Oral Fluorescence Imaging Using 405-nm Excitation, Aiding the Discrimination of Cancers and Precancers by Identifying Changes in Collagen and Elastic Breakdown and Neovascularization in the Underlying Stroma

Pierre Lane, PhD[1]; Sylvia Lam, PhD[1]; Michele Follen, MD, PhD[2]; and Calum MacAulay, PhD[1]

[1]Department of Integrative Oncology, Section of Cancer Imaging, The British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada; and [2]Department of Obstetrics and Gynecology, Drexel University of College of Medicine; Philadelphia, Pennsylvania

ABSTRACT

Background: Optical spectroscopy and imaging devices are being developed and tested for the screening and diagnosis of cancer and precancer in multiple organ sites.

Objective: The aim of the study reported here is to optimize the capability of an optical imaging device to discriminate precancerous tissue from other lesions by identifying ideal excitation wavelengths.

Methods: The studies reported here used a prototype of a direct fluorescence imaging device that uses 405-nm illumination to excite tissue.

Results: There is ample evidence in the literature that 405 nm can distinguish oral cancers from normal tissue. Higher wavelengths may be necessary to differentiate potential confounding lesions, such as abrasions, burns, viral infections, inflammation, and gingivitis.

Conclusions: Imaging at 405 nm could help doctors detect precancerous and cancerous oral lesions. Such imaging could be used by dentists, family practitioners, otorhinolaryngologists, general surgeons, obstetrician gynecologists, and internists, and could greatly increase the number of patients who have lesions detected in the precancerous phase. (*Gend Med.* 2012;9:S78–S82) © 2012 Elsevier HS Journals, Inc. All rights reserved.

Key words: cancer screening, fluorescence imaging, oral cancer diagnosis, oral intraepithelial neoplasia, sensitivity and specificity.

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INTRODUCTION

Almost all cancers are easier to treat successfully if caught early in their growth. This principal is particularly true for 85% of cancers that are epithelial in origin. Most of these cancers do not originate in fully malignant form. They typically arise from normal tissues that progress through one or more preinvasive pathologically recognizable steps, such as dysplasia or carcinoma in situ (CIS), for which there are limited detection methods.[1] Oral cancer is an especially tragic example of an epithelial cancer.[2] Although the mouth is easily accessible, oral cancers are often diagnosed at a late stage, beyond treatability, that brings with it high morbidity and mortality. Fluorescence spectroscopy and imaging may provide means of early detection and have been studied in many organ sites.[3]

Robyler et al[4] reported on the use of 67 autofluoresence images that were taken of 56 patients who were divided into a training and validation set of 46 measurements each and applied to a test set of 21 images. An automated algorithm using 405-nm illumination discriminated normal from neoplastic tissue with a sensitivity of 96% and specificity of 96% in the training and in the validation sets and with a sensitivity of 100% and specificity of 91% in the test set. A previous report based on quantitative analyses suggested that imaging at this wavelength would be useful for diagnosis.[5] These results were confirmed by other investigators and found applicable to other organ sites.[6–11] This wavelength was shown to demonstrate intracellular activities of interest by several investigators.[12–14]

This article presents the reader with a series of images showing the varied presentation of squamous cell carcinoma and dysplasia when observing these lesions with 405-nm excited direct fluorescence imaging, such as a clinician or dentist might see in an oral dysplasia referral clinic. We hope these images will be a useful visual aid as 405-nm excitation illumination fluorescence imaging devices are introduced into the market.

MATERIALS AND METHODS

Overview of Study Procedures

Men and women who were ≥18 years of age were eligible to participate in the study, which began in 2008 and is ongoing. Patients are recruited from the oral cancer clinics of the British Columbia Cancer Research Centre and Agency. A research nurse or research assistant describes the study to eligible patients and written informed consent is obtained from those agreeing to participate, with emphasis that nonparticipation will not affect their care. A biomedical engineer assists with trial conduct, including assisting the provider with taking the photographic images included in the trial.

All patients seen in the clinic receive: (1) a complete history and an oral cancer risk factors questionnaire, (2) a comprehensive head and neck examination, (3) a comprehensive oral white light examination, and (4) referrals for outstanding medical problems. Those patients in the trial undergo an additional examination using the device, which has violet light-excited fluorescence at 405 nm. Fluorescence images are taken of all lesions observed under white light or fluorescence. Suspicious areas are biopsied if the clinical impression suggest dysplasia or cancers. Each patient's history of lesions, including precancers and cancers are noted, and the clinical impression is entered into the database. Lesions that are biopsied are submitted for permanent section. All tissue biopsies are reviewed by histopathologists who are blinded to the imaging data and who specialize in oral cavity pathologic diagnosis.

The study protocol was reviewed and approved by the Institutional Review Board at The British Columbia Cancer Agency. The studies were carried out at The British Columbia Cancer Agency, Vancouver, British Columbia, Canada.

