Brogan & Partners

Native Cellular Fluorescence and Its Application to Cancer Prevention
Author(s): Stimson P. Schantz, Howard E. Savage, Peter Sacks, Robert R. Alfano
Source: *Environmental Health Perspectives*, Vol. 105, Supplement 4 (Jun., 1997), pp. 941-944
Published by: Brogan & Partners
Stable URL: http://www.jstor.org/stable/3433308
Accessed: 14/04/2009 12:41

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at http://www.jstor.org/page/info/about/policies/terms.jsp. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/action/showPublisher?publisherCode=brogpart.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit organization founded in 1995 to build trusted digital archives for scholarship. We work with the scholarly community to preserve their work and the materials they rely upon, and to build a common research platform that promotes the discovery and use of these resources. For more information about JSTOR, please contact support@jstor.org.

Brogan & Partners is collaborating with JSTOR to digitize, preserve and extend access to *Environmental Health Perspectives.*

http://www.jstor.org

Native Cellular Fluorescence and Its Application to Cancer Prevention

Stimson P. Schantz,[1] Howard E. Savage,[1] Peter Sacks,[1] and Robert R. Alfano[2]

[1]Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Department of Physics and Electrical Engineering, City College of New York, New York, New York

Native cellular fluorescence (NCF) represents the innate capacity of tissues to absorb and emit light of specified wavelengths. Recent advances in optical engineering and computer technology have provided the opportunity to measure NCF characteristics of various tissues *in vivo*. This report will briefly review the current status of NCF analysis of various neoplastic tissues. The status of investigations involving the upper aerodigestive tract will be discussed. Though initial results demonstrate that neoplastic tissues can be discriminated from normal mucosa by NCF analysis, the biologic basis of this difference remains uncertain. This report will also emphasize that the ability to screen for cancer in aerodigestive mucosa may be enhanced through the assessment of multiple emission and excitation wavelengths. The true nature of the cellular fluorophores responsible for these mucosal spectral characteristics should be more fully defined in coming years. — *Environ Health Perspect* 105(Suppl 4):941–944 (1997)

Key words: native cellular fluorescence, screening, tobacco, head cancer, neck cancer

The capacity to effectively screen for premalignant and early invasive cancers of the upper aerodigestive tract and lung should lead to improved survival. Numerous strategies developed over the past 2 decades to achieve this goal include novel immune and molecular cytological techniques, the development of circulating tumor markers, and advances in diagnostic radiology (*1–7*). It is evident from the review of these various approaches that optimal methods must still be established.

The need for improved sensitivity and specificity in cancer diagnosis has led to interest in native cellular fluorescence (NCF), i.e., the innate capacities of tissues to absorb and transmit light, as a means of distinguishing normal from neoplastic tissue. It has been well recognized for many years that numerous subcellular components, termed fluorophores, were capable of emitting light of specified wavelengths. Such fluorophores include various proteins, coenzymes, micronutrients, and even DNA (*8*). The concept that qualitative and quantitative differences in cellular fluorophores would distinguish diseased from normal tissue *in vivo* had been explored since the 1950s. Schaffer and Sacks, for instance, noted that assessment of oral cavity mucosa by means of a Wood-Light ultraviolet lamp would distinguish individuals with riboflavin deficiency (*9*). By providing nutritional supplements, those authors noted a reversal of the abnormal mucosal fluorescence and thus demonstrated the clinical utility of this approach. The spectral characteristics of riboflavin have been previously described (*8,10*).

In the mid-1980s investigators began to apply the principle of NCF to the assessment of neoplastic tissue. Alfano et al. (*11,12*) and Glassman et al. (*13*) analyzed breast and lung tissues for NCF patterns *in vitro*. Glassman et al. (*13*) utilized a xenon lamp to generate a 300-nm excitation beam and then quantitated 340:440-nm emission ratios. They noted that neoplastic tissue contributed significantly elevated 340:440 nm ratios compared to nonmalignant tissues. Similar observations regarding the 340:440-nm ratio were noted for cervical cancers (*13*). Numerous investigations over the past several years have explored the use of NCF technology for screening of diseases of the colon, cervix, and lung (Table 1) (*14–19*). Various laser sources were utilized, including helium–cadmium and nitrogen-pulsed lasers. The analysis of fluorescence emission has pointed to abnormalities at various wavelengths, including 380-, 450-, 520-, and 680-nm emission. Overall sensitivity and specificity of NCF have ranged from 85 to 95% and 80 to 85%, respectively. Indeed, several authors claim that NCF analysis was superior to more standard approaches, including physical examination and routine light-based endoscopy (*17,18*).

