Intraoperative Use of Wide-Field Optical Coherence Tomography to Evaluate Tissue Microstructure in the Oral Cavity and Oropharynx

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IMPORTANCE
Involvement of deep margins represents a significant challenge in the treatment of oropharyngeal cancer, and given practical limitations of frozen-section analysis, a need exists for real-time, nondestructive intraoperative margin analysis. Wide-field optical coherence tomography (WF-OCT) has been evaluated as a tool for high-resolution adjunct specimen imaging in breast surgery, but its clinical application in head and neck surgery has not been explored.

OBJECTIVE
To evaluate the utility of WF-OCT for visualizing microstructures at margins of excised oral and oropharyngeal tissue.

DESIGN, SETTING, AND PARTICIPANTS
This nonrandomized, investigator-initiated qualitative study evaluated the feasibility of the Perimeter Medical Imaging AI Otis WF-OCT device at a single academic center. Included participants were adults undergoing primary ablative surgery of the oral cavity or oropharynx for squamous cell carcinoma in 2018 and 2019. Data were analyzed in October 2019.

EXPOSURES
Patients were treated according to standard surgical care. Freshly resected specimens were imaged with high-resolution WF-OCT prior to routine pathology. Interdisciplinary interpretation was performed to interpret WF-OCT images and compare them with corresponding digitized pathology slides. No clinical decisions were made based on WF-OCT image data.

MAIN OUTCOMES AND MEASURES
Visual comparisons were performed between WF-OCT images and hematoxylin and eosin slides.

RESULTS
A total of 69 specimens were collected and scanned from 53 patients (mean [SD] age, 59.4 [15.2] years; 35 [72.9%] men among 48 patients with demographic data) undergoing oral cavity or oropharynx surgery for squamous cell carcinoma, including 42 tonsillar tissue, 17 base of the tongue, 4 buccal tissue, 3 mandibular, and 3 other specimens. There were 41 malignant specimens (59.4%) and 28 benign specimens (40.6%). In visual comparisons of WF-OCT images and hematoxylin and eosin slides, visual differentiation among mucosa, submucosa, muscle, dysplastic, and benign tissue was possible in real time using WF-OCT images. Microarchitectural features observed in WF-OCT images could be matched with corresponding features within the permanent histology with fidelity.

CONCLUSIONS AND RELEVANCE
This qualitative study found that WF-OCT imaging was feasible for visualizing tissue microarchitecture at the surface of resected tissues and was not associated with changes in specimen integrity or surgical and pathology workflow. These findings suggest that formal clinical studies investigating use of WF-OCT for intraoperative analysis of deep margins in head and neck surgery may be warranted.
The achievement of negative resection margins has important prognostic implications in the treatment of head and neck squamous cell carcinoma (SCC). Patients found to have involved margins after surgery are known to have increased risk of local recurrence,1-5 poorer rates of progression-free survival,5-10 and a need for adjuvant treatments, such as radiotherapy and chemotherapy, and additional surgery. These interventions themselves were associated with increased risk of morbidity and mortality.11-13

Anatomical constraints inherent to the head and neck play a role in the difficulty of achieving local control, especially at the posterior, deep margin of the tonsils, where resectable tissue is limited and critical nervous and vascular structures are at risk of surgical injury. With so little room for error, confidence in margin status is critical and is further complicated by limitations of current methods for intraoperative pathological assessment of tissue samples.14

An emerging approach is the use of optical coherence tomography (OCT). This nondestructive imaging modality, first described in the 1990s,15 uses the principle of near-infrared interferometry to produce high-resolution, cross-sectional, and volumetric images of tissues of interest in a manner analogous to the use of sound waves for ultrasonography imaging.16-19 It was previously shown that trained readers can use OCT images to identify heterogenous and disorganized patterns in tissue microarchitecture, including discrepancies in epithelial layer thickness, from a variety of tissue types.20 This capability suggests that OCT may be useful as a tool to distinguish suspicious regions of interest at the surfaces of surgically resected tissue.

While OCT was originally developed for ophthalmologic applications,15,20 a summary of research from 201516 demonstrated the potential application of the technology in numerous other tissue types. There have also been preliminary studies21-26 exploring OCT’s utility for imaging soft tissues of the head, neck, and oral cavity. To date, the form factor of investigational OCT tissue-scanning devices has been handheld probes of various designs.25,27-33 While handheld scanners can allow for in situ imaging of a tumor or cavity, this design has thus far been unable to provide images at sufficient power, resolution, and detail to be clinically useful.

