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# Oncologic Applications of Biophotonics

*Prospects and Problems*

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## Abstract

The understanding of various intrinsic photobiophysical processes has prompted researchers to develop different types of biodevices for health care. In the recent past, because of extensive contributions from various groups in the field of biophotonics, several important biomedical applications are emerging in the fields of both diagnostics and therapy. In this brief review, we discuss a few specific applications related to early detection and characterization of premalignant and malignant lesions using optical spectroscopic techniques, namely, fluorescence and Raman, and in management of cancer, the emerging scene of photodynamic therapy.

Index Entries: Fluorescence spectroscopy; Raman spectroscopy; tissue characterization; cancer detection; photodynamic therapy.

## Introduction

The quest for understanding the biochemical basis of human disease is the most important area in the development of modern medicine. Biochemical changes within tissue may either initiate disease or occur as a result of disease process. In physiologic systems, molecular changes often precede the onset of disease. These changes, if detected and interpreted properly, could provide vital information regarding the stages of disease progression and the effect of therapy. Malignant tumor causes clinical symptoms when it is usually large enough and can be detected by various conventional methods such as X-ray, computerized tomography, magnetic resonance imaging, ultrasound, and mammography. These techniques have their own advantages and limitations. Most of the advanced stage cancers are difficult to treat effectively, and, therefore, it is important to detect cancers at an early stage *(1)*. Many invasive cancers are preceded by premalignant

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alterations, such as dysplasia or carcinoma *in situ* (CIS) *(2)*, and no radiologic imaging technique is available to detect such physiologic alterations.

For example, in the bladder, small papillary lesions and *cis* are difficult to detect by conventional white-light cystoscopy, requiring random tissue biopsy to test for persistent or recurrent disease after treatment *(3,4)*. In the gastrointestinal (GI) tract *(2)* and tracheobronchial tree *(5)*, conventional white-light endoscopy has a low sensitivity for detecting dysplastic lesions. Patients who are at high risk owing to the presence of preexisting chronic conditions (e.g., ulcerative colitis) require more frequent monitoring of therapy and multiple biopsies. Assessment of the extent of dysplastic or superficial neoplastic disease is also important for optimal treatment and is often not adequately delineated by white-light imaging. Breast cancer has a high incidence among women and conventional X-ray and mammography cannot detect early stage tumors *(6)*. Oral cancer also constitutes a major share of cancers, and particularly in India, its incidence is very high because of bad chewing habits. Oral cancer passes through three stages: initiation, promotion, and progression. Leukoplakia, a premalignant condition of the oral cavity, appears as a white patch during the initiation stage and if not treated turns into malignancy. Oral cancer is symptomless in the early stages, and, therefore, it is necessary to develop a suitable method that will detect oral precancerous and cancerous conditions at an early stage.

Present methods for monitoring various stages of cancerous disease are mainly by pathology or clinical biochemistry coupled with various radiologic imaging modalities. Pathologic examination depends on morphology and is often subjective. Clinical evaluation depends on immuno- or biochemical methods, is time-consuming, and often is not sensitive in the early stages of disease *(7)*. Furthermore, in various cases, removal of tissues becomes very difficult or not possible. In addition, conventional histopathologic methods lack both the capability of providing immediate feedback and data for quantifying the extent of disease.

These clinical problems, combined with improvements in medical endoscopes, laser sources, optical fibers, imaging, nonimaging detectors, and most important, because of the analytical sensitivity of spectroscopic tools, have driven the development and evaluation of fluorescence and Raman spectroscopic methods for detection, characterization, and differential diagnosis of malignant and premalignant lesions in various body sites *(8–10)*. Laser-induced fluorescence (LIF) and Raman spectroscopic techniques are now being investigated extensively to derive qualitative and quantitative information on composition, morphology, biochemical interactions, and so forth in cells, tissues, and even body fluids *(7,11–13)*. Additional potential applications of these techniques are in providing guidance in locating the optimum sites for biopsy *(14)* to define the surgical margins for tumor resection and to design a light-delivery system for photodynamic therapy (PDT) of cancers *(15)*.

## Biologic Nature of Tissue Composition

Tissues are cooperative assemblies of different cells of multicellular organisms and, as such, exist in various compositions. The cells are held together by extracellular macromolecules in the matrix. Tissues depending on the location (e.g., epithelial, connective, smooth muscle) and the composition of extracellular matrix vary extensively. For example, the matrix can vary from the calcified, rock hard substance of bone and teeth to the transparent corneas or even ropelike structures of tendons. The extracellular matrix primarily consists of fibrous proteins, namely collagen, elastin, and fibronectin. The other major constituents of the extracellular matrix are polysaccharides and glycosaminoglycans. The structural proteins, collagen, and elastin can undergo both quantitative and qualitative change under pathologic conditions; for example, normal aortic wall is composed of up to 20% elastin and 30% collagen by dry wt, whereas in atherosclerotic lesions, collagen can account for up to 60% of the dry wt with <10% of elastin. In addition, cells contain a number of structural and functional macromolecules such as DNA, proteins, enzymes, nicotinamide adenine dinucleotide (NADH), heme proteins, and porphyrins. Since cancer is a disease of the cell, it may appear that information on these extracellular matrices may not be of much importance. But the matrix plays an important and complex role in regulating the behavior of the cells, influencing their development, migration, proliferation, and metabolic functions. In view of this, information on both cellular and extracellular matrix is equally important in understanding the biochemical processes that precede and accompany the onset of disease *(7)*.

Several constituents of proteins, namely amino acids such as tryptophan, tyrosine, and phenylalaline, are fluorescent in nature. In addition, NADH, flavins, porphyrins, lipopigments, as well as various extracellular constituents such as collagen and elastin have distinct emission characteristics. Tissue contains a mixture of these fluorescent molecules at different concentrations. The relative distribution of these molecules varies both with the type and origin of tissues and with the stages of disease. Tissue autofluorescence is owing to the presence of these fluorophores. The fluorescence spectroscopy of tissues thus depends on the origin of the tissues. Fluorescence properties of these molecules are sensitive to various physicochemical properties of the microenvironment. The detection of early stage cancer using tissue autofluorescence spectroscopy will therefore depend on the relative concentration, spatial distribution, metabolic status, and nature of the tissue. Tissue fluorescence has been used to differentiate normal and abnormal tissue in human breast and lung *(16,17)*, bronchus, oral mucosa *(18)*, and GI tract *(19–21)*. The initial results of fluorescence spectroscopy have shown to be promising for clinical diagnosis of precancer and cancer of these organs *(22–27)*.