Table 1. Demographic characteristics of cases and controls.

Organ system, reference	Native cellular fluorescence characteristics: Excitation source	Native cellular fluorescence characteristics: Wavelength analysis	Sensitivity, %/specificity, %
Colon			
Kapadia et al. (*15*)	HC	Multiwavelength	100/99
Cothren et al. (*14*)	PN	680 versus 460 nm	100/94
Schomacker et al. (*16*)	PN	12 wavelengths	80/92
Lung			
Lam et al. (*17*)	HC	630:520-nm ratio	73/94
Cervix			
Ramanujam et al. (*18*)	PN	450-nm emission	87/75
Esophagus			
Vo Dinh et al. (*19*)	PN	480-nm emission	–
		640-nm emission	NS
Head and neck			
Franchesci et al. (*21*)	X	450-nm emission	NS
Kolli et al. (*22*)	X	Multiwavelength	NS

Abbreviations: HC, helium cadmium; PN, pulsed nitrogen; NS, not stated; X, xenon.

This paper is based on a presentation at the symposium on Mechanisms and Prevention of Environmentally Caused Cancers held 21–25 October 1995 in Santa Fe, New Mexico. Manuscript received at *EHP* 16 April 1996; accepted 13 August 1996.

This research was supported in part by grants 47349-A from the Office of Naval Research and 49547-C from NASA, and by a gift from the Mediscience Technology Corporation.

Address correspondence to Dr. S.P. Schantz, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Room C973, New York, NY 10021. Telephone: (212) 639-6033. Fax: (212) 717-3302. E-mail: schantz@mskcc.org

Abbreviations used: EGF, epidermal growth factor; NADH, reduced nicotinamide-adenine dinucleotide. NCF, native cellular fluorescence; NMBA, *N*-nitrosomethyl benzylamine; NOE, normal oral epithelia.

In Vivo Analysis of Upper Aerodigestive Mucosa

Initial investigations on upper aerodigestive cavity mucosa, in both humans and animal model systems, have recently been reported (*20–24*). The oral cavity lends itself to such studies for several reasons (*20*). One is that the relationship between environmental exposures and diseases of the oral cavity has been well documented. Approximately 90% of individuals with cancer at this site will give a past or current history of tobacco exposure. The oral cavity is readily examined during a routine office or dental visit, thus requiring no special preparation or patient discomfort. Multiple examinations on a large population can then be performed to derive the significance of a particular screening strategy. In addition, a well-defined premalignant state, termed leukoplakia, can be identified within the oral cavity. This condition has facilitated our understanding of neoplastic progression not only within the oral cavity but in other epithelial tissues as well. Finally, it is well known that patients with one oral cavity cancer are at risk of a second cancer. This has given rise to the concept of field cancerization, i.e., the theory that all tissues exposed to a particular carcinogen will be at risk for malignant transformation and that such tissues will progress in various stages toward invasive disease (*25,26*). The question remains as to the most appropriate way to screen for multifocal diseases within such individuals.

To our knowledge, only a few studies have addressed the NCF characteristics of oral cavity neoplasias *in vivo*. Franchesci et al. assessed 13 tongue cancers and contralateral normal mucosa by means of a xenon-lamp excitation source (*21*). An excitation scan at 450-nm emission was utilized. This scan varies the excitation wavelength over a range of 280 to 430 nm. Optimal excitation wavelengths capable of generating 450 nm emission can then be established. Using Fourier transform analysis, the authors noted a significant difference between tongue lesions and contralateral normal mucosa (*21*). A recent study by Kolli et al. (*22*) has extended this observation and showed that fluorescence characteristics of oral cavity cancers also involved the abnormal absorption and emission of other wavelengths, including 340-, 380-, and 440-nm wavelengths. The choice of these wavelengths was based in part on previous *in vitro* and *in vivo* studies, which demonstrated that each was capable of distinguishing neoplastic from nonneoplastic tissues. Furthermore, each wavelength can be ascribed to particular fluorophores, which may differ in both quantitative and qualitative terms in tissues in varying stages of progression from normal to invasive disease (Table 2) (*8*).

