HIGH RESOLUTION IMAGING OF NEOPLASIA USING OPTICAL COHERENCE TOMOGRAPHY

by

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They were filming the Hitchcock episode where the outline of his profile is drawn on the wall. He must walk up to it and fit it exactly. He keeps failing to do so. They retake the scene ...

We don’t all dress to suit the work we do. I, for example was in a straitjacket for the cooking lesson dream …

From the poem Signal-to-Noise
by Miriam Goodman

Καρτερούμε μέρα νύχτα να φυσήσει ένας αέρας
Στον τόπο που ν' αναμένει τζι εν τοιχία ποτέ δροσιάν
Για να φέρει καρτερούμε το φως τζιήνης της μέρας
Πο'ν να φέρει στον καθ' έναν τζι δροσιάν τζιαί ποσπασιάν

Από το ποίημα “Καρτερούμε Μέρα Νύχτα”
tου Δ. Λιπέρτη
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ABSTRACT

Diagnostic imaging technologies for the detection of cancer include CT, MRI, ultrasonography, and endoscopy. However, many early neoplastic changes remain beyond their detection limits. A modality capable of imaging at or near the cellular level could detect disease at earlier stages than currently possible and thus improve patient prognosis. Optical coherence tomography (OCT) can achieve resolutions in the cellular and subcellular range (1-15 \( \mu \text{m} \)) and could improve the diagnostic range of clinical imaging procedures.

The research described in this thesis includes the ex vivo imaging surveys, technology development and clinical studies necessary to demonstrate the feasibility of OCT imaging for the in situ diagnosis of premalignant and neoplastic lesions. The first step in this endeavor was to evaluate the performance of OCT using ex vivo tissue specimens and identify the features in the OCT images which can be used for the diagnosis of disease. The development of the technologies necessary to introduce OCT to clinical settings, including a portable system and imaging devices, ensued. Enhancements in the post processing and visualization algorithms were introduced and the feasibility of ultra-high resolution and spectroscopic imaging was evaluated. In vivo OCT imaging, to assess the performance of the technology in clinical scenarios, followed in two systems, the cervical and oral mucosa.

OCT can function as a type of optical biopsy to yield image information with resolutions approaching that of conventional histopathology \textit{in situ}, without the need for excision and processing. OCT can be integrated to a wide range of clinical instruments including endoscopes, catheters, laparoscopes, and surgical probes. A role for OCT is envisioned in clinical scenarios such as the follow-up management of patients with cervical intraepithelial neoplasia, on tamoxifen, with gastro-esophageal reflux or Barrett’s and also guidance of surgical procedures in sensitive areas. Further improvements of the OCT technology will most likely enhance the abilities of the technique and may eventually lead to its establishment as a powerful imaging modality for the diagnosis and management of cancer.

Thesis Supervisor: James G. Fujimoto
Professor of Electrical Engineering and Computer Science
Dedication

Θα ήθελα να αφιερώσω την διατριβή αυτή στην μητέρα και τον πατέρα μου που περιμένουν ακόμα την αποφοίτησή μου. Σας υπόχωμα να τελειώσω σε δύο χρόνια!

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PART I - INTRODUCTION

CHAPTER 1 - NEOPLASIA, CANCER AND IMAGING

Every year 1.2 million new cases of cancer are detected in the US alone, resulting in 560,000 deaths with lung, prostate, breast and colon leading in mortality. [1] One in three of all people living in the US now will develop cancer and one in five will die of the disease. These numbers have doubled over the past 30 years and are surpassed only by the number of deaths due to cardiovascular disease. [2] These alarming statistics have prompted much research effort in the diagnosis and treatment of cancer with a clear understanding that early diagnosis is paramount to increase the survival chances of the patient. A number of imaging techniques have been employed in the fight against cancer to better understand the disease process, features and consequences and to more effectively guide diagnosis and treatment. Optical Coherence Tomography (OCT), which can image tissue microstructure in vivo and in situ, could be a powerful addition to the armamentarium of diagnostic tools for early detection of cancer. [3, 4]

Biology of cancer

According to R. A. Wills' formal definition, a neoplasm "is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissues and persists in the same excessive manner after the cessation of the stimuli which evoked the change." [5] The current understanding is that cancer begins at the level of individual cells with a multi-step process which includes the mutation of proto-oncogenes and tumor suppressor genes leading to abnormal, incomplete and uncontrolled proliferation and differentiation. Genetic factors, chemical carcinogens, radiation and viruses have all been implicated in the development of cancer. [6] Although the likelihood that a given cell will accumulate all the mutations which lead to neoplasia is infinitesimal, the large number of divisions and cell cycles in an individuals lifetime make the chance of developing some form of cancer a definite possibility. Once proper control of the cell cycle is impaired, the cell proliferates into an ever growing colony of abnormal, usually monoclonal cells. [7] These colonies expand around capillaries. Due to O₂ diffusion limitations they can only grow up to a few hundred microns around the existing vascular network to a total of about 2 mm diameter. [8] For the tumor to increase in size above 2 mm, neovascularization, the proliferation of a new vascular supply, initiated and promoted by growth factors secreted by the neoplastic cells, must occur. [9] Pressure from the growth of the tumor mass, decreased tumor cell cohesiveness and increased tumor cell motility, coupled with the destruction of host tissue by tumor enzymes, lead to invasion of adjacent blood and lymph vessels by tumor cells and metastasis to distant organs. [10] Survival rates significantly decrease once metastasis has occurred. Although heredity does plays a role in the development of cancer (accounting for about 5% of all cancer cases) a large fraction of malignancies is actually due to avoidable factors. [11]

Management

Cancer management involves more than treatment. Rather it refers to a spectrum of care that includes prevention, screening, detection, diagnosis, counseling, psychosocial support, specific therapeutic interventions, rehabilitation and terminal care.

According to the American Cancer Society, "one way to reduce the human suffering, early deaths and economic loss due to cancer, is to avoid exposure to known carcinogens. Through early detection and diagnosis of cancer, the prospect of cure using available treatment modalities can be improved." [2] Cancer prevention is by far the easiest and most effective way to fight cancer. Diet and smoking habits, hormone control and/or replacement have all been implicated in enhancing the carcinogenic process and
can all be modified to minimize the risk of disease. An addition to our weapons against cancer is also the possibility of using chemopreventive agents such as proper diet, retinoids, carotenoids and NSAIDS. [12] Where prevention fails, screening can be employed to detect cancer at an early and treatable if not curable stage. [7] However, for a screening test to be successful it must detect cancer earlier AND there must be evidence that earlier diagnosis will result in an improved outcome. [10] The types of neoplasias that are amenable to screening are those characterized by substantial morbidity and mortality, high prevalence in a detectable preclinical state, the possibility of effective and improved treatment because of the early detection, the availability of a good screening test with high sensitivity and specificity, low cost and little inconvenience and discomfort. [7] Unfortunately, to this date, only cancers of the breast, cervix, skin, colon-rectum, prostate and testes have widely accepted screening interventions and there is still controversy over some aspects of each. [13]

In cancer diagnosis, signs and symptoms, cytological and imaging tests have all been employed but the medical community still feels that "there is no foolproof test for malignant disease. The diagnosis of cancer may be suspected from the history and physical examination and supported by subsequent investigations, but it can be confirmed only by histologic investigation of a representative sample of the lesion. The importance of microscopic proof of malignant disease can not be overemphasized." [14] To that end, a variety of biopsy techniques have been developed to confirm the diagnosis of malignancy. They include fluid cytology, needle aspiration cytology/biopsy, core needle biopsy and open (excisional or incisional) biopsy. The choice of the technique depends on the amount of the material required by the pathologist and the location of the tumor. Radiologic, endoscopic or sonographic techniques can be used to guide biopsies targeting smaller tumors to enhance the likelihood of successful tissue harvest.

Once a histologic cross-section is available, an attempt is made to grade the lesion and classify the cellular proliferation as benign or malignant or perhaps list it in the nebulous gray zone of "atypical" or "borderline malignant". The histopathologic grade is the degree to which the tumor resembles normal tissue of its histopathologic type as well as degree of differentiation and number of mitoses, which presumably correlate with the tumor’s aggressiveness. This assessment is based on morphologic criteria and additional subjective features that can help predict the possible biologic behavior of a particular growth process. The differentiation of benign from a malignant tumor can be made morphologically based on cell differentiation and anaplasia (i.e. lack of normal differentiation), rate of growth, local invasion and/or metastasis. Differentiation refers to the extent to which parenchymal cells resemble comparable normal cells, both morphologically and functionally. In general, all benign neoplasms are well differentiated, whereas, in contrast, malignant neoplasm range from well differentiated to undifferentiated. One of the signs of abnormal differentiation is pleomorphism (i.e. variation in cell size and shape) characterized by cells ranging from giant to small, primitive-looking with disproportionately large nuclei and nuclear-to-cytoplasmic ratios (NCR) approaching 1:1 instead of the normal 1:4 to 1:6. Large degree of cell division and proliferation marked by an increased number of mitoses is also a sign of abnormal differentiation. Most benign tumors grow slowly over a period of years, whereas most cancers grow rapidly, sometimes at an erratic pace, and eventually spread and kill their host. Nearly all benign tumors grow as cohesive, expandable masses that remain localized, sometimes encased in a fibrous capsule. Cancers grow by progressive infiltration, invasion and destruction of the surrounding tissue. Metastases, tumor implants discontinuous with the primary tumor, unequivocally mark a tumor as malignant since benign neoplasms do not metastasize. [15] For malignant tumors, one of the more pivotal determinants of behavior is the tumor cell lineage. Morphologic criteria for identifying the origin have included cell size and shape, growth pattern and presence of pigment. Lately immunohistochemistry has made the identification of the cell lineage of some tumors easier. A significant proportion of tumors defy classification and are relegated to the "undifferentiated" category. [14]

Once a tumor is detected and graded, there is an attempt to determine the stage of the disease. The objective of staging in cancer is to provide a basis for planning appropriate therapy, estimating prognosis,
evaluating the results of therapy, determining the biologic behavior of tumors and also provide a common framework for comparing patient populations and outcome between different centers. [14] The standard of staging throughout the world is now based on the TNM system which describes the anatomic extent of a cancer. T refers to the extent of the primary tumor, N to the presence of regional lymph node metastases and M to the presence of distant metastases. The histologic type and grade (G) and the patients overall medical condition (H) are also documented and taken into consideration in determining the life expectancy and deciding the most suitable treatment. [14, 16, 17] Most therapies, however, have been empirical and we are just now discovering the biologic basis of the malignancy. Treatment of established cancers is determined by the stage and anticipated behavior of the tumor. [16]

Histopathologic grading may be the “golden standard” of cancer diagnosis but it suffers from certain limitations. The success of the diagnosis depends on adequately sampling the tissue under investigation and providing a representative sample to the pathologist. If the number of biopsies or their location are inadequate, the result will be a false negative reading, with the ramifications that follow a missed diagnosis. [18, 19] Research has been motivated by the need to improve the performance of biopsy-based diagnosis, including studying the optimal number of biopsies required to maximize the likelihood of correct diagnosis of certain malignancies, the amount of tissue material required, the effect of proper personnel training etc. [20, 21] Some imaging modalities are also employed to more effectively guide biopsies to suspect areas, if such areas have been identified. The choice of imaging modality depends on the size and nature of the neoplasia under investigation. Endoscopy, Ultrasound, CT and MRI are now routinely used to direct the harvest of tissue from neoplastic areas. [22] Another area imaging is influencing is the effort to minimize the number of biopsies taken from each site. This effort is driven by financial and resource constraints but it is also necessitated by the need to minimize the damage to the parenchymal tissue, especially when sampling cardiovascular or neural organs. [23, 24]

**Imaging techniques in cancer management**

Several imaging modalities have now become mainstay in cancer diagnosis and management. Unfortunately, the only technology that has been proven effective enough for screening and is now widely accepted as such, is plain film radiography of the female breast (mammography). Other modalities are, however, routinely used for staging cancer in affected individuals. Most are used for detecting or delineating the disease locus and have developed to the point where they have a significant impact on patient management. The diagnostic accuracy of each technique varies depending on the type of neoplasia being imaged but also on expertise and equipment. The interpretation of some of these imaging results is made even more difficult by the lack of baseline imaging studies, except in the rare cases where the same imaging study was performed before for, perhaps, a different reason. Surveillance imaging studies are also now becoming more common in an attempt to monitor tumor response to treatment, guard against relapse and detect the presence of residual disease. A common problem that plagues all the currently available imaging systems is their inability to detect the tissue origin and grade the lesion, mass, cyst or other architectural anomaly, that they have detected. Some imaging modalities have, however, emerged as powerful diagnostic tools and are briefly described below, indicating the limitations of each that a high resolution imaging technique such as OCT may address.

**Endoscopy**

Endoscopy, the direct imaging of the surface of luminal structures, is an expanding field which has revolutionized the diagnostic ability of clinicians to identify and treat more effectively respiratory and gastrointestinal malignancy. The utilization of state of the art video and fiberoptic technology allows detailed survey of luminal surfaces and, in association with biopsies, can lead to diagnostic accuracy of 95% in the case of some gastrointestinal cancers. [25] However, the effectiveness of endoscopy depends on the number of biopsies taken, which sometimes has to be quite high, and on other factors such as personnel training and expertise. [26, 27] Endoscopy also fails to detect the subsurface margins of tumors,
rendering a complete evaluation of the degree of infiltration of the disease in real-time, impossible. [28] The effectiveness of endoscopy also relies on the superficial features of the lesion, resulting in high yields for raised lesions but poor performance for other types. [29, 30] The size of the endoscope is also prohibitive when it comes to the investigation of small lumens such as the finer branches of the bronchial tree. [31] Laparoscopy is the minimally invasive use of an endoscope, usually solid, to probe into internal body cavities via small incisions on the surface. It is extensively used in surgical interventions and has resulted in impressive progress in the area of minimally invasive surgery, resulting in significantly reduced post-operative morbidity. Diagnostic laparoscopy, however, is indicated only in a small number of cases which include suspected liver tumors and metastases, palpable abdominal masses, staging of tumors and ascites of unknown origin. [32]

**Endoscopic Ultrasonography**

The development of miniature ultrasound transducers in the late 80s led to the introduction of Endoscopic Ultrasonography (EUS). Ultrasound probes, forward or side imaging, with diameters less than 3 mm are introduced through the accessory port of an endoscope and cross-sectional images of the tissue layer structure are obtained. The source of contrast, as in regular ultrasound, is the density of the layer. EUS was applied extensively in the gastrointestinal tract with an accuracy of preoperative tumor depth of invasion staging in the range of 80-90% which even surpassed CT. [33, 34] The recent introduction of high frequency (20 MHz) ultrasound transducers with improved resolution could further improve the diagnostic utility of EUS. [35] Unfortunately, EUS relies on the presence of a transducing medium and the presence of air renders the technique totally ineffective. This limitation is particularly restrictive in the pulmonary tract where the introduction of a transducing medium may be detrimental to an already compromised patient and limits the application of EUS only to imaging of lung tissue from the mediastinal site. [36] Inflammation and scarring make the interpretation of the EUS images more severe and make the use of EUS as a tool to monitor tumor response to therapy problematic. [37] EUS is also not able to differentiate between mucosal and submucosal tumors due to inadequate resolution. [38] EUS is also very user dependent although recent advances in technology and the introduction of more complete databases of abnormal findings have made the operation and interpretation of the images less difficult. [39]

**Imaging techniques with whole body penetration**

The advent of imaging apparatus that can penetrate through several layers of tissue and image the entire human body has revolutionized cancer management and diagnosis. As mentioned above, mammography, a unique variation of plain radiography, has become an increasingly accepted and effective screening tool for breast cancer. [40] Plain radiography and x-ray Computed Tomography (CT) are the imaging modalities of choice when it comes to cancer diagnosis. [41] Plain radiography is very effective in certain cases and is also very cost effective and widely available. CT combines the benefits of radiographs with higher resolution, lower radiation doses and 3-dimensional spatial information. Unfortunately those systems are still quite expensive and are not available at small, rural hospitals. The place of Magnetic Resonance Imaging (MRI) remains to be clearly defined. At present, it is probably the imaging modality of choice for the central nervous system and possibly for staging some musculoskeletal tumors. [42] For lesions in the abdominal or thoracic sites, MRI and CT scan appear to be equal in diagnostic and staging efficacy. Ultrasonography (US) is a target oriented imaging technique which is very effective for detecting biliary or renal obstruction, abdominal masses and ascites. It is also efficient in guiding percutaneous biopsy and drainage. This modality is very safe but very operator dependent and cannot be used whenever there is gas, bone or previous surgical scarring present or where the probe can not come in contact with the tissue. [14] Lack of organ specific contrast agents further limits US applications. Nucleotide imaging, like PET, has only found limited application in bone and thyroid tumor visualization. Along with Magnetic Resonance Spectroscopy (MRS), PET can also provide information on biochemical constituents and metabolism, but there is no clear clinical use as of these modalities at present time. All
the above techniques offer a unique advantage in their ability to aid staging and investigate and determine nodal involvement. Although OCT has shallow imaging depths and will not image deep tissue that these modalities can image, OCT has significantly higher resolution and provides a powerful adjunct which could be used for guiding excisional biopsy, grading early dysplastic or neoplastic changes, or identifying tumor margins. [43]

OCT image properties and early neoplastic changes

Optical coherence tomography (OCT) is analogous to ultrasound B mode scanning, except that measures the intensity of back-reflected infrared light rather than sound waves [3]. OCT can achieve resolutions of 1 to 10 μm, one to two orders of magnitude better than conventional ultrasound. Optical coherence tomography was first applied to image optically transparent structures such as the anterior eye and retina. [44] Subsequent advances have enabled high resolution imaging in nontransparent tissue, including the identification of pathology in the cardiovascular system, skin, and nervous system. [45-47] Numerous studies have been performed which investigate the application of OCT for imaging the gastrointestinal, pulmonary, urinary, female reproductive tracts [48-51]. The application of OCT for in surgery and surgical guidance has also been explored. [52] OCT imaging devices have been developed including catheters, endoscopes, laparoscopes, and hand held surgical probes [53, 54] Recently, real time, in vivo endoscope/catheter based imaging of the gastrointestinal tract, pulmonary tract, and arteries, have been performed in an animal model. [4, 55] Preliminary studies of imaging neoplasia have been performed in patients. [56, 57] More extensive studies including both cross sectional and horizontal studies are currently underway in a number of research groups.

Several features of optical coherence tomography suggest it will be a powerful imaging technology for the diagnosis of a wide range of pathologies.

1. OCT can image with axial resolutions of 1-10 μm, one to two orders of magnitude higher than conventional ultrasound. This resolution approaches that of histopathology, allowing architectural morphology as well as cellular features to be resolved. Unlike ultrasound, imaging can be performed directly through air without requiring direct contact with the tissue or a transducing medium.

2. Imaging can be performed in situ, without the need to excise a specimen. This enables imaging of structures in which biopsy would be hazardous or impossible. It also allows better coverage, reducing the sampling errors associated with excisional biopsy.

3. Imaging can be performed in real time, without the need process a specimen as in conventional biopsy and histopathology. This allows pathology to be monitored on screen and stored on high resolution video tape. Real time imaging can enable real time diagnosis, and coupling this information with surgery, can enable surgical guidance.

4. OCT is fiber optically based can be interfaced to a wide range of instruments including catheters, endoscopes, laparoscopes, and surgical probes. This enables imaging of organ systems inside the body.

5. Finally, optical coherence tomography is compact and portable, an important consideration for a clinically viable device.

Diagnostic indicators of early neoplastic changes include accelerated rate of growth, mass growth, local invasion, lack of differentiation and anaplasia and metastasis. Evaluation of all of these structural and cellular features is necessary for the correct identification and grading of neoplasias and should be addressed by OCT. Macroscopic changes, such as microstructural changes, architectural morphology, and tumor growth, are relatively easy to identify since they fall within the resolution limits of most of today's OCT systems. Imaging cellular features, however, is more difficult but its diagnostic importance can not be over emphasized.

There are three general application scenarios that are envisioned for OCT in neoplastic diagnosis. First, guiding standard excisional biopsy to reduce sampling errors and false negative results. This can improve
the accuracy of biopsy as well as reduce the number of biopsies that are taken, resulting in a cost savings. Second, after more extensive clinical studies have been performed, it may be possible to use OCT to directly diagnose or grade early neoplastic changes. This application will be more challenging since it implies making a diagnosis on the basis of OCT rather than conventional pathology. Other applications include situations where OCT might be used to grade early neoplastic changes or determine the depth of neoplastic invasion. Third, we hope to find scenarios where diagnosis can be made by OCT alone, enabling diagnosis and surgical guidance to be performed in real time. This would enable OCT diagnostic information to be immediately coupled to treatment decisions. The integration of diagnosis and treatment could reduce the number of patient visits, yielding a significant reduction in health care costs and improve patient compliance.

Each of these application scenarios requires a different level of OCT performance not only in its ability to image tissue pathology, but to achieve the required level of sensitivity and specificity in clinical trials for a given clinical situation. Generally speaking OCT can be used to resolve morphological features on several dimension scales ranging from architectural morphology or glandular organization (10-20 μm) to cellular (2-10 μm). Since cancer is a highly heterogeneous disease, characterized by a spectrum of morphological changes, etiologies, etc., we expect that the viability of OCT to be highly dependent upon the details of the specific clinical application. In the following sections some examples of neoplasias in different types of tissue and organ systems where OCT promises to have a clinical or scientific impact will be investigated.

**Imaging epithelial surfaces and mucosal layers**

Cells in tissue form organized structures that serve different functional purposes. Some cells specialize in forming epithelial layers. These are arranged on luminal sides of organs and play a role in the maintenance and equilibrium of the external milieu and serve as a barrier between the environment and the body. Epithelial surfaces are usually squamous, columnar or cuboidal. Squamous epithelium is characterized by flattened, plate-like cells and can be multi-layered (stratified) and sometimes keratinized. Columnar epithelium is composed of tall cells and can be stratified or pseudostratified (i.e. nuclei are at different levels and cells are variable in shape giving the appearance of stratified epithelium.) Epithelial structures of columnar origin exhibit another degree of variability between tissues. Epithelia in different organs may form crypts or villi which increase the surface area of the organ or form glands with secretory properties. These glands sometimes become occluded and when filled with fluids form cysts. Cuboidal epithelium as the name implies is made of cells that have a cubical shape. There are cases, some physiologic and some malignant, when epithelium changes from squamous to columnar, a stage described as transitional epithelium. This process is referred to as metaplasia. Most epithelial structures sit on a collagenous layer called the basal membrane. Cell proliferation and differentiation usually start at the basal membrane and as cells mature they migrate toward the luminal surface. The high mitotic activity close to the basal layer makes it a prime location for the development of cancer. Below the epithelium usually lies a loose connective tissue layer, the lamina propria, followed by layers of muscle and connective tissue which vary from organ to organ. Within those layers run vessels and nerves, and different types of lymphoid or inflammatory cells may also reside there. Necrotic tissue and scar formation are usually present in areas of injury.

Epithelia are prime locations for neoplastic changes which result in subtle or marked changes in tissue structure depending on the lesion type stage and degree of infiltration. A very common histologic type of carcinoma is what is referred to as adenocarcinoma. Adenocarcinoma is a derived from glandular tissue or other tissue in which tumor cells form recognizable glandular structures. Adenocarcinomas may be classified according to the predominant pattern of cell arrangement, as papillary, alveolar, etc., or according to a particular product of the cells, as mucinous adenocarcinomas. Squamous carcinoma
develops from squamous epithelia and has cuboid cells and is characterized by keratinization and often by preservation of intercellular bridges, but lacks glandular or cystic features.

The ability of OCT to accurately delineate and perhaps grade cancers will be partly based on its ability to detect the structural changes associated with neoplasia. The thickness of squamous epithelium can vary from organ to organ, but it is not unusual to see epithelial layers of up to 0.5 mm thick. Those structures are within the imaging capabilities of OCT and their thickness, as it relates to abnormal proliferation and differentiation, should be readily assessed. Even more important is the evaluation of the basal membrane. Its integrity is often used as the defining feature of in situ vs. invasive carcinoma and the distinction between the two types changes both the management of the disease and the prognosis of the patient. Although that membrane is less than a few micrometers thick it may be possible to evaluate the interface between the epithelium and the underlying lamina propria and deduce the membrane integrity. Columnar epithelium is very hard to evaluate since it is usually only one cell layer thick. (See discussion below on cellular imaging.) However, it is possible to image and evaluate the microstructure, the glands and crypts, which are usually associated with columnar epithelium. These structures can have dimensions up to a few millimeters, so changes in tissue architecture are well within the resolution limits of OCT systems. An epithelial variation that may actually prove more challenging, but which is very important to identify when establishing the diagnostic capabilities of OCT, is metaplasia. The epithelial changes associated with metaplasia have to be differentiated from early neoplastic changes to avoid an increased number of false positive diagnoses. To date, there has not been an adequate number of studies to evaluate such situations.

**Imaging Cellular features**

Although there is great variability in cell and nuclear sizes and shapes between different tissue types, each tissue is usually characterized by a unique cell population with defined morphological characteristics. These attributes may be common to all cells in a population or change as the cells mature. However, size, shape and orientation are conserved and exhibit common variation patterns between similar cells. The nuclear microstructure, such as degree of granularity or the presence of nucleoli, should also be similar. The rate of differentiation and proliferation of cells in certain tissues is also well defined. The number of mitoses (the presence of condensed DNA chromatin and the division of the nuclei and cells) is characteristic of the proliferative activity of the cells and should not deviate from the tissue norm. Any deviation in these morphological characteristics as well as mitotic failure, i.e. incomplete or abnormal cell division, is usually an indicator of uncontrolled proliferation and differentiation and abnormal transcriptional activity. These features are also indicative of the presence of neoplasia. While changes in architectural morphology and other larger scale features are often used as a first order diagnostic criteria for cancer, cellular and subcellular features are used to confirm the diagnosis. It is therefore important for OCT to prove its capability to image cellular and subcellular changes to increase its potential as a diagnostic tool and better aid the grading of malignancies.

Studies have been performed in an animal model (*Xenopus laevis*) which demonstrated the capability of OCT to perform cellular level imaging. [58] The observations of greatest clinical relevance were the ability to identify active cell division and assess nuclear-to-cytoplasmic ratios, two important markers of malignant transformation. The presence of multiple nuclei and cell membranes was also confirmed. The OCT image also illustrated cells with varying size and nuclear-to-cytoplasmic ratios. These undifferentiated cells ranged in size from 100 μm down to sizes below the resolution of OCT. Based on image measurements, cells as small as 10 μm in diameter could be imaged. A number of cells show sub-nuclear morphology such as the regions of increased optical backscatter within both the nucleus and cytoplasm. The OCT images also demonstrated the high backscattering observed from neural crest melanocytes and tissue structures. These findings are of great importance in cancer diagnosis but further improvement in OCT technology is needed for the extension of these results to human cell imaging.
Limitations of OCT

A very important shortcoming of OCT is the limited penetration depth. Attenuation and multiple scattering will probably limit the penetration to only a few millimeters for most scattering tissues, imposing an important constraint on OCT applications for the assessment of cancer. [59] Superficial imaging will probably be adequate for the evaluation of most epithelial cancers since early neoplastic changes begin in the superficial layers. The evaluation of deep invasion, though, may prove more challenging. The determination of invasion, especially beyond the submucosa/muscularis interface, changes both the patient prognosis and the aggressiveness of management considerably. It is very important for any diagnostic utility, including OCT, to be able to make that distinction. The limited penetration depth of OCT implies that its application will be limited to a specific class of diagnostic scenarios.

The current resolution limits of most OCT systems are adequate for the evaluation of tissue microstructure but cannot evaluate subcellular features. Since these features are of paramount importance for the identification and correct classification of cancer the resolution of OCT has to be improved. Recent technological advances in laser technology have allowed resolutions of the order of ~1 μm and may prove adequate for high resolution imaging of neoplasias. However, implementing even smaller resolutions becomes exceedingly difficult and the technological requirements exceedingly more severe. New novel techniques must be developed to allow better visualization of cellular characteristics.

The current OCT systems are also limited in speed of acquisition and limited delivery devices. Since these are mostly instrumentation problems and not a fundamental limitation of the technique, these limitations should be overcome in the near future.

Scope of the Thesis

The main goal of this thesis is to investigate the hypothesis that OCT can be applied for the in situ diagnosis of pre-malignant and neoplastic lesions. The research described here is part of several collaborative projects between Prof. James G. Fujimoto, of the Massachusetts Institute of Technology, Dr. Mark Brezinski, of the Brigham and Women’s Hospital, Dr. Annekathryn Goodman, of the Massachusetts General Hospital and Dr. James Mulshine, of the National Cancer Institute.

The contents of this thesis span a spectrum of topics that cover the transition of OCT imaging of neoplastic and cancerous tissues from the laboratory to clinical testing. They range from initial ex vivo imaging and technology development to clinical feasibility studies. The first step in this endeavor was to evaluate the performance of OCT using ex vivo tissue specimens. These experiments elucidated the potential and limitations of the technique and established the micro-structural and cellular markers, visible in OCT images, that are characteristic of neoplastic tissue. The development of the technology necessary to introduce OCT to clinical settings ensued. This included developing a portable system and delivery apparatus to seamlessly integrate OCT with current diagnostic modalities. The technology was tested both in the lab and in animal models. Enhancements in the post processing and visualization techniques used for OCT were introduced and the feasibility of ultra-high resolution and spectroscopic imaging was evaluated. In vivo OCT imaging to assess the performance of the technology in clinical scenarios ensued in two systems, the cervical and oral mucosas. The markers of neoplasia identified earlier were re-evaluated in vivo and the potential and limitations of OCT for in vivo neoplastic imaging were investigated.
Chapter 1. Neoplasia, Cancer And Imaging

Correspondingly, this thesis is organized in five parts. The first part is this introduction and a description of the theory of OCT. (Chapters 1 & 2) The second part is the \textit{ex vivo} evaluation of OCT imaging using normal, pre-malignant and neoplastic tissues from the female reproductive, gastrointestinal, respiratory and urinary tracts. (Chapters 3-6) In the same section, a study on tissue preservation is included, work resulting from the need for better preservation of tissue that became apparent as the OCT resolution improved. (Chapter 7) Part three is devoted to technology and includes the design of a clinical system as well as delivery devices. A forward imaging probe, an OCT colposcope and the concept of an imaging needle are introduced. The results of testing the high speed system in an animal model are also presented. (Chapters 8 & 9) The fourth part of the thesis introduces possible enhancements to the OCT technology, including post-processing and visualization as well as ultra-high resolution (1-2 \textmu m range) and spectroscopic imaging. (Chapters 10 & 11) The remaining of the thesis is devoted to the clinical evaluation of OCT and describes the feasibility studies for \textit{in vivo} imaging of human cervical and oral mucosa. (Chapters 12-14) The last chapter summarizes the current work and includes comments on the performance and future of OCT imaging of neoplasias. (Chapter 15)


Chapter 2 — Optical Coherence Tomography

Introduction

Optical Coherence Tomography (OCT) is analogous to ultrasound imaging but is based on the detection of infrared light waves, instead of sound, back-scattered (reflected) from different layers and structures within the tissue. Unlike ultrasound however, the speed of light is very high, rendering electronic measurement of the echo delay time of the reflected light (time for the signal to return) impossible. Similar measurements can be performed, however, using a technique known as low coherence interferometry. Within the interferometer, the beam leaving the optical light source is split into two parts, a reference and a sample beam. The reference beam is reflected off a mirror at a known distance and returns to the detector. The sample beam reflects off different layers within the tissue and light returning from the sample and reference arms recombines. If the two light beams have traveled the same distances (optical pathlength), the two beams will interfere. OCT measures the intensity of interference obtained from different points within the tissue by moving the mirror in the reference arm which changes the distance light travels in that arm. Low coherence can be used to localize backreflection sites and provide the desired high resolution by limiting the interference pattern to a coherence length of mismatch between the two arms. Two or three dimensional images are produced by scanning the beam across the sample and recording the optical backscattering versus depth at different transverse positions. The resulting data is a two or three dimensional representation of the optical backscattering of the sample on a micron scale. The logarithm of the backscattering signal is represented as a false color or gray scale image.

