of OCT as a non invasive alternate imaging modality for screening and diagnostic purposes. Further studies may improve the resolution of the system and the probe capabilities which shall provide valuable information on the development and progression oral precancers and cancers.

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ANNEXURE

ANNEXURE –I

COLLABORATIVE CLINICO-PATHOLOGICAL CASE RECORD SHEET

Places of study:- Department of Oral & Maxillofacial Pathology, GNIDSR, Kolkata

&

Human Genetics Unit, Indian Statistical Institute, Kolkata.

SL.NO: OPD CARD NO: SAMPLE NO:
DATE:

- PATIENTS' PARTICULARS:-
  - **I. NAME:**
  - **II. AGE:**
  - **III. SEX:**
  - **IV. RELIGION:**
  - **V. MOTHER TONGUE:**
  - **VI. ADDRESS:**
  - **VII. PHONE NO:**
  - **VIII. POLICE STATION:**
  - **IX. MARITAL STATUS:**
  - X. **EDUCATIONAL STA**TUS:
  - XI. OCCUPATION:
  - XII. ECONOMIC STATUS:

PERSONAL HABIT:-

(A) Food	Veg. / Non Veg. (Fresh / Refrigerated / Dried / Smoked / Preserved)
	Spicy Food
(B) Alcohol	Yes / No
	Country Liquor / Foreign Liquor / any other ( )
	Intake: amount in volume:
	How many times/day:
	Duration in years:
(C) Smoking	Yes / No
	Beedi / Cigarette / Ciger / Chuta/Hookli
	Nature direct or reverse
	How many times/day:
	Duration in years:
(D) Khaini / Snuffing	Yes / No
	Place of keeping khaini: labial sulcus/Buccal sulcus
	How many times/day:
	Duration in years:
(E) Chewing	Yes / No
	Pan / Nut / Tobacco/ Pan + Nut / Pan + Tobacco / Pan + Nut + Lime / Pan + Nut + Lime + Tobacco / Others (…….)
	How many times/day:
	Duration in years:
	Quid placement: Yes / No
	Duration in years:……
	Quid in LBM / RBM / Both
	When: Morning / Night / Always

- CHIEF COMPLAINTS:-

- HISTORY OF PRESENT ILLNESS:-

- PAST MEDICAL HISTORY:-

- PAST SURGICAL HISTORY:-

- PAST DENTAL HISTORY:-

- FAMILY HISTORY:-

(a) General history:-

(b) History of malignancy:-

CONSENT OF THE PATIENT:- **TAKEN:** **NOT TAKEN:**

- PHYSICAL EXAMINATION OF THE PATIENT:-
- GENERAL SURVEY OF THE PATIENT

i. BUILT OR STATE OF NUTRITION:
ii. WEIGHT:
iii. ATTITUDE:
iv. CONSCIOUSNESS STATUS:
v. GAIT:
vi. FACIES:
vii. ANAEMIA:
viii. OEDEMA:
ix. CLUBBING:
x. SKIN ERUPTION:
xi. VITAL SIGNS:
    a. PULSE
    b. BLOOD PRESSURE
    c. RESPIRATION
    d. TEMPERATURE
xii. GENERAL ASSESMENT OF THE PATIENT'S VITAL SYSTEMS
    a. C.N.S
    b. C.V.S
    c. G.I.T
    d. OTHERS

- EXTRAORAL EXAMINATION

a. EXAMINATION OF THE FACIAL SKIN:

b. EXAMINATION OF NECK VEINS:

c. EXAMINATION OF PERINASAL REGION:

d. EXAMINATION OF MAXILLA & MANDIBLE:

e. EXAMINATION OF TMJ:

f. EXAMINATION OF THE LYMPH NODES OF HEAD NECK AND FACIAL REGION:

g. JAW MOVEMENTS:

- INTRA ORAL EXAMINATIONS

1. EXAMINATION OF THE LIP
   a. vermillion border:
   b. commissural area:
   c. labial mucosa:
   d. labial frenum:
2. EXAMINATION OF THE CHEEK
   a. Buccal mucosa:
   b. Minor salivary glands :
   c. Orifices & papilla of the parotid duct & gland:
   d. External oblique ridge:
   e. Internal oblique ridge:
3. EXAMINATION OF THE TEETH