Only a limited number of biological molecules are fluorescent in nature. Therefore to improve diagnostic capability, a more sensitive tech-

nique based on the vibrational motions of the molecule, known as Raman spectroscopy, has been considered, because a wide range of biomolecules including intracellular molecules such as DNA and protein have Raman active fingerprint vibrational spectra *(8)*. This spectral information can be used to identify and quantify changes up to the molecular level during disease processes with much greater detail. As a result, several groups have undertaken studies with the aim of detecting precancer and cancer *(28–30)*. In general, both fluorescence and Raman spectroscopic techniques are highly sensitive, specific, and objective, and measurement can be performed in a noninvasive mode in many cancers.

## Interaction of Light with Biomolecules in Relation to Fluorescence and Raman Spectroscopy

Light, an electromagnetic radiation, travels in space in the form of an oscillating electric field. When light interacts with atoms or molecules, the electric field of radiation tends to disturb or change the electron density of interacting atoms or molecules. A molecule, depending on its symmetry, has different electronic states, and on interaction with an electromagnetic field of light, either absorption or emission of radiation will occur and the system can reach another stationary state. The interaction of light with molecules leads to absorption only if a dipole moment is created, and during the process of emission, the dipole is destroyed. The stationary states are basically different electronic energy levels comprising various vibrational and rotational components. The order of energy associated with each electronic level is of the order of 160–600 kJ/mol, and the different sublevels, vibrational and rotational, are 20–40 and about 0.08 kJ/mol, respectively.

Figure 1 shows the different light-scattering processes for fluorescence and Raman spectroscopy. When a molecule absorbs a light photon, it interacts with the dipole moment of the molecule and undergoes various physical processes in different time scales ranging from subpicoseconds ($10^{-12}$) seconds to nanoseconds ($10^{-9}$). The first event is the instantaneous absorption of light energy ($10^{-15}$ s) by the interacting molecule in which the molecule is excited to the first singlet electronic state ($S_1$) from the ground electronic state ($S_0$), and in the excited state the molecule remains for a definite but short time period ranging from picoseconds to a few nanoseconds and then deactivates to the ground state ($S_0$) either by radiative or nonradiative processes. The radiative decay process is called as fluorescence and can be detected and analyzed by a conventional fluorescence spectrometer. Since the energy associated with fluorescence is always lower than the excitation energy, the wavelength of emission is always red shifted. Fluorescence spectral data are generally presented as an emission spectrum, which is a plot of the fluorescence intensity vs scanning wavelength. The spectral position of emission bands is very sensitive to the microenvironment and generally independent of excitation wavelength. Usually, fluorescence emission take place from the lowest vibrational level of the

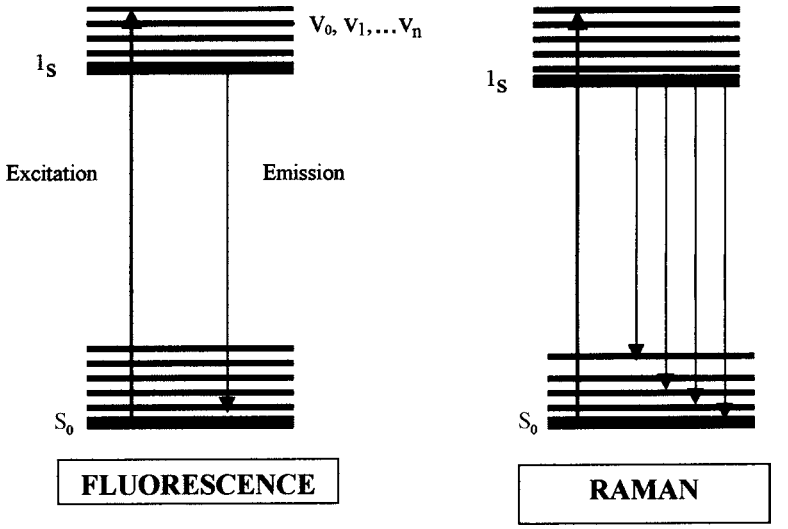


Fig. 1. Schematic diagram showing the various transitions involving interaction of light with molecules in fluorescence and Raman scattering processes. Thick and thin lines represent electronic ($^1S$, $S_0$) and vibrational levels ($v_0$, $v_1$, … $v_n$), respectively.

first excited state ($S_1$) to the lowest vibrational level of $S_0$. However, often some hyperfine transitions are observed in the form of sharp spectral bands owing to transition from lowest vibrational level of the excited state to different vibrational levels $v_0$, $v_1$ … $v_n$ of the ground electronic level ($S_0$). The intensity and spectrally allowed transitions are dictated by the molecular symmetry of the molecule under investigation. The hyperfine transitions, if present, are observed as sharp bands overriding the main emission band and provide additional information about the microenvironment. Emission properties of a molecule therefore depict the characteristic transitions of a molecule in the form of spectral bands in which each individual band is a marker of the molecule under investigation.

Raman scattering takes place owing to the changes in the polarizability component of the molecule during interaction with light. When an electromagnetic radiation interacts with a molecule, a dipole is induced and the oscillating dipole instantaneously generates a secondary wave that interferes with the incident wave, giving rise to secondary emission of different wavelengths: this phenomenon is called the Raman effect. Usually, Raman scattering involves transition between different vibrational levels of excited and ground electronic states. The spectral positions of different bands and their relative intensity in the Raman spectrum are strongly related to a group of theoretically allowed specific transitions corresponding to various vibrational motions arising from a group of atoms of the molecule under investigation. These vibrational motions are basically different types of interatomic vibrations such as stretching and angular motions from a group of atoms in a molecule constituting characteristic vibrational modes. The total number of these modes that are Raman active can be theoretically

calculated and have distinct spectral positions in a spectrum. Subsequently, these spectral positions can be used to generate fingerprint or marker bands for different biomolecules. In comparison to fluorescence spectroscopy, Raman spectral bands are very sharp and correspond to specific vibrational motions of a molecule. These vibrational features can be used to differentiate a particular chromophore of a molecule in the presence of other molecules such as DNA, protein, and various other interfering molecules. Most important, by using Raman spectroscopy, it is possible to selectively enhance specific vibrational modes of a molecule, and because of this advantage, Raman spectroscopy can be used to detect multianalytes from a single measurement. The fluorescence emission crosssection of a tissue fluorophore is always a million times larger than that of a Raman scattering cross section. Because of this, fluorescence severely interferes with weak Raman signals. This interference often prevents collection of good-quality Raman spectra. However, this problem can be circumvented by either using near-infrared (IR) excitation wavelengths or exciting the required sample at a wavelength away from its absorption maximum. Several groups have used near-IR Fourier transform Raman spectroscopy to record fluorescence-free Raman spectra from human tissues.

