How close are we to real time optical margin control in head and neck oncologic surgery?

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The quest for negative margins during head and neck oncologic surgery continues to challenge surgeons worldwide. Current technological advances offer significant promise to advance the field and improve operative margin detection. This review focuses on current optical technology, which has been evaluated for intraoperative margin control in head and neck oncologic surgery. Many of the current systems have technical limitations and there is no current standard technology being applied in the field of head and neck oncology to enhance intraoperative margin control. Surgeons continue to rely on intraoperative frozen section analysis, however many optical systems have shown high rates of sensitivity and specificity when discriminating benign from malignant tissue. These technologies show significant promise for intraoperative margin control and may be enhanced by targeted molecular imaging, or spectral analysis, in the future. Translational trials are rare and are the key to the path of independence from frozen section analysis.

Keywords: Optical technology; microscopy; oral cancer; neoplasia; surgical margins; squamous cell carcinoma

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Introduction

Oncologic surgical resection remains the standard of care for many malignancies of the head and neck. One of the most challenging issues that continue to plague surgeons is obtaining negative margins during surgery. The current “gold standard” is frozen section analysis of submitted margins by the pathologist [1-3]. This technique is fraught with several technical and conceptual limitations, which have been recognized in the literature. Of course the concept of a surgical margin is a refection of the surgeons goal to surgically resect all malignant tissue, while preserving normal tissues, resulting in adequate oncologic control with favorable functional outcomes. That being said, the actual definition of the surgical margin varies by tumor histology, anatomic location, histologic criteria, and surgical tradition. In fact even the concept that tumors grow radially and that resecting a certain distance from the tumor will “ensure negative margins” is a flawed concept [4, 5]. Even the concept of “negative margins” while seeming to be conceptually obvious, is technically highly variable in real practice due to sampling issues during specimen submission, pathological processing errors, and the fact that tumor cells may often be left within the surgical bed despite the fact that the final pathology report has a negative margin designation [6, 7]. What has been shown, with significant reliability, is that
malignant disease left behind after ablative oncologic surgery leads to recurrent disease and adversely affects outcomes [8, 9].

The literature is clear that in order to maximize survival, reduce recurrence, and improve quality of life for patients undergoing oncologic head and neck surgery, negative margins are paramount [10-14]. In fact, positive margin rates are associated with tremendous variability and have been postulated as a useful quality measure for early oral cavity cancer therapy [15]. Therefore while the goal of modern oncologic surgery is to achieve a true negative margin resection, this goal is currently hampered by significant technical limitations in margin evaluation. This fact has prompted numerous scientific and clinical investigations regarding novel optical technology, which may allow for improved accuracy in margin detection. The field of image-guided oncologic surgery is an emerging area of research, and a variety of optical imaging modalities have been proposed during head and neck ablative oncologic surgery [16-18]. The development of cost-effective technology that would allow the surgeon to establish immediate, real-time margin information that is consistent with the histologic diagnosis is the ultimate objective of the field. With growing interest in minimally invasive surgical techniques in the head and neck advanced imaging modalities are likely to play an increasingly important role in oncologic surgery. This review will provide background information on the current status of the variety of optical systems that are currently being studied for the use of optical

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**Table 1. Current technology for Intraoperative Margin Control in Head and Neck Surgery**

