Chapter 1

The Optical Detection of Cancer: An Introduction

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Part I: An Introduction to Optical Detection Technology

Introduction

During 2008, approximately 1.4 million new cases of cancer were diagnosed in the United States, leading to more than 1500 deaths every day. Even with remarkable technological advancements and extraordinary efforts from cancer advocates, scientists, and clinicians, the diagnosis of cancer often occurs at a late stage conferring a dismal prognosis. Importantly, the improvement of patient outcomes is clearly related to the detection of cancerous or precancerous lesions at early stages of disease. Optical detection technology offers the promise to not only detect disease at early stages, but also to improve the monitoring of disease progression or regression during treatment.

The field of optical diagnostics comprises a variety of techniques designed to characterize the relationship between the optical and biological properties of tissue. Through the detection of changes in light after interaction with tissue, optical technologies provide information on the physiologic condition of the tissue at a molecular level. Early research in optical diagnostics suggested that alterations in light-tissue

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interactions can be used differentiate normal from malignant tissue. Subsequent advances in molecular biology, genomics, and proteomics, have vastly improved our scientific understanding of the complex biochemical and morphological changes that occur as tissue undergoes the transformation from normal to neoplasia. Many of these early biological events have been shown to alter the optical properties of pre-cancerous and cancerous tissue. Light based detection systems identify these “optical signatures” created during tissue transformation to provide a real-time assessment of tissue structure and metabolism.

Although the pathways responsible for the development of cancer are complex, it is widely accepted that cancer arises through the accumulation of DNA mutations that tip the cell cycle toward proliferation. The proliferation of squamous cancer cells forms a morphologically distinctive spectrum of disease ranging from mild dysplasia to invasive carcinoma. The current identification and diagnosis of precancerous and cancerous lesions relies on the histological and cytological examination performed by a pathologist after suspicious tissue is biopsied. While these methods represent the gold standard for cancer diagnosis, they have several limitations. Tissue biopsy is invasive, expensive, and often time-consuming. The diagnostic interpretation of the tissue sample has been shown to vary amongst pathologists, and the pathologic criteria for the identification of precancerous lesions are not well defined. In addition, early precancerous changes are frequently undetectable by conventional visual inspection, leading to missed opportunities for diagnosis. Optical technologies have the potential to improve these limitations in several ways. Though the benefits provided vary with each technology, optical techniques offering objective data analysis may reduce the variation in pathologic diagnosis. Furthermore, optical technologies show the potential to provide a real-time tissue assessment through a minimally invasive route, eliminating lengthy waits and the need for tissue biopsy. While the benefits of optical technologies are currently limited in clinical practice, the achievement of a highly sensitive and specific optically determined histopathologic diagnosis, an optical biopsy, has the potential to revolutionize medical practice.
Optical detection technology applications

The majority of cancers (~85%) arise in the epithelial tissues that line the interior and exterior surfaces of the human body. Representative cases include cancers of the oral cavity and pharynx, respiratory system, digestive system, genital system, and urinary system. The majority of these cancers are detected visually, generally through the application of endoscopic techniques. Endoscopy involves a fiber based optical device directed by a physician to visualize tissue surface abnormalities. The surveillance and detection of pre-cancerous and cancerous lesions is achieved through images captured by the endoscope, and tissue biopsied after suspicious sites have been identified. The addition of optical technology to conventional endoscopic visualization techniques allows for the identification of lesions often unidentifiable through conventional endoscopy. In addition, the application of optical technology extends beyond the detection of surface cancer. Techniques have been applied to detect cancer in breast and prostatic tissue, along with molecular contrast agents designed to target specific biochemical pathways in the development of cancer.

