OPTICAL COHERENCE TOMOGRAPHY OF ORAL MUCOSA

by

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ABSTRACT

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Oral cancer causes 120000* deaths annually around the world and 280000* new cases are diagnosed every year.

Technology has developed and the death rate of oral cancer has improved over the last few decades; hence to improve the survival rate, development of a technique able to diagnose carcinomas in its early stages is very important.

OCT is a non invasive imaging technique capable to provide high resolution microstructure images of tissue. A development of in vivo OCT; capable to image tissue with micrometer resolution and able to identify pre cancerous morphological changes non invasively could improve survival rate in cancer patients and also quality of life.

The work was started with ex vivo OCT imaging of extracted tissue samples. Tissue extraction and storing methods have major impact on optical properties of tissues, which diminishes the quality of OCT images of such samples. Hence, there is a

need for a technique which performs in vivo imaging. In vivo OCT system was developed to perform real time imaging. Images of normal healthy oral mucosa were taken and compared with standard histological images. The OCT images were taken from various regions of the mouth like lip, gingival, tongue, buccal mucosa and mapping was done. Further, image processing module was developed to provide better quality real time images. OCT images were compared with standard histological images. The OCT images of oral cavity shows distinct layers of epithelium, lamina propria and basal membrane.

The capability of OCT images to distinguish different tissue layers like epithelium and other sub epithelial layers supports the possible application of OCT imaging in early detection of carcinomas.

* Cancer Mondial: http://www-dep.iarc.fr/

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CHAPTER 1

RESEARCH OVERVIEW

1.1 Introduction

This thesis describes a high resolution optical coherence tomography technique to image human oral cavity. The primary objective of this thesis was to use spectral domain optical coherence tomography (SD-OCT) system to image oral tissue. Ex-vivo tissue imaging was performed to verify the feasibility of OCT system to extract morphological information.

Primary goals were:

1. To probe the feasibility of SD-OCT system for ex-vivo imaging of oral tissue and to compare the images obtained with the histology images.
2. To probe the feasibility of SD-OCT to differentiate normal and malignant oral tissue, using ex-vivo tissue samples.

Acquired results suggest tissue preservation procedures effect the morphological information obtained from the images. This led us to an alternate approach.

Detour in research path:

1. Since SD-OCT images of ex-vivo oral tissue samples didn't show much structure we decided to acquire in-vivo OCT images of oral tissue.
2. To develop a miniature scanning probe for video rate acquisition of OCT images from human volunteers.
3. Acquisition and processing of OCT images and movies from various sites in the

oral cavity.

Scanning probe for video rate in-vivo imaging was developed and used to image the oral cavity of healthy human volunteer. The thesis describes these experiments and the results obtained.

1.2 Organization of The Thesis

Following this chapter of research overview, the next chapter provides with the epidemiology of oral cavity cancer statistics, our primary motivation for trying to develop a non-invasive imaging tool for screening oral cavity lesions.

Chapter 3 gives an overview of the OCT system principle, the hardware used to carry out the experiments, the system set up for ex vivo and in-vivo imaging and gives an outline of the system used.

Chapter 4 elaborates on the imaging protocol followed during the experiments. It also describes the image processing algorithm that was used in order to implement background subtraction and obtain videos of the images that were received from the OCT system.

Chapter 5 is the last chapter of the thesis and concludes with a discussion of the results and the translation of these results into clinically relevant applications.

CHAPTER 2

ORAL CANCER

2.1 Motivation

One in four people will get cancer at some stage in life. The early detection and complete cure of cancer are one of the most burning questions in medical researchers. Hence, thanks to all new developed instrumentations, progress in diagnostics and therapeutic techniques has lead to a drop in morbidity and mortality rates of many cancers. But not all cancers are detected early and show obvious symptoms in the early stages. Cancer of oral cavity is one of the highest occurring cancer sites in southern and central Asia.

Worldwide approximately 280,000 new cases of oral cancer are found every year. And only half of the people diagnosed with oral cancer lives in next 5 year[1]. This year American Cancer Society estimates 30,990 new cases of the disease in USA.[2] The death rate in oral cancer is higher than that of cervical cancer, Hodgkin's disease, cancer of the brain, liver, testes, kidney, or malignant melanoma and these numbers are not much improved over the last few years.

Most of the time oral cancer is preceded by a pre-malignant lesion in oral cavity, but not always and not all those oral lesions progress to be malignant. Due to this uncertainty the current early diagnosis of oral lesions needs to be modified for more precise early detection of oral cancer.

Optical techniques have been used in medicine since 18th centaury. From the high quality examination light source, and counting cells to complicated image guided surgeries; optics has now become crucial part of the medicine. Optical coherence tomography is the high resolutions two dimensional imaging technique, which enables us to image microstructures of tissue beyond the scope of available bright field and confocal microscopes.[3] OCT can image high scattering tissue and can image blood vessels and other structures beneath the surface as much as 1-2 mm deep. OCT gives high resolutions images with an advantage of simple system and comparatively low cost of the hardware.

Despite all the medical diagnoses improvement and technical developments, the survival rate in head and neck cancer has not improved significantly over the last 30 years. Treatment advances have been undermined and a significant percentage of patients cured from head and neck cancer develop second primary tumors.[4]

2.1.1 Oral Cancer Statistics

Cancer of oral cavity is amongst the leading cancer sites in the world. Intake of tobacco in the form of pipe smoking and also as a puff (inhalation) is very common in some developing countries of southern and central Asia.[5] Chewing of areca nuts with betel quid leaf is a very popular habit and is a predominant factor for having very high statistics of oral cancer. Cancer of the oral cavity and pharynx is the first and third commonest cancer in Indian men and women, respectively.[6]

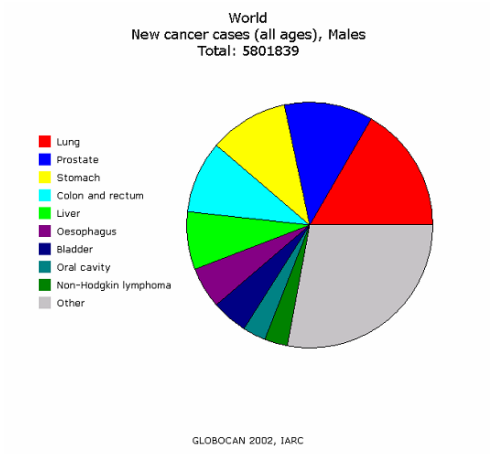

Figure 2-1: World Map: New Cases of Cancer,
Courtesy: CANCER Mondial http://www-dep.iarc.fr/