Fluorescence Imaging

A handheld device emitting light at variable wavelengths was used. An image and detailed description of the imaging system can be found in Lane et al.[15] A light source with significant energy around 405 nm was positioned behind a high-performance, narrow band-pass interference filter designed to transmit predominately 405 nm light. This was used to excite the interior of the mouth, allowing the entire field of view of the camera to be illuminated. Each measurement takes approxi-

mately an additional ≤3 minutes; however, documentation with photography adds an additional ~2 minutes. A clinician using a similar device views the interrogated tissue through either filtered glasses or a variable magnification scope, both designed to highlight tissue fluorescence. Photographs presented here were taken using a filter to present the reader with an image akin to what a device operator sees. The total light exposure is less than the American National Standards Institute standards described in Roblyer et al.[4,5] All images were reviewed 4 times by 4 independent investigators blinded to the histology. These investigators included dentists, optical engineers, and clinicians familiar with fluorescence imaging.

RESULTS

One hundred nineteen patients participated in this study of 405 nm imaging. From each of these patients, several images were captured using fluorescence at 405 nm. Ninety percent of these patients had a concurrent biopsy at the time of their clinical visit; the remaining 10% were biopsied within 6 months of the time the image was taken. These images represent all surfaces areas in the oral cavity. Among the data set are 23 lesions of carcinoma (19 squamous and 2 verrucous); 2 CISs, 9 severe dyplasias, 18 moderate or mild dyplasias, 15 leukoplakias, 1 erythroplakia, 13 lichen planus, 5 hyperplasias, 2 mucositis or gingivitis, 10 traumatic lesions, 19 scars, 5 areas of pigmentation, including grafts and biopsy sites, and several other confounders (see **Supplemental Appendix** in the online version at doi: 10.1016/j.genm.2011.11.006). For this article, we limited demonstrative images to invasive cancers, CIS, dyplasias, and erythroplakias and leukoplakias. The confounding lesions and scars will be published in a separate article outlining strategies for their separation from cancerous lesions.

We chose 24 pairs of images (see **Supplemental Figures 1–6** in the online version at doi: 10.1016/j.genm.2011.11.006). On the left side, images are presented as seen by a clinician at the time of examination. On the right side, lesions are delineated to highlight regions of interest. Images of cancers, severe dysplasias, mild to moderate dysplasias, and 1 erythroplakia are presented. In most of these images, the delineated area is seen as a darker blue than the surrounding region. In some images, neovasculature can be noted. In those images where the delineated area is near the teeth, porphyrins can be noted, which fluoresce as pink, bright red, or pale green.

There are several observations that can be made at 405 nm. The first observation is that areas of neoplasm that have outgrown their blood supply are often full of bacteria. Bacteria make porphyrins as a by-product, and porphyrins are seen as bright pink- and blue-colored areas using violet light fluorescence imaging. The dark areas demonstrate collagen and elastin breakdown in the stroma, and these areas can be very large, surrounding the white light visible cancers. Often, one can identify a plexus of vessels in the stroma that accompanies the breakdown of collagen and elastin. This neovascularization occurs as lesions grow; the process becomes increasingly deregulated in carcinogenesis.

DISCUSSION

Laser-induced fluorescence imaging has been used to identify precancerous and cancerous tissues for 20 years. When the field began, there were a limited number of lasers available: argon ion, helium–neon, and helium–cadmium. Besides being large and bulky instruments, they had limited lifetimes. Over the last 20 years, significant advances have made laser use more practical for clinical applications. Diode lasers at 405 nm have been used extensively in many forms of fluorescent microscopy. Their use has allowed cell biologists to image intracellular structures in the 20 to 50 nm range. They have also been used to turn on and off green fluorescent protein markers at the cellular level, facilitating a whole new field in science.

This article shows a large number of images of 405 nm taken in a high-risk population in which there were a large percentage of dysplasic and neoplastic lesions. This gives the viewer some experience with images akin to the experience of the

imaging scientists and clinicians who performed the trials. It takes time to become accustomed to seeing the fluorescence images at this wavelength, and we provide a gallery of images for the viewer, to serve as a type of atlas.

Most of the images show the lesion and an area of darkening surrounding it. Some of the images shown are quite dark in printed format. That is not so when viewed on the computer. This darkened image makes discrimination of the lesion harder when printed than when viewed on the computer.

Early results suggested that the addition of the fluorescence examination at 405 nm was helpful in identifying characteristics indicative of precancer and cancer over that seen solely by comprehensive white light examination. Images of the violet-induced autofluorescence at 405 nm showed the deeper neovascularization of the stromal changes that accompanied the progression to neoplasia. Biologic studies of tissue slices demonstrated that there was loss of autofluorescence consistent with breakdown of collagen matrixes in the connective tissues as lesions progressed from normal to neoplastic.[3] Elastin breakdown is less well studied, but also strongly suspected in the carcinogenic pathway involving the epithelial–stromal interface. Type 1 collagen is a major component of the extracellular matrix that exhibits both optical scattering and endogenous fluorescence. These optical properties render the study of the tumor microenvironment amenable to optical spectroscopy and imaging techniques.