## LIF Spectroscopy in Oncology

The approach to characterize tissues by LIF spectroscopy can be classified depending on the type of fluorescence molecules investigated. The fluorescence molecules can be classified into three main categories: endogenous fluorophores that are responsible for native tissue fluorescence and are commonly known as tissue autofluorescence *(31)*, fluorescence owing to prodrug 5-aminolevulinic acid (5-ALA), in which a fluorophore protoporphyrin-IX (PP-IX) is synthesized in vivo after external administration of a precursor drug, 5-ALA, and whose characteristic fluorescence can be useful in differentiating between normal and abnormal tissues, because of the concentration of 5-ALA-induced fluorophore (i.e., PP-IX can be markedly different in these tissues and can be cell type dependent) *(32)*; and, finally, exogenous fluorescence owing to various externally administered fluorochromes. Currently, the last category of molecules has gained more importance because of the recent approval of photofrin II, a porphyrin-based fluorescent molecule for PDT of cancers. Photofrin II is a hematoporphyrin derivative that fluoresces strongly from cancer cells in the red spectral region (>600 nm) *(33)*. This fluorescence can play a significant role not only in detecting tumor tissues but also in defining the lesion margins for PDT, in assessing the effective dose, and in evaluating therapy. Several new photosensitizers have been developed for improving both detection and therapy of cancers, and many of these are in the advanced stage of clinical trials at various centers *(34)*.

The possible advantages of the fluorescence spectroscopic technique over routine clinical methods are high signal sensitivity, speed, and reduc-

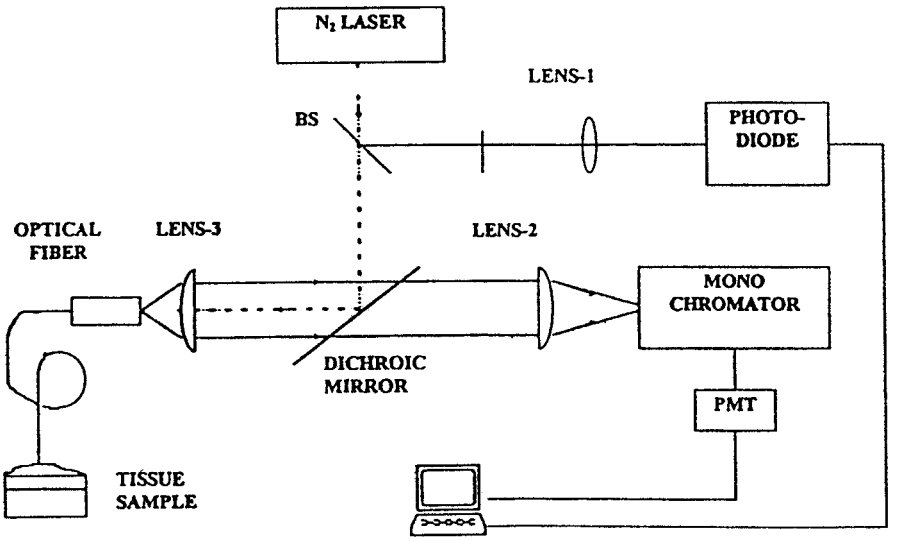


Fig. 2. Schematic diagram of the experimental setup for autofluorescence spectroscopy of tissues. BS, beam splitter; PMT, photomultiplier tube. (Reprinted with permission from ref. *35*.)

tion in the use of random tissue biopsies, especially when optical fiber coupled with a catheter-based system for sample handling is used. A fluorescence setup using nitrogen laser for biopsy sample is shown in Fig. 2 *(35)*.

### *Tissue Autofluorescence Owing to Endogenous Fluorophores*

Most of the endogenous fluorophores are associated with the various proteins, namely collagen and elastin, present in the structural matrix of tissue. Fluorescence also arises owing to intracellular biomolecules such as aromatic acids, porphyrins, and lipopigments. Metabolic processes in cells that include reduced NADH and flavins also contribute to the fluorescence spectrum, as does red porphyrin fluorescence owing to the presence of certain bacteria in body sites or in lesions. These fluorophores have distinct absorption and emission characteristics. Any given tissue contains a mixture of many fluorophores of different concentrations, and these fluorophores are not evenly distributed over the tissue depth. Thus, the fluorescence spectrum measured at the tissue surface will be different from that measured at a certain depth. For example, in hollow organs such as the bronchus, GI tract, and bladder there exist distinctly different layered structure, mucosa, and submucosa, each of which has a different fluorophore composition. The detection of premalignant lesions or early cancers using autofluorescence, then, depends on the changes in concentration, spatial distribution, metabolic status, biophysical and biochemical microenvironment, and tissue architecture. Sometimes these fluorescence characteristics also depend on the wavelength of light. A few selected examples for oncologic applications are discussed in the following sections.

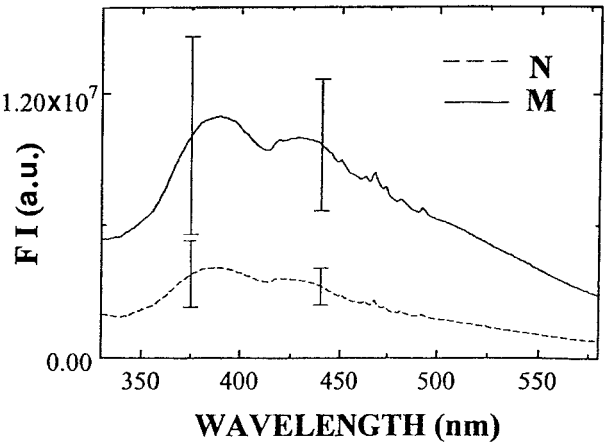


Fig. 3. Mean fluorescence spectrum of normal (N) and malignant (M) tissues with 310 nm excitation. The malignant and the normal tissue spectra are the average of the spectra of tissue samples from 11 patients. a.u., arbitrary units; FI, fluorescence intensity. (Reprinted with permission from ref. *35*.)

### *Breast Cancer*

Breast cancer is the most common type of cancer in women, and current breast cancer assessment is based on either voluntary breast examination or inpatient screening using conventional techniques. The diagnostic path follows mammography depending on whether the suspected lesion is palpable or nonpalpable and confirmation through biopsy. The physical and psychologic trauma associated with biopsy, the high cost per malignancy detected, and frequent exposure to ionizing radiation during mammography have motivated the current interest in developing fluorescence spectroscopy for characterization of tissues and diagnosis of lesions.

Figure 3 shows the 310-nm excited mean fluorescence emission spectra of malignant and normal tissue samples *(35)*. Autofluorescence from malignant and normal human breast tissues excited in the ultraviolet region at 310 nm showed a significant difference: the malignant tissues were reported to be more fluorescent compared with the normal tissues *(35)*. The individual spectrum depicted peaks at 390 and 440 nm, and these peaks were assigned to structural proteins and coenzyme NADH. The significantly larger fluorescence yield for the cancerous tissues may arise owing to either an enhanced concentration of fluorophores or a difference in the fluorescence decay kinetics.