In India, the number of newly diagnosed tobacco related cancers has been estimated at approximately 250 000 out of a total of 700 000–900 000 new cancers diagnosed each year. [7]

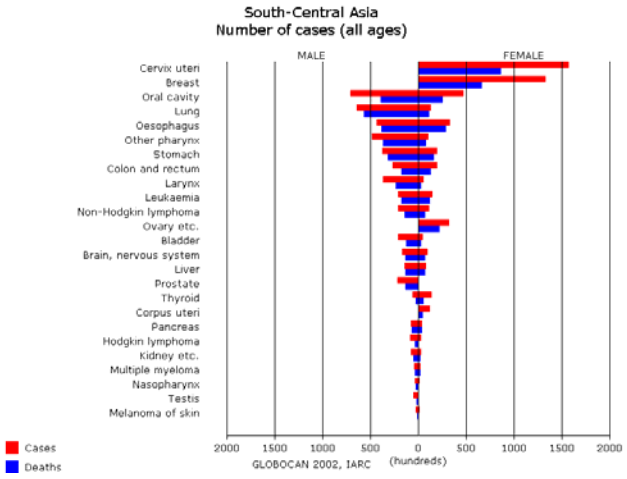

Figure 2-2: Cancer: Cases & Deaths South Central Asia,
Courtesy: CANCER Mondialhttp://www-dep.iarc.fr/

Most of the times, oral cancer is not diagnosed in the early stages and thus the death ratio is not much improved over the last few decades. Often at the time the cancer is diagnosed, it has metastasized or it is too late for local treatment. These numbers can be improved if the cancer is diagnosed in its early stage. Goal of screening oral cancer in the early stages is to find premalignant and malignant lesions before they cause symptoms. The early detection raises the possibility to cure and prevent cancer.

2.1.2 Abnormal Cell Growth

Cancer or neoplasia means the process of new growth, which is typically uncontrolled. "A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change."[8] Many normal body cells grow, divide and die. Until person becomes an adult these cells grow and divide more rapidly, after that cells in most parts of the body grow and divide only to replace worn-out dying cells or to repair injuries.

Because cancer cells continue to grow and divide, they are different from normal cells. Instead of dying, they outlive normal cells and continue to form new abnormal cells. Cancer cells develop because of mutant damage to DNA, the substance in every cell that directs all activities. Most of the time when DNA becomes damaged the body is able to repair it. But in cancer cells, the damaged DNA is not repaired. People can inherit damaged DNA, which accounts for inherited cancers.

Cancer usually forms as a tumor, lump of cells. Some cancers, like leukemia, do not form tumors. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow. Often, cancer cells travel to other parts of the body where they begin to grow and replace normal tissue. This process is called metastasis. Regardless of where a cancer may spread, however, it is always named for the tissue of origin. Not all tumors are cancerous. Benign (non-cancerous) tumors do not spread (metastasize) to other parts of the body and, with very rare exceptions, are not life threatening.[9]

2.1.3 Cancer of Oral Cavity

Cancer is a common term used for all malignant tumors.[10] Cancer of the oral cavity is known as oral cancer. This term is widely used for carcinomas of oral cavity and lesions of oropharyngeal regions. All these include lips, buccal mucosa, tongue, floor of the mouth, hard and soft palate, upper and lower gingiva, pharynx and larynx.

The identification and appropriate management of premalignant mucosal lesions are important aspects of patient management in the oral cancer. At the time of diagnosis, the extent (stage) of disease is the most important factor for prognosis. All these factors have major impact on the survival rate in head and neck malignancies.

2.2 Premalignant Lesions

2.2.1 Normal Oral Mucosa

Mucous membrane of the oral cavity consists of stratified squamous epithelium and connective tissue called lamina propria. In some regions, this mucosa directly lies over the bone. In other regions it contains submucosa with fat and salivary glands. In the oral cavity, there is not a clear distinction between lamina propria and sub mucosa.

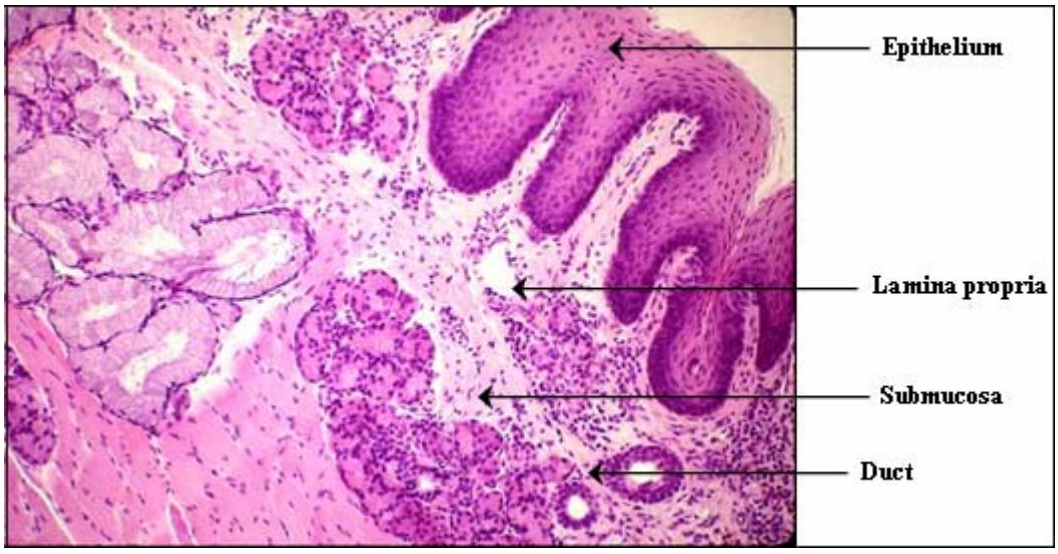

Figure 2-3: Normal Oral Mucosa Histology Image

The oral mucosa varies from site to site within the oral cavity, but the epithelium is stratified squamous at all the sites. This epithelium is partially keratinized on gingiva and hard palate and on tongue; it is non-keratinized elsewhere. Lamina propria is unspecialized. Oral epithelial tissue continuously undergoes reproduction and new cells replace the dead or injured cells. All the pathological changes observed in oral epithelium may not necessarily become cancerous. These conditions include:

hyperorthokeratosis, hyperparakeratosis, acanthosis and atrophy. Keratin is the outermost layer of epithelium as seen under the microscope and is seen in two forms: orthokeratin and parakeratin. Orthokeratin has no visible nuclei within the outer layer, whereas in parakeratin nuclei are present. Hyperorthokeratosis is the presence of excess orthokeratin. Hyperparakeratosis is presence of excess parakeratin. Acanthosis is a skin disorder characterized by dark, thick, velvety skin in body folds and creases. Atrophy means wasting or decrease in size of a body organ. Epithelium undergoing malignant transformation shows changes at the cellular level and this abnormal growth of epithelium is known as epithelium dysplasia. Epithelial dysplasia is the premalignant stage and primary features include: Loss of basal cell polarity, parabasilar hyperplasia, increased nuclear cytoplasmic ratio, drop shaped rete ridges, abnormal epithelial maturation, increased mitotic activities,mitoses in the superficial half of the surface epithelium ,nuclear hyperchromaticity, enlarged nucleoli, loss of cellular cohesiveness. [11]