Interferometry

\[
\begin{pmatrix}
    -r_s & \mu_s \\
    \mu_s & -r_s
\end{pmatrix}
\]

Source

Beam Splitter

Reference

Sample

Detector

\[
h_1 = -\frac{i}{\sqrt{2}} a_i, \quad \frac{1}{\sqrt{2}} r_i e^{-A t_i}
\]

\[
h_2 = \frac{i}{\sqrt{2}} a_i, \quad -\frac{1}{\sqrt{2}} r_i e^{-A t_i}
\]

\[
E_i = \frac{1}{\sqrt{2}} r_i e^{-A t_i}, \quad E_i = \frac{1}{\sqrt{2}} r_i e^{-A t_i}
\]

\[
\delta t = \Delta t, \quad dz
\]

Figure 2-1. a. Michelson interferometer. b. Interferometric pattern resulting from moving the reference arm and using a coherent source. c. Interferometric pattern resulting from moving the reference arm and using a low coherence source. The peak of the pattern corresponds to the position of the reference mirror at which the pathlengths of the two arms match.
Chapter 2. Optical Coherence Tomography

The heart of an OCT system is the Michelson interferometer. (Figure 2-1 A) [60, 61] Assuming the simplest case first, we can consider a plane wave \( a_i \) leaving the source and entering the interferometer, described by:

\[
a_i = A e^{-j\mathbf{k} \cdot \mathbf{r}}
\]

where

\[
k = \frac{2\pi}{\lambda}
\]

This plane wave is split into two parts by a beam splitter (\( r = 1/2, t = 1/2 \)) and reflected at the reference and sample arm mirrors. The beam splitter is represented by the scattering matrix:

\[
\begin{bmatrix}
  b_1 \\
  b_2
\end{bmatrix} =
\begin{bmatrix}
  -\frac{1}{\sqrt{2}} & \frac{j}{\sqrt{2}} \\
  \frac{j}{\sqrt{2}} & \frac{1}{\sqrt{2}}
\end{bmatrix}
\begin{bmatrix}
  a_1 \\
  a_2 = 0
\end{bmatrix}
\]

The reference and sample fields recombine at the detector resulting in a field of the form:

\[
E_d = E_r + E_s = \frac{j}{2} [a_r r e^{-j\mathbf{k} \cdot \mathbf{r}} + a_s r e^{-j\mathbf{k} \cdot \mathbf{r}}]
\]

The detector is sensitive to the intensity of the fields:

\[
I_d = |E_d|^2 = \frac{1}{4} |E_r|^2 + \frac{1}{4} |E_s|^2 + \frac{1}{4} E_r^* E_s + \frac{1}{4} E_s^* E_r
\]

\[
I_d = \frac{1}{4} |Ar_r|^2 + |Ar_s|^2 + \text{real} \{E_r E_s^*\}
\]

\[
I_d = \frac{1}{4} |Ar_r|^2 + |Ar_s|^2 + 2 A^2 r_r^* r_s \cos(2k\Delta l)
\]

where

\[
\Delta l = l_r - l_s
\]

Equation 2-7 implies that for a monochromatic plane wave in free space the interferometric signal consists of a DC term which is proportional to the reflectance from each arm and an AC component which is modulated sinusoidally as a function of pathlength difference (\( \Delta l \)) between the two arms.

The part of this equation that contains backscattering information is the AC component. The OCT signal can therefore be represented by:

\[
I_{\text{int}_{\text{AC}}} \propto A^2 r_r^* r_s \cos(2k\Delta l)
\]

The above treatment assumes a monochromatic source. In OCT, a low coherence source is used so that an interference signal will only be present when the two arms are matched within a coherence length. A low coherence source can be represented as the sum of monochromatic sources:

\[
a_i = \int_{-\infty}^{+\infty} A(k) e^{-j\mathbf{k} \cdot \mathbf{r}} \, dk
\]

The field at the detector for a low coherence source will, therefore, be:

\[
E_d = E_r + E_s = \frac{j}{2} \left[ \int_{-\infty}^{+\infty} A(k) y_r(k) e^{-j\mathbf{k} \cdot \mathbf{r}} \, dk + \int_{-\infty}^{+\infty} A(k) y_s(k) e^{-j\mathbf{k} \cdot \mathbf{r}} \, dk \right]
\]

As shown before:

\[
I_{\text{int}_{\text{AC}}} \propto \text{real} \{E_r E_s^*\}
\]
\[ I_{\text{int}, \text{er}} \propto \text{real} \left\{ \int_{-\infty}^{+\infty} A(k) r_x(k) A^*(k) r_y^*(k) e^{-j k \Delta l} \, dk \right\} \]  
\[ I_{\text{int}, \text{er}} \propto \text{real} \left\{ \int_{-\infty}^{+\infty} |A(k)|^2 r_x(k) r_y^*(k) e^{-j k \Delta l} \, dk \right\} \]  
\[ I_{\text{int}, \text{er}} \propto \text{real} \left\{ \int_{-\infty}^{+\infty} S(k) e^{-2j k \Delta l} \, dk \right\} \]

where
\[ S(k) = |A(k)|^2 r_x(k) r_y^*(k) \]

If the medium is non-dispersive, the propagation constants in each arm can be considered equal and can be re-written using a Taylor series expansion around \( \omega_0 \), resulting in:
\[ k(\omega) = k(\omega_o) + k'(\omega_o)(\omega - \omega_o) \]
\[ I_{\text{int}, \text{er}} \propto \text{real} \left\{ e^{-2j k(\omega_o) \Delta l} \int_{-\infty}^{+\infty} S(\omega - \omega_o) e^{-2j k'(\omega_o) \Delta l} \, d(\omega - \omega_o) \right\} \]

If we let the phase delay (\( \Delta \tau_p \)) and group delay (\( \Delta \tau_g \)) mismatches be:
\[ \Delta \tau_p = \frac{2 \Delta l}{v_p}, \quad \text{where} \quad v_p = \frac{\omega_o}{k(\omega_o)} \quad \text{and} \]
\[ \Delta \tau_g = \frac{2 \Delta l}{v_g}, \quad \text{where} \quad v_g = \frac{1}{k'(\omega_o)} \]

Then \( I_{\text{int}, \text{er}} \) can be expressed as:
\[ I_{\text{int}, \text{er}} \propto \text{real} \left\{ e^{-j \omega_0 \Delta \tau_p} \int_{-\infty}^{+\infty} S(\omega - \omega_o) e^{-j \Delta \tau_g (\omega - \omega_o)} \, d(\omega - \omega_o) \right\} \]

Since the spectrum of the source and the spectral reflectivities of the source and sample are conjugate-symmetric, the Fourier transform is real, resulting in an interferometric signal of the form:
\[ I_{\text{int}, \text{er}} \propto \mathfrak{R} \{ S(\omega) \} \cos(\omega_0 \tau_p) \]

**Origin of Back-scattering Signal**

As the resolution of OCT becomes higher and clinical studies get under way, it will be very important to understand what the contrast in the OCT images implies about the tissue under investigation. Several investigators have attempted to identify the origins of scattering from cells, both with experiments and simulations. [62-65] Mie theory has been employed in the past in order to understand the optical properties of tissue. [62] However, this method suffers from the inherent limitation that it assumes collections of only spherical scatterers. Recently, more sophisticated methods such as the Finite Difference Time Domain (FDTD) technique, which is a numerical solution of Maxwell’s equations in space and time, were used to shed further inside into cellular scattering. [66-68] A common finding of all investigators is the fact that most of the scattering is a result of differences in index of refraction between the cell, its organelles and the environment. A summary of the latest results is presented here:
Influence of the Nucleus
The scattering cross section of the tissue increases with increasing size, i.e. nuclear-to-cytoplasmic ratio (NCR), and increasing index mismatch. The most notable increases are at small angles, i.e. forward. The scattering at larger angles is more influenced by the wavelength and sub-nuclear index of refraction variations. The nucleus also appears to be the origin of the increased backscattering after the administration of acetic acid. Acetic acid is routinely applied to the cervix since it has been found to increase the scattering from abnormal areas. It has been suggested that this effect is due to the coagulation of nuclear proteins. Investigators have found that the nuclear index of refraction is not homogeneous but exhibits variations which increase both in frequency and in amplitude after the application of acetic acid.

Influence of Organelles
The presence of organelles, in the range of \(\sim 0.5 \, \mu m\) diameter, strongly increases the scattering at angles above 40°. In some cases, such as the liver, it was estimated that mitochondria may generate up to 70% of the scattering. [69] It is also interesting to note that melanin, which has been traditionally viewed as an absorber, at cellular level, where distances are small and absorption negligible, is actually a very strong backscatterer due to its large index of refraction. It is also important to note that the absolute position of the organelles does not seem to be important. Rather, the number and index mismatch is what determines the scattering pattern. Scattering from high spatial frequency index variations, which would correspond to very small scatterers, is particularly apparent at high angles.

Influence of the Extracellular Medium,
The effect of the extracellular medium is often ignored in studies of cells, as in flow cytometry. In tissues, however, the cells are in an environment of high index of refraction due to the presence of extracellular fluid and other cells. As a matter of fact, in confocal microscopy, the membranes of cells are very often invisible and nuclear size vs. internuclear spacing is measured instead of NCR. Also, when considering the effects of osmotically active agents both the change in index of refraction of the fluid and volume changes of the cell have to be considered. [70]

Scattering from Collagen
Collagen fibers, which have approximately a cylindrical geometry, have been shown to significantly favor backscattering, i.e. scattering at large angles. This could actually become a problem when trying to investigate the optical properties of a cell layer over collagenous matrix. The backscattering properties of collagen bundles with coherent detection have not been investigated and the role of the geometry and fiber density remains unclear.

Degradation of the Scattering Signal
Some investigators have also identified mechanisms of degradation of backscattered signals. One such mechanism is destructive interference between the front and back sides of cells, e.g. red blood cells, which may or may not be a problem in OCT depending on the coherence length of the source. They have also observed severe degradation of the point spread function (PSF) of a gaussian beam which has been focused through a layer of cells with inhomogeneous nuclei.

Speckle
Speckle in OCT images has recently been the subject of more rigorous analysis. [71] Waves backscattered from any pair of point scatterers separated by an optical distance close to an odd multiple of one half of the wavelength can generate speckle, provided that the optical distance does not exceed the coherence length of the source in the medium. Waves backscattered from different facets of a single large particle can generate speckle in a similar manner. Recent studies suggest that closely packed subwavelength-diameter particles contribute a large fraction of the total optical cross section of tissue. [64, 72]
Therefore, the likelihood of finding a pair of scatterers or cluster of scatterers within the sample volume that satisfy the conditions for speckle generation is high.

The simulation results in Figure 2-2 illustrate how interference noise caused by multiple backscattering can distort the envelope of an OCT signal generated as the sample beam scans along a single line (an OCT "A-Scan"). [71] The coherent A-Scan shown in Figure 2 was computed by convolving a random sequence of scatterers (top line) with the theoretical coherent point-spread function (PSF) of an OCT scanner; the incoherent A-Scan was computed by convolution with the envelope of the PSF. [73] Notice that, unlike those of the incoherent A-Scan, the variations in the magnitude of the coherent A-Scan track the density of scatterers poorly. In two-dimensional OCT images formed from a series of A-Scans, this type of distortion manifests itself as speckle. Speckle is multiplicative noise and is magnitude can reach that of the signal.

![Distribution of scatterers](image)

![Incoherent signal](image)

![Coherent signal](image)

![Coherent signal envelope](image)

**Figure 2-2.** Simulation of the distortion of an OCT A-Scan caused by the coherent detection process. The uppermost plot shows the ideal backscatter profile generated by a dense random distribution of point particles with cross sections modulated by a cosinusoidal function. Below this plot is the incoherent signal that was formed by convolving the backscatter profile with the Gaussian envelope of the simulated OCT point-spread function (15 \( \mu \)m FWHM). The coherent signal was formed in a similar way by convolving the backscatter profile with the coherent PSF containing the optical carrier frequency. The lowermost plot is a low-pass filtered version of the coherent signal. [71]
Speckle-reduction techniques fall into four main categories: polarization diversity, spatial compounding, frequency compounding, and digital signal processing. Polarization diversity in OCT can be achieved simply by illuminating the sample with unpolarized light and interfering the backscattered light with an unpolarized reference beam. Most OCT scanners based on interferometers built with non-polarization-preserving single-mode fibers automatically implement a form of polarization diversity. In spatial compounding, the absolute magnitudes of signals derived from the same sample volume or slightly displaced sample volumes are averaged to form a new signal with reduced speckle noise. [74] The effectiveness of this approach depends on the number of signals averaged and their mutual coherence. The incoherent average of N uncorrelated signals, each with the same signal-to-noise ratio, SNR=S_R, yields a combined signal with a SNR equal to S_R\sqrt{N}. Any correlation among the signals reduces the SNR gain. If the sample is moving just enough to render speckle in consecutive images uncorrelated, then frame average can significantly improve signal quality. However, it also increases acquisition time. Frequency compounding takes advantage of the reduced correlation between speckled images recorded within different optical frequency bands. To ensure sufficient decorrelation for effective averaging of the images, the overlap of the bands should be as small as possible. However, the axial resolution would degrade at the same time. In OCT, this loss of resolution is usually unacceptable. Most signal processing methods are applied after an image is formed and are commonly referred to as image postprocessing methods. The remaining methods are applied directly to the complex interference signal before the image is recorded and are referred to as complex-domain processing methods. Among the most popular image postprocessing methods for speckle reduction are median filtering, homomorphic Weiner filtering, multi-resolution wavelet analysis, and adaptive smoothing. [75-79]

**Axial Resolution and Low Coherence Sources**

In deriving the axial resolution of an OCT system we can assume, for simplicity, that the OCT source has a gaussian spectrum. This makes the development of the theory more manageable without compromising the physical understanding of the system. For a source with a gaussian power spectrum, normalized to unit power:

$$S(\omega - \omega_o) = 2 \left( \frac{\pi}{w_o^2} \right)^{\frac{1}{2}} e^{-\frac{2(\omega - \omega_o)^2}{w_o^2}} \tag{2-23}$$

which results in an interference pattern:

$$I_{int\,err} \propto e^{-\frac{2\Delta r^2}{w_l^2}} \cos(\omega_o \Delta \tau_p) \tag{2-24}$$

where

$$w_l = \frac{4}{w_o} \tag{2-25}$$

If we define $\Delta \omega$ and $\Delta \lambda$ to be the FWHM of the frequency and wavelength spectra respectively, with a center wavelength $\lambda$, then we can calculate the radius of the a gaussian spectrum in free space ($v_p = c$):

$$w_o = \frac{\Delta \omega}{\sqrt{2 \ln 2}} = \frac{2\pi c \Delta \lambda}{\lambda^2 \sqrt{2 \ln 2}} \tag{2-26}$$

We can further express the signal intensity in terms of pathlength mismatch ($\Delta l$) results in:

$$I_{int\,err} \propto e^{-\frac{2\Delta l^2}{w_l^2}} \cos(\omega_o \Delta \tau_p) \tag{2-27}$$

where the radius interferogram in terms of distance, $w_r$, is:

$$w_r = \frac{v_g}{2} \frac{w_l}{w_o} \tag{2-28}$$
Therefore the FWHM of the interferogram, i.e. the OCT axial resolution (dz), in free space ($v_e = c$) is:

$$FWHM = dz = \frac{2\ln 2 \lambda^2}{\pi \Delta \lambda} \quad (2-29)$$

In tissue, the presence of a material with an index of refraction, $n > 1$, results in a resolution of $dz/n$ since:

$$FWHM \propto \frac{\lambda^2}{\Delta \lambda} \quad (2-30)$$

The inverse relationship of the bandwidth of the source to the point spread function of the system makes development of increasingly better resolution systems more difficult as the sources are pushed to their broad band limit. (Figure 2-3)

Real sources are rarely perfect gaussians. For non-gaussian sources the resolution will still be the FWHM of the Fourier transform of the source spectrum but it may not be symmetrical or have severe sidelobes which degrade the signal quality. Two common problems with source spectra are a flattened top and modulation of the spectrum. The first case is simply a gaussian source convoluted with a rectangle and the second is a gaussian multiplied by a sinusoid added to the original gaussian (gaussian + gaussian * sinusoid). The spectra and FFT’s of example sources with equivalent $\Delta \lambda$’s are shown in Figure 2-4. In the case of the flattened gaussian the resulting OCT point spread function is, as expected the product of a gaussian and a sinc function resulting in severe sidelodes and reduced dynamic range. In the case of the modulated source the dynamic range is unchanged but there are severe sidelobes as a result of the convolution of the Fourier transforms of the gaussian and the sinusoid. When evaluating sources for OCT the shape of the spectrum should, therefore be carefully evaluated. Spectral shaping techniques should also be considered if necessary and possible.

![Figure 2-3. FWHM vs. bandwidth at some common wavelengths. Large changes in source bandwidth are required to achieve small changes in resolution in the broad bandwidth limit of the curves.](image-url)
Chapter 2. Optical Coherence Tomography

Figure 2-4. Spectra (a,d,g) and autocorrelations (linear scale (b,e,h) and log scale(c,g,i)) for a perfect gaussian source (top row), a gaussian with a flat top (middle row) and a modulated gaussian (bottom row.) All sources have the same spectral bandwidth ($\Delta \lambda$) and would theoretically result in the same axial resolution.

The presence of group velocity dispersion (GVD) causes different frequencies to propagate at different velocities. [61, 80, 81] Significant GVD broadens short pulses but, more importantly for OCT, broadens the interferometric autocorrelation causing a degradation in the point spread function of the system. GVD is defined as:

$$\frac{d\nu_g}{d\lambda} = \frac{\omega^2 v_g^2 k''(\omega_o)}{2\pi c} \propto k''(\omega_o)$$  \hspace{1cm} (2-31)

and can be included in our analysis by further expanding k:

$$k(\omega) = k(\omega_o) + k'(\omega_o)(\omega - \omega_o) + \frac{1}{2} k''(\omega_o)(\omega - \omega_o)^2$$  \hspace{1cm} (2-32)

Assuming the GVD mismatch exists over a distance L in the two arms of the interferometer, then the intensity of the OCT signal has an additional term which includes $\Delta k''$, the GVD mismatch:

$$I_{int \ err} \propto \frac{\text{real}}{W(L)^2} \left[ e^{-j\omega_o\Delta \tau_p} e^{-\frac{1}{2} \Delta k''(\omega_o)(\omega - \omega_o)^2L} e^{-j\Delta k''(\omega - \omega_o)} d(\omega - \omega_o) \right]$$  \hspace{1cm} (2-33)

Assuming, again, a gaussian spectrum the resulting interferometric intensity is:

$$I_{int \ err} \propto \frac{W}{W(2L)} \frac{2\Delta \tau_p^2}{W(2L)^2} \cos(\omega_o \Delta \tau_p)$$  \hspace{1cm} (2-34)

where

$$W(2L)^2 = w_t^2 + 4j\Delta k''(\omega_o)(2L)$$  \hspace{1cm} (2-35)

It is obvious from the complex nature of W(2L) that the gaussian function has now a real and imaginary part defining an envelope change as well as a chirp. Separating the two parts we can get, from the real part, an expression of the new radius of the gaussian:
\[ w_d(2L) = w_t \sqrt{1 + \frac{(4\Delta k''(\omega_0)(2L))^2}{w_t^2}} \]  \hspace{1cm} (2-36)

Significant broadening will be present when the second term in the square root becomes greater than 1, which implies that in the case of air vs. some other material even a few mm are enough to significantly broaden the point spread function.

From (2-33) we see that a dispersion mismatch also causes a decrease in the peak intensity of the OCT interferogram which is inversely proportional to the square root of the degree of broadening:

\[ \frac{w_t}{\sqrt{W(2L)}} = \frac{1}{\sqrt{1 + \frac{(4\Delta k''(\omega_0)(2L))^2}{w_t^2}}} \propto \frac{1}{\sqrt{\frac{w_d(2L)}{w_t}}} \]  \hspace{1cm} (2-37)

Currently Available Low Coherence Sources

A variety of sources are available for OCT imaging. Superluminescent diodes (SLDs) are semiconductor devices, similar to laser diodes but with their facets cleaved at an angle. They run below threshold thus emitting a broad bandwidth light rather than lasing. There is a range of these devices available and they are very compact and inexpensive but lately they have fallen out of favor since their low power precludes their use in real time systems. AFC Tech offers an alternative, where a semiconductor amplifier and filters are used to deliver 20 mW of broad bandwidth light into a single mode fiber from an SLD.

Mode-locked solid state lasers can also provide broad bandwidth light. Kerr-lens modelocked (KLM) lasers, which can be used for OCT, include a the Chromium:Forsterite (Cr4+:Mg2SiO4) emitting at 1.28 \( \mu \)m and the Titanium:Sapphire (Ti:Al2O3) laser emitting at 0.8 \( \mu \)m. [82, 83] Since the spectral and temporal characteristics of KLM lasers are related through the Fourier transform, shorter pulse duration (\( \tau_p \)) results in proportionally broader spectra, governed (assuming a gaussian spectrum) by the relation:

\[ \Delta \lambda \geq \frac{2\ln(2) \lambda^2}{\pi \tau_p c} \]  \hspace{1cm} (2-38)

Further broadening of the spectrum can be achieved by inducing self-phase modulation in a dispersion shifted fiber. [83]

The choice of wavelengths used depends on the nature of the tissue under investigation, the required resolution but also availability. Figure 2-6 shows a figure of tissue properties and available sources. For imaging the eye, for example, water absorption is the limiting factor which means that a wavelength has to be chosen to minimize that absorption. For highly scattering tissue longer wavelengths are desirable to reduce scattering.

A list of devices available in our lab and used for the experiments described in this thesis is given in Table 2-1.
Figure 2-6. The absorption spectra of melanin, water and hemoglobin vs. wavelength. The regions where the penetration of light is limited by absorption or multiple scattering are also indicated along with the wavelength range of three solid state lasers. [84]

Table 2-1. Sources for OCT

<table>
<thead>
<tr>
<th>Source</th>
<th>Power (mW)</th>
<th>Wavelength (μm)</th>
<th>Wavelength (nm)</th>
<th>Wavelength (μm)</th>
<th>Cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG&amp;G SLD</td>
<td>1</td>
<td>1.3</td>
<td>45</td>
<td>16.6</td>
<td>1,000</td>
</tr>
<tr>
<td>AFC</td>
<td>30</td>
<td>1.3</td>
<td>50</td>
<td>14.9</td>
<td>22,000</td>
</tr>
<tr>
<td>Cr:Forsterite</td>
<td>300</td>
<td>1.28</td>
<td>120</td>
<td>6.02</td>
<td>100,000</td>
</tr>
<tr>
<td>Ti:Sapphire</td>
<td>300</td>
<td>0.80</td>
<td>250</td>
<td>1.1</td>
<td>100,000</td>
</tr>
</tbody>
</table>

Implementations of the Michelson Interferometer

Linearly Scanning System

The simplest way to implement a Michelson interferometer is to use a fiber optic coupler and a galvanometer to scan the reference arm in a linear fashion. This scanning will introduce a Doppler shift in the frequency of the light in the reference arm. [85] This frequency will be:

$$ f_d = \frac{2v}{\lambda_0} \quad (2-39) $$

and will result in a frequency bandwidth of:

$$ \Delta f_d = 2v \frac{\Delta \lambda}{\lambda_0^2} = f_d \frac{\Delta \lambda}{\lambda_0} \quad (2-40) $$

Phase Delay Line

The main limitation of a linearly scanned galvanometer is speed. Due to the large mass of the optics, conversion of rotational motion to linear motion is limited to slow speeds. This makes linear galvanometers unsuited for real-time, in vivo imaging. In an attempt to remedy the situation, Tearney et al have developed an optical phase delay line which is based on rotation of a mirror and can perform individual A-Scans at up to 2 kHz. [4, 86] This device uses pulse shaping techniques developed for ultrafast optics to introduce a phase delay which is proportional to the angle (γ) at which the galvanometer is
scanned. (Figure 2-5) [87, 88] Qualitatively, the light enters the delay line in a collimated fashion and its component wavelengths are separated using a grating. A lens collimates the light after the grating. This wavelength-expanded beam hits a rotating galvanometer with the wavelengths spread over the length of its mirror. The mirror tilt introduces a variable travel path for each wavelength resulting in a variable phase differences for each one. This phase difference translates to a time delay in the time domain. The light is then reflected and coupled back into the interferometer.

![Figure 2-5. Block diagram (left) and schematic (right) of the optical phase delay line.](image)

The phase introduced by this system is:

$$\phi(\omega) = -2\frac{\omega}{c} f \tan(\gamma) \tan\left(\theta_o - \sin^{-1}\left(\frac{2\pi c}{\omega f} + \sin(\theta_o)\right)\right)$$  \hspace{1cm} (2-41)

resulting in a group delay:

$$\Delta \tau_g = \frac{d\phi}{d\omega}_{|\omega_0} = \frac{2 f \lambda_o \tan(\gamma)}{c d \cos(\theta_o)}$$  \hspace{1cm} (2-42)

and, given the double pass nature of the device, the scan length is:

$$L(\gamma) = 2 \Delta \tau_g c = \frac{4 f \lambda_o \tan(\gamma)}{d \cos(\theta_o)}$$  \hspace{1cm} (2-43)

When the angle $\gamma$ is scanned at small angles $\gamma(t) = \omega_s t$ such that $\tan(\gamma) \approx \gamma$, the phase at the center wavelength becomes:

$$\phi(\lambda_o, t) = \frac{4 \pi \gamma(t) x_o}{\lambda_o}$$  \hspace{1cm} (2-44)

which implies that the OCT interferogram is modulated by a $\cos(2 \pi f_s t)$ with a frequency of:

$$f_s = \frac{2 \omega_s x_o}{\lambda_o}$$  \hspace{1cm} (2-45)

The modulation frequency then depends on the offset from the center of the galvanometer, $x_o$, and can be adjusted independently of $\omega_s$, the frequency at which the galvanometer is scanned. This property is very advantageous when scanning at high frequencies since it allows the demodulation frequency to be adjusted down to more manageable levels.

The group velocity dispersion (GVD) of this device has been well studied and determined to be: [89]

$$\left|\frac{d^2 \phi}{d\omega^2}\right|_{\omega_0} = -\frac{\lambda_o^3 (t - f)}{\pi c^2 d^2} \left[\cos(\theta_o)\right]^3$$  \hspace{1cm} (2-46)
This setup, therefore, provides positive (l<f) and negative (l>f) dispersion compensation by simply adjusting the distance between the grating and the lens.

Delivery Optics

A variety of devices have been designed to deliver the OCT beam to the tissue under investigation. Since OCT is amenable to integration to most diagnostic devices, the diversity of delivery optics is expected to continue to expand.

The simplest implementation, the one used for most of experiments in the lab, consists of a pair of translation stages (to move the sample) or orthogonal galvanometers (to scan the beam) and a combination of a collimator and objective to focus the beam on the sample. (Figure 2-7)

Our group has also developed a fiber optic catheter / endoscope for imaging luminal structures. [4, 54] The catheter/endoscope consists of a rotating hollow cable carrying a single-mode optical fiber. The beam from the distal end of the fiber is focused by a graded index (GRIN) lens and is directed perpendicular to the catheter axis by a microprism. The distal optics are encased in a transparent housing. (Figure 2-8) The beam is scanned circumferentially by rotation of the cable inside a transparent, static housing. The catheter/endoscope has a diameter of ~1 mm, which is small enough to allow imaging in a human coronary artery or access through the flush port of a standard endoscope. It can also be made significantly smaller by using smaller components.

![Figure 2-7. Benchtop OCT systems. A: Stationary lens with two translation stages to scan the sample. B: Stationary sample with a pair of orthogonal galvanometers to scan the beam. This system is faster and can scan arbitrary patterns much more easily than A. C: Definitions: b is the confocal parameter, l is the distance to the imaging plane and D is the diameter of the beam at the focusing lens.](image-url)
Transverse Resolution

The transverse resolution of the OCT systems depends on the delivery optics. Assuming a gaussian profile for the sample beam the diameter of the beam is given by:

\[ 2w = \frac{2b \lambda}{\pi} \]  
(2.47)

where \( b \) is the confocal parameter of the system and \( \lambda \) the center wavelength. We have conventionally called \( 2w \), i.e. the diameter of the beam at which the intensity falls by \( e^2 \), the "resolution of the system". Strictly speaking the resolution should be the FWHM of the gaussian which is related to the radius by:

\[ FWHM = w \sqrt{2 \ln(2)} \]  
(2.48)

however, to avoid any confusion in this thesis all the transverse resolution measurements will refer to \( 2w \) unless otherwise indicated. The confocal parameter \( b \) is defined as twice the Rayleigh range \( (z_R) \) which is the distance away from the position of the minimum radius \( (w_o) \) to where the radius becomes \( \sqrt{2} \) \( w_o \).

The system resolution can be measured experimentally by measuring either \( b \) or \( w_o \) directly. Although measuring \( b \) directly is very easy it is not very accurate and it does not provide any measure of the symmetry or the shape of the beam. The radius of the beam can be directly measured using the knife edge method which is harder but more accurate technique. This involves the recording of the intensity while a sharp edge is scanned through the focal plane. The resulting function, called an "error function" is related to the gaussian profile by:

\[ \text{erf}\left(\sqrt{2} \frac{x - x_o}{w_o}\right) \approx \frac{1}{\sqrt{\pi}} \int_{-w_o}^{w_o} e^{-t^2} \, dt \]  
(2.49)

A fit of the "error function" to the data should result in values for \( w_o \) which would only be limited by the resolution of the knife translation. The beam shape can also be recovered by integrating the "error function" while keeping in mind that differentiation is very sensitive to noise. The transverse resolution can also be approximated by: [90]

\[ 2w_o = \frac{4A_l}{\pi D} \]  
(2.50)
Detection and Digitization

The OCT detection electronics are described in Figure 2-9. They consist of a photodetector which is connected to a transimpedance amplifier. The signal is then passed through a bandpass filter centered at the doppler frequency. The bandwidth of this filter must be sufficiently broad to accommodate all the signal frequencies but at the same time remove as much of the background noise as possible. Theoretically, for a source with a gaussian spectrum an infinitely broad filter is required. However, in practice a $2\Delta f_d$ filter is usually adequate. [85]

![Block diagram of the OCT electronics.](image)

The signal must then be demodulated using either a linear envelope detector, such as a combination of a rectifier and low pass filter, or a logarithmic detector. Demodulator circuits are also available as monolithic ICs. The signal is then digitized using an analog-to-digital converter (ADC) and a computer.

The circuitry can take a variety of forms. It has to be optimized for the specific OCT system based on the center wavelength, bandwidth and speed or Doppler frequency. In general, higher frequency circuits are more prone to noise pick-ups and are harder to optimize.