Further study of fluorescence imaging has the potential to improve clinical outcomes by giving providers more tools for the early detection of cancer. Similar technologies are being developed to employ fluorescence imaging in diverse organ sites, including the cervix, throat, and lungs.

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CONFLICTS OF INTEREST

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SUPPLEMENTAL MATERIAL

Supplemental appendix and figures accompanying this article can be found in the online version at doi: 10.1016/j.genm.2011.11.006.

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Address correspondence to: Michele Follen MD, PhD, Drexel University College of Medicine 245 N. 15th St., Philadelphia, PA 19102. E-mail: Michele.Follen@Drexelmed.edu.

Supplemental Appendix 1. Study patients, date of measurement, location of lesions, and clinical impression

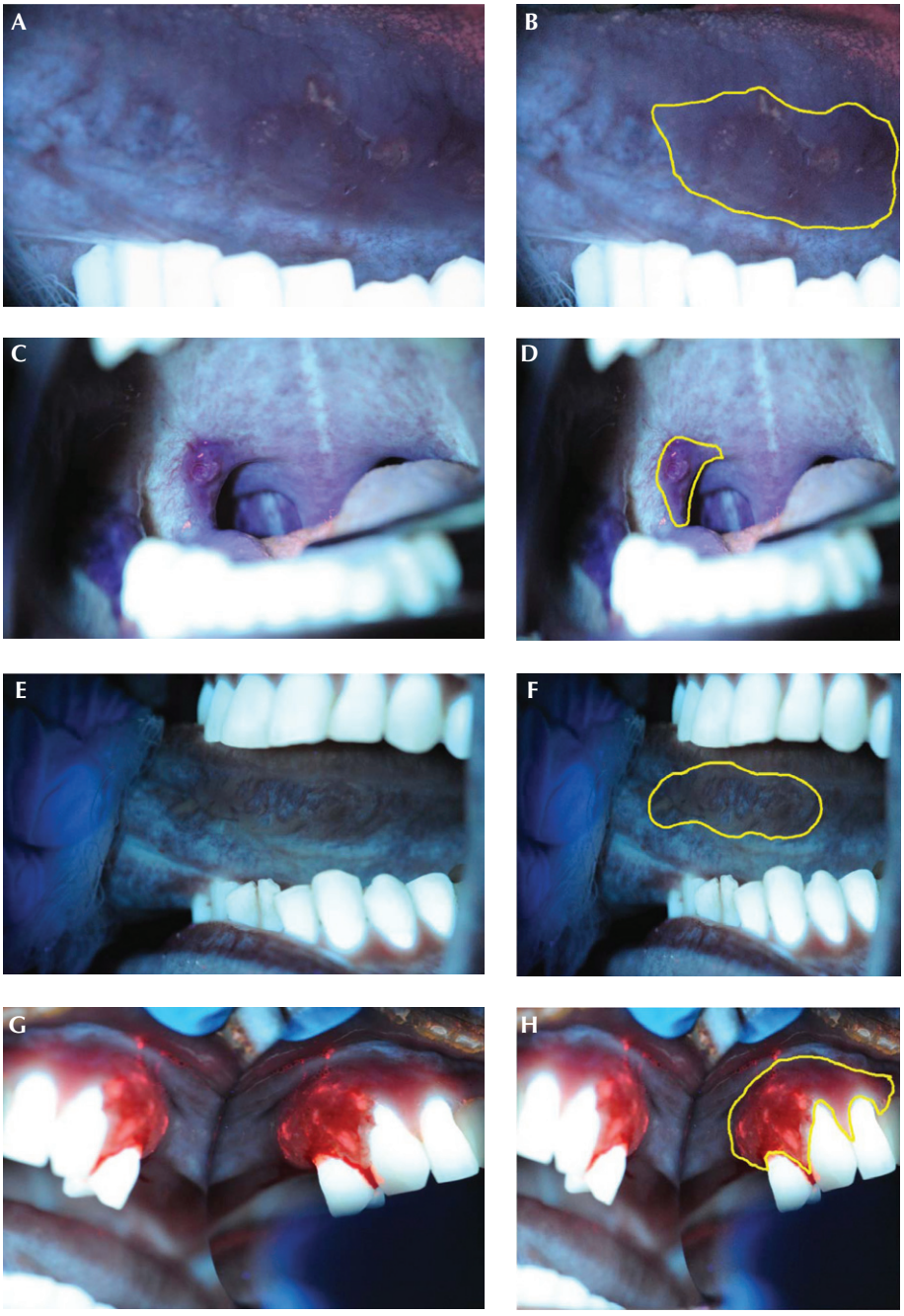
Study ID	Date	Location	Clinical Impression
1488	20090605	left lateral tongue	> 6mos scar for s/t of CIS
1971	20091002	right lateral tongue	1 yr old scar
1878	20090710	left ventral tongue	2 yr post s/t scar
1829	20090904	right lateral tongue	2.5 yr old scar, post s/t SCC
1846	20091016	right buccal mucosa	4 days wound, cheek biting
1414	20090918	lower left gingiva	6 mos post s/t graft
2016	20091106	right lateral tongue	6 mos post s/t scar for cancer
2068	20091120	left tongue	background lichen planus
2057	20091113	right lateral tongue	biopsy
resident1	20090702	left buccal mucosa	biteline
1362	20090626	right gingiva	C1FV1 TB2
7006-0014	20100219	floor of mouth	cancer, pre-surgery
7011-0011	20100326	left tongue	cancer, pre-surgery
confounder	20090605	left inside upper lip	canker sore
1738	20090710	right buccal mucosa	cheek biting
1362	20090327	right lower dentulus ridge	CIS, concurrent bx
1720	20090313	left floor of mouth	leukoplakia (D1-2)
1985	20090327	right posterior soft palate	Squamous cell carcinoma
3040	20090416	right buccal mucosa	confounder, contact lichenoid mucositis
1985	20090327	right lateral tongue	mild dysplasia
1585	20090612	left lateral tongue	moderate dysplasia
1884	20090828	right lateral tongue	moderate dysplasia
2024	20091002	right lateral tongue	moderate dysplasia
3037	20091113	right lateral tongue	moderate dysplasia
1365	20100122	right lateral tongue	moderate dysplasia
1466	20100122	right lateral tongue	moderate to severe dysplasia
2010	20090416	left lateral tongue	severe dysplasia
3178-0010	20100219	floor of mouth	C
7002-0007	20100129	right tongue	severe dysplasia
1449-0003	20100302	left tongue	severe dysplasia
1402	20091009	left lateral tongue	dysplasia, hx of CIS
2043	20090904	right lateral tongue	erythroplakia
3090	20090904	right lateral tongue	fluorescence lost
1811	20090612	left lateral tongue	geographic tongue
2019	20090925	tip of tongue	geographic tongue
1178	20091023	left posterior tongue	leukoplakia
1362	20090626	left ventral tongue	leukoplakia
1662	20091120	left soft palate	leukoplakia
1732	20100205	left ventral tongue	leukoplakia
1827	20091113	right lateral tongue	leukoplakia
1869	20100212	left tongue	leukoplakia
1971	20091002	left lateral tongue	leukoplakia
2016	20090724	left post maxillary retromolar	leukoplakia
1888	20090828	right lateral tongue	moderate dysplasia
1585	20090612	right buccal mucosa	lichen planus
1914	20091204	right buccal mucosa	lichen planus
1998	20091009	left posterior buccal mucosa	lichen planus