These cytological changes appear in the different extent in different lesions, also the entire lesion may not show dysplasia in early stages. The presence of these features and numbers of features appearing decides the severity of the dysplasia.It is assumed that all the malignant transformations are the result of progression from normal epithelium to dysplasia to malignant changes. But unfortunately there is no confirmation that all malignancy progress gradually or serially progress through different stages of premalignancies.

2.2.2 Premalignant Lesions

Premalignant is a term used to describe a condition that may (or is likely to) become cancer. Typically classification of lesions of the oral cavity is based on the appearance of the lesions. Most common premalignant lesions of oral cavity include:

(1) Leukoplakia

(2) Erythroplakia

The basic features of the most common premalignant lesions in oral cancer development are tabulated below.

Table 2-1: Clinical features of Leukoplakia

Feature	Leukoplakia
Definition	white plaque that does not rub off and cannot be clinically identified as another entity
Etiology	Tobacco Chronic hyperplastic candidosis Idiopathic leukoplakia (heterogeneous)
Clinical Feature	• Age : middle aged and elderly • Sex: Male predilection • Common Sites: Alveolar mucosa, buccal mucosa • Dynamic process, shows continuous histological changes
Clinicopathologic correlation	Dysplasia Carcinoma in situ Squamous cell carcinoma
Other designations	Leukoplakia simplex, Leukoplakia verrucosa, Leukoplakia erosive, Verrucous hyperplasia, Leukoplakia speckled, Leukoplakia nodular, Leukoplakia ulcerative, Erythroleukoplakia

The clinical features of the other main premalignant lesion are tabulated below.

Table 2-2: Clinical features of Erythroplakia

Feature	Erythroplakia
Definition	Red velvety plaque that cannot be clinically or pathologically identified as another entity
Etiology	Unknown
Clinical Feature	• Age : elderly • Sex: Male predilection • Common Sites: floor of mouth , ventral and lateral tongue • Often well demarcated from surrounding mucosa • More likely to develop malignancy compared to Leukoplakia
Clinicopathologic correlation	Epithelial dysplasia Carcinoma *insitu* Epithelium is mostly non keratinized and shows atrophy

There are many other conditions which show a relationship to the cancer at more or less extent. These include Syphilis, Sideropenic Dysphagia, Oral submucous fibrosis, Erosive lichen planus, chronic immunosuppression.

2.3 Diagnosis of Oral Cancer

Most early premalignant changes or in situ carcinomas of the oral mucosa occur as patches of Erythroplakia or Leukoplakia which should be readily apparent on visual examination. In areas less easily visualized directly, such as the larynx and hypopharynx, visualization of these lesions requires direct or indirect laryngoscopy.[12]

2.3.1 Biopsy

Biopsy is removal of cells or tissue for microscopic examination. In oral cavity lesions, tissue samples from lesions suspected for malignancy are removed and histologic examination determines the possible malignancy.

Most of the time oral cancers are far advanced by the time they are detected because cancerous changes in the mouth are not always visible to the naked eye. There are many limitations of biopsy method used for detection of the cancer.

The invasive nature of biopsy prevents it from being used repeatedly to study a micro-tumor or multiple tumor sites on the same organ. Only conformational diagnosis is carried out using biopsies. Primary screening of tumors is clinically done by visual inspection. The results of primary screening depend on clinician's skills and experience. In early stages of cancer, it is possible that the entire lesion does not show dysplasia. The results of a biopsy depend upon the site of biopsy. False negative rates in this scenario tend to get inflated. Excisional biopsy imposes problems like the risk of infection and haemorrhage. Biopsy is an expensive surgical procedure and is an invasive with all risks of surgical procedure.

2.3.2 Optical Coherence Tomography Technique

OCT is a non invasive imaging modality which provides 2D or 3D images with very high resolutions compared to other high frequency imaging modalities like ultrasound. The obvious advantages of the OCT promises the possibility of detection of cancer in early stage.

Being noninvasive OCT provides us with an opportunity to use it repetitively on a subject to study the entire region (suspected cancer site). It also does away with a need for primary screening. Subjective and personal errors are reduced. This technique does not increase any further infection or spreading of cancerous cells.

Optical imaging promises to assess tissue morphology non invasively in situ.[1] Early diagnosis of cancer requires a high resolution imaging technique which is repetitive and therefore the choice of OCT is an important step in optical biopsy (non invasive tissue imaging) for early detection of carcinoma.

In the following chapters, I have described the OCT system setup, ex-vivo imaging experiments and in-vivo imaging experiements.

CHAPTER 3

OPTICAL COHERENCE TOMOGRAPHY: TECHNIQUE OF CHOICE

Optical Coherence Tomography (OCT) is a non invasive 2D/3D imaging technique which is capable of producing high resolution images of tissue microstructures. OCT is based on low coherence interferometry. The interferometry is the basic principle of OCT. Initially, interference signal detection technique was used in finding faults in fiber optic connections[13]. Later, the usefulness of the technique in medical diagnosis was realized and today OCT is successfully used in a wide variety of medical fields. Most of the components in OCT system are optical components, so this is relatively low cost and simple system. Today, many researchers are working on verifying possible applications of OCT in medical diagnosis.

3.1 FD OCT

Michelson interferometer is the heart of Fourier Domain OCT (FDOCT) system. Coupler splits the source light in to reference and sample arm and light reflected back is detected by spectrometer. Individual wavelength components are detected by array of detectors in the spectrometer camera. In FD OCT system, spectrometer measures the interference pattern as a function of frequency. The discrete Fourier transform of the interference pattern provides information about the object's structure.

3.1.1 Theory: Low Coherence Interferometry

OCT is based on low coherence interferometer. Figure2.1. shows the basic schematic diagram of OCT system based on Michelson interferometer. The broadband light source Ein illuminates the interferometer. A 50-50 beam splitter splits light in reference path Er and the sample path Es. The light is reflected back from the mirror in the reference path and from the tissue sample from the sample path. Electrical field of the light in the sample arm is modified when reflected back. Light reflected back from the mirror in the reference arm, interferes with the modified light in the sample arm and is detected by the spectrometer.

