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Fluorescence Emission-Based Detection and Diagnosis of Malignancy

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Abstract Over the past decades, laser use in medicine has expanded from its initial application as a light-based scalpel to a plethora of clinical uses, ranging from surgical treatment through composite polymerization, dental ablation, vision correction, and skin resurfacing to diverse diagnostic modalities. Recently, the concept of light-based diagnostics and therapy has come under investigation. Low light intensities are used to excite endogenous or exogenous fluorophores, some of which have characteristic fluorescence emissions in pathological tissues. Thus, premalignancy and malignancy potentially can be detected and diagnosed. Photosensitized superficial lesions can subsequently be destroyed selectively by using higher intensities of laser light. The application of fluorescence emission-based detection and diagnosis of precancer and cancer is reviewed, based on its application to the oral cavity—the author's primary anatomical area of expertise. This approach is justified as the same principles apply throughout the human body; to any area accessible to the clinician either directly or by some sort of fiber-optic probe. J. Cell. Biochem. Suppl. 39: 54–59, 2002.

Key words: fluorescence; malignancy; photosensitizer; diagnosis

Fifteen million people worldwide will be diagnosed with cancer this year. Cancer cure and survival relate directly to the stage of the cancer at the time of diagnosis. Early detection permits minimally invasive treatment and greatly improves the patient's chances of long-term survival. In many anatomical sites, early recognition of malignancy is problematic due to the frequent lack of gross signs/obvious symptoms, exacerbated by poor visual access in many cases, and the inability to perform adequate or regularly repeated screening in high-risk patients. The non-invasive nature of fluorescence-based diagnosis renders it applicable to almost any areas accessible either directly or by an endoscopic system.

Oral cancer will claim approximately 10,000 lives in the U.S. this year. Accounting for 96% of all oral cancers, squamous cell carcinoma is usually preceded by dysplasia presenting as white epithelial lesions on the oral mucosa (leukoplakia). Leukoplakias develop in 1–4% of the population. Malignant transformation, which is quite unpredictable, occurs in 1–40% of patients over 5 years. Tumor detection is complicated by a tendency towards field cancerization, leading to multicentric lesions. Current techniques detect malignant change too late. Of all oral cancer cases documented by the National Cancer Institute Surveillance, Epidemiology and End Results Program, advanced lesions outnumbered localized lesions at more than 2:1. Five-year survival rate is 75% for those with localized disease at diagnosis compared to only 16% for those with cancer metastasis.

Oral cancer can be detected by dentists and physicians, but physicians do not routinely inspect their patients for suspicious oral lesions, and dentists are also remiss in the early diagnosis and referral for oral cancer. Since 11% of dentists and 45% of physicians do not feel

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adequately trained to complete an effective oral cancer examination, this results in their failure to examine for oral cancer. The current approach to detecting the transformation of leukoplakia to squamous cell carcinoma is regular surveillance combined with biopsy or surgical excision. However, these techniques are invasive and unsuitable for regular screening of high risk sectors of the population. Adequate visual identification and biopsy of all such lesions to ensure that they are all recognized and diagnosed is difficult. Several studies have investigated use of vital staining with agents such as Lugol iodine and Toluidine blue for detection of oral malignancy [Silverman et al., 1984; Epstein et al., 1992]. Although the sensitivity of these agents in the hands of experts generally approximates 90%, specificity of these agents is poor; sensitivity rapidly decreases when this modality is used by non-experts. Moreover, clinical experience is necessary to perform these examinations adequately. Therefore, new techniques for non-invasive early detection and diagnosis of oral dysplasia and squamous cell carcinoma are required.

