MEDICAL DIAGNOSTICS: A NEW OPTICAL FRONTIER

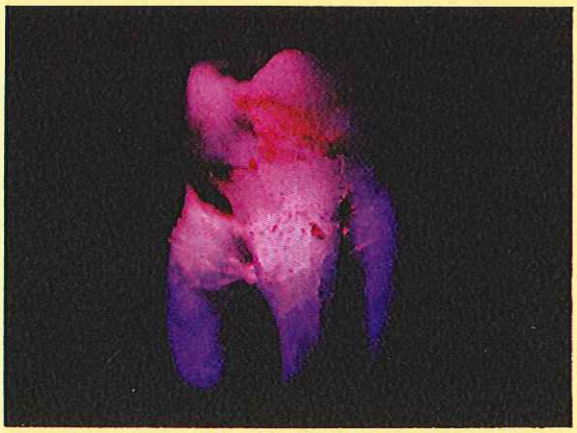
BY R.R. ALFANO AND MICHELE ANNE ALFANO

The diagnosis of diseases ranging from cancer and heart ailments to tooth decay and blood disorders has relied mainly on nuclear and x-ray radiation, or on invasive methods based on chemical laboratory analysis. In recent years, sound waves have been added to this diagnostic arsenal, with video and computer imaging using these modalities to enhance the detection of pathological changes caused by disease.

In view of the possible dangerous side effects of x-rays and nuclear radiation, a definite need exists for new techniques in disease detection that can either eliminate or reduce their use in examinations. Optical techniques are probably safer than x-rays and nuclear approaches, and are noninvasive. The visible and infrared regions of the electromagnetic spectrum have not yet been called upon for duty by the medical profession, but optical spectroscopy and laser technology offer techniques for the detection and characterization of physical and chemical changes that occur in diseased tissue.[1-3] The light energy lost from an incident beam upon its interaction with matter can be dissipated in a variety of radiative and nonradiative processes, such as luminescence, absorption, light scattering, internal conversion, vibrational relaxation and photochemical changes.

The successful application of spectroscopic techniques to the detection of cancer, atherosclerosis and dental caries indicates that optics could play an important role in the war against these diseases.

Most recently, there have been major breakthroughs in the use of luminescence spectroscopy as a diagnostic tool. Alfano and Yao[4] first introduced this weapon to the medical arsenal in 1981, when tooth decay was detected for the first time using luminescence spectroscopy instead of x-rays. Differences in the luminescence spectra of carious and noncarious regions of teeth were clearly detected.



A tooth illuminated by blue light from a lamp, and viewed through optical filters, shows dental caries and bacteria as red areas. Photograph by Yury Budansky and Philip Carvalho.

This pioneering work was later extended to the diagnosis of cancer by Alfano et al.[5] in 1984, and to atherosclerosis by Kittrel et al.[6] in 1985. Other optical approaches using light scattering and absorption spectroscopies were also employed to show the differences between the spectra of normal and abnormal tissues.[7]

This paper, after summarizing the research on the detection of cancer,[5] tooth decay[4,7] and atherosclerosis[6] using optical spectroscopy, will describe conceptual designs of optical systems to be used for such diagnostic applications.

Optical spectroscopy methods

The experimental arrangement used to measure the luminescence spectra from the various teeth and tissues is shown in Figure 1.[4,5,7] An argon-ion laser operated at 488nm was focused on the front surface of the samples.