The preponderance of studies, therefore, have demonstrated that neoplastic tissues can be discriminated from normal epithelial tissues within an individual patient. The optimal wavelengths for either exciting tissue or measuring emission, however, have yet to be defined. Likewise, considerable variance in the means of analyzing spectral profiles exists among studies (*13–18,22*). Methods include the utilization of absolute intensity, various transformations of data, multivariate logistic regression models, and other normalization procedures to best predict the presence of disease. No doubt, part of the confusion stems from the complexity of both the carcinogenisis process and fluorescence determinants. It is clear, for instance, that wide variance in NCF characteristics exists in the normal mucosa of various individuals (*18,21,22*). The biologic basis for these differences is unclear. However, differences within an individual will be more informative than differences between individuals. Attempts to define a particular algorithm for predicting a diseased state, though informative, will have limited clinical value if analysis is confined to the use of a single excitation wavelength.

Current results support the use of multiple excitation and emission profiles in defining a particular tissue. Composite results may more accurately represent the state of health of a given tissue than will the analysis of a single wavelength. The clear application of this principle to cancer screening *in vivo* needs to be defined. Our efforts have been to explore the biologic basis for NCF within aerodigestive mucosa through the use of various laboratory model systems.

Animal Studies on Upper Aerodigestive Mucosa

Several animal models have been utilized to assess the ability of NCF to distinguish carcinogen-induced alterations within exposed tissue (*23,24*). Glasgold et al. utilized a Sprague-Dawley rat model to assess alterations (*24*). In this well-defined cancer model, Sprague-Dawley rats are injected ip with a 5% solution of *N*-nitrosomethyl benzylamine (NMBA). Following 15 weeks of injections, the majority of rats will develop gross evidence of esophageal disease consisting of either adenomas or invasive cancers. In the initial studies by Glasgold et al. (*24*), NCF characteristics of the rat esophageal mucosa were demonstrated at varying times after initial exposures. Two spectral profiles were discussed—the 380- and 450-nm excitation scans. Spectral alterations become consistently apparent after 5 weeks of NMBA exposure. Indeed, in several instances fluorescence alterations were noted as early as 2 weeks (*24*).

The above study provided evidence regarding the determinants of spectral alterations as well. Though changes in both the 380- and 450-nm excitation profiles occurred at approximately the same time, each reflected a different component of the esophageal wall. In the 380-nm scan, continued carcinogen exposure led to progressive increases in the 290:330-nm ratio (Figure 1). This decrease was directly proportional to the increasing thickness of the esophageal mucosa, which occurred during malignant transformation. We suggested that the loss of the 330-nm excitation peak

Table 2. Spectral characteristics of major fluorophores.

Fluorophores	Wavelength, nm	
	Excitation	Emission
Proteins and amino acids		
Tryptophan	287	348
Collagen	360	405
Elastin	290	340
Porphyrin	440	630
Enzymes and coenzymes		
NADH	325–350	440–462
Riboflavin	450	520–535

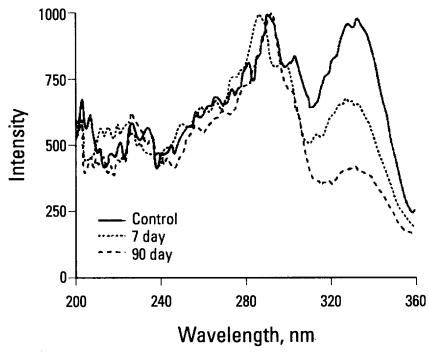

Figure 1. Representative excitation scan of an NMBA-induced esophageal carcinogenesis model within Sprague-Dawley rats. Rats were exposed to saline (control) or NMBA for 7 and 90 days, respectively. Fluorescence analysis reveals progressive loss of intensity of the 330-nm peak relative to the 290-nm peak with continued exposure. Reprinted from Glasgold et al. (*24*), with permission.