Topical or systemic application of chemical agents called photosensitizers can render pathologic tissues fluorescent when exposed to specific wavelengths of light [Benson, 1985; Shomacker et al., 1992]. While several studies have demonstrated the use of various porphyrins as photosensitizers [Benson, 1985; Hayata et al., 1982; Divaris et al., 1990], their accumulation in skin after systemic administration can cause phototoxic reactions upon exposure to sunlight. An alternative approach is to stimulate synthesis of photosensitizing agents in situ with a photoinactive precursor. The photosensitizer, protoporphyrin IX (PpIX) is an immediate precursor of heme in the biosynthetic pathway for heme. In certain types of cells and tissues, the rate of PpIX synthesis is determined by the rate of synthesis of 5-aminolevulinic acid (ALA), which in turn is regulated via a feedback control mechanism governed by the concentration of free heme. The presence of exogenous ALA bypasses the feedback control of this process, inducing the intracellular accumulation of photosensitizing concentrations of PpIX. A selective accumulation of PpIX occurs in areas of increased metabolism such as tumor cells [Benson, 1985; Kennedy and Pottier, 1992; Shomacker et al., 1992]. The resulting tissue-specific photosensitization provides the basis for using ALA-induced PpIX for photodynamic diagnosis and therapy, whereby far lower light doses are used for photodynamic diagnosis.

Using ALA-induced PpIX fluorescence in the rat colon [Bedwell et al., 1992] and in the bladder [Iinuma et al., 1995; Jichlinski et al., 1997], malignant tissues were found to fluoresce in the red spectral region with significantly greater intensity than the surrounding healthy tissues. The ratio of florescence levels in normal mucosa vs. that of the viable tumor was 1:6 [Iinuma et al., 1995]. Four hours post-ALA application was the optimal detection timepoint regardless of administration method in both the studies cited. Photosensitization using ALA-induced PpIX was shown to be effective for mapping tumour presence and for photodestruction of bladder carcinoma [Iinuma et al., 1995; Jichlinski et al., 1997]. An investigation by Svanberg et al. [1995], on head and neck cancer determined that the dosage of ALA must be kept low for maximal tumor demarcation. A higher dosage can cause excess bioavailability in normal tissue. An ALA-dose of 5–7.5 mg/kg was determined to be optimal for tumor differentiation.

Abels et al. [1994] investigated time courses in the type of porphyrin accumulation in neoplastic and surrounding tissue by measuring the kinetics and spectra of ALA-induced fluorescence in vivo. Using amelanotic melanoma A-Mel-3 cells grown in the Golden Syrian hamster the optimal time point for photodynamic diagnosis and therapy post-I.V. administration of 500 mg/kg of ALA was found to be 90 and 150 min. Maximum fluorescence intensity in neoplastic tissue occurred at 150 min.

The use of ALA-induced PpIX fluorescence for the early detection of laryngeal and lung cancer is also promising [Baumgartner et al., 1996; Mehlmann et al., 1999]. For this application, the preferred method of administration is via the medical nebulizer. Investigators found a strong ALA-induced fluorescence in carcinoma, carcinoma in situ, and dysplasia [Baumgartner et al., 1996; Mehlmann et al., 1999]. Both of these studies determined a correlation between red fluorescence intensity and histological diagnosis.

Studies in the hamster cheek pouch model have demonstrated significantly earlier and more intense PpIX fluorescence after topical ALA application in malignant lesions than in dysplastic and healthy oral mucosa. Fluorescence

intensity is on average 8–15 times greater in malignant lesions than in healthy mucosa [Ebihara et al., 2002]. Fluorescence development rate and intensity in dysplastic oral mucosa also exceed those in healthy tissue, but to a lesser degree than in malignant tissue, fluorescing on average 3–5 times more brightly than healthy tissue [Ebihara et al., 2002]. Clinical studies have confirmed these results, demonstrating very similar ALA-based pharmacokinetics in human oral mucosa as in the hamster cheek pouch model [Leunig et al., 1996, 2000; Kulapaditharom and Boonkitticharoen, 1998; Zenk et al., 1999; Betz et al., 2002]. The sensitivity and specificity of ALA-induced PpIX fluorescence diagnostics can further be enhanced by combining these data with autofluorescence measurements [Betz et al., 2002].

