Detection of Squamous Cell Carcinoma and Corresponding Biomarkers Using Optical Spectroscopy

H. Wolfgang Beumer, MD1, Karthik Vishwanath, PhD2, Liana Puscas, MD1,3, Hamid R. Afshari, DDS3, Nimmi Ramanujam, PhD2, and Walter T. Lee, MD1,3

Abstract
Objectives. Investigate the use of optical reflectance spectroscopy to differentiate malignant and nonmalignant tissues in head and neck lesions and characterize corresponding oxygen tissue biomarkers that are associated with pathologic diagnosis.

Study Design. Cross-sectional study.

Setting. Tertiary Veterans Administration Medical Center.

Subjects and Methods. All patients undergoing panendoscopy with biopsy for suspected head and neck cancer were eligible. Prior to taking tissue samples, the optical probe was placed at 3 locations to collect diffuse reflectance data. These locations were labeled “tumor,” “immediately adjacent,” and “distant normal tissue.” Biopsies were taken of each of these respective sites. The diffuse reflectance spectra were analyzed, and biomarker-specific absorption data were extracted using an inverse Monte Carlo algorithm for malignant and nonmalignant tissues. Histopathological analysis was performed and used as the gold standard to analyze the optical biomarker data.

Results. Twenty-one patients with mucosal squamous cell carcinoma of the head and neck were identified and selected to participate in the study. Statistically significant differences in oxygen saturation ($P = .001$) and oxygenated hemoglobin ($P = .019$) were identified between malignant and nonmalignant tissues.

Conclusion. This study established proof of principle that optical spectroscopy can be used in the head and neck areas to detect malignant tissue. Furthermore, tissue biomarkers were correlated with a diagnosis of malignancy.

Keywords
optical spectroscopy, squamous cell carcinoma, diagnosis

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Head and neck malignancies affect an estimated 50,000 people annually. Early detection of these malignancies results in improved outcomes. However, clinical differentiation between malignant and nonmalignant lesions of the upper aerodigestive tract can be challenging. Furthermore, prior surgery, trauma, or radiation may make a clinical diagnosis even more difficult. In these cases, biopsy of the lesion is performed to establish a diagnosis.

On the basis of a review at our institution between January 2009 and December 2009, 256 patients underwent 305 biopsies of a suspected malignancy, of which 192 samples were nonmalignant. These biopsies did not represent margin biopsies during tumor resection. Thus, nearly 2 of 3 clinically indicated biopsies were later pathologically determined to be nonmalignant. This statistic is likely representative of other tertiary care centers throughout the nation and emphasizes the variability and uncertainty inherent in current clinical examinations.

In addition, surgical biopsy is not without its issues. Some lesions are difficult to biopsy in a clinical setting, such as those located in the oropharynx, hypopharynx, and larynx.
These biopsies often require general anesthesia and biopsy in the operating room. According to the American Hospital Directory, the national average charge of laser endoscopy with biopsy (CPT 31535) is $1176 per patient. Significant amounts of labor, facility, and monetary resources are expended on patients who ultimately may have no malignancy. Consequently, patient selection is important to minimize risks and costs and maximize yield. To address this 63% negative biopsy rate at our institution, we have collaborated with the Duke biomedical engineering department to explore an optical method as a potential tool to improve patient selection.

This method is based on reflectance optical spectroscopy technology. Optical spectroscopy is the use of light to evaluate the composition of materials. Spectroscopy is a broad field. In this instance, diffuse reflectance spectroscopy using light in the visible ultraviolet spectrum is used. Reflected light is analyzed, providing absorption and scattering data that reflect underlying tissue composition, particular tissue oxygen saturation, total hemoglobin concentration, and morphology.

Because carcinogenesis alters the structural and biochemical makeup of cells, the metabolic needs of the tissues are altered, often leading to increased microvascularization and differing oxygen utilization. This, in turn, affects optical properties of dysplastic and cancerous tissue. Numerous studies have demonstrated differences between normal tissue and tumors in their respective diffuse reflectance spectra. These prior studies have relied on qualitative waveform pattern matching across a broad wavelength range.