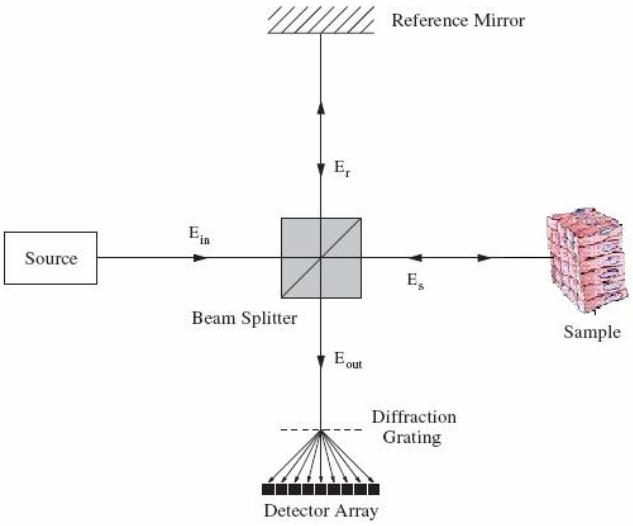

Figure 3-1: Basic System Setup of OCT

Due to broadband nature of the source, when path length of sample arm and reference arm matches within the coherence length of the source; interference signal is observed. Sharp refractive index variations between layers in the sample medium manifest themselves as corresponding intensity peaks in the interference pattern.[14] The amplitude of the interference depends on the refractive index differences at the interfaces.[15] Two or three-dimensional OCT image is obtained by making multiple depth scans. This can be achieved by scanning the beam in either one or two orthogonal directions. The axial resolution of the OCT system depends on the coherence length of the source and the transverse resolution depends on the focusing system. The depth (axial) resolution of an OCT system is determined by the temporal coherence of the light source. In OCT imaging any tissue property which changes amplitude, phase or polarization of the signal, gives rise to diagnostically informative signals.

3.1.2 Practical Aspects: OCT Hardware

OCT system instrumentation consists of the source, reference arm and sample arm and a detector. All of these plays very important role in deciding performance of the system and quality of OCT images.

In OCT, source (Laser) is very important factor that decides the general performance of the system. High Irradiance, short temporal coherence and Emission in near infrared are basic requirements for OCT source. Light reflected back from deep tissue is very weak and thus high irradiance is required while imaging tissue samples. Temporal coherence has inverse relation with bandwidth. Shorter coherence that is

higher bandwidth provides better resolution contrast in imaging. OCT source wavelength should be good enough to provide better depth resolution. Light at UV frequencies is able to image at only superficial layers, at higher than 2500 nm wavelength vibrational absorption by water limits the depth resolution. Hence these wavelength ranges are not useful. Also the window between 950 and 1000 nm wavelengths should be avoided because the absorption of water in this range is the highest and it would cause tissue surface burns. Thus far, wavelength rages from 1200nm to 1600nm have been proven the best for tissue imaging.

Fiber based Michelson interferometer is one of the most common configuration of OCT system set up. Light from the source is conducted through a single mode fiber to 50-50 coupler. From coupler half of the power is conducted in reference arm and half of the power is conducted into the sample arm, via single mode fiber. Reflected light from sample and reference arms interferes at coupler and is detected by the spectrometer.

The interference pattern, detected at the spectrometer, contains light intensities from reference and sample arms and also contains the depth information. Because the path length of reference arm and the sample arm are the same the depth information is coming only from the tissue sample which is light reflecting back from the different layers of tissue. The spectrometer measures the intensities as function of wavelength. To construct an axial scan from the wavelength component, k space transformation is performed.

According to the Nyquist's criteria, the maximum measurable frequency and hence the depth is one half of the sample frequency of the photo diode array. Thus the maximum measurable depth, Δz is $\Delta z = \frac{1}{4n}\frac{\lambda 0^2}{\delta\lambda}$ where $\delta\lambda$ is the resolution of the spectrometer. In FD OCT axial resolution depends on the source coherence length. The maximum achievable axial resolution is $axialresolution = \frac{2\ln 2}{n\pi}\frac{\lambda o^2}{\lambda FWHM}$

According to this, shorter wavelength, broader bandwidth, will provide higher resolution. Transverse resolution of FD OCT system depends on the beam waist on the sample, which depends on the numerical aperture of the lens which focuses the beam on sample and also depends on the mean wavelength of the spectrum. We can not increase transverse resolution by using higher NA lens because it reduces depth of focus.

3.2 OCT System Setup

For imaging tissue samples two FD OCT systems were used. 1) Bulk system 2) In-vivo OCT probe, both the systems are based on the principle explained above. The bulk system is used for ex vivo imaging in the lab, but it cannot be used in clinical application because of its size and bulky hardware. To overcome this limitation, in vivo OCT probe was designed in the lab and was used to get images in vivo from human subjects.

3.2.1Bulk FD OCT System Set up

Ex vivo OCT imaging was done using FD OCT bulk system. The schematic of the FD OCT bulk system is shown in the figure below.

