clinical feature

Optical Diagnostics for Early Detection of Oral Cancer

By **Darren Roblyer, PhD,** and **Rebecca Richards-Kortum, PhD**

Introduction

Oral cancer is deadly if diagnosed at a late stage. Surgery to remove cancers of the oral cavity can be highly disfiguring, and patients often experience recurrence. Precancerous lesions are difficult to identify by visual examination, and medical imaging technologies such as computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET), are prohibitively costly for most dental and clinical settings and may lack sensitivity for screening.

These challenges have stimulated research to develop optical technologies to improve early detection of cancer in the oral cavity. Optical technologies use light to reveal anatomic and biochemical information about the tissue. Unlike X-rays, they do not use potential harmful ionizing radiation, and they are relatively inexpensive compared to other medical imaging modalities. Recent pilot studies have shown that simple, commercially available optical devices have the potential to improve the detection of early precancerous lesions compared to visual inspection.[1,2] These technologies are designed to be used as part of a standard oral examination, and dental hygienists can play an important role in their adoption. This article describes technologies that are currently available or show promise to be available in clinical practice in the next few years.

Background

Oral cancer causes over 7,000 deaths a year in the United States and over 127,000 deaths worldwide.[3,4] Patients with oral cancer face a high mortality rate, which escalates when diagnosis is made at a late stage. In the U.S., the five-year survival rate for localized disease is 81 percent, but it falls to 17 percent for those with distant metastases.[5] If not identified at an early stage at a routine dental or medical visit, lesions are often diagnosed at a later stage only after a patient complains of pain or irritation. Early detection is complicated by the fact that it is difficult to discriminate benign lesions, which account for the vast majority of oral lesions, from malignant lesions when relying on visual inspection alone. Common visible lesions such as leukoplakia and erythroplakia, which appear as white or red areas in the oral cavity respectively, typically do not transform into malignancies but must be carefully monitored.[6] These ambiguities in visual appearance have traditionally required that suspicious lesions be confirmed by tissue biopsy and pathology analysis, processes that are both invasive and costly.

New optical technologies show promise to improve the early detection of premalignant and malignant lesions and to more accurately discriminate premalignant and benign lesions in the oral cavity. By measuring the biochemical composition of tissue or imaging below the tissue surface, optical technologies can provide diagnostic information not available with standard visual inspection. To achieve this, optical technologies interrogate tissue using light from the visible and nonvisible portions of the electromagnetic spectrum, including the near-infrared and ultraviolet regions. Illumination photons enter tissue where they may be scattered or absorbed by tissue chromophores (molecules that interact with light); photons exiting the tissue are collected by optical instruments and then analyzed in a variety of ways. Spectroscopic methods analyze the spectrum (or color content) of the collected photons from a small area of tissue. Imaging systems display the amount of light emitted from each point on the surface of the tissue, allowing comparisons between adjacent tissues. Optical coherence tomography (OCT) and confocal microscopy use sophisticated optical sectioning techniques to view the layered structure of tissue in three dimensions, revealing details of cellular and subcellular anatomy throughout the entire epithelium (top tissue layer). Table I shows the field of view, advantages, disadvantages and stage of development of each of these techniques, all of which are discussed in detail below.

Spectroscopy

Spectroscopic techniques illuminate tissue with one or more different wavelength bands (or colors) of light and then analyze the color spectrum of the remitted light. Most

Table I. Properties of the optical technologies discussed in this article.

Technology	Field of View	Advantages	Disadvantages	Stage of Development
Spectroscopy	1-2 millimeters	High data content	No image, small sample size	Clinical studies
Fluorescence imaging	1-10 centimeters	Can be used to scan entire oral cavity quickly	Requires dark room	Non-imaging systems are commercially available
Optical coherence tomography	1-10 millimeters	Tissue layers can be viewed	Small sample size	Clinical studies
Confocal microscopy	10-1000 microns	Individual cells can be viewed	Small sample size	Clinical studies

experimental devices use a probe held in the operator's hand and placed against suspicious areas in the oral cavity. Light is both transmitted and collected through a fiber optic light guide attached to an equipment box containing optical components. The tissue in the oral cavity can scatter, absorb or emit fluorescence photons differently depending on the anatomical location (e.g. the tongue compared to the gingiva) or whether the tissue is healthy, precancerous or cancerous. These differences in collected signals can be analyzed using a computer algorithm to discriminate healthy and benign tissue from malignant tissue. Spectroscopy has the advantage of being able to quickly measure how tissue interacts with many wavelengths of light.