(continued)

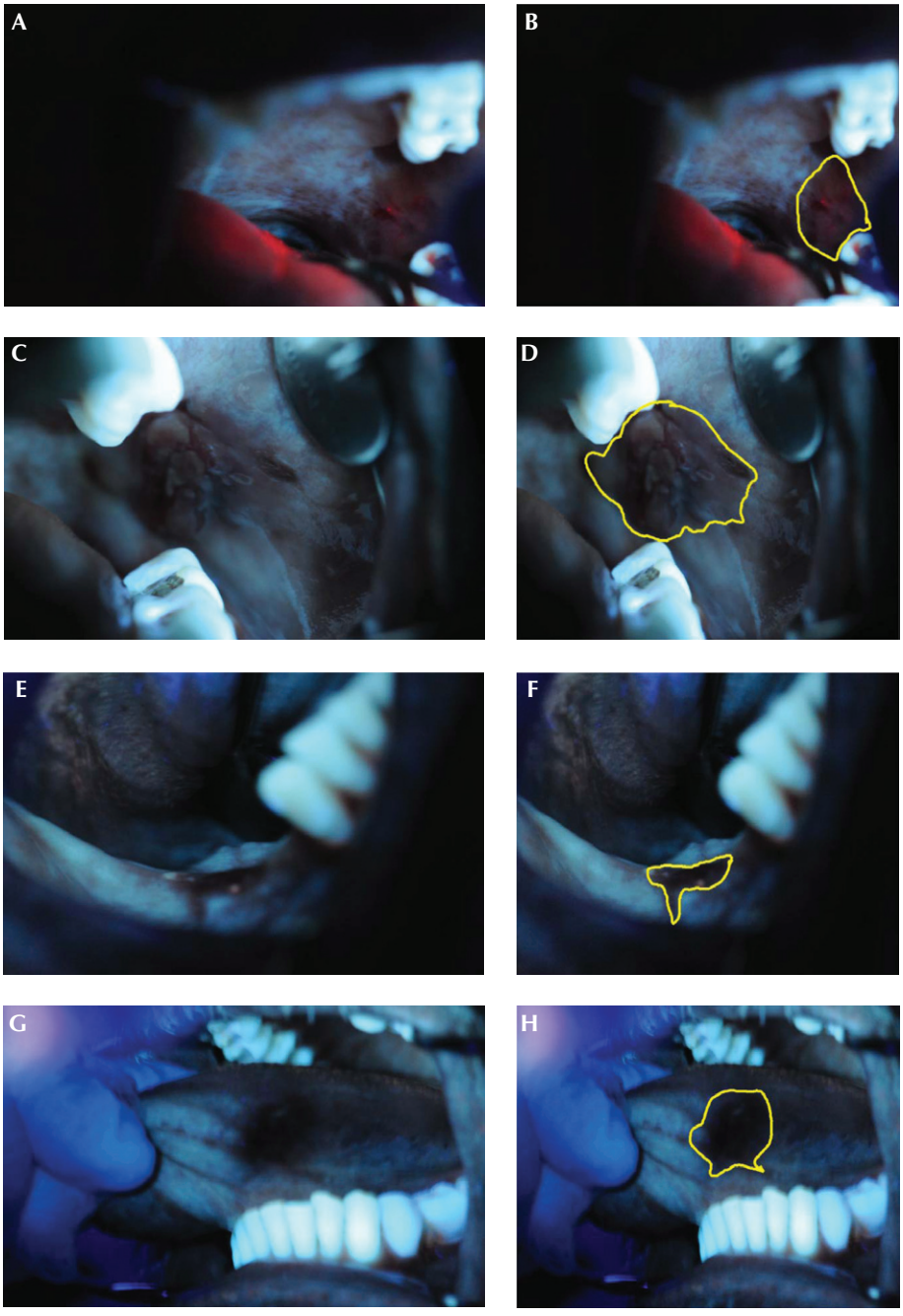
Supplemental Appendix 1 (continued).