The effects of the signal digitization must also be considered. [91, 92] The Nyquist criterion specifies that to adequately sample a signal with a maximum frequency $\omega_{\max}$ the sampling frequency $\omega_s$ has to be:

$$\omega_s > 2\omega_{\max} \quad (2-51)$$

If we consider a time limited signal, or a continuous signal sampled over a limited time ($T_m$) then the resolution in the frequency domain will depend on the FWHM ($\Delta \omega$) of the Fourier transform of the window $h(t)$. (Figure 2-10) For a rectangular window, i.e. $\text{rect}(t/T_m)$, where $\Delta \omega = 2\pi/T_m$. The sampling time, $T_m$ should, therefore, be chosen to insure that the desired resolution, $\Delta \omega$, can be achieved:

$$T_m \geq \frac{2\pi}{\Delta \omega} \quad (2-52)$$

It is obvious from the above that the number of samples in the digitized signal will be:

$$N = \frac{T_m}{T_s} \quad (2-53)$$

where $T_s = 2\pi/\omega_s$ is the time between samples. If at some point the Fourier transform of the signal is required, then the issue of the length of the Fourier transform has to be considered. Since for the discretized signal $\Omega = \omega T_s$ the Fourier sampling frequency, $\Omega_s = 2\pi/N_{FFT}$, which will adequately sample frequency resolutions of $\Delta \omega = 2\pi/T_m$ has to be:

$$\Omega_s < \frac{\Delta \omega T_s}{2} \Rightarrow N_{FFT} > 2N \quad (2-54)$$

Further keeping in mind that if two digital signals are to be convoluted without aliasing the FFT's used have to be at least $2N_{FFT}$-1 long.
It is also important to consider the digitization depth, i.e. the number of bits of the ADC. This determines both the resolution of the digitization:

\[ q = \frac{V_{\text{max}}}{2^N} \]  \hspace{1cm} (2- 55)

and the dynamic range of the digitization:

\[ DR_{\text{dB}} = 10 \log(2^N) \]  \hspace{1cm} (2- 56)

For a 12-bit (N=12) or a 16-bit (N=16) ADC the dynamic range is 35 and 48 dB respectively. This may not be adequate and digitization of the logarithm of the signal is preferred. In the later case the dynamic range is limited by the dynamic range of the demodulator, usually in the range of 80-90 dB.

**Noise**

There are several sources of noise in an optical system. Starting with the photodetector, we have to consider shot noise. This is the fluctuation of carriers produced by light of a given power reaching the detector. \[N\] Shot noise is given by:

\[ \left\langle i_N^2(f) \right\rangle = 2e < i > \Delta f \]  \hspace{1cm} (2- 57)

where \(e\) is the electronic charge (1.602e-19 C) and \(\Delta f\) the bandwidth of the signal. The resulting shot-noise-limited (SNL) signal-to-noise ratio (SNR) is therefore:

\[ SNR_{\text{SNL}} = \frac{n_P_s}{2h\nu\Delta f} \]  \hspace{1cm} (2- 58)

where \(n\) is the efficiency of the detector, \(h\nu\) is the energy of the incoming photons and \(P_s\) the power from the sample. The optimal dynamic range of the electronics is therefore determined by the above relationship:

\[ DR_{\text{dB}} = 10 \log(SNR_{\text{SNL}}) \]  \hspace{1cm} (2- 59)

and can be as high as 125 dB. Shot noise defines the minimum detectable signal since, even if all other sources of noise are removed, shot noise is produced by the signal and it is unavoidable. In reality, however, there are other sources of noise such as Johnson (or thermal), 1/f and, in semiconductor detectors, generation-recombination (gr) noise.

Johnson noise is a result of fluctuations in charge density due to the thermal energy of the carriers. It depends on temperature and the bandwidth of the signal as defined by:
\[ \langle i_n^2(f) \rangle = \frac{4kT}{R} \Delta f \]  
(2-60)

where k is the Boltzmann constant (1.38e-23 J/K) and R is the noisy resistor in the circuit. Johnson noise can be reduced by cooling the circuit but can be completely removed only at absolute zero. It is also very important to keep the Johnson noise constant through out a given experiment. This can be achieved by maintaining the circuit temperature constant.

Noise that depends on the inverse of frequency, hence the name 1/f, also exists. Its origins are not clearly understood but it is believed that it originates from a variety of sources including diffusion of charge carriers, presence of impurities and lattice defects in materials and interaction of charge carriers with the semiconductor surface. This noise can be described as:

\[ \langle i_n^2(f) \rangle = \frac{K < i^2 > \Delta f}{f} \]  
(2-61)

where K is some constant.

Generation-recombination noise is also present in semiconductors. It is a result of random electron hole recombinations and depends on the lifetime of the recombinations (\(\tau_0\)) and the time of a charged carrier to travel from the detector to the circuit (\(\tau_d\)):

\[ \langle i_n^2(f) \rangle = \frac{4e < i^2 > \tau_0 / \tau_d}{1 + (2\pi f \tau_0)^2} \]  
(2-62)

When choosing a detector one should also consider the “dark current” or “dark charge” and “readout charge” of the device. The dark current is the current output with no signal present and can accumulate to large values especially with long exposures. Readout charge is the noise of the buffers used to transfer the charge from the CCDs to the electronics. This variable must also be accounted for when designing a circuit for photodetection.

Another source of noise is the one originating from fluctuations in the source called excess intensity noise. It can be a result of source noise or other instabilities in the system such as vibrations and motion. This type of noise can be suppressed to large degree by using dual balanced detection.

Most noise source are random but have stationary properties so the total effect of all noise sources can be calculated in a mean square fashion, i.e.:

\[ \langle i_n^2(f) \rangle = \sum_{AllSources} \langle i_n^2(f) \rangle \]  
(2-63)

For more information on noise in optical systems and reduction of noise in electronic circuits references [90, 93, 94] provide useful information and suggestions for further reading.
PART II – EX Vivo Feasibility Studies

CHAPTER 3 - Imaging of the Reproductive Tract

Cancers of the Female Reproductive Tract

Most cancers of the cervix are squamous cell carcinomas (80-90%), and adenocarcinoma (10-16%). [95] The current understanding is that the malignancy starts from squamocolumnar junction and is preceded by cervical dysplasia and carcinoma in situ. [96] Cervical cancer precursors can be divided into dysplasias (mild, moderate, severe) or, using an alternative scheme, into intraepithelial neoplasias ranging from CIN I, with minimal morphologic changes, to CIN III, where the entire epithelium from the basement membrane to the surface is composed of malignant cells. Invasion is defined as the spread of malignant cells below the basal membrane. Screening for cervical cancer is performed by periodic cytology, the pap smear, and annual gynecologic examinations. [97, 98] Once atypical cells are seen in the cytological smear the cervix is further evaluated with colposcopy and biopsies and also CT scans if macroinvasion is suspected. It is interesting to note that all deaths due to cervical cancer are preventable but screening is less frequent in the underserved population, resulting in increased fatalities from the disease.

Cancer of the uterus, the most common manifestation of which is adenocarcinoma, is the most common female genital cancer, with a peak incidence between 55 and 60 years of age. It has been shown that it is closely associated with and preceded by hyperplasia of the endometrium. [99, 100] Hyperplasia is described by hyper-proliferation and changes in architectural morphology as well as cytologic atypia. It is classified as atypical when any proliferation pattern is associated with cellular atypia, simple or complex. The classification of simple (cystic) vs. complex (adenomatous) is based on glandular complexity or crowding. [101] The diagnosis of endometrial cancer is based on dilatation and curettage, with chest x-ray, CT and cystoscopy recommended for staging and discovering distant metastases. The 5 year survival rates for uterine cancer is high, in the range of 80-95%, mainly due to early diagnosis of the abnormal bleeding associated with 70-75% of all cases which is easily identifiable especially if the patient is postmenopausal. [102]

Fallopian tube cancer is very rare, comprising only 0.5-1.1% of gynecologic malignancies. [103] Most cancers fall in the pathologic category of adenocarcinomas and present as dilated tubes with papillary and solid tumor. Degeneration with hemorrhage and necrosis may also be present. [103] The cancer is evaluated and treated following the same guidelines that apply to ovarian cancer.

Ovarian carcinoma can be epithelial carcinoma (80%-90%) and germ or stromal cell carcinomas (10-20%). Of all ovarian cancers 60% are adenocarcinomas, 17% are undifferentiated, 15% are endometrioid carcinoma and 6% are clear cell carcinoma. [104] Extraovarian spread by transperitoneal insemation is common. Once ovarian cancer is suspected x-rays, ultrasound or transvaginal ultrasound may be employed for the evaluation of the pelvic area. [105] Therapy is usually surgery, radiation and/or chemotherapy. Despite the relatively low number of ovarian cancer incidence, more patients die from ovarian cancer than cervical and endometrial combined. In 1998, more than 50% of the deaths from cancers of the female reproductive system were due to ovarian cancer. [1] The high mortality rate is largely due to poor techniques for early detection. The 5 year survival rate drops from 74-98% for benign, to 91-93% for borderline malignant, to a dramatic 29-34% for malignant making the need for early diagnosis all the more obvious. [106]
Imaging Of the Female Reproductive Tract

Reproductive structures can be accessed either using a colposcope or a hysteroscope from a vaginal approach or using a laparoscope during a minimally invasive laparoscopic procedure. Each technique offers access to different sides of the reproductive tract allowing the investigation of various pathologies that may localize to different areas. Structures that would be visible in a vaginal approach scheme include the ectocervix, endocervix, uterine mucosa and the fallopian tubes. Approaching the reproductive tract transabdominally allows access to the pelvic side of the reproductive organs, including the ovaries, the myometrium and the serosal layer of the fallopian tubes. The feasibility of OCT for high resolution imaging of gynecological tissue from a vaginal approach was explored with studies on human cervical and uterine tissue ex vivo. Both normal and pathologic microstructure was imaged and the images were correlated with histopathology to confirm tissue identity.

Methods

The uterine and cervical tissue, both normal and neoplastic, were obtained from surgically excised specimens and were imaged fresh. Sixty five sites from seven different patients were imaged for this study. (Cervix: 21 normal, 2 neoplastic/in situ and 9 carcinoma. Uterus: 11 normal, 22 adenocarcinoma.) The tissue specimens were placed in a Petri dish and irrigated with isotonic saline to prevent dehydration during imaging. Imaging was performed using a bench-top system. The acquisition of each image required between 10 and 30 sec depending on the size (number of pixel elements) of the image. Since the OCT beam is invisible, tissue registration was performed with a visible light guiding beam. The orientation of the imaging scan was marked on the specimen using the microapplication of India ink. Small variations between the cross sectional plane of the tissue imaged and the plane sectioned histologically do exist. Slight mismatch between histology and imaging plains as well as changes in physical dimension of the tissue associated with fixation and sectioning account for the minor differences observed. The samples then underwent routine histologic processing. Samples were immersed in 10% buffered formalin for 48 hours. The tissues were then processed for standard paraffin embedding. Five micron thick sections were cut at the marked imaging sites and stained with hematoxylin and eosin. The stained histologic sections enabled verification of tissue identity and allowed identification of sources of tissue contrast in the OCT images.

Results

Human cervix was imaged ex vivo using OCT at a resolution of 6 μm. In Figure 3-1, microstructure associated with both the ectocervix and the endocervix were demonstrated. The epithelial layer of the ectocervix and the basal membrane were clearly identified. Deep endocervical glands, some of which developed into fluid filled cysts, were also visible and are a finding common in postmenopausal women. In Figure 3-2, cervical samples with carcinoma in situ and poorly differentiated carcinoma were examined. In Figure 3-2a, the carcinoma in situ is characterized by a thick, irregular epithelial layer in addition thickening of the basement membrane. With invasive carcinoma(3-2b and 3-2c), the tissue surface is now heterogeneous and the basement membrane is no longer defined. Images were correlated with histopathology.

Figure 3-3 demonstrates ex vivo OCT imaging of postmenopausal endometrium. The OCT images demonstrate sparse uterine glands, consistent with postmenopausal uterine atrophy, which has been confirmed on the histopathology. In Figure 3-3c, larger fluid filled cysts can be identified. Ex vivo OCT images of neoplastic changes in the uterine endometrial adenocarcinoma are demonstrated in Figure 3-4. In both 3-4a and 3-4c, defined epithelial layers and glands are no longer present. The interlacing of cellular and non-cellular tissue results in a layered appearance, with the presence of a rare hyperplastic gland.
Figure 3-1. Human cervix imaged *ex vivo*. A) Microstructure associated with the ectocervix: epithelial layer (e) and basal membrane (b). B) Microstructure associated with the ectocervix: deep endocervical glands (g), some of which developed into fluid-filled cysts. The epithelial layer has been partially denuded in the histologic photograph. (Image size: 3 mm x 1.5 mm, 500 A-Scans; Resolution: 10 μm x 6 μm)

Figure 3-2. Cervical diseases imaged *ex vivo*. A) Carcinoma *in situ*, characterized by a thick, irregular epithelial layer and thickening of the basement membrane (b). B, C) Invasive carcinoma. The tissue surface is now heterogeneous, and the basement membrane is no longer defined. Distinct back-scattering patterns can be seen in cellular (c) and noncellular (n) regions. (Image size: 1.5 mm x 1.25 mm, 250 A-Scans; Resolution: 10 μm x 6 μm)
Figure 3-3. Postmenopausal endometrium imaged *ex vivo*. A&B) Sparse uterine glands (g), consistent with postmenopausal uterine atrophy; epithelial layer (e); and larger fluid-filled cysts (c) can be identified. (Image size: 3 mm x 1.5 mm, 500 A-Scans; Resolution: 10 μm x 6 μm)

Figure 3-4. Endometrial adenocarcinoma imaged *ex vivo*. In both 5A and 5C, defined epithelial layers and glands are no longer present. The interlacing of cellular and noncellular tissue results in a layered appearance, with the presence of a rare hyperplastic gland (g). (Image size: 3 mm x 1.5 mm, 500 A-Scans; Resolution: 10 μm x 6 μm)
Conclusions

This work demonstrates the feasibility of OCT for imaging clinically relevant microstructures of the uterus and cervix. Normal tissue and neoplastic lesions were examined. Sharp differentiation of structures included the epithelium, glands, supportive tissue, and intramural cysts. The epithelial layer could be clearly identify and, although the basal membrane is beyond the resolution limits of our system, the highly backscattering and sharp basal layer provided the means of evaluating its integrity. The increase in immature cells at the basal layer, which resulted in a thicker band of high backscatter in that area, was a clear sign of dysplasia. Other structures such as cysts, crypts and glands were also distinctly identified. However, it is difficult to separate true cysts from glands at the current resolution since that would entail identifying the presence of a single cell thickness epithelial layer around the lumen. Growth formation both in the cervix and uterus was also identified including the presence of necrotic tissue in more advanced stages. Features not distinguished in these images, due to the lack of adequate resolution, included subcellular markers of dysplasia such as nuclear to cytoplasmic ratio (NCR), pleomorphism and mitotic figures.

It is clear from the images included here that OCT is capable of identifying normal from abnormal tissue both in the cervix and the uterus. Such distinction would be useful if a screening tool was to be devised using OCT. However, in order to successfully grade dysplasia one must be able to assess cellular features such the NCR, pleomorphism and mitotic figures. An indirect measure of the NCR and the fraction of the epithelium that is occupied by immature cells could be the thickness and intensity of the highly backscattering layer closer to the basal membrane. Such measurements could result in the ability to grade cervical neoplasias, from CIN I to CIN III, based on the extend that the epithelium is occupied by these immature cells, to invasive cancer, when the sharp borders of this layer are interrupted. Unfortunately, features on this scale would be inadequate for the early identification of endometrial cancer and especially atypia in the presence of hyperplasia. The abnormal glandular formations in these cases would most likely be easily assessed but the classification of atypia depends on subcellular features and the number of mitotic cells. The resolution for successfully imaging such structures is currently unavailable but new sources and spectroscopic OCT hold some promise for the future.

The next step in the clinical evaluation of OCT is to assess the feasibility of imaging the reproductive tract in vivo in humans. From the findings of this study, it is obvious that resolution in the range of at least 5-15 μm is needed with even higher resolution required for grading some dysplasias especially in the uterus. New imaging devices are also required for access to the relevant tissues. For the cervix, such devices can either take the form of a forward imaging probe or an integrated OCT colposcope. The first would allow imaging from distances closer to the cervical surface and would therefore be easier to handle with high numerical apertures which would allow higher transverse resolution. The second option would image from a large distance but would be integrated with and would not interrupt the normal colposcopic procedure. Imaging of the endometrium can be achieved either by the blind insertion of an OCT catheter or the integration of OCT with standard solid hysteroscopes with trade offs similar to the ones described above for cervical imaging. The efforts to develop a clinical system and relevant imaging devices will be described later in this thesis.

It is improbable that an imaging technology such as OCT would be cost effective for screening the general patient population for early cervical neoplasias. The development of the Papanicolaou smear has reduced the number of cases of cervical cancers per year in the US to 12,000 and the number of deaths to approximately 3000. The Papanicolaou smear has also significantly reduced the cost of screening by making such a test available to a broad range within the general public. However, a role for OCT is envisioned in scenarios such as the follow-up management of cervical intraepithelial neoplasia (CIN) lesions in addition to its potential for the reduction of cone biopsies is patients with CIN II, CIN III, and microinvasive lesions.
Chapter 3. Imaging of the Reproductive Tract

The cervix is also an excellent model system for the study of both neoplasia and OCT imaging. The cervix is easily accessible and exhibits well defined pathology. The progression and endpoints of neoplastic changes are clearly defined and validated with colposcopic and histopathologic findings. Neoplastic changes in the cervix can be used to quantitatively evaluate the system. Also, the conclusions drawn from studies of cervical neoplasia can be extended to guide the study of other squamous carcinomas that share similar features.

The study suggests the feasibility of ultimately applying this technique to the female reproductive tract. By integrating OCT with small imaging endoscopes, the uterine surface could be scanned and pathology imaging *in situ* at high resolution and in real time. This suggests the potential for future applications in screening patients for endometrial carcinoma. Endometrial cancer is one of the most common neoplasms of women in many industrialized societies. The majority of patients have postmenopausal bleeding and are identified at early stages. Unfortunately, 90% of dilation and curettage procedures performed for postmenopausal bleeding show no abnormality. A minimally invasive technique with a high sensitivity but low false-positive rate would be a powerful modality in selecting patients who would benefit from invasive investigation. This is particularly relevant to patients on tamoxifen, who demonstrate an increase incidence of endometrial abnormalities 6.3 per 1000 women (at 5 years), a factor of 2-3 increase over the general population. [107] Therefore a screening approach is needed for this patient population, especially if therapy will be extended prophylactically to patients at high risk for breast cancer. In should be noted that current recommendations leave screening to the discretion of the individual gynecologist. [108]
CHAPTER 4 - IMAGING OF THE GASTROINTESTINAL TRACT

Cancers of the Gastrointestinal Tract

The tissue structure of most of the gastrointestinal tract is quite similar and follows the same histologic paradigm. It consists of a mucosa with an epithelial layer, lamina propria (areolar connective tissue with vascular structures) and muscularis mucosa, a submucosa with loose connective tissue, blood vessels, lymphatics and glands, a muscularis propria with inner circular and outer longitudinal smooth muscle and an adventitia. There are differences, however, in some organs. For example the esophageal and ductal lumens are covered with a layer of squamous epithelium. Most other structures in the gastrointestinal tract are covered with columnar epithelium. The morphology of the epithelium also varies, especially in the stomach and small and large intestine where the function of those organs necessitates and depends on the presence of villi, crypts and/or pits. One exception to the gastrointestinal histologic structure paradigm is the gallbladder which lacks a muscularis mucosa and submucosa and consists only of a mucosal lining with a single layer of columnar cells, a fibromuscular layer, a layer of subserosal fat with blood vessels and nerves and a peritoneal covering. The great variability of gastrointestinal tissue, its continuous exposure to the external environment and rough enzymatic activity and the cyclic and rapid cell proliferation and epithelial turn-over result in an increased number and variety of malignancies.

Most esophageal cancers fall within two histologic types, squamous, also called epidermoid, carcinoma and adenocarcinoma with some occurrences of other rare cancers and metastases. The patterns of esophageal cancer are dramatically changing in the United States. Three decades ago the large majority of these cancers were squamous cell carcinomas, but the incidence of esophageal adenocarcinoma has been steadily increasing. By the early 1990s, adenocarcinoma had become the most common cell type of esophageal cancer among white patients, although squamous cell cancers still predominated among black patients. [109] However, in certain endemic regions squamous carcinomas of the esophagus are extremely prevalent. [110, 111] In some regions of Northern China, for example, they account for 20% of all deaths. [110] The initial diagnosis is usually based on the findings of chest x-rays (CXR) and/or a barium swallow study but endoscopy and biopsy are always used to confirm the findings. Cytological brushing, in addition to excisional biopsy, was found to improve the overall accuracy of the procedure. [112] A group at high risk for esophageal cancer are patients with Barrett’s esophagus, a form of esophageal metaplasia, who have at least a 30-fold increased chance of developing adenocarcinoma of the esophagus compared to the general population. [113] These patients are advised to seek regular endoscopic evaluations to monitor for the development of dysplasia and to ensure that they receive treatment at the earliest stage of the disease if needed.

Gastric cancer can be divided into two major groups, intestinal, which is a differentiated cancer with the tendency to form glands, and diffuse, which has a predilection for extensive submucosal spread and metastasis and is characterized by very little cell cohesion. [114] Approximately 95% of all malignant gastric neoplasms are adenocarcinomas, with some other rare tumors comprising the remaining 5%. [115] The Borrmann classification divides gastric cancer into five types depending on macroscopic appearance, including polypoid or fungating cancers, ulcerating lesions surrounded by elevated borders or infiltrating the gastric wall, diffusely infiltrating tumors, and unclassified. [116] In Asian and South American countries the incidence of gastric cancer is very high, reaching epidemic proportions in Japan (100:100,000). [117] The evaluation of gastric cancer can be performed with a barium study or flexible upper endoscopy. The likelihood of positive yield on biopsy is greater than 95% when 6 to 10 tissue samples are obtained. [26] EUS has been studied extensively for the evaluation of depth of penetration and was proven more effective than CT in identifying primary tumors but not in establishing nodal...
involvement. [33] Mass screening programs have been successful in Japan but gastric cancer remains the number one cause of death. This fact may be reflecting limitations if the entire risk population is not effectively screened. [118]

Pancreatic cancers can be divided into malignant, uncertain malignant potential, and benign. The normal pancreatic architecture is markedly altered in carcinoma, with the predominant histologic feature being a dense collagenous stroma with atrophic acini, remarkably preserved islet cell clusters and a slight to moderate increase in ducts, both normal-appearing and cancerous. Pancreatic cancers are difficult to diagnose and very aggressive. The evaluation of the disease depends mainly on CT. Laparoscopy is limited only to patients with localized disease. The diagnosis of ductal adenocarcinoma rests on biopsies and the identification of mitoses, nuclear and cellular pleomorphism, and discontinuity of ductal epithelium. [119] The five year survival rate of pancreatic cancers is very low, in the order of 1-4%. Unfortunately, there are no good therapies and sometimes the treatment is worse than the disease and offers little benefit. [120]

The major types of cancer of the biliary tree are carcinoma of the gallbladder, which includes adenocarcinoma (80% of all gallbladder cancers) and other rare types, and cholangiocarcinoma, i.e. carcinoma of the bile duct manifested mainly as adenocarcinoma of the bile duct (90%) or other rare types. [121] Gallbladder cancers exhibit varying histologic forms, including papillary, tubular, nodular and combinations of those, with increased likelihood of invasion. The incidence of gallbladder cancer is very high in Mexico, especially among native American women, and in other Latin American countries. Japanese, American Indians and Israelis, on the other hand, have a 5 to 7x increase in incidence of cholangiocarcinomas. Evaluation of biliary tree carcinomas is based on ultrasonography, CT, MRI, cholangiography and angiography, but there is no good screening technique. Five year survival rates for gallbladder carcinoma are less than 5% mainly because most patients have unresectable tumors at presentation.

There are more than 35 histologic variants of small bowel neoplasms. They can be divided into benign and malignant and classified according to their cell of origin. [122] Small intestine malignancies are rare considering the size and surface area of the small intestine. [123] The incidences in the US in 1998 were 4,500 (1-3% of all gastrointestinal malignancies). The evaluation of patients presenting with these malignancies consists of upper GI series and CT.

Colon cancers can be classified according to gross appearance as ulcerating (2/3 of all), fungating or exophytic (1/3 of all but usually right sided), stenosing and constricting (annular, circumferencial, usually left sided). On a histologic basis they can be divided into adenocarcinomas (90-95%), some of which are mucinous adenocarcinomas (17%) which retain mucin within the tumor crypts and glands, squamous cell carcinomas, undifferentiated carcinomas, carcinoids and non-epithelial tumors (leiomyosarcomas, etc). [124] Grading of colon cancer is based on histologic type, overall cell differentiation, nuclear polarity, tubule configuration, pattern of growth, lymphocytic infiltration and amount of fibrosis. [125] Endoscopy with biopsies are commonly applied to evaluate colon cancer. If there is occlusion or edema, which block access to the cancer, a barium enema x-ray evaluation can be performed. Endoscopic screening for colon cancer is recommended for high risk populations, such as patients suffering from ulcerative colitis who have a 6x increase risk of developing cancer. [126, 127] Screening is also recommended for asymptomatic individuals over the age of 50. [128] Furthermore, digital rectal exams and occult blood testing should be part of the general population annual physical exam. [129] The 5 year survival rates of colon carcinomas range from 30-90% depending on how advanced the cancer is.
Imaging of the Gastrointestinal Tract

The endoscopic diagnosis and management of gastrointestinal malignancies has advanced considerably over the past two decades offering patients better management and more treatment alternatives. [130] High resolution video endoscopy using distal CCDs provides image resolutions that are impossible or prohibitively expensive to achieve using fiber bundle endoscopes. [131] Endoscopic ultrasonography provides information on tissue structure and pathology complementary to endoscopic image information. [37, 132, 133] However, many diseases, such as early neoplastic changes, remain beyond the resolution limit of these approaches. The dysplasia associated with esophageal columnar metaplasia (Barrett’s Esophagus) for example, can go undetected using conventional endoscopy and ultrasonography. [133] The early stages of colon adenocarcinoma can also present with microstructural changes below the resolution limit of these imaging modalities. [132]

Previous OCT experiments of gastrointestinal tissue, by Tearney et al, showed great promise in identifying tissue microstructure. [48, 134] This study examined the feasibility of OCT for high resolution imaging of gastrointestinal malignancies. In addition to normal tissue, pathologic microstructure was imaged ex vivo and the OCT images were correlated with histopathology to confirm tissue identity.

Methods

Tissue, both normal and neoplastic, was obtained from surgically excised specimens and was imaged fresh, prior to fixation which might change optical properties. Samples were obtained from 14 patients and included tissue from normal (3 samples), Barrett’s (2 samples) and squamous carcinoma (2 samples) of the esophagus, as well as normal colon (2 samples), ulcerative colitis (1 samples) and adenocarcinoma of the colon (4 samples). The specimens were placed in a Petri dish and irrigated with isotonic saline to prevent dehydration during imaging. Imaging was performed using a bench-top system. The acquisition of each image required between 10 and 30 sec depending on the size (number of pixel elements) of the image. Since the OCT beam is invisible, tissue registration was performed using a coincident, visible-light, guiding beam. The orientation of the OCT imaging scan was marked on the specimen using a micro-application of India ink. The slight mismatch between histology and OCT imaging planes as well as changes in physical dimension of the tissue associated with fixation and sectioning account for the minor differences between the OCT images and histology which are observed. After OCT imaging, samples then underwent routine histologic processing. Specimens were immersed in 10% buffered formalin for 48 hours. The tissues were then processed for standard paraffin embedding. Five-micron-thick sections were cut at the marked imaging sites and stained with hematoxylin and eosin. The stained histologic sections enabled verification of tissue identity and allowed identification of sources of tissue contrast in the OCT images.

Results

The stratified squamous epithelium of the normal esophagus is clearly visible in the OCT images. It presents as a uniform, highly backscattering layer as would be expected for that epithelial structure. The lamina propria appears loose and less optically backscattering. Blood vessels in the lamina propria are also identified in the OCT images. (Figure 4-1.A) In the case of esophageal metaplasia (Barrett’s Esophagus) the uniformly layered structure is destroyed and is instead replaced by columnar epithelium (Figure 4-1.B). OCT images of Barrett’s esophagus clearly demonstrate the disorganized and non-uniform nature of the mucosal layers. Lymphoid aggregates are also visible. Squamous cell carcinoma of the esophageal can also be visualized using OCT. The tumors appear as abnormal growths that interrupt the normal structure of the esophageal layers. The formation of malignant cell nodules is evident in the OCT images. (Figure 4-1.C)
Figure 4-1. OCT images and associated histology of normal esophagus (A), Barrett’s esophagus (B) and esophageal cancer (C). The epithelium (e), lamina propria (lm) and muscularis mucosae layers (mm) are visible in A & B. Vessels (v), lymphoid aggregates (l), as well as tumor nodules (t) are also present. (Image size: 6 mm x 2 mm, 600 (A) or 3 mm x 2 mm (B&C); 600 (A) or 300 (B&C) A-Scans; Resolution: 30 μm x 18 μm)
Imaging of the colon produced distinct images of the layers characteristic of colonic microstructure. The mucosa of the normal colon is clearly visible. Ordered, narrow crypts and villi are present, as expected and confirmed by histology. The submucosa appears as a loose, less optically backscattering layer. (Figure 4-2.A) In contrast, destruction of the normal mucosal layers and ulcerative lesions were identified in cases of ulcerative colitis. (Figure 4-2.B) In the case of early cancer, OCT was able to detect changes in the mucosal/submucosal microstructure. The crypts were more dilated and disorganized, sharply contrasting to the appearance of normal colon described previously. (Figure 4-2.C)

**Conclusions**

This study suggests the feasibility of OCT for imaging structural changes in the gastrointestinal tract. Significant differentiation of structures, including the epithelium, crypts, supportive tissue, and tumors was observed. In the case of the esophagus, features similar to those seen in the cervix, another squamous epithelial structure, were identified. The epithelial layer could be clearly demarcated and, although the basal membrane is beyond the resolution limits of our system, the highly backscattering and sharp basal layer provided the means of evaluating its integrity. However, squamous esophageal cancer is rarely identified at the pre-invasive stages and the lack of samples of early lesions does not allow the evaluation of the appearance vs. grade of neoplastic changes in the esophagus. Mass growth and nodularity was
evident in the case of squamous cancer of the esophagus. Crypts and villi were also clearly identify, both in normal tissue, such as the colon, and in cases of pre-malignant conditions, such Barrett’s esophagus. In adenocarcinoma of the colon, the regular structures of those crypts and villi were distorted and dilated. Lesions were also clearly visible in samples of ulcerative colitis. It should also be noted here that this study was performed at lower resolution, 18 μm instead of 6 μm due to the unavailability of laser sources at the time. As a result, a loss in image contrast and feature sharpness can be seen compared to the images presented in the previous chapter. As before, features not distinguished, due to the lack of adequate resolution, included subcellular markers of dysplasia such as nuclear to cytoplasmic ratio (NCR), pleomorphism and mitotic figures.

Normal, pre-malignant and cancerous changes can be identified in the images collected in this study. Variations in the squamous epithelial layer of the esophagus, including metaplasia and neoplastic changes, can be distinguished. The size, distribution and regularity of crypts and villi in the colon can also be assessed. Such distinctions can form the basis of OCT screening tools. However, an issue of great importance in the evaluation of Barrett’s esophagus is the identification of dysplastic areas. Cellular features, pleomorphism and mitotic features are necessary to identify dysplasia in a background of metaplasia. Such features were beyond the resolution limits of OCT but future work on ultra-high resolution and spectroscopic imaging could result in direct or indirect, based on wavelength depended scattering, detection and assessment of dysplastic areas.

Access to the luminal structures of the gastrointestinal system for OCT imaging can be achieved by fiber optic catheters similar to the one described in Chapter 2. Such devices can be used in two scenarios. First, they can be inserted through the accessory port of an endoscope and thus provide an adjunct for conventional endoscopy. Alternatively, the catheter can be advanced to the esophagus through a transnasal or transoral tube. In such cases, anesthesia is not required and the procedure can be conducted in an office setting thus reducing costs and establishing OCT as a screening tool. Direct integration with endoscopes is not feasible since most modern endoscopes have the their CCD chips and optics distally.

