# Intraoperative In Vivo Imaging Modalities in HNC Status

Subjects: Otorhinolaryngology

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Surgical margin status is one of the strongest prognosticators in predicting patient outcomes in head and neck cancer, yet head and neck surgeons continue to face challenges in the accurate detection of these margins with the current standard of care. Advances in intraoperative imaging techniques have been developed to address these limitations in determining cancer margins in head and neck cancer (HNC). These modalities include optical coherence tomography, narrow band imaging, autofluorescence, and fluorescent-tagged probe techniques. Studies have demonstrated encouraging sensitivity and specificity in detecting HNC margins and cancer from normal tissue.

Keywords: fluorescence lifetime imaging ; head and neck cancer ; hyperspectral imaging

## 1. Introduction

A clear surgical margin is one of the strongest prognosticators in head and neck cancer (HNC) [1], and positive margins have been shown to drastically raise the rates of local recurrence and all-cause mortality in these cohorts [2][3]. Despite this understanding, surgical extirpation with clear margins can be particularly difficult to achieve in certain HNC subsites due to their close proximity to vital structures, irregular tumor invasion patterns, and occult or undetectable spread of disease. Indeed, the rate of positive margins in HNC is among the highest across cancers, ranging between 10% and 30% [4].

The current standard of care in HNC surgery often utilizes frozen section analysis for intraoperative margin assessment. However, histopathologic analysis of frozen sections for surgical margins is limited by time constraints, architecture distortion, and inability to assess certain tissues, with decreased accuracy pertaining to close and positive margins [5]. Furthermore, key histologic features, including lymphatic, vascular, and perineural invasion, and assessment of the true depth of invasion and invasive tumor fronts are limited with frozen sections, [6] and may not be known until permanent pathological sections after surgery. Additionally, in instances of positive frozen margins requiring intraoperative surgical re-excision, there exist significant challenges in tissue reorientation and identifying appropriate locations to re-excise, increasing the propensity for error and missed residual cancer [7]. Consequently, head and neck surgeons continue to be challenged in accurately identifying surgical margin features in the present day. This issue is of particular importance since the presence of positive margins can lead to substantial changes in treatment plans and survival outcomes [8][9].

Advances in intraoperative imaging techniques have been developed to address these limitations in determining cancer margins in HNC. These modalities include optical coherence tomography, narrow band imaging, autofluorescence, and fluorescent-tagged probe techniques. Initial studies have demonstrated encouraging sensitivity and specificity in detecting HNC margins and cancer from normal tissue. However, these studies remain preliminary and in smaller cohorts. Additionally, the different techniques have not been compared with one another.

## 2. Optical Coherence Tomography

Optical coherence tomography (OCT) measures the echo time delay and intensity of light, which is reflected and captured with low-coherence interferometry. OCT is a noninvasive and label-free diagnostic tool that can deliver high-fidelity imaging in real-time intraoperative settings, providing vital information on tumor margins. Since its inception in 1991, OCT was largely relegated to ophthalmological use [10]. However, technological advances over the past couple of decades have subsequently broadened the application of OCT across several disciplines, including in head and neck surgery [11]. This technology is comparable to that of ultrasound imaging, but OCT relies on captured data from reflected light instead of sound [12]. The first investigations utilizing OCT in head and neck tissues were conducted on the larynx, which was an optimal location due to its relatively thin epithelium [13][14][15]. In their prospective series with 33 subjects, Englhard et al. incorporated OCT technology together with surgical microscopy, allowing for a hands-free imaging process with improved surgical precision [16]. This investigation reported that microscope-integrated OCT imaging was able to correctly differentiate malignant (17/18) from benign laryngeal lesions (4/5), with poorer performance regarding premalignant

processes (1/5). Nonetheless, this also suffered from similar drawbacks including inferior image penetration and provided little insight regarding surgical margins. Indeed, the reported maximum penetration depth of 1.2 mm (average = 0.6 mm) poses potential limitations in the applicability of this imaging modality on bulkier, deeply invasive, or submucosal lesions and across other cancer subsites [15][16].