<table>
<thead>
<tr>
<th>Imaging Technology</th>
<th>Surface Resolution</th>
<th>Depth</th>
<th>Targeted vs. Morphologic</th>
<th>Ex-Vivo</th>
<th>In-Vivo</th>
<th>Clinical Trial</th>
<th>Reported Accuracy</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Coherence Tomography (OCT)</td>
<td>15-20µm</td>
<td>1-2mm</td>
<td>Morphologic</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Sens/Spec 81.5%/87% PPV 61.5% NPV 95% [19-21]</td>
<td>No contrast required</td>
<td>Not designed for surface imaging of cellular architecture</td>
</tr>
<tr>
<td>Confocal Microscopy</td>
<td>150-400 µm</td>
<td>50-500 µm</td>
<td>Targeted/Morphologic</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Sens/Spec 90% /59-100% [22]</td>
<td>Excellent cellular resolution</td>
<td>Limited depth</td>
</tr>
<tr>
<td>Narrow Band Imaging (NBI)</td>
<td>HD 1280x1024 1.5-2X magnification</td>
<td>100-300 µm</td>
<td>Morphologic</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Sens/Spec 72.5% -100%/66.7-88.9% [23-26]</td>
<td>Excellent vascular visualization</td>
<td>No cellular resolution</td>
</tr>
<tr>
<td>High Resolution Microendoscopy (HRME)</td>
<td>50-100</td>
<td>100-200 µm</td>
<td>Targeted/Morphologic</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Sens/Spec 96%/95% PPV 91% NPV 98%. Mean Accuracy 95.1% (CI 94-96%) [27]</td>
<td>Nuclear contrast agent</td>
<td>Non-specific</td>
</tr>
<tr>
<td>Tissue Auto fluorescence</td>
<td>150-200 µm</td>
<td>50 µm</td>
<td>Morphologic</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes*</td>
<td>Sens/Spec33.3%/8 6.6% PPV 33.3% NPV 88.6% [28] [28]** N/A[30-32]****</td>
<td>No contrast agent required</td>
<td>Poor specificity in vivo</td>
</tr>
<tr>
<td>Near Infrared Imaging (NIR)</td>
<td>25-50 µm</td>
<td>2-3mm</td>
<td>Targeted/Morphologic</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>N/A[33, 34]</td>
<td>Specific Targeted Fluorescence</td>
<td>Gross Margin Assessment Only</td>
</tr>
<tr>
<td>Targeted Fluorescent Imaging</td>
<td>Whole Tumor</td>
<td>Whole Tumor</td>
<td>Targeted</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>N/A[35]</td>
<td>No contrast Molecularly specific</td>
<td>Laboratory only at this point</td>
</tr>
<tr>
<td>Spectral Imaging (Raman, HIS, laser spectroscopy)</td>
<td>Molecular/ nm</td>
<td>N/A</td>
<td>Targeted</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>N/A[36]</td>
<td>Excellent depth capability</td>
<td>No surface imaging ability</td>
</tr>
<tr>
<td>Ultrasound Imaging</td>
<td>N/A</td>
<td>3mm-5cm</td>
<td>Morfids</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Sensitivity 91%/36, 37**** N/A [38-41]</td>
<td>Robotic Feasibility</td>
<td></td>
</tr>
</tbody>
</table>

* Specificity varied when combining targeted and morphologic criteria vs. either alone. ** Phase III results pending. *** Reduction in local recurrence rate from 11 of 28 patients (39.3%) to 5 of 62 patients (8.1%) (P < .001) ****50% increased survival in animal model ***** Consistency ratio 91.4% to 98.2%, and the mean difference between US thickness and histologic thickness was 1.21mm.
margin control in head and neck oncologic surgery.

Research methods

The literature from 2000-2015 was reviewed via online databases including OVID/MEDLINE, PUBMED using a combination of medical subject heading (MeSH) terms and free text words. These MeSH terms and phrases included, optical technology; optical microscopy; oral cancer; oral neoplasia; larynx cancer, surgical margins; squamous cell carcinoma, high resolution microscopy, optical coherence tomography, spectroscopy, pathologic margins, optical margins, head and neck squamous cell carcinoma. Articles that reported results of intraoperative evaluation using ultrasound, while not technically an optical technology, were included for completeness of the review and clinical relevance.

The primary focus of this review is to determine the current status of technology when used specifically for surgical margin evaluation during oncologic head and neck surgery (in vivo discrimination of benign versus malignant tissue) therefore all references which were pertaining to diagnostic optical technologies, screening technologies, or other imaging technologies not designed specifically to assist the surgeon during oncologic surgery were excluded. In addition, reports regarding the identification of malignant cells via optical systems in a laboratory setting, which have not been successfully translated to systems that could be adapted to the operating theater, were excluded. Reports regarding cytological techniques such as brush biopsy, buccal smears, and oral rinses were excluded, as these techniques are not suitable for determining the status of epithelium at the margin of a resection. Also investigations reporting dyes, stains, or other topical agents designed to assist the naked eye in surgical margin identification (i.e. not coupled with an optical system) were excluded. Animal studies performed with optical margin control were referenced for informational purposes but not included in the review of current technology available for optical margin control in vivo. It should also be noted that reports pertaining to optical or fluorescent technologies for the identification of lymph node metastasis were also excluded, as they are not subjects of this review.