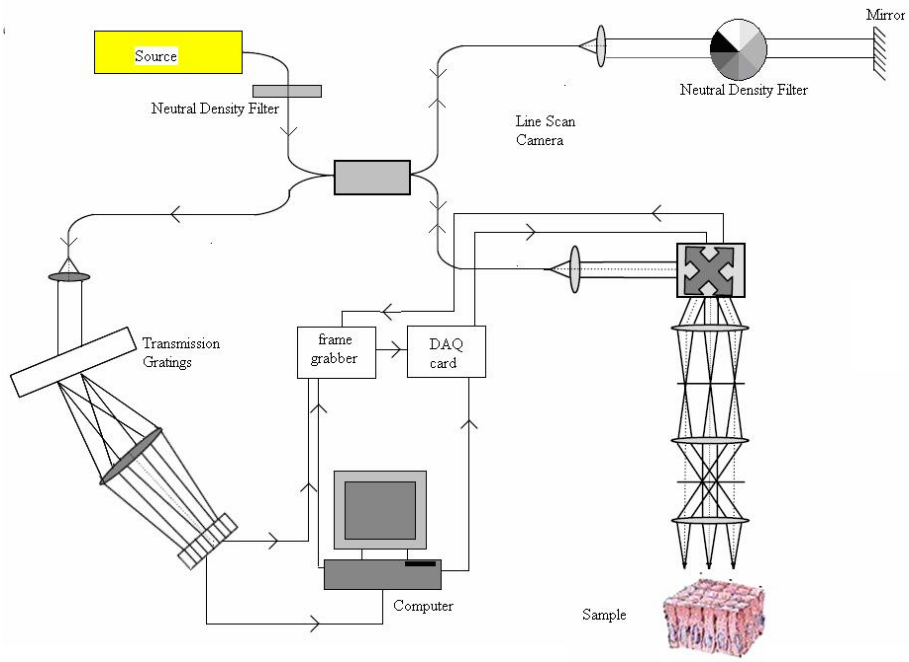

Figure 3-2: OCT System Setup

The source is a broadband Ti-Sapphire laser (Kapteyn- Murnane Laboratories, Boulder, CO) which is pumped using a green laser with center wavelength of 532 nm. The source is capable of lasing in the wavelength range of 700-900 nm. Before imaging the laser is mod-locked at 810nm center wavelength and with broad spectrum from 730nm to 860nm. The laser output is attenuated using neutral density filters and is coupled into the fiber based Michelson's interferometer setup. A 50:50 coupler splits light in to sample and the reference path.

Reference path has a reflecting mirror and neutral density filter. Light in the reference path reflects back from a mirror. Neutral density filter is used to manipulate the light reflected back to detector.

The sample arm consists of XY galvo scanning assembly and light is focused using lens on to the sample. Light reflected back from sample combines with the light reflected back from the reference mirror and is detected by spectrometer.

The spectrometer has lens, diffraction grating and line scan camera. The camera has array of 2048 pixels and scans 18587 lines per second. Image data is displayed and acquired using LabView VI.

3.2.2 In Vivo OCT Probe

The bulk FD OCT system has its limitations for in vivo imaging sue to its size. In Vivo OCT probe was designed to facilitate OCT imaging in vivo. The figure below shows the OCT system with in vivo probe.

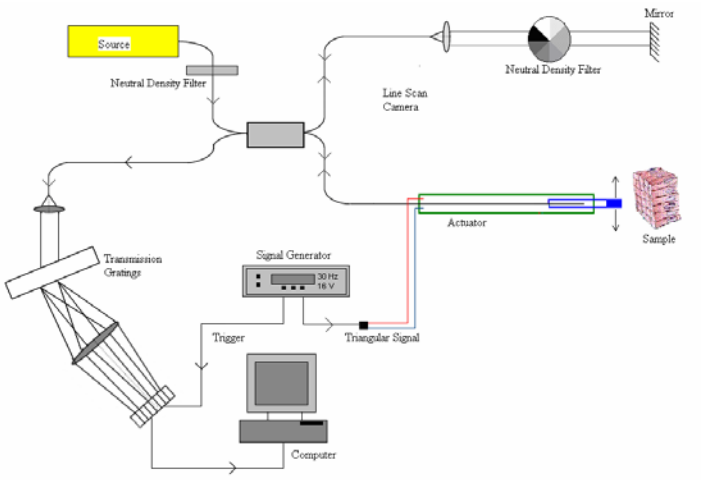

Figure 3-3: OCT System Setup, Invivo Probe

The only change in the system is sample path. The in vivo probe was designed in the lab. The piezo is a scanning device in the probe. The optical system of the probe has angle cleaved fiber with green lens in a glass ferule. The optical system is placed on the piezo actuator. When the voltage is appliedto an actuator, it moves and beam scans the sample. The figure 3-4 shows the design of sample probe.

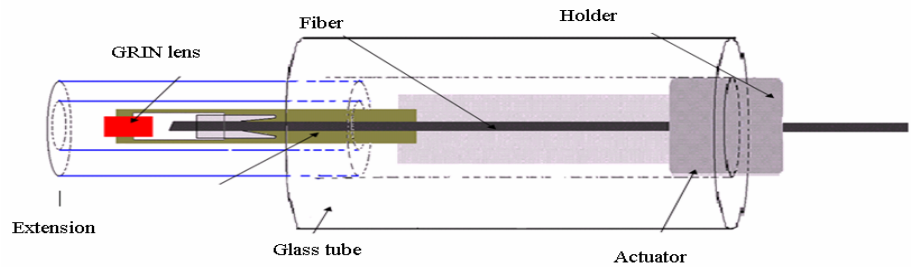

Figure 3-4: In Vivo Probe

The size of this probe is approximately 20mm wide and it is attached on the flexible arm of a lamp. This probe assembly reduces the size of the sample arm and enables fast rate in vivo imaging Due to its compact size; it is possible to use this probe for imaging human oral cavity.

The bulk FD OCT system was used to perform ex vivo tissue sample. OCT imaging of human oral cavity was done using in vivo OCT system. The following chapter describes the sample preparation and experimental set up for tissue imaging.

CHAPTER 4

IMAGING PROTOCOL

Optical coherence tomography OCT is an emerging biomedical imaging modality that can generate micron resolution, cross-sectional images of tissue microstructure *in situ* and in real time.[16]-[17] Initially we focused on *ex vivo* OCT imaging to establish correlation with histology and for feasibility in *in vivo* OCT imaging. *Ex vivo* imaging of tissue of larynx and oral lesions was done. With the development of fiber optic imaging probes, *in vivo* OCT imaging of human oral cavity was possible. Since, exact correlation between *in vivo* OCT images and histology is difficult, the images were compared with standard histology images of the same tissue sites.

4.1 Tissue Selection And Preparation

Optical Coherence Tomography was performed on ex vivo tissue. The tissue were obtained in the RPMI tissue media and were images within 48 hours of tissue extraction.

4.1.1 Tissue Optics Interaction

Biological tissues are optically inhomogeneous and act as absorbing medium whose average refractive index is higher than that of an air. This causes partial reflection of light or radiation at tissue air interface. The remaining light penetrates and

multiple scattering and absorbance occurs. Major part of the light is back reflected and dispersed due to bulk scattering. Light incident on tissue may be transmitted, reflected, refracted, scattered and/or absorbed.

Absorption of light may cause heat, chemical/conformational change, fluorescence, phosphorescence. Absorbance of light greatly depends on water content of tissue and predominant absorption centers. Light from Laser is coherent and laser tissue interaction can cause thermal effect, mechanical effect, Photo-ablative effects and or Photodynamic effects.