Since 2017, a novel OCT imaging system based on a different approach to tissue scanning has been in use by our institution’s breast surgical service.34,35 This system differs from those based on handheld probes in that it uses a flatbed scanner designed for rapid acquisition of wide-field OCT (WF-OCT) images of whole, excised (ex vivo) surgical specimens prior to processing for standard permanent pathology. The goal of this study was to evaluate the feasibility of using this WF-OCT system to visualize microstructures at the margins of tissue excised from the oral cavity or oropharynx with the aim of investigating whether benign and suspicious features could be observed in WF-OCT images in a way that corresponded to use of permanent hematoxylin and eosin (H&E) pathology slides.

Methods

The protocol for this nonrandomized, investigator-initiated qualitative study evaluating feasibility was approved by the institutional review board of the Icahn School of Medicine at Mount Sinai, and all patients gave signed, informed consent before participating. This study was reported using an adaptation of the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline for randomized feasibility and pilot trials.36 Specimens were deidentified, and surgeons (M.Y., M.S.T., E.M.G., and B.A.M.) were blinded to results of the imaging process. Only the principal investigator (B.A.M.) was able to link each patient’s WF-OCT image data to their final pathology report for image comparisons. This was a prospective, single-center feasibility study.

Wide-Field Optical Coherence Tomography

Principles of OCT have been described previously.17,37 Briefly, low-coherence light from the near-infrared spectrum (1250-1350 nm) is split into sample and reference beams; these are then focused at the target tissue or at a reference interferometer, respectively. Backscattered and reflected light recombine at the beam splitter, and the resulting interference patterns allow digital reconstruction of 2-dimensional optical slices at an axial resolution of 6 to 15 μm and a penetration depth of up to 2 mm. Slices can be further stacked to create a volumetric representation of the specimen.

The WF-OCT platform used in this study (Otis version 2.0; Perimeter Medical Imaging AI) is a stand-alone, cart-mounted system. User-operable parts are a flatbed scanning window and a console with a touch screen user interface system for setting parameters, observing image acquisition in real time, and performing postacquisition image review, analysis, and annotation. After excision and prior to imaging, specimens are placed within a single-use, lidded tissue-handling tray that connects to the console to enable application of gentle vacuum pressure to hold the specimen in place against the scanning window during imaging.

At the time of this study, the system was an investigational device. It was subsequently approved by the US Food and Drug Administration for general use as an imaging tool.
in the evaluation of excised human tissue by providing 2-dimensional, cross-sectional, real-time visualization of human tissues with image review manipulation software for identifying and annotating regions of interest. The system does not have a specific clinical indication for use in oropharyngeal tissue.

**Patient Selection**
Consecutive adult patients (aged ≥18 years) referred for primary ablative surgery of the oral cavity or oropharynx for biopsy-proven squamous cell carcinoma from 2018 to 2019 were prospectively enrolled in the study. Patients undergoing revision surgery and those previously treated with oral cavity radiation or chemotherapy were excluded. Target enrollment was up to 100 patients.

**Study Procedures**
Patients were treated according to institutional standards of care (SOC) for surgical management and specimen pathology (Figure 1). Surgical excision of tissue was performed under general anesthesia using electrocautery or an ultrasonic surgical dissection device (Harmonic Scalpel, Ethicon).

Immediately after excision, fresh tissue specimens were passed out of the sterile field to the circulating nurse in the operating room (Figure 1). Additional or supplemental tissue was taken based on the surgeon’s detailed gross visual and physical inspection of the resected specimen and the resection cavity per SOC. Then, prior to frozen section analysis (FSA) or fixation in neutral buffered formalin, primary specimens were marked with ink or sutures for orientation, then placed in a disposable specimen-handling tray and then the scanning window of the WF-OCT imaging system (Video 1).

Vacuum was applied to gently hold the specimen in place during scanning. Predetermined system settings for low or high vacuum were selected for each tissue depending on the fragility or density of the specimen. The superior, inferior, medial, lateral, and deep specimen margins, corresponding to those marked with ink and examined during routine pathology sectioning, were scanned for each oropharyngeal specimen (Video 2). Volumes were captured at approximately 15 μm resolution, up to 8.5 × 8.5 cm of tissue surface, and to a penetration depth of up to 2 mm. The WF-OCT scanning system also captured optical photographs of each specimen for orientation and identification.

