Published in final edited form as:
*Otolaryngol Head Neck Surg*. 2011 December ; 145(6): 956–960. doi:10.1177/0194599811416773.

# Assessment of Tissue Autofluorescence and Reflectance for Oral Cavity Cancer Screening

**Larissa Sweeny, MD**[1], **Nichole R. Dean, DO**[1], **J. Scott Magnuson, MD**[1], **William R. Carroll, MD**[1], **Lisa Clemons**[1], and **Eben L. Rosenthal, MD**[1,*]

[1]Department of Surgery, Division of Otolaryngology – Head and Neck Surgery, University of Alabama at Birmingham, Birmingham, Alabama.

## Abstract

**OBJECTIVE—**Although approved by the U.S. Food and Drug Administration for clinical use, the utility of hand-held tissue reflectance and autofluorescence devices for screening head and neck cancer patients is poorly defined. There is limited published evidence regarding the efficacy of these devices. We investigated the sensitivity and specificity of these modalities compared to standard exam.

**STUDY DESIGN—**Prospective, cross sectional analysis.

**SETTING—**Tertiary care medical center.

**SUBJECTS AND METHODS—**Patients who were treated previously for head and neck cancer (n=88) between 2009-2010 were included. Patients were screened using white light visualization (standard of care) and compared to tissue reflectance and autofluorescence visualization. Screening results were compared to biopsy or long term follow-up.

**RESULTS—**Autofluorescence visualization had inferior specificity (81%) and equivalent sensitivity (50%), for detecting oral cavity cancer, when compared to white light visualization (98% specificity, 50% sensitivity). Tissue reflectance visualization had poor sensitivity (0%) and good specificity (86%). The positive and negative predictive values for standard white light exam (50% and 98% respectively) were superior to either tissue reflectance or autofluorescence.

**CONCLUSION—**Standard clinical lighting has a higher sensitivity than tissue reflectance and autofluorescence visualization for detection of disease in patients with a history of head and neck cancer. This study does not support the added costs associated with these devices.

### Keywords

Aerodigestive squamous cell carcinoma; head and neck; survival; recurrent disease; autofluorescence imaging; screening

## Introduction

Oral cavity cancer accounts for almost 130,000 deaths annually world-wide and is the 6$^{th}$ most common cancer worldwide.[1,2] In the U.S., the overall five year survival for all stages is 59% and decreases to 52% for those with regional disease and 27% for those with distant

[*]**Corresponding Author:** UAB - Division of Otolaryngology Volker Hall G082 1670 University Blvd Birmingham, AL 35233 Tel: (205) 934-9766 Fax: (205) 934-3993 oto@uab.edu.

No potential conflicts of interest were disclosed.

**Conflicts of Interest:** None

disease.[3] Screening strategies to detect cancers at an early stage or in the premalignant phase may improve statistics. Furthermore, approximately 5% of those patients that do survive their initial disease are at risk of developing a second primary and approximately 19% are at risk of developing a local recurrence.[4] Therefore, a routine follow-up schedule is recommended to promote early detection of new lesions. However, oral cavity exam in previously treated patients is often complicated by diffuse mucosal changes from field cancerization, prior surgery, and radiation therapy. Non-invasive screening techniques balanced to optimize detection rates with cost effectiveness are needed for detection of both primary and secondary oral cavity cancer.

Currently the standard of care for the oral cavity cancer screening is visual inspection under white light (traditional exam light) and palpation by a physician or dentist. The sensitivity of this technique is highly dependent on the experience of the examiner and therefore more objective methods were developed. In recent years it was discovered that oral cavity tissues contain fluorophores, such as nicotinamide adenine dinucleotide dehydrogenase and the cross-links between collagen or elastin. These fluorophores absorb UV photons and emit lower energy, longer wavelength photons. These longer wavelength photons can be visualized as fluorescence.[5,6] Neoplastic lesions have decreased number of collagen and elastin cross-links therefore fewer of the longer wavelength photons are emitted, leading to a decrease in the amount of fluorescence visualized.[6-8] In addition, it was found that 540-575 nm wavelengths are able to detect changes in vasculature by enhancing the reflective properties of the mucosa. Tissue reflectance appears darker in areas of increased vasculature. Hand held devices have been designed which include filters that allow clinicians to visualize tissue fluorescence and reflectance in the clinic. In recent years, these hand held tissue autofluorescence[9,10] and reflectance[10] screening devices have been marketed to primary care and dental offices. These devices have been approved by U.S. Food and Drug Administration for oral cavity screening. Although these instruments have the potential to improve detection of mucosal dysplasia and cancer in situ, their efficacy as screening tools remains unproven.