Study ID	Date	Location	Clinical Impression
2041	20090703	right lateral tongue	lichen planus
9219	20090702	dorsal tongue	lichen planus
9219	20090702	right buccal mucosa	lichen planus
9219	20091008	left buccal mucosa	lichen planus
resident2	20090702	left gingival buccal	lichen planus
1983	20090626	left buccal mucosa	lichen planus, possible dysplasia
2020	20090417	right gingiva	lichen planus, plaque type
1860	20090605	right buccal mucosa	lichenoid dysplasia
1978	20090612	left lateral tongue	mild dysplasia
2039	20090703	right lateral tongue	mild dysplasia
1595	20090619	left lateral tongue	mod dysplasia
1827	20090807	right lateral tongue	mod dysplasia
2038	20090703	left lateral tongue	mod dysplasia
2024	20090605	right lateral tongue	mod dysplasia, leukoplakia
1208	20090327	left gingiva	gingivitis
1365	20090710	right lateral tongue	pigmentation
1365	20090710	left latetal tongue	pigmentation
2048	20090828	right buccal mucosa	pigmentation, lichen planus
1843	20090828	right ventral tongue	post s/t scar
2049	20091120	right lateral tongue	pre-surgery
2053	20091021	right tongue	pre-surgery
2054	20091021	tongue	pre-surgery
2055	20091021	right tongue	pre-surgery
2059	20091021	left tongue	pre-surgery
2010	20091120	left lateral tongue	severe dyplasia
1452	20090807	right buccal mucosa	red, white
1452	20090807	right tongue	red, white
1888	20091127	right lateral tongue	red, white leukoplakia
1983	20090626	right FOM	residual lesion
1178	20090612	left ventral tongue	scar
1432	20090605	left buccal mucosa	scar
1735	20090807	right tongue	scar
1798	20090612	right lateral tongue	scar
1811	20090612	right lateral tongue	scar
2033	20090619	left lateral tongue	scar
2048	20090828	right lateral tongue	scar
2019	20090925	right lateral tongue	scar 3 mos rad tx severe dysplasia
1869	20091009	left lateral tongue	scar, 6 mos post s/t