Research staff immediately reviewed WF-OCT imaging data in the operating room to ensure that images were of sufficient quality for subsequent detailed analysis. No actions or

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**Table. Patient Baseline Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value, No. (%) (N = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>59.4 (15.2)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>35 (72.9)</td>
</tr>
<tr>
<td>Women</td>
<td>13 (27.1)</td>
</tr>
<tr>
<td>p16 Test status</td>
<td></td>
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<tr>
<td>Positive</td>
<td>21 (43.8)</td>
</tr>
<tr>
<td>Negative</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td>Not tested</td>
<td>26 (54.2)</td>
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<tr>
<td>HPV test status</td>
<td></td>
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<tr>
<td>Type 16</td>
<td>20 (41.7)</td>
</tr>
<tr>
<td>Type 35</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td>Type 69</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td>Not tested</td>
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<tr>
<td>Diagnosis</td>
<td></td>
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<tr>
<td>SCC</td>
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</tr>
<tr>
<td>HPV related</td>
<td>23 (47.9)</td>
</tr>
<tr>
<td>Not HPV related</td>
<td>12 (25.0)</td>
</tr>
<tr>
<td>Otherb</td>
<td>10 (20.8)</td>
</tr>
<tr>
<td>No tumor or residual invasive disease found</td>
<td>3 (6.3)</td>
</tr>
</tbody>
</table>

Abbreviations: HPV, human papillomavirus; SCC, squamous cell carcinoma.

a Data were available for 48 patients.

b Other diagnoses included reactive lymphoid hyperplasia (3 patients), cellular pleomorphic adenoma (1 patient), diffuse large B cell lymphoma (1 patient), epithelial hyperplasia (1 patient), invasive malignant melanoma (1 patient), lymphangioma (1 patient), squamous dysplasia (1 patient), and squamous papilloma (1 patient).
changes to SOC were taken based on WF-OCT image assessment. Scanning procedures were completed within the cold ischemic window. In some patients undergoing tonsillectomy in which the healthy, contralateral tonsil was also resected as SOC, that tissue was also imaged for comparative purposes. After scanning was completed, specimens were placed in neutral buffered formalin for transport to pathology and processed for sectioning, H&E staining, slide digitization, and histopathological analysis according to institutional SOC.

Data Analysis
An author (A.K.B.) and a trained image reader assessed WF-OCT images of each scanned margin postoperatively. Observed tissue architecture and features were annotated, and regions of interest identified in the WF-OCT images were compared with corresponding regions in digitized histology slide images for each specimen. These were located based on orientations recorded on the device during scanning. A head and neck pathologist (B.A.V. or W.H.W.) then reviewed annotated comparisons and confirmed that images could be used to visibly discern muscle, lymphatic vessels, and other regions from each other on WF-OCT and H&E images. Data were analyzed in October 2019. Analyses of visual comparisons were qualitative, and no formal measures of performance are presented.

Results
Between June 2018 and May 2019, 69 specimens from 53 patients (mean [SD] age, 59.4 [15.2] years; 35 [72.9%] men among 48 patients with demographic data) were collected from a variety of tissue types within the oral cavity and oropharynx and successfully scanned using WF-OCT before being analyzed per SOC (Table; eTable in the Supplement). Analyzed specimens included 42 tonsillar tissue, 17 base of the tongue, 4 buccal tissue, 3 mandibular, and 3 other specimens. There were 41 malignant specimens (59.4%) and 28 benign specimens (40.6%). These were successfully analyzed by SOC histology after WF-OCT scanning. It took approximately 1 to 2 minutes per margin to scan excised specimens, which was not associated with changes in the integrity of specimens for downstream pathology.

Representative comparisons of WF-OCT and H&E images from patients with invasive, human papillomavirus (HPV)-negative and -positive, and p16-negative and -positive SCC are shown in Figure 2, Figure 3, and Figure 4 and eFigures 1 through 6 in the Supplement. Areas suggestive of malignancy as well as benign structures within the healthy surrounding tissue could be observed in WF-OCT images and matched with fidelity within corresponding H&E slides by the trained reader. Benign tonsillar tissues and structures identified in these images included normal tonsillar lymphatic tissue (Figure 2A and Figure 3B), crypts (Figure 2A and Figure 4B), squamous epithelium (Figure 2B and Figure 4B), the submucosal layer (Figure 2B), skeletal muscle (Figure 2B and Figure 3A), a dilated lymphatic channel (Figure 2B), blood vessels (Figure 3C), and a lymph node (Figure 4B).