As these devices become more common in the clinic, their reliability to differentiate a benign oral cavity lesion from a premalignant or malignant lesion must be assessed. Additionally, the added cost of these screening modalities must be evaluated relative to their potential benefit. To this end, we evaluated patients undergoing routine head and neck cancer surveillance using the Identafi® 3000 device manufactured by Trimira® (Houston, Tx). This device combines the technologies of tissue autofluorescence and reflectance making it unique from other hand held oral cavity screening devices.

## Materials and Methods

### Patient Selection

Following Institutional Review Board approval, a prospective study was performed at the University of Alabama at Birmingham. Patients who presented to the Otolaryngology clinic between November 2009 and October 2010 for follow-up (n=88) following management of primary head and neck cancer, were included. Data obtained included patient age, gender, race, social history, original cancer stage (if applicable), other comorbidities, and prior treatments. Tumors were staged according to the American Joint Committee on Cancer (AJCC)[11] guidelines and histology was confirmed by pathology.

### Imaging Procedure

Oral cavity and oropharynx sites were initially screened by a registered nurse and then by a fellowship trained head and neck surgeon using visualization with white light illumination

(traditional exam light) followed by visualization of tissue autofluorescence and tissue reflectance. The Trimira® Identafi® 3000 ultra, multi-spectral oral cavity screening system was used. Any abnormality was assessed by the fellowship trained head and neck surgeon and if there was concern for malignancy or recurrence the abnormal lesion was biopsied. Screening results were compared to histological biopsy results or a three month follow up screening.

### Histopathologic Correlation

Any area of abnormality found by visualization with traditional white light illumination and/or by tissue autofluorescence or reflectance was biopsied and evaluated by a pathologist using standard histopathologic analysis. The location of the biopsy was noted in the patient's chart.

### Statistical Analysis

The statistical analysis included calculation of sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) for outcomes of the different screening visualization modalities (white light, tissue autofluorescence, and tissue reflectance). The Wilson score interval or method was used to calculate 95% confidence intervals for the sensitivity and specificity values.

## Results

### Screening in Previously Treated Patients

We evaluated patients during routine surveillance visits. All patients had undergone a previous treatment for head and neck cancer. The patients were primarily elderly (mean age 64, range 41-85), Caucasian (61%, n=54) and male (74%, n=65). All patients were in the first five years of follow-up. The most common location of the primary was the oral cavity (47%, n=41), followed by the oropharynx (25%, n=22) and larynx (24%, n=21). The remaining sites included the maxillary sinus (n=2), nasopharynx (n=1), and temple (n=1). The majority of patients had undergone treatment for stage IV (57%, n=50) or a stage III (19%, n=17) head and neck cancer. The majority had a history of tobacco use (81%, n=71) or alcohol consumption (66%, n=58). The majority of patients had undergone surgical excision of their primary lesion (85%, n=75), and 73% (n=64) had undergone previous radiation therapy, while only one-third had received chemotherapy (38%, n=33). A summary of patient characteristics can be found in.

Patients were evaluated by direct visualization of the oral cavity with white light (traditional exam light), tissue autofluorescence and tissue reflectance. The patients experienced no harmful or adverse side effects from the screening. There were a total of 17 lesions identified by tissue autofluorescence and/or reflectance, of which 9 underwent biopsy. There were no lesions detected by tissue autofluorescence or reflectance which were not detected by white light. There were nine lesions identified on autofluorescence which were determined to be benign by the clinician and were followed for six months without change. Screening with white light illumination had the best results overall with a specificity of 98% (95% confidence intervals [CI], 92-99%), a sensitivity of 50% (95% CI, 15-85%), a PPV of 50% and a NPV of 98% (Table 1). Screening with tissue autofluorescence visualization had a specificity of 81% (95% CI, 71-88%), a sensitivity of 50% (95% CI, 15-85%), a PPV of 11% and a NPV of 97%. Screening with tissue reflectance illumination had poor sensitivity (0%; 95% CI, 0-49%) and PPV (0%) and good specificity (86%; 95% CI, 77-92%), and NPV (95%). A summary of screening results is found in Table 1.