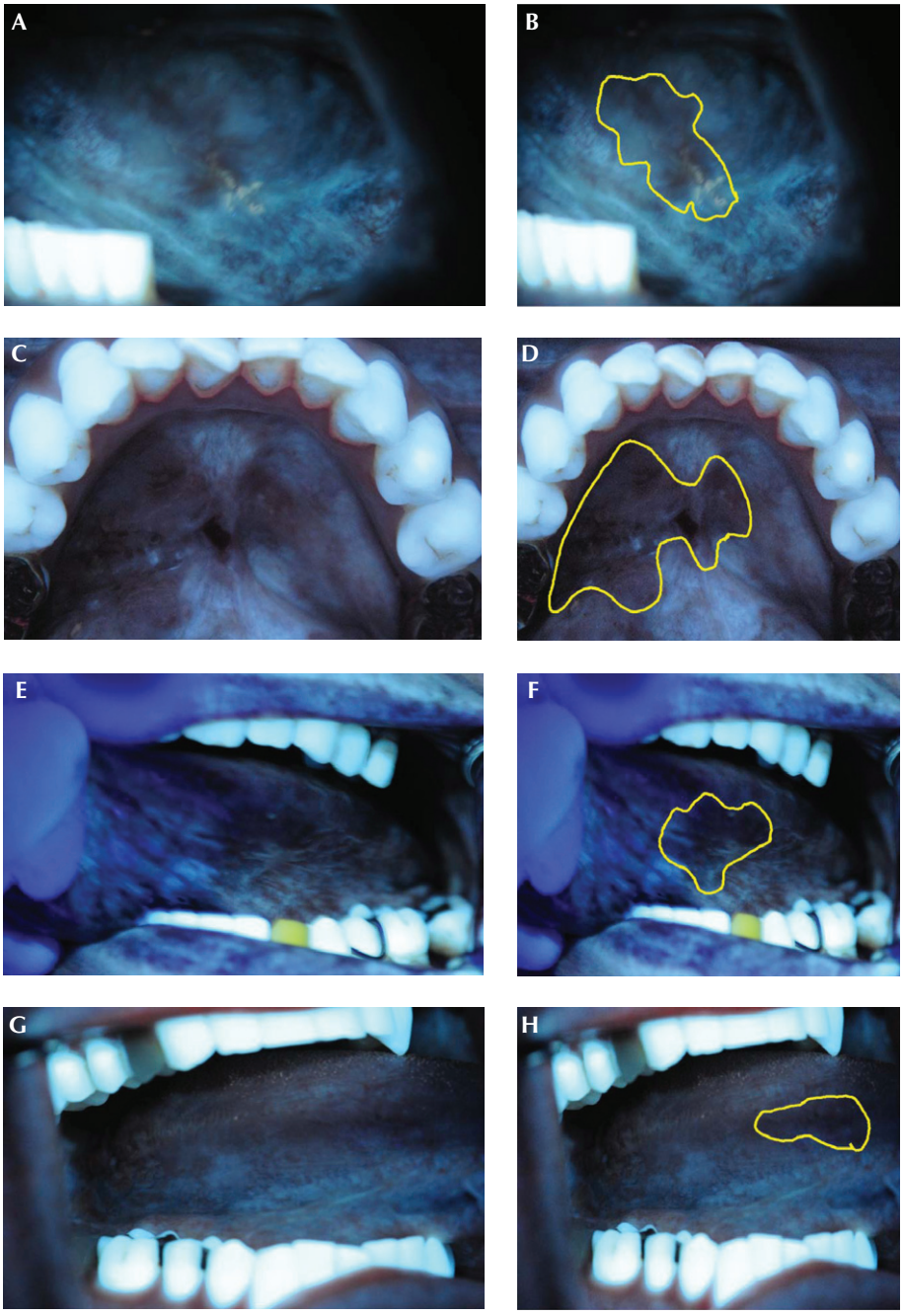
CIS carcinoma in situ; hx = history; SCC = squamous cell carcinoma; s/t = surgical treatment; rad tx = radiotherapy.



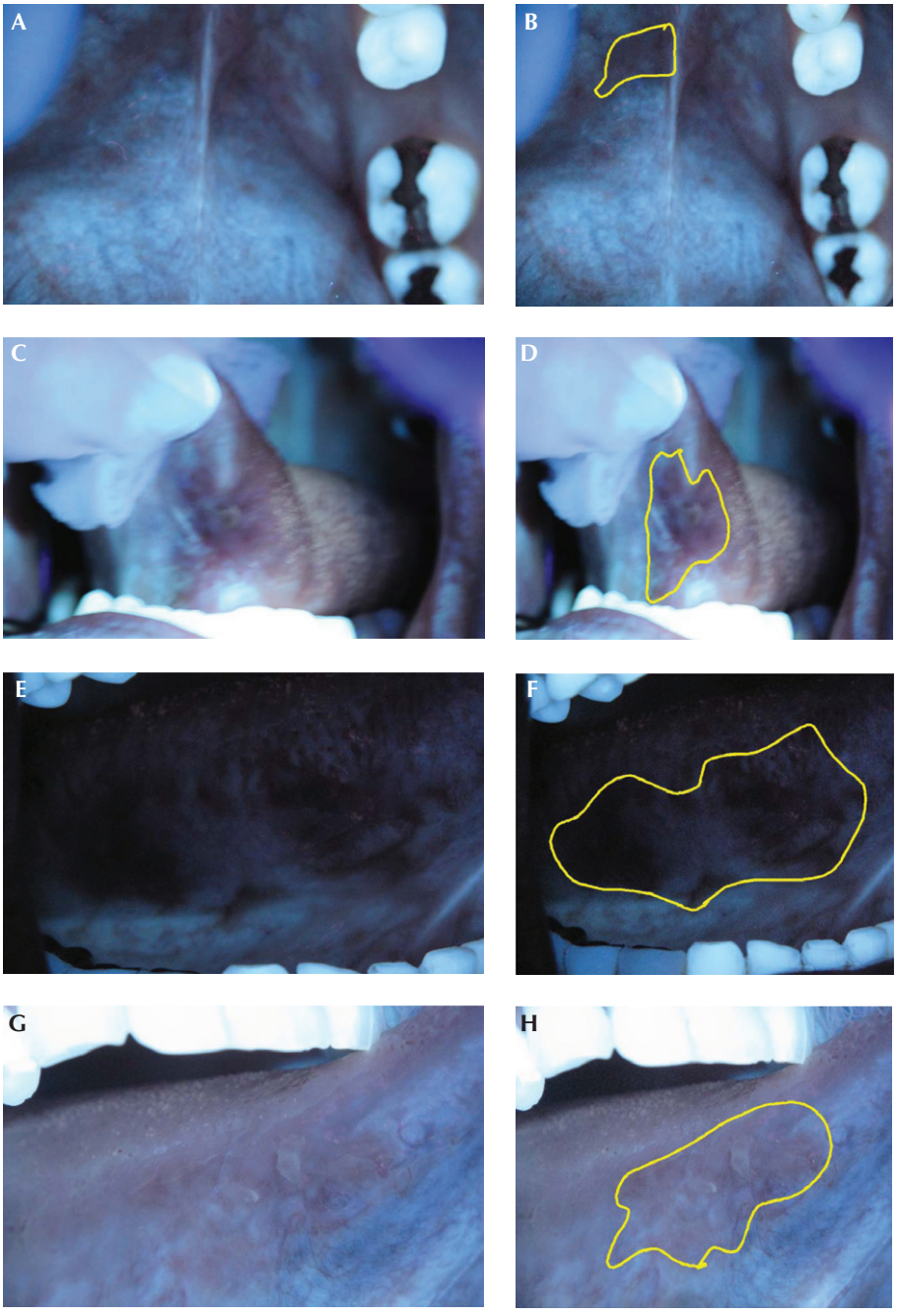
Supplemental Figure 1. Oral cavity illuminated at 405nm excitation. Areas of interest are delineated in panels on the right.