Techniques for the optical detection of cancer

Spectroscopy

The utilization of spectroscopic techniques for the detection of cancerous and pre-cancerous lesions is based on the analysis of specific light-tissue interactions to assess the state of biological tissue. As tissue undergoes the carcinogenic sequence from normal to neoplasia, complex morphological and molecular transformations occur that modify the manner in which light is absorbed and reflected in the tissue. With the delivery of specific wavelengths of light to tissue through an optical probe, a spectral pattern is collected that contains diagnostic information for tissue classification. Using histologically confirmed tissue specimens from benign and neoplastic tissue, scientists have assembled a spectral database of known light-tissue interactions. The spectra collected from an unknown tissue sample
can then be analyzed through various empirical and statistical techniques to produce a histological diagnosis. Spectroscopic techniques such as fluorescence spectroscopy, light scattering spectroscopy and Raman spectroscopy, utilize the unique spectral patterns that are created as tissue progresses towards cancer to offer the potential to detect diseased tissue during the initial stages of carcinogenesis.7

Fluorescence spectroscopy

Fluorescence spectroscopy is based on the biological emission of fluorescent light from tissue exposed to ultraviolet (UV) or short wavelength visible (VIS) light. To better understand fluorescence, a brief review of the interaction of light and tissue is warranted. Light is formed by packages of energy termed photons. When tissue is exposed to light, photons may be absorbed, reflected, or scattered by specific molecules within the tissue. As light illuminates the targeted tissue, these biomolecules, termed fluorophores, absorb the energy in the illuminating light and respond by emitting fluorescent light of lower energy (and longer wavelength). The change in wavelength then allows fluorescent light to be differentiated from illuminating light (UV or VIS light). Each group of fluorophores will respond to specific excitation wavelengths, and in turn, emit a different range of wavelengths resulting in a spectral pattern that ideally represents the biochemical and metabolic status of the tissue undergoing optical interrogation.7–10

Fluorescent light may be generated by the administration of an exogenous agent such as in drug-induced fluorescence or by the excitation of endogenous fluorophores (autofluorescence). As tissue undergoes the biochemical and morphological progression to neoplasia, the concentration and distribution of the fluorophores is transformed. Known fluorophores include components of the connective matrix (collagen, elastin), metabolic coenzymes (reduced nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN)), aromatic amino acids (tryptophan, tyrosine, phenylalanine), byproducts of the heme biosynthetic pathway (porphyrins) and lipopigments (lipofuscin, ceroids). Factors
influencing tissue autofluorescence include tissue architecture, light absorption and scattering properties of each tissue layer, the distribution and concentration of the fluorophores in the different tissue layers, the biochemical environment, and the metabolic status of the tissue. Though complex, tissue autofluorescence patterns reflect changes in tissue composition and have been shown to be capable of distinguishing benign from malignant tissue.\textsuperscript{7–10,15}

Elastic scattering (reflectance) spectroscopy

Elastic scattering spectroscopy, also known as diffuse reflectance spectroscopy, utilizes the principle of white light (400–700 μm) reflectance to determine the structural characteristics of illuminated tissue. Elastic scattering occurs when photons from visible light are reflected from tissue constituents without a change in wavelength (or energy). The intensity of this back scattered light is measured resulting in a reflectance spectrum that describes the interaction of white light with tissue following multiple scattering events. As tissue transitions to dysplasia or neoplasia, the relative concentrations, density, and size of endogenous scatterers is affected. The measurement of the intensity of back scattered light is then influenced by the characteristics of the scatterers (i.e. nuclei, mitochondria, connective tissue) and absorbers (i.e. hemoglobin). For example, dysplastic change is often characterized by enlarged nuclei, crowding, and hyperchromacity. These changes lead to characteristic reflectance spectra used to identify the structural composition of tissue and aid in clinical diagnosis.\textsuperscript{7–10,15}

Raman spectroscopy

Raman spectroscopy is a novel optical technique employed to provide detailed information about the molecular composition of tissue. In contrast to elastic scattering spectroscopy, Raman spectra are generated from the molecule-specific inelastic scattering of light. Following exposure to a light source (generally near-infrared light 700–1300 μm), a minute fraction of the scattered light undergoes a
wavelength shift due to the energy transfer between incident photons and tissue molecules. The wavelength shift (and change in energy) is achieved when the incident photon alters the vibrational state of an intramolecular bond. A Raman emission spectrum is generated from the combination of the wavelengths scattered by the molecules in a tissue sample. These spectral features provide detailed and specific information about the molecular composition of tissue. Though Raman spectroscopy is sensitive to a wide range of specific biomolecules such as proteins, lipids and nucleic acids, the Raman effect only compromises a small fraction (1 in a million) of scattering events and signals may be weak and difficult to implement.\textsuperscript{8,10,40} Other modifications of the Raman technique, such as surface enhancing Raman spectroscopy and coherent anti-stokes Raman scattering are designed to amplify the signal.