In breast cancer, fluorescence-based diagnosis can be made during needle biopsy. This method can be particularly advantageous over X-ray mammography because the adverse effects of ionizing radiation can be minimized.

### *Oral Cancer*

Lesions in the oral cavity are mostly superficial in nature and can be very easily accessible to optical fiber systems. The LIF technique is particu-

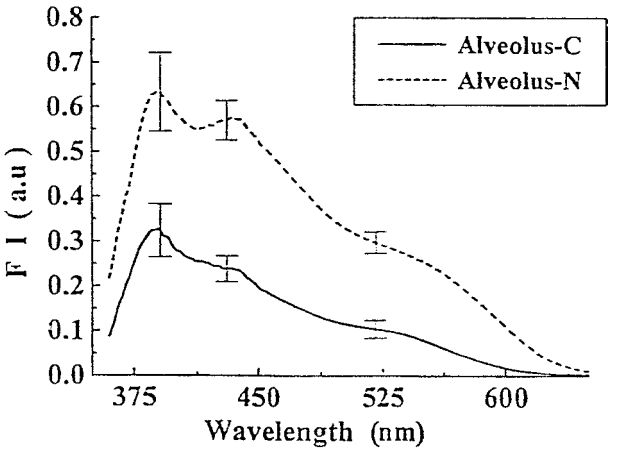


Fig. 4. Mean spectra of the cancerous and normal alveolus tissues with nitrogen laser (excitation wavelength of 337 nm). The cancerous tissue spectrum is the average of the spectra over 144 sites, and the normal tissue spectrum is the average of the spectra over 138 sites. a.u., arbitrary units; FI, fluorescence intensity. (Reprinted with permission from ref. *36*.)

larly suitable for early detection of lesions in the oral cavity owing to the easy accessibility of affected sites. Both excitation and the scattered signals from tissue sites can be collected and guided to the spectrometer by using same optical fiber. Studies performed on oral tissue samples by Majumder et al. *(36)* have shown that the fluorescence spectra are in general characterized by two bands centered around 390 and 430 nm and a shoulder at 520 nm (Fig. 4). The mean value of spectral intensity from normal tissue sites has been reported to be higher by a factor of 2 compared with that of cancerous tissues. The sensitivity and specificity of discriminating cancerous over normal tissues have been reported by various groups to be >90%. Significant differences have been observed in the spectrally integrated intensity from cancerous and normal oral tissue sites. The LIF-based approach is expected to provide early diagnosis and allow detection of premalignant alteration for which presently no effective noninvasive method exists.

### Tissue Fluorescence Owing to Prodrug (5-ALA)

5-ALA is a rate-limiting precursor in heme biosynthesis *(32)*. Oral administration of 5-ALA increases the synthesis of PP-IX, a photosensitizer, leading to generation of heme. Although excess PP-IX is metabolized within an hour, but remains for a longer duration in mucosal epithelium, on excitation at about 400 or at the 600-nm region, a typical red emission from PP-IX is observed. For example, in hollow organs, the accumulation of PP-IX is higher than in submucosal and muscular layers after topical application. Furthermore, 5-ALA-induced PP-IX tumor fluorescence has been observed to increase faster in tumor tissue and also decay rapidly; therefore, the observation time is extremely important *(37)*. In addition, the

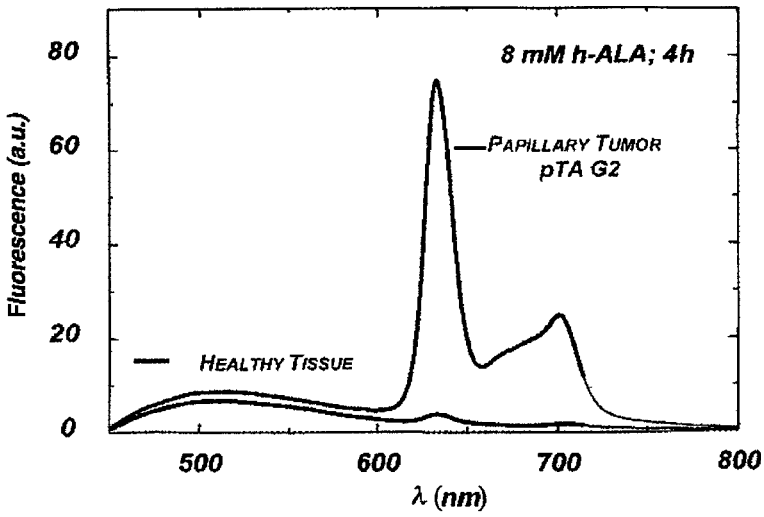


Fig. 5. Fluorescence spectrum of a small papillary tumor (pTA G2, top line) compared to the surrounding healthy tissue (bottom line) measured in vivo after 4 h of instillation of 8 m*M* h-ALA, a more effective prodrug than 5-ALA. The absence of PP-IX fluorescence in the healthy tissue indicates the high selectivity of 5-ALA. a.u., arbitrary units. (Reprinted with permission from ref. *40*.)

fluorescence intensity of 5-ALA-induced PP-IX between normal and cancerous tissues depends on pharmacologic and physiologic factors as well as disease stage. Considerable variation in PP-IX synthesis in tumor and normal cells leading to a difference in PP-IX fluorescence is reported in vitro studies. 5-ALA has also been investigated for fluorescence detection and localization of dysplastic and early stage malignant lesions *(38)*. In addition, variation in PP-IX fluorescence from lesion to lesion in the same patient in the case of cutaneous basal carcinoma has also been reported *(38)*. Such differential concentration in premalignant/malignant tissues may be owing to intrinsic difference in cells or the tissue microenvironment.