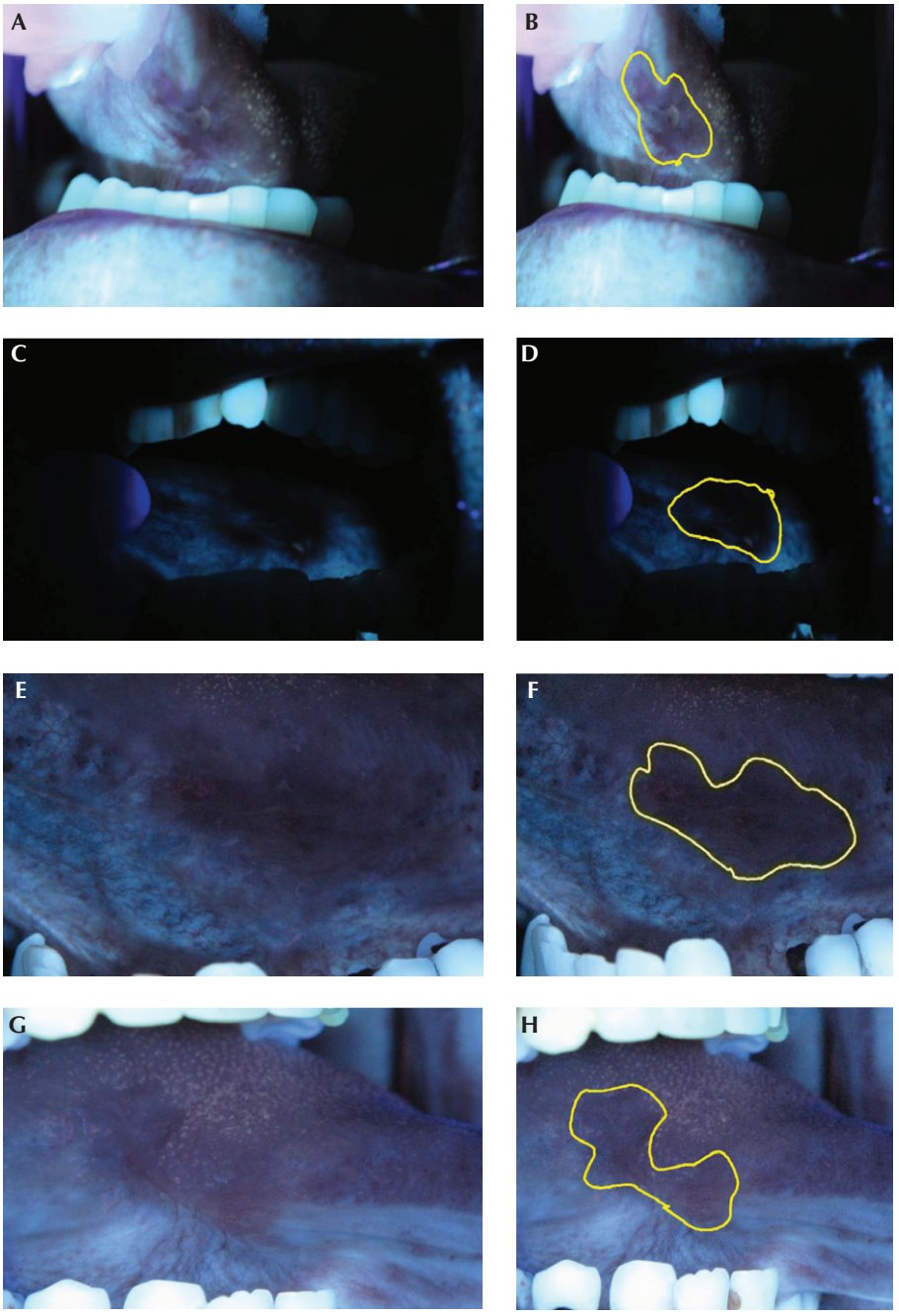
Supplemental Figure 2. Oral cavity illuminated at 405nm excitation. Areas of interest are delineated in panels on the right.