Adipose tissue, connective tissue, and cysts were also observed. In the tongue, squamous metaplasia and dysplasia were identified. In buccal tissues, benign structures included minor salivary glands and salivary ducts.

Discussion
This nonrandomized, investigator-initiated qualitative study evaluated the feasibility of using WF-OCT as an adjunct imaging technique. The technique demonstrated the ability to distinguish malignant from benign tissue, and the information obtained was consistent with traditional histopathological analysis. This finding supports the potential use of WF-OCT as a complementary tool in surgical oncology, particularly in the evaluation of margins and the identification of high-risk areas that may require additional sampling or further treatment.

The feasibility of using WF-OCT for margin assessment was confirmed, and the data analysis indicated that the technique could be used to discern tissue architecture and features, which was consistent with histopathological findings. The integration of WF-OCT into clinical practice may offer several advantages, including real-time visualization of surgical margins, improved accuracy in identifying margins, and potentially reduced sampling of healthy tissue.

In conclusion, the study demonstrated the potential of WF-OCT as a valuable tool in the evaluation of tissue margins. Further research is needed to evaluate the performance of WF-OCT in a larger and more diverse cohort of patients, as well as its impact on patient outcomes and surgical decision-making.

Figure 2. Representative Comparison of Images for Patient A

A. Posterior margin of right tonsil
B. Posterior-inferior additional margin

Comparison is shown between wide-field optical coherence tomography and permanent histology of invasive, moderately differentiated squamous cell carcinoma (SCC, p16 negative) with lymphatic invasion. A. The posterior margin of the right tonsil is shown, with wide-field optical coherence tomography in the top panel showing SCC as an area with decreased light penetration depth compared with the adjacent normal tonsillar lymphatic tissue. In the bottom panel, a hematoxylin and eosin slide from the corresponding region is shown.

B. A crypt (C) is also seen on the right side of the image. The posterior-inferior additional margin is shown, with wide-field optical coherence tomography in the top panel showing squamous epithelium (SE), submucosal layer (S), and skeletal muscle (SM). In the bottom panel, a hematoxylin and eosin slide from the corresponding region is shown. A dilated lymphatic channel (DLC) is also seen on the right side of the image.
modality for the visualization of microstructures at the margin of excised oral cavity or oropharyngeal tissue specimens. We found that the imaging resolution was sufficient to allow identification of specific, pertinent microarchitectural features within WF-OCT images of benign and diseased tissue and that these features could be observed on permanent histology slides from the corresponding location within the specimen. The brief elapsed time necessary to scan the excised specimen (approximately 1-2 minutes per margin) would not have been associated with changes in the timing of the procedure and was not associated with the integrity of the specimens for downstream pathology.

Involvement of deep soft-tissue margins represents a major problem in oropharyngeal cancer.38–40 Current intraoperative practices to increase the likelihood of gaining local control include visual gross examination and palpation of the surgical specimen and resection cavity, as well as FSA. While FSA was previously found to be associated with reduced positive surgical margins and decreased re-excision rates,4 the associated time needed for processing and assessment can add as much as 30 minutes to the surgical procedure, requires a pathologist to be on call during the surgery, and is not readily available outside large academic centers. In addition, FSA is a destructive method, so it can be performed on only a small subsection of the primary specimen. It is thus subject to sampling bias, and results of FSA cannot be compared directly with those of permanent histology.5,40

Other avenues of development for real-time, nondestructive intraoperative margin analysis have focused on adapting various forms of confocal microscopy, fluorescence microscopy, and imaging of tissue autofluorescence, fluorescent nuclear contrast agents, or immunofluorescent probes targeted against specific biomarkers.41 While study is ongoing, to date there is insufficient evidence that any 1 of these techniques has sufficient sensitivity and specificity for widespread clinical utility in rapid intraoperative margin assessment.

Significant effort has gone into attempting to develop OCT for this purpose, as well, but handheld probe designs have been...
insufficient given that they provide low-power images of narrow bands of tissue, which lack sufficient resolution at a clinically relevant depth. The nature of images produced by WF-OCT is very different; WF-OCT is performed ex vivo on a flatbed scanner and has optical sectioning capability similar to that of confocal microscopy. In our study, it rapidly provided high-resolution (approximately 15 μm), en bloc imaging of specimen margins. A surgeon or other trained reader could view and interpret these images before a specimen is sent to pathology. We also found WF-OCT to be flexible and readily adaptable to surgical and pathology workflows.