Although a number of variants responsible for 5-ALA-induced fluorescence are being studied in several centers, characteristic PP-IX fluorescence is being used to characterize tissues. Since PP-IX can be a potential drug for PDT of tumors, considerable research is also being conducted in 5-ALA-PDT for both detection of and therapy for cancers. Particular attention is being focused on 5-ALA-induced fluorescence to attain a differential effect between normal and cancerous tissues. Various aspects starting from drug delivery, distribution, time interval between administration and light irradiation, depth dose distribution, and synthesis of better 5-ALA derivatives are being studied in a few PDT research centers *(39,40)*. The 5-ALA-hexyl-ester (5-ALA), a new derivative of 5-ALA, has been used clinically to detect early carcinoma in urinary bladder. Figure 5 depicts the differential effect of 5-ALA on fluorescence from normal and papillary tumor for diagnosis *(40)*. Since 5-ALA-induced fluorescence depends on

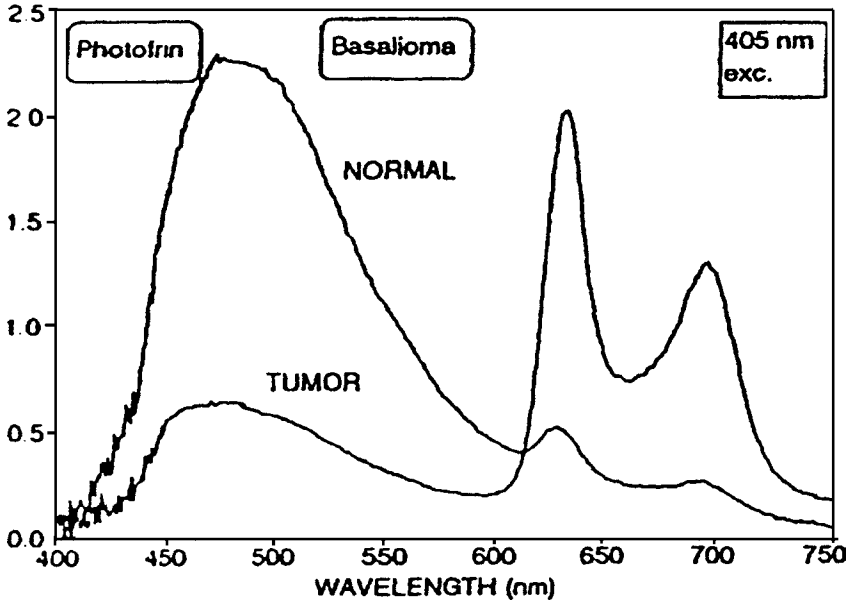


Fig. 6. Fluorescence spectra of superficial basal cell carcinoma after 48 h of administration of photofrin II. (Reprinted with permission from ref. *33*.)

various factors such as concentration, time interval between administration, and metabolic status of cells, more studies are required to quantify and utilize this prodrug fluorescence for the diagnosis of cancers.

### Fluorescence Owing to Exogenous Fluorophores

Most of the fluorophores in this category that are currently under investigation have been developed primarily as photosensitizers for PDT for cancers. Examples of such photosensitizers are photofrin II, a hematoporphyrin derivative, pheophorbide-a, meso-tetrahydroxyphenylchlorin (m THPC), benzoporphyrin derivatives (BPDs), and sulfonated aluminum phthalocyanines *(41–43)*. All these photosensitizers are known to localize and be retained for a longer duration in tumor as compared to the surrounding normal tissues, and on exposure to light, they emit a characteristic fluorescence signal that is usually higher than the tissue autofluorescence, thus increasing the possibility of detecting cancer tissues. This fluorescence is only from tumor volume, and, therefore, it can be helpful in demarcating/differentiating the treatment volume, sparing all the important surrounding normal tissues. Because of increasing acceptance of PDT for cancer management, fluorescence photodetection of cancer is becoming a very important area of research in clinical oncology. At present, the major focus is on the design and synthesis of a better drug that can be considered for more effective detection and therapy in PDT. Since the fluorescence signal from any of these photosensitizers is so high, large tissue surfaces can be easily scanned, by using fiber optic based endoscopy.

Several groups are working in the area of photodynamic technology to develop noninvasive fluorescence spectroscopy for detection of superficial lesions in hollow organs such as the lungs, bladder, GI tract, and esophagus. Figure 6 shows the fluorescence spectral scan from basal cell carcinoma and the surrounding normal tissues. The tumor area showed a distinctly different spectrum owing to accumulation of preadministered photosensitizer, photofrin II, which has characteristic emissions at 620 and 680 nm, whereas the surrounding normal areas showed a spectrum owing to tissue autofluorescence *(33)*.

Several other new photosensitizers synthesized for PDT could be suitable for fluorescence detection of early stage cancer. Dets et al. *(44)* fluorescence from orally administered hypercin (emission at 603 nm) for detection of GI and esophageal cancers and they demonstrated that this fluorescence from photosensitizer can be effectively manipulated with tissue autofluorescence (emission at 555 nm) for better tumor detection. Texaphyrin, a new infrared (IR) absorbing photosensitizer (732 nm), has been used for fluorescence imaging through overlying skin in rodent animal models *(45)*.

## Raman Spectroscopy in Diagnosis of Cancer

Raman spectroscopy in comparison to fluorescence spectroscopy is more versatile because many biomolecules have distinct fingerprint spectra and provide specific molecular information that can be applied to diagnose diseased tissue. Raman spectroscopy is a very powerful technique for vibrational spectroscopy of molecules. Vibrational spectra consist of bands that are sensitive to molecular structure, conformation, and the microenvironment. Various extracellular proteins can undergo qualitative and quantitative changes during pathologic conditions. These proteins (e.g., collagen and elastin) have marker bands in Raman spectra. For example, collagen shows two amide III bands at 1271 and 1284 $cm^{-1}$, and these two bands have been attributed to amide vibrations in the polar and nonpolar regions of collagen. Elastin shows spectral features characteristic of disorder protein conformation *(8)*. Many more molecules, such as amino acids and DNA, have Raman active bands with fingerprint spectra, providing specific molecular information that can be applied to diagnose diseased tissues. Molecular vibrations from these biomolecules (e.g., DNA and protein), can be selectively enhanced by resonance excitation in the wavelength region of 210–260 nm. This will result in strong enhancement of Raman spectral signals of aromatic acids such as tryptophan, tyrosine, and phenylanaline *(46)*. Nucleic acid bases such as adenine and guanine residues dominate the spectra with excitation above 250 nm *(47)*. Furthermore, using resonance Raman spectroscopy, more complicated studies such as growth kinetics using DNA contents as marker can also be used to diagnose disease stage in lesions. Raman spectroscopy thus has potential to provide quantitative information on biochemical aspects of tissue composition *(48)*. The possibility of direct detection of biochemical tissue constituents using