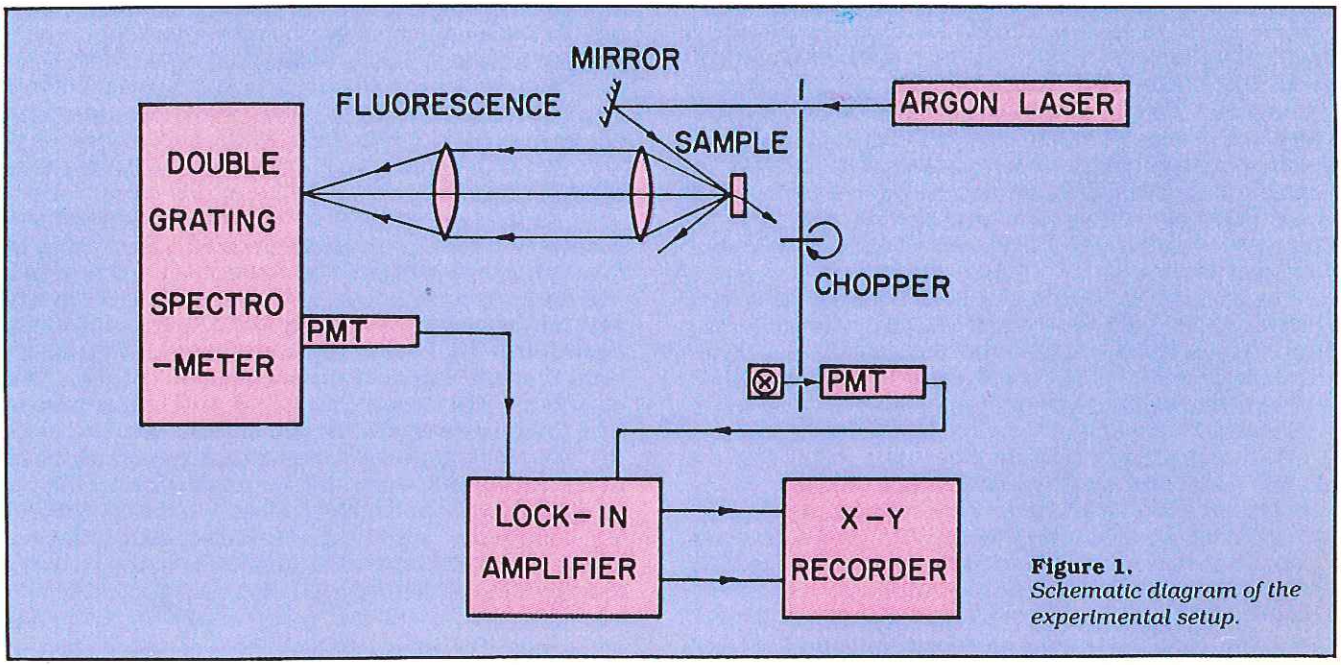


Figure 1. *Schematic diagram of the experimental setup.*

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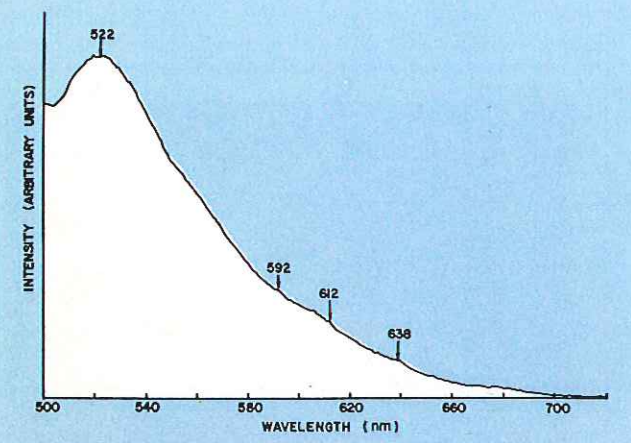

Figure 2a.
Fluorescence spectrum of a kidney tumor in a rat.

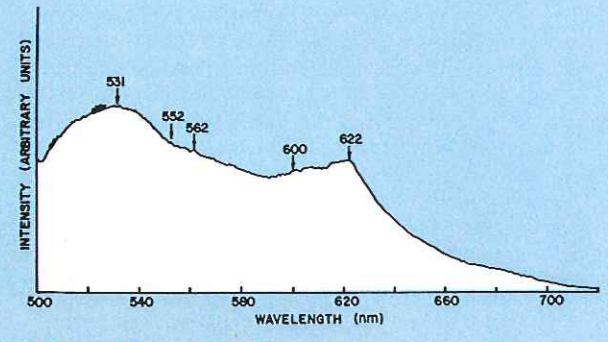

Figure 2b.
Fluorescence spectrum of a normal rat kidney.

The luminescence from the surface was collected into a double Spex-½m scanning spectrometer blazed at 500nm, and a photomultiplier tube (PMT) located at the exit slit of the spectrometer measured the intensity at different scattered wavelengths. The output of the PMT was connected to a lock-in amplifier/recorder combination to display the scattered intensity at each wavelength. Light from the scatterplate was used to calibrate the spectral response of the system.