Results

Review of the current literature revealed 824 references that were identified using the above noted search criteria. The majority of investigations were related to squamous cell carcinoma in a variety of anatomical sub-sites including oral cavity, oropharynx, larynx, and hypopharynx. Despite a plethora of ex-vivo laboratory and animal investigations, as well as numerous investigations regarding variety of optical technologies for detection, delineation and discrimination of head and neck malignancy in vivo in humans, only 26 articles were identified which actually tested novel technology during oncologic head and neck surgery for the specific goal of real-time surgical margin assessment. The most common technologies evaluated in a clinical setting were autofluorescence (4 articles), narrow band imaging (NBI) (5 articles), confocal microscopy (CFM) (5 articles) and ultrasound (7 articles). Current optical technologies that are being investigated for intraoperative margin detection during head and neck ablative oncologic surgery are summarized in Table 1. Each of the available modalities has specific advantages and limitations and the findings of these investigations are summarized in the Discussion below.

Discussion

Several studies have indicated that close, or positive margins after oncologic head and neck surgery results in poor outcomes, in terms of disease free survival and overall survival [42]. There continues to exist significant technical and oncological limitations regarding the current standard of care, namely histologic frozen section surgical margins. There are multiple factors that lead to inaccurate margin assessment, which include non-standardized technique, processing issues such as orientation and tissue shrinkage, and institutional variability when reporting close or negative margins. Maxwell et al, examine the role of margin sampling techniques during glossecotomy in 5 tertiary care centers and reported that margins which were obtained from the tumor resection specimen were more accurate than those sampled from the tumor bed. The authors noted that reliance on margin sampling from the tumor bed is associated with worse local control [43]. This finding was also noted in a single center trial that noted a statistical improvement in local control, disease-free, and overall survival with increasing radial margin distance from the tumor [44]. Currently there are no recommended standards to guide surgeons regarding appropriate surgical margin sampling.

The inability to detect microscopic disease intra-operatively can lead to additional surgery, local recurrence, and decreased survival rates. These issues related to the current standard of care have prompted the examination of optical technology and molecular diagnostics as a way to improve margin accuracy during head and neck oncologic surgery [45]. Certainly these technological advancements have been applied widely in the field of cutaneous malignancy, but have not been evaluated as thoroughly in mucosal disease of the head and neck [46]. Several innovative optical imaging technologies have been developed for intraoperative surgical guidance and have been
examined for use in head and neck oncologic surgery. While these technologies all have significant promise in this setting, they each have individual technical advantages and limitations. A discussion regarding each of these current technologies examined for the potential for intra-operative margin detection in head and neck oncologic surgery is as follows:

**Optical coherence tomography (OCT)**

Optical coherence tomography has been utilized extensively in the medical field in a variety of applications. It is a non-invasive imaging technology that uses low-coherence interferometry, typically employing near infrared and light from a variety of laser sources that have a relatively long wavelength allowing it to penetrate tissues and to produce cross-sectional images of tissue microstructure. This essentially acts like a low energy laser based “optical ultrasound” when evaluating tissue. OCT has been applied in intraoperative margin detection in cutaneous, vulvar, breast, and gastro-intestinal malignancy. OCT has been examined in several diagnostic/detection applications in the head and neck such as larynx, oral, and esophageal cancer however studies examining the ability of OCT to determine the accuracy intra-operative margins are less common. Hamdoon et al., examined the use of OCT for intraoperative margin evaluation in twenty-eight T1-T2 N0M0 oral cavity squamous cell carcinoma patients. After tumor resection, the specimen margins were evaluated with an OCT scanner to evaluate the margins, which were then confirmed by standard histopathology. The overall sensitivity and specificity of OCT was found to be 81.5% and 87%, respectively. Whilst the positive predictive value was 61.5% and the negative predictive value was 95%. The authors reported that positive margins could be identified by architectural changes and an increase in epithelial layer thickness with OCT technology. The use of OCT for intraoperative margin detection remains investigational and several advancements such as polarization-sensitive OCT, or contrast enhances OCT (gold nanoparticles) may offer superior imaging characteristics for this application in the future.