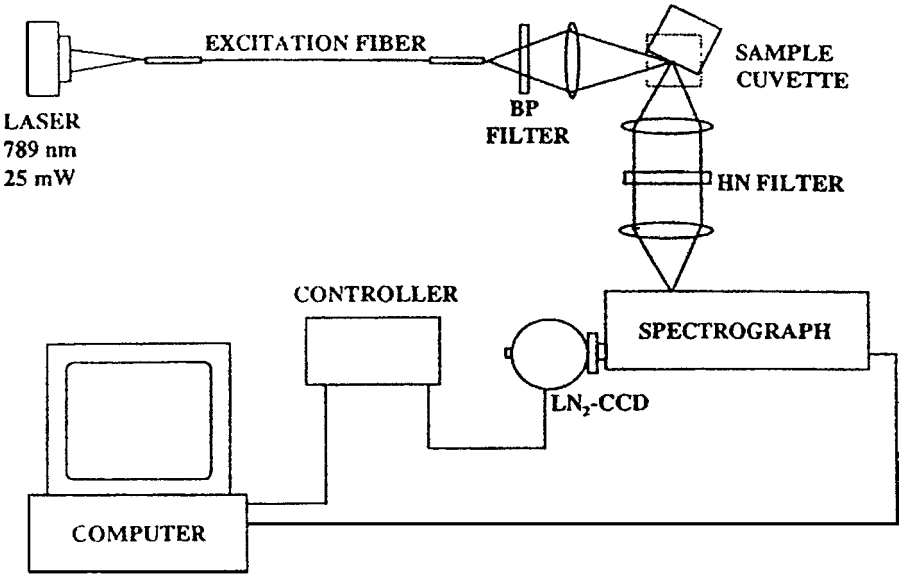


Fig. 7. Schematic diagram showing experimental setup to measure near-IR Raman spectra of tissues. (Reprinted with permission from ref. *50*.)

Raman spectroscopy can provide important information for classification, grading, and progression of disease.

Liu et al. *(9)* used Fourier transform Raman spectroscopy for detection of gynecologic cancer. Raman spectroscopy has also been applied to detect breast cancer *(29)*, colon cancer and atherosclerosis *(49)*, and even pre-cancers. All these studies have emphasized the molecular specificity of Raman spectroscopy. A typical schematic diagram of the near-IR Raman spectroscopic setup is shown in Fig. 7 *(50)*.

### *Breast Cancer*

Several groups have investigated the near-IR Raman spectroscopy *in situ* technique as an alternative method for characterizing and diagnosing breast cancer. Alfano et al. *(51)* first reported Fourier transform (FT) Raman spectra of breast tissue in 1989. Raman spectroscopy is being extensively used for biochemical characterization of lesions of breast tissues. Manoharan et al. *(29)* examined normal, benign, and malignant samples using 1064-nm laser excitation and correlated the disappearance of certain protein bands with pathology. Figure 8 shows the average spectrum of normal samples, which is dramatically different from those of benign and malignant lesions. Normal tissue spectra are dominated by the characteristic peaks of fatty acids (mostly triolein) with Raman shifts of 1657, 1442, and 1300 $cm^{-1}$, whereas cancer lesions are dominated by structural protein modes at 1667, 1452, 1260, 890, and 820 $cm^{-1}$. These spectral features can provide a simple empiric diagnostic algorithm that can be useful in clinics.

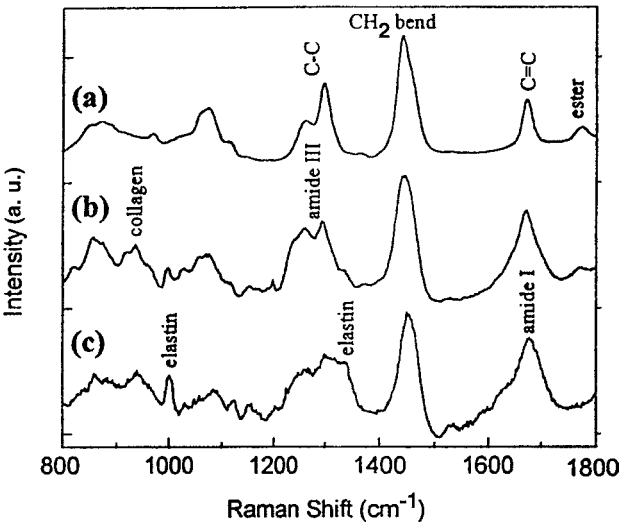


Fig. 8. Near-IR Raman spectra of three histopathologically distinct types of breast tissues: (a) normal, (b) benign, and (c) malignant. a.u., arbitrary units. (Reprinted with permission from ref. *29*.)

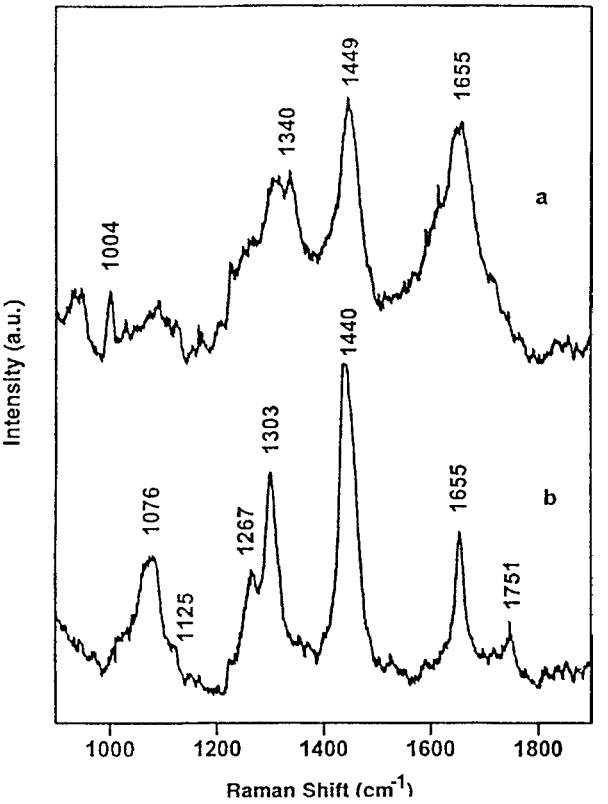


Fig. 9. Raman spectra of buccal mucosa: (a) malignant and (b) normal. a.u., arbitrary units. (Reprinted with permission from ref. *52*.)

Note that near IR-excited FT-Raman spectra of normal breast tissue samples reported by Alfano et al. *(51)* are dominated by proteins. This has been correlated with collagen, the major protein component in tumor. Many important spectral features can be used to differentiate normal tissue from lesions. For example, the C=C stretching band of lipid at 1757 $cm^{-1}$ in normal tissue is sharper compared to the amide I band of protein at 1660 $cm^{-1}$ in lesions. Similarly, there are many other marker bands that are characteristic of lesions. Most important, Raman spectroscopy can be performed using a minimally invasive needle probe for the diagnosis of lesions in breast tumors.