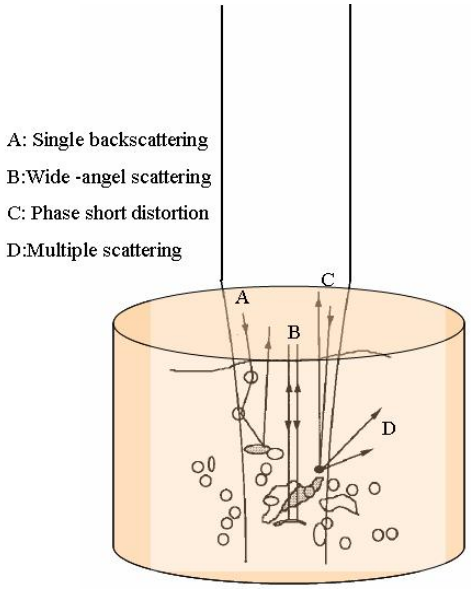

Figure 4-1: Light Propagation In Tissue

In complex materials, any combination of interactions is possible. The exact nature of each process depends on the physical and chemical structure of the tissue.

4.1.2 Tissue Sample and Subject Preparation

Initially ex vivo imaging was performed on tissue sample. For ex vivo imaging tissue samples were obtained from Co-operative human tissue network (CHTN), a non profit tissue bank. The tissue samples were extracted from surgery or autopsy and were kept in to RPMI medium (Roswell Park Memorial Institute medium), after extraction. Imaging was done within 48 hrs of tissue extraction. For OCT imaging tumor of larynx and matched normal tissue was obtained. Tissue samples were kept in Petri dish while imaging. The average tissue scanning time for a tissue sample of average size 10mmx10mm was 3 hours. Tissue was immediately kept into 10% Formalin solution after imaging. To avoid desiccation, few drops of RPMI were added on tissue during the experiment.

Another set of tissue sample from benign oral lesions were obtained for ex vivo imaging. These tissue samples were remaining part of the excision biopsy samples and were kept into 10% formalin solution prior to imaging. Since, tissue structure remains intact in formalin, the time period between tissue extraction and imaging was not fixed. This tissue samples were kept in to Petri dish during imaging. The average size of benign tissue samples were 5mmx5mm and average imaging time was 1 hr. The tissue samples were suspected oral malignancies. Pathology report of tissue condition was obtained for samples for ex vivo imaging and no further patient or tissue related information was collected.

The in vivo OCT system was developed to perform in vivo imaging. In vivo imaging was done on the healthy human oral cavity and images were obtained from various sites of the oral cavity. No chemicals were applied or introduced prior to or after imaging in vivo tissue.

4.2 Experimental Setup

4.2.1 System Setup Before Imaging

Ti:sapphire (Kapteyn - Murnane Laboratories, Boulder, CO) laser source capable of lasing from wavelength range from 700-900nm is the source of OCT system used for imaging experiments. This laser is pumped using 1032 frequency doubled Nd:Yag solid state. The output of Ti:sapphire can be manipulated using software controlled prism movement within laser cavity. Using the prism position, Ti:sapphire laser output was mod locked at center wavelength around 810 nm and with broad spectrum with FWHM of 40 nm. Part of the beam is coupled into the spectrometer to monitor and see the spectrum of light. Before laser output is coupled in to Michelson's interferometer, the light is attenuated using neutral density filters. Three different neutral density filters with transmission of 4.6%, 15 % and 52% respectively were used to manipulate the light power coupled into the system. 80% light was coupled into the fiber based Michelson Interferometer system using tip and tilt stage.

The fiber based Michelson's Interferometer system consists of SM750 fiber with a mode field diameter of 5.9μm and a 50:50 fiber coupler assembly (Canadian Instrumentation & Research Ltd.). Angle polished fiber connectors (FC/APC) were used to prevent any back reflection of light at the connector end into the interferometer.

The sample path comprises of a collimator, XY Galvo assembly, an optical relay, and a microscope objective. To maintain common optical axis of all the components; the optical components were mounted on rods and cage assembly were made.

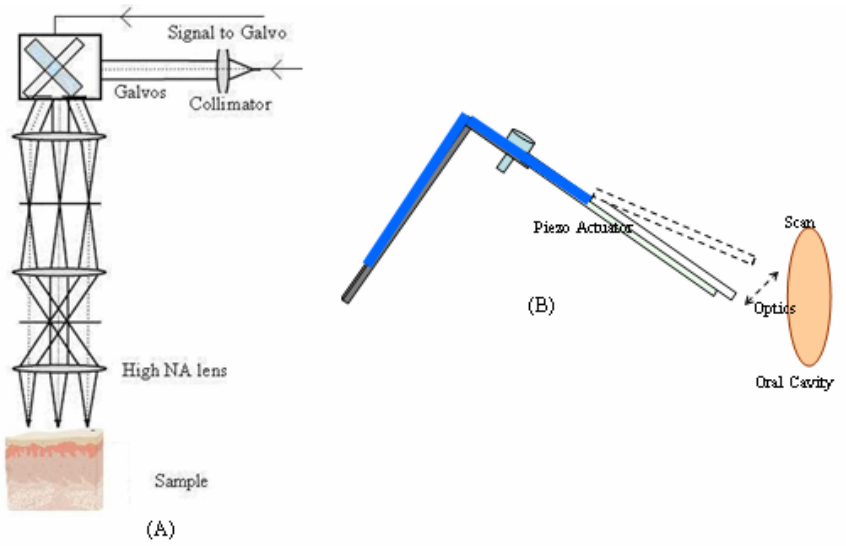

Figure 4-2: Sample Path, (A) Bulk OCT System, (B) Invivo OCT System

A galvo or galvanometer is an electromechanical voltage sensitive device that deflects the mirror mounted on a shaft in accordance to the voltage provided to it. Triangular wave drives the signal and amplitude and frequency of the signal is controlled using the Lab view software. Amplitude to the Galvo decides the scan length and frequency decides the frame rate of imaging. The back reflected light from the sample is collected by the objective lens with high NA and coupled back to the collimator.

For ex vivo imaging tissue sample is placed in a Petri dish on XYZ stage system which enables to position the sample and to change the sample position with micrometer precision in 3 dimensions.

Reference path consists of collimator and a mirror. Light reflected back from the mirror is coupled back in to the collimator. To control the power of light from the reference path, a variable neutral density filter is placed between mirror and the collimator.

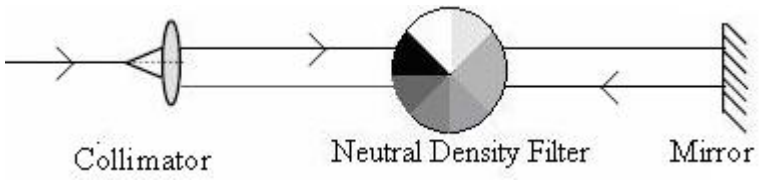

Figure 4-3: Reference Path of OCT System

Spectrometer built in the lab using a collimator lens, diffraction grating and camera lens and a line scan camera makes the detector of the system. Line scan camera is positioned on the XYZ stage system to enable to direct the separated wavelengths on the pixels of the camera.