In contrast, Shih et al have demonstrated similar quantitative optical spectroscopy techniques in vitro. Brown et al demonstrated the use of quantitative optical spectroscopy in breast cancer. Using a novel inverse Monte Carlo model, specific biomarker parameters were extracted from the optical reflectance data. This allowed for a quantitative analysis to measure concentrations of these specific biomarkers and their association with the pathologic diagnoses of disease. This study investigated the use of optical reflectance spectroscopy and the scalable inverse Monte Carlo model to differentiate malignant and nonmalignant tissues specifically in head and neck lesions. Furthermore, characterization of corresponding oxygen tissue biomarkers that predict pathologic diagnosis was performed. The hypothesis was that optical reflectance spectroscopy can differentiate malignant and nonmalignant tissues in head and neck lesions. Furthermore, these differences can be correlated with tissue oxygen biomarkers.

**Subjects and Methods**

This study, approved by the Durham Veterans Administration Institutional Review Board, was open to all patients who were scheduled for panendoscopy and biopsy for suspected head and neck cancer. Patients were approached and consented to undergo noninvasive evaluation by the optical probe of sites to be biopsied. These sites included “tumor,” “immediately adjacent to tumor,” and “distant normal.”

**Optical Spectroscopy Probe**

An optical spectroscopy probe was designed and manufactured to final form in the laboratory of Dr. Nimmi Ramanujam at Duke University. This device is composed of optical fibers that are epoxied together and covered by a stainless steel tube. The fiber-optical probe contains 19 individual source fibers surrounded by 18 collection fibers. Each individual fiber was 200 microns in diameter, and they were arranged such that the collection fibers formed a ring with a diameter of ~1 mm around a centrally, uniformly illuminated core. Thus, the active sensing area of the probe was a circular area of ~1 mm diameter. The fiber is 2 mm in diameter, and the handheld probe is 5 mm in diameter and is ensheathed in a stainless steel tube that is amenable to sterilization. Probe design details have been described in prior publications by the Ramanujam group. In brief, light from a 450-Watt xenon source is passed through a monochromator, then down a collection of optical fibers within a cable to the probe tip. The monochromator selects for wavelengths in the range of 350 to 600 nm. Light reflected off the tissues is then passed along a set of different fibers within the cable to a spectrometer. A laptop computer is used to both control the light source and spectrometer as well as analyze the incoming data.

**Clinical Study Design**

Consented patients had the optical probe placed on the surface of at least 3 sites (tumor, immediately adjacent to tumor, and distant normal). By this design, the patients served as their own control. Immediately adjacent to tumor sites were included in an attempt to capture more dysplastic lesions. The vast majority of them came back as normal tissue. The rest were malignant. There were no dysplastic samples from these sites. Distant normal sites were matched to contralaterally symmetrical locations with normal-appearing mucosa. If biopsy of other areas was clinically indicated, then the probe was also used to analyze that site prior to biopsy. Diffuse reflectance scans were performed, and data were collected. The data acquisition program allowed for multiple scans to be obtained at the same site, and thus 2 to 4 optical scans were routinely obtained at each biopsy site. Each scan was obtained in approximately 5 seconds. Immediately after the scan, the probe was removed, and the underlying tissue was sampled with biopsy forceps such that the reflectance data could be directly paired with the histopathological diagnosis.

**Extraction of Tissue Biomarkers from Optical Probe Data**

A quantitative inverse Monte Carlo model was used to extract scattering and absorption properties of the measured tissues. Diffuse reflectance spectra measured from the tissue were fit over the wavelength range of 450 to 600 nm. Using this inverse Monte Carlo model, concentrations of oxygenated hemoglobin (HbO2), deoxygenated hemoglobin (dHb), and total hemoglobin (THb), as well as oxygen saturation (SO2%), were calculated in µM. These biomarkers were extracted from the absorption coefficient spectra across all measured subjects and sites. All fits and subsequent data analysis were performed using MATLAB version 7.8, release 2009 (MathWorks, Natick, Massachusetts).
### Statistical Analysis

For data analysis, tissue samples were grouped into 2 groups, malignant and nonmalignant, based on histopathological diagnosis. Mean concentrations per site for HbO₂, dHb, and THb and SO₂% were then compared using unpaired Wilcoxon rank-sum test to determine if there were statistically significant differences between malignant and nonmalignant tissues.