Cancer diagnosis

Fluorescence from cellular macromolecules had been studied in the 1950s and 1960s.[9,10] It was discovered that the fluorescence spectra of intact cells coincided with tryptophan containing proteins, and that the most intensely fluorescing component in the cell was the mitochondrion. There are several natural fluorophors within the cells that fluoresce in the visible regions. Flavins, for example, are known to fluoresce and exhibit spectral changes when transforming from the oxidized to the reduced state. Riboflavins are known to fluoresce in the visible spectrum and are part of the coenzyme flavin adenine dinucleotide that is responsible for oxidation reduction in the mitochondria. Chance et al.[10] demonstrated flavin fluorescence in mitochondria of pigeon heart cells with the fluorescence peaked at 522nm. It has been shown that the spectra maxima of flavins span from 520 to 535nm, depending on the environment.[11] Furthermore, porphyrins have been found in nature to be highly fluorescent, with naturally occurring porphyrins found in hemoglobin, urine, stool and in erythrocytes. The principal porphyrins' fluorescence bands lie between 590 and 640nm.[3]

The visible luminescence spectra from native animal tissues show different spectral characteristics of emission from cancerous and normal tissues of kidney, bladder and prostate,[5] and there appear to be well-defined spectral fingerprints for both the cancerous and normal tissues.

The spectral curves[5] for the cancerous and normal rat kidney tissues are displayed in Figure 2. The differences are very noticeable. The prominent maxima in the spectra from rat prostate tumor (not shown) and normal rat prostate are located at 521 and 533nm, respectively. The prostate tumor spectrum has two subsidiary maxima located at 552 and 593nm, while no additional maxima are recorded in the normal prostate spectrum.

The main maxima in the spectra from rat kidney tumor, Figure 2a, and normal rat kidney, Figure 2b, are also located at 521 and 531nm, respectively. After the first prominent peak, the spectrum from the rat kidney tumor decreases monotonically, with three small peaks.

The most salient differences between the cancerous and the normal tissues' spectra are that the spectral profiles are very different, and that the cancerous prominent maxima are blue-shifted and located around 521nm, whereas the prominent maxima of the normal tissue's spectra are located at about 531nm. These differences should occur in humans with their own maxima.

Visible luminescence spectra emitted by natural fluorophors, within the intact cells, are most intriguing and clearly display a unique set of spectral features that characterize the state of cells making up the tissues. Salient differences in the spectral profiles of malignant and normal tissues are probably attributable either to changes in the environment of the fluorophors, or perhaps to production of new fluorophors induced by changes within the cellular environment. It is well documented that the internal environment of cancer cells is physiologically and biochemically different from their normal counterparts. Depending on the environmental conditions, flavins are known to fluoresce at 520 to 535nm. If no new fluorophors are produced (ones that fluoresce at 520 to 535nm) due to the environmental transformations, then one may speculate that the most likely fluorophors giving rise to our spectral signatures must be flavins in the mitochondria.

When protein containing fluorophors either gains positive or loses negative ions, the fluorescence from the fluorophors is blue-shifted. The prominent maxima of all cancerous spectra in our results exhibit a 10nm blue shift, suggesting an accumulation of positive or a depletion of negative ions in the mitochondria of cancerous cells, thus causing the flavins to emit at 521nm instead of 531nm. It is known that tumor cells entrap positive ions to a greater degree than do normal cells.[12]

The secondary peaks common to all tissue spectra between 590 and 640nm may be attributable to the porphyrins. Heme proteins containing metalloporphyrins are commonly found within hemoglobin and cytochrome in the mitochondria. When iron or copper are removed from the heme porphyrins, they fluoresce in distinctive principal and secondary bands. Depending on the environment, the principal band of various porphy-