was secondary to decreased excitation of collagen within stroma. As the mucosa thickens, less of the excitation beam penetrates the stromal–mucosal interface. In support of this conclusion, layers of the esophageal wall were separated by treatment with versene. The 330-nm excitation peak emitted solely from the pure stromal preparation. No peak could be generated by exciting only mucosal cells. Data supported previous observations by Schomacker et al., who examined human colon tissue (*15*). These researchers demonstrated that *in vitro* layering of mucosal tissue on a stromal bed decreased collagen signaling.

In contrast to the 380-nm excitation scan, alterations in the 450-nm excitation induced by carcinogen exposure emanated entirely from esophageal mucosa (*24*). These changes became evident as early as 2 weeks after NMBA exposure and long before gross pathologic changes were noted. These data demonstrate that multiwavelength assessment of esophageal mucosa, i.e., the use of multiple excitation wavelengths and the measurement of multiple emission characteristics, will define different histoarchitectural elements within diseased mucosa. The study also demonstrates that NCF alterations occur very early in malignant transformation and long before gross malignancy or even premalignant changes can be identified. These results support the use of NCF characteristics as a means of screening for preclinical disease. They also provide a basis for monitoring the impact of prevention strategies designed to halt or reverse the neoplastic process.

In Vitro Studies of Upper Aerodigestive Mucosa

The biologic basis for NCF characteristics of upper aerodigestive mucosa has been explored through *in vitro* analysis (*27–29*). The premise of these studies is that precancerous alterations within aerodigestive mucosa that precede clinically apparent disease will be characterized by abnormal proliferation and differentiation. Our approach has been to characterize fluorescence abnormalities that reflect that process. Using three cell systems consisting of 3T3 fibroblasts, A431 squamous carcinoma cells, and short-term cultures of normal oral epithelium, proliferation rates were altered by various growth conditions (*28*). Proliferation rates were measured by cell count, tritiated thymidine incorporation, and cell-cycle analysis by flow cytometry. A characteristic spectral profile was identified in each instance, independent of the cell line utilized, and involved emission at 450 nm. Figure 2 shows a characteristic profile of the 450 nm excitation scan. In this example, A431 cells were growth inhibited by medium supplementation with epidermal growth factor. Scans revealed a hypochromic shift of the primary maximum in cells that were more rapidly proliferating. Furthermore, the primary excitation peak maximum:370-nm ratio was consistently greater in the more rapidly proliferating cells. Although one may assume that the fluorophore that contributes to these alterations is NADH (*8*), further characterization of the biochemical basis of this fluorescence profile is in order.

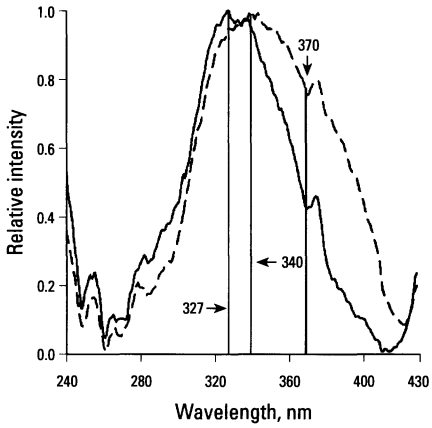

Figure 2. Representative normalized excitation scan (excitation 240–430 nm, 450 nm) of control (solid line) and treated (dotted line) A431 carcinoma cells. A431 cells were treated in the presence (solid line) or absence (dotted line) of 100 ng/ml of epidermal growth factor (EGF). EGF induced inhibition of growth at this concentration. Reprinted from Zhang et al. (*28*), with permission.