### *Oral Cancer*

Raman spectroscopy can be carried out *in situ* from the oral tissue site. However, most of the present studies are based on biopsy tissues. Figure 9 shows Raman spectra of oral tissues *(52)*. The bands at 1750, 1656, and 1304 $cm^{-1}$ and medium-intensity bands in the 1120 to 1065-$cm^{-1}$ region have been attributed to unsaturated lipid derivatives, and the intense bands arise from the membrane layer of epithelial cells. These include disappearance of the band at 1751 $cm^{-1}$ and broadening of the band at 1650 $cm^{-1}$. By comparing spectra from normal and malignant tissues, several changes can be observed. After thorough analysis, it has been concluded that the normal spectrum is predominantly of lipid, while the malignant sample spectrum is mainly owing to protein *(52)*. The data obtained from these studies suggested that the epithelial membrane structure might be damaged at the onset of malignancy. Spectroscopic studies performed with several normal and malignant samples have supported this conclusion. The available data demonstrated that the spectral differences could be owing to considerable damage/alteration as a consequence of malignancy.

### *Cervical Cancers*

Cervical cancer is the second most common malignancy among women worldwide. Management of cervical cancers is difficult because of the nonavailability of effective screening and detection techniques *(53,54)*. Liu et al. *(9)* have conducted near-IR Raman spectroscopy for diagnosis of malignant lesions in the gynecologic tract. The results of their studies and those of Mahadevan-Jansen et al. *(50)* suggest that Raman spectroscopy can be used for the diagnosis of cervical precancers and may be able to accurately separate samples with inflammation and metaplasia from precancers. The approach has been to identify the specific markers and then to formulate a suitable algorithm for characterization of a large number of tissues and cross validation with a histopathologic report. Figure 10 depicts the typical Raman spectra of normal, benign, and malignant tissues *(9)*. Spectra from these tissues exhibit peaks owing to amide I (~1656 $cm^{-1}$), $CH_2$ and $CH_3$ bending modes (~1445 $cm^{-1}$), and amide III (1240–1260 $cm^{-1}$). The relative intensities of amide I and C-H bending modes have been used to differentiate the different types of tissues.

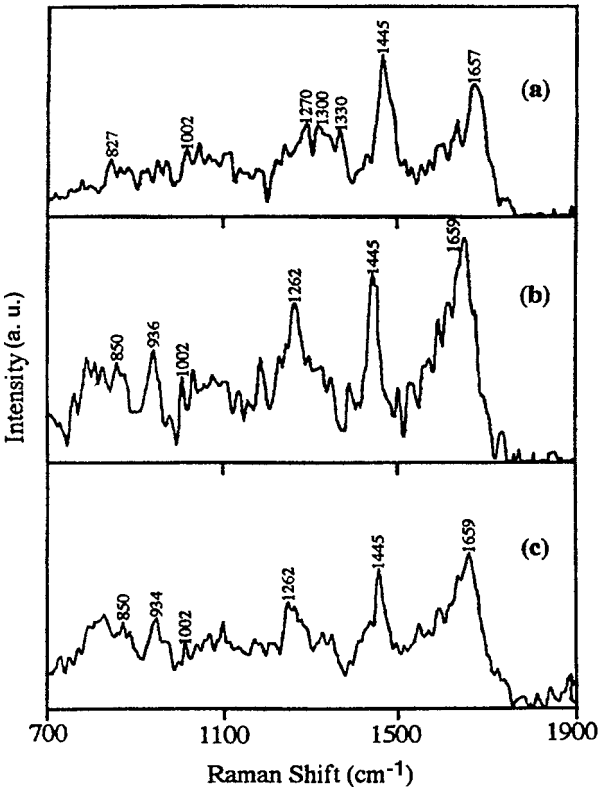


Fig. 10. FT Raman spectra of (a) normal, (b) benign, and (c) malignant cervical tissues. a.u., arbitrary units. (Reprinted with permission from ref. *9*.)

The changes in Raman spectra thus can be effectively utilized for early detection and identification of cancer, as well as for marking surgical boundaries. Already detection algorithms based on spectral changes have been developed in many centers and evaluated relative to histopathologic examination *(26,50)*.

## Prospects and Problems of Optical Spectroscopic Techniques

Optical spectroscopy is becoming a very powerful diagnostic tool. However, to develop a cost-effective system for routine clinical uses, an enormous amount of research still needs to be conducted. Development of fluorescence spectroscopy–based diagnostics in vivo will depend on several factors, such as the availability of fluorescence agents specifically for cancer detection, cost, and clinical suitability of the technology. In the case of Raman spectroscopy, build-up cost is always much higher because of the need for a high-quality laser source, spectrometer, and detection systems. The best advantage point in these spectroscopic techniques is that both the excitation and scattered optical signals from the target tissues can be guided through optical fiber, which can be suitably coupled to the spectrometer. Most important, the softwares necessary for quick diagnosis of specific

lesions need to be developed and evaluated at several centers. Cost-effectiveness is another important aspect that needs particular attention. At present, prototype systems are under development in various centers for surface studies in hollow organs. Since fluorescence properties are very sensitive to physicochemical characteristics, handling of tissues will play a very important role in diagnostic accuracy. A great amount of concerted research work is necessary to achieve the primary objective—tissue characterization prior to obtaining histopathologic information.

By contrast, Raman spectroscopy has greater potential for biochemical information that can be relatively more quantitative, and since already the diagnostic algorithms have been developed at different centers on an experimental basis, analysis of data from prototype systems needs to be critically evaluated with data from many patients. Raman spectroscopy, because of the complexity in instrumentation, will cost considerably more than the fluorescence-based technique.

## PDT of Cancers

One of the most significant advances in medicine in the last decade has been the development of PDT for cancers. PDT is now becoming an important weapon in the management of cancers for oncologists. PDT is a relatively simple two-step process. The patient is injected with a photosensitizer, and after a number of days or hours, the tumor to be treated is exposed to nonthermal light of the specific wavelength needed to activate the drug. The process by which light interacts with the photosensitizer and molecular oxygen in tumor tissue is known as photosensitization. PDT is perhaps the only application of biophotonics that deals with both detection and treatment. Photodynamic action requires the simultaneous presence of light, photosensitizer, and oxygen, which makes the treatment very selective and localized in nature.

