Conf-950226--18

LA-UR- 95-479

Title: DETECTION OF GASTROINTESTINAL CANCER BY ELASTIC SCATTERING AND ABSORPTION SPECTROSCOPIES WITH THE LOS ALAMOS BIOPSY SYSTEM

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Submitted to: SPIE, San Jose, CA, February 2-10