LA-UR- 93-2764

Title: OPTICAL DIAGNOSTICS BASED ON ELASTIC SCATTERING: RECENT CLINICAL DEMONSTRATIONS WITH THE LOS ALAMOS OPTICAL BIOPSY SYSTEM

Author(s): I. J. Bigio, T. R. Loree, J. Mourant, T. Shimada, K. Story-Held, R. D. Glickman, and R. Conn

Submitted to: Biomedical Optics Europe '93, Budapest, Hungary, September 1-5, 1993

Los Alamos
NATIONAL LABORATORY

Form No. 836 R5
ST 2629 10/91

Optical diagnostics based on elastic scattering: recent clinical demonstrations with the Los Alamos Optical Biopsy System.

Irving J Bigio, Thomas R. Loree, Judith Mourant, Tsutomu Shimada, K. Story-Held*, R.D. Glickman* and Richard Conn**

Los Alamos National Laboratory
CLS-5, MS-E543, Los Alamos, NM 87545, USA
*University of Texas Health Sciences Center, Department of Ophthalmology
San Antonio, TX 78284-6230 USA
**Department of Urology
Lovelace Medical Center, Albuquerque, NM 87108 USA

ABSTRACT

A non-invasive diagnostic tool that could identify malignancy *in situ* and in real time would have a major impact on the detection and treatment of cancer. We have developed and are testing early prototypes of an optical biopsy system (OBS) for detection of cancer and other tissue pathologies. The OBS invokes a unique approach to optical diagnosis of tissue pathologies based on the elastic scattering properties, over a wide range of wavelengths, of the microscopic structure of the tissue. The use of elastic scattering as the key to optical tissue diagnostics in the OBS is based on the fact that many tissue pathologies, including a majority of cancer forms, manifest significant architectural changes at the cellular and sub-cellular level. Since the cellular components that cause elastic scattering have dimensions typically on the order of visible to near-IR wavelengths, the elastic (Mie) scattering properties will be strongly wavelength dependent. Thus, morphology and size changes can be expected to cause significant changes in an optical signature that is derived from the wavelength-dependence of elastic scattering. The data acquisition and storage/display time with the OBS instrument is ~1 second. Thus, in addition to the reduced invasiveness of this technique compared with current state-of-the-art methods (surgical biopsy and pathology analysis), the OBS offers the possibility of impressively faster diagnostic assessment.

The OBS employs a small fiber-optic probe that is amenable to use with any endoscope, catheter or hypodermic, or to direct surface examination (e.g., as in skin cancer or cervical cancer). It has been tested *in vitro* on animal and human tissue samples, and clinical testing *in vivo* is currently in progress.

2. BACKGROUND AND SIGNIFICANCE

Early diagnosis appears to be an essential requirement for successful treatment of cancer, and tissue/cell analysis is the *sine-qua-non* key to diagnosis in oncology. The analyses are always accomplished *in vitro*: a biopsy is performed (or a sputum sample or swab is collected) and the tissue (or a cell group from a swab) is microscopically

assessed by a pathologist. The results are then related to an *in vivo* condition. The physician is limited in the number of sites he/she can biopsy, and in many cases is only likely to choose sites where visible gross changes exist. If the macroscopic manifestations of cancer (hyperplasia, vascular proliferation, etc.) are not yet obvious to the naked eye, the physician is more likely to detect early malignancy with a system that can make rapid measurements on a larger number of sites.

Internationally, there is a strong emphasis on early cancer detection and on the reduction in the cost of providing medical care. Both of these issues would be directly addressed by the OBS technology.

There are obvious clinical benefits in an ability to determine malignancy of various tissues *in situ* and instantly. For example, during routine colonoscopy (especially in the presence of ulcerative colitis) it is envisioned that the physician could simply touch the tip of the optical fiber probe to any suspect tissue and the OBS would immediately display the signature, allowing the physician to recognize early malignant conditions "on the spot". In this manner more sites could be assessed than would be possible with conventional biopsy techniques. It should be noted, however, that the non-invasive benefit of the OBS technique is limited to detecting malignancy that is at, or near, an organ surface that can be reached by fiber. (The use of a syringe needle would be required to reach a suspected interstitial site such as liver or prostate.) When compared with some of the other optical techniques under development, the proposed OBS technique would have the additional advantage of not requiring the administration of drugs such as hematoporphyrin derivatives (HPD's) or other targeting fluorescers with their potential concomitant side effects.