Leunig et al. [2000, 2001] examined the effectiveness of imaging 5-ALA using laser light in clinical diagnosis of squamous cell carcinoma in fifty eight oral cancer patients, and found that the tumerous tissues were demarcated from the normal tissues by a strong red fluorescence after 1–2.5 h of incubation with the ALA; while normal mucosa showed a bright green fluorescence. The fluorescence contrast between tumors and healthy mucosa was quantified by spectral analysis and the authors state that the high contrast of cancerous tissues (red) to healthy mucosa (green) after 5-ALA application could be due to both the accumulation of PpIX and a decrease of green autofluorescence within the tumor.

In a clinical study, oral use of a 15-min ALA mouth rinse resulted in varying levels of PpIX fluorescence. Differing levels of intensity were attributed to variations in rinsing procedure in each patient and to varying localization and histopathology of the tumors under investigation. Optimal conditions for fluorescence-based tumor localization occurred within 2 h of ALA-rinsing. PpIX fluorescence was limited to the surface layers of the epithelium (penetration depth less than 1 mm) [Leunig et al., 2000, 2001]. This leaves unresolved issues pertaining to tumor depth. Moreover, effects of common coexisting clinical pathologies such as candidal superinfection, hyperplasia, atrophy, ulceration have not been determined comprehensively.

The safety of ALA when used as a topical or systemic photosensitizer has been established in multiple clinical trials. ALA and PpIX are normally present in certain tissues of the body. ALA-induced PpIX is cleared from the body within 24 h of its induction, whether the route of administration is systemic or topical [Kennedy and Pottier, 1992]. Thus protection from exposure to sunlight is only necessary for 24 h after ALA application. ALA-induced PpIX fluorescence constitutes the main form of photosensitizer-based diagnostics which has been investigated in the oral cavity.

Another approach to fluorescence-based oral diagnosis uses endogenous emissions, or autofluorescence. Light exposure of cells and tissues in the visible and the UV range results in excitation of naturally occurring fluorophores. Deactivation occurs in part via fluorescence emission. Studies have shown that autofluorescence spectroscopy may be useful for distinguishing cancerous from normal tissues in a variety of organ systems, such as human lung and breast [Alfano et al., 1991; Tang et al., 1989], bronchus [Hung et al., 1991; Bottiroli et al., 1995], colon [Romer et al., 1995], cervix [Mahadevan et al., 1993; Ramajunam et al., 1994], esophagus [Vo-Dinh et al., 1995], and head and neck [Kolli et al., 1995; Wang et al., 1999]. Factors that contribute to autofluorescence changes in human neoplasia range from changes in tissue architecture [Kulapaditharom and Boonkitticharoen, 1998] to intrinsic changes in fluorophores [Hung et al., 1991]. Proteins and amino acids (including tryptophan, tyrosin, phenylalanine), purines, pyrimidines, and nucleic acids absorb UV light near 280 nm and have autofluorescence peaks at 340, 360, and 390 nm: the onset and progression of malignancy can affect these peaks [Alfano et al., 1991; Silberberg et al., 1994]. Alternately, an excitation wavelength near 340 nm can identify the collagen-, NADH-, and hemoglobin-related changes occurring in cancer tissues, whereby collagen fluorescence decreases while NADH fluorescence and hemoglobin re-absorption increase [Hung et al., 1991; Schomacker et al., 1992; Lam et al., 1993; Harries et al., 1995; Ramajunam et al., 1996] all demonstrated malignancy-related reduced lung tissue autofluorescence in the green region after excitation at 442 nm. This is attributed to thickening of epithelium or overlying tumor tissue and a reduction in concentration of fluorophores such as flavins [Qu et al., 1994].