## Discussion

Despite the widespread proliferation of approved devices using tissue autofluorescence or reflectance for screening of oral cavity cancer, there is little data to support their use. We present the first prospective study addressing the application of this technology for oral cavity cancer screening in previously treated patients. Examination with white light and pathological confirmation remain the gold standard for early detection of oral cavity malignancies. However, clinical exam remains subjective. Several noninvasive fluorescence screening modalities have been developed to improve malignancy detection rates. These technologies range from devices requiring contact with tissue, such as fiber-optic spectrometers[12] and depth-sensitive optical spectroscopy[13] to full field visualization aids such as Velscope and Identafi[14,15]. Subtle changes in early cancer cellularity and blood flow are the basis for development of light-based screening strategies to improve the sensitivity and specificity of routine exam. Although previous case reports have identified the utility of this technology in evaluation of known lesions,[6,8,16,17] our data suggest that addition of tissue autofluorescence or reflectance illumination to routine clinical exam of high risk patients has very limited benefit and does not justify the additional time or expense associated with their use.

Even though screening with white light illumination provides a cost-effective means of screening for oral cancer, this technique remains subjective and has limited sensitivity. In low risk patient populations, screening for oral cavity cancer with white light visualization was found by systematic review to have a weighted pooled sensitivity of 85% and specificity of 97%[18], while others found a sensitivity of 64% and positive predictive value of 74%.[19] Variables affecting the sensitivity and specificity of traditional white light visual examinations include index of suspicion and experience of the examiner.[6] The difficulty in detecting oral cavity neoplastic lesions is confounded by field cancerization in which up to 58% of biopsies of normal appearing mucosa are found to have histologic abnormalities (cellular atypia, dysplasia, squamous cell carcinoma).

In an effort to improve detection rates of new primaries and recurrence several autofluorescence devices have been introduced to aid detection. Some studies have found oral cavity visualization under autofluorescence to be advantageous for assessment of known neoplastic disease[16,17,20] when compared to white, incandescent light alone. Marketing materials state that “Dentists, oral Surgeons, primary care physicians, and otolaryngologist now have the technology to detect oral cancer earlier and save lives” (www.trimira.net). However, there is limited evidence in the peer reviewed literature that tissue autofluorescence or reflectance can be used to detect cancer during screening visits and no data it saves lives. The lack of consensus on the potential benefit of this technology is related to study design flaws and insufficient data found within the literature.[18,19,21,22]

One group performed 50 biopsies from areas with changes in tissue autofluorescence and showed a sensitivity of 98% and specificity of 100% for identification of dysplasia, carcinoma in situ or invasive cancer[16]. However, this broad classification of success does not significantly improve clinical decision making or suggest that it can be used for screening, or make comparisons to white light exam. More objective measures have been developed to assess tissue autofluorescence and have demonstrated sensitivity of 90% and specificity of 87%, however this are requires a quantitative computer based algorithm[23]. Furthermore, this study used clinical consensus rather than pathology or clinical follow-up as the gold standard to determine sensitivity and specificity. Other studies have evaluated autofluorescence visualization as a diagnostic aid for oral cavity lesions not suspicious for neoplasia and requiring only serial follow-up[15]. In this setting, autofluorescence was found to be of no benefit. In addition, no lesions were detected with autofluorescence that were not

already apparent on white light examination. Similarly, we found use of tissue autofluorescence in previously treated head and neck cancer patients was equivalent to standard white light exam. In the current study we demonstrate a sensitivity of 50% and specificity of 98% for white light visualization compared to tissue autofluorescence, which had a lower specificity (81%) and equivalent sensitivity for screening these high risk individuals. There were only two false negative screenings by white light examination; one lesion was in a location out of the range of visualization by the naked eye (the base of tongue and detected by fibroptic scope exam) and the second lesion was initially screened a few weeks post-operatively and was found to be local recurrence in the surgical site a few weeks later. The low sensitivity of screening in this patient population may be attributed to post-operative and post-radiation changes in the oral mucosa resulting in pigmentation and fibrosis. When examining oral cavity mucosa using white light or tissue autofluorescence or reflectance, dysplastic lesions were difficult to differentiate from benign changes in the mucosa, such as inflammation, ulceration or radiation changes. Inflammatory lesions were detected (Figure 2), but differentiation from malignancy require biopsy.

Our study was unique in that it evaluated the population most likely to benefit from screening. However, patients previously treated for head and neck cancer, particularly those with a history of radiation, may suffer diffuse changes that make tissue autofluorescence a poor tool for early diagnosis of a new primary or recurrence. Areas of inflammation, ulceration or radiation damage cause changes to the underlying stroma and basement membrane which result in loss of tissue autofluorescence, making differentiation between these benign changes and dysplastic changes difficult. Changes in stromal fluorescence observed in dysplastic lesions is similar to loss of stromal fluorescence associated with inflammatory changes.[7] It is possible that post-radiation changes or xerostomia are sufficient to cause low level inflammation which limits the value of autofluorescence screening. It would appear in this setting autofluorescence does not enhance visualization of neoplastic lesions and would increase the false positive rate and as a result lead to unnecessary biopsies when compared to white light examination. Ultimately, use of autofluorescence (much like the standard of care) relies on subjective interpretation of visual inspection and therefore it is a qualitative in nature and will vary depending the examiner and level of experience.