**Confocal microscopy (CFM)**

Confocal microscopy is an optical imaging technique that uses laser energy that excites a specific plane within the tissue. The illumination intensity rapidly falls above and below the plane of focus thus reducing interfering of tissue the focal plane being examined. The reflected signal then passes through an aperture situated in the conjugate focal plane of the specimen. Any light that emanates from a region other than the illuminated point is blocked by the aperture. This provides decreased background noise and superior resolution of cellular morphology when compared to OCT, albeit with less penetration. CFM has been widely applied in the field of dermatology when determining the margins of lesions of the skin. With regards to head and neck oncology studies are somewhat more limited. Clark, et al examined CFM for use in the detection and evaluation of oral cavity neoplasia. Confocal images were successfully acquired from 15 biopsy pairs from 17 patients. Depth-related changes in cell diameter and nuclear density were observed at multiple anatomical sites within the oral cavity. In squamous cell carcinomas, densely packed, pleomorphic tumor nuclei could be visualized with distinct differences in nuclear density and morphology distinguishable between confocal images of neoplastic and non-neoplastic oral cavity. Other features of noncancerous and cancerous oral tissue that could be identified in the confocal images included areas of inflammation, fibrosis, muscle fibers, and salivary glands. Additional studies have evaluated the use of CFM for detection of oral malignancy although to the best of the authors knowledge, no clinical trials evaluating the technology for intraoperative margin control have been reported to date. In a recent review, Maher et al noted that current device developments for oral CFM include improved probe design and did suggest that improvements could allow CFM to play a role in the intra-operative mapping for cancer surgery.

**Narrow-band imaging (NBI)**

Narrow-band imaging technology has also been evaluated as a potential technology for intra-operative margin detection. This technology uses a switch filter and light wavelengths of 440 to 460 nm (blue) and 540 to 560 nm (green). By taking advantage of the peak absorption of hemoglobin the system can image surface characteristics of mucosal tissue as well as vascularity differences between tissues.

Orita et al., reported the use of NBI system to delineate intraoperative margins in a 62-year-old female undergoing partial hypopharyngectomy. The surgical margins were sufficient after resection due to visualization of the microvasculature of the lesion and the authors hypothesized that this system could be used for intraoperative margin detection. Tirelli et al., reported on the use of NBI margin mapping in 16 patients with head and neck squamous cell carcinoma after traditional macroscopic margin delineation. The authors reported that margins delineated with NBI technology were frequently positive for dysplasia (25%) or malignancy (75%) and that the technology was superior to traditional macroscopic margin delineation. While NBI was reported to be highly sensitive (100%) in this selected
group of patients, the author did note that the specificity performance was approximately 88%.

Vicini et al., reported their use of NBI during transoral robotic surgery (TORS) in order to improve on the positive margin rate in a population of patients undergoing TORS for squamous cell carcinoma. The authors performed a retrospective review of 32 patients who underwent TORS and intra-operative NBI evaluation (NBI-TORS) and compared them to 21 patients who underwent standard TORS with white light evaluation. The authors reported a higher rate of negative superficial lateral margins in the NBI-TORS group compared with the standard group (87.9% vs. 57.9%, respectively, p = 0.02)\[^{23}\]. Despite this promising data, the specificity of NBI in this study was reported as 66.7%. Despite this, the authors have shown that NBI has potential for intraoperative margin detection during TORS.