Spectroscopic signals can be analyzed using mathematical models to calculate functional parameters and concentrations of biochemical components, such as oxy- and deoxyhemoglobin, water and fat content. Fluorescence spectroscopy has been shown to reveal important information about the integrity of the extracellular matrix, a layered component of tissue made of collagen and elastin that provides shape and support. All of the above-mentioned parameters are known to alter as disease progresses from normal to cancerous and can therefore be used as markers to detect precancers.

A recent study from our group, which included 124 subjects (60 patients with precancerous or cancerous lesions and 64 normal volunteers), demonstrated that spectroscopy could successfully identify 100 percent of the abnormal lesions and 73 percent of the normal or benign regions, relative to the gold standard of histopathology. This result compared favorably to an expert head and neck surgeon in the study who was able to correctly identify 94 percent of the abnormal lesions and 75 percent of the normal lesions using visual inspection.[7]

Spectroscopy methods have the disadvantage of measuring only small areas of tissue (millimeters) at any one time due to the nature of the probe design. This limitation makes screening the entire oral cavity impractical; however, spectroscopy can be used to measure suspicious areas previously identified by dental hygienists or patients.

Fluorescence Imaging and Visualization

Fluorescence imaging techniques illuminate tissue with blue or UV light and display the tissue fluorescence on a monitor or through a viewing port on the instrument. These techniques are convenient since the screener can quickly examine the entire oral cavity for signs of abnormal growth, also called neoplasia. Tissue fluorescence generally appears green and results from fluorophores associated with collagen and elastin in the stroma (deeper, supportive tissue matrix) or mitochondria in the epithelial cells. Neoplastic lesions appear significantly darker than normal tissue; this loss of fluorescence is believed, in part, to result from interactions between the neoplastic cells and the underlying matrix, which results in loss of collagen and elastin fluorescence. Figure 1 shows images of the buccal mucosa of a patient with severe dysplasia and cancer. Figure 1a shows the tissue in white light (equivalent to ambient room light). Although some darker areas are apparent in the white light image, it is difficult to determine whether they are due to a benign condition, such as inflammation, or to cancer. Figure 1b shows the same tissue in fluorescence mode. The neoplastic areas appear very dark compared to the normal surrounding areas.

Computer algorithms are being implemented to help identify abnormal regions in fluorescence images. Through the process of machine learning, a computer program determines how to discriminate images of normal tissue and cancer by analyzing examples with known normal and neoplastic regions. A program can then estimate the probability that a newly acquired image contains precancerous lesions or cancer. An example of this method is shown in Figure 1c. A superimposed color scale is used to indicate areas the computer program has calculated as having a high likelihood of underlying precancer or cancer. The algorithm used in this example was trained on images from 39 patients with precancerous or cancerous lesions.[8]

At least two fluorescence visualization devices are commercially available and Food and Drug Administration approved. The Velscope® uses a specialized lamp to illuminate tissue; the operator looks though an eyepiece to view tissue fluorescence. The Identafi® 3000 ultra consists of a battery-operated handpiece that illuminates the oral cavity using a specialized light-emitting diode (LED). The operator wears specially designed glasses in order to view fluorescence. Both systems are pictured in Figure 2.

Optical Coherence Tomography

OCT is a relatively new technique that provides detailed images of the structured layers of tissue beneath the surface of the oral cavity. The technique is analogous to ultrasound imaging with light and provides superior spatial resolution. A near-infrared light source is used to illuminate the tissue, and a cross-sectional image is made by analyzing the time delay of light reflected from various depths within the tissue. Images can be displayed in real-time and can show tissue at depths 1 to 2 millimeters below the surface. This technique is well suited to oral cancer detection since most neoplastic lesions in the oral cavity begin in the epithelial lining. As tissue progresses from normal to premalignant to malignant, the fraction of the epithelial layer containing neoplastic cells increases, and eventually the normal layered structure disappears completely. These changes, previously visible only by