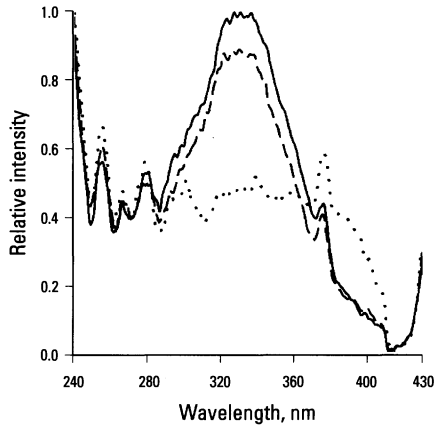

Figure 3. Representative normalized excitation scans (excitation 270–500 nm, emission 520 nm) of normal oral epithelia (NOE). Short-term cultures of NOE were grown in media supplemented with (dotted line) or without (solid line) 0.8-M NaCl. The high-salt containing media induced cellular differentiation. Reprinted from Sacks et al. (*30*), with permission.

Next, short-term cultures of normal oral epithelia were established to modulate cellular differentiation (*26*). Differentiation in these experiments was induced by culturing oral epithelium in high salt concentrations, and was noted through the acquisition of cornified envelopes and high molecular weight keratin, cytokeratin 13. Distinct from the proliferation model described above, alterations in the 520-nm excitation scan could be identified (Figure 3). Compared to less-differentiated cells, cells grown in high salt solution demonstrated an increased secondary absorption maximum at 380 nm. Thus, two systems that measure cellular proliferation and differentiation, respectively, provide unique changes in fluorescence emission patterns, again pointing to the value of multiwavelength analysis. These results also support the use of NCF characterization as a means of screening for subclinical phenotypic alterations in tissues at high risk for cancer development.

REFERENCES

1. McDougall JC. Lung cancer. To screen or not to screen? [Editorial comment]. Arch Intern Med 154:945 (1994).
2. Keogan MT, Tung KT, Kaplan DK, Goldstraw PJ, Hansell DM. The significance of pulmonary nodules detected on CT staging for lung cancer. Clin Radiol 48:94–96 (1993).
3. Patz EF Jr. Positron emission tomography imaging of the thorax. Radiol Clin North Am 32:811–823 (1994).
4. Tochman MS, Gupta PK, Myers JD, Frost JK, Baylin SB, Gold EB, Chase AM, Wilkinson PH, Mulshine JL. Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: a new approach to early lung cancer detection. J Clin Oncol 6:1685–1693 (1988).
5. Mao L, Hruban RH, Boyle JO, Tockman M, Sidransky D. Detection of oncogene mutations in sputum precedes diagnosis