Our study also evaluated tissue reflectance, which has not been previously evaluated for head and neck cancer screening, but is also marketed in this capacity. Tissue reflectance in the setting of these multi-spectral devices is based on the premise of detecting changes in angiogenesis with green-amber light (540-575 nm wavelength) illumination. The amber light is thought to enhance the reflective properties of the oral mucosa allowing a distinction between normal and abnormal tissue vasculature.[10] Surprisingly, there are no published results evaluating this technology. Our study found its ability to detect recurrence or a second primary to be poor with a sensitivity of 0%, specificity of 86% and PPV of 0% and a NPV of 95%. We therefore found tissue reflectance visualization to be inferior to traditional whit light examinations.

This study focused on a narrow patient population being evaluated by physicians with extensive experience examining the oral cavity for malignancies. Therefore the utility of these devices for screening the primary care setting remains unknown and still needs to be investigated. While all lesions found by tissue autofluorescence and/reflectance were not biopsied they were evaluated for at least 6 months and were still considered to demonstrate no evidence of disease by current standards of care. Therefore, we feel the added discomfort and cost to the patient were not justified. When examining the oral cavity with tissue autofluorescence or reflectance visualization there is a clear distinction between positive and negative areas. Therefore, we do not feel that the examiners experience or training biased the interpretation of the screening results. However, for the white light examination there is

a clear advantage in favor of the examinees in the environment evaluated in this study. Fellowship trained head and neck surgeons have extensive training and experience allowing them to easily differentiate malignancy or dysplasia from mucosal abnormalities caused by irradiation changes or inflammation.

## Conclusion

In the setting of trained physicians and health care staff, our data suggests that the addition of tissue autofluorescence or reflectance illumination to routine clinical exam of high risk patients has limited benefit in detecting lesions. The cost of device itself is quoted at $3,390.00 and various dental offices advertise the cost to the patient for a one time screening to range from $25-$70 dollars. To our knowledge, these screening modalities are not currently covered by insurance companies. The added cost associated with the tissue reflectance and autofluorescence screening devices does not appear to be justified in this patient population.

## Acknowledgments

**Financial Disclosure:** This work was supported by grants from the National Institute of Health (2T32 CA091078-09).

## References

1. Furness S, Glenny AM, Worthington HV, et al. Interventions for the treatment of oral cavity and oropharyngeal cancer: chemotherapy. Cochrane Database Syst Rev. 2010:CD006386. [PubMed: 20824847]
2. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009; 45:309–16. [PubMed: 18804401]
3. Society, AC. Available at: http://www.cancer.org/acs/groups/content/@nho/documents/document/2008cafffinalsecuredpdf.pdf
4. Gonzalez-Garcia R, Naval-Gias L, Roman-Romero L, Sastre-Perez J, Rodriguez-Campo FJ. Local recurrences and second primary tumors from squamous cell carcinoma of the oral cavity: a retrospective analytic study of 500 patients. Head Neck. 2009; 31:1168–80. [PubMed: 19408289]
5. Richards-Kortum R, Sevick-Muraca E. Quantitative optical spectroscopy for tissue diagnosis. Annu Rev Phys Chem. 1996; 47:555–606. [PubMed: 8930102]
6. Roblyer D, Kurachi C, Stepanek V, et al. Objective detection and delineation of oral neoplasia using autofluorescence imaging. Cancer Prev Res (Phila). 2009; 2:423–31. [PubMed: 19401530]
7. Pavlova I, Williams M, El-Naggar A, Richards-Kortum R, Gillenwater A. Understanding the biological basis of autofluorescence imaging for oral cancer detection: high-resolution fluorescence microscopy in viable tissue. Clin Cancer Res. 2008; 14:2396–404. [PubMed: 18413830]
8. Shin D, Vigneswaran N, Gillenwater A, Richards-Kortum R. Advances in fluorescence imaging techniques to detect oral cancer and its precursors. Future Oncol. 2010; 6:1143–54. [PubMed: 20624126]
9. VELscope. Available at: http://www.velscope.com/
10. [Accessed Accessed January 14, 2011] Trimira. Available at: http://www.trimira.net/identafi
11. Edge, SB.; American Joint Committee on Cancer. AJCC cancer staging manual. 7th ed. Springer; New York ; London: 2010. p. xivp. 648
12. Mallia RJ, Subhash N, Mathews A, et al. Clinical grading of oral mucosa by curve-fitting of corrected autofluorescence using diffuse reflectance spectra. Head Neck. 2010; 32:763–79. [PubMed: 19827122]
13. Schwarz RA, Gao W, Redden Weber C, et al. Noninvasive evaluation of oral lesions using depth-sensitive optical spectroscopy. Cancer. 2009; 115:1669–79. [PubMed: 19170229]
14. Fedele S. Diagnostic aids in the screening of oral cancer. Head Neck Oncol. 2009; 1:5. [PubMed: 19284694]