**Fluorescence imaging**

Fluorescent imaging systems utilize spectroscopic principles to capture fluorescence emission spectra from a larger tissue sampling area than is possible with point spectroscopy. The acquisition of an image requires tissue illumination with a light source, often in the near-UV to green range. The subsequent fluorescence produced from the absorption and scattering events is recorded with a camera and results in a real-time image. In addition, fluorescence imaging systems are capable of sampling larger tissue areas and provide two-dimensional information allowing for the detection of lesion-specific features such as homogeneities.\textsuperscript{13}

**Optical coherence tomography (OCT)**

Optical coherence tomography is an innovative optical imaging technique designed to provide high resolution (~10–20 µm) cross-sectional images of microscopic sub-surface tissue structures. As the optical analogue of high frequency B-scan ultrasonography, a imaging technique that detects back scattered sound waves, OCT images are generated by measuring the intensity of back scattered light after tissue is probed with a low-power near-infrared light source.
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(wavelengths ranging from 750 to 1300 µm). Based on the principle of low-coherence interferometry, OCT is able to provide real-time images at a resolution 10 times greater than endoscopic ultrasound, thereby allowing for the identification of microscopic tissue features such as villi, glands, crypts, lymphatic aggregates, and blood vessels. Despite this high resolution, OCT imaging is limited by a depth of penetration of 1–3 mm.

To obtain images, infrared light is delivered to tissue through an optical probe, typically 2 to 2.4 mm in diameter. Various OCT system designs allow for tissue to be scanned a linear, tranverse, or radial fashion and are easily interfaced with endoscopes, laparoscopes, catheters, and hand-held probes. Initially applied to obtain images in the field of ophthalmology, technological advancements have resulted in the clinical application of OCT imaging in a diverse set of medical specialties, including gastroenterology, dermatology, cardiology and oncology.11

Narrow-band imaging (NBI)

Narrow-band imaging is a recently developed optical technique designed to enhance the visualization of microvasculature on the mucosal surface. Developed to improve the quality of endoscopic images, NBI systems limit the depth of light penetration into tissue through red, green, and blue optical interference filters. These three filters divide the visible wavelength ranges into three shorter wavelength bands, while increasing the relative contribution of blue filtered light. The increased contribution of blue light is fundamental to creating a narrow-band image, as blue light corresponds to the peak absorption of hemoglobin. The resulting image demonstrates preferential enhancement of the vascular network of the superficial mucosa. To differentiate normal from dysplastic tissue, the microvascular patterns present in the narrow band image are analyzed. Areas of nondysplastic tissue generally have fine capillary patterns of normal size and distribution; while areas harboring dysplasia demonstrate abnormal capillary patterns with increased size, number, and dilation. The clear visualization of vascular patterns through NBI has been shown to enhance the diagnostic capability of endoscopy and offers promise for the early detection of cancer.10,25
Multimodal optical imaging

Advances in bioengineering and the continued refinement of optical detection techniques have led to the development of multimodal optical detection systems. These multimodal devices often function in real-time to provide complementary diagnostic information and wide tissue surveillance capability. The ultimate goal of the optical detection systems centers on the achievement of an “optical biopsy”. This achievement would allow clinicians to determine a tissue diagnosis based on \textit{in vivo} optical measurements and would eliminate the need for conventional biopsy and histopathological interpretation. Furthermore, the reliable detection of malignant change through the “optical biopsy,” will provide the clinician the ability to immediately determine definitive treatment and optimally improve patient outcomes.