of lung cancer. Cancer Res 54:1634–1637 (1994).
6. Van der Gaast A, Schoenmakers CHH, Kok TC, Blijenberg BG, Cornillie F, Splinter TAW. Evaluation of a new tumor marker in patients with non-small–cell lung cancer: Cyfra 1.1. Br J Cancer 69:525–528 (1994).
7. Ferrigno D. Serum tumor markers in lung cancer: history, biology, and clinical applications. Eur Respir J 7:186–197 (1994).
8. Underfriend S. Fluorescence Assay in Biology and Medicine, Vol 1. New York:Academic Press, 1962.
9. Schaffer AB, Sacks SW. Observations on the fluorescent tongue pattern and its relations to the deficiency state. Oral Surg Oral Med Oral Pathol 6:1425–1434 (1953).
10. Ghisla S, Massey V, Lhoste J-M, Mayhew SG. Fluorescence characteristics of reduced flavins and flavoproteins. In: Reactivity of Flavins (Yagi K, ed). Tokyo:University of Tokyo Press, 1975;15–24.
11. Alfano RR, Tata D, Cordero J, Tomashefsky P, Longo F, Alfano M. Laser induced fluorescence spectroscopy from native cancerous and normal tissues. Inst Electr Electron Eng J Quant Electr QE-20:1507–1511 (1984).
12. Alfano RR, Das BB, Cleary J, Prudent R, Celmer E. Light sheds light on cancer-distinguishing malignant tumors from benign tissues and tumors. Bull NY Acad Med 67:143–150 (1991).
13. Glassman WS, Liu CH, Tang GC, Lubicz S, Alfano RR. Ultraviolet fluorescence spectra from nonmalignant and malignant tissues of the gynecological tract. Lasers Life Sci 5:49–54 (1992).
14. Cothren RM, Richards-Kortum R, Sivak M, Fitzmaurice M, Rava R, Boyce G, Doxtader M, Blackman R, Ivanc T, Hayes G et al. Gastrointestinal tissue diagnostic by laser-induced fluorescence spectroscopy at endoscopy. Gastrointest Endosc 36:105–111 (1990).
15. Kapadia CR, Cutruzzola FW, O'Brian KM, Stetz M, Enriquez R, Deckelbaum L. Laser-induced fluorescence spectroscopy on human colonic mucosa. Gastroenterology 99:150–17 (1990).
16. Schomacker KT, Fusoli JK, Compton CC, Flotte T, Richter J, Nishioka N, Deutsch T. Ultraviolet laser induced fluorescence of colonic tissue: basic biology and diagnostic potential. Lasers Surg Med 12:63–78 (1992).
17. Lam S, MacAulay C, Hung J, LeRiche JC, Profio AE, Palcic B. Detection of dysplasia and carcinoma *in situ* by a lung imaging fluorescence endoscope (LIFE) device. J Thorac Cardiovasc Surg 105:1035–1040 (1993).
18. Ramanujam N, Mitchell MF, Mahadevan H, Thomsen S, Silva E, Richards-Kortum R. Fluorescence spectroscopy: a diagnostic tool for cervical intraepithelial neoplasia (CIN). Gynecol Oncol 52:31–38 (1994).
19. Vo Dinh T, Panjehpour M, Overholt BF, Farris C, Buckley FP, Sneed, R. *In vivo* cancer diagnosis of the esophagus using differential normalized fluorescence indices. Lasers Surg Med 16:41–47 (1995).
20. Schantz SP, Alfano RR. Tissue autofluorescence as an intermediate endpoint in cancer chemoprevention trials. J Cell Biochem Suppl 17F:199–204 (1993).
21. Franchesci D, Savage HE, Alfano R, Schantz S. *In vivo* fast Fourier transform analysis of the autofluorescence of normal and neoplastic upper aerodigestive mucosa. Surg Forum 45:488–493 (1994).
22. Kolli V, Savage HE, Yao TJ, Schantz SP. Native cellular fluorescence of neoplastic upper aerodigestive mucosa. Arch Otolaryngol Head Neck Surg 121:1287–1292 (1995).
23. Kluftinger AM, Davis NL, Quenville NF, Lam S, Hung J, Palcic B. Detection of squamous cell cancer and pre-cancerous lesions by imaging of tissue autofluorescence in the hamster cheek pouch model. Surg Oncol 1:183–188 (1992).
24. Glasgold R, Glasgold M, Savage H, Pinto J, Alfano R, Schantz S. Tissue autofluorescence as an intermediate endpoint in NMBA-induced esophageal carcinogenesis. Cancer Lett 82:33–41 (1994).
25. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium. Cancer 6:963–968 (1953).
26. Schottenfeld D, Gantt RC, Wynder EL. The role of alcohol and tobacco in multiple primary cancers of the upper digestive system, larynx, and lung: a prospective study. Prev Med 3:277–293 (1974).
27. Silberberg MB, Savage HE, Tang GC, Sacks PG, Alfano RR, Schantz SP. Detecting retinoic acid induced biochemical alterations in squamous cell carcinoma using intrinsic fluorescence spectroscopy. Laryngoscope 104:278–283 (1994).
28. Zhang JC, Savage HE, Sacks PG, Delohery T, Katz A, Alfano RR, Schantz SP. Innate cellular fluorescence reflects alterations in cellular proliferation. Lasers Surg Med (in press).
29. Sacks PG, Savage HE, Levine J, Kolli V, Schantz SP. Tissue autofluorescent identification of *in vitro* epithelial differentiation: a potential chemopreventive biomarker. Proc Am Assoc Cancer Res 36:588 (1995).
30. Sacks PG, Savage HE, Levine J, Kolli VR, Alfano RR, Schantz SP. Native cellular fluorescence identifies terminal squamous differentiation of normal epithelial cells in culture: a potential chemopreventive biomarker. Cancer Lett 104:171–181 (1996).