3. OTHER APPROACHES TO OPTICAL BIOPSY

Other attempts at *in situ* real-time optical diagnostics have generally invoked single-color UV illumination and recording of the autofluorescence signal from the tissue.[1] Laser-induced fluorescence spectroscopy (LIF) has limitations of when applied to complex and heterogeneous biological media since patient-to-patient variations can mask the differences between similar tissues (or subtle changes in a given tissue). Thus, LIF has shown limited reliability in detecting malignancy[2] except with the (invasive) application of exogenous fluorescent dyes or drugs such as HPD used as targeting fluorescers[3,4,5] or under highly-controlled conditions with sophisticated multicolor detection.[6] The limitation is due primarily to broadly-absorbing chromophores with broadband and relatively featureless fluorescence emissions that overlap, compounded by variations in patient conditions (blood pH, hemoglobin saturation, etc.), which can add to the confusing variety in the "normal" condition.

A partial solution to the problem is to collect fluorescence spectra for a sequence of illumination wavelengths, from the UV through the visible. The different excitation wavelengths might be expected to variously excite different chromophores, resulting in more complex emission patterns with more information. Recently this approach, sometimes called "fluorescence contour mapping", has been used to generate more informative fluorescence spectroscopy signatures.[7] The technique is based on earlier developments in the field of chemical engineering.[8,9] In this method fluorescence

spectra are taken for a sequence of different excitation wavelengths. The data are then presented in a two-dimensional contour plot, graphing the fluorescence wavelength against the excitation wavelength for a given intensity. This matrix can be viewed as a topographic spectroscopic map whose topography comprises a group of "hills". The displayed shapes constitute an enhanced (more detailed) autofluorescence signature of the tissue, resulting from biochemical changes, with greater likelihood of distinguishing malignancy from normal conditions.[10]

4. OUR APPROACH

But what does a pathologist normally look for? After preparing a slide, a pathologist performs a microscopic assessment (histopathology) of the cell architecture or morphology: the sizes and shapes of cells, the ratio of nuclear to cellular volume, the form of the bilipid membrane, clustering patterns, etc. These changes will have an effect on the elastic scattering properties, separate from any inelastic scattering due to fluorescence. Thus, a more direct optical solution to the problem is to generate a signature that comprises data from the elastic scattering and absorption - the optical transport properties of the tissue. In cases where useful fluorescence information is also available, an instrument with the most general capabilities would also collect that data.

We have constructed and demonstrated an optical biopsy system (OBS) that is fiber-optic mediate; it collects and displays data in approximately 1-3 seconds (depending on what range of data is collected). The OBS invokes a special fiber-bundle design that allows the system to collect data on the elastic scattering and absorption properties of the tissue, in addition to the multiple-excitation-color fluorescence data if desired. Our laboratory and clinical tests indicate that these data result in a more reliable diagnostic signature than is possible with fluorescence data alone, in the organ areas tested. We have designed the OBS fiber probe to be used in optical contact with the tissue expressly to facilitate the collection of the tissue multiply-scattered signal. The fiber bundle is less than 1 mm in diameter, being easily reduced further in size, and can be passed readily through the working channel of most endoscopes.

Currently we are developing a simpler system that is limited to collecting only the elastic-scatter/optical transport signal. This system is inexpensive, smaller and faster. Although we sacrifice the fluorescence data with this system, preliminary measurements have indicated that in many applications the "backscatter" signal alone is reliable for cancer detection, and in some cases it may provide the only useful signature.

It should be noted that the OBS does not image a tissue surface: rather, it provides a large amount of information about the optical transport and fluorescence properties of the tissue, for one spot at a time (where the fiber probe contacts the tissue).

5. SYSTEM DESCRIPTION

Light incident on tissue may be reflected from the surface, scattered or absorbed. Because our probe is designed to be placed in direct contact with the tissue surface, as illustrated in Figure 1, we avoid collecting light from simple surface reflection. Both the

illuminating light delivered by the fiber(s) from the light source, and any fluorescence generated in the tissue, must scatter through the tissue (thus experiencing the elastic scattering and absorption properties of the tissue) before reaching the separate collection fiber(s) that lead to the spectrometer.