Prior to imaging tissue sample or subject, mirror is placed in the sample path. To get the sample position at focus; reference path is blocked initially and sample mirror is moved until maximum light is coupled back into the same. Reference mirror position is then adjusted to get maximum intensity interference fringes. Thus system is optimized before imaging tissue.

4.3. Imaging Tissue Sample And Subject

Once the system is ready for imaging experiment, tissue sample is placed in the Petri dish for imaging. It is important to take all preliminary precaution like wearing gloves, cleaning place with acetone, before imaging to reduce imaging time.

4.3.1 Scanning Tissue Sample

For ex vivo imaging tissue is placed in the sample path. Figure shows the tissue scanning method.

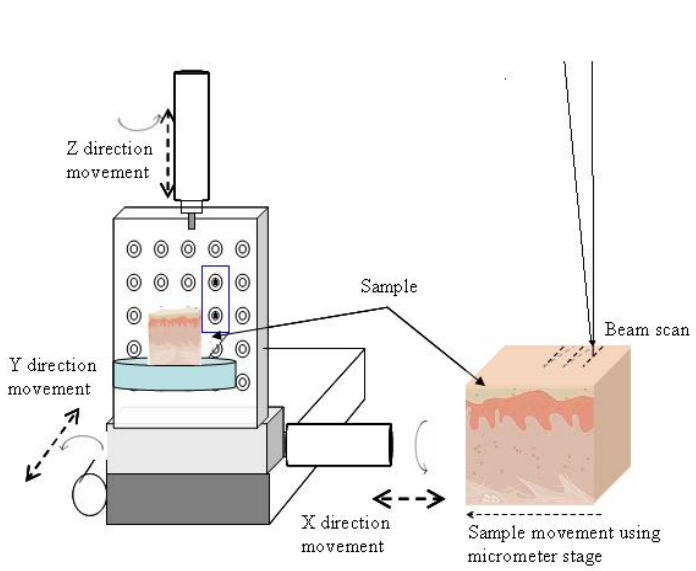

Figure 4-4: Sample Scanning During Imaging

Sample is placed on the platform attached with XYZ stage system. During imaging Y galvo scans the tissue in one direction, and image of that scan is recorded. After each Y scan, tissue was moved using micrometer stage in X direction, and another Y scan was recorded as shown in the figure. Each scan is a 2d image which contains depth

information. During ex vivo imaging tissue was marked with Tissue Marking Dye (TMD Cancer Diagnosis) for the scanning location. This can be helpful to obtain histology image from the exact site of OCT imaging. Each sample was scanned line by line.

4.3.2 Imaging Subject

For in vivo imaging compact size OCT probe, able to image directly from subject, was developed in the lab by colleague. Movement artifact was the major problem while doing in vivo imaging. The figure below shows the method of in vivo imaging. The probe was directly placed on the site of imaging. For each image 1 second data was recorded.

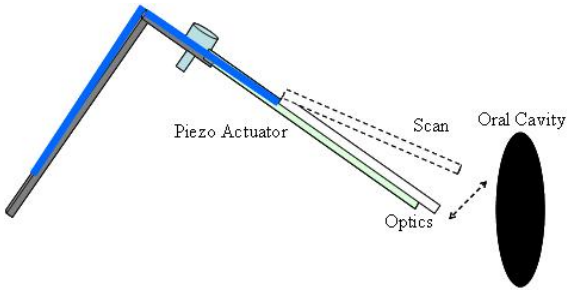

Figure 4-5: Scanning Using In Vivo Probe

Images were acquired and displayed using Labview software. Each scan was 1 sec data and was stored as binary file.

4.4 Image Processing

Image Processing of OCT images was done using MATLAB 7.0. The spectrometer designed in the lab acquires the OCT data and is saved as binary data.

4.4.1 Acquisition Parameter

The CCD line scan camera is main component of the spectrometer. There are 2048 pixels in the CCD camera array and each pixel is a 14 micron square with a 12 bit resolution. Each pixel saved 2 byte of data. The pixel array can be read out at rate of 40MHz or 60 MHz when run on the internal clock of the camera (free run mode). In the free run mode, the maximum number of line scans that can be acquired is calculated using the formulae below:

Line period = data rate period x (No. of pixels + No. of periods for charge transfer)

Line Frequency f_L = 1/Line period.

To start camera acquisition exactly at the time galvo or piezo actuator device starts scanning the sample, - galvo position signal and piezo trigger signal was used to trigger acquisition. As shown in figure 4.6 a trigger to camera acquisition was synchronized with galvo position. Signal to galvo decides the acquisition parameters.

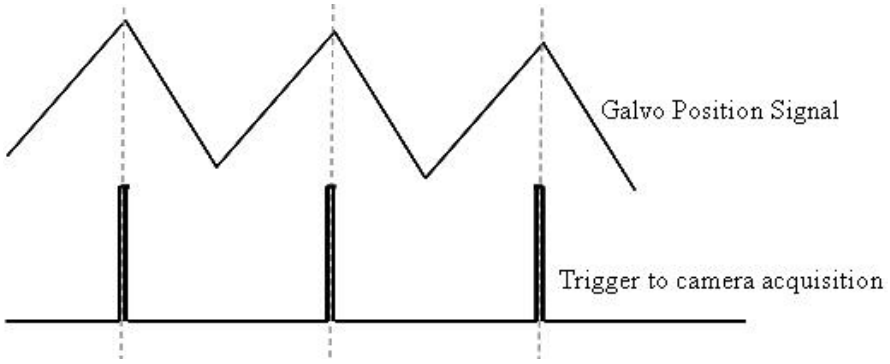

Figure 4-6: Galvo Position and Camera Acquisition Synchronization

For example, at 30Hz galvo frequency, the total number of frames acquired in one second is 60. Camera acquired total of 18587 lines per scan. From this

Total numbers of lines per frame = total lines acquired by camera in one second/ total frames. For 30Hz galvo frequency, camera acquires 310 lines per frame. During data acquisition and processing it was observed that the actual galvo frequency is little bit different from the signal fed, because of the mechanical friction and motion lag.