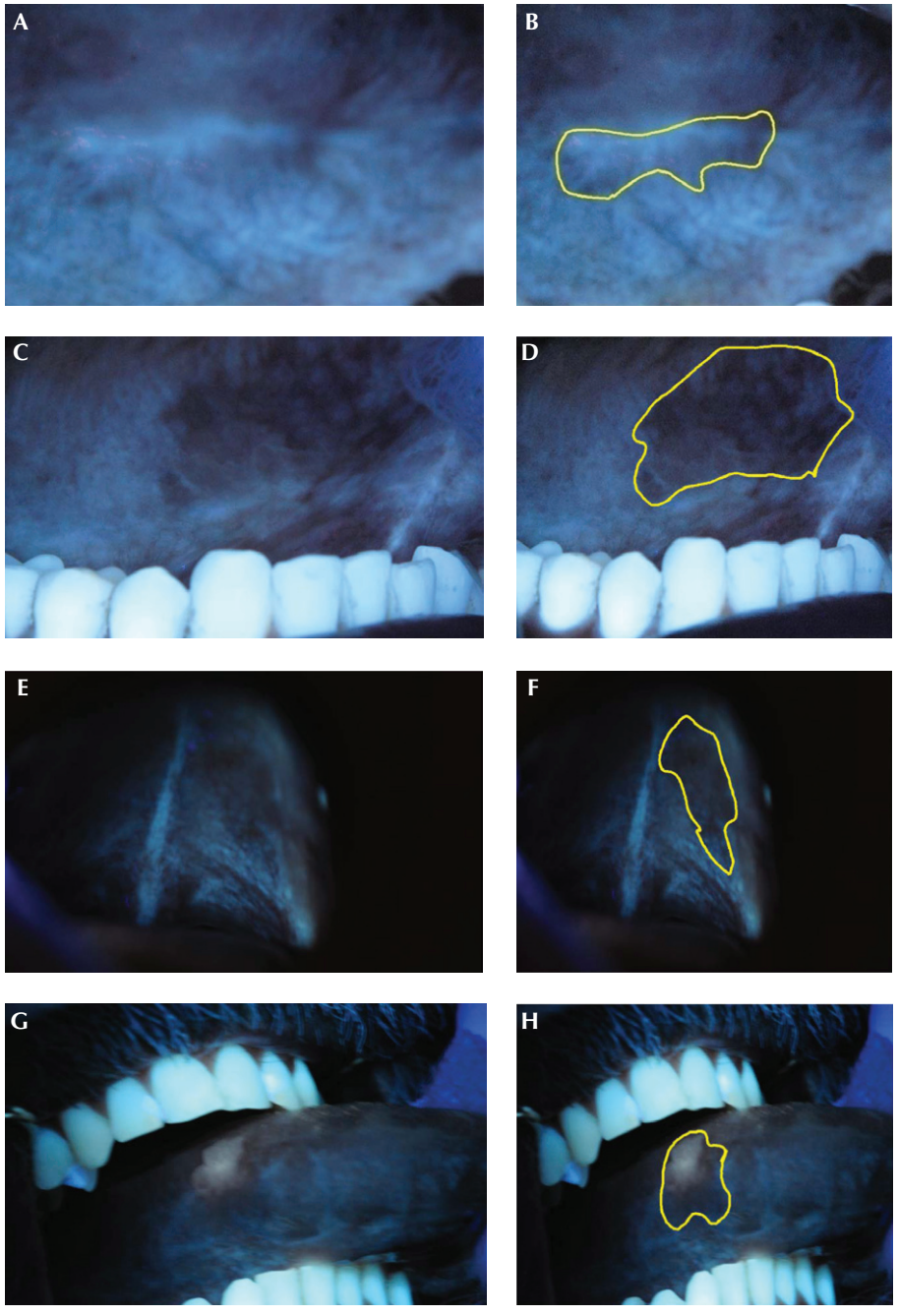
Supplemental Figure 3. Oral cavity illuminated at 405nm excitation. Areas of interest are delineated in panels on the right.



Supplemental Figure 4. Oral cavity illuminated at 405nm excitation. Areas of interest are delineated in panels on the right.



Supplemental Figure 5. Oral cavity illuminated at 405nm excitation. Areas of interest are delineated in panels on the right.



Supplemental Figure 6. Oral cavity illuminated at 405nm excitation. Areas of interest are delineated in panels on the right.