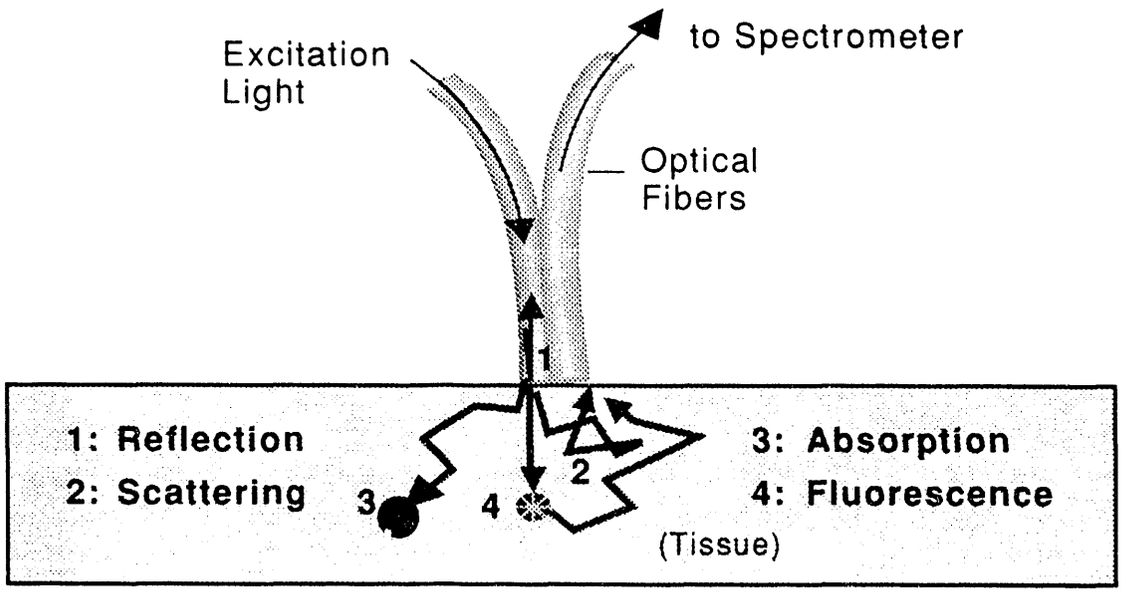

Figure 1. Light that enters the tissue from the illuminating fibers is modified by both elastic (optical transport) and inelastic scattering processes before being collected by the fibers that lead to the spectrometer.

<u>The major elements of the Optical Biopsy System:</u>

- The most general manifestation of the OBS employs a rapidly-tunable light source. Light from a pulsed xenon point-arc lamp is collimated and focused on the entrance slit of a rapidly-tunable mini-monochromator. The source is capable of generating light pulses at ≥30 different wavelengths per second over the range <280nm to >1000 nm. (In typical use the range utilized is ~280-850 nm, with sequential pulses every 10 nm.)

- The tuned light source is imaged onto one end of the "illumination" fibers that then join with the "collection" fibers to form the small (~0.8 mm diameter) fiber bundle of the optical probe.

- The collection fibers transmit the "backscattered" signal from the tissue back to the entrance slit of a small spectrometer. The spectrally dispersed signal at the exit plane is read by a multiple-element detector, such as an intensified diode array or CCD detector.

- The detector is interfaced, through a buffer/controller, to a PC.

- System software controls the illumination source and the data acquisition process, and then displays the signature on the computer's CRT. The signature can be presented in various ways, including false-color and quasi-3D displays.