It is unclear if an imaging technology such as OCT would be cost effective for screening the general patient population for early neoplasias. However, future applications of OCT might be identified in screening high-risk patient populations. Such target populations could be patients who suffer from chronic gastro-esophageal reflux and are at a high risk for developing esophageal metaplasia. Patients with metaplasia have at least a 30-fold increased chance of developing adenocarcinoma of the esophagus compared to the general population. [113] Other high risk populations who could benefit from OCT screening could be people suffering from ulcerative colitis or older patients, above 50 years of age, who are usually advised to undergo regular evaluations for colon cancer. The presence or absence of pre-malignant or neoplastic changes can be identified using OCT and followed by the standard endoscopy and biopsy evaluation. OCT could also be used to provide adjunct diagnostic information to conventional endoscopy by imaging the internal microstructure to assess lesions identified endoscopically. An area where current endoscopic techniques are lacking is the identification of dysplasia in Barrett’s esophagus, resulting in random biopsy assessment of metaplastic tissues. If in the future OCT is capable of identifying dysplasia, it could be used to guide conventional biopsy, reduce sampling errors, and achieve higher sensitivity using fewer biopsies to identify areas of dysplasia, thus increasing the diagnostic yield of endoscopy and histology. [26] Furthermore, in analogy to endoscopic ultrasound, OCT could aid in identifying the invasion depth of early neoplasias and monitor response to therapy.
CHAPTER 5 - IMAGING OF THE RESPIRATORY TRACT

Cancers of the Respiratory Tract

Considering the rarity of tumors of the larynx, laryngeal carcinomas receive disproportional attention in the literature. The major reason for this disparity is the effect of this cancer on the patients quality of life and functional skills in society. [135] Significant alteration of laryngeal anatomy, by surgery or cancer, can have noticeable impact on communicative skills and digestive and respiratory physiology. There were 10,100 cases of laryngeal cancers in the US last year and of those 8,100 were men. [1] The ratio of 4:1, as disproportional as it may seem, is down from 15:1 in the 1950s. [136] Major risks factors for laryngeal cancer are smoking and alcohol. [137, 138] Other factors such as environmental exposures also appear to predispose people to laryngeal cancer. More than 95% of all primary laryngeal malignancies are squamous carcinomas with the remainder being sarcomas, adenocarcinomas, neuroendocrine tumors and other types. [139] The majority of the cancers of the larynx are diagnosed endoscopically by gross appearance and the diagnosis is confirmed by biopsies and histology. Only a small percentage are obscure to endoscopy. Once a tumor is discovered then the status of lymph nodes can be further evaluated with CT or MRI. Therapy for laryngeal cancer consists of surgery and/or radiation. The 5-year survival rates are high for early lesions (70-90%) but drop significantly for more advanced cases (20-50%) depending on the site and type. [135] After laryngectomy, extensive rehabilitation is required to return the patient to a level of vocal communication skills that would enable a functional role in society. [140]

Lung cancers are common with 164,100 cases and 156,900 deaths in the United States. They are some of the deadliest forms of tumors with an overall 5-year survival rate of only 13%. [1] The evidence of a causal relationship between smoking and cancer is overwhelming. [141] It is estimated that of all the deaths due to lung cancer 80% in men and 75% in women are a result of smoking. [2] Other environmental exposures, e.g. asbestos, also increase the chances of developing lung cancer. [142, 143] Lung cancers are separated into small cell (SC) and non-small cell (NSC) lung cancers. NSC cancers can take the form of squamous carcinomas (30%) or adenocarcinomas (40%) as well so called large cell carcinomas (10%). Most squamous carcinomas are found in the trachea and bronchi whereas adenocarcinomas occur in the middle or periphery. Large cell carcinomas are histologically undifferentiated. SC carcinomas occur as hilar or mediastinal masses and are rapidly invasive. Lately they have been increasing in frequency especially in women. Sputum cytology, X-rays and CT are used for diagnosis and staging is completed with bronchoscopy and/or thoracoscopy with biopsies. Therapy for lung cancer includes surgery when the tumor is resectable, or confined to a small area of the lung, and radiation and/or chemotherapy. The 5-year survival rates of NSC cancers range from 60-80% for small defined lesions with no extrapulmonary involvement to less than 5% when the mediastinum and lymph nodes are involved. [144] The 5-year survival rate of SC carcinoma is 1-4%. [145] Of all treated patients 5-7% will develop a new primary lesion. [2]

Imaging of the Respiratory Tract

The upper respiratory tract is the site of a wide range of disorders extending from infectious to neoplastic etiologies. [15] Early detection of many of these disorders could greatly improve patient morbidity and mortality, especially the recognition of pre-malignant lesions. Current imaging modalities including chest X-ray, Computed Tomography (CT), Magnetic Resonance Imaging (MRI), bronchoscopy, and catheter-based endoluminal ultrasound, have resolutions of 100 µm or greater, which cannot identify changes in architectural morphology, especially in early stage neoplastic changes. Although fiber optic bronchoscopy greatly improves the yield of diagnostic information, it cannot image subsurface morphology. Bronchoscopic ultrasound requires special procedures to remove the air between the
ultrasound transducer and target, and resolutions are limited to \(~100 \mu m\). In addition to the need for early cancer diagnosis, a high resolution imaging modality is also highly desired to determine tumor margins and guide treatment. The laser treatment of endobronchial disorders has become increasingly accepted, but the operation requires a high degree of skill. [146] A portable imaging method with high resolution has the potential to improve the precision in differentiating tumor margins and in monitoring laser exposure in real time.

This study examined the feasibility of OCT for ultra-high resolution imaging of the upper respiratory tract by \textit{ex vivo} studies of human tissue from the epiglottis to the secondary bronchi. Images were obtained that demonstrated the ability of OCT to image microstructural features and demarcate tissue layers. Microstructure was correlated with histopathology to confirm the image interpretation and verified the ability of OCT to delineate features that could be used as pathological markers. The ability of OCT to generate image resolution in the range close to that of histopathology in real time, supports the hypothesis that this optical technology will become a powerful modality in the diagnosis and management of a wide range of clinical respiratory pathology.

**Methods**

The tissue samples were obtain from cadavers and were refrigerated and maintained 0°C in 0.1% sodium azide. After dissection, the tissue specimens were placed in a Petri dish and irrigated with isotonic saline to prevent dehydration during imaging. The acquisition of each image required between 10 and 30 sec depending on the size (number of pixel elements) of the image. Since the OCT beam is invisible, tissue registration was performed with a visible light guiding beam. The orientation of the imaging scan was marked on the specimen using the microapplication of India ink. The samples then underwent routine histologic processing. Samples were immersed in 10% buffered formalin for 48 hours. The tissues were then processed for standard paraffin embedding. Five micron thick sections were cut at the marked imaging sites and stained with hematoxylin and eosin (H&E) or Trichrome Blue. The stained histologic sections enabled verification of tissue identity and in most instances allowed identification of sources of tissue contrast in the OCT images.

**Results**

All the images are 6 mm by 3 mm at 18 or 6 \mu m resolution, and the scale bar (bottom left of each OCT image) is 500 \mu m, unless otherwise indicated. They were plotted in a logarithmic intensity scale with the least back-scattering areas being white and the most back-scattering areas black as indicated by the intensity bar (bottom of each OCT image.)

![OCT image of a human epiglottis with associated histology. The different layers of the tissue of the epiglottis were clearly visible. The epithelium, lamina propria and cartilage were demarcated as well as several glandular structures.](image)

The images of human vocal and ventricular chords (Figure 5-2) illustrate the ability of OCT to image the epithelium and lamina propria of tissue as well as characteristic structural features such as glands and vessels. Muscle layers appeared as uniform amorphous regions in the OCT image. Higher resolution should enable identification of individual fiber bundles.
Figure 5-1. *Ex vivo* OCT image of human epiglottis (A) and associated histology (B). The epithelial (e) and lamina propria (l) layers are clearly identified as well as cartilaginous (c) and glandular (g and arrows) structures. (Image size: 6 mm x 3 mm, 300 A-Scans; Resolution: 30 µm x 18 µm)

Figure 5-2. *Ex vivo* OCT image of human vocal (A) and ventricular chords (B) and associated histology (C and D respectively). Areas of epithelium (e) of different thickness can be identified as well as the lamina propria (l), muscular (mm) and glandular (g) layers of the tissue. (Image size: 6 mm x 2 mm, 500 A-Scans; Resolution: 30 µm x 18 µm)
Figure 5-3. *Ex vivo* OCT image of human trachea (A, C) and associated histology (B and D respectively). The lamina propria (l) and cartilage (c) as well as the damage caused from the patient’s intubation are visible. Several glands (g) and a gland neck (gn) can also be identified. The perichondrium (p), interterritorial (i) and territorial matrix of the tracheal cartilage are visible without staining. There is also some debris and mucus deposits (d) on the surface. (Image size: 6 mm × 3 mm, 300 A-Scans; Resolution: 30 μm × 6 μm)

Figure 5-4. *Ex vivo* image of a secondary bronchus. Once again, cartilage (c), glands (g), and lamina propria (l) are noted. There is also some debris and mucus deposits (d) on the surface. (Image size: 6 mm × 2 mm, 500 A-Scans; Resolution: 30 μm × 18 μm)
Trachea damaged from intubation was also imaged. (Figure 5-3, A and B) The damage to both the epithelial and mucosal layers was visible in the images and associated histology. Several glands and gland ducts were also observed. Structures within the cartilaginous layer could also be identified, with layers such as the perichondrium, interterritorial and territorial matrix clearly demarcated without any staining. In Figure 5-3 C and D, an image of a secondary bronchus and the corresponding histology are shown. Cartilage, lamina propria, and glands were noted as well as the fact that OCT was able to image through the entire thickness of the bronchus. A closer look at an undamaged specimen revealed the presence of a highly scattering epithelial layer under mucus and debris deposits which had possibly accumulated there \textit{ex vivo}. (Figure 5-4, A and B) The lamina propria was highly vascularized which could be an indication of inflammation.

\section*{Conclusions}

This work demonstrates the feasibility of OCT for intraluminal imaging of the upper respiratory tract. In addition to sharp differentiation of the epithelium, subsurface structures were identified including glands, supportive tissue, and cartilage. OCT imaging was also able to successfully assess the epithelium, which appeared as a highly backscattering structure as well as damage to superficial layers due to intubation. Glandular structures were sharply visible and their ducts could be followed to the surface of the tissue. In addition, the perichondrium and different layers of cartilage matrix were visible in the OCT images without any special staining.

The primary focus of this study was to demonstrate the feasibility of OCT imaging on relatively normal tissue of the respiratory tract. Whether OCT can reproducibly identify respiratory pathology both \textit{ex vivo} and \textit{in vivo} is still to be determined. However, successful delineation of features such as the epithelium, glandular, cartilaginous and muscular structures suggest that detection of changes in these structures should be possible. In addition, the ability of OCT to identify carcinomas in other tissues with structural morphology similar to the respiratory tract indicates that imaging of neoplasias of the airways should be feasible. More work is needed to evaluate the ability to assess different pathologies, including early neoplastic changes.

The application of OCT imaging to the respiratory tract is in many ways similar to gastrointestinal imaging. A fiber optic OCT catheter has to be used to access the airway tree through the accessory port of a bronchoscope. Direct introduction of the OCT catheter is also possible but will most probably be limited to the upper airway structures since the complexity of the bronchial conduits will make blind guidance of the OCT device almost impossible. This complexity also introduces the additional difficulty of successfully imaging large luminal areas in the respiratory tract. A fast, real-time, system with a large imaging area would most likely be required to successfully achieve good coverage. If quantitative evaluation of the tissue is possible in the future then imaging of the respiratory tract will be simplified. It would allow reduction of the OCT data to simpler forms, such as topographic maps, allowing easier and faster imaging and evaluation of such large areas.

Many applications of OCT can be envisioned, from the identification of early neoplastic changes, both of the larynx and conduit airways, to the assessment of non-neoplastic disorders such as tracheal malacia and chronic bronchitis. The feasibility of OCT for the diagnosis of respiratory disorders is further supported by the easy integration with small, relatively inexpensive endoscope, low cost, and lack of a need for a transducing medium an issue of great importance in patients with respiratory distress. However, screening applications are rather unlikely, due to the large area of the respiratory tract lumens and inaccessibility of the smaller airways. On the other hand, surgical guidance of excisional procedures in sensitive areas, such
as the vocal chords, where tissue preservation is imperative to patient social and functional adjustment, could have a significant impact. Furthermore, with the advent of miniature OCT needle devices, OCT imaging will likely be directly extended to transbronchial and transcutaneous techniques, areas where the diagnosis of pathology is often unsuccessful due to limitations in the number of excisional biopsies which can be performed.
CHAPTER 6- IMAGING OF THE URINARY TRACT

Cancers of the Urinary Tract

Bladder cancers usually fall into the pathologic categories of transitional cell carcinomas (95%), squamous cell carcinomas (3%) and adenocarcinomas (2%). Approximately 30% are multifocal and 75% are superficial. [147] They are graded on a scale from 1 to 3, ranging from well differentiated tumors with limited atypia and mitoses to tumors with marked increase in cell layers, size and mitoses and noticeable pleomorphism. [148] The depth of penetration is very important in staging since it directly correlates with metastatic potential. [149] There were 54,400 new cases and 12,500 deaths in the US, with a peak incidence in the seventh decade of life and a male to female ratio of 3:1, although the disparity is not as great in populations where smoking is equally prevalent in men and women. Evaluation consists of x-ray or CT imaging and cystoscopy. Therapy is usually transurethral resection (TUR) or partial or total cystectomy with radiation and chemotherapy depending on the degree of invasion. For superficial tumors the goal is to prevent the morbidity of recurrence, which can be anywhere between 30-85%, and can be treated by TUR. [150, 151] Grade progression occurs in 10-30% of all treated superficial tumors. [152] More invasive disease is treated more aggressively. [153] Despite the recurrence early bladder cancer is completely treatable. Unfortunately, the 5 year survival rate of muscle invasive cancer is only 20-50%. [153]

Although many aspects of the management of transitional cell carcinoma (TCC) are now well established, significant challenges remain that influence patient morbidity and mortality. Among the most important of these challenges involve the inability to identify early neoplastic changes and to assess the degree of tumor invasion into the bladder wall. [154] For example, patients who have undergone local resection of superficial disease have recurrence rates as high as 60%. [155] Early detection and treatment of recurrent disease is required to maximize bladder preservation and patient survival. [156] However, current diagnostic methods have only a limited ability to detect early neoplastic lesions, such as carcinoma in situ and dysplasia, resulting in some patients presenting with invasive disease. Similarly, the optimal management of patients with TCC requires that, during initial local staging, superficial tumors must be distinguished from those which have become invasive (T2). Once the tumor has spread into the muscular layer, both prognosis and therapy differ substantially from that of superficial disease. [157] Accurate methods of assessing, during endoscopic evaluation, both the degree of tumor invasion and the presence of early neoplasia would substantially improve patient management.

Imaging of the Urinary Tract

Cystoscopy, the direct visualization of the bladder surface via optical fiber bundles, is the current ‘gold standard’ for the diagnosis of TCC. [158] Under cystoscopic evaluation, those tumors raised above the bladder surface can be readily detected. Furthermore, cystoscopy can be used to guide the transurethral resection of superficial tumors. [156] However, some clinically relevant pathology, which appears benign by direct visualization, may go unobserved. This would include multiple site involvement at the time of local resection or the presence of recurrences during subsequent screening. In addition, cystoscopy does not allow subsurface microstructural information to be obtained, such as the extent of tumor invasion into the bladder wall. Blind biopsy or cytology may be used in conjunction with cystoscopy, but they are only of limited value. [159]

The limitations of cystoscopy have led investigators to examine other methods for interrogating the bladder wall. MRI, transabdominal ultrasound, and CT are powerful imaging technologies for a wide range of medical applications, including the assessment of distant metastasis. Unfortunately, their relatively low
resolution (>500 μm) prevents assessments of microstructural changes within the bladder wall. High frequency endoscopic ultrasound (20 MHz) has been applied experimentally to the assessment of bladder carcinomas. However, its axial resolution of greater 150 μm is also not sufficient for reliably identifying layers within the bladder wall, which has prevented its adoption into routine clinical use.[160, 161] Both diffuse reflectance and fluorescence techniques have been explored, but high false positive rates have raised questions on their ultimate utility. [162] An imaging technology capable of assessing the bladder wall near the resolution of histopathology would be a powerful tool to overcome these limitations in the management of TCC.

**Methods**

For *ex vivo* studies, bladder tissue (both normal and neoplastic) was obtained from patients after surgical resection. The tissue specimens were placed in a Petri dish and irrigated with isotonic saline to prevent dehydration during imaging. The acquisition of each *ex vivo* image required between 10 and 40 sec depending on the size (number of pixel elements) of the image. Since the OCT beam is invisible, tissue registration was performed with a visible light guiding beam. The orientation of the imaging scan was marked on the specimen using the microapplication of India ink. The samples then underwent routine histologic processing. Samples were immersed in 10% buffered formalin for 48 hours. The tissues were then processed for standard paraffin embedding. Five micron thick sections were cut at the marked imaging sites and stained with hematoxylin and eosin (H&E). The stained histologic sections enabled verification of tissue identity and in most instances allowed identification of sources of tissue contrast in the OCT images.

![Figure 6-1. OCT image of normal and corresponding histology. In 6-1a, the red arrow identifies the mucosa/submucosal interface while the black arrow identifies the submucosa/muscularis interface. Figure 6-1c demonstrates the mucosa/submucosa interface (white arrow) and submucosa/muscularis interface (black arrow). High muscle bundles (m) and vessels (v) are noted. Bar represents 500 μm in all images. Figures 6-1b and 6-1d represent corresponding histology. (Image size: 6 mm x 3 mm, 500 A-Scans; Resolution: 30 μm x 18 μm)](image-url)
Figure 6-2. OCT image of normal bladder and corresponding histology. In 6-2a, the mucosa/submucosa interface (green arrow) and submucosa/ muscularis interface (white arrow) is delineated. Vessels (v) are noted within the wall. In 6-2c, a section of bladder is noted. This section with relatively little mucosal layer has been included since it confirms that the submucosa/muscularis layer on previous images was correctly defined (black arrow). The green arrow identifies capillaries within the submucosa. (Image size: 6 mm x 3 mm, 500 A-Scans; Resolution: 30 μm x 18 μm)

Figure 6-3. OCT images of invasive transitional cell carcinoma and corresponding histology. In 6-3a and 6-3c, the mucosa/submucosal and submucosal/muscularis boundaries are no longer observed. In addition, there are no capillaries noted, which were present in all normal images. There are also distorted sections of muscle bundles (black arrows). In figure 6-3b and 6-3d the structure of the tumors are confirmed by histopathology. (Image size: 6 mm x 3 mm, 500 A-Scans; Resolution: 30 μm x 18 μm)
Chapter 6. Imaging of the Urinary Tract

Results

OCT images of normal bladder obtained post surgical resection were taken and are shown in figures 6-1 and 6-2. The mucosa/submucosal interface as well as the submucosa/muscularis interface were differentiated (Figure 6-1a). High muscle bundles were seen. Figure 6-1c also demonstrates the mucosa/submucosa interface (white arrow) and submucosa/muscularis interface (black arrow). High muscle bundles (m) and vessels (v) are noted. Bar represents 500 μm in all images. The corresponding histology (6-1b and 6-1d) was included.

Figure 6-2 is also images of normal bladder. In figure 6-2a, the mucosa/submucosa interface (green arrow) and submucosa/muscularis interface (white arrow) are delineated. Vessels (v) are also noted within the wall. In figure 6-2c, a section of bladder is noted. This section with relatively little mucosal layer has been included since it confirms that the submucosa/muscularis layer on previous images was correctly defined (black arrow). The green arrow identifies capillaries within the submucosa.

Figures 6-3a and 6-3c are OCT images of invasive transitional cell carcinoma. The mucosa/submucosal and submucosal/muscularis boundaries are no longer clearly observed. In addition, there are no capillaries noted, which were present in all normal images. There are also distorted sections of muscle bundles (black arrows). In figure 6-3b and 6-3d the structure of the tumors are confirmed by histopathology.

Conclusions

The feasibility of OCT is demonstrated for high resolution assessment of the bladder. OCT was able to delineate normal microstructure of the bladder, such as the mucosa, submucosa, and muscularis layers. This was in contrast to specimens of invasive carcinoma, where a disruption of the normal bladder wall architecture was seen. Other structures such as blood vessels could also be identified.

The results in this study are very preliminary. The primary focus was to demonstrate the feasibility of OCT imaging of bladder tissue. The lack of samples of early lesions limits our understanding of the appearance and grading of neoplastic changes at different stages. However, the ability of OCT to identify carcinomas in the bladder and other tissues indicates that imaging of neoplasias should be feasible. Further work is needed to evaluate the ability to assess different pathologies, including early neoplastic changes in more detail.

Imaging of the urethral wall can be achieved using an OCT catheter similar to the one described earlier. However, imaging of the bladder poses additional challenges. First, the access to the bladder, i.e. the urethra, is small so the OCT imaging apparatus will most likely have to be inserted either blindly without the benefit of endoscopic guidance or through a very small port of a cystoscope. Second, to successfully image the wall of the bladder, a forward imaging device is needed and, given the access restrictions just stated, the size and consequently the scanning range of such a device will be limited. Third, to image certain areas, such those around the opening of the urethra in the bladder, the OCT catheter will have to turn 180° which is very difficult with such a small fiber optic device with moving (scanning) parts. Fourth, the surface of the bladder is fairly large and the tissue is somewhat mobile which will make thorough imaging of the entire area challenging. However, all of these hurdles are mainly technical in nature and, if an application for OCT imaging of the bladder is identified, additional engineering work should resolve most of them.

A role for OCT is envisioned as an adjunct to conventional cystoscopy. In particular, this technology would show its greatest utility in the management of TCC, where the degree of tumor invasion and the presence of early neoplastic disease currently can not be effectively characterized. Even more important could be monitoring TCC patients after the completion of therapy to allow early detection of recurrences.
This high risk population, who are usually advised to undergo regular evaluations, could benefit from OCT screening. The presence or absence of pre-malignant or neoplastic changes can be identified using OCT and followed by the standard cystoscopy and biopsy evaluation. OCT should provide an advantage over high resolution endoscopic ultrasound in this respect since that technique is not very effective in identifying tissues with radiation scarring.

Acknowledgements

The results in this chapter are to a large part a product of the work of Christine Jesser, to whom I am very grateful. [163]
CHAPTER 7- TISSUE PRESERVATION FOR EX VIVO IMAGING

Once tissue is removed from the circulation, the effects of ischemia become immediately evident. The end result are metabolic and structural changes that lead to tissue edema and necrosis. As OCT imaging technology advances, with marked improvement in its resolution capabilities, inconsistency between in vivo and ex vivo images of the same tissue are observed. If ex vivo experiments are to be a good demonstration of what one should expect from in vivo studies, every effort has to be made to preserve the tissue close to its in vivo state. A lot of the work on this subject has been performed as a response to the need for tissue preservation in anticipation of transplantation. The goal of this study was to understand the origin of OCT contrast in vivo and examine the changes in the OCT images associated with tissue excision and ischemia under different storage conditions in the hamster cheek pouch. This tissue site was chosen both because it is very easy to access and it is scientifically relevant since it will be the focus of future high resolution imaging studies of carcinogenesis. An understanding of the changes induced by ischemia at the tissue level should result in improved preservation methods and imaging results and enable safer transitions from ex vivo to in vivo imaging.

Effects of Ischemia

When tissue is removed from the circulation it is subject to two types of stresses. First, the lack of an oxygen and nutrient supply and, second, the accumulation of potentially toxic metabolic waste. Most of the body’s energy supply (80-1800 moles of ATP/day) comes from the oxidative metabolism of glucose. [164] The depletion of oxygen, which is almost complete within seconds, results in a cessation of the electron transport chain with anaerobic glycolysis remaining as the sole source of energy production. [165] ATP stores are quickly depleted and most energy dependent functions stop. Anaerobic glycolysis proceeds and as a result the tissue pH drops. At the same time glycogen stores are depleted. As NADH and H+ accumulate, NAD is depleted and glycolysis ceases completely resulting in cell death. [166]

The depletion of the cellular energy stores has several detrimental consequences. The cessation of the activity of the energy-dependent Na+/K+ ATPase, which normally regulates the osmolarity of the intracellular space and the membrane potential, causes an uncontrolled redistribution of ions, Na+ and Cl- enter the cell but little K+ exits due to the presence of large anionic proteins. With the ions water enters the intracellular space which causes cell swelling. At the same time the impermeable ionic and non-charged proteins in the cell compound the problem by exerting an additional onotic load and attracting more water. [167, 168] The swelling of the intracellular space can weaken the cell membrane and can cause rapture. [169] The dependence of cell on glycolysis for energy production also leads to an accumulation of H+ and lactic acid and a drop in the cell’s pH. Values below 6.7 have been found to be harmful to the cells. [170] The combination of low pH and swelling can also cause the release of enzymes from lysosomes which can digest the structural and functional components of the cells. Self digestion of a cell can occur as early as 30 minutes after the induction of ischemia. [171] The lack of circulation and endogenous enzymatic activity also leads in the accumulation of metabolic waste and especially free oxygen radicals which can further damage the cells. [172]

Methods of Preservation

Organ preservation, in anticipation of transplantation, has improved considerably in the last 10 years due to the introduction of new solutions and the better understanding of preservation damage. [173]

Hypothermia (4°C to 10°C) remains the essence of preservation today and tissues can either be placed in hypothermic storage or under hypothermic pulsatile perfusion. Currently, hypothermic storage is more
common, because of its practicality and reduced cost. [174, 175] Under hypothermic conditions metabolic demand drops to about 10% but not all enzymes are affected the same way. At room temperature (20°C) the metabolic activity is probably around 25%. [176] The degradation of intracellular components is reduced, albeit not completely. Beneficial functions, such as glycolysis, are also impaired and intermediaries are shunted to other pathways. Ironically, hypothermia also increases free oxygen radicals due to the increase in xanthine oxidase function at lower temperatures, a problem which becomes transiently very severe with reperfusion. [172] Despite the decreased metabolic demands, at low temperatures, the cell remains minimally functional, with restitution of adenosine triphosphate (ATP) at reperfusion being critical. [174] The details of the protective effects of hypothermia are not completely understood.

**Preservation Solutions**

The type of solution, the length of preservation, and the preserved organ are important factors to be considered when determining preservation damage. Cell swelling and acidosis are two end results of ischemia that must be overcome in order to improve organ viability. [164-166] The permeability of the cell membrane, exchange of ions between the cell and the environment and the activity of the Na⁺/K⁺ pump have to be controlled. The membrane potential is also diminished. [164] Significant changes in these parameters will cause cell damage, which could be prevented to a certain extent by using the right preservation solution. [164-169]

Various solutions have been formulated in an attempt to balance the ionic and osmotic pressures in the intracellular and extracellular compartments and control swelling. (Tables 7-1 to 7-3) [173, 177-179] The buffering capacity of these solutions is strong to keep the pH around 7.4. (Table 7-2) [180] Colloid molecules have also been introduced to provide intravascular oncotic pressure and prevent fluid from enteric the tissue vessels and causing organ edema. [181] Active pharmacological additives in some of these solutions include: Oxygen free radical scavengers (reduce tissue damage), Prostaglandins (improved vasodilation during reperfusion and inhibition of platelet aggregation), Ca²⁺ channel blockers (reduced influx of Ca²⁺ which can be toxic and reduce contractility and energy demands), substrate enhancements such as Adenosine and Adenosine nucleotides, amino acids, glucose and glycogen, vasoactive drugs and vasodilators and finally steroids (anti-inflammatory and membrane stabilizing). More recently, experimental membrane stabilizing substances (Trehalose, which binds to the phospholipid bilayer) and apoptosis inhibitors (phospholipids made from soybeans) were also tested. [166] The most common, clinical solutions are Collins, University of Wisconsin, Histidine-Tryptophan-Ketoglutarate and Celsior.

**Collins solution**

One of the first solutions for kidney preservation was developed by G.M. Collins in laboratories in Los Angeles.[182] The Collins solution was modified by the Eurotransplant Organization by taking out magnesium without negative effect on preservation results. [183] This Eurocollins solution was cheap but not quite simple to use because sterilization of the glucose-containing fluid had to be done separately from the electrolyte part and mixing before use was necessary. The Eurocollins solution was standard in Europe for about 15 years. The basic principle of this solution is based on is the philosophy of "intracellular electrolyte composition" where the electrolyte concentration mimics the intracellular milieu. There is a strong phosphate buffer and, as a carrier for the osmotic load to reach hypertonic condition of 420 mOsmol, glucose is used. This in fact may be the major disadvantage of this solution, because even under hypothermic conditions, glucose is enzymatically broken down, at least to the level of lactate, which results in doubling of intracellular substrate molecules followed by cell swelling.

**University of Wisconsin solution**

Continuous research effort headed by one of the pioneers of organ preservation, Folkert O. Belzer, resulted in a development of a new preservation solution that introduces three new philosophies: (a)
osmotic concentration is no longer maintained by metabolically active glucose, it is rather achieved by the administration of metabolically inert substrates like lactobionate and raffinose; (b) much attention is paid to the additional administration of colloid carrier hydroxyethylstarch (HES); (c) addition of oxygen radical scavengers, glutathione, allopurinol, and adenosine. [184] This solution has proven to guarantee extreme long cold ischemia tolerance, 72 hours in kidneys and certainly 48 hours in livers. This solution was also tested prospectively in a multi-center European trial, where University of Wisconsin solution (UW) has been found superior to Eurocollins by decreasing the rate of delayed graft function from 33% to 23%. [185] At the moment, UW is considered the standard preservation solution for livers, kidneys, and pancreases. Attempts to use UW for heart preservation were not convincing.

**Histidine-Tryptophan-Ketoglutarate solution**

Histidine-Tryptophan-Ketoglutarate solution (HTK) was introduced by H.J. Brettschneider from Göttingen, Germany, who was interested in developing a physiologic cardioplegic solution. [186] The basic philosophy is the introduction of a very potent buffer system, which is Histidine, along with the two substrates. The HTK solution has a very low viscosity and according to Brettschneider, high volumes at a low flow rate should be applied to guarantee "equilibration." HTK has been tested in clinical trials. Eurotransplant conducted a multi-center randomized prospective trial comparing UW versus HTK, and the results show same efficacy at the endpoint of delayed graft function. [187] Clinical use in liver preservation is documented by single-center experiences, which show the same safety and efficacy profile as UW, at least in the time range of below 24 hours cold ischemia time. [188]

**Celsior**

The Celsior preservation solution created by Pasteur-Merieux is a mixture of the impermeant inert osmotic carrier philosophy from the Belzer solution, using lactobionate and mannitol, and the strong buffer philosophy from the HTK solution using 30 mmol of histidine buffer. (Table 7-3) In addition, Celsior also contains oxygen radical scavengers. From the electrolyte point of view, Celsior is a high sodium, low potassium solution that is unique in this respect. Celsior has been used clinically in cardiac conservation. [189] Exclusively experimental approaches to other organs are under current investigation.

**Summary**

Eurocollins has almost been abandoned because of the glucose disadvantage. UW is certainly the most used preservation solution for livers, kidneys, and pancreases with excellent clinical and experimental preservation data. UW can certainly be considered the current golden standard solution. However, the disadvantage of high viscosity, high price, uneasy handling, and the fact that the radical scavenger glutathion cannot be detected in the bags by chemical analysis (presumably due to diffusion) encourage competitors to produce new compounds with better cost to effect ratios.

HTK has a firm place in cardiac preservation by demonstration of equal safety and efficacy in preserving livers and kidneys, at least in the middle and lower range of cold ischemia time, HTK will be used more frequently, particularly with the consideration of lower price and more easy handling aspects. The suggested high volume perfusion is not really necessary, calculation based on a total volume of 10 L for a multiorgan donor show significant cost reductions.