Similarly Garofolo et al., presented data using NBI in the setting of transoral laser microsurgery (TLM) in early glottic cancer. The authors compared a cohort of 82 patients undergoing cordectomies for laryngeal malignancy, using lesion delineation with a NBI system. This group was compared to a historical cohort of patients who had similar procedures with standard microscopic evaluation during surgery. The rate of positive superficial margins was reported as 3.6% in the present group and 23.7% in the control cohort (P<.001). The authors concluded that NBI of laryngeal lesions dramatically decreased the rate of final positive margins\[^{25}\].

Farah et al., recently reported on the molecular divergence among surgical margins delineated by white light versus NBI-defined margins. The authors reported that the numbers of differentially expressed genes indicated that NBI-defined margins exhibited less genetic abnormalities indicated superior margin placement. In this study of 18 patients, 4 patients (22%) benefited directly from the NBI margin delineation. The authors concluded that resection to NBI-defined margins will leave less dysplastic and malignant residual tissue in situ, improving outcomes\[^{26}\].

**High resolution microendoscopy (HRME)**

The high-resolution microendoscope (HRME) is a noninvasive imaging technology that utilizes a fluorescence microscope coupled to a flexible fiber optic probe to obtain images in real-time of tissue stained with a topical fluorescent nuclear contrast agent (0.01% proflavine)\[^{66, 67}\]. Nuclear and cellular morphology including, allowing visualization of epithelial architecture and cellular
morbidity such as nuclear size, nuclear-to-cytoplasmic ratio, and nuclear dispersion. This device has been utilized in gastroenterology applications to evaluate and screen for lesion of the esophagus [86, 88]. Previously reported data has validated this device for the detection of head and neck squamous cell carcinoma (HNSCC) ex vivo, with a sensitivity and specificity of 98% and 92% respectively [69].

A recently reported clinical trial by the author noted the ability of HRME to distinguish benign versus malignant tissue in 33 adult subjects with squamous cell carcinoma of the oral cavity. The reported mean accuracy was 95.1% (95% CI, 94–96%). The mean sensitivity and specificity were 96% (95% CI, 94–99%) and 95% (95% CI, 90–99%), respectively. The negative predictive value was 98% (95% CI, 97-99%) and PPV was 91% (95% CI, 85-98%). The authors concluded that HRME had a reliability of high-to-distinguish benign versus malignant mucosa in these selected images (Figure 1).[27] The investigators did note that issues with image acquisition were problematic including severe inflammation, keratin debris, contrast artifact, bleeding, and the significant limitation of the technology that at the current time is limited to surface imaging (50µm). The authors concluded that HRME may offer promise in the intraoperative evaluation of surgical margins if more specific targeted contrast agents (i.e. fluorescent targeted contrast) were coupled with the device (Figure 2).[27]

Fluorescent imaging technology

The use of a variety of fluorescent technology for the detection, screening, and delineation of head and neck squamous cell carcinoma has received a lot of attention in the literature. Tissue autofluorescence, near infrared imaging (NIR), and a variety of fluorescent probe imaging systems have been investigated for this application.

Tissue auto fluorescence

Human mucosal tissues exhibit an inherent fluorescence spectra, which can be used to discriminate malignant from benign tissue due to loss of fluorescence in dysplastic or malignant tissues. Klatt et al, measured time-resolved autofluorescence in tumors and healthy tissues of the oral cavity ex vivo and calculated the corresponding fractal dimension that was significantly higher in malignant tissue compared to healthy mucosa. The authors described the method and the potential applications for head and neck oncologic surgery [70].

Tissue auto fluorescence for intraoperative margin control is somewhat limited by issues related to specificity [71]. This is due to the fact that all malignant tissues exhibit decreased auto fluorescence, but a variety of other conditions such as inflammation, keratosis, and benign lesions may also exhibit this loss of signal. Poh et al, recently published a retrospective observational study including 246 patients undergoing surgery for high-grade dysplasia or oral carcinoma. 154 patients underwent surgery with fluorescent visualization of intraoperative tumor margins, with 92 undergoing traditional surgery serving as controls. The fluorescent system was the VELscope system (VELscope; LED Medical Diagnostics, Inc.) Patients were followed for after surgery and patients who underwent surgery with fluorescence visualization had a dramatic reduction in the 3-year local recurrence rate. This publication has validated the concept that image guided margin delineation can result in improved oncologic outcomes [28]. While this investigation is an excellent example of the concept of fluorescent-guided imaging to guide surgical resection, the VELscope system relies on tissue autofluorescence with the associated issues of specificity as noted previously. However in the setting of known dysplastic lesion, or frank malignancy, which has been biopsied, tissue autofluorescence may provide sufficient specificity to improve outcomes as reported in the noted study. Currently the group is currently finalizing data from a multicenter, phase 3 trial to validate their results [28].