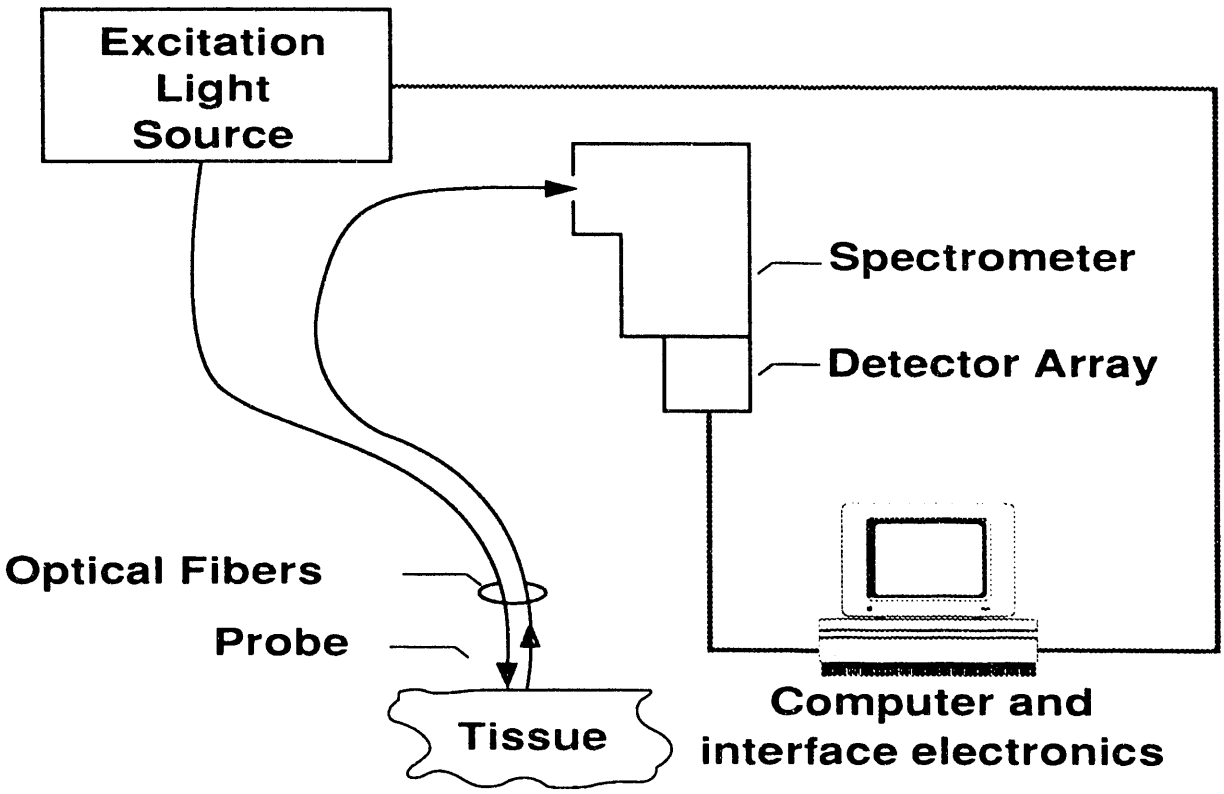

Figure 2. A schematic of the major elements of the OBS system.

6. PRELIMINARY RESULTS

Testing of the first prototype OBS was carried out *in vitro* on tissue samples, both normal and malignant, from various canine organs (liver, lung, spleen, kidney and pancreas) and from human prostate.

The animal samples were preserved in formalin. In all organ types distinctive signature differentiation between malignant and normal tissue was generated by the OBS. Moreover, the differentiation between malignant and healthy tissue was clearly much greater than any variation among signatures from healthy sites for the same organ type. All results were corroborated by traditional pathology analyses. Figures 3 and 4 show the clearly distinguishable signatures of canine normal and malignant samples, in this case for the liver. These plot the spectrally-dispersed collected light for each illuminating wavelength in a pseudo-three-dimensional display. Other canine organ tissues exhibited similarly large changes between normal and malignant conditions, although, as expected, the general character of the signatures varied among the organ types. Since these malignancies were tumors that were obvious to the naked eye, it is not surprising that signature changes are manifested in both the elastic scattering and fluorescence.

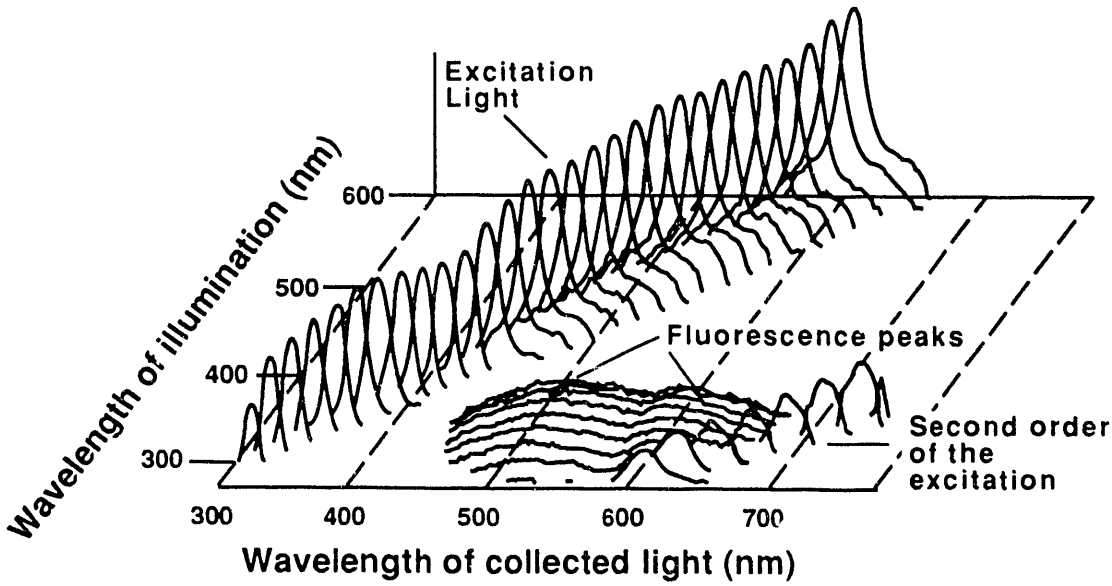

Figure 3. OBS spectral signature of a normal site of canine liver. The major "mountain range" is the elastic scattered light.