Celsior is currently only used for cardiac preservation. Other solutions have been tried for a variety of tissues with varying success. Examples include cell culture media for bladder tissue and simple phosphate buffered sucrose (PB S140.) [178, 179]
<table>
<thead>
<tr>
<th>Content (mmol/L)</th>
<th>Saline 0.9%</th>
<th>PBS</th>
<th>EC</th>
<th>UW</th>
<th>HTK</th>
<th>CEL</th>
<th>PB-S140</th>
<th>Kerb</th>
<th>Dex 40</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>198</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High molecular weight impermeables (↑ osm)</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mannitol also ↑ collateral perfusion.</td>
</tr>
<tr>
<td>Lactobionate⁻</td>
<td>100</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Enhance the TCA cycle for ↑ anaerobic energy production</td>
</tr>
<tr>
<td>Raffinose</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran 40</td>
<td></td>
<td>30</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20g/l</td>
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<td></td>
</tr>
<tr>
<td>Mannitol</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Glutamate</td>
<td></td>
<td>20</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoglutarate</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_2PO_4^-$</td>
<td>10</td>
<td>58</td>
<td>25</td>
<td></td>
<td>69</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Buffer to keep the pH constant</td>
</tr>
<tr>
<td>$HCO_3^-$</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td></td>
<td>198</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Free radical scavenger</td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ATP precursor and more?</td>
</tr>
<tr>
<td>Alupurinol</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Block xanthine oxidase, ↓ free rad.</td>
</tr>
<tr>
<td>Insulin</td>
<td>100 u/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloidal HES</td>
<td></td>
<td>50g/l</td>
<td></td>
<td></td>
<td></td>
<td>↑ osm intravascular space, ↓edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Na^+$</td>
<td>155</td>
<td>137</td>
<td>10</td>
<td>30</td>
<td>15</td>
<td>100</td>
<td>125</td>
<td>140</td>
<td>155</td>
<td>Preserve the ion balance and prevent swelling and toxicity</td>
</tr>
<tr>
<td>$K^+$</td>
<td>2.7</td>
<td>115</td>
<td>120</td>
<td>10</td>
<td>15</td>
<td>10</td>
<td>4.7</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Mg^{2+}$</td>
<td>5</td>
<td>4</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Ca^{2+}$</td>
<td>0.015</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$SO_4^{2-}$</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Cl^-$</td>
<td>155</td>
<td>139.7</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td>128</td>
<td>155</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>4.60</td>
<td>6.8</td>
<td>7.4</td>
<td>7.4</td>
<td>7.3</td>
<td>7.3</td>
<td>7.4</td>
<td>7.3</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>OSM</td>
<td>310</td>
<td>305</td>
<td>406</td>
<td>320</td>
<td>310</td>
<td>360</td>
<td>330</td>
<td>310</td>
<td>314</td>
<td></td>
</tr>
<tr>
<td>Viscosity</td>
<td></td>
<td>2.5</td>
<td>4.8</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EC: Eurocollins; UW: University of Wisconsin Solution; HTK: Custodiol Bretschneider Solution; CEL: Celsior; HES: hydroxyethylstarch.
Table 7-2. Tolerance of preservation in various organs under hypothermic conditions (hrs) [173, 177-179]

<table>
<thead>
<tr>
<th></th>
<th>Clinical</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC</td>
<td>UW</td>
</tr>
<tr>
<td>Kidney</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Liver</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Heart</td>
<td>4</td>
<td>4 (24)*</td>
</tr>
<tr>
<td>Lung</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Small Bowel</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 24 hrs with Cardiosol solution (UW with polyethylene glycol (PEG) instead of HES.)

Table 7-3. pH (Mean ± SD) of Different Preservation Solutions at 4°C to 37°C [180]

<table>
<thead>
<tr>
<th></th>
<th>37°C</th>
<th>20°C</th>
<th>10°C</th>
<th>4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>7.47 ± 0.06</td>
<td>7.46 ± 0.02</td>
<td>7.36 ± 0.08</td>
<td>7.38 ± 0.06</td>
</tr>
<tr>
<td>HTK</td>
<td>6.89 ± 0.04*</td>
<td>7.01 ± 0.15</td>
<td>7.33 ± 0.06</td>
<td>7.35 ± 0.06</td>
</tr>
<tr>
<td>UW</td>
<td>7.33 ± 0.03</td>
<td>7.36 ± 0.06</td>
<td>7.38 ± 0.11</td>
<td>7.35 ± 0.01</td>
</tr>
</tbody>
</table>

*: Different from UW and EC, P < .01.

When considering the conservation and preservation potencies of all these fluids, it must not be forgotten that cold ischemia itself is a risk factor for organ function. Therefore, cold ischemia time should be kept as short as possible. People are willing to accept 24 hours or more cold ischemia time in kidney transplantation because organ failure can be treated by dialysis. In other organs, where immediate organ function is essential, like in clinical heart transplantation, cold ischemia is hardly ever extended beyond 6 hours. Why are hearts and kidneys so different? Very likely, there is no difference, and the outstanding results in living unrelated kidney transplants is mostly due to short cold ischemia time. [190]

**Tissue Fixation**

The goal of tissue fixation is to prevent the processes of autolysis, deter bacterial attacks and maintain the tissue volume and shape while at the same time preserving the chemical and antigenic composition of the cells. [191] No single fixative can usually fulfill all of those aims so a variety of agents have been formulated for specific tissues and applications. Fixatives can be divided into coagulants and non-coagulants depending on their mode of action. A very common class of fixatives are the aldehydes which work by cross-linking amino acids of tissue proteins. This class includes, among others, glutaraldehyde and formaldehyde which fix the tissue by cross linking the amine groups of lysine. They act on the residues closer to the surface of the folded proteins which account for 40-60% of the total number of lysine groups in proteins. [192] The effect of glutaraldehyde is fast and irreversible and was found to be better at preserving the tissue structure. On the other hand formaldehyde is reversible, since it can be almost completely removed with excess water up to 24 hrs post fixation, which makes it attractive for
histrochemical and antigenic studies. Formaldehyde is usually in solution where it can reach 40 % by weight in water. This solution is also known as 100 % formalin (formalin being a trade name) which implies that 10% formalin is a solution of 4% by weight formaldehyde in water. Of note is that formalin and formaldehyde are not interchangeable terms.

Fixation is pH sensitive. Studies have shown that satisfactory fixation occurs between a pH of 6 and 8. Fixation is also faster at higher temperatures although the effect of increasing autolysis at higher temperatures also has to be considered. The nature of fixative penetration is probably complex especially considering that fixed tissue itself becomes a barrier to fixation of deeper layers. However, it was empirically determined that fixatives usually penetrate at rates proportional to the square root of time:

\[ d = K \sqrt{t} \]  

(7-1)

where \( d \) is the depth of penetration and \( K \) a constant: \(-0.8 \text{ mm/hr}^{1/2}\) for 10% formalin and \(-0.30 \text{ mm/hr}^{1/2}\) for glutaraldehyde. [191] These numbers imply that formalin will fix tissue down to 0.5 mm within 23 min, penetrating to 1 mm at 1 hr 30 min.

Artifacts of tissue fixation have been studied extensively over the past years, especially in relation to fixation for electron microscopy and histochemical analysis, but the processes are still ill-understood. [193, 194] Volume changes have been documented. The tissue usually swells in plain 10% formalin. However, it was estimated that subsequent processing and paraffin embedding for histology can result in shrinkage of approximately 33%. [195] Most researchers agree that artifacts are minimized for iso- to slightly hyper-osmolar solutions, i.e. in the range of 300-400 mOsm. Index of refraction changes, as recorded by phase contrast microscopy, were also reported.

Imaging of the Hamster Cheek Pouch

In order to evaluate the origin of contrast in OCT images and the optimal conditions for tissue storage before and during OCT imaging a series of calibrated experiments are required. As a first step in that direction, in vivo imaging of the hamster cheek pouch was performed and the effects of three different solutions, temperature and 10% formalin on OCT images were investigated.

Methods

The experiments begun with the preparation of solutions according to Table 7-4 and the instructions in the references. The animals were then anaesthetized according to Committee on Animal Care (CAC) guidelines. Several areas were marked on the cheek pouch with India ink and imaged \textit{in vivo} using a high resolution Ti:Al\textsubscript{2}O\textsubscript{3} system. This system provided enhanced resolution, 5 \( \mu \)m transverse and 2 \( \mu \)m axial. Each image extended 1 x 0.75 mm and was composed of 1000 A-scans. The acquisition time per image was approximately 15 sec. The performance of this system allows evaluation of the tissue at unprecedented resolution. After the \textit{in vivo} imaging was complete, the animal was euthanised and tissue harvested. The pre-marked sites were resected and the samples placed in saline, saline in an ice bath, PB S140, modified UW and 10% formalin. Registration was maintained by imaging the locations marked previously and marking the relative coordinates from the translation stages. The same areas were imaged at 30, 50, 70 min post mortem. After the conclusion of the experiments, the tissue was fixed in formalin and histologically processed. The image quality, details and contrast for each solution were compared between solutions and histology.
Table 7-4. Composition of solutions used for the hamster cheek pouch experiments
(quantities in mM, mg/l and cost)

<table>
<thead>
<tr>
<th></th>
<th>Saline (0.9%)</th>
<th>Modified PB S140</th>
<th>Modified UW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>g/l</td>
<td>S/l</td>
</tr>
<tr>
<td>NaCl</td>
<td>155</td>
<td>9.0582</td>
<td>0.10</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>56</td>
<td>7.94976</td>
<td>0.19</td>
</tr>
<tr>
<td>Sucrose</td>
<td>140</td>
<td>47.9220</td>
<td>0.79</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>13</td>
<td>1.76917</td>
<td>0.04</td>
</tr>
<tr>
<td>NaOH</td>
<td>30</td>
<td>1.19991</td>
<td>0.02</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>5</td>
<td>0.60185</td>
<td>0.02</td>
</tr>
<tr>
<td>Lactobionic acid</td>
<td>100</td>
<td>35.8299</td>
<td>42.64</td>
</tr>
<tr>
<td>KOH</td>
<td></td>
<td>5.61056</td>
<td>0.34</td>
</tr>
<tr>
<td>Raffinose</td>
<td>30</td>
<td>17.8350</td>
<td>25.47</td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
<td>3.02615</td>
<td>20.74</td>
</tr>
<tr>
<td>Glutathione</td>
<td>3</td>
<td>0.92199</td>
<td>27.66</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>1</td>
<td>0.13610</td>
<td>0.53</td>
</tr>
<tr>
<td>osm</td>
<td>310</td>
<td>310</td>
<td>320</td>
</tr>
<tr>
<td>pH</td>
<td>4-6.0</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Total $</td>
<td>0.10</td>
<td>1.02</td>
<td>117.50</td>
</tr>
</tbody>
</table>

Notes: pH = pKa + log([HPO₄²⁻]/[H₂PO₄⁻])
pKa=6.8 for HPO₄²⁻:H₂PO₄⁻

Results

The results of these experiments are shown in Figures 7-1 to 7-3. Figure 7-1 is compilation of the in vivo images and comparison with histology. Figure 7-2 compares the effect of different preservation solutions at 30 and 70 min postmortem and also includes the associated histology for comparison. Figure 7-3 contains the same images as Figure 7-2 but at higher magnification. A small section of each of the Figure 7-2 images was expanded 5x to allow evaluation of the finer details in the epithelial layers. Figure 7-4 contains the images acquired in vivo and then after fixation in 10% formalin over a period of 30 min to 16 hrs.
Figure 7-1. Images of 5 different sides on the hamster cheek pouch imaged \textit{in vivo} (a,e,i,m,q) with magnified areas at 5x (b,f,j,n,r) and compared to histologic cross-sections stained with Hematoxylin and Eosin (c,g,k,o,s) and Trichrome (d,h,l,p,t) stains. The histology slides were viewed at 40x and were cropped and scaled to match the image size. These areas were subsequently imaged \textit{ex vivo} while preserved in a saline ice bath (CS: a-d), room temperature saline (RS: e-h), phosphate buffered sucrose 140 (PBS140: i-l), University of Wisconsin solution (UW: m-p) and 10% Formalin (F: q-t).
(Image size: 1 mm x 0.75 mm, 1000 A-Scans; Resolution: 5 \(\mu\)m x 2 \(\mu\)m)
Figure 7-2. Images of the same sides as in Figure 7-1 imaged in vivo (a,e,i,m,q) and ex vivo at 30 min (b,f,j,n,t) and 70 min (c,g,k,o,s) post mortem, compared with histologic cross-sections stained with Trichrome (d,h,l,p,t) stain. The histology slides were viewed at 40x and were cropped and scaled to match the image size. These areas were imaged ex vivo while preserved in a saline ice bath (CS: a-d), room temperature saline (RS: e-h), phosphate buffered sucrose 140 (PBS140: i-l), University of Wisconsin solution (UW: m-p) and 10% Formalin (F: q-t). (Image size: 1 mm x 0.75 mm, 1000 A-Scans; Resolution: 5 μm x 2 μm)
Figure 7-3. Magnified (5x) images of the same sides as in Figures 7-1 and 7-2 imaged in vivo (a,e,i,m,q) and ex vivo at 30 min (b,f,j,n,r) and 70 min (c,g,k,o,s) post mortem, compared with histologic cross-sections stained with Trichrome (d,h,l,p,t) stain. These areas were imaged ex vivo while preserved in a saline ice bath (CS: a-d), room temperature saline (RS: e-h), phosphate buffered sucrose 140 (PBS140: i-l), University of Wisconsin solution (UW: m-p) and 10% Formalin (F: q-t). (Image size: 0.2 mm x 0.15 mm, 200 A-Scans; Resolution: 5 μm x 2 μm)
Figure 7-4. OCT images of tissue imaged *in vivo* (a & d) and in 10% formalin at different time intervals post mortem: 30 min (b & e), 70 min (c & f), 2:30 hrs (g & j), 3:30 hrs (h & k) and 16 hrs (l & l). Images (d,e,f,j,k,l) are the same as (a,b,c,g,h,i) but magnified (5x). (Image size: 1 mm x 0.75 mm (a,b,c,g,h,i) or 0.2 mm x 0.15 mm (d,e,f,j,k,l), 1000 (a,b,c,g,h,i) or 200 (d,e,f,j,k,l) A-Scans; Resolution: 5 μm x 2 μm)
Chapter 7. Tissue Preservation for Ex vivo Imaging

Conclusions

The origin of contrast in the OCT images

Figure 7-5 summarizes the results of the investigation of the origin of contrast in OCT images. The epithelium and stratum corneum of the buccal mucosa are consistently visible and of uniform intensity. The stratum corneum, consisting mostly of layers of keratin, is highly backscattering. A region of low backscatter underneath, at the interface with the younger epithelial cells, appears to correspond histologically to a thin single cell layer of maturing cells before they become completely keratinized. The epithelial layer itself appears uniformly backscattering. The individual cells, cellular membranes and nuclei are not discernable at the current resolution and dynamic range probably due to their small size, small differences in index of refraction with the cytoplasm and their mostly forward scattering nature.

Connective tissue appears variably backscattering in OCT images with the density of packing emerging as the probable dominant factor of the scattering intensity. Highly dense connective tissue, as the one directly under the epithelium, is low backscattering where as less dense tissue in the deeper layers of the tissue is highly backscattering. Unfortunately, the resolution of our device does not allow precise quantification of the density and nature of the connective tissue fibers, so it is impossible to claim with certainty the reason for these variations. It could be a result of interference effects from individual fibers or the effect of index matching between the fibers when they are closely packed. If this theory is indeed true it could partially explain the variations in backscattering intensity ex vivo with tissue edema and volume expansion.

Smooth muscle cells also appear uniform of intermediate to low backscattering intensity. The intervening collagen is also visible making the individual fibers discernible. Collections of fat cells appear as clear, rounded regions of almost no backscatter. Blood vessels may appear clear in standard, i.e. amplitude, OCT images, since the Doppler shift of the interferometric signal, induced by the blood flow, may be enough to shift the frequency outside the supported bandwidth of the electronics. In frequency encoded imaging, i.e. Doppler or spectroscopic, the effects of the frequency shift will be present.

![Image of OCT images](image)

**Figure 7-5.** Summary of the origin of contrast of *in vivo* OCT images of the hamster cheek pouch. sc: stratum corneum, e: epithelium. dc: dense connective tissue. lc: loose connective tissue. m: muscle. v: vessels. f: fat. The arrow in the left images points to the layer of cells between the stratum corneum and the epithelium. (Note that these images are at different scales.)
**Image Degradation**

A qualitative evaluation of these images indicates that most changes in optical properties and OCT contrast in tissue after excision occur on a fast time scale for all preservation solutions but formalin. In the case of saline, for example, it appears that all the changes occur within 30 min. In the case of PBS140 and UW the changes appear to occur within 90 min and finally with formalin the changes occur in the order of a day. (Figures 7-2 to 7-4) The lack of data points between euthanasia and 30 min post mortem precludes a finer understanding of the time scale. Unfortunately, the process, i.e. euthanasia, tissue harvest and preparation takes almost 30 min, thus no imaging is feasible in between. Also, small variations between the cross sectional plane of the tissue imaged and the plane sectioned histologically do exist. Slight mismatch between histology and imaging planes as well as changes in physical dimension of the tissue associated with fixation and sectioning account for the some of differences observed between the OCT images and histology.

The contrast between the epithelial cells and the stratum corneum as well as the underlying connective tissue is lost rapidly in almost all solutions, with the exception of formalin. The low backscattering nature of the dense connective tissue is not preserved. The reason is hard to confirm but could be leakage of intracellular material from the cells into the extracellular space and/or expansion of the spaces between the normally dense collagen fibers. In formalin the two layers above and below the epithelial cells increase in intensity. The relation between this change and protein cross-linking will be hard to explain without knowledge of the exact protein structure. Modification of the backscattering intensity of the deeper, looser collagen fibers is also visible *ex vivo*. These changes are also hard to predict, which is not surprising considering the observation that the backscattering intensity of connective tissue appears to be related to its packing density. The osmolarity changes and related volume changes in the solutions are expected to cause variations in the density of the tissue and not only change its intensity but even cause variations over time. More detailed experiments are needed to confirm this theory. However, it will be difficult to calibrate these experiments. Muscle backscattering intensity usually decreases. If the mitochondria in the muscle are presumed to be the major source of the backscattering signal then these changes can be explained by the lack of energy and degradation of these organelles *ex vivo*. The contribution to backscattering of the actin-myosin filaments and other structural proteins is unclear so pending further investigation no assumptions can be deduced as to the degree which they affect the appearance of muscle in OCT images.

The epithelial layer, which is often the region of interest in neoplastic imaging, appears to be very sensitive to *ex vivo* conditions. The epithelial volume changes even in situations where the solution is considered iso- or even a little hyper-osmolar compared to intracellular space. The contrast with adjacent layers is also blurred. The cellular changes associated with prolonged ischemia are not clearly understood so it is hard to explain these features of *ex vivo* imaging. However, the problem remains and it is important to recognize when imaging sensitive tissue. Formalin, long considered a poor option when it comes to optical imaging, may actually be a useful preservative for *ex vivo*, morphological imaging. The observation that the index of refraction and absorption/fluorescence properties of tissue change with fixation may not be a problem for microstructural imaging. If the goal of the study is to image and evaluate variations in tissue morphology then fixation may change the contrast of some compartments but it also appears to maintain the architectural features of interest. Although in this study plain 10% formalin was used, buffered and osmotically corrected to ~300 mosm solutions can be utilized. For example, a combination of 10% formaldehyde in PB S140 should be superior in preventing swelling and preserving microstructure. Such solutions are trivial to make by just adding phosphate buffer and sucrose to 10% formalin. However, this type of preservation will probably be unsuitable for spectroscopic imaging. In
such cases, if in vivo access is impossible the best technique would be preservation in PB S140 and immediate imaging.

Our understanding of the origin of contrast in OCT images is still limited. Although the present studies of the origin of cellular backscattering offer a framework for forming hypotheses, none of these can be confirmed without further simulations and experiments. The effect of the overall structure of tissue, and not just the cellular components, has to be further evaluated to provide a complete picture of tissue backscattering. In the mean time, when imaging tissue ex vivo, a step which is very often necessary during the planning stages of a study, special care should be taken to image the tissue as soon as possible while maintaining its milieu at the best attainable preservation level. Also, the role of formalin in microstructural imaging has to be reevaluated. Considering the fairly innocuous nature of the changes in image quality seen in this study when comparing iv vivo and fixed morphology, it may be the only viable alternative in imaging sensitive tissue ex vivo. The temporary nature of formaldehyde fixation makes it ever more interesting for further study. Since the fixative can be washed out with excess preservation solution, it can be completely removed during the actual imaging process.
PART III - DEVICES

CHAPTER 8 – CLINICAL SYSTEM DESIGN

The transition from bench-top studies to a clinical situation, involving patients and the strict operating conditions of a clinic or a surgical suite, requires significant modifications to the OCT technology. A portable and robust system is required to allow transportation of the imaging apparatus to the hospital. Smooth and uninterrupted operation must also be assured to avoid unnecessary and prolonged delays to the procedure time. Further, the safety of the patient and the operator must be assured according to the American National Standards Institute (ANSI) safety standards. This chapter describes the design and construction of a prototype clinical OCT device.

Requirements for a clinical device

In order to turn an optical device into a viable clinical tool certain issues have to be addressed that would allow the device to be successfully introduced to medical practice. These issues include:

1. Robustness and compactness. The device has to be compact enough to allow transportation to the site of imaging. At the same time it has to be robust enough to withstand such transportation with no or minimal readjustments needed afterwards.
2. Electrical safety. The system has to follow the strict standards of electrical safety of medical devices, including low capacitance and leakage current. Medical grade power cords, supplies and isolation transformers are usually necessary.
3. Optical Safety. Care must be taken to ensure both the patient’s and operators safety from exposure to optical radiation. Guidelines are published by the American National Standards Institute (ANSI Standard Z136.1-1993) and are explained briefly below.

Maximum Permissible Exposure (MPE)

The current ANSI standard defines the MPE values. Those exposures, in the wavelength range of interest for OCT imaging, are listed in Table 8-1.

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Exposure Duration (s)</th>
<th>MPE $E$ (J/cm$^2$)</th>
<th>MPE $P$ (W/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.400-1.400</td>
<td>$10^{-9}$ to $10^{-7}$</td>
<td>$2 C_A \times 10^{-2}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^{-7}$ to 10</td>
<td>$1.1 C_A \times t^{1/4}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 to $3 \times 10^4$</td>
<td>$0.2 C_A$</td>
<td></td>
</tr>
</tbody>
</table>

The correction factor for $\lambda$ = 1.050 to 1.400 $\mu$m is $C_A = 5$. For high repetition rates, pulses can be summed and their total effect calculated. The standard defines:

For repetition rates which are so high that multiple pulses occur in a time frame less than 18 $\mu$s (0.4 to 1.05 $\mu$m), or less than 50 $\mu$s (1.05 to 1.4 $\mu$m), pulse energies delivered within those time frames are summed directly such that one can assume the energy acts as if it were delivered as a single pulse... For short accidental exposures... the limiting case is a pulse repetition frequency of... 20 KHz for wavelengths between 1.04 and 1.4 $\mu$m.
Both the Cr:Forsterite and the Ti:Sapphire laser repetition rates are high enough to allow summation of the effects of their radiation for MPE calculation purposes. The MPE for multiple exposures of the same area of tissue is modified by a factor of \( n^{1/4} \), where \( n \) is the number of pulses.

One should also keep in mind that for short accidental ocular exposures the minimum aversion response time is 0.25 s for visible (0.4 to 0.7 \( \mu \)m) or 10 s for other wavelengths. The risk of injury to the operator should be calculated assuming the above exposure durations.

In summary, the MPE for \( \lambda = 1.05 \) to 1.4 \( \mu \)m is:

\[
MPE = 5.5 I_{\text{exp}}^{1/4} \text{ J/cm}^2
\]  

(8-1)

Assuming continuous or high repetition rate pulses the equation can be modified to:

\[
MPE = 5.5 I_{\text{exp}}^{-3/4} \text{ W/cm}^2
\]  

(8-2)

**In vivo OCT System MPE Limitations**

![Diagram of OCT system](image)

**Figure 8-1.** Scan range and exposures of an OCT system.

For a system where the beam is scanned over the tissue using a galvanometer, the area of tissue exposed can be approximated by a series of non-overlapping spots over the total length of the transverse scan \( L \). (Figure 8-1) The number of those spots, \( N \), can be calculated by \( N = L/dx \) where \( dx \) is the transverse resolution of the system. Note that this number does NOT necessarily correspond to the number of A-scans in the image. The exposure time for each spot is \( 1/(fpsN) \) where fps is the frames per second at which the imaging system operates. The MPE for each of those spots (in W/cm\(^2\)) can be calculated by formula (8-2) and the total power into the delivery optics (in W) can be estimated by multiplying the single spot MPE by the area covered by the beam, i.e. \( -dx^2 \).

\[
MPE = 5.5 L^{3/4} fps^{3/4} dx^{5/4} \text{ W}
\]  

(8-3)

This number further drops, by a factor of \( n^{-1/4} \), if the same area is scanned repeatedly over certain time. Table 8-2 shows some examples of MPEs at different rates and resolutions at \( \lambda = 1.3 \) \( \mu \)m.
Table 8-2. MPE values for $\lambda=1.3\mu m$.

<table>
<thead>
<tr>
<th>Transverse Scan Length (L) (mm)</th>
<th>Frame Rate (fps)</th>
<th>Transverse Resolution (\mu m)</th>
<th>NPE (single exp) (W/cm²)</th>
<th>MPE (total exp) (mW)</th>
<th>MPE (total exp) (mW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4</td>
<td>30</td>
<td>491.935</td>
<td>4.427</td>
<td>2.633</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>30</td>
<td>610.396</td>
<td>5.494</td>
<td>3.266</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>30</td>
<td>721.596</td>
<td>6.494</td>
<td>3.862</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>30</td>
<td>827.333</td>
<td>7.446</td>
<td>3.723</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>30</td>
<td>1026.560</td>
<td>9.239</td>
<td>4.620</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>30</td>
<td>1213.575</td>
<td>10.922</td>
<td>5.461</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>15</td>
<td>827.333</td>
<td>1.861</td>
<td>1.107</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>15</td>
<td>1026.560</td>
<td>2.310</td>
<td>1.373</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>15</td>
<td>1213.575</td>
<td>2.731</td>
<td>1.624</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>15</td>
<td>2340.050</td>
<td>5.265</td>
<td>2.214</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>15</td>
<td>2903.549</td>
<td>6.533</td>
<td>2.747</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>15</td>
<td>3432.509</td>
<td>7.723</td>
<td>3.247</td>
</tr>
</tbody>
</table>

Re-engineering of the OCT system

Construction of the delay line

Figure 8-2 shows a block diagram of a compact delay line. The theory of operation of the phase delay line has been described in Chapter 2. The original high speed system was developed by Gary Tearney, a former Ph.D. student and Brett Bouma, a former postdoctoral associate. This version has been modified from the original design into a folded configuration to make alignment easier. Also, three translation stages added allow independent adjustment of the delay line length, dispersion and modulation frequency. Each component in this system was mounted so that tilt and translational degrees of freedom would be available during alignment. This level of adjustability may not be necessary, since several of these degrees of freedom are redundant. Reduction of the system’s flexibility could introduce more compact mounts and facilitate a smaller device.

The double pass nature of the phase delay line makes alignment complicated. A method that follows a systematic approach to the problem is the alignment of a single pass cavity with one on-axis beam and the subsequent translation of the lens off axis to deflect the beam and allow return on a second plane at the stationary mirror. The alignment is also made easier when the beam out of the collimator is not perfectly collimated. This configuration makes the system less sensitive to alignment while reducing the coupling efficiency of the delay line. The loss of power here, however, is not significant since the reference arm is usually overpowered.

A problem arises when one attempts to scan the galvanometer in the phase delay line at progressively higher speeds with a triangular waveform. It is difficult to perform scanning without non-linearity and hysteresis because this involves high speed mechanical motion. The galvanometer is driven with a triangle wave, but the motion has a time delay and an overshoot. The overshoot gives an asymmetry in the motion which causes the “zipper” problems seen in some OCT images. (Figure 8-3) One can measure the error in the z scan and correct for it in the acquisition software. Another approach is to drive the galvanometer with a more complicated function to compensate for the non-linearity. (Figure 8-4) One way to compensate the overshoot is to subtract a delta function and an exponential from the triangle when it reverses direction. The delta function reverses the direction of motion of the galvanometer fast and the
leading edge of the exponential attempts to compensate the overshoot. When the galvanometer mirror begins to respond, the exponential approaches the desired triangle waveform.

![Block diagram of the delay line](image)

**Figure 8-2.** Block diagram of the delay line. This setup allows tilt and rotational degrees of freedom in almost any direction. The majority of these could be eliminated making the system alignment harder but the device even more compact.

![OCT image with the “zipper” effect](image)

**Figure 8-3.** OCT image with the “zipper” effect. The galvanometer non-linearities cause scans taken on the forward and reverse not to match exactly with each other. As the galvanometer bearings are worn down the problem becomes significantly more pronounced.
Chapter 8. Clinical System Design

Figure 8-4. Compensation of the galvanometer motion non-linearities. The top graph (A) shows the ideal drive and motion waveform (triangle) and the actual mechanical motion (shifted and overshoot). Graph B is the correction factor applied. The modified drive is shown in graph C.

Software improvement

The software is an integral part of the system and could significantly enhance, or conversely diminish, the utility of the device. The user interface and underlying processing code are currently being redesigned to improve user friendliness and speed and make future modifications easier. Although abandoned at this time, the idea of using a frame grabber with integrated Field Programmable Gate Array (FPGA) technology should be considered. Unfortunately, the current lack of support for high density analog-to-digital conversion did not allow use of such a device in the current system. However, several enhancements were implemented using the existing code as the basis. One such enhancement was the implementation of arbitrary scanning, both computer and manually (joystick) controlled. The system was modified to scan the OCT beam in any arbitrary position or pattern.

Another enhancement to the system was the introduction of automated volume scanning. The major issue there was the choice of Cartesian vs. Polar scanning. Cartesian scanning allows better coverage and uniform volume sampling but requires a large number of images. Polar scanning allows good coverage of a central region with more sparse sampling of the periphery but requires less cross-sections. Polar scanning was chosen and implemented in the current system mainly because of the speed limitations that would preclude acquiring the large number of images required by Cartesian scanning in a reasonable time.

System Integration

The compact delay line and computer and software described above were integrated into a single system unit. The necessary power supplies and function generators were included as well as an SVHS tape recorder to save the entire imaging session. The complete system is shown in figure 8-5. The system and especially the associated electronics were further optimized with the help of Dr. Xingde Li, a postdoctoral associate at MIT.
Testing in an animal model

As with any new medical technology, the prototype clinical OCT system was tested for in vivo use in an animal model to prepare and ensure a successful transition to human studies. The system performance was evaluated to confirm that the apparatus would function as expected and that no unforeseen technical difficulties would hinder its operation in vivo. The question whether there is close correspondence between in vivo and ex vivo images of the same tissues or whether the notion of contrast in OCT needs to be reevaluated was also investigated. The data presented in this chapter was part of a larger survey of several tissue areas in a rabbit which was the first demonstration of in vivo OCT imaging. A summary of the results pertaining to the respiratory and urinary systems is presented here.

Methods

Imaging for this study was performed on New Zealand white rabbits. The animals were anesthetized in accordance with NIH animal welfare guidelines under a protocol approved by the MIT CAC committee. After the animals were fully anesthetized, they were intubated (> 1mm inner diameter tube.) An imaging catheter (similar to the one described in chapter 2) was inserted through the tube and manually moved towards the lungs. Images were collected at 4 frames/sec and saved on video tape and in digital format using a system based on a phase delay line mechanism. The catheter was also inserted in the bladder and imaging was repeated as above. After the imaging was complete, tissue was harvested from the bladder for histology.

Results

As the catheter was shifted towards the lungs, images from several layers of the respiratory tract were collected. (Figure 8-6) Several micro-structural components of the respiratory tract were visible in the OCT images. The cartilaginous and muscle layers were delineated. Glands were also discernible. As the catheter progressed lower into the respiratory tree, the cartilage regressed giving way to more muscle while at the same time the lumens got smaller. The bifurcation of the major bronchi was visible with the smaller and more numerous lumens of the bronchioles following deeper insertion of the catheter.
Figure 8-6. *In vivo* imaging of the rabbit respiratory tract. A. Trachea. B. Major bronchus. C. Bifurcation of the bronchus. D. Bronchioles. Microstructural features visible include: cartilage (c), muscle (m), glands (g) and several lumens (L). The images were acquired at 4 fps at a resolution of 10 μm axial, 30 μm transverse.