Nearly a decade later, this technique has been expanded to oral malignancies as well in intraoperative ex vivo settings [17][18]. Here, a malignant architecture detected by OCT was found to correspond to the histopathologic imaging counterparts with high sensitivity (81.5%) and specificity (87.0%) in detecting tumor margins [18]. In a recent investigation, Sunny et al. found an excellent concordance between OCT imaging and histopathologic analysis of oral cavity HNC, with a sensitivity and specificity both of 100% [19]. The pilot study with 14 patients was the first to utilize in vivo OCT intraoperatively to delineate tumor margins in the oral cavity, suggesting the potential for equivalency of this methodology to permanent histopathological analysis. In summary, nevertheless, larger prospective studies are necessary to validate these findings.

## 3. Narrow Band Imaging

NBI utilizes an optical filter that only permits a narrow range of wavelengths to be emitted as light, with varying levels of tissue penetrance dependent on the selected spectra [20]. This technology highlights hemoglobin-carrying regions and relies on the presumption that cancers have higher density and aberrant vascularity. Although several prior investigations have confirmed the utility of NBI in screening for oropharyngeal and hypopharyngeal cancers [21][22], these data were acquired in an outpatient setting and were not used to guide intraoperative management. One of the first investigations utilizing NBI intraoperatively to determine surgical margins was conducted by Garofolo et al. in 2015 [23]. Here, 67 subjects with laryngeal lesions were intraoperatively assessed with NBI, and final pathological analysis yielded a 3.6% rate for positive margins compared with 23.7% for a historical control group. Furthermore, Klimza et al. reported that NBI had an accuracy and a sensitivity of 85.7% and 100%, respectively [24]. Although these findings were superior to that of white light alone, more information on specificity was unavailable due to the inclusion criteria of biopsy-proven cancer, precluding the identification of false-positive results. Piersiala et al.'s investigation mirrored these results in their sample of 98 patients with laryngeal lesions in 2018 [25]. Here, the researchers performed cordectomies using a combination of white light and NBI endoscopy. One subset of 10 patients underwent NBI endoscopic imaging alone due to suspected peripheral margins, revealing several cases of moderate dysplasia (4), severe dysplasia (2), carcinoma in situ (3), and hyperkeratosis (1). The significance of these results was that these aberrant specimens were invisible on white light endoscopy, indicating a possible advantage of NBI in surgical margin detection. After final histopathological analysis, all tumor margins were determined to be clear. The investigators found an accuracy, sensitivity, and specificity of 99.0%, 100%, and 99.0%, respectively. Although these investigations were limited to neoplasms of the larynx, it is important to note that other limited studies across other HNC subsites have been performed [26]. However, extralaryngeal NBI imaging is often limited by the presence of lymphoid-tissue-dense regions, differing degrees of tumor thickness, and tissue keratinization [27]. While NBI shows promising results in surgical margin delineation, all studies were restricted by the need for an endoscopic camera for imaging. Larger controlled trials and prospective studies are required before formally incorporating NBI in the clinical setting.

## 4. Storz Professional Image Enhancement System

The Storz Professional Image Enhancement System (SPIES) utilizes a high-definition camera system with image-enhancing technology to improve the appearance of the mucosal surface and the vascular architecture across five spectral ranges [28][29]. This system also highlights contrast for vascular arrangements. Initial studies with SPIES found that it was comparable to NBI in the recognition and analysis of vascular patterns in typical benign and malignant lesions of laryngeal and hypolaryngeal pathologies [30]. Abdullah et al. investigated SPIES endoscopy with Ni et al.'s classification system for the detection of upper aerodigestive tract tumors and found a sensitivity and a specificity of 97.5% and 94.7%, respectively, regarding both benign and malignant lesions [31][32], enabling complete tumor resection by accurately delineating between healthy and tumorous tissue. This was found to be greater than the sensitivity and specificity of white light endoscopy, at 77.5% and 84.2%, respectively.