Several clinical studies have measured fluorescence spectra in normal and neoplastic areas of oral mucosa using fiber-optic probes [Kolli

et al., 1995; Schantz et al., 1998; Gillenwater et al., 1998]. Schantz et al. [1998] measured fluorescence excitation spectra at 450 nm emission, finding that average maximum fluorescence intensities of poorly differentiated tumors were significantly lower than those of well-differentiated tumors, although there was considerable overlap. Kolli et al. [1995] measured fluorescence spectra in healthy and malignant oral tissues after excitation/detection at 300, 340 nm excitation and 380, 450 nm detection. Mean ratios of intensities between neoplastic and normal sites varied significantly at 300, 340 nm excitation and 380 nm emission. Decreased blue fluorescence and increased red fluorescence were observed when using an in vivo fiber-optic probe after 410 nm excitation [Gillenwater et al., 1998]. At 337 nm excitation, overall fluorescence intensities in healthy tissues greatly exceeded those in abnormal sites [Gillenwater et al., 1998]. This ability for spectral characterization of specific histopathological diagnoses can provide a basis for potential future non-invasive differential diagnosis of oral leukoplakia as well as monitoring of suspect lesions and screening of high-risk populations using laser-induced autofluorescence.

Another approach has been the measurement of fluorescence emission spectra at many excitation wavelengths from biopsy specimens to identify optimum excitation, detection wavelengths. After using excitation in 10 nm steps at 270–400 nm, Chen et al. [1996] reported spectral peaks at 330, 470 nm emission. The average ratio of fluorescence intensities at 330 nm emission to that at 470 nm emission was significantly greater than in normal tissues. Measurement of fluorescence emission spectra from biopsy tissues after multiple wavelength excitation identified most marked differences between healthy and malignant tissues at 410 nm excitation, with cancerous tissues showing a stronger red fluorescence at 635 nm [Roy et al., 1995; Ingrams et al., 1997]. Based on this information, fluorescence spectra were measured in vivo in an animal model [Dhingra et al., 1996] and in human subjects [Dhingra et al., 1996]. Neoplastic lesions typically showed a stronger red fluorescence, permitting clinical diagnosis of 45 out of 49 cancerous lesions [Dhingra et al., 1996]. Another study compared the effectiveness of 370 vs. 410 nm excitation. Using red fluorescence again as diagnostic criterion, 17 of 19 malignant lesions were correctly identified in another study, with two false positives [Dhingra et al., 1996].

A different approach has been the use of imaging systems, which record the spatial distribution of fluorescence intensity at specific excitation/detection wavelengths. These systems permit screening of large areas of the oral mucosa for neoplastic change. The LIFE (laser induced fluorescence endoscopy) system, originally developed for pulmonary diagnostics, compares red-to-green fluorescence intensities at 442 nm excitation to identify areas of neoplasia. When this system was applied to the oral cavity, malignancy was associated with increased red/green fluorescence intensity ratio, providing a sensitivity of 100% and a specificity of 87.5% [Kulapaditharom and Boonkitticharoen, 1998]. Using fluorescence photography at 360 nm excitation and emission $>$ 480 nm, Onizawa et al. [1996] achieved 88% sensitivity and 94% specificity in distinguishing between healthy and neoplastic oral tissues.

These studies indicate that fluorescence is a promising tool for detection and screening of oral dysplasia and malignancy. However, most studies were performed on small numbers of small biopsy samples. In the few in vivo human trials reported, a limited number of excitation and detection wavelengths were investigated due to the limited range of excitation and detection sources suitable for clinical use. Moreover, fluorescence measurements performed in biopsy samples do not necessarily translate directly or accurately to the clinical situation. Relevant factors include:

1. The absence of blood flow in the biopsy sample. Lack of perfusion in biopsy specimens overlooks the contribution of hemoglobin (re)absorption to emission spectra. This leads to, for example, reduced measured fluorescence emission in the blue/green range in perfused vs. non-perfused samples [Schomacker et al., 1992].
2. Oxidation of electron carriers (such as reduced NAD, reduced FAD) can affect spectra considerably. This is because fluorescence emissions from the oxidized vs. reduced states vary enormously [Cothren et al., 1990].