In 1958, Peck et al. *(55)* first observed the accumulation of a photosensitizer, hematoporphyrin, in various types of cancer cells. Subsequently, Lipson et al. *(56)* synthesized hematoporphyrin derivatives, which have shown better localization in cancer tissues in various organs *(56,57)*. Profio and Doiron *(58)* and others *(59)* have demonstrated instrumentation for fluorescence-based detection of lesions. Finally, because of dedicated and systematic studies on animal tumor models and clinical studies performed by the group led by Dougherty et al. *(60)*, PDT has reached the stage of accepted treatment modality for several types of cancers. Using photofrin II as a photosensitizing drug, PDT is now in clinical practice in at least 10 countries, and in 1999, 8 more European countries applied for permission for clinical practice of PDT for the treatment of cancers *(61)*. The most promising treatment sites may be those where there is limited thickness of tumors, such as in superficial skin lesions, and in early stage carcinomas involving the aerodigestive tract, bronchus, urinary tract, and bladder. PDT offers several advantages over conventional modalities, such as chemotherapy, radiotherapy, and surgery. Tissue-sparing benefits

over surgery appear to be an important advantage of PDT. This treatment modality is minimally invasive, can be repeated several times, and apart from skin photosensitivity, it is not accompanied by significant morbidity. Compared to surgery or radiotherapy, PDT is a relatively benign procedure that produces good results from both from a cosmetic and a functional point of view. The cost-effectiveness of PDT is very high, and with the availability of new light sources, such as portable diode lasers in the red wavelength region, the cost is expected to decrease further.

PDT has been particularly successful in the treatment of lung cancers and of GI malignancy including gastric, pancreaticobilary, and colorectal cancer *(62)*. More than 1000 lung cancer patients have been treated with PDT in Japan and The Netherlands *(63)*. Skin cancer is one of the most common malignancies in the United States and Australia. Over the past 10 yr, PDT has been used for multiple types of cutaneous and subcutaneous (sc) malignancies. For primary lesions, investigators have reported high complete response (>80%) rates that are often durable and combined with excellent results. In the upper aerodigestive tract, PDT is being used for the treatment of malignant neoplasms and occasionally for benign lesions such as papillomas of the larynx. More recently, owing to the availability of better photosensitizers and light-delivery systems, clinical trials have also been initiated to treat larger dysplasia in Barret esophagus. Long-term survival after PDT for esophagus cancer suggests that this treatment modality is effective in high-risk patients with small tumors *(64)*. PDT appears to be effective up to a tumor depth of about 5 mm, depending on the nature of the photosensitizer and light source and the delivery systems used.

The potential of PDT has been greatly increased by the introduction of laser light and optical fibers. The optical fibers allow different modes of light delivery depending on the target sites and shape. For example, laser light can be guided through the fiberoptics in an endoscope in body cavities. For treatment of superficial areas, a simple lens producing uniform illumination may be used. The spatial distribution can be controlled by modifying the fiber end. Already a few specific light-delivery systems are available commercially.

## Limitations of PDT

Photodynamic action requires the simultaneous presence of light in a tumor volume containing photosensitizer, and because of very little penetration depth of light in skin, a few millimeters, the treatment volume is limited to about 1 $cm^3$. Photofrin II, the photosensitizer that is currently in clinical use, has a very low absorption coefficient in the red wavelength region and thus requires higher power to be delivered at the tumor volume. This also increases the laser irradiation time. Moreover, Photofrin II also causes skin phototoxicity in the presence of direct sunlight, which requires patients to be kept in subdued light for several weeks.

## Current Activities in Pharmaceutical Industries

Several pharmaceutical companies are conducting clinical trials with newly developed photosensitizers, and it is expected that many of the present limitations will be eliminated by these photosensitizers. QLT Phototherapeutics, Canada, is developing second-generation photosensitizers, BPD, a benzoporphyrin derivative of PP-IX, and sulfonated aluminum phthalocyanine. These photosensitizers have a longer absorption band above 650 nm with a much higher extinction coefficient. Other important companies that are actively involved in the development of PDT drugs are Scotia (United Kingdom) and PDT of California (United States). Scotia has developed m THPC under the trade name Foscan, for the treatment of head and neck cancers. This drug is in phase III clinical trial in Europe. PDT is studying Sn ET2 (tin etiopurpurin) for the treatment of cutaneous and breast cancer and autoimmune deficiency syndrome related Kaposi sarcoma. All these new photosensitizers absorb strongly in the red wavelength region (700–900 nm). DUSA (United States) is developing 5-ALA for treatment of a variety of skin disorders.

All these photosensitizers in PDT can also serve the purpose of detecting even small tumors. They emit red fluorescence when excited with blue light, and since these photosensitizers clear out from normal tissues, only tumor areas will emit the characteristic fluorescence. Although several groups are exploiting this fluorescence property for early detection of small tumors with different types of photosensitizers, the photodiagnosis using 5-ALA, a precursor for the generation of PP-IX, appears to be more promising.

Most important, owing to the ease in light-delivery systems and administration of photosensitizers, this treatment modality is rapidly expanding not only in different types of cancers but also in various other nononcologic disorders including microbial infections. Cost-effectiveness and simplicity of use of PDT make it very useful in developing countries such as India.

## PDT in Oral Cancers

Oral cancer encompasses cancers of the lip, tongue, and inner cavity of the mouth, and at present surgery and radiotherapy in combination or alone are the only modes of treatment available. These modalities are always associated with limitations and functional risks. Oral cancers can arise in clinically normal mucosa and often are preceded by malignant lesions. PDT has shown particular promise in local treatment of early malignancies. Different types of photosensitizers are currently being studied in clinical trials in several centers *(65,66)*. 5-ALA, a precursor for in vivo synthesis of PP-IX, a photosensitizer, appears to be very promising in the treatment of precancerous and cancerous conditions in the oral cavity. This prodrug is usually administered orally, and after 24 h of delay treat-

ment is initiated by delivering a light dose from a laser source. This new treatment modality for oral cancers is becoming successful in precancerous and cancerous conditions. At present, different types of topical formulations of 5-ALA for applications in lesions are being investigated in several PDT centers.

## Prospects of PDT in Management of Cancers

The major cost in terms of investment is in the procurement of laser sources such as dye lasers coupled with either Nd-YAG or argon ion lasers. These lasers require appropriate infrastructure and trained personnel for operation. The recurring expenditure toward maintenance is also substantially high. However, with the rapid developments in the field of solid-state semiconductor lasers, diode lasers are now becoming available in the red wavelength region, which is, in principle, most suitable for PDT applications with most of the new photosensitizers. Since bandwidth of emission from these diode lasers is generally very narrow, selection of the appropriate combination of laser and photosensitizer will be more critical. This will not only decrease the cost by an order of magnitude but also reduce the technical complexity involving the operation and maintenance of light sources. Light-delivery systems and *in situ* light dosimetry systems are still being developed at different centers, and, therefore, the present light dose estimation is rather empirical. At present only a selected type of light-delivery systems are commercially available for hollow organs (Diomed Limited, The Jafreys Building, Cowley Road, Cambridge CB4 4WS, UK). However, for treatment of skin cancers and lesions in oral cavity tumors, a simple lens that produces a uniform illumination can be used. Since photosensitizers can play a dual role in clinical practice as diagnostics and therapeutics, the same technology can be useful for better management of cancers with minimal invasiveness.

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