Figure 8-7. *In vivo* imaging of the rabbit bladder. This figure shows an *in vivo* image of the rabbit bladder (left) and the corresponding histology (right). The mucosa (m), muscularis mucosa (mm) and muscularis propria (mp) layers are visible in both the OCT image and histology. The image was acquired at 4 fps at a resolution of 10 μm axial, 30 μm transverse.
In vivo imaging of the bladder ensued. The OCT images showed clear distinction between the mucosa (m), muscularis mucosa (mm) and muscularis propria (mp) layers. (Figure 8-7) Even the connective tissue beyond the muscularis propria was visible with blood vessels sometimes entering the imaging plane. There was good correspondence between these images and histology cross-sections.

Conclusion

A portable and robust system suitable for clinical use was constructed. It allows introduction of OCT technology in the clinic or operating room and acquisition of in vivo animal and human data. The current system was field tested extensively and was proven to withstand transportation and rough handling. The process of constructing a phase delay line was also reorganized into a more systematic approach which allows a step by step implementation. In vivo images were acquired using this system both in animals and in humans and will be presented later in this thesis.

Successful imaging of tissue microstructure in vivo was demonstrated for the first time during these rabbit studies. Tissues in the respiratory and urinary tracts, among others, were imaged. The OCT images exhibited clear delineation of micro-structural morphology including mucosal and muscular layers and structures such as glands, cartilage and vessels. No unusual findings in the backscattering properties of different tissue layers were observed at this stage which led to the belief that the OCT image attributes of tissue in vivo should be those expected from ex vivo studies. The penetration and contrast of the in vivo images was qualitatively evaluated to be superior to ex vivo ones collected at equivalent signal to noise ratio. This finding was later reconfirmed in studies of tissue preservation, which were presented in Chapter 7, but the exact reasons for this discrepancy are still under investigation.

This study also indicated that further system improvements were needed. The resolution (10 μm) would not be adequate to perform cellular level imaging and would therefore have to be improved if such a fine detail is needed for diagnostic purposes. The speed of the system was not up to video rate and motion artifacts sometimes appeared in the images. Further, the transverse imaging catheter was not the optimal device for bladder imaging. It was hard to direct to the wall of the bladder and its circular scan pattern which works well for lumens is not efficient in imaging bigger, flatter surfaces. These instrumentation deficits would not present in the human studies planned since new delivery devices, described in Chapter 9, were designed to specifically meet the needs of imaging the particular target tissues.

Further improvements to the system are still needed. The speed of acquisition has to improve to allow the collection of images at faster, video rates and digitization of higher pixel density and volumes of images without severely impairing the frame rate. Such a system could be possible with the introduction of resonant galvanometers and state of the art analog to digital acquisition boards and hardware digital processors. Further reduction of the overall size and weight of the system would also be beneficial, especially since the system has to be transported to different hospitals. As mentioned above, the software could also be further improved, a task which is currently underway.

Overall, the system performed as expected and was deemed suitable for clinical use. The imaging results were more than encouraging and the image quality suggested that in vivo human imaging should be feasible. In vivo imaging studies of the human cervix and oral cavity ensued, the results of which will be presented in later chapters.
CHAPTER 9 – DEVELOPMENT OF NOVEL IMAGING DEVICES

The success of Optical Coherence Tomography (OCT) in clinical applications will depend on a large part on the design and availability of delivery mechanisms that would allow seamless integration with current and new diagnostic modalities. Such integration would lead to the introduction of OCT in mainstream diagnostic applications and allow clinicians to use the enhanced imaging capabilities of this technique to benefit the patients without the need of extensive re-training or significant increase in procedure time. Such interplay between imaging technologies and clinical diagnostics would both improve outcome and reduce the cost of therapy. For the purposes of this thesis two devices were developed, an improved forward imaging probe and an integrated OCT colposcope. The concept of an OCT imaging needle was also introduced.

Requirements

As in any other medical device design, OCT imaging devices have to fulfil certain requirements. Briefly, these conditions include:

1. Safety. Probably the most important concern is the safety of the patient without, of course, overlooking the safety of the operator. The light exposure limits, which were explained in the previous chapter, are but one concern. Electrical safety, for devices which use electrical components, and physical trauma, for devices that come or could come into contact, should also be considered carefully. Contamination and infection are an additional risk concern especially when imaging under sterile conditions and/or with devices which contact the tissue.

2. Robustness. The device has to be robust. Failures during imaging not only result in failure to obtain OCT images but also may unnecessarily prolong the procedure time and even endanger the patient. Broken devices and detached small components could be released and cause serious complications.

3. Compactness. The device has to be as small as possible to allow easy handling and minimal obstruction of the clinicians field of view.

4. Interference. The imaging tools have to be introduced seamlessly to avoid any interference with the clinicians operations or other devices.

5. Integration. Where possible OCT imaging should be integrated with existing imaging or surgical devices to allow easier introduction to the clinical routine and minimize the training needed for use by the medical personnel.

Design of a Forward Imaging Probe

Applications such as imaging during open surgery, laparoscopic imaging and imaging of the skin or oral cavity require forward imaging probes. Such devices can have a variety of shapes and sizes sharing the main characteristic of imaging in the forward direction. Scanning can be achieved with one of a number of different methods. The previous prototype, available in our lab, employed a PZT cantilever which bend under high voltage and deflected an optical fiber. It was designed by Stephen A. Boppart, a former Ph.D. student, and a working prototype constructed by Ravi K. Ghanta, an MD student from Harvard Medical School. This design, although compact, is not very flexible in its scanning abilities, its movement is highly non-linear and it requires a 300 Volt drive waveform which raised safety concerns. This device was never used for imaging in clinical situations because of these safety issues. A new design was implemented to remedy these limitations.

New Design

The new design was based on a galvanometer to scan a collimated beam and a relay of lenses to guide and focus the beam forward. (Figure 9-1) This design converts a deflection after the first collimator to a linear
displacement after the last objective. The extra stationary mirror was used to change the orientation of the collimator and galvanometer in such a way that both the fiber optic cable and the galvanometer wires run along the axis of the probe. This was deemed necessary to make handling the device simpler and allow easy draping with sterile plastic bags for imaging in the sterile field of open surgery. The tube which houses the series of lenses was designed to be detachable and sterilizable. The stationary mirror can easily be replaced with another galvanometer to allow two-dimensional scanning with, of course, an increase in the size of the device.

Figure 9-1. Block diagram (top view) of the forward imaging OCT probe.

Figure 9-2. Optics of the forward imaging probe (top view) with a beam scanned off the axis of the system.

The angle of deflection is limited by the size of the lenses and the cylindrical housing tube. At large angles the beam will be clipped by the tube and the quality of the focal spot will degrade due to spherical and chromatic aberrations which will result from focusing at the periphery of the lenses. (Figure 9-3) The deflection at the second lens, \( x_1 \), will be:

\[
x_1 = l_1 \tan(\theta_1)
\]  \hspace{1cm} (9-1)

Continuing through the system, the deflection at the third lens and consequently at the imaging plane, \( x_2 \), will be:

\[
x_2 = \tan(\theta_1) \frac{l_1 f_3}{f_2}
\]  \hspace{1cm} (9-2)

In the mean time the radius of the beam at the second lens, \( r_1 \), will be:

\[
r_1 = f_1 \frac{NA}{2} + r_{core}
\]  \hspace{1cm} (9-3)
where NA is the numerical aperture of the fiber and \( r_{\text{core}} \) is the radius of the beam in the fiber core. At the third lens the radius, \( r_3 \), will be:

\[
r_3 = r_1 \frac{f_3}{f_2}
\]  

(9-4)

Therefore, a good estimate for the resolution of the system, from equation 2-49, is:

\[
FWMH = w_o \sqrt{2 \ln(2)} = \frac{\lambda f_2 f_3 \sqrt{2 \ln(2)}}{\pi f_3 \left[ f_1 \frac{NA}{2} + r_{\text{core}} \right]}
\]  

(9-5)

**Performance and Testing**

A forward imaging probe like the one described above was constructed with \( f_1 = 11 \) mm, \( f_2 = 38.1 \) mm, \( f_3 = 25.4 \) mm, \( f_4 = 25.4 \) mm lenses and a Cambridge Technology 6800 galvanometer with the assistance of Dr. Xingde Li and Pei-Lin Hsiung. (Figure 9-3) The resolution of that system was measured to be FWHM \approx 18 \mu m, which is in close agreement with the estimated one (FWHM = 15.8 \mu m.) The linearity of the system was confirmed by imaging a calibration grating.

![Figure 9-3. Photograph (side view) of the forward imaging OCT probe.](image)

**Design of an Integrated OCT Colposcope**

A study of the uterine cervix is an ideal clinical scenario where OCT can be systematically investigated. The diagnosis of neoplasia and early cancer is based on colposcopy and biopsy, i.e. the visual examination of the surface of the cervix under high magnification and white light illumination from a distance of about 30 cm and the simultaneous acquisition of biopsies.

OCT imaging could be performed with a forward imaging probe but that would require disruption of the colposcopic procedure and imaging without the benefit of direct visual guidance. On the other hand, an OCT system directly integrated with a standard colposcope would allow seamless integration with the current diagnostic modality without a significant increase in procedure time or personnel training. It would also permit simultaneous OCT and *en face* video imaging.
New Design

The OCT colposcope system was based on a standard Zeiss colposcope which has a long working distance of 300 mm. (Figure 9-4) The OCT beam was delivered via an optical fiber and focused with an 80 mm focal length, spherically and chromatically corrected, doublet lens. Two orthogonal mirrors, actuated by galvanometers, were used to scan the transverse position of the OCT beam in the colposcope field of 40x40 mm. This design has the advantage of permitting en face viewing of pathology on the surface of the cervix, while simultaneously controlling the scan pattern of the OCT image. The input of Dr. Wolfgang Drexler, a visiting scientist from the University of Vienna, was critical to the development of this device.

![Diagram of OCT colposcope](image)

Figure 9-4. Block diagram (top view) and photograph of the integrated OCT colposcope.

The distance from the lens to the imaging plane, \( l_2 \), was set by the colposcope and OCT optics. In the case of the OCT colposcope described here \( l_2 \) was 325 mm. The distance between the incoming fiber and the lens, \( l_1 \), is therefore defined by:

\[
l_1 = \left[ \frac{1}{f} - \frac{1}{l_2} \right]^{-1} \tag{9-6}
\]

and the diameter of the beam at the imaging plane can be approximated by:

\[
2w_o = \frac{4 \lambda l_2}{\pi NA l_1} \tag{9-7}
\]

The long working distance of the colposcope (30 cm) posed an interesting challenge for the optical design in order to maintain the required focused spot size. To achieve a focused spot diameter of \( 2w_o \sim 20 \mu m \) (FWHM of \( \sim 12 \mu m \)) at a 300 mm working distance, a beam diameter of \( 2w_o \sim 16.5 \) mm was required at the colposcope. This necessitated relatively large 19 mm clear aperture steering mirrors to accommodate the beam. The OCT beam was delivered an angle of \( \sim 5^\circ \) from the colposcope viewing axis. (Figure 9-5) Although this results in a slight parallax between the OCT beam and the colposcope view, this approach was used rather than a beamsplitter and collinear delivery because it did not introduce any optical elements into the colposcope optical path and also minimized the aberration of the OCT beam. The focal plane of the OCT beam and the colposcope were commensurate, and the small parallax angle resulted only in a slight angular deviation of the OCT scan plane from the viewing perspective.
Since the working distance was long, the precision and the noise of the galvanometers and driving waveforms were important considerations for maintaining beam positioning stability.

**Performance and testing**

The diameter of the focused spot was measured experimentally, using the knife edge method, to be 21.7 \( \mu \text{m} \), in close agreement with the theoretically predicted value of 19.3 \( \mu \text{m} \). The shape of the beam also appeared symmetrical, implying minimal aberrations. (Figure 9-6)

The incident power of the OCT beam was approximately 3.5 mW at 1310 nm. A visible aiming beam was used to visualize the OCT scan pattern. Since colposcopic examination requires bright, white-light illumination, a 532 nm green, rather than a red aiming beam was used to achieve better visibility.

A clinical feasibility study was performed to test this apparatus and evaluate the performance of OCT as a clinical diagnostic tool for early cervical neoplasia. The results of that study will be described in later chapters.
Chapter 9. Development of Novel Imaging Devices

Concept of an OCT Imaging Needle

Current catheter and laparoscope based devices depend on pre-existing lumens to deliver the OCT beam. An OCT imaging needle device would be small enough to be delivered using a standard size, very small diameter needle to be introduced anywhere with minimal disruption to the tissue or structure. (Figure 9-7) It could therefore be used to image or deliver light practically anywhere in a living body or non-biological tissue, even in solid masses.

![Diagram of an OCT imaging needle](image)

**Figure 9-7.** Diagram of an OCT imaging needle.

This small diameter and ease of delivery of this device would make it an obvious candidate for a number of applications. This system could be utilized to image tissue, *in vivo*, at areas otherwise inaccessible without major, invasive procedures. (Figure 9-8) This probe could also be employed to perform spatially resolved spectroscopic analysis of hidden structures which has also been proven to be of clinical importance in the diagnosis of disease but until this point required an open access to the area under investigation. It could also be integrated with existing devices for tissue ablation during microsurgery or for photo-dynamic therapy.

![Introduction of the imaging needle into otherwise inaccessible volumes](image)

**Figure 9-8.** Introduction of the imaging needle into otherwise inaccessible volumes. The needle on the left is scanned in a translational pattern whereas the one on the right in a rotational one.

The core of this system is an optical fiber with an integrated spherical lens at its tip, cleaved at an angle greater than the critical angle, $\theta_c$ (where $\theta_c = \sin^{-1}(1/n)$ ) required for total internal reflection. As shown in figure 9-9, this will result in a focused beam with the focal point at a location transverse to the fiber axis. The spherical lens can be integrated in one of several methods, including tapering or attaching commercially available, grated index (GRIN) or spherical micro-lenses. This fiber can then be rotated or translated to move the focal point in any direction. Rotational, translational and spiral movement, as well as any combination of the three is possible using commercially available motors and/or translational devices. This flexibility allows coverage and/or imaging of complete volumes.
The fiber, described above, can then be integrated into a delivery mechanism consisting of a needle or other solid or flexible guiding device. (Figure 9-10) The device can be transparent, to allow imaging in all directions, or non-transparent with transparent windows, to increase the mechanical strength and durability of the device while limiting the visible area. The imaging needle can also be integrated directly with existing biopsy devices, such as those used in the prostate or the breast. (Figure 9-11) Such a device would allow the introduction of a very small diameter, hypodermic, needle to perform imaging, a "first look," and subsequently biopsy only those areas which look suspicious. (Figure 9-12) This technique would allow more areas to be sampled, thus increasing the diagnostic yield of procedures, without increasing the trauma to the patient. Dr. Xingde Li, a postdoctoral associate at MIT, has recently demonstrated a working prototype of an OCT imaging needle.
Chapter 9. Development of Novel Imaging Devices

Figure 9-11. Imaging needle integrated with a prostate biopsy device. The needle can image either through the device or, as illustrated here, protrude out of the device and image before the biopsy.

Figure 9-12. The concept of "first look" imaging. The imaging needle is inserted first. If the tissue looks suspicious for malignancy then a biopsy follows. This technique could improve the diagnostic yield of biopsies of large tissues or organs without increasing the actual number of biopsies or associated trauma.

Conclusions

The devices presented here constitute a new generation of OCT imaging tools. The improved imaging probe enables scanning in any pattern in a forward direction. It is constructed using interchangeable components which make the design flexible and allow easy modification of the imaging characteristics of the device. Its components are also detachable and sterilizable. This probe can be used in any application
that requires high resolution imaging of accessible tissue. The drawbacks of this design are that the scan length is limited by the size of the probe and the working distance has to decrease to improve the transverse resolution. The integrated OCT colposcope combines highly linear galvanometers and large diameter optics to achieve high resolution imaging at a distance of 30 cm. It allows simultaneous, en face, view of the tissue surface and does not interfere with the normal colposcopic procedure. Unfortunately, the long working distance makes improvements in transverse resolution increasingly difficult thus requiring either bigger optics or shorter working distances. The imaging needle has great potential in the minimally invasive imaging of solid tumors or organs. Its miniature size, however, makes the construction of this device very difficult.

The apparatuses constructed and illustrated here, as well as the concept of the imaging needle described, offer a preview the diversity of designs that OCT imaging devices can take. They can assume different forms and allow imaging of almost any site in the human body. Examples of the studies performed and images acquired with these devices will be extensively described in later chapters. One has to keep in mind that these are but examples of the possibilities. We can, with certainty, expect more devices, of probably increasing complexity, to appear and allow OCT integration with a variety of existing and novel imaging and diagnostic modalities as the need arises to image new tissue sites.
PART IV – OCT IMAGING ENHANCEMENTS

CHAPTER 10 – POST PROCESSING AND VISUALIZATION OF OCT IMAGES

As with any successful imaging system, presentation and visualization of the OCT images will become increasingly important as the clinical applications of the technique expand. Further, with the advent of clinical studies, quantitative measurements will have to be extracted from the images. These measurements will require the identification and segmentation of features of interest and will introduce further challenges both in processing the data and presentation of the results. Efficient and effective techniques for visualizing multivariate data will be crucial to the clinical success of the system.

Over the past few years there was a large effort in the OCT community in terms of post processing for enhancing the resolution. [196, 197] A gain of about a factor of two has been shown by some groups. However, there was little effort in terms of visualization with the exception of one case of segmentation and rendering of vessels. [198] Although a full treatise of image processing techniques is beyond the scope of this thesis, this chapter is an attempt to identify the optimal post-processing techniques for the OCT images to allow the best possible presentation of the data until more specialized and complicated algorithms are developed for specific applications.

Image Processing Applications

Irrespective of their actual content OCT Images share certain unique attributes. The noise in the images collected using a properly optimized system is mainly shot noise and as such is proportional to the signal and multiplicative. However, OCT images are usually either digitized or processed for displaying in logarithmic form. In that form, the noise is still proportional to the signal but it is now additive. Another form of “noise” is speckle noise which has been described in Chapter 2. The intensity of the OCT images also decreases exponentially with respect to the depth of penetration as a result of the decay of the signal mainly due to scattering and to a lesser degree absorption. Post processing and visualization of OCT images has to be performed with these features in mind.

Filtering

Filtering to reduce the noise of the images or smooth the image for segmentation requires an efficient filter that preserves the features of the image while reducing the local variations. Four types filters were compared in this study by application on a test image. This image included 3 rectangles (100x100 and 5x5 pixels) and noise which was taken from a real OCT image and was scaled with the signal. The filters compared were a mean filter (3x3 applied several times), a median filter (3x3 applied several times), a large median filter (6x6 applied once), a hybrid median filter (7x7 applied several times) and a linear estimator filter (3x3 neighborhood applied several times.) Each filter was applied to the image until approximately the same level of variation in the noise was reached. (Table 11-1) The results are shown in Table 11-1 and Figures 10-1 to 10-4. Contrast is defined as

\[ Contrast = 1 - \frac{\text{NoiseMean}}{\text{SignalMean}} \]  

(11-1)

and Signal-to Noise Ratio (SNR) as

\[ SNR = 20 \log \left( \frac{\text{SignalMean}}{\text{NoiseStd}} \right) \]  

(11-2)

Some of these filters were also applied to real OCT images for comparison. (Figures 10-5 to 10-6)
Table 11-1: Results of Filtering

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<td>19.2118</td>
<td>16.3889</td>
<td>21.5487</td>
<td>17.9785</td>
<td>18.9888</td>
</tr>
</tbody>
</table>

Figure 10-1. Filtering of a test OCT image (A) consisting of three objects: A large rectangle (100x100 pixels) and two smaller ones (5x5 pixels.) That image was filtered with a 3x3 mean filter, 4 iterations (B), a 6x6 median filter, 1 iteration (C), a 3x3 median filter, 7 iterations (D), a 7x7 hybrid median, 3 iterations (E), and a linear estimator with a 3x3 neighborhood, 8 iterations (F). The choice of size and/or iterations was such that all filtered images contain similar noise variations.

The mean filter works by assigning each pixel the average value of all the pixels in the neighborhood surrounding it. It is the simplest of all filters but unfortunately, it also blurs sharp edges of the image. The median filter, in contrast, replaces each pixel by the value which ranks in the middle of all sorted values in the pixel’s neighborhood. This action preserves the edges but rounds sharp corners and erases lines or features which are smaller than half its width. The hybrid median filter is a modification of the median that corrects for this tendency. The hybrid median filter is a three-step operation which takes the median of the median of the pixels on the diagonals (“x” pixels), the median of the pixels on the vertical and horizontal center lines (“+” pixels), and the center pixel. The last filter used was one based on a linear...
estimator of the signal which uses the local variance and mean of the degraded image and of the noise, calculated from a relatively signal free area of the image, to calculate an estimate of the original signal. This method is very efficient at reducing signal dependent noise and improving the signal to noise ratio (Table 11-1) but does not preserve sharp edges. In the simple test image used here it appeared that the hybrid median filter would be the best method to denoise the OCT images with minimal distortion. This type of filtering was used for all subsequent processing.

Figure 10-2. Details of the images of Figure 10-1. The smaller features and edges are better preserved with the hybrid median filter despite the fact that its dimensions are 7x7 pixels.

Figure 10-3. Single cross-sections (row 103) from the images of Figure 10-1. The linear estimation filter (F) is very efficient at removing noise variations but the hybrid median (E) better preserves the image features.
Figure 10-4. Application of the filters discussed above on real OCT images. A. Image of a mouse prostate acquired at high resolution using a logarithmic demodulator to directly digitize the logarithmic signal. B. Image filtered with a 5x5 median filter. C. Image filtered with a 7x7 hybrid median. D. Image filtered with a linear estimator from a 3x3 neighborhood. The black and white levels of the images were chosen such that the images would be equivalently scaled within the grayscale colormap. (Image size: 1 mm x 0.75 mm, 1000 A-Scans; Resolution: 5 μm x 2 μm)

Figure 10-5. Details of the images of Figure 10-4. The original (A) compared with median (B), hybrid median (C) and linear estimation (D) filtering. The details are better preserved with hybrid median filtering. (Image size: 0.125 mm x 0.094 mm, 125 A-Scans; Resolution: 5 μm x 2 μm)
Segmentation

Segmentation of the images is necessary for any quantitative measurement and in some cases for efficient visualization. The simplest case is intensity based segmentation, where all the pixels of the image of a given intensity value or range of values are separated and grouped together. Figure 10-6 shows an example of an image, with its corresponding histogram of values. Most of the information is grouped in a single distribution of pixels and the noise is spread over the entire range of values. Filtering that image, to reduce the noise and make the pixel distributions narrower, does not reveal any additional pixel groupings, confirming the assumption that all the information lies within a given range of values. (Figure 10-6, C) That image is subsequently filtered, thresholded and converted to binary. (Figure 10-7, A&B) Morphological operations on the binary image, i.e. erosion and dilation, reduce the extraneous pixels and result in smooth regions. (Figure 10-7, C&D) The edges of those regions can be separated by convolution with a horizontal edge kernel and detection of the maxima and minima of the resulting data. (Figure 10-7, E&F) As this example shows, this technique is certainly adequate to segment simple images such as this one and even works well with noisy images.

However, there are times when simple intensity thresholding is not adequate. Figure 10-8 shows an example of an image where the features are grouped in at least 3 different distributions with distinct mean values. The histogram of the original image shows clearly two of these distributions but filtering of the image results in a better visualization of all three regions. (Figure 10-8, B&D) Segmentation of the image into groups of pixels in the 100%-75%, 75-55%, 55-40% and 40%-0% percentiles intensity results in the four regions shown figure 10-9.

![Figure 10-6. A. OCT image of the human cervix. B. Histogram of the values contained in the OCT image. Most of the information appears to be in a single distribution. C. Filtering of the image in A with a 7x7 hybrid median filter makes the intensity distribution narrower but does not reveal any other significant features. (Image size: 5 mm x 2.5 mm, 512 A-Scans; Resolution: 15 µm x 15 µm)
Figure 10-7. A. The image of figure 10-6 filtered and thresholded to remove the noise. B. The same figure converted to binary by setting all the background pixels to 0 and all remaining data pixels to 1. C. An eroded version of the image with less extraneous pixels remaining outside the regions of interest. D. Dilation smooths the regions of interest. E. Convolution with a horizontal edge kernel results in maxima at the positive edges (black lines) and minima at the negative edges (white lines). E. The four lines separating the two regions calculated from E and overlaid over the original image.

Figure 10-8. OCT image of a mouse prostate (A) with its corresponding histogram showing a bi-modal distribution. When the same image is filtered (C), its histogram (C) shows a tri-modal distribution with the peaks at values 75, 125, 160. The vertical lines in the histogram indicate the values at which the image was segmented.
Figure 10-9. The OCT image of figure 10-8 segmented into four regions (a-d) with region (a) being the noise level. The technique used was similar to that used for figure 10-6 with the exception that multiple intensity regions were segmented. Since the exponential decay of the image has not been corrected, region (d) contains areas that are characterized by the same mean intensity but visually do not appear to be part of the same tissue. Exponential correction of the image should result in elevated mean intensity for those areas and their segmentation into the correct region.

Figure 10-10. A. The variance of the values in a 5x5 neighborhood around each pixel in region b of the image show and segmented in figures 10-8 and 10-9. B. The range of the values in a 5x5 neighborhood in that same area. In neither case there is any additional distinction made between different areas of the region under investigation.

Segmentation is not, of course, limited to intensity. Other variables can be calculated, such as range and variance. Also, convolution with specific kernels can separate an OCT image into areas of similar “texture.” Unfortunately, the noise in most OCT images, which can reach the level of the signal in the case of speckle, makes texture thresholding very challenging. Figure 10-10 shows the region (b) of the OCT image of figures 10-8&9 with the variance and range values of a 5x5 neighborhood around each pixel. The variation is so large that no additional features can be extracted. Texture mapping may prove useful, however, for larger images with small features, although some filtering and exclusion of outlier data values may be required, and should be further explored if the need arises in the future.
Decay correction

As mentioned already earlier, OCT images are characterized by an exponential decay in intensity with depth which is a direct result of the scattering and absorption within the tissue. Although the capacity of the human visual system to accommodate for such changes may make the problem seem not so significant when visually inspecting the images, it may create difficulties when images are segmented or quantitative measurements are performed. To correct this problem the degree of decay for the image has to be calculated and an appropriate correction factor has to be applied. (Figure 10-11) In this fashion, features deeper in the image are much more visible and more amenable to segmentation and measurements. (Figure 10-12) Problems may arise when areas of noise, especially deeper into the tissue, are multiplied by this correction factor and are amplified. Thresholding and setting most of the noise to zero before the application of the correction factor may alleviate some of these problems, but amplified noise deeper in the image will probably still be present.

Figure 10-11. A. Example of an A-scan from an OCT image which decays exponentially with depth, plotted on a logarithmic scale. B. The scan of A multiplied by a correction factor to adjust for the exponential decay.
Figure 10-12. A. OCT image of a mouse prostate with clear signs of exponential decay. B. The same image corrected. The noise level in the lower part of the image appears elevated despite the thresholding and filtering. However, features deeper in the tissue (arrows) are also much more clear which makes them amenable to segmentation and measurement. (Image size: 1 mm x 0.75 mm, 1000 A-Scans; Resolution: 5 μm x 2 μm)

Mapping and Visualization

For the OCT data to be clinical useful the features pertinent to diagnosis must be presented in a meaningful and concise manner so as not to overwhelm the user and allow quick, accurate and efficient diagnosis. One way of achieving this goal is to extract certain information from features of the volume of images and present that data in a topographic map. This technique was employed in ophthalmology where, for example, the thickness of the nerve fiber layer, which is of clinical importance, was measured and mapped. The situation of course is different for various tissues and the diagnostic relevance of each feature has to be determined in appropriate clinical studies. However, segmentation and mapping of some structural morphology can be demonstrated as an initial step in the examination of measurements of clinical relevance.

One issue that arises when dealing with mapping of a certain area is the density and form of slicing. The density determines the spatial resolution in the direction perpendicular to the OCT scanning and is usually limited by the acquisition capabilities of the system. In vivo imaging imposes additional constrains on volume scanning, especially concerning the speed of the acquisition, since each volume scan has to be completed before there is significant subject movement. Motion artifacts are often an issue in in vivo imaging and may have to be corrected post-imaging. The scans can be arranged in a Cartesian grid, where all slices are taken in parallel, or polar grid, where the slices are acquired by rotating around a single origin. Cartesian scanning offers more uniform coverage of an entire area but requires a large number of images. Polar scanning over-samples the center region and under-samples the periphery but requires a smaller number of images.
Figure 10-13. Example of *ex vivo* volume scanning of a 4x4 mm area of cervical tissue. The tissue was imaged at 25 μm intervals, requiring 160 images. Some of the OCT images are shown on the left. The epithelium was segmented and its thickness measured and mapped in the topographic map shown on the right. (Image size: 4 mm x 2 mm, 500 A-Scans; Resolution: 30 μm x 15 μm)

Two examples of mapping of cervical epithelium thickness are presented here. The first case (Figure 10-13) is a 4x4 mm area of cervical tissue imaged *ex vivo*. Images were collected in a Cartesian manner at 25 μm intervals, requiring 160 images to cover the area of interest. The epithelium was segmented in each image and its thickness measured and mapped using a false color scale for intensity.

The second example is a case of a 5 mm diameter circular area of cervical tissue imaged *in vivo*. Six images were collected in a polar fashion since the combination of system speed and patient movement did not allow better coverage of the area. (Figure 10-14) Again, the epithelium was segmented and measured for each image and plotted in a topographic map, interpolating between the images to fill in the pixels in the periphery. (Figure 10-5)
Figure 10-14. Example of in vivo volume scanning of the human cervix. The top image is a colposcopic picture of the cervix with the position and direction of the imaging illustrated by the arrows. The collected OCT images are shown below with relevant features marked. (e: epithelium, c: endocervical canal, g: glands and m: mucous and debris.) Notice that in the 90° image there is a bend in the epithelial surface which is not clear whether it is real or a motion artifact. (Image size: 5 mm x 2.5 mm, 512 A-Scans; Resolution: 15 μm x 15 μm)

Figure 10-15. Epithelial thickness corresponding to the area imaged in figure 10-4. The arrow points at a step probably due to some motion between the first (0°) and last (150°) images.
There are cases when visualization of just a single variable may not be adequate and a view of the complete OCT data volume may be necessary or preferable. In those cases, the OCT images may be presented in 3 dimensions, either as slices or rendered volumes. An interactive program that would allow the user to scan through the OCT images in different directions and even re-slice the data to suit his/her needs would be a very efficient way to examine the data. Figure 10-16 shows an example of viewing slices in 3 dimensions. If a more realistic 3 dimensional view of certain well defined features, e.g. the epithelium, is required for some reason, this can be achieved by segmentation of the features and rendering of the resulting surfaces. (Figure 10-17) This is a time and processing power consuming option which may not necessarily result in diagnostically useful images and should be used in moderation.

**Figure 10-16.** Two of the slices from the OCT imaging volume of figure 10-4 presented in 3 dimensions. This scheme can be extended to include any number of slices or parts of slices, as well as allowing re-slicing in arbitrary directions.