Thus, further studies are necessary in an in vivo model to permit direct and valid translation of data to the clinical situation.

The concept of fluorescence-emission-based detection and diagnosis of premalignancy and malignancy is very promising, with autofluorescence and photosensitizer-mediated fluorescence each providing their specific strengths and weaknesses. These can be exploited to meet specific clinical needs.

With the capability for directly identifying when biopsies of suspect lesions are needed, the need for traumatic, often repeated biopsies is greatly reduced, and early detection and treatment are facilitated. Large-scale screening of high-risk populations and monitoring of suspect lesions also becomes possible. This multi-use clinical capability will immediately produce a sharp drop in cancer-related suffering, death, and cost.

REFERENCES

Abels C, Heil P, Dellian M, Kuhnle GEH, Baumgartner R, Goetz AE. 1994. In vivo kinetics and spectra of 5-aminolevulinic acid-induced fluorescence in an amelanotic melanoma of the hamster. Br J Cancer 70(5):826–833.

Alfano RR, Das BB, Cleary J, Prudent R, Celmer EJ. 1991. Light sheds light on cancer-distinguishing malignant tumors. Bull N Y Acad Med 67:143–150.

Baumgartner R, Huber RM, Schulz H, Stepp H, Rick K, Gamarra F, Leberig A, Roth C. 1996. Inhalation of 5-aminolevulinic acid: A new technique for fluorescence detection of early stage lung cancer. J Photochem Photobiol 36:169–174.

Bedwell J, MacRobert AJ, Phillips D, Bown SG. 1992. Fluorescence distribution and photodynamic effect of ALA-induced PpIX in the DMH rat colonic tumor model. Br J Cancer 65:818–824.

Benson RCJ. 1985. Treatment of diffuse transitional cell carcinoma in situ by whole bladder hematoporphyrin derivative photodynamic therapy. J Urol 134:675–678.

Betz CS, Stepp H, Arbogast S, Grevers G, Baumgartner R, Leunig A. 2002. A comparative study of normal inspection, autoflourescence and 5-ALA-induced PPIX fluorescence for oral cancer diagnosis. Int J Cancer 97:245–252.

Bottiroli G, Corce AC, Locatelli D, Marchesini R, Pignoli E, Tomatis S, Cuzzoni C, Palma SD, Dalfante M, Spinelli P. 1995. Natural fluorescence of normal and neoplastic human colon. Lasers Surg Med 16:48–60.

Chen CT, Wang CY, Kuo YS, Lee M. 1996. Light-induced fluorescence spectroscopy. Proc Natl Sci Counc Repub China, Part B, Life Sci 20:123–130.

Cothren RR, Richards-Kortum R, Sivak M. 1990. Gastrointestinal tissue diagnosis by laser-induced fluorescence spectroscopy at endoscopy. Gastrointest Endosc 36:105–111.

Dhingra JK, Perrault K, McMillan I, Rebeiz EE, Kabani S, Manoharan R. 1996. Early diagnosis of upper aerodigestive cancer by auto fluorescence. Arch Otolaryngo/Head Neck Surg 122:1181–1186.

Divaris DXG, Kennedy JC, Poittier RH. 1990. Phototoxic damage to sebaceous glands and hair follicles of mice after systemic administration of ALA correlates with localized PpIX fluorescence. Am J Pathol 136:891–897.

Ebihara A, Liaw L-H, Krasieva TB, Messadi D, Osann K, Wilder-Smith P. 2002. Detection and diagnosis of oral cancer by light-induced fluorescence. Lasers Surg Med (in press).

Epstein J, Scully C, Spinelli U. 1992. Toluidine blue and Lugol's iodine solution for the assessment of oral malignant disease and lesions at risk of malignancy. J Oral Pathol Med 21:160–163.