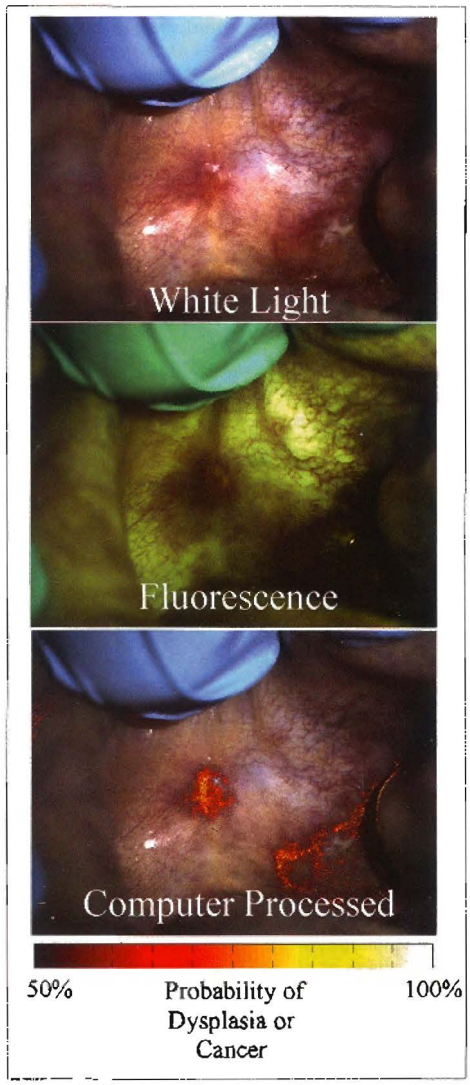

Figure 1. The buccal mucosa of a patient with severe dysplasia and cancer. The white light image represents what is normally seen by visual inspection. The fluorescence image is darker in areas of dysplasia and cancer. The computer processed image shows the probability of areas having dysplasia and cancer over the white light image.

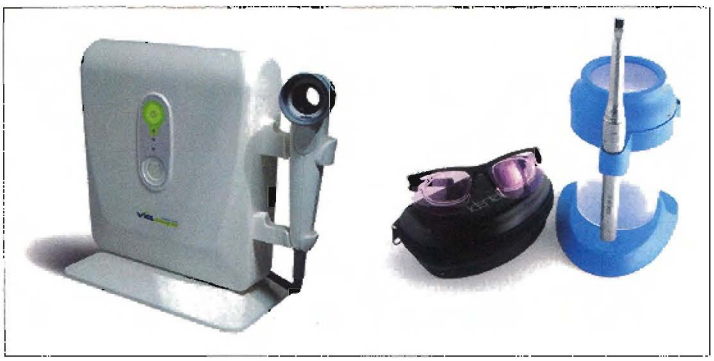

Figure 2. The Velscope® system is shown on the left and the Identafi® 3000 ultra is shown on the right. Both systems are currently commercially available. Images courtesy of LED Dental Inc. and Remicalm Inc., respectively.

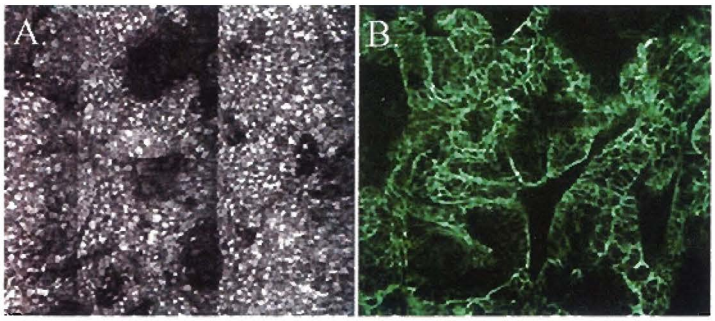

Figure 3. Confocal images of cancerous oral cavity tissue. (A.) A reflectance confocal image which shows a high density of individual nuclei, indicative of cancer. (B.) A fluorescence confocal image of the same field. A fluorescent dye was used that binds specifically to EGFR, a cellular receptor overexpressed in cancerous tissue. The brightness and contrast of these images were altered for maximized visualization in print (adapted from Carlson et al. 2007., with permission).

physically removing tissue with a biopsy and viewing under a microscope, can now be detected by OCT imaging noninvasively. A recent preliminary study of 50 patients showed that OCT could discriminate normal tissue from precancerous and cancerous tissue with a sensitivity of 93 percent and a specificity of 93 percent.[9] OCT systems are still being tested in laboratories and clinics, and no devices for oral cancer screening are yet commercially available.