4. EXAMINATION OF GINGIVA

5. EXAMINATION OF TONGUE

a. General considerations:-

- Size:
- Shape:
- Movement:
- Status of papilla:
- Colour:
- Consistency:
- Taste perception:
- Bidigital examination of tongue:

b. Lingual mucosa in general:

c. Dorsum of tongue:
d. Lateral border of tongue:
e. Foramen caecum-Lymphoid follicles:
f. Ventral surface of the tongue:
g. Mucosa:
h. Fimbriated folds:
i. Superficial veins & varicosities:
j. Anterior lingual gland and ducts:

6. EXAMINATION OF RETROMOLAR REGION

7. EXAMINATION OF THE FLOOR OF THE MOUTH:-
    a. Bidigital palpation of the floor of the mouth:
    b. Mucosal surface:
    c. Subgingival folds:
    d. Submandibular duct:
    e. Orifices of the submandibular & sublingual glands:
    f. Lingual vestibules:
    g. Genial tubercles:
    h. Mylohyoid ridge:

8. EXAMINATION OF PALATE
    a. Shape & size:
    b. Colour of the mucosa:
    c. Fovea palatine:
    d. Maxillary tuberosity:
    e. Uvula:
    f. Movement of the soft palate:
    g. Lateral /medial pterygoid muscle:
    h. Change of voice:

9. EXAMINATION OF PHARYNX/TONSILS

a. Palatine tonsils:

b. Tonsillar crypts:

c. Posterior pharyngeal wall:

d. Lateral pharyngeal wall:

SUMMARY OF INTRA-ORAL CLINICAL FINDINGS:

Lesion	Present	Absent
Duration of the lesion		
Site/ sites of the lesion		
Size of the lesion		
Shape of the lesion		
Colour of the lesion		

- PROVISIONAL DIAGNOSIS

- ROUTINE INVESTIGATIONS

ROUTINE IMAGING

a. Extra oral:

b. Intra oral:

ADVANCE IMAGING

a. Ultrasound
b. C.T scan:
c. MRI:
d. Others (if any):

ROUTINE HAEMOGRAM

a. Haemoglobin%:
b. T.L.C:
c. D.L.C:
   - Lymphocytes:
   - Neutroplils:
   - Basophils:
   - Eosinophils:

- Monocytes:

d. E.S.R:

e. SUGAR:
   - PP:
   - FASTING:

f. PLATELETS:

URINE EXAMINATION

STOOL EXAMINATION:

MICROBIAL ASSAY

CYTOLOGY:

EXFOLIATIVE
FNAC

BIOPSY

INCISIONAL
EXCISIONAL

LIGHT- MICROSCOPIC FINDINGS(H/P REPORT):-

***mi*RNA ASSAY : PERFORMED: NOT PERFORMED:**

ANY OTHER SPECIAL INVESTIGATIONS:

- DIFFERENTIAL DIAGNOSIS:
- CONFIRMATIVE DIAGNOSIS:
- TREATMENT PLAN:

- REFERRAL:

_____

SIGNATURE OF THE P.G STUDENT

_____ _____

SIGNATURE OF THE CO-GUIDE SIGNATURE OF GUIDE

ANNEXURE –II

DEPARTMENT OF ORAL AND MAXILLOFACIAL PATHOLOGY

GURUNANK INSTITUTE OF DENTAL SCIENCES AND RESEARCH, PANIHATI, KOLKATA

CONSENT OF THE PATIENT

PLACE :
DATE:
I _____
son/wife/daughter of
_____ address
_____ P.S._____do
hereby declare that I have been explained the necessity of the physical examination and/ or the surgical procedure required for performing biopsy from the lesion / growth in my orofacial region , or for collection of blood by venupuncture or aspiration of body fluid , as case may be , in the language , I understand , by the doctor concerned , for establishment of diagnosis , for treatment or for research purposes.
I do hereby give my consent in full alertness of my mind for performing the above procedures in my body.

Full signature/ left thumb
Impression of the patient party

Full signature / left thumb
Impression of the patient

Signature of the P.G student

Signature of the Guide/Co-Guide