Figure 1. Example of targeted contrast imaging near infrared (NIR) images of human cell line tissue culture (20x, 800nm dye) from the author’s collection. A. RBFN labeled SCCA cells. B. EGFR labeled SCCA cells.

Figure 2. Example of targeted contrast imaging near infrared (NIR) images of human cell line tissue culture (20x, 800nm dye) from the author's collection.
the laboratory results of examining tissue autofluorescence have shown significant promise in the discrimination of malignant tissues, actual application in clinical trials has been extremely variable in terms of specificity.\cite{29, 72}

Near infrared imaging (NIR)

Near Infrared imaging (NIR) is a technology that encompasses a variety of techniques, however the underlying principle is to use near infrared light between 650 and 950 nm to interrogate tissue. This wavelength window takes advantage of a gap in the tissue absorption wavelengths and results in increased tissue penetration of light as well as improved signal-to-background when contrast agents are used. There are a variety of contrast agents such as nanoparticles, molecular targeted agents which may allow for the detection and delineation of head and neck cancer.\cite{18}

Atallah, et al, tested a novel NIR system in nude mice using a fluorescent probe targeting v3 integrin molecules. The authors noted that optical imaging-guided surgery with this system increased the recurrence-free survival rate by 50%. The system could detect cancer deposits as small as 185 microns which resulted in improved quality of cancer resections in this model.\cite{322} This study group also pointed out in a recent review of NIR fluorescence guided surgery that although the technology has significant promise for optical margin control, that current use is limited by the small number of fluorescent probes approved for clinical use.\cite{73}

Tanca et al, reported results of a mouse model examining NIR imaging technology with iRFP protein probes compared to visible fluorescent molecular imaging with EGFR targeted probes and found that while the iRFP cell line produced better results than cells emitting visible light when studying local, distant, and deep tumors in the mouse model, the EGFR-targeted probe conjugated with IRDye800 accurately detected tumor perimeters.\cite{754} This work may indicate a role for targeted tumor margin delineation in real time with targeted optical systems guided by fluorescent-targeted contrast agents.

Targeted fluorescence image guided surgery

Zheng et al, has reported the development of the use of this type of approach (near-infrared fluorescence nano liposomal agent - CF800) for targeted tumor delineation. Combining these agents with preoperative computed tomography, three-dimensional surgical planning and intraoperative target mapping as well as near-infrared fluorescence guided resection. The authors reported a >5-fold tumor-to-background ratio with the system and proposed a model for imaging guided surgery with margin delineation.\cite{75} The research group has yet to publish a clinical trial using the system. Rosenthal et al, have reported a dose escalation study with IRDye800 in 12 patients undergoing resection of head and neck squamous cell carcinoma. The authors reported that fluorescence imaging with an intraoperative, wide-field device successfully differentiated tumor from normal tissue during resection with an average tumor-to-background ratio of 5.2 in the highest dose range with sub-millimeter resolution.\cite{34} This work demonstrated the first clinical use of fluorescence guided imaging and the identification of head and neck cancer, however the study was not designed to evaluate margin control specifically.

Spectroscopy

Optical imaging technology which utilizes the inherent molecular spectra of tissue, or contrast enhance spectra have been evaluated for margin delineation in head and neck surgical applications as another promising technology. There are a variety of optical techniques to determine the molecular spectra of tissue and several investigations have shown some promise for this technology.