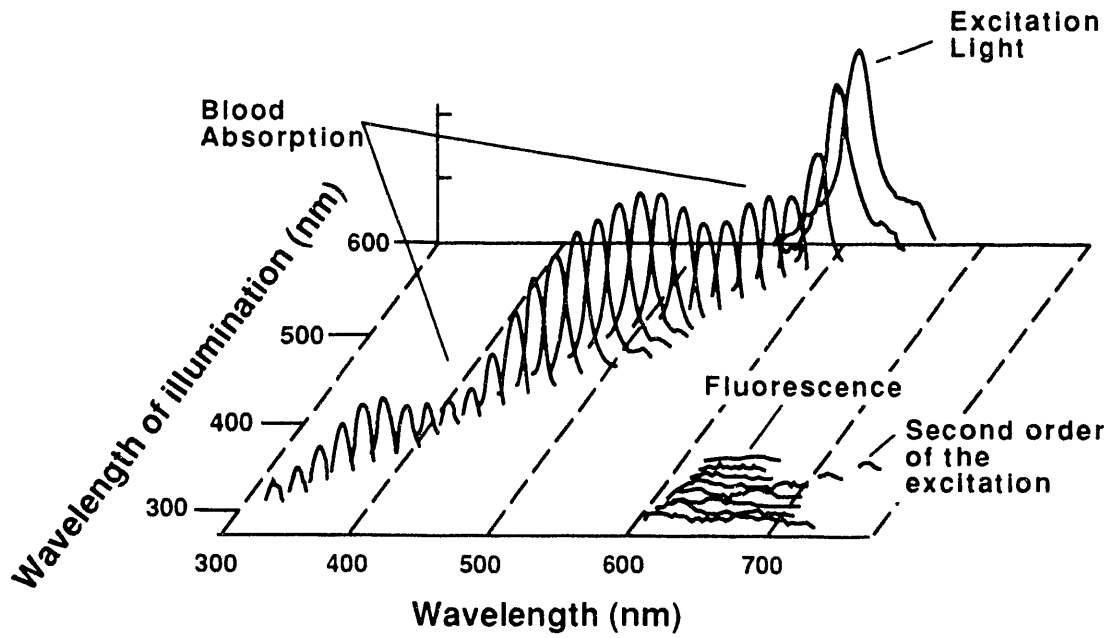

Figure 4. OBS spectral signature of a tumor site in canine liver. Changes from the normal signature in Figure 3 are readily observable.

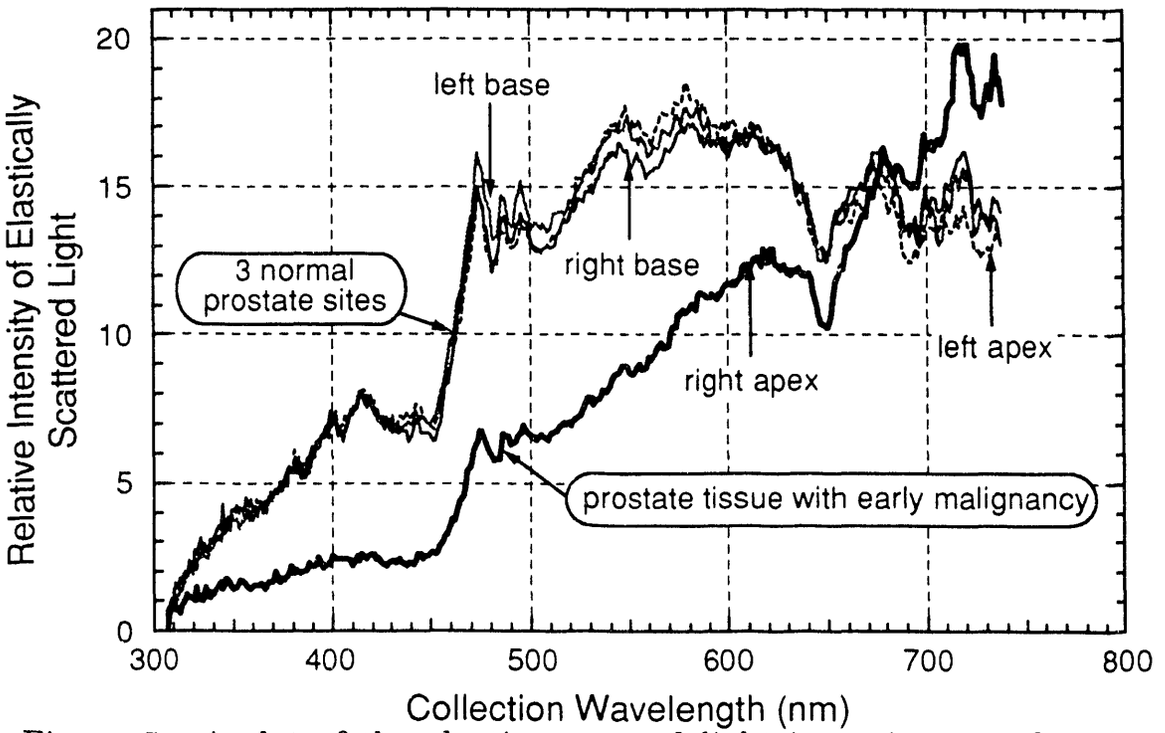

Figure 5. A plot of the elastic scattered light intensity as a function of wavelength, for various sites measured in freshly excised human prostate. The diagnoses as provided by the pathologist are indicated in the circled annotations.