**Figure 10-17.** The epithelial layer of the cervical tissue area imaged in figure 10-4 segmented and rendered in a 3-dimensional view. The 60° scan line is shown for orientation purposes. The epithelial thickness (e), which is thicker at the periphery and thinner closer to the endocervical canal (EC) as expected, is visible. The blue arrows point to image processing artifacts and the black arrow points to the bend visible in the 90° image in figure 10-4.
Chapter 10. Post Processing And Visualization Of OCT Images

Conclusions

This chapter is by no means a complete treatise of image processing, mapping and 3-dimensional visualization. It does however contain some important points. The first is that noise reduction in OCT images is possible without degradation of the details and small features using hybrid median filtering. Also, the correction of the exponential decay is feasible and may be necessary when processing the images but probably not advisable for direct, visual inspection of the OCT data since it significantly increases the noise level of parts of the image. Intensity based segmentation of the OCT images is feasible and, in most cases, will probably be more than adequate to extract quantitative information. Mapping of the epithelial thickness or other variables is an efficient way of presenting measurements extracted from OCT volumes but the nature and predictive value of the variables has to be carefully determined. The role of rendering in OCT imaging is still in question. It is not clear yet whether visualization of the 3-dimensional relationship of the features in the OCT images is necessary so further work on rendering should be reserved for cases were it would offer a clear advantage.
CHAPTER 11 –HIGH RESOLUTION AND SPECTROSCOPIC IMAGING

Introduction
The previous chapter has dealt with post-processing techniques that can reduce the noise of the OCT images. Other groups, have also labored with methods to improve resolution with deconvolution. [73, 196, 197] However, the quality of the OCT images is ultimately limited by the capabilities of the system and any effort to improve the images after the acquisition of the data will eventually be constrained by the system properties. The best way to improve the quality of the OCT images is to improve the apparatus. New sources, with very broad spectra, are required to improve the resolution. Such sources would allow cellular level imaging and improved identification and grading of neoplasias. The extended wavelength range of broad sources would also allow the implementation of spectroscopic analysis of the images which had only limited success in the past. [199] Spectroscopy could provide a form of “optical staining,” therefore identifying, in the biochemical or sub-resolution contents of tissue, diagnostically useful markers of disease.

High Resolution Imaging
As explained in Chapter 2, the axial resolution of OCT is inversely proportional to the bandwidth of the source spectrum. Efforts in ultra-fast optics by Dr. Franz Kaertner, a visiting Professor, and Dr. Uwe Morgner, a visiting scientist, including state of the art dielectric double-chirp mirrors and dispersion balancing, resulted in a sub-5 fs pulse-width Titanium doped Sapphire (Ti:Al₂O₃) solid state laser with a very broad spectrum. [200] (Drs. Kaertner and Morgner are currently at the University of Karlsruhe, Germany.) When that source was interfaced with a specifically designed and optimized, broad bandwidth, dispersion balanced OCT system, by Dr. Wolfgang Drexler, a visiting scientist from the University of Vienna, resolutions of 1.5 μm in air (or ~1 μm in tissue) were possible. [201] (Figure 11-1)

![Figure 11-1](image-url) The spectra of the sub-5 fs laser and a superluminescent diode are compared on the left. The signals resulting from using the two sources for OCT imaging are also compared on the right. The use of the solid state laser results in almost an order of magnitude improvement of the resolution.

The ultra-high resolution imaging system was used to image gynecological and gastrointestinal tissue ex vivo. In the case of the cervix, the OCT images demonstrated, for the first time, the presence of human cells. (Figure 11-2) At this resolution and dynamic range, the index of refraction mismatch between the cytoplasm and cell membranes was enough to outline the extend of individual cells in the superficial and intermediate layers of the epithelium. The image correlated well with histology, confirming the presence
of cells ranging from 10 to 30 \( \mu \text{m} \). The closely packed smaller cells closer to the basal membrane were not individually resolved but an increase in the backscattering intensity was observed in that region. The same system was also used to image samples of Barrett’s esophagus \textit{ex vivo}. The resolution of the images was markedly improved over the previous \textit{ex vivo} images. Enhanced visualization of the glandular structure of the metaplastic esophagus was possible. There was however an absence of distinct cellular features which could be a result of not enough contrast between the cells and paenchyma with the problem worsened by delays between tissue removal and imaging and sub-optimal storage.

![Image A](image_url)

**Figure 11-2.** \textit{Ex vivo} high resolution imaging of the human cervix. The image was acquired with 5 \( \mu \text{m} \) transverse and 1.5 \( \mu \text{m} \) axial resolution at 800 nm center wavelength using the sub-5 fs Ti:Al\(_2\)O\(_3\) laser. The effects of tight focusing of the OCT beam are evident in the form of the signal decay above and below the plane of focus which is approximately in the middle of the image. (Image size: 0.7 mm x 0.4 mm, 700 A-Scans; Resolution: 5 \( \mu \text{m} \) x 1.5 \( \mu \text{m} \))

![Image B](image_url)

**Figure 11-3.** \textit{Ex vivo} high resolution imaging of the Barrett’s esophagus. The image was acquired with 5 \( \mu \text{m} \) transverse and 1.5 \( \mu \text{m} \) axial resolution at 800 nm center wavelength using the sub-5 fs Ti:Al\(_2\)O\(_3\) laser. Visualization of the glandular structure characteristic of metaplastic tissue has improved (g). However, no clear cellular features can be resolved. (Image size: 1.5 mm x 0.75 mm, 1500 A-Scans; Resolution: 5 \( \mu \text{m} \) x 1.5 \( \mu \text{m} \))

**Spectroscopic Imaging**

All the OCT images presented so far are grayscale maps of the envelope of the demodulated interferogram. Demodulation removes any phase information and results in images of the backscattered intensity from the different tissue layers. The complete interferogram, however, contains additional information. Changes in the modulation frequency, for example, correspond to Doppler shifts due to flow in the tissue and were the subject of several studies by other groups. [202-204] In addition, spectral information can be extracted from the OCT signal. Dr. Uwe Morgner, a visiting scientist and currently at
the University of Karlsruhe, Germany, developed a system to digitize and extract such spectroscopic information.

As shown in Chapter 2, the interferogram can be represented by:

$$I_{\text{int}} \propto \Re \left\{ A(\omega, z) \right\} R_r(\omega, z) \cos(\omega_c \tau_p)$$  \hspace{1cm} (11-1)

where $A(\omega)$ is the spectrum of the source and $R_r(\omega)$ and $R_s(\omega)$ the reflectance as a function of wavelength from the reference and sample arms respectively. Therefore, the reflectance from within a given depth in the tissue can be calculated from the inverse Fourier transform of the intensity, assuming the source spectrum and reference mirror reflectance are known:

$$\prod_{0}^{z} R_s(\omega, z) = \frac{\Re^{-1} \{ I(\omega, z) \}}{|A(\omega, z)|^2 R_r(\omega, z)}$$  \hspace{1cm} (11-2)

where the convolution with the delta functions resulting from the Fourier transform of the cosine factor has been ignored since it has no effect on the shape of the spectrum. The contribution of a small layer of thickness $dz$ is:

$$R_s(\omega, z) = \frac{\Re^{-1} \{ I(\omega, z) \}}{|A(\omega, z)|^2 R_r(\omega, z) \prod_{0}^{z-dz} R_s(\omega, z)}$$  \hspace{1cm} (11-3)

When the entire interferometric signal is collected, the spectroscopic data can be extracted by one of several methods. One possibility is the use of the short fast Fourier transform (short FFT) which involves taking the FFT of short, overlapping sections of the signal. Another method for computing the spectroscopic signal is the use of the Morlet wavelet transform which allows calculation of the spectral response at any given point and reduces the windowing artifacts as compared to the short FFT. [205] This transform is given by:

$$W(\omega, \tau) = \left| \int I(t + \tau) e^{-\left(\frac{t}{\tau_p}\right)^2} e^{i\omega t} dt \right|^2 = \Re \left\{ \int I(t + \tau) e^{-\left(\frac{t}{\tau_p}\right)^2} dt \right\}^2$$  \hspace{1cm} (11-4)

Figure 11-4 shows this process schematically. The wavelength resolution of this transform is limited by the FWHM of the gaussian window in the time domain.

**Figure 11-4.** Morlet wavelet transform. Wavelets at different frequencies are convoluted with the signal and the center of mass of the resulting spectra determines the hue (color) of the image. The amplitude of the envelope of the OCT signal determines the saturation (i.e. quantity of that color) of the signal. The luminance is kept constant.
A simple spectroscopic analysis can be performed by calculating the deviation of the center of mass of the spectrum away from the center wavelength. This value is an indicator of the spectral shifts caused by wavelength depended scattering or absorption. The resulting values can be plotted in a hue-saturation-luminance space (HSL) with the hue, varying from green to red, representing short to long wavelength shifts and saturation representing the intensity of the envelope of the signal, in analogy to the amplitude tomograms collected normally. The luminance can be kept constant. Figures 11-5 and 11-6 show examples of such images from gastrointestinal and gynecological tissues. Green areas in the image represent sections of the tissue where the shorter wavelengths were predominantly backscattered whereas red indicates longer wavelengths. As expected, layers deeper in the tissues backscatter mainly longer wavelengths, since those are the least backscattered and therefore the ones reaching deeper into the tissue. However, there are other areas where certain wavelengths appear to be preferentially backscattered. These regions include layers of cervical tissue closer to the surface backscattering longer wavelengths unlike deeper ones which backscatter shorter wavelengths. Also, in the case of the esophagus, there are islands of long wavelength shifts in the regions of metaplasia.

More detailed information can also be extracted from the spectroscopic OCT images. Figure 14-7 shows an example of the spectral content of the OCT signal at different depths within the tissue of figure 14-6. When the spectra are normalized with respect to the source and the effects of each layer separated there is distinct differences between the resulting reflectance functions. The origin of those differences is most likely wavelength dependent backscattering from the different layers which strongly depends on the cellular and supportive structures of the underlying tissue. Other groups have recently suggested that nuclear sizes can be inferred from the shape of the backscattered signal in the visible range and these results are very likely to extend for spectroscopic OCT. [206, 207] This is, however, very preliminary work and there is a lot to be done before this technique proves itself in the medical field.

![Figure 11-5](image-url)

**Figure 11-5.** *Ex vivo* amplitude tomograms and spectroscopic images of normal (A&C) and Barrett’s (B&D) esophagus. The spectroscopic image of Barrett’s esophagus exhibits, in addition to the structural changes in the tissue, a spectroscopic disorganization in the form of islands that preferentially backscatter longer wavelengths (red.) Although the origin of these changes is not clear, they may still form the basis of an important marker for metaplastic or dysplastic tissue.
Figure 11-6. *Ex vivo* spectroscopic image (A) and amplitude tomogram (B) of cervical tissue. The wavelength dependent scattering from the different layers of the tissue is evident. The basal and parabasal layers of the cervical tissue appear to preferentially backscatter longer wavelengths (red) in contrast to the connective tissue below which predominantly backscatter shorter wavelengths (green.)

Figure 11-7. Spectra (A) form different layers of the image of figure 14-5. (black: surface, blue: superficial layer, red: basal/parabasal layer) The reflectance of the superficial (B) and the basal/parabasal (C) layers, extracted from the above spectra. The differences in the shape of the reflectance from the different layers is presumably a result of the wavelength-dependent backscattering from the cellular and supportive tissue structure.
Conclusion

The results of ultra-high resolution imaging are very encouraging. Enhanced resolution may be the key to successful grading of neoplasia with OCT and will definitely provide unprecedented quality images which will truly approach those provided by histopathology. Some problems, however, do exist. One is the trade off between lateral resolution and penetration. The large NA required for high resolution imaging precludes deep penetration and even results in degradation of the resolution away from the focus. To remedy the situation it may be necessary to scan the focus, while scanning the delay of the OCT system, in and out of the tissue or perform the equivalent of C-mode scanning in ultrasound where images are acquired with the focus at progressively deeper planes within the tissue and the results combined into a single image. As already suggested, the absence of cellular features in some of the ex vivo images is also an issue of concern which raises questions about proper tissue preservation and even the validity of ex vivo studies. The best solution to this dilemma would be, of course, to perform in vivo ultra-high resolution studies. Unfortunately, these are not currently possible since there is no appropriate portable source. Solid state laser technology, however, is advancing rapidly and such a device can be expected in the near future.

Despite the preliminary nature of the spectroscopic results, this new area of OCT research is one of the most exciting. It introduces the prospect of combining the traditional micro-structural imaging provided by OCT with spatially resolved molecular information on organelles and structures otherwise not resolved. The wavelength dependence of the backscattering signal could be used to extract information on the shape orientation and relative position of particles that affect the propagation of the laser light. Such information would be a powerful marker for changes associated with disease and provide an “optical staining” technique to be used in vivo and in situ without the need for additional chemical dyes. It could also be combined with OCT “contrast agents” to stain and delineate certain populations of cells or tissues. Spectroscopic images are, however, sensitive to noise so complete and thorough optimization of the system is needed. Further, it is difficult to identify the origin of the spectroscopic signals and separate the effects of scattering, absorption and shifts due to Doppler. There is a lot of work to be done before the spectroscopic images are completely understood and contrast agents are invented, but this aspect of OCT is certainly one which has the potential to have a significant impact and reshape OCT imaging.
PART V – *IN VIVO* OCT IMAGING

CHAPTER 12 – *IN VIVO* IMAGING OF CERVICAL NEOPLASIA

Introduction

Diagnostic imaging methods available to the gynecologist include computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and direct visualization via colposcopy and hysteroscopy. These technologies allow for the assessment and treatment of a wide range of female reproductive tract disorders. However, many diseases, such as early neoplastic changes, remain beyond the detection limit of these technologies. An imaging modality capable of imaging the female reproductive tract, at or near the cellular level, could lead to the detection of pathology at earlier stages than currently possible. The current clinical technology with the greatest resolution is high frequency ultrasound (30 MHz). It has a maximal resolution of 110 μm, but is insufficient to identify epithelial or other microstructure. [208, 209]

In contrast, Optical Coherence Tomography (OCT) has been shown to achieve resolutions in the cellular and sub-cellular range (4 - 20 μm), and could improve the diagnostic capabilities of clinical imaging procedures.

It is improbable that an imaging technology such as optical coherence tomography would be cost effective for screening the general patient population for early cervical neoplasias. The development of the Papanicolaou smear has reduced the number of cases of cervical cancers per year in the US to 12,000 and the number of deaths to approximately 3000. The Papanicolaou smear has also significantly reduced the cost of screening by making such a test available to a broad range within the general public. However, a role for optical coherence tomography is envisioned in scenarios such as the follow-up management of cervical intraepithelial neoplasia (CIN) I lesions in addition to its potential for the reduction of cone biopsies in patients with CIN II, CIN III, and micro-invasive lesions. Also, in order to develop clinical devices based on OCT, the ability of the technique to differentiate and grade abnormal pathologies has to be developed, extended and tested. An area where OCT can be systematically investigated *in vivo* is imaging of the dysplastic human cervix. The cervix is an excellent model system because it is accessible, exhibits well defined pathology and progression and clear endpoints, colposcopic and histopathologic, can be used to quantitatively evaluate the system.

Protocol

The aim of this study was to investigate the feasibility of using Optical Coherence Tomography for high resolution, non-contact, real-time imaging of the cervix for the identification of early neoplastic changes. We intend to investigate this hypothesis following the procedure described below:

1. Informed consent was obtained from patients previously diagnosed with cervical neoplasia or carcinoma *in situ* following a Pap smear and/or colposcopy and scheduled to undergo loop excision of the cervix.
2. After obtaining informed consent, the patient was prepared for loop excision following the normal guidelines of the Massachusetts General Hospital.
3. Immediately prior to loop excision, the 12-o'clock position of the excised part of the cervix was marked with india ink to assure proper orientation during subsequent sectioning and histological processing.
4. When the relevant point was marked, real time imaging of cervical tissue was performed using the portable OCT system integrated with a standard colposcope described in Chapters 8 & 9. The imaging was aligned with the sectioning planes of the standard post-excision processing protocol for
cervical tissue. This permitted the registration of OCT images with histological sections without interfering with normal diagnostic procedures.

5. The OCT images were taped and the most interesting images were saved digitally. Colposcopy images of the cervix, and the areas which were imaged with OCT were also acquired in digital and video form.

6. The loop excision procedure then continued as usual and the patient allowed to recover according to standard protocol.

After the completion of this procedure, several questions were addressed. First, whether diagnostically useful structures such as the epithelium could be identified and differences between normal and disease tissue could be assessed in vivo. Second, whether the OCT images correlated well with the histological cross-sections taken from the imaged areas after excision. Third, whether the current system's speed and resolution were adequate for diagnostic purposes.

Risks and Discomforts

The risk to the patient from the light is minimal since the light is non-ionizing and within ANSI standards as described in Chapter 8. The MPE is an established radiation power limit that is roughly an order of magnitude lower than powers which have been experimentally determined to induce damage. Hence, the MPE limit represents an appreciable margin of safety. The light exposures used in this study were within the limits described by ANSI and imaging was confined within the area of tissue to be excised. The risk to the device operator from ocular light exposure was also minimal. The OCT beam is diverging so that the power density decreases rapidly with distance from the focal plane of the colposcope. Furthermore, the intensity is below the ANSI standard for continuous ocular exposure at this wavelength. To further safeguard the operator an infrared-blocking filter was placed between the colposcope and the OCT apparatus.

The risk of infection associated with the imaging study was extremely low. The OCT imaging procedure was non-contact. Marking of the cervix in order to correlate images with results pathology was performed with sterile disposable applicators and sterile india ink. The main india ink reservoir never came in contact with the applicator to avoid possibility of transmission of disease from one patient to the next. In addition, the imaged tissue was excised after imaging, further reducing the risks for complications. The OCT instrumentation that was attached to the colposcope was be amenable to the standard disinfecting procedures employed for that instrument. The overall risk of infection was not increased beyond the normal risk associated with cervical loop excision. The procedure for performing pathology was not changed and the diagnostic effectiveness of the pathology results was not affected.

The overall risk and discomfort to the patient was not increased beyond the risk associated with the standard treatment procedure, cervical loop excision. The imaging study did not affect the clinical effectiveness of the patient's treatment or the patient's recovery.

Results

During this phase of the study, 42 sites in 18 patients were imaged. The imaging studies were performed at the Massachusetts General Hospital in collaboration with Dr. Annekathryn Goodman, a gynecologic oncologist. Pei-Lin Hsiung, a current Ph.D. student assisted with the imaging.

The OCT images clearly delineated the epithelial and subepithelial structure of both normal and abnormal tissue. The epithelium, sub-epithelial glands and the endocervical canal were visible. Normal tissue was characterized by sharply distinct bands of high, low and high backscattering intensity and a sharp basal layer. (Figure 12-1) The top zone appears to correspond to the superficial epithelial cell layer, which consists of flat, mature cells with small, dense nuclei and large cytoplasmic volume. The middle layer consists of intermediate cells which are characterized by large cytoplasm and a "basket weave"
appearance. In the lower layer, parabasal and basal cells with high nuclear-to-cytoplasmic ratios dominate. (Figure 12-2) This indicates that the origin of the contrast in OCT images probably depends upon the differences in the size and shape of both the cell and the nuclei and possibly connective tissue too. It is interesting to note that the flat, layered structure of the mature superficial cells is highly backscattering, a feature shared with loose connective tissue. (See Chapter 7) Glycogen, which accumulates significantly as the cells mature, does not appear to have a significant role in the scattering properties of the layers as this accumulation is more of a gradual process. Mitochondria and other organelles, which are highly backscattering, become more sparse as the cells mature a finding that could partially explain why the cytoplasm of intermediate cells although abundant does not result in a high backscattering signal.

Figure 12-3 shows examples of mild, moderate and severe dysplasia. In dysplasia, the epithelial layers were irregular with no clear boarders between the intra-epithelial layers. Higher backscattering intensity was also observed in areas with dysplasia or cancer similar to the intensity level of the basal layer. This may be the result of the increased nuclear size in dysplastic cells, although its origin must be further investigated. One could argue that the loss of the maturation layers in the OCT images is a gradual one and could be used as a marker for dysplasia grading but at the current resolution and dynamic range this is very hard to quantify.

Figure 12-1. Examples of colposcopic images (A, B & C), OCT images of those areas (D, E & F) and associated histology (G, H & I) of normal cervical tissue. The OCT images of normal cervix is characterized by layers of high and low backscattering intensity which correspond to layers of cells of different maturation. (s: superficial layer, i: intermediate layer, p/b: parabasal/basal layers.) (Image size: 2.5 mm x 2 mm, 256 A-Scans; Resolution: 15 μm x 15 μm)
 Chapter 12. In vivo Imaging of Cervical Neoplasia

Figure 12-2. High magnification (400x) histologic cross-sections of normal cervical tissue (A&C) and associated OCT images. (B&D) Although the resolution of these OCT images is poor compared to the histologic cross-sections and the planes of the OCT imaging and tissue sectioning may not exactly match, we can still see a correspondence between the layers in the OCT images and the histologic appearance. (s: superficial layer, i: intermediate layer, p/b: parabasal/basal layers.)

Metaplasia was also present in some images. (Figure 12-4) Metaplasia is defined as the gradual replacement of columnar epithelium in areas which somehow have been exposed to the ectocervical environment by squamous tissue. It is a physiologic process which does not warrant any intervention. The appearance of immature metaplastic tissue in OCT images is as a uniformly backscattering layer of intermediate intensity something that would be expected by cells that have not matured yet and are resembling something between the parabasal and intermediate cells of the normal epithelium (i.e. metaplastic active cells that will eventually mature.) Metaplasia can be discriminated from dysplasia by the presence of a higher intensity, sharp basal layer.

Cervicitis and chronic inflammation, although not considered a major clinical problem, could introduce an added challenge in the interpretation of cervical OCT images. The accumulation of inflammatory or lymphocytic cells in ad below the epithelial layer results in a loss of the sharp appearance of the basal layer and an increase in the backscattering intensity overall. (Figure 12-5) This may result in a false classification of the tissue as dysplastic.

Other benign features such as nabothian cysts, endocervical glands and vessels were also identified in the OCT images. (Figure 12-6) With the exception of vessels in areas of severe dysplasia, most of these findings are not of direct clinical significance. They can, however, help identify adjacent features if they are usually associated with each other, e.g. glands, metaplasia and the endocervical canal. Of course, it has to be stressed again that these features can only serve as clues that will help to orient and concentrate the investigation on certain areas but can not in themselves be the basis of diagnosis.
Figure 12-3. In vivo colposcopic and OCT images of mild (A&D), moderate (B&E) and severe (C&F) cervical dysplasia and associated histology (G,H & I respectively.) The images are characterized by a progressive loss of the layered appearance present in the images of normal tissue. (Image size: 2.5 mm x 2 mm, 256 A-Scans; Resolution: 15 μm x 15 μm)

Figure 12-4. In vivo colposcopic (A & B) and OCT (C & D) images of squamous metaplasia and associated histology (E & F). The epithelium appears uniformly backscattering but clearly distinct from the sharp basal layer. (m: metaplastic epithelium, b: basal layer.) (Image size: 2.5 mm x 2 mm, 256 A-Scans; Resolution: 15 μm x 15 μm)
Figure 12-5. *In vivo* colposcopic and OCT images of cervical epithelium with cervicitis (with metaplasia) (A & C) and chronic inflammation (B & D) and associated histology (E & F). The sharp distinction between the basal layer and the stroma is diminished in these images. (b: basal layer.) (Image size: 2.5 mm x 2 mm, 256 A-Scans; Resolution: 15 μm x 15 μm)

Figure 12-6. *In vivo* colposcopic and OCT images of nabothian cysts (A & D), glands (B & E) and vessels in a region of moderate dysplasia (C & F) and associated histology (G, H & I respectively). (c: cysts, g: glands, v: vessels, ec: endocervical canal.) (Image size: 4 (D) or 2.5 (E,F) mm x 2 mm, 410 (D) or 256 (E,F) A-Scans; Resolution: 15 μm x 15 μm)
The integrated OCT colposcope also enables the collection of OCT image data covering large areas of tissue. Multiple imaging planes can be scanned using radial or Cartesian scanning patterns. (Figure 12-7) Similar techniques are used in ophthalmic retinal imaging, to extract the retinal or retinal nerve fiber layer thickness, or in three-dimensional surgical imaging. Data covering extended areas of the cervix can be collected and analyzed for features of diagnostic significance. Unfortunately, the current version of the system was too slow to allow the collection of large volumes without patient motion become a severe limitation. This is a technical issue, however, which will be solved in the very near future with the introduction of faster acquisition and processing power systems.

Figure 12-7. Example of in vivo volume scanning of the human cervix. The top image is a colposcopic picture of the cervix with the position and direction of the imaging illustrated by the arrows. The collected OCT images at each angle are shown below with relevant features marked. Notice that in the 90° image there is a bend in the epithelial surface which is not clear whether it is real or a result of motion. (e: epithelium, c: endocervical canal, g: glands and m: mucous and debris.)
In order to analyze images it is helpful to have image processing algorithms which can recognize areas of abnormal pathology. For the cervix, we developed and applied imaging processing algorithms which examine features such as epithelial thickness and texture, as well as signal intensity and variance. Figure 12-8 shows an example of image processing to detect dysplasia. The OCT imaged was segmented using filtering and edge detection algorithms. Measurements of the epithelial layer median intensity and standard deviation differences are shown in Figure 12-8b. Preliminary results suggest that image processing can identify areas of dysplasia and cancer, however the ability to differentiate different grades of dysplasia has not been established.

![Figure 12-8. A segmented OCT image of the human cervix. The epithelium is divided for measurement purposes into two regions following conventional histopathology definitions, including the Superficial layer in one region and the Intermediate/Parabasal/Basal layers in the second. b. Graph of the median intensity of the top region of the epithelium (solid line) and the differences of standard variance of the intensity (dash line) between the two layers.](image_url)

**Conclusions**

This study has shown that OCT can successfully be applied to *in vivo*, near real time imaging of the human cervix. The epithelial layer can be successfully evaluated and distinctions can be made between normal and abnormal tissue. Benign conditions, such as nabothian cysts, and other structure such as glands, vessels and metaplastic epithelium were also visible in the OCT images. It is interesting to note that *in vivo* OCT cervical images differ from *ex vivo* OCT images. *Ex vivo* images generally exhibit lower contrast and poorer differentiation between different tissue structure which is probably the result of post mortem degradation of the epithelial cells. After cell death, cell membranes lose integrity, causing leakage of intracellular contents, and changes of cell dimensions and optical contrast.

Perhaps the most significant question governing the clinical viability of OCT colposcopy is its ability to differentiate varying grades of dysplasia. These preliminary results demonstrate OCT imaging of architectural morphology and suggest an ability to differentiate normal versus dysplasia or cancer.
However, differentiating between grades of dysplasia appears to be more challenging. Although certain morphological feature which correspond to cell maturation can be used, grading dysplasia usually requires subcellular level resolution. The axial image resolution of OCT can be improved to ~1 μm using ultrahigh resolution OCT. (Chapter 11) However, the long working distance of the colposcope yields low transverse resolutions and high resolution would require a high numerical aperture, short working distance imaging device. In addition the lack of detailed histologic cross-sections that would guarantee a close match between the OCT images and pathology is expected to make evaluation of even a high resolution system harder. One alternative is to require more dense sectioning of the tissue, which would significantly increase the cost and processing time and would not be seen favorably by any hospital administration. The other alternative is to have the blocks resectioned at an external lab after the pathologists are done evaluating the usual sections. Spectroscopic features provide another approach for enhancing contrast between different tissue pathologies. Light scattering measurements have recently emerged as a powerful approach for characterizing nuclear size by examining spectral features which are dependent upon the size of the scatterers. Using these techniques in combination with spectroscopically resolved OCT, it may be possible to perform cross-sectional image and extract information on cellular and nuclear features.

Image processing techniques can be a powerful method for analyzing OCT image data. They allow complex image information to be reduced to quantitative variables which can be statistically analyzed, quantified, and interpreted. In addition, the ability to interpret image data analytically enables larger volumes of data to be more rapidly analyzed, thus permitting better coverage and visualization of large areas. Some preliminary results have been introduced here but quantitative results will require the accumulation of larger volumes of better resolution and dynamic range images.

In summary, this study reports the first demonstration of integrated OCT colposcopy for in vivo imaging of cervical neoplasia in patients. The cervix is an excellent model system for investigating imaging modalities and cancer. Integrating OCT with colposcopy, or low numerical aperture microscopy in general, is a powerful technique since it permits simultaneous en face viewing of structural features while allowing precise registration of the OCT scan plane. This study indicates that distinction between normal and abnormal tissue, as well as identification of other structures such as cysts, vessels and glands, is possible although the validity of this claim has to be substantiated by future blinded evaluation of the results. However, grading of dysplasia has not been demonstrated and the interpretation of the OCT images is currently based on qualitative features. Future technology improvements should make the identification and grading of dysplasia possible and provide a tool that would be equivalent to in situ, non-invasive, real time histopathology evaluation. Also, image processing techniques can be used for the analysis and interpretation of the OCT images, enabling statistical interpretation and visualization of image data as well as rapid analysis of large volumes of data.
CHAPTER 13 – IN VIVO IMAGING OF CERVICAL NEOPLASIA – ATLAS OF RESULTS

Introduction

This chapter contains the data collected during the imaging study described previously. All imaging sessions, for which matching histology was available to confirm the diagnosis, are included. For each session, colposcopic images, OCT images and histologic cross-sections are presented. The OCT images are 5mm x 3.0mm or 5mm x 2.5mm (in air) and were collected at a resolution of 15 μm axial and 21 μm transverse. The histologic processing was performed at MGH as part of the standard diagnostic procedure following LEEP. Sections stained with Hematoxylin and Eosin at 12.5x and 100x are presented. The low magnification images match the size of the OCT image whereas the higher magnification images are included to allow better evaluation of the pathology. Also included is the pathology report as obtained from MGH and which pertains to the overall diagnosis. For each image, the diagnosis of the specific histology slides is included as well as the comments recorded during colposcopy. These results are summarized in Table 13-1.

The quality of the OCT images was not uniform throughout the study. The system was modified after patient # 010 to improve the signal to noise ratio. A Faraday rotator-based dual balanced implementation was used. Also, the length of the delay line scanning was changed from 3.5 to 2.5 mm since the structures imaged where very superficial and a smaller scanning range would allow increased sampling.

The procurement and interpretation of histology is still a problem for this study. As mentioned earlier, only a limited number of cross sections was obtained from each LEEP specimen thus limiting the extend to which OCT images could be adequately matched to histology. This was a result of the desire not to interfere with the standard diagnostic procedures but severely limited the number of OCT images the nature of which was confirmed by histopathology. Two possible solutions to that problem are either to image the entire volume of the section of the cervix to be excised thus guaranteeing that at least some of the images will match or to section the tissue more densely. More dense sectioning of the tissue would significantly increase the cost and processing time and would not be seen favorably by any hospital administration. The other alternative is to have the blocks resectioned at an external lab after the pathologists are done evaluating the usual sections. Whichever approach is chosen is important to ensure good histologic correlation before any quantitative studies are undertaken.