Gillenwater A, Jacob R, Ganeshuppa B, Kemp B, El-Naggar AK, Raimer JL, Clayman E, Mitcheu MF, Richards-Kortum R. 1998. Noninvasive diagnosis of oral neoplasia based on fluorescence spectroscopy and native tissue autofluorescence. Arch Otolaryng Head Neck Surg 124:1251–1258.

Harries ML, Lam S, MacAuley C, Qu J, Palcic B. 1995. Diagnostic imaging of the larynx. J Laryngol Otol 109: 108–110.

Hayata Y, Kato H, Kanaka C, Ono J, Takizawa N. 1982. Hematoporphyrin derivative and laser photoradiation in the treatment of lung cancer. Chest 81:269–277.

Hung J, Lam S, LeRiche JC, Palcic B. 1991. Autofluorescence of normal and malignant bronchial tissue. Lasers Surg Med 11:99–105.

Iinuma S, Bachor R, Flotte T, Hasan T. 1995. Biodistribution and toxicity of ALA-induced PpIX in an orthopotic rat tumour model. J Urol 153:802–806.

Ingrams DR, Dhingra JK, Roy K, Perrault DF, Bottrill FD, Kabani S, Rebeiz EE, Pankrator MM, Shapshay SM, Manoharan R, Feld MS. 1997. Autofluorescence characteristics of oral mucosa. Head Neck 19:27–32.

Jichlinski P, Forrer M, Mizeret J, Glanzmann T, Braichotte D, Wagnieres G, Zimmer G, Guillou L, Schmidlin F, Graber P, van den Bergh H, Leisinger H-J. 1997. Clinical evaluation of a method for detecting superficial transitional cell carcinoma of the bladder by light-induced fluorescence of protoporphyrin IX following topical application of 5-aminolevulinic acid: Preliminary results. Lasers Surg Med 20:402–408.

Kennedy JC, Pottier RH. 1992. Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. J Photochem Photobiol 14:275–292.

Kolli VR, Shaha AR, Savage HE, Sacks PG, Casale MA, Schantz SP. 1995. Native cellular fluorescence can identify changes in epithelial thickness in vivo in the upper aerodigestive tract. Am J Surg 170:495–498.

Kulapaditharom B, Boonkitticharoen V. 1998. Laser-induced fluorescence imaging in localization of head and neck cancers. Ann Otol Rhinol Laryngol 107:241–246.

Lam S, MacAuley C, Hung J, LeRiche J, Profio AE, Palcic B. 1993. Detection of dysplasia and carcinoma in situ with a lung imaging endoscopic device. F Thorac Cardiovasc Surg 105:1035–1040.

Leunig A, Rick K, Stepp H, Gutmann R, Alwin G, Baumgartner R, Feyh J. 1996. Fluorescence imaging and spectroscopy of 5-aminolevulinic acid-induced protoporphyrin IX for the detection of neoplastic lesions in the oral cavity. Am J Surg 172(6):674–677.

Leunig A, Betz CS, Mehlmann M, Stepp H, Arbogast G, Baumgartner R. 2000. Detection of squamous cell carcinoma of the oral cavity by imaging 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. The Laryngoscope 110:78–83.

Leunig A, Mehlmann M, Betz CS, Stepp H, Arbogast G, Grevers G, Baumgartner R. 2001. Fluorescence staining of oral cancer using a topical application of 5-aminolevulinic acid: Fluorescence microscopic studies. Photochem Photobiol 60:44–49.

Mahadevan A, Mitchell MF, Silva E, Thomsen S, Richard-Kortum RR. 1993. A study of the fluorescence properties of normal and neoplastic human cervical tissue. Lasers Surg Med 13:647–655.

Mehlmann M, Betz CS, Stepp H, Arbogast S, Baumgartner R, Grevers G, Leunig A. 1999. Fluorescence staining of laryngeal neoplasms after topical application of 5-aminolevulinic acid: Preliminary results. Lasers Surg Med 25: 414–420.