Li et al. conducted a pilot study involving SPIES technology in the assessment of sinonasal inverted papilloma (SIP) [33]. The study involved a total of 115 patients, including 80 patients with SIPs or nasal polyps, and 35 healthy controls. Of the 80 patients, 44 patients were found to have nasal polyps, and 36 were found to have SIPs on histopathologic examination. White light endoscopy successfully detected 41 of the 44 cases with nasal polyps and 24 of the 36 cases with SIPs. Using SPIES endoscopy, 43 of the 44 cases with nasal polyps and 33 of the 36 cases with SIPs were successfully identified. The researchers reported a sensitivity and a specificity of 91.7% and 95.5%, respectively. The results further demonstrate SPIES as a rapid and noninvasive, accurate, real-time modality that can be used to detect

SIPs. Englhard et al. conducted another study using SPIES to detect nasal and paranasal sinus diseases intraoperatively with the objective of evaluating its feasibility in clinical practice [34]. Twenty-seven patients with varied pathology and 10 healthy individuals were examined with both SPIES and white light endoscopy. Two questionnaires were provided: the first evaluated the surgeon's subjective experience with SPIES technology; the second evaluated whether specific advantages exist between SPIES and white light endoscopy. Results of the study show that SPIES subjectively facilitated the assessment of tumor extension, particularly in vascularized tumors, and it proved to be superior to white light endoscopy via the results of the questionnaires in the study.

## 5. Autofluorescence Imaging

Fluorescence lifetime imaging (FLIM) has been a burgeoning area of research since it was first discovered in decades prior [35]. Here, endogenous fluorophores are excited by a pulsed laser, and subsequent fluorescent lifetimes are measured and quantified. One of the first intraoperative applications of this technique in HNC was performed by Sun et al., where an endoscopic apparatus was utilized to measure in vivo autofluorescence [36]. Here, FLIM was able to identify different patterns of intensity and lifetime between cancerous tissue, margin, and normal tissue, demonstrating the potential of this modality to be utilized in the intraoperative setting for oral cancers. However, the preliminary study was limited by a small sample of 10 patients and slow image capture rates. FLIM was further investigated in 10 patients undergoing transoral robotic surgery (TORS) at this same institution, although information regarding margins was not available since all tumor beds were clear of residual disease [37]. More recently, the same researchers conducted a larger prospective study using either TORS or an endoscope FLIM scanning method with 53 subjects diagnosed with either oral or oropharyngeal HNC [38]. Similar to the aforementioned studies, cancer tissues were found to have significantly weaker spectral intensities and shorter lifetimes in comparison with their healthy counterparts. The sensitivity (86%) and specificity (87%) of free-handed FLIM in differentiating malignant from healthy tissues were excellent, and remained high when challenged with point-measurement classifier outputs. While all of these findings are founded on studies with limited sample sizes, the potential for FLIM in the intraoperative identification of tumor margins is encouraging.

Following a similar fluorophore-dependent mechanism as FLIM, dynamic optical contrast imaging (DOCI) was developed to bypass the complex mathematical models required in FLIM [39]. This method offers shorter imaging time frames while still producing scalable, proportionally accurate fluorescence lifetimes. In a preliminary ex vivo study with 81 patients with primary hyperparathyroidism undergoing parathyroidectomy, DOCI was able to clearly differentiate the parathyroid glands from surrounding tissues [40]. When DOCI was applied in a smaller in vivo intraoperative setting for HNC, researchers were able to clearly differentiate malignant from healthy tissues through measuring fluorescent lifetimes [41]. However, this did not quantify fluorescent intensity as was done in the aforementioned FLIM studies. The literature regarding this technique is still limited, and additional studies on the applicability of DOCI in HNC must be performed to accurately gauge its potential role in defining surgical margins.

## 6. Hyperspectral Imaging

Although first developed in the context of improving space exploration [42], HSI has been readily adapted to head and neck surgery. This methodology collects reflected light across a continuum of spectral bands, generating objective surface analysis data in a noninvasive, tracer-free process [43]. Halicek et al. were among the first to utilize this imaging modality to determine cancer margins in ex vivo HNC specimens [5][44][45]. In an ex vivo study of 102 patients with oral cavity HNC, these investigators compared the accuracy between HSI, autofluorescence, 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose (2-NBDG), and proflavin dye [5]. Here, HSI and autofluorescence demonstrated greater accuracy than when compared with their dye-based counterparts. However, while HSI was found to be more accurate in detecting cancer margins in conventional squamous cell HNC across nearly all contexts, autofluorescence proved to be superior in most aspects of HPV-positive HNC margin detection. In a smaller in vivo experiment of 24 subjects utilizing HSI imaging coupled with a 3D reconstructive algorithm, the accuracy, sensitivity, and specificity in determining tumorous tissues against healthy samples were found to be 81.3%, 83.3%, and 79.2%, respectively [46]. Similarly with other in vivo studies, limitations of this investigation included motion artifact and image noise from unamenable causes, such as patient pulse. Despite the clear promise of hyperspectral imaging in the intraoperative setting, more extensive prospective trials are needed to confirm these initial findings.