The first tests on human tissue samples were made on freshly-excised human prostate tissue that was not yet fixed. (It was placed in saline.) In one set of measurements the prostate, cross sectioned by the pathologist, showed no sign of malignancy that was visible to the unaided eye. However, the OBS data clearly distinguished one site from among several sites chosen randomly. That site was later found by pathology, under microscopic examination, to have contained early malignant changes. This distinction found by the OBS is illustrated in Figure 5, which displays only the elastic optical-transport component from the OBS data.

Clinical testing on superficial ocular lesions:

We began our first *in vivo* human clinical tests in November 1992 at the University of Texas Health Science Center in San Antonio, in a clinical study of superficial lesions of the eye. The basic question that the study is designed to answer is whether the OBS signatures can be used to distinguish benign from malignant lesions. Initial comparisons with histopathology are very encouraging, and additional testing is generating a larger patient sample and good statistical correlations between the optical and physical biopsies. The OBS signatures are reliably different for lesions and normal tissue, with obvious differences among the backscattering spectra for various types of lesions and the normal tissues, whether the underlying tissue was conjunctiva/sclera or cornea/iris. (There was no useful fluorescence signal in any of these ocular measurements.) Most of the lesions were pigmented to some degree, and it was quickly apparent that the OBS signature could be used to locate the margins of the lesion with an

accuracy limited only by the size of the handpiece tip. Interestingly, however, the OBS-determined margins were often well outside the borders of visible pigmentation. This has correlated with histopathology reports, which show where that malignant changes in the cellular architecture were found outside the pigmented area. Figure 6 shows one example of the differentiations that were seen.

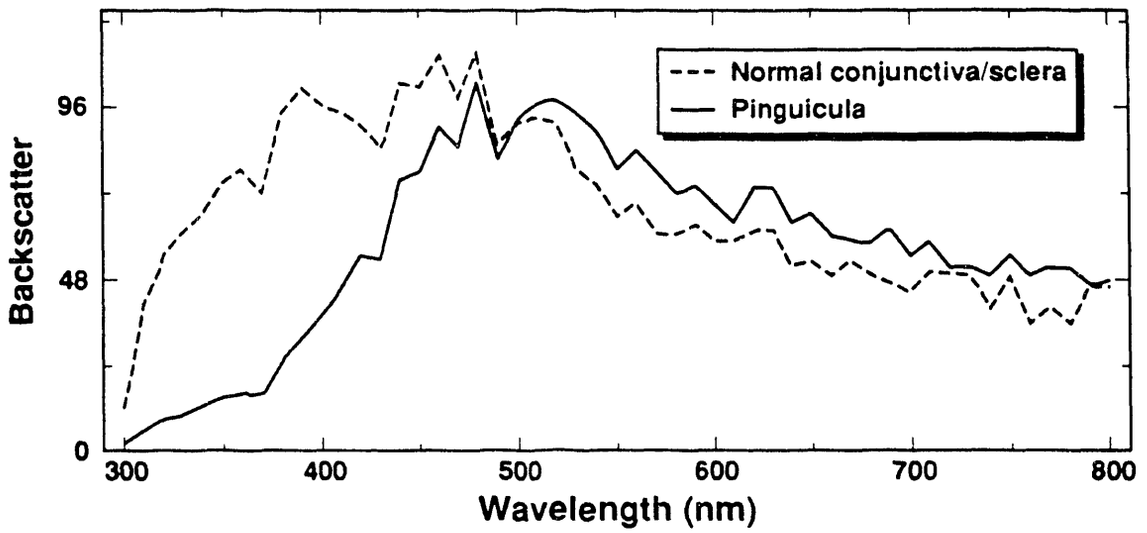

Figure 6. A comparison of the OBS spectral signature of a pinguicula lesion with that from normal conjunctiva/sclera.

An interesting check can be made on the variability, from patient to patient and also for different physicians performing the measurement, of the OBS signatures for normal tissue. A total of 27 measurements were made on a number of normal sites in three patients by three different physicians. As can be seen in Figure 7, the reproducibility of the spectral signature for normal sites is remarkable.