Francisco et al, reported the use of fluorescence spectroscopy to compare oral squamous cell carcinoma lesions to surgical margins and the mucosa of healthy subjects. 214 spectra of oral squamous cell carcinoma margins were compared to 200 healthy control spectra. These spectra were compared to histopathology to determine fluorescence efficiency for diagnostic discrimination of tumors. The authors reported significant qualitative differences in the fluorescence spectra of benign and malignant tissue and concluded that this technology had some promise for intraoperative margin delineation and the discrimination of altered mucosa.\cite{35}

Several research groups have evaluated one particular type of spectroscopy, Raman spectroscopy, in this setting. Raman spectroscopy is a spectroscopic technique that uses the property of inelastic scattering, or Raman scattering, of monochromatic light in a variety of visible, NIR, or NUV wavelengths. The light interacts with the tissue molecular vibrations or other interactions and the resulting energy information can discriminate between tissue types. The primary advantage of this type of imaging is that the molecular discrimination does not rely on contrast media or labeling of the tissue. Cals et al, compared the Raman spectra of 10 patients and examined the spectral findings compared to histopathologic examination. They created annotated spectra were used as input for linear discriminant analysis (LDA) models to discriminate between malignant spectra and healthy tissue spectra. These analytic models could distinguish malignant spectra from the spectra of squamous epithelium in 75% of the cases. Structures that were most often confused with OCSCC were dysplastic epithelium,
basal layers of epithelium, inflammation and capillary-rich connective tissue. Certainly this study offered evidence that Raman spectroscopy can distinguish a variety of tissues, but the performance was insufficient to design an intraoperative margin protocol given the issues with spectra adjacent to the malignancy and the lack of specificity with other tissues in the region. Barroso et al, reported on 14 patients who underwent tongue resection for squamous cell carcinoma. Using high-wavenumber Raman spectroscopy to detect the water content of malignant tissue compared to surrounding normal tissue the authors reported that the water content values from squamous cell carcinoma measurements were significantly higher than from surrounding healthy tissue and tumor tissue could be detected with a sensitivity of 99% and a specificity of 92%. The authors noted several advantages of the technique as Raman measurements are fast and can be carried out on freshly excised tissue without any tissue preparation. The authors did note some limitations in this small preliminary study, including the lack of information about the margin of the tumor and the transitional zone water content.

Lu et al, performed an evaluation of Hyperspectral Imaging (HSI), which is a technique the captures spectral information as well as spatial resolution data without the necessity of contrast agents or molecular labeling. The authors used histological images to provide a platform to validate tumor margins detected by HIS and optimize imaging parameters. The study group then applied the system to a xenograft model and was able to delineate tumor margins in the animal model with the HIS system, demonstrating feasibility. The authors did note some technical limitations in terms of being able to map the HSI images and the fact that the images acquired are a 2D representation of a 3D tumor. This study examined the ability of the system to identify the margin of a tumor, but the experiment was not specifically designed to test HSI for intraoperative margin detection.

**Ultrasound**

Ultrasonic platforms (US), while technically not optical in nature, offer another non-invasive real time technology for the detection of the tumor interface and may have promise for the intraoperative detection of tumor margins. Perhaps one of the most advantageous characteristics of ultrasound imaging is the ability to image the depth of the tumor with a reasonable level of accuracy. This ability is often limited in optical imaging technology which provides high resolution surface imaging but little information about the depth of the tumor beyond 1-2mm. Yuen et al, published results of a large study of 45 patients in which intraoral ultrasonography was used to document tumor thickness prior to resection and noted that the accuracy of US in tumors from 3-15mm thick was >91%, although this study was performed as a preoperative evaluation rather than a real time operative technique for margin control.