15. Mehrotra R, Singh M, Thomas S, et al. A cross-sectional study evaluating chemiluminescence and autofluorescence in the detection of clinically innocuous precancerous and cancerous oral lesions. J Am Dent Assoc. 2010; 141:151–6. [PubMed: 20123872]
16. Lane PM, Gilhuly T, Whitehead P, et al. Simple device for the direct visualization of oral-cavity tissue fluorescence. J Biomed Opt. 2006; 11:024006. [PubMed: 16674196]
17. Poh CF, Ng SP, Williams PM, et al. Direct fluorescence visualization of clinically occult high-risk oral premalignant disease using a simple hand-held device. Head Neck. 2007; 29:71–6. [PubMed: 16983693]
18. Downer MC, Moles DR, Palmer S, Speight PM. A systematic review of test performance in screening for oral cancer and precancer. Oral Oncol. 2004; 40:264–73. [PubMed: 14747057]
19. Brocklehurst P, Kujan O, Glenny AM, et al. Screening programmes for the early detection and prevention of oral cancer. Cochrane Database Syst Rev. 2010:CD004150. [PubMed: 21069680]
20. De Veld DC, Witjes MJ, Sterenborg HJ, Roodenburg JL. The status of in vivo autofluorescence spectroscopy and imaging for oral oncology. Oral Oncol. 2005; 41:117–31. [PubMed: 15695112]
21. Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol. 2008; 44:10–22. [PubMed: 17825602]
22. Rethman MP, Carpenter W, Cohen EE, et al. Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. J Am Dent Assoc. 2010; 141:509–20. [PubMed: 20436098]
23. Rahman MS, Ingole N, Roblyer D, et al. Evaluation of a low-cost, portable imaging system for early detection of oral cancer. Head Neck Oncol. 2010; 2:10. [PubMed: 20409347]

**Table I**

Detection of Head and Neck Cancer Recurrence in Previous ly Treated Patients by Examination with White Light, Tissue Autofiuorescence. and Tissue Refiectance Alone

| **Visualization** | **White Light, %** | **Autofiuorescence. n** | **Refiectance, n** |
|---|---|---|---|
| True positives | 2 | 2 | 0 |
| False negatives | 2 | 2 | 4 |
| True negatives | 82 | 68 | 72 |
| False positives | 2 | 16 | 12 |
| | White Light, % | Autofluorescence, % | Refiectance, % |
| Sensitivity | 50 | 50 | 0 |
| Specificity | 98 | 81 | 86 |
| Positive predictive value | 50 | 11 | 0 |
| Negat ive predictive value | 98 | 97 | 95 |

## Table 2

Detection of Head and Neck Cancer Recurrence in Previously Irradiated Patients by Examination with White Light, Tissue Autofluorescence, and Tissue Reflectance Alone

| Visualization | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) |
|---|---|---|---|---|
| White light | 50 (13-99) | 98 (92-100) | 50 (13-99) | 98 (92-100) |
| Autofluorescence | 50 (13-99) | 79 (67-89) | 71 (18-34) | 98 (90-100) |
| Reflectance | 0 (0-98) | 98 (92-100) | 0 (0-98) | 98 (92-100) |

Abbreviations: CI, confidence interval; ppv, positive pred ict ive value; NPV, negative predictive value.

## Table 3

Detection of Head and Neck Cancer Recurrence in Previously Nonirradiated Patients by Examination with White Light, Tissue Autofiuorescence, and Tissue Refiectance Alone

| Visualization | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) |
|---|---|---|---|---|
| White light | 0 (0-98) | 100 (80-100) | 0 (0-98) | 94 (72-100) |
| Autofiuorescence | 0 (0-85) | 93 (66-100) | 0 (0-98) | 87 (60-98) |
| Refiectance | 0(0-98) | 93 (68-100) | 0 (0-98) | 93 (68-100) |

Abbreviations: CI. confidence interval; PPY, positive predictive value; NPY, negative predictive value.