Included here are a series of images grouped by pathology and severity. This work, however, is by no means a complete atlas of OCT imaging of cervical pathology. It is limited to the experience gathered during this study and it lacks the breadth to cover all possible pathologies and inter-patient variability. As more and better quality images are collected in the future a more complete atlas should become available to assist in the training of personnel and interpretation of the OCT images as has happened with endoscopic ultrasound and other similar imaging modalities.
Table 13-1. Summary of in vivo cervical imaging results

<table>
<thead>
<tr>
<th>Patient #</th>
<th>OCT Images</th>
<th>Histo Images</th>
<th>Histologic Diagnosis</th>
<th>Cytoscopic Diagnosis</th>
<th>Pathology Report (from MGH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>002</td>
<td>3,6,7</td>
<td>1,2</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>005</td>
<td>3,4,5</td>
<td>1,2</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>011</td>
<td>0,1</td>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>012</td>
<td>1,2</td>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>014</td>
<td>7,8,9</td>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>016</td>
<td>7,8,9</td>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>017</td>
<td>9,10,11</td>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>003</td>
<td>4,5,14,15</td>
<td>1,2</td>
<td>Moderate squamous dysplasia</td>
<td>Before (4,5) and after (14,15) acetic acid</td>
<td>Mild to moderate squamous dysplasia with koilocytosis</td>
</tr>
<tr>
<td>005</td>
<td>8,9,10</td>
<td>3,4</td>
<td>Chronic inflammation</td>
<td>Abnml</td>
<td>Chronic inflammation, no dysplasia</td>
</tr>
<tr>
<td>006</td>
<td>14,15</td>
<td>1,2</td>
<td>Mild dysplasia and inflammatory infiltrate color to the canal</td>
<td>Transition Nml-Abnm</td>
<td>Focal mild squamous dysplasia with koilocytosis</td>
</tr>
<tr>
<td>007</td>
<td>3,4,5</td>
<td>1,2</td>
<td>Nabothian cysts</td>
<td>Nabothian cysts</td>
<td>Nabothian cysts</td>
</tr>
<tr>
<td>009</td>
<td>8,13,14</td>
<td>1,2</td>
<td>Focal mild dysplasia</td>
<td>Abnml in the middle only</td>
<td>Focal mild squamous dysplasia in a background of squamous metaplasia and reactive atypia</td>
</tr>
<tr>
<td>010</td>
<td>2,6</td>
<td>1,2</td>
<td>Metaplasia, mild inflammation</td>
<td>Questionable tissue</td>
<td>Focal reactive squamous changes, chronic cervicitis and squamous metaplasia</td>
</tr>
<tr>
<td>011</td>
<td>9,10,11</td>
<td>2</td>
<td>Nabothian cysts, koilocytosis and mild(?) dysplasia</td>
<td>Nabothian cyst next to abnormal</td>
<td>Moderate dysplasia with koilocytosis</td>
</tr>
<tr>
<td>Volume</td>
<td>Images</td>
<td>Volumes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>012</td>
<td>17,20,21</td>
<td>2 Chronic cervicitis with squamous metaplasia</td>
<td>Abnormal</td>
<td>Chronic cervicitis with squamous metaplasia and reactive atypia.</td>
<td></td>
</tr>
<tr>
<td>012</td>
<td>24,23,22</td>
<td>3 Chronic cervicitis with squamous metaplasia</td>
<td>Abnormal</td>
<td>Chronic cervicitis with squamous metaplasia and reactive atypia.</td>
<td></td>
</tr>
<tr>
<td>013</td>
<td>1,2</td>
<td>1 Moderate dysplasia, metaplasia</td>
<td>Dysplasia</td>
<td>Focal moderate dysplasia.</td>
<td></td>
</tr>
<tr>
<td>014</td>
<td>0,4,5</td>
<td>1 Moderate dysplasia, vascularization, inflammation</td>
<td>Abnormal, biopsy?</td>
<td>Moderate to severe squamous dysplasia with koilocytosis.</td>
<td></td>
</tr>
<tr>
<td>015</td>
<td>1,2,3</td>
<td>1 Mild dysplasia</td>
<td>Abnormal</td>
<td>Focal mild squamous dysplasia.</td>
<td></td>
</tr>
<tr>
<td>016</td>
<td>1,17,18</td>
<td>2 Moderate to severe dysplasia</td>
<td>Abnormal</td>
<td>Moderate to severe squamous dysplasia. Cauterized dysplasia with nuclear features suggestive of high-grade dysplasia is present at the endocervical margin. The other margins are negative.</td>
<td></td>
</tr>
<tr>
<td>017</td>
<td>4,7</td>
<td>1 Severe dysplasia</td>
<td>Abnormal</td>
<td>10 oclock biopsy: Severe squamous dysplasia. 3 oclock biopsy: Squamous metaplasia with koilocytosus. Endocervix curettage: Fragments of unremarkable endocervical epithelium.</td>
<td></td>
</tr>
</tbody>
</table>

**Volumes**

<table>
<thead>
<tr>
<th>Volume</th>
<th>Volumes</th>
</tr>
</thead>
<tbody>
<tr>
<td>008</td>
<td>5-10</td>
</tr>
<tr>
<td>010</td>
<td>8-13</td>
</tr>
</tbody>
</table>
Normal

Colposcopy:
Normal cervical tissue
Histology: Normal cervical tissue

Pathology Report:
Mild to focally moderate squamous dysplasia with koilocytosis extending very near the endocervical margin. Multiple levels examined

Colposcopy:
Normal cervical tissue
Histology: Normal cervical tissue

Pathology Report:
Predominantly exocervix with slight chronic inflammation. There is no evidence of dysplasia margin. Multiple levels examined
Colposcopy:
Normal cervical tissue
Histology: Normal cervical tissue

Pathology Report:
Moderate dysplasia. The margins were negative of dysplasia. Koilocytosis.

Colposcopy:
Normal cervical tissue
Histology: Normal cervical tissue

Pathology Report:
Anterior: chronic cervicitis with squamous metaplasia and reactive atypia. Posterior: chronic cervicitis with squamous metaplasia and chronic atypia.
Colposcopy:
Normal cervical tissue
Histology: Normal cervical tissue

Pathology Report:
10 o'clock biopsy: Severe squamous dysplasia. 3 o'clock biopsy: Squamous metaplasia with koilocytosis.
Endocervix curettage: Fragments of unremarkable endocervical epithelium.

Colposcopy:
Normal cervical tissue
Histology: Normal cervical tissue

Pathology Report:
Moderate to severe squamous dysplasia. Cauterized dysplasia with nuclear features suggestive of high-grade dysplasia is present at the endocervical margin. The other margins are negative.
Colposcopy:
Normal cervical tissue

Histology: Normal cervical tissue

Pathology Report:
Predominantly exocervix with slight chronic inflammation. There is no evidence of dysplasia
Dysplasia

**Pathology Report:**
Focal mild squamous dysplasia with koilocytosis, extending to the endocervical margin (in the region between 4 and 9 o'clock)

**Pathology Report:**
Focal mild squamous dysplasia in a background of immature squamous metaplasia and reactive atypia. The margins of the specimen are free of dysplasia.
**Case 1:**

Colposcopy: Questionable
Histology: Metaplasia, mild inflammation

Pathology Report:
Focal reactive squamous changes, chronic cervicitis and squamous metaplasia.

---

**Case 2:**

Colposcopy: Mostly abnormal
Histology: Mild dysplasia

Pathology Report:
Anterior: Fold mild squamous dysplasia. The margins are negative.
Posterior: Focal moderate squamous dysplasia. The margins are negative.
Endocervix leep: no diagnostic abnormality recognized.
Chapter 13. *In vivo Imaging of Cervical Neoplasia – Atlas of Results*

**Before acetic acid**

**After acetic acid**

Colposcopy:
Abnormal

Histology:
Moderate squamous dysplasia

Pathology Report:
Mild to moderate squamous dysplasia with koilocytosis, present at the cauterized endocervical margin in the 12-2 o'clock region.

Colposcopy:
Abnormal

Histology:
Moderate dysplasia, metaplasia

Pathology Report:
Focal to moderate dysplasia. No dysplasia present at the margins. (Negative)
Colposcopy:
Abnormal, punctuation

Histology:
Moderate dysplasia, vascularization, inflammation.

Pathology Report:
Moderate to severe squamous dysplasia. The margins are negative. Koilocytosis.

Colposcopy:
Dysplasia

Histology:
Moderate to severe dysplasia

Pathology Report:
Moderate to severe squamous dysplasia. Cauterized dysplasia with nuclear features suggestive of high-grade dysplasia is present at the endocervical margin. The other margins are negative.
Colposcopy: Small area of dysplasia, cysts, glands
Histology: Severe dysplasia
Pathology Report:
Severe squamous dysplasia
Nabothian Cysts

Colposcopy:
Nabothian cysts

Histology:
Nabothian cysts, slight inflammation

Pathology Report:
Anterior:
Squamous metaplasia and chronic cervicitis.

Posterior:
Squamous cell carcinoma-in situ involving endocervical glands. Margins free.

Colposcopy:
Abnormal and nabothian cysts

Histology:
Nabothian cysts, koilocytosis and mild(?) dysplasia

Pathology Report:
Moderate dysplasia. The margins were negative of dysplasia. Koilocytosis.
Metaplasia, Cervicitis and Inflammation

Colposcopy:
Abnormal
Histology: Chronic cervicitis with squamous metaplasia

Pathology Report:
Chronic cervicitis with squamous metaplasia and reactive atypia.

Colposcopy:
Abnormal
Histology: Chronic cervicitis with squamous metaplasia

Pathology Report:
Chronic cervicitis with squamous metaplasia and reactive atypia.
Colposcopy:
Abnormal
Histology: Chronic Inflammation

Pathology Report:
Predominantly exocervix with slight chronic inflammation. There is no evidence of dysplasia.
CHAPTER 14 – IN VIVO IMAGING OF ORAL TISSUES

Introduction
The experiments described in this chapter are the pilot work designed to evaluate and initiate a study which will investigate the feasibility of using OCT to assess the morphology of precancerous oral lesions and their response to treatment. They were performed at MIT and in collaboration with the National Cancer Institute. This study was very preliminary and only a first experiment on imaging feasibility was performed. The work is ongoing and will be continued in the future.

Head, neck and oral cancer jointly result in over 30,000 new cases with 8,400 deaths annually. [1] The oropharynx is the entrance to the aerodigestive tract and is one of the primary sites of neoplastic injury from tobacco combustion products. The management of these cancers typically involves surgery for local disease. Various combinations of surgery and radiation therapy with or without chemotherapy are used to manage the more advanced stages of these cancers. Unfortunately, these management approaches can often result in considerable morbidity, which is unavoidably related to the critical location and functions of this site. The frequent unsatisfactory outcomes with treating cancers of the oropharynx are an important part of the profound public health cost of tobacco consumption. [210]

New approaches to the management of oropharyngeal cancer include chemoprevention approaches that attempt to interrupt precursors of oropharyngeal cancer before they become malignant. Several pilot studies have shown that conditions such as leukoplakia, which predispose patients to oral cancer, appear to be responsive to chemoprevention approaches using retinoids. [211-214] Standard methods to evaluate the response of oropharyngeal tissue to pharmacological intervention include measurement of leukoplakia lesion surface area and repeated punch biopsies to monitor histological change from baseline to study completion and resolution. Serial oral cavity biopsies, which are the standard for monitoring and evaluation in chemoprevention trials, cause discomfort to the patient and are associated with side effects. Biopsy also suffers from sampling errors and processing artifacts. OCT has the potential to non-invasively and serially evaluate the effectiveness of pharmacological intervention at a level approaching that of standard biopsy and histology. OCT might be useful as a screening technique as a “first look” screening prior to biopsy for assessing precancerous oropharyngeal changes.

Protocol
The hypothesis of this study is that OCT can be used for high resolution, non-invasive, real-time, in situ imaging of oropharyngeal tissue and for monitoring pharmacological treatment in patients with oral leukoplakia. We investigated this hypothesis by imaging normal oral mucosa at 800 and 1300 nm, at different acquisition speeds, to determine the optimal system requirements. Imaging was also performed in a patient with leukoplakia at 800 nm.

Imaging of normal oral mucosa at 1300 nm (2000 scans per second) and 800 nm (80 scans per second) was performed at MIT on normal volunteers. The resolution of the systems were 18x15 μm for the 1300 nm system and 18x3 μm or 6x2 μm for the 800 nm system. The power at the tissue was 3.5 and 3.0 mW respectively resulting in lower signal to noise ratio for the faster device. A forward imaging probe (similar to the one described in Chapter 9) was used, with different objective lenses to achieve various resolutions. A contact guide of fixed length was attached at the distal end of the probe which allowed alignment of the tissue surface with the
imaging plane of the probe simply be placing the probe, and contact guide, on the site of interest. The probe was disinfected with alcohol between imaging sessions. This experiments were completed with the help of Dr. Wolfgang Drexler, a visiting scientist from the University of Vienna, Dr. Xingde Li, a postdoctoral associate, Pei-Lin Hsiung and Tony Ko, Ph.D. students and Ravy Ghanta, an MD student from the Harvard Medical School.

Imaging was also performed in a patient with leukoplakia using a prototype, portable, 800 nm system at the NCI campus in Bethesda, MD. This session was accomplished with the collaboration of Dr. James Mulshine of the National Cancer Institute. Pei-Lin Hsiung and Tony Ko also assisted with the imaging. Ravi Ghanta and Tony Ko were responsible for developing the PC-based version of the OCT software used in this study. Informed consent was obtained from the patient identified through the NCI investigation. He had objective evidence of leukoplakia of the oropharynx that could be measured. Images of the patient were acquired in the area of the leukoplakia lesions and adjacent normal areas. Each individual image required several seconds, adding ~5-10 minutes overall to the scheduled oral exam. No biopsies were taken since they were not allowed for in the protocol at this time so no correlation with histopathology was performed.

**Results**

The results of the imaging of normal oral tissues are presented in Figures 14-1 to 14-6. The wavelength at which the imaging was performed as well as the resolution and acquisition speed of the system are indicated for each image.

Imaging of the lip mucosa revealed that both systems, the 800 and 1300 nm, were able to delineate the squamous epithelial layer and some of the vessels and glands characteristic of such tissue. (Figures 14-1 & 14-2) The uniform squamous epithelium and the highly back-scattering layer of connective tissue below it were a common characteristic of almost all images. Lumens of varying diameters were visible below that layer, most likely vessels and glands. Unfortunately, it is hard to distinguish the two, especially with the lack of extensive histological knowledge of the specific region, but some empirical rules on their appearance allows us to make some educated guesses. (See the images for more information.) The thickness of the epithelium and the architectural morphology of the sub-epithelial structures varied at different sites on the lips. The enhanced resolution and signal to noise ratio of the 800 nm system appeared to significantly improve the images of lip mucosa. The high resolution images revealed much finer detail and smaller structures which could potentially be of diagnostic significance. The penetration did not appear to be significantly different between the 1300 and 800 nm systems.

![Figure 14-1](image-url)  
**Figure 14-1.** Lower lip. The epithelial layer (*e*) and connective tissue (*c*) are visible in this image. Lumens of superficial vessels and/or glands are also present (arrows). (Wavelength: 1300nm; Image size: 5 mm x 2.5 mm, 512 A-Scans; Speed: 4fps; Resolution: 18 µm x 15 µm)
Figure 14-2. Lower lip. The epithelial layer (e) and connective tissue (c) are visible. Glands (g) and vessels (v) are also present. The distinction between glands and vessels is empirical and is based on observation of features common to vessels (i.e. round/oval shape, shadowing etc) and glands (more arbitrary shapes, no shadowing etc.) The top image (A) was acquired at a resolution and signal to noise ratio (SNR) equivalent to that of Figure 14-1. The lower four images (B-E) were collected at higher resolution and SNR. (A: Wavelength: 800nm; Image size: 2.3 mm x 1.3 mm, 400 A-Scans; Speed: 0.25 fps; Resolution: 18 μm x 3 μm. B-E: Wavelength: 800nm; Image size: 1.3 mm x 1.1 mm, 600 A-Scans; Speed: 0.25 fps; Resolution: 6 μm x 2 μm.)

Imaging of the tongue superior and inferior surfaces illustrates the capability of OCT to demarcate the mucosal structure of the tissue, including taste buds, epithelium, lamina propria and tendons. (Figures 14-3 & 14-4) The taste buds are more clearly delineated when the resolution is highest at 6 x 2 μm which is what one would expect given their size. As before, the penetration did not appear to be significantly different between the 1300 and 800 nm systems.
Figure 14-3. Superior (A) and inferior surface (B&C) of the tongue. The arrows in the top image point to taste buds. The squamous epithelium (e) of the inferior surface of the tongue is visible in images B & C as is a tendon (t) in C. (Wavelength: 1300 nm; Image size: 5 mm x 2.5 mm, 512 A-Scans; Speed: 4 fps; Resolution: 18 μm x 15 μm)
Figure 14-4. Superior surface of the tongue. The arrows point to taste buds. The top image (A) was acquired at a resolution and signal to noise ratio equivalent to that of Figure 14-3. The lower image (B) was collected at higher resolution. (A: Wavelength: 800nm; Image size: 2.3 mm x 1.3 mm, 400 A-Scans; Speed: 0.25 fps; Resolution: 18 μm x 3 μm. B: Wavelength: 800nm; Image size: 1.6 mm x 0.8 mm, 600 A-Scans; Speed: 0.25 fps; Resolution: 6 μm x 2 μm.)

Imaging of the buccal mucosa clearly showed the squamous epithelial layer and the underlying lamina propria including some superficial glands and/or vessels. (Figures 14-5 & 14-6) The epithelium appeared consistently uniform with underlying connective tissue of varying backscattering intensity depending on the location. The intensity, which might correspond to concentration or packing density of the connective tissue, increased as the imaging plane moved from the mouth towards the gum line. Improving the resolution, at 800 nm, did not appear to significantly enhance the features in the OCT images although the improved signal to noise ratio made the images clearer. As before, the penetration did not appear to be significantly different between the 1300 and 800 nm systems.

Images of leukoplakic tissue in a patient exhibited marked differences as compared to normal buccal mucosa. (Figures 14-7 & 14-8) Instead of the smooth and uniform epithelial layer characteristic of the normal tissue, a rough surface was visible in the images of the lesion. The penetration in the leukoplakia images was reduced to ~150 μm. The lower resolution and signal to noise ratio of these images are evident. This compromise, however, was necessitated by the need for a portable system which could be transported to Bethesda, MD.
Figure 14-5. Different sites of normal buccal mucosa of a single volunteer (A-C) and an area of a whitish lesion (D). Images A to C were acquired by progressively imaging closer to the gum line. The epithelial layer (e) and connective tissue (c) are visible in all images. The arrows point to some superficial glands and/or vessels. The discoloration in the area imaged in D appears to be a result of disorganization of the connective tissue. (Wavelength: 1300 nm; Image size: 5 mm x 2.5 mm, 512 A-Scans; Speed: 4 fps; Resolution: 18 μm x 15 μm)

Figure 14-6. Normal buccal mucosa (A-C). The epithelial layer (e) and connective tissue (c) are visible in all images. The arrows point to some superficial glands and/or vessels. (A: Wavelength: 800 nm; Image size: 4.5 mm x 1.3 mm, 400 A-Scans; Speed: 0.25 fps; Resolution: 18 μm x 3 μm. B-C: Wavelength: 800 nm; Image size: 1.3 mm x 1 mm, 600 A-Scans; Speed: 0.25 fps; Resolution: 6 μm x 2 μm.)
Figure 14-7. Normal buccal mucosa (A) imaged in vivo. The epithelial layer (e) which is visible in the normal image does not appear in images of leukoplakia. (Wavelength: 800nm; Image size: 3 mm x 1.5 mm, 400 A-Scans; Speed: 0.25 fps; Resolution: 18 μm x 10 μm)

Figure 14-8. Leukoplasic lesions imaged in vivo (A&B). The leukoplasic surface appears rough with limited penetration. The biopsies included here were taken during the patient’s previous visit. C&E: Biopsy from the left cheek showing acantholitic squamous mucosa with no hyperkeratosis. D&F: Biopsy from the left lower ridge with mildly hyperatotic squamous mucosa and bacteria present on the surface. This lesion could represent a squamous papilloma. The grid in the high magnification histology images is 100 μm. (Wavelength: 800nm; Image size: 3 mm x 1.5 mm, 400 A-Scans; Speed: 0.25 fps; Resolution: 18 μm x 10 μm)
Conclusions

The results of this imaging study offer a glimpse into the capabilities of OCT imaging of oral tissues. The thickness and structure of the epithelial layer, which is of clinical importance in certain cases, can be assessed. Further, subepithelial structures, such as glands and vessels, can also be examined in vivo and in situ. These features should prove very powerful in neoplastic imaging of oral cancers. Although screening of such an accessible area can be easily performed with standard techniques, OCT imaging could provide a powerful tool for monitoring malignant and pre-malignant response to therapy. It could also provide a tool for evaluating pharmacological approaches to therapy and prevention and minimize the trauma associated with repeated biopsies. This is an area where OCT could have a significant impact since oral cancers are still a major health care issue due to lack of progress in the reduction of smoking and use of tobacco products in general.

Further technology improvements, however, could benefit and expedite the deployment of OCT for imaging oral pathologies in vivo. Although motion artifacts are rarely a problem when imaging with the forward imaging probe with a contact guide, faster acquisition rates would significantly decrease procedure time. Also, the probe has to be redesigned to allow easier access to the areas of the oral cavity closer to the gum line and throat. This will most likely require a pivoting head to accommodate for the large variations in the tissue morphology.

The increased penetration associated with imaging at longer wavelengths, such as at 1300 nm vs. 800 nm, did not appear to be a significant benefit in imaging of oral tissues. Most of the structures imaged were superficial enough to be adequately assessed even with the 800 nm system. On the other hand, enhancing the resolution of the imaging apparatus resulted in marked improvement in the images of lip and tongue mucosa. It appears that resolutions in the range of \( \sim 5 \) \( \mu \)m will be necessary for successfully imaging those tissue sites. Assessment of the epithelial layer of the buccal mucosal does not require such high resolution, since it lacks small morphological features, so OCT imaging could effectively be performed at either wavelength.

Imaging oral leukoplakia proved more challenging than initially expected. The leukoplakic tissue is very scattering and as a result the penetration of the OCT imaging was limited. It is rather unlikely that increasing the signal to noise ratio of the system would significantly improve the penetration. Instead, it may be advisable here to switch to a longer wavelength. This move will not significantly impair the portability of the system since a compact 1300 nm source already exists. There is, however, a good chance that this may not be adequate either. In that case monitoring of leukoplakia response to therapy should take a different form, such as monitoring of the penetration depth of OCT or measuring the change in the scattering parameters using diffuse light.

Imaging oral leukoplakia proved more challenging than initially expected. The leukoplakic tissue is very scattering and as a result the penetration of the OCT imaging was limited. It is rather unlikely that increasing the signal to noise ratio of the system would significantly improve the penetration. Instead, it may be advisable here to switch to a longer wavelength. This move will not significantly impair the portability of the system since a compact 1300 nm source already exists. There is, however, a good chance that this may not be adequate either. In that case monitoring of leukoplakia response to therapy should take a different form, such as monitoring of the penetration depth of OCT or measuring the change in the scattering parameters using diffuse light.
CHAPTER 15 - CONCLUSION

Summary

The research described in this thesis includes the ex vivo imaging surveys, technology development and clinical studies necessary to demonstrate the feasibility of OCT imaging for the in situ diagnosis of pre-malignant and neoplastic lesions. The contents of this thesis span a spectrum of topics that cover the transition from the laboratory to the clinic.

The first step in this endeavor was to evaluate the performance of OCT using ex vivo tissue specimens. These experiments elucidated the potentials and limitations of the technique and established the micro-structural features, visible in the OCT images, that are characteristic of neoplastic tissue. Sharp differentiation of structures included the epithelium, glands, supportive tissue, crypts, villi and cysts. The epithelial layer could be clearly identified and its integrity assessed in most tissues. Distortion of normal morphology and even growth formation were also recognized including the presence of necrotic tissue in more advanced stages of cancer. Features not distinguished in the ex vivo images, due to the lack of adequate resolution, included subcellular markers of dysplasia such as nuclear to cytoplasmic ratio (NCR), pleomorphism and mitotic figures. These experiments indicated that OCT can, based on morphological features, distinguish normal from abnormal tissue. The potential therefore existed for further investigation of OCT imaging for pre-malignant and neoplastic tissue in an attempt to assess if identification and, more importantly, grading of neoplasias would be feasible in vivo. Studies comparing the in vivo and ex vivo appearance of tissue were also performed in an animal model to enable a better understanding and meaningful interpretation of ex vivo results.

The development of the technologies necessary to introduce OCT to clinical settings ensued. This included developing a prototype clinical system and imaging devices to integrate OCT with current diagnostic modalities. A portable and robust system suitable for clinical use was constructed. It allows introduction of OCT technology in the clinic or operating room and acquisition of in vivo animal and human data. OCT imaging devices can assume different forms and allow imaging of almost any site in the human body. For the purposes of this study an integrated OCT colposcope and an improved forward imaging probe were constructed. The OCT colposcope allows seamless integration with standard diagnostic and interventional procedures and simultaneous acquisition of colposcopic and OCT images at a distance of 30 cm from the surface of the cervix. The forward imaging probe utilizes a galvanometer and a series of lenses to facilitate imaging in the forward direction with high precision and linearity. The clinical system and imaging devices were tested both in the lab and in animal models.

Enhancements in the post processing and visualization techniques used for OCT were introduced and the feasibility of ultra-high resolution and spectroscopic imaging was evaluated. Image processing of the OCT images was demonstrated and included algorithms for denoising without degradation of the details and small features of the images using hybrid median filtering. Correction of the exponential decay, intensity based segmentation, mapping of the quantitative results and 3-d visualization were also demonstrated. The results of ultra-high resolution imaging were very encouraging. For the first time, human cells were imaged using OCT. Enhanced resolution may be the key to successful grading of neoplasia with OCT and will definitely provide unprecedented image quality which will truly approach that provided by histopathology. Spectroscopic imaging results were also very interesting. Wavelength dependent scattering changes were observed in different tissues which may provide a form of "optical staining" that
will enhance the ability of OCT to grade dysplasias. Despite the preliminary nature of the spectroscopic results, this new area of OCT research is one of the most exciting. It introduces the prospect of combining the traditional micro-structural imaging provided by OCT with spatially resolved molecular information. However, a better understanding of the nature of the wavelength depended scattering is necessary before any progress can be made.

In vivo OCT imaging to assess the performance of the technology in clinical scenarios followed in two model systems, the cervical and oral mucosa. The markers of neoplasia identified earlier were re-evaluated in vivo and the potential and limitations of OCT for in vivo neoplastic imaging were investigated. This research has shown that OCT can successfully be applied for in vivo, near real time imaging of the human cervix. The epithelial layer could be successfully evaluated and distinctions could be made between normal and abnormal tissue. Benign conditions, such as nabothian cysts, and other structure such as glands, vessels and metaplastic epithelium were also visible in the OCT images. OCT was able to detect differences between normal and abnormal tissues but there is no clear indication whether it can grade neoplasias. Some features in the images may provide such potential but the feasibility of grading is unclear and may require additional enhancements to the system. Imaging oral leukoplakia proved more challenging than initially expected. Although imaging of normal oral tissues resulted in figures rich in micro-structural features, the leukoplasic tissue was very scattering and as a result the penetration of OCT was limited. It is rather unlikely that increasing the signal to noise ration of the system would significantly improve the penetration. Instead, it may be advisable here to switch to a longer wavelength. There is, however, a good chance that this may not be adequate either. In that case monitoring of leukoplakia response to therapy should take a different form, such as monitoring of the penetration depth of OCT or measuring the change in the scattering parameters using diffuse light.

**Future Studies**

Given the findings described in this thesis, it seems prudent to suggest that further improvement of the OCT technology will most likely enhance the abilities of the technique and may eventually lead to its establishment as a clinical tool. The speed and portability of the system could both be advanced. New delivery devices could also be developed to allow effortless integration with current and new diagnostic modalities. More importantly however, developments in laser sources should result in the introduction of portable high resolution OCT systems which may prove to be the critical element in grading dysplasias. Another aspect of OCT which holds great promise, is spectroscopic analysis of OCT images and possibly the introduction of OCT contrast agents. As the understanding of spectroscopic features in the OCT images progresses, this technique can be developed to identify pre-malignant and neoplastic tissues otherwise invisible to OCT. Although it is currently unclear how one would introduce an OCT “contrast agent,” it is evident that such a development would have a significant impact in the clinical utility of OCT.

A role for OCT is also envisioned in clinical scenarios such as the follow-up management of cervical intraepithelial neoplasia (CIN) I lesions in addition to its potential for the reduction of cone biopsies is patients with CIN II, CIN III, and microinvasive lesions. Patients on tamoxifen, who show an increased incidence of endometrial abnormalities (6.3 per 1000 women [at 5 years]), an increase of 2-3 times over the general population, could also benefit from OCT monitoring. Future applications of OCT in the gastrointestinal tract might be identified in screening high-risk patient populations. Such target populations could be patients who suffer from chronic gastro-esophageal reflux and are at a high risk for developing esophageal metaplasia. Patients with metaplasia have at least a 30-fold increased chance of developing adenocarcinoma of the esophagus compared to the general population. Other high risk
populations who could benefit from OCT screening could be people suffering from ulcerative colitis or older patients, above 50 years of age, who are usually advised to undergo regular evaluations for colon cancer. Also, surgical guidance of excisional procedures in sensitive areas, such as the vocal chords, where tissue preservation is imperative to patient morbidity, could have a significant impact. Feasibility studies should be pursued in these areas.

Conclusions

OCT can function as a type of optical biopsy to yield image information with resolutions approaching that of conventional histopathology. It can also perform micron-scale, real time imaging of tissue pathology in situ, without the need for excision and processing. OCT can be integrated to a wide range of clinical instruments including endoscopes, catheters, laparascopes, and surgical probes. The ability of OCT to imaging epithelial and other architectural morphology as well as the possibility of cellular level imaging can be used to identify and grade many type of early neoplastic changes. The features and performance of OCT imaging technology suggest that it will become a powerful imaging modality for the diagnosis and management of cancer.

In summary, we believe that there are three general application scenarios that are envisioned for OCT in neoplastic diagnosis.

1. Guiding standard excisional biopsy. Since excisional biopsy is a sampling procedure, there are many cases where it suffers from sampling errors and yields false negative results. We believe that the initial applications of OCT will be for guiding conventional excisional biopsy. This can improve the accuracy of the procedures as well as reduce the number of biopsies that are taken, resulting in cost savings. In addition, diagnosis and decisions about treatment are still made on the basis of the gold standard, biopsy and pathology.

2. Screening or direct diagnosis. After more extensive clinical studies have been performed and more data is available, it may be possible to use OCT to directly diagnose or grade early neoplastic changes. This application will only be possible for certain types of neoplasias where OCT would demonstrate sufficient sensitivity and specificity. It will also be more challenging since it implies making a diagnosis on the basis of OCT rather than conventional pathology. Perhaps the most intriguing application of OCT in this scenario is screening. In screening, the initial OCT finding can be followed up and confirmed using standard excisional biopsy and pathology, thus avoiding the demanding requirement that the diagnosis be made on the basis of OCT imaging alone. Other applications include situations where OCT might be used to grade early neoplastic changes or determine the depth of neoplastic invasion. In these cases, treatment decisions would be made on the basis of OCT information, placing stringent requirements on the sensitivity and specificity.

3. Real time diagnosis and surgical intervention and guidance. After more extensive clinical studies, we hope to find scenarios where diagnosis can be made by OCT alone, enabling the diagnosis to be performed in real time. This would provide the means for OCT diagnostic information to be immediately coupled to patient management decisions. The immediate availability of this information could reduce the number of patient visits, yielding a significant reduction in health care costs and improve patient compliance. Finally the highest level of OCT application would be for real time guidance of surgical or other intervention. In this case OCT imaging information could be coupled directly with interventional procedures to show the extent of neoplastic or dysplastic tissue that should be surgically resectioned or otherwise removed.
As with any other diagnostic tool, technique, or method, the ultimate proof of effectiveness and diagnostic utility is its ability to change patient outcomes in a more beneficial or effective manner than currently possible. Good correlation with histology is not enough to prove clinical effectiveness. As in vivo imaging systems become available and human clinical trials are performed, studies must be designed to correctly assess the value of OCT from a large scale perspective. As target applications are identified, investigators must keep in mind that alternative diagnostic techniques may be available. In order to justify the use of a new diagnostic technique, it must be cost effective and offer increased sensitivity and specificity over alternative techniques. In addition, the diagnostic must have clinical utility, that is the information must be relevant to clinical decision making regarding treatment options or management of the disease.

OCT is still in its infancy but despite the current lack of extensive clinical studies, the possibility of application in the detection and management of early neoplasia and cancer remains very high and opens a new, exciting field of research. In the near future as OCT becomes more standardized and new faster and portable systems emerge, we expect to see a surge in the amount of research in this area. This will spark not only new applications of OCT in the diagnosis of cancer but also new innovations in technology that will move OCT towards clinical acceptance.
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