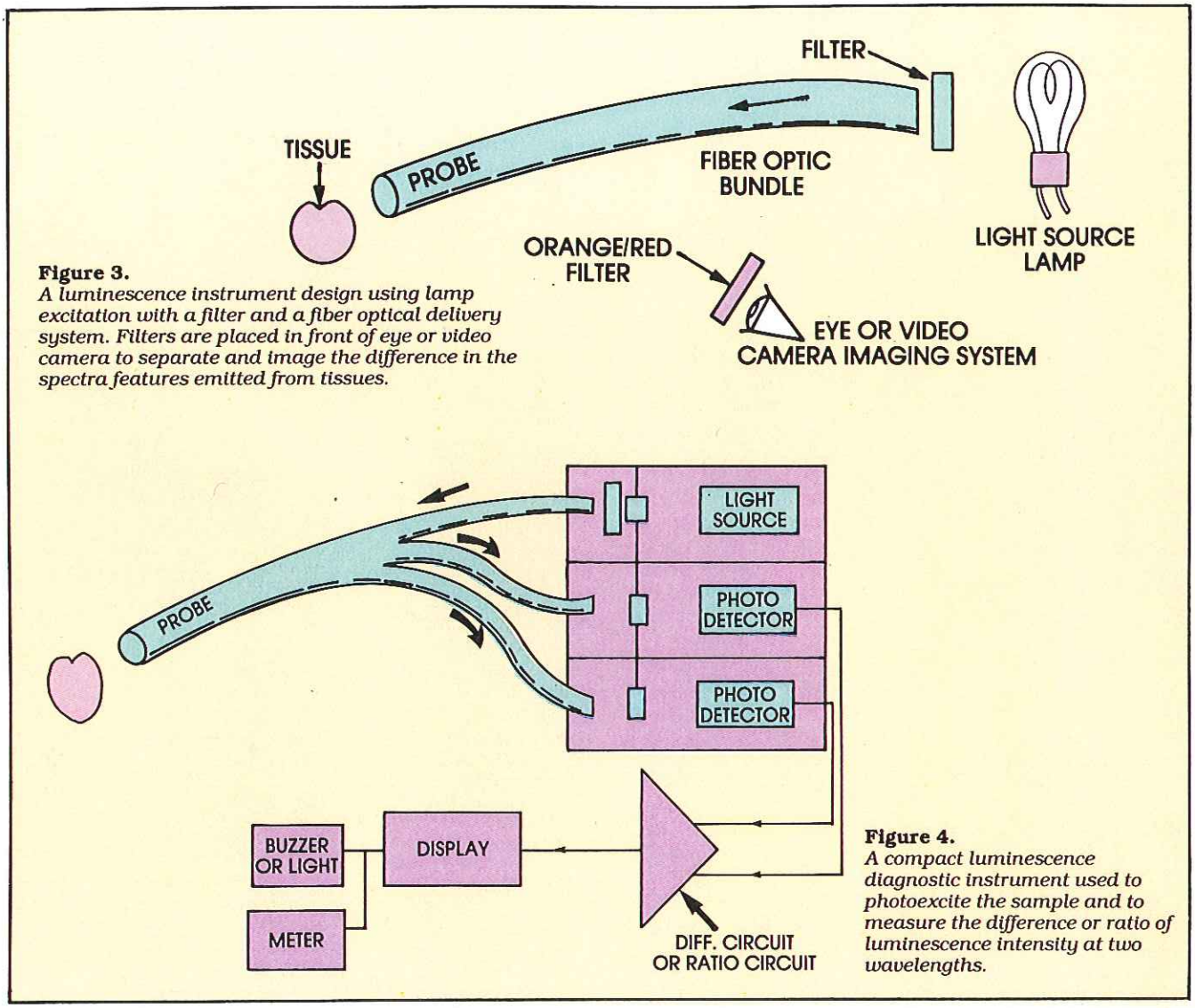

Figure 3.
A luminescence instrument design using lamp excitation with a filter and a fiber optical delivery system. Filters are placed in front of eye or video camera to separate and image the difference in the spectra features emitted from tissues.

Figure 4.
A compact luminescence diagnostic instrument used to photoexcite the sample and to measure the difference or ratio of luminescence intensity at two wavelengths.

rins lies between 590 and 634nm. In an acidic environment, coroporphyrin and protoporphyrin have their bands at 597 and 603nm, respectively. In view of these results, spectra display signatures in 590 to 640nm may arise from porphyrins, with the intensity of porphyrins' peaks weaker in cancerous samples. This would suggest that the environment within the cancerous cells may be transformed to such a degree that the metal components dissociate from the porphyrin part, yielding different characteristic fluorescence of various porphyrins that indicates their regional environment.

The visible luminescence spectra from native cancerous and normal tissues have been measured, and the emission spectra profiles of the cancerous and normal tissues found to be substantially different, each displaying their own characteristic prominent maxima and other spectral marks. Currently, research in this area is in its embryonic stage, and further research is warranted in order to check the consistency and reproducibility of these spectral fingerprints of human tissues, with the hope that they may become a viable diagnostic tool in cancer diagnosis and research.