### Results

A total of 21 patients were enrolled into the study, of whom all were men. During the study, no adverse events were encountered. Patient demographics and biopsy locations are represented in Table 1. A total of 266 scans were performed on 68 tissue biopsy sites—on average, 4 scans per site (range, 1-8 scans/site). Histopathologically, 43 biopsies were normal tissue, 18 biopsies were tumor tissue, and 7 biopsies demonstrated dysplasia. These 7 dysplastic specimens represented 23 total scans (Table 2). The dysplasia samples were integrated into the nonmalignant category for analysis. Figures 1A-C demonstrates the calculated molar concentrations of the biomarkers dHb, HbO₂, and THb for malignant and nonmalignant samples. Figure 1D shows the calculated tissue SO₂%. Statistically significant differences (defined as \( P < .05 \)) were noted in the HbO₂ concentration (\( P = .019 \)) and in SO₂% (\( P = .001 \)). Figure 2 is a representative reflectance scan demonstrating differences in overall reflectance between malignant and nonmalignant tissues.

### Discussion

Our study established proof of principle that optical spectroscopy can be used in head and neck lesions to detect tissue biomarkers associated with malignancy. Data demonstrated that tissue reflectance differences between malignant and nonmalignant mucosal lesions of the head and neck can be delineated with optical spectroscopy. Specifically, 4 biomarkers were extracted from the reflectance data using an inverse Monte Carlo model and compared between malignant and nonmalignant tissues. Of these, the concentration of HbO₂ and the tissue SO₂% showed a statistically significant difference.

Optical reflectance spectroscopy has previously been demonstrated to show different reflectance, scatter, and absorption patterns between malignant and nonmalignant tissues. This can be attributed to different structural and metabolic properties of the tissues in question. This is in line with many prior studies that have demonstrated significant differences in oxygen tension in normal and metabolically active solid tumor tissues.

In the head and neck, prior studies focused on waveform pattern matching to demonstrate qualitative differences between normal and malignant tissues. For example, Mallia et al have used laser induced autofluorescence and diffuse reflectance spectroscopy spectral differences with curve fitting to show differences between dysplastic tissues and squamous cell carcinoma in oral cancers.

Furthermore, many study methods have relied on extrinsic contrast materials such as dyes or stains or more complex instrumentation in the case of laser-induced autofluorescence. Tsui et al used direct fluorescence visualization to evaluate surgical margins in oral cavity carcinoma. Their study did highlight that optical technologies clearly define surgical margins better than direct visualization. Other studies have used autofluorescence to distinguish normal and abnormal tissues in the oral cavity, but no standardization has been established. These methodologies require additional skill sets or bulky equipment and may rely on subjective interpretation. A quantitative approach, such as the methods described in this study, may aid in defining surgical margins more accurately and objectively.

A limitation of this study is the small sample size. A larger study may better define and identify other biomarkers. Also, it is recognized that determining dysplasia versus malignancy is a more clinically relevant question. To address this, patients are being recruited for a study to expand this data set with the goal of establishing good specificity and sensitivity data of optical biometric signatures. As part of this upcoming larger study, the goal would be to stratify findings by site. Other studies have also identified probe pressure to be a variable that affects spectroscopy readings. Probe pressure may affect the contents of vascular channels, artificially reducing measured hemoglobin or oxygen saturation. Standardizing the probe readings in the future will be accomplished by integrating pressure sensors into the probe design.
This study suggests that specific biomarkers can be identified via this method of analyzing optical spectroscopy data. The clinical impact of this approach should improve patient selection for biopsy. The technology used to demonstrate this has the advantage of being low cost, portable, noninvasive, and, most important, quantitative.

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**Author Contributions**

H. Wolfgang Beumer, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published; Karthik Vishwanath, substantial contributions to conception and design, acquisition of data or analysis and interpretation of data, revising article critically for important intellectual content, and final approval of the version to be published; Liana Puscas, acquisition and...
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**Disclosures**

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