Summary

To briefly review the diagnostic information provided by various optical techniques, fluorescence spectroscopy and Raman spectroscopy offer diagnostic information about the biochemical composition of tissue, while reflectance spectroscopy probes changes in light scattering and absorption to characterize tissue morphology. Optical coherence tomography provides high resolution images of tissue morphology, and NBI offers complementary information about tissue microvasculature.

Part II: The Application of Optical Diagnostic Technology in the Upper Aerodigestive Tract

Introduction

Approximately 281,000 cancers of the upper aerodigestive tract were estimated to be diagnosed in 2008.\textsuperscript{1} These sites include the oral cavity, larynx, pharynx, hypopharynx, trachea, bronchus, lung, and esophagus. Importantly, the 5-year survival rates for cancers of the upper aerodigestive tract are dismal, and efforts aimed at the early
detection of these cancers have yet to significantly improve clinical outcomes.\textsuperscript{1} The promise of optical detection technologies centers on the ability to perform the inexpensive, rapid, and accurate diagnosis of early cancers leading to improved survival rates. As with all new medical technologies, the employment of optical diagnostic technologies in clinical practice must meet or exceed the current standards of care. Recent research in optical diagnostic technology has focused on the translation of these technologies from the laboratory to the clinic. Initial research in optical diagnostics described the properties of normal, dysplastic, and cancerous tissue, and provided a foundation for the \textit{in vivo} diagnosis of suspicious areas. The following segments provide a brief introduction into the current knowledge and clinical application of optical detection systems for the early detection of cancers of the upper aerodigestive tract. Subsequent chapters provide further background and detailed descriptions of each technology. Due to the limited nature of this introduction, a comprehensive review of the applications of each optical technology is excluded and will be discussed in other chapters of the book.

\textbf{Oral cavity}

Cancers of the oral cavity account for approximately 3\% of malignancies diagnosed annually in the United States. As with other upper aerodigestive tract cancers, 5-year survival rates for oral cavity cancers decrease significantly with delayed diagnosis (Local: 81.8\%, Distant 26.5\%).\textsuperscript{1} Cancers of the oral cavity are believed to progress from premalignant/precancerous lesions, beginning as hyperplastic tissue, with subsequent increasing levels of dysplasia (mild, moderate, severe) into carcinoma \textit{in situ} (CIS), and finally developing into invasive squamous cell carcinoma.\textsuperscript{12} Several optical imaging techniques have shown promise in identifying these lesions, and several systems have gained FDA approval for clinical use.

As the most thoroughly investigated optical techniques for the detection and characterization of oral lesions, autofluorescence spectroscopy and imaging systems have been shown to be capable of
distinguishing normal oral mucosa from cancerous lesions. In addition, research suggests that autofluorescence techniques are capable of discriminating between lesion types, though sensitivities and specificities reported by researchers have varied. Research suggests that autofluorescence spectroscopy is exceedingly accurate in distinguishing lesions from healthy oral mucosa (sensitivity 82–100%, specificity 63–100%), though there is a lack of compelling evidence for the discrimination between lesion types.\textsuperscript{13} Autofluorescence imaging systems, such as the commercially available VELscope, allow the clinician to probe oral cavity tissue for the direct visualization of precancerous and cancerous lesions. Studies demonstrate that oral cancer and precancerous lesions show a characteristic decrease in green fluorescence when probed with autofluorescent imaging systems. This fluorescent pattern allows for the clinician to visualize malignant changes of oral tissue that manifest as darkened areas surrounded by healthy, green fluorescent tissue.\textsuperscript{12}

In addition to fluorescence spectroscopy and imaging techniques, several additional optical diagnostic systems have demonstrated potential for the successful evaluation of the oral cavity. A recent study using a multispectral imaging system (fluorescence, narrow-band imaging, and orthogonal polarized reflectance) demonstrated that oral lesion borders change with each imaging modality, suggesting that multimodal imaging can provide important diagnostic information not available through conventional white light examination or through the use of a single imaging mode alone.\textsuperscript{14} Trimodal spectroscopy (Fluorescence spectroscopy, Elastic scattering spectroscopy, Raman spectroscopy) has been shown to be capable of diagnosing malignant/precancerous tissue with a sensitivity and specificity of 96%.\textsuperscript{15} Despite the diagnostic advantages created by the combination of optical technologies, these complementary techniques may prove to be time-consuming and expensive, limiting clinical utility.