Optical technology offers techniques for the detection and characterization of physical and chemical changes that occur in calcified tissue. Differences in the visible luminescence spectra and elastic light scattering of carious and noncarious regions of the teeth exist, with carious regions scattering and emitting more light for a wavelength greater than 560nm than noncarious regions.[4,7] Three different experimental methods were employed in these investigations: absorption, light scattering and fluorescence. We will review the latter two.[7]

Typical light-scattered spectra measured from carious and noncarious regions on the same tooth for two different teeth were examined. Normalizing the peak of the scattered spectrum of the cavity equal to the peak of the noncavity at 530nm, and correcting for the spectral response of the detection system, we find that carious regions (relatively) scattered more light in the red area than noncarious regions with respect to the peak (at 530nm). For different teeth investigated, the scattered intensities from carious lesions were found to be smaller by 0.8 to 5 times the intensities from the same size noncarious regions. The sizes of caries regions ranged from 0.5 to 3mm.

Fluorescence spectra of teeth can be excited at 488nm with either a noncoherent (lamp) or coherent (laser) beam. At a given wavelength, the differences in the intensities of fluorescence between the carious and noncarious spectra vary across the spectrum, with the normalized spectral curves showing more light emitted from caries in the red portion of the spectrum relative to

the fluorescence peak (see photograph). This result is consistent with our light scattering data. The fluorescence probably arises from traps of unknown origin. Similar fluorescence spectra were measured for both laser and lamp excitation at 488nm; and although laser-induced fluorescence is more enhanced at a longer wavelength as compared to incoherent excitation, the spectral shapes are quite similar.

Our light scattering and fluorescence measurements found that caries scatter and emit more red light relative to its peak than do adjacent noncarious regions — a result traceable to the fact that caries absorb more light than noncarious regions in the 400-to-600nm spectral domain, as indicated by reduction in the scattered light. The optical techniques may offer a method to observe incipient decay by measuring changes in the fluorescence or scattering intensities and changes in the spectral shapes emitted by a carious region as compared to adjacent noncarious regions.[6]

These optical techniques are quite sensitive, being microscopic in origin, and therefore may allow for *early detection of decay and preventive maintenance.* If the origin of the fluorescence from tooth (or bone) were understood at the molecular level, perhaps a better understanding of the process of dental caries (and bone disease) would be attained.

Atherosclerosis diagnosis

Atherosclerosis is a major medical problem for the middle-aged and elderly, and x-ray visualization of arteries followed by injection of radiopaque material via a catheter is the common method of diagnosis. Recently, Kittrel et al.[6] used the fluorescence spectra on extracted carotid artery to study plaque formations. Normal and diseased samples exhibit two peaks near 550 and 600nm. The intensity of the 600nm peak was smaller than the 550nm peak in the diseased samples. The valley between the peaks at 580nm was used to normalize the spectra from the tissues. The ratio of intensity at 600nm to 580nm for normal artery was 2 and for diseased artery was unity. Thus optical spectroscopy offers a means to determine whether a given arterial wall is diseased.

Optical diagnostic instruments

Possible design of a diagnostic instrument[8] that can detect the presence of caries in teeth or cancer in tissues is shown in Figures 3 and 4. This type of instrument, which we have called DECALUS — an acronym for detection of cancer (or caries) by luminescence spectroscopy — illuminates a sample region to be examined with a beam of monochromatic light, say 410 or 488nm, by a laser or lamp with a narrowband filter, and then measures the intensity of luminescence at different wavelengths. The simplest way to accomplish this is to use two optical filters and measure the intensity at two wavelengths where the luminescence from normal or abnormal tissue is different (see Figures 3 or 4). The intensity ratio or difference at two wavelengths is measured to determine whether a tissue is diseased. The human eye (Figure 3) or photodetectors (Figure 4) can be used to detect the luminescence. The types of photodetectors that can be used are photodiodes with amplifiers, photomultipliers or video SIT cameras. The difference, or ratio, between the signals at two wavelengths is stored and displayed to indicate the presence of disease.

A better system couples a spectrograph to a video camera-computer in order to display and compare the difference of the entire spectra profiles. Optical multiple-channel video systems are available from EG&G PAR and Hamamatsu. A video imaging system coupled to filters or a spectrograph can be used to detect breast cancers for optical mammography. The spectral image can be stored in a computer and differences in spectral profiles of the tissues displayed to show cancerous regions.

Much more research will be required to measure the characteristic spectral features in tissues and catalog the luminescence spectra from different human organ tissues. One can envision a strategic defense against disease using optical fiber spectroscopic instruments[8] for diagnostics that offer high speed and spatial resolution for *in vivo, in vitro,* and surgical use. □

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This article is an invited contribution.