Confocal Microscopy

Reflectance confocal microscopy is another high-resolution imaging technique that is just beginning to be explored for oral cancer detection. A laser is used for illumination and is scanned over the tissue surface using miniature mirrors that vibrate at high speeds. Images of cellular detail can be obtained at specific tissue depths. The technique is particularly useful for oral cancer screening because it can optically "section" the tissue so that only a single layer of cells is in focus. This is similar to the physical tissue section pathologists use to make histology slides. A typical image may contain tens to several hundreds of cells. The outer membrane and nucleus of each cell can be observed. Changes in nuclear size and morphology associated with neoplastic transformation are observable using confocal imaging.[10] Figure 3a shows a reflectance confocal image of cancerous tissue from a human tongue. Individual cells can be identified and are highly crowded, a common observation in cancerous tissue.

Fluorescence confocal microscopy can be used to analyze the metabolic behavior of cells, which is known to change in cancer. Cancer cells proliferate at an accelerated rate and may be identified by measuring the fluorescence intensity ratio of different fluorescent cofactors contained in cells.[11] Fluorescent contrast agents can also be applied to tissue to aid in the identification of precancerous and cancerous tissue. Fluorescent dyes can be chemically attached to antibodies that specifically target cancer cells.[12] Figure 3b shows an image of cancerous tongue tissue captured using fluorescence confocal microscopy. In this image, a green fluorescent dye is attached to an antibody for the human epidermal growth factor receptor (EGFR). EGFR is a cellular receptor highly overexpressed in many cancers, including oral cancer. Growth factors that bind to this receptor signal cells to divide and multiply. Cancer cells expressing EGFR appear bright green; normal cells are dark. This type of imaging allows a user to precisely identify cancerous cells and could aid in determining the margins of lesions during surgery. Other areas of confocal microscopy development include miniaturizing systems so they may be used in a clinical setting.

Nanotechnology

Nanotechnology is being explored to improve some of the above-mentioned imaging techniques. Electrons on the surface of metal nanoparticles interact with photons to produce unique effects. Gold and silver nanoparticles can be designed as spheres, rods, cubes, triangles and stars; each scatter and absorb light at different wavelengths. The surface of these nanoparticles can be functionalized with probe molecules that bind to cancer-related biomarkers. Nanoparticles may be injected into tissue, applied topically onto tissue or injected intravenously into the bloodstream. One group has experimented with gold nanoparticles using OCT imaging for oral cancer detection. By using a microinjection technique, they were able to deliver the nanoparticles under the surface of the oral tissue. The contrast of OCT images taken after the injection was significantly improved.[13] Nanoparticles are also being explored to improve confocal imaging to help identify malignant cells. When conjugated to antibodies that bind specifically to proteins present on the surface of cancer cells but not on normal cells, thousands or millions of nanoparticles will attach to a single cancerous cell, and this high density causes the cell membrane to appear many times brighter than normal cells when viewed using confocal microscopy.

Conclusion

Optical techniques are ideally suited for early detection of oral cancer, and new technologies are expanding rapidly. Some optical devices are already available for use by practitioners, while others will take years to further develop before they come to the market. Optical technologies are safe and inexpensive compared with other medical imaging technologies and can easily be integrated into clinical practice. Dental hygienists can play an active role in the adaptation of these new technologies. By learning how to operate these new devices, patients can be screened quickly during routine checkups, increasing the likelihood of detecting disease and potentially saving lives.

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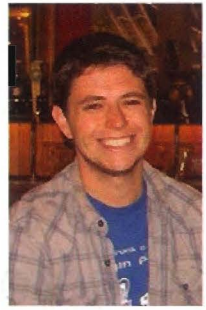

Darren Roblyer, PhD, received his BS in Biomedical Engineering from Johns Hopkins University in 2004. He received his PhD in Bioengineering from Rice University in 2009. During his graduate work, he investigated fluorescence imaging for the early detection of oral cancer through a collaboration with the M.D. Anderson Cancer Center. He is currently a postdoctoral fellow at the Beckman Laser and Medical Clinic at the University of California, Irvine, investigating optical methods to monitor chemotherapy in breast cancer patients.

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