Optical coherence tomography imaging is widely used for ophthalmologic and endovascular imaging. The use of OCT has also been investigated in numerous other tissues, including breast, lung, cardiac, genitourinary tract, gastrointestinal tract, and head and neck tissues. However, the use of WF-OCT is currently principally under study as an adjunct tool for detecting tumor-involved margins in real time during breast-conserving surgery. To our knowledge, this is the first reported study of WF-OCT in the oral cavity and oropharynx.

Validating the technology specifically for head and neck is important because breast tissue is relatively homogenous compared with the multipart structures of the oral cavity and oropharynx, and disease manifests differently. For example, transitional, premalignant, or in situ disease patterns common to breast tissue are not generally relevant to head and neck cancers; head and neck SCC manifests as starkly different from normal tissue, with little to no architectural transitional morphology. With WF-OCT, we found that epithelium was readily distinguishable from submucosal and muscular layers of the tongue, with a clear and well-delineated transition. This suggests that WF-OCT may therefore be especially useful for analysis of head and neck SCC tumor margins given that they penetrate deeper tissue layers.

Margin assessment is critical at the posterior deep margin of the tonsils and the base of the tongue, and here, every millimeter of tissue is critical to outcomes. In these cases, better margin management may be associated with complete local control, de-escalation of treatment, and the possibility of surgical cure, especially for HPV-associated head and neck SCC. For the population of individuals with HPV who are young and otherwise healthy and who do not smoke, a surgical cure for HPV-associated oral cancer may yield meaningful long-term benefits in health, quality of life, and economic measures that may more than offset the incremental cost of the technology.

More rigorous studies will be necessary to validate the clinical utility and performance of WF-OCT in intraoperative margin assessment during head and neck procedures. However, based on our experience, we hypothesize that WF-OCT imaging may have a place as an adjunct tool with which to collect valuable image data regarding the deep margin during the time in which the surgeon is performing gross assessment of the resection bed. The WF-OCT workflow may be associated with reductions in certain limitations of FSA (e.g., sampling bias and sample destruction), but this study was not designed to evaluate superiority or noninferiority. Furthermore, we do not envision WF-OCT as a substitute for standard pathology; it is intended as an adjunct intraoperative imaging modality with data that may serve to give the surgeon an additional level of confidence in the completeness of margin resection during primary surgery.

Limitations

This study has several limitations. It was a device feasibility study with no formal control group and was not designed to make direct comparisons between WF-OCT and other emerging technologies for intraoperative margin assessment, nor was it designed to measure performance. However, it was designed as a prospective study, surgeons were blinded to image assessment at the time of surgery, and at least some portion of the study population formed a self-control group when healthy and diseased contralateral tonsils were removed.
A clinically relevant limitation of OCT in general is that it has a maximum light-penetration depth in biological tissue of 1 to 2 mm. While that depth is generally sufficient for margin assessment in breast oncology, tumors of the oral cavity are typically defined as negative if the margin is clear to a depth of 5 mm or more. At present, OCT is not capable of assessing tissue microarchitecture at that depth unless multiple scans are acquired from different specimen aspects. This is an area for further study and refinement.

As with other methods of margin analysis, artifacts caused by surgical electrocauterization during transoral robotic surgery present a potential technical limitation because they may complicate image interpretation at the deep margin. Ultrasonic or other low-temperature dissection methods may eventually solve the problem of cautery artifacts, but no such method has yet reached SOC. Further work is needed to solve the problem of margin destruction during electrosurgical dissection.

We also identified a workflow limitation related to standardization of margin orientation and specimen processing. Specifically, we found that specimens were often being serially sectioned along a different axis than the direction of OCT optical slices (eg, sectioned posterior to anterior, while OCT was scanned inferior to superior). This impaired the ability of pathologists to make direct comparisons between WF-OCT images and corresponding histology in some cases.

### Role of the Funder/Sponsor
The funder had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

### Meeting Presentations
Some data in this study were previously presented as a poster (P366) at the American Head and Neck Society 10th International Conference on Head and Neck Cancer; July 22-25, 2021; virtual meeting. An update was presented as a poster at the annual meeting of the College of American Pathologists; October 9, 2022; New Orleans, Louisiana.

### Additional Contributions
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### Statistical Analysis
Badhey, Schwarz, Genden.

### Administrative, technical, or material support
Badhey, Schwarz, Genden.

### Conflicts of Interest Disclosures
Dr Veremis reported receiving personal fees from Perimeter Medical Imaging as a consultant on breast cancer projects (funding was not received for this project) during the conduct of the study. No other disclosures were reported.

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