## 7. Near-Infrared Fluorescence

Near-infrared (NIR) fluorescent probes have been developed with the goals of improving cancer detection and characterization, lymphatic imaging, and intraoperative surgical guidance [47]. Near-infrared light (650–900 nm) has a

deeper tissue penetration than visible-range light, making it a favorable imaging agent to guide tumor resection [47]. Furthermore, there are available surgical instruments allowing for the intraoperative detection of NIR fluorescence. Since tissues absorb and emit light at different wavelengths, surgeons can use this imaging modality to help differentiate between normal and cancerous mucosa based on fluorescence patterns [48]. The two major forms of NIR fluorescence, fluorescence reflectance imaging (FRI) and tomographic fluorescence imaging, each have unique properties [47]. While FRI displays high spatial resolution, cost and time efficiency, portability, and color flexibility, the tomographic fluorescence imaging can create three-dimensional images that have greater depth sensitivity but lower spatial resolution [47].

Several studies have investigated the use of label-free NIR fluorescent dyes in the delineation between cancerous and healthy tissues. Stubbs et al. explored how free indocyanine green (ICG) dye could be infused 24 h prior to surgery and still provide benefit to surgeons in 3/14 surgeries for squamous cell carcinoma and adenoid cystic carcinoma [49]. However, Scott-Wittenborn et al. noted that untagged ICG NIR fluorescence failed to help surgeons differentiate between cancerous and normal mucosa for 6 patients with oropharyngeal squamous cell carcinoma [50]. These findings could be attributed to poor fluorescence tumor target and greater vasculature for the oropharyngeal region. Despite this, in a separate investigation comparing the off-line analysis of ICG NIR imaging with histopathologic results in various mucosal HNCs, the sensitivity and specificity for malignant tissue were found to be 90.5% and 90.9%, respectively [51]. In regard to oral cavity HNC, another clinical trial of 20 patients discovered 4 tumor beds with abnormal fluorescence, leading to the excision of 2 additional pathology-confirmed residual malignant specimens [52]. However, the researchers draw attention to an important caveat: inflammatory processes, regardless of etiology, may influence dye uptake, leading to increased chances of false-positive results.

## 8. Near-Infrared Fluorescence (Tagged Probes)

NIR fluorescently labeled probes targeting EGFR, which is overexpressed in the majority of HNC, have been investigated as an intraoperative imaging technique for HNC [48]. Van Keulen et al.'s study found that NIR fluorescently guided surgery using anti-EGFR antibodies conjugated to a NIR probe (panitumumab-IRDye800CW) helped improve surgeon decision making for 3/14 head and neck squamous cell carcinoma resections [48]. Additionally, 10/10 patients had deep margins that were negative for fluorescence, with all final pathologic specimens demonstrating clear tumor margins >3 mm. The same researchers conducted another study with the same tagged anti-EGFR antibodies with 20 patients with HNC and demonstrated that in situ tumors were associated with higher fluorescent intensities than healthy tissue, regardless of age, sex, tumor site, or size [53]. Furthermore, fluorescent intensities were not significantly altered by ambient lighting or variations in EGFR expression levels, giving encouragement to the further investigation of fluorescently labeled probes targeting EGFR as a valuable surgical imaging modality in HNC. However, it should be noted that Zhou et al. reported that cellular EGFR expression, tumor cell density, and plasma antibody concentrations can affect the distribution of tissue fluorescence [54]. Additionally, the researchers reported a sensitivity and a specificity of panitumumab-IRDye800 NIR imaging in detecting malignant tissues in the head and neck at 97% and 86%, respectively. Several fluorescent nanoprobes have also been developed to rapidly react to acidic environments encountered in malignancy-associated metabolic acidotic states, releasing conjugated ICG dye [55]. An investigation by Voskuil et al. identified tumor-positive margins with 100% sensitivity [56]. However, the specificity for HNC was unclear due to the pooling of findings across different cancer types. However, similarly to their tag-free counterparts, the use of these specific acid-activated probes may be complicated by inflammatory processes.

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