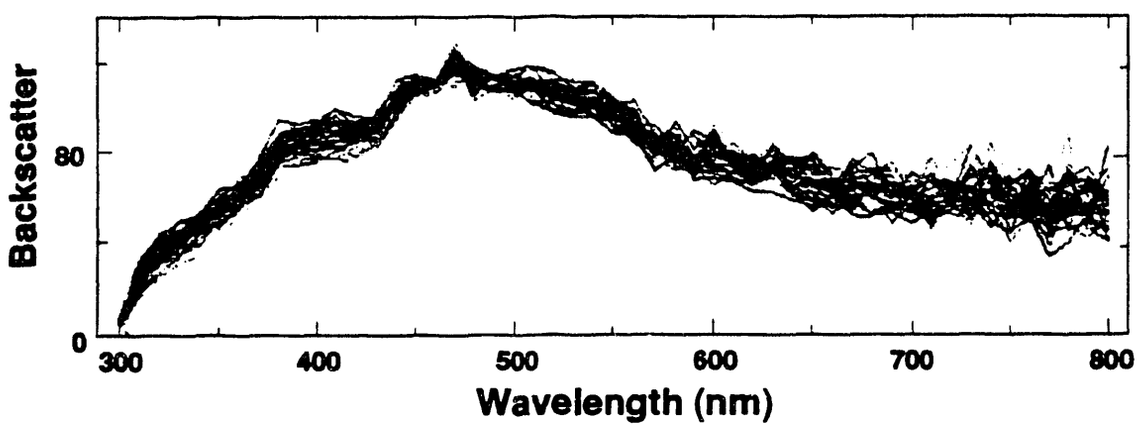

Figure 7. Normal sclera/conjunctiva reproducibility test: a set of 27 measurements performed by three different physicians on three different patients, all on presumptive normal sites.

Clinical testing with bladder cancer:
In situ measurements of human bladders with malignancies have also been performed. These tests have been conducted at the Department of Urology of the Lovelace Medical Clinic in Albuquerque. We devised an OBS fiber optic probe compatible with the endoscope used to view the bladder interior wall, and obtained data from various normal and malignant sites. Each site that was interrogated optically was then biopsied for comparison with histopathology. Again, no useful fluorescence was observed from any site. The backscatter signatures from the normal bladder wall and tumor sites were reproducible and contained significant differences. With ~100 measured sites in 10 patients pathology diagnosis has been completely consistent. We also saw differences at sites of (presumptive) premalignant changes, but we are awaiting confirmation by histopathology. Fig. 8 shows an example of these data. This study is also continuing.

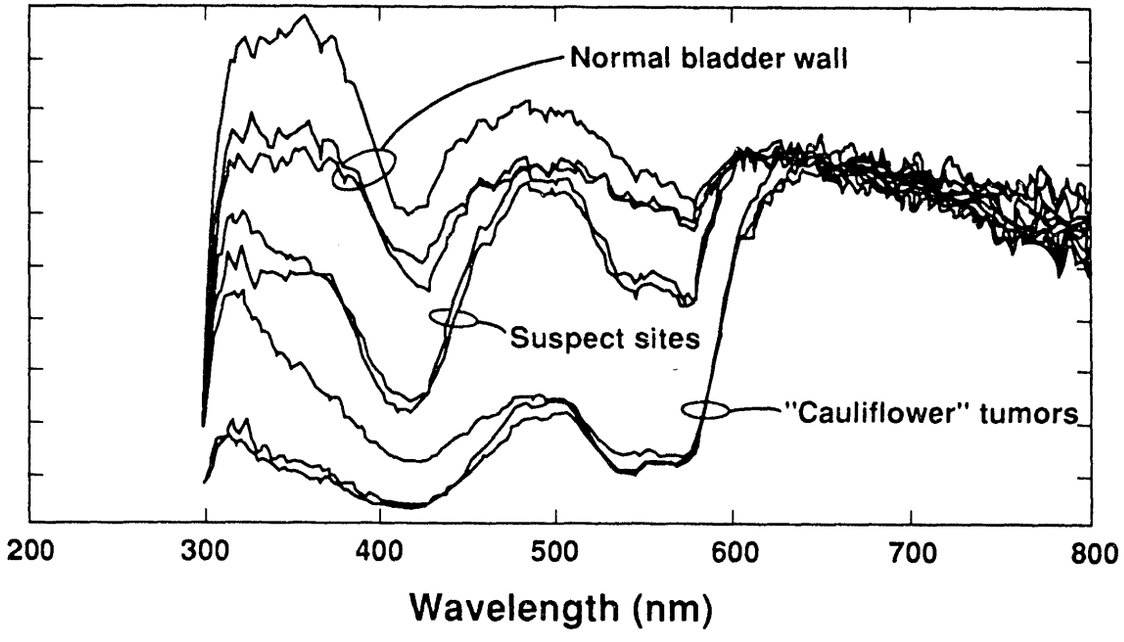

Fig. 8. Bladder cancer: an example of a set of OBS signatures for various sites on one patient.