Given the accuracy of ultrasound in preclinical studies, several investigators have explored the use of ultrasound in head and neck cancer to improve operative margin control. Helbig et al, examined five patients with intraoperative endosonography for tongue cancers via filling the oral cavity with water and immersing the transducer, which allowed for delineation of the tumor and marking the margins with sutures. The investigators reported that the histologic margins corresponded to the sonographic margins, and the technique provided a more precise and individual definition of resection margins during surgery. Ng et al, tested this concept by reporting on ultrasound assisted tumor delineation during cases where skin involvement does not predict the underlying tumor volume. In 2 cases of tumors involving facial skin, the ultrasound was used to outline the deep tumor margin to more accurately guide the tumor resection and this was found to be superior to CT scan to delineate the periphery of the tumor in these cases. Kodama et al, reported a prospective study of 13 patients with T1-T2 tongue squamous cell carcinomas who underwent a partial glossectomy. Ultrasonography was used to assess tumor thickness prior to resection. In four cases the deep surgical margin was marked with elastic needles 10mm deep to the tumor front in order to guide resection. When compared to histologic sectioning, the ultrasonographic tumor thickness corresponded to the histologic thickness, with a consistency ratio of 91.4% to 98.2% (Pearson correlation coefficient = 0.981, P < .05) tumor. Tominaga et al, proposed a simple method by resecting the tumor, immersing the tumor in a gelatin solution and performing immediate ultrasound in three cases of tongue squamous cell carcinoma. The authors reported excellent correlation of the ultrasound depth with the histologic depth in these tumors, and noted that this method allowed for real time assessment of adequacy of deep margin resection. Baek et al, investigated intraoral sonography assisted resection in 20 stage-matched patients undergoing surgery for T1-T2 tongue squamous cell carcinoma. The authors found that intraoral sonography resulted in a more adequate deep margin when compared to conventional resection. (9.8 +/- 5.2 mm vs. 4.0 +/- 2.03 mm) (P < 0.001). It was also observed that the mucosal margins were not improved with intraoral sonography compared to visual inspection. This study illustrates the fact that although many surface based optical technologies may offer improved mucosal tumor margin identification, these technologies may require coupling with other technology such as ultrasound to ensure adequate deep margin resection. Ultrasound also offers the advantage of real
time information at the time of resection, as traditional imaging modalities such as CT or MRI often occur several weeks prior to surgical resection.

More recently, Clayburgh et al reviewed their experience using ultrasound intra-operatively in 10 patients undergoing TORS for oropharyngeal carcinoma. They reported that US provided anatomic information such as vessel location, but also provided accurate definition of deep tumor margins (4 patients, accurate to 1-2mm compared to gross section) [41]. This data is very preliminary however, and a rigorous testing of the ability of the US sound system to reliably define tumor margins and offer significantly improved outcomes compared to traditional frozen section margins was not performed in this investigation.

Conclusions

The application of optical technology in the quest for real-time margin evaluation during oncologic head and neck surgery continues to be evaluated by investigators around the world. Significant progress has been made in the last decade, which may allow the realization of this goal. Currently however, no system offers the appropriate profile of reasonable cost, ease of clinical use, and high levels of sensitivity and specificity, which would be required to obviate the need for frozen section histopathology. That being said, several technologies are primed for use in the operating room and the capability to image oncologic margins in real time is likely closer than realized by the majority of clinicians. Optical technologies such as optical coherence tomography, confocal microscopy, spectroscopy, special frequency domain imaging, and a variety of targeted fluorescence imaging technologies are extremely likely to play a role in the future of head and neck oncologic surgery. Currently available data from clinical trials evaluating these optical systems is lacking. The continued development of novel optical systems and targeted contrast agents in the laboratory will not be enough to transform the practice of oncologic head and neck surgery. The key lies in the translation of the known accuracy and specificity of optical imaging systems available in the laboratory to systems that are appropriately designed for clinical trials evaluating the presence of positive margins in real time during oncologic surgery. It is only with these translational efforts that the need for frozen section analysis will become obsolete.

Conflicting interests

The authors have declared that no conflict of interests exist.

Abbreviations

CFM: confocal microscopy; EGFR: epithelial-like growth factor receptor; HNSCC: head and neck squamous cell carcinoma; HRME: high-resolution microendoscopy; HSI: hyperspectral imaging; NBI: narrow-band imaging; NIR: near infrared; OCT: optical coherence tomography; SCC: squamous cell carcinoma; TORS: transoral robotic surgery

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