Onizawa K, Saginoya H, Furuya Y, Yoshida H. 1996. Fluorescence photography as a diagnostic method for oral cancer. Cancer Lett 108:61–66.

Qu J, MacAuley C, Lam S, Palcic B. 1994. Optical properties of normal and carcinoma bronchial tissue. Appl Opt 33:7397–7405.

Ramajunam M, Mitchell MF, Mahadevan A, Thomsen S, Silva E, Richards-Kortum RR. 1994. Fluorescence spectroscopy: A diagnostic tool for CIN. Gynecologic Oncol 52:32–38.

Ramajunam M, Mitchell MF, Mahadevan A, Thomsen S, Maloica A, Wright T, Atkinson N, Richards-Kortum R. 1996. Spectroscopic diagnosis of CIN in vivo using LIF spectra at multiple excitation wavelengths. Lasers Surg Med 19:63–74.

Romer TJ, Fitzmaurice M, Cothren RM, Richards-Kortum R, Petra S, Sivak MV, Kramer LR, Romer TJ. 1995. Laser-induced fluorescence microscopy of normal colon and dysplasia in colonic adenomas. Am J Gastroenterol 90(1):81–87.

Roy KI, Bottrill ID, Ingrams DR. 1995. Diagnostic fluorescence spectroscopy of oral mucosa. SPIE 2395:135–142.

Schantz SP, Kolli V, Savage HE, Yu G, Harris DE, Katz A, Alfano RR, Haros AG. 1998. In vivo native cellular fluorescence and histological characteristics of head and neck cancer. Clin Cancer Res 4:1177–1182.

Schomacker KT, Frisoli JK, Compton CC, Flotte TJ, Richter JM, Nishioka S, Deutsch TF. 1992. UV laser-induced fluorescence of colonic tissue. Lasers Surg Med 12:63–78.

Shomacker KT, Frisoli JK, Compton CC, Flotte TJ, Richter JM, Nishioka NS, Deutsch TF. 1992. Ultraviolet laser-induced fluorescence of colonic tissue. Lasers Surg Med 12:63–68.

Silberberg MB, Savage HE, Tang GC, Sacks PG, Alfano RR, Schantz SP. 1994. Detecting retinoic-acid induced biochemical alteration in squamous cell carcinoma using intrinsic fluorescence spectroscopy. Laryngoscope 104: 278–282.

Silverman S, Migliorati C, Barbosa J. 1984. Toluidine blue staining in the detection of oral precancerous and malignant lesions. Oral Surg Oral Med Oral Pathol 57:379–382.

Svanberg K, Wang I, Rydell R, Elner A, Wennerberg J, Pais Clemente L, Cardosa E, Pratas R, Pais Clemente M, Andersson-Engels S, Svanberg S. 1995. Fluorescence diagnostics of head and neck cancer utilizing oral administration of δ-amino levulinic acid. SPIE 2371:129–141.

Tang GC, Prodhan A, Alfano RR. 1989. Spectroscopic differences between human cancer and normal lung and breast tissues. Laser Surg Med 9:290–295.

Vo-Dinh T, Pamjehpour M, Overholt BF, Farris C, Bukley FP, Sneed R. 1995. In vivo cancer diagnosis of the esophagus using DNF indices. Lasers Surg Med 16:41–47.

Wang C-Y, Chiang HK, Chen C-T, Chiang C-P, Kuo Y-S, Chow S-N. 1999. Diagnosis of oral cancer by light-induced autofluorescence spectroscopy using double excitation wavelengths. Oral Oncol 35:144–150.

Zenk W, Dietel W, Schleier P, Gunzel S. 1999. Visualizing carcinomas of the mouth cavity by stimulating synthesis of fluorescent protoporphyrin IX. Mund Kiefer Gesichtschir 3(4):205–209.