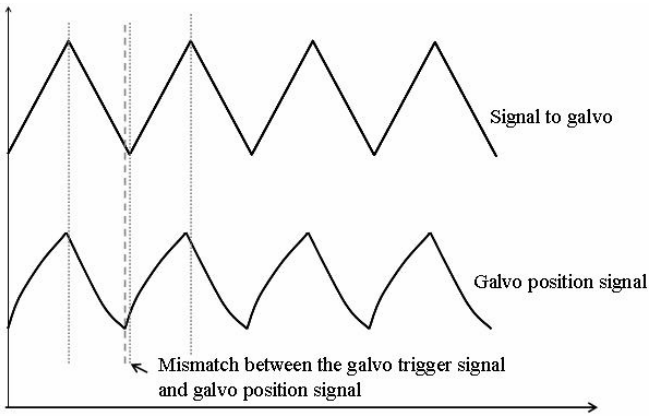

Figure 4-7: Mismatch between Galvo Trigger Signal and Galvo Position Signal

While processing data, images from each frame data was created. To compensate for the mismatch in the galvo position and galvo trigger signal, actual time to complete one galvo cycle was calculated using oscilloscope. From the galvo position signal, time of the one frame is calculated. During processing data of one frame is read using this time calculations.

To read data of one frame following calculations are considered. One line has 2048 pixels and each pixel reads 2bytes of data.

Data in one line = 2048* 2 bytes

Thus one image is made of; total lines in one frame*4096 bytes of data. For example, if galvo is run at 30 Hz frequency, to total numbers of frames achievable in one second will be 60. Each frame is one scan and there are 310 lines /frame. And 310*4096 bytes makes one scan or frame of data.

4.4.2 Image Processing Algorithm

For each tissue position 1 second data was recorded and processed. While processing one frame data is read and processed. Background noise and back reflection of light creates major noise problem in images. This noise reduction is done in image processing modules. In addition to tissue scan data, image data of only sample arm and only reference arm are recorded separately. To image sample only data, light from reference arm is blocked so light from sample arm only reaches to detector. Similarly, to image reference only data, light from reference arm is blocked so that light only from the reference arm reaches to detector.

Image processing was done using MATLAB. There are 3 data set, tissue scan, reference only data and sample only data is selected. One frame data is selected and intensity values at different wavelengths λ; are converted into k space. Linear interpolation is performed using following equation.

This equation is achieved doing spectrometer calibration. FFT is performed on k values and sample only and reference only data are subtracted from image data. This gives background noise subtraction. Further 4 frame rolling average is performed during

the imaging to reduce the speckle noise from the final image. Movie is created for each scan.

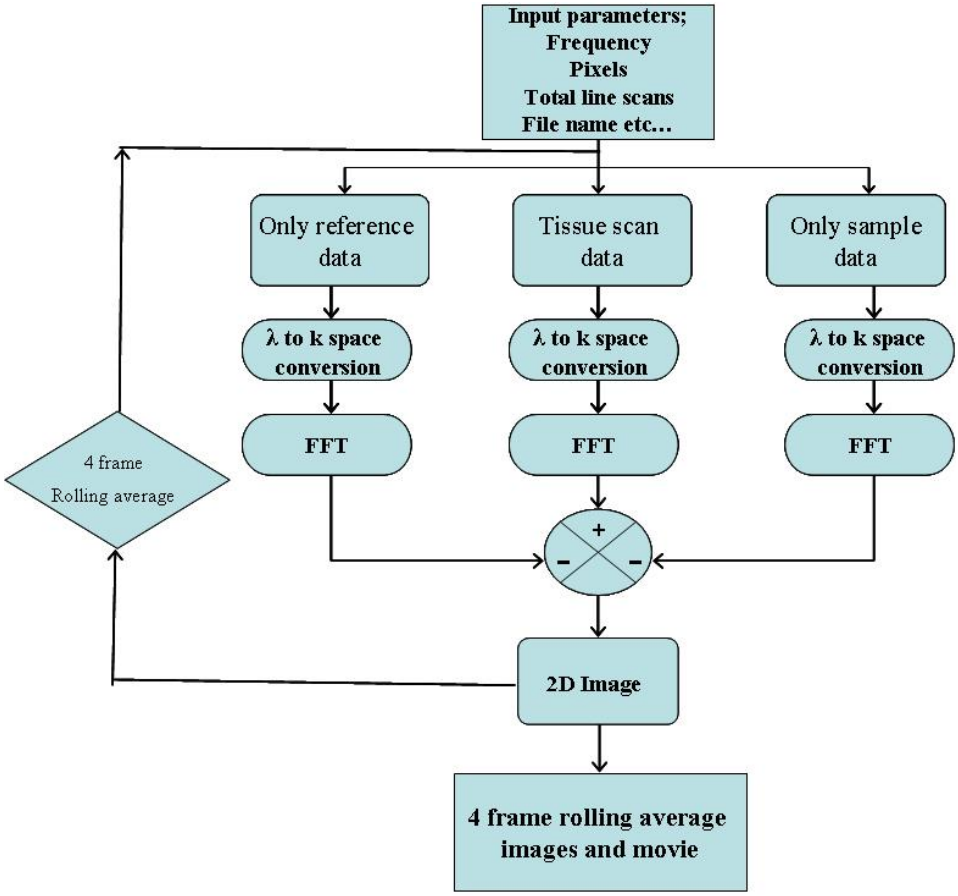

Figure 4-8: Image Processing Algorithm

OCT imaging of ex vivo sample of oral benign lesions were done. This tissue samples were stored in 10% formalin solution prior imaging. Also in vivo images of healthy human oral cavity were obtained.

CHAPTER 5

RESULTS AND DISCUSSION

SD_OCT system capable to take 2d images of tissue was designed . Image processing module was improved to enable video rate imaging. The SD OCT system capable to take real time in vivo images is demonstrated. OCT imaging experiments were performed on ex vivo tissue samples. The resulting images had very little morphological information present in them. Since tissue preservation methods, RPMI and 10% formalin solution, change optical properties of tissues, the lack of morphological information in ex-vivo images was attributed to these techniques. This led us into conducting in-vivo OCT experiments.

Miniature OCT probe was designed in the lab for in vivo imaging.

The system is capable to give real time in vivo images.

The future work of this project involved real time imaging of human tissue and comparison of OCT images with gold standard video rate images. The ability of OCT to provide tissue morphology structure shows its potential applications in early detection of cancer and disease monitoring.

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BIOGRAPHICAL INFORMATION

Nidhi Mehta was born on 22nd April, 1981 in Rajkot – Gujarat, India. She completed her schooling from Alembic Vidyalaya in Vadodara, India and received her Bachelor's degree from Saurashtra University, India in 2003. She served as lecturer in U V Patel College of Engineering for short period of time.

In fall 2004, she started her graduate studies in Joint Program of Biomedical Engineering at the University of Texas at Arlington and University of Texas Southwestern Medical Center at Dallas. She joined the biomedical optics lab as a Graduate Research Assistant and worked of application of Optical Coherence Tomography on tissue imaging.