The application of optical coherence tomography (OCT) for the evaluation of oral cavity disease began as early as 1998 when researchers obtained images of the human tooth and oral mucosa.\textsuperscript{16,17} In 2004, OCT images captured from varying states
of pathology in hamster cheek mucosa were used to study the feasibility of OCT scanning for oral disease diagnosis. Recent improvements in OCT technology have led researchers to study the clinical utility of OCT for oral cancer diagnosis. Images obtained from in vivo benign and malignant oral tissue demonstrated that OCT is capable of recognizing differences in mucosal and submucosal tissue structures allowing for image correlation with known histological features. As OCT technology continues to evolve, faster scanning speeds and higher-resolution images will improve characterization of tissue structure with the hope that this optical modality could improve the detection and management of early stage oral disease.

**Pharynx, hypopharynx, and larynx**

The upper aerodigestive tract descends from the oral cavity into the pharynx, hypopharynx, and larynx where these anatomical structures contribute to human functions such as swallowing and speech. Similar to the other cancers of the upper aerodigestive tract, pharyngeal, hypopharyngeal, and laryngeal cancers are frequently diagnosed at advanced stages of disease often conferring a dreary prognosis. In particular, pharyngeal and hypopharyngeal cancers are often asymptomatic and difficult to detect until late stages, leading to extensive and sometimes disfiguring surgical and medical interventions. Presently, the detection of these cancers is achieved through endoscopic visualization performed with indirect and direct white-light laryngoscopy, with direct laryngoscopy under general anesthesia and biopsy representing the gold standard for diagnosis. Despite the diagnostic accuracy of laryngoscopy, assessment of precancerous and cancerous lesions may be limited by physician experience, difficult anatomy, and patient discomfort.

Efforts to improve the diagnostic information provided during the evaluation of pharyngeal, hypopharyngeal, and laryngeal surfaces incorporate several of the novel optical diagnostic techniques. Adjunctive optical techniques for the detection of these lesions
include autofluorescence laryngoscopy, narrow-band imaging, and optical coherence tomography. Indirect autofluorescence endoscopy has recently been shown to improve routine white-light endoscopy in the evaluation of suspected precancerous and cancerous laryngeal lesions. This imaging technique improved sensitivity by 7% and specificity by 18% and may easily be implemented as an outpatient procedure.\textsuperscript{20-22} Additionally, the fluorescence staining of laryngeal neoplasms after the topical application of photosensitizer 5-aminolevulinic acid (ALA) has been described as a promising diagnostic procedure for differentiating tumor from normal mucosa during microlaryngoscopy.\textsuperscript{24} Research investigating the value of narrow band imaging for the early detection of laryngeal cancer demonstrates that NBI can provide high sensitivities and specificities on the basis of abnormal intraepithelial microvasculature changes.\textsuperscript{24} Furthermore, research suggests that NBI may improve the sensitivity for the discovery of pharyngeal lesions over conventional methods in addition to serving as an ideal surveillance method after chemoradiation therapy for oropharyngeal and hypopharyngeal cancers.\textsuperscript{25,26} Optical Coherence Tomography has also shown promise as an imaging device for laryngeal cancer, reliably identifying the loss of basement membrane integrity in patients with laryngeal cancer.\textsuperscript{27} However, the diagnostic utility of OCT for laryngeal lesions requires further research. Ongoing investigation into the implementation and refinement of these optical techniques demonstrates increasing potential for the role of optical diagnostics in the detection and diagnosis of pharyngeal, hypopharyngeal, and laryngeal cancers.