7. CURRENT ONGOING DEVELOPMENTS

1) A simplified version of the OBS that is limited to detecting only a spectrally dispersed backscatter signal (but not fluorescence) is being developed. This version differs in that the light from the xenon point-arc lamp is focused directly onto the delivery fiber(s). The light from the collection fiber(s) is spectrally dispersed onto a low-cost linear CCD array which is interfaced to a PC. Such single spectra can be collected in milliseconds.

2) Development is planned of special probes and software to detect the depth of a lesion.

3) Measurements on bladder cancer at Lovelace Medical Center are continuing.

4) As data are gathered from both *in vitro* and *in vivo* evaluations, the accumulated experience will be used to further improve and iterate the system design, especially the software.

5) We will begin to establish a data library of signatures for the various organ tissues studied. The purpose is to allow for a determination of the sensitivity and reliability of the tissue signatures generated. At this time we are developing the OBS with the intent that actual diagnosis should be made by the physician, or consulting pathologist, upon examination of the displayed signatures. However, for certain applications it is expected that system software itself would be capable of establishing a diagnosis.

8. OTHER POSSIBLE APPLICATIONS IN MEDICINE

We have begun testing the OBS to assess its ability to detect ocular pathologies other than cancer. Preliminary measurements of human eye lenses indicate that the biochemical breakdown products of UV photo-damage, which eventually lead to cataract formation, can be detected in lenses at relatively young ages[11]. There is a correlation between the fluorescence signal and the age of human lenses. Recently, we have begun a study to determine the utility of the OBS technology in diagnosing corneal infections of the types typically experienced by contact lens wearers. Among gynecological applications, we speculate that diagnosis of endometriosis could be made quick and easy. (Although this is not cancer, cellular architecture differences are the key to diagnosis.) Finally, the diagnosis of gum disease may yield to OBS technology.

9. REFERENCES

[1] "Diagnosis of tumors by fluorescence", A.E. Profio, Proceedings of the SPIE Conferences on Biomedical Optics, January 14-19, 1990 (Los Angeles).

"Gastrointestinal tissue diagnosis by laser-induced fluorescence spectroscopy at endoscopy", R.M. Cothren et al., Gastrointestinal Endoscopy **36**, 105 (1990).

"Optical spectroscopic diagnosis of cancer and normal breast tissues", R.R. Alfano et al., J. Opt. Soc. Am. **B6,** 1015 (1989).

[2] "Ultraviolet laser-induced fluorescence of colonic tissue: basic biology and diagnostic potential," K. T. Schomacker, et al., Lasers in Surg. and Med. **12**, p.63 (1992).

[3] "Tumor localization and photosensitization by derivatives of hematoporphyrin: a review", D. Kessel, IEEE J. Quantum Electron. **QE-23**, p.1718 (Oct. 1987).

[4] "Some instrumental and chemical developments in the therapy and photodetection of tumors", H.Van den Burgh, et al.;
"Laser-induced fluorescence in medical diagnostics", S. Andersson-Engels et al.;
"Targeting of cancer cells with molecular delivery systems", T. Hasan,
Proceedings of the SPIE Conferences on Biomedical Optics, January 14-19, 1990 (Los Angeles).

[5] "Fluorescence localization of early colonic cancer in the rat by hematoporphyrin derivative," D. J. Bjorkman, et al., Lasers in Surg. and Med. **11**, p.263 (1991).

[6]"Tissue diagnostics using laser-induced fluorescence", S. Svanberg et al., Proceedings of the SPIE Conferences on Biomedical Optics, January 14-19, 1990 (Los Angeles).

[7] "Fluorescence contour mapping: applications to differentiation of normal and pathologic human tissues", R. Richards-Kortum, et al., 1989 Conference on Lasers and Electro-Optics, April 1989 (Baltimore).

[8] "Multidimentional luminescence measurements", I.M. Warner et al., Analytical Chem. **57**, 463A (1985).

[9] Design considerations for a two-dimensional rapid scanning fluorimeter", I.M. Warner et al., Analytica Chemica Acta **109**, 361 (1979).

[10] "Fluorescence contour mapping: applications to differentiation of normal and pathologic human tissues", R. Richards-Kortum, et al., 1989 Conference on Lasers and Electro-Optics, April 1989 (Baltimore).

[11]"Rapid noninvasive optical characterization of the human lens," J.A. Zuclich, I.J. Bigio et al., Lasers in the Life Sciences, (1993, in press).

END

DATE
FILMED
10/6/93