**Trachea, bronchus, and lung**

The application of optical detection modalities in the diagnosis of airway disease is continually evolving. As the most common cause of cancer death worldwide, lung cancer typically presents at a late stage and with a dismal prognosis.\textsuperscript{1} The focus of various optical detection techniques (ODT) in the diagnosis of airway disease is in the identification and discrimination of benign (i.e. bronchitis), pre-invasive, and malignant lesions in the central airway.
Autofluorescence bronchoscopy (AFB) has shown considerable promise in the evaluation of pre-invasive airway disease, as evidenced by improved sensitivity compared to white-light bronchoscopy. However, despite this improved sensitivity, screening for pre-invasive lesions with AFB has not been recommended outside controlled clinical trials. In addition to AFB, optical coherence tomography has been shown to be capable of discriminating invasive cancer from CIS, and dysplasia from metaplasia, hyperplasia, and normal tissue, and has the potential to serve as an optical biopsy technique as an adjunct to AFB. The use of Narrow-Band Imaging in the evaluation of airway disease is also under investigation. Research indicates that NBI is capable of improving detection of bronchial dysplasia compared to WLB alone, but requires further evaluation as a stand-alone technology.

**Esophagus**

The incidence of esophageal cancer has shown a recent dramatic rise in both Europe and the USA, a result mainly attributed to Barrett’s esophagus. Barrett’s esophagus is a condition in which the normal lining of the upper GI tract is replaced by an epithelium that is associated with an increased risk of developing adenocarcinoma. Unfortunately, the five year survival rate for all stages of esophageal cancer is only 15.6%, and improving survival for these cancers must focus on the early detection of cancerous changes. The detection of esophageal cancers is achieved through endoscopic evaluation with biopsy, and several optical techniques have recently been developed to increase the diagnostic accuracy of endoscopic evaluation.

The use of autofluorescence spectroscopy and raman spectroscopy are each promising optical techniques for the detection of upper GI cancers. Data from studies performed to evaluate autofluorescence spectroscopy shows a wide range of sensitivities (75–100%) and specificities (65–95%), and this technique demonstrates promise as an adjunctive optical technique. Raman spectroscopy appears capable of identifying high grade dysplasia/early adenocarcinoma
with a reported sensitivity of 88%, specificity of 89%, and accuracy of 89%.  

Several optical imaging systems have been developed to enhance endoscopic diagnosis of esophageal lesions. Recent studies have shown that autofluorescent imaging systems may increase the detection rate of early esophageal cancer from 63 to 91%, though autofluorescent imaging is often limited by a high false positive rate. Trimodal imaging systems are also under investigation for the optical detection of esophageal cancer. Incorporating high resolution endoscopy, autofluorescence imaging, and narrow band imaging, the trimodal system was able to increase the detection of early neoplasia with the addition of autofluorescence, and reduce the false positive rate with the addition of narrow band imaging.

Optical coherence tomography has also been investigated for the detection of upper GI cancerous changes with promising results. A recent study using ultrahigh resolution OCT demonstrated the feasibility of carrying out OCT imaging in conjunction with standard endoscopy for the evaluation of Barrett’s esophagus, dysplasia, and adenocarcinoma, though future research will need to focus on evaluating its clinical utility. The recent research and emphasis on enhancing optical techniques for the detection of early esophageal cancerous changes shows significant promise as clinicians seek to alter the poor prognostic implications of cancer diagnosis.

**Conclusion**

Optical diagnostic technologies utilize the interaction of light and tissue to provide objective data that describes the biological, chemical and morphological composition of the tissue under interrogation. While many of these techniques have only recently been implemented in medical settings, they offer scientists a highly sought after method for the early detection of cancer. Many of the optical diagnostic techniques are still in the research and development stage and
before they can be implemented in widespread clinical practice, further research in large clinical trials must confirm the initial experimental data provided by researchers across the globe. With further research and optimization of optical diagnostic technology, the early detection of cancer is an increasingly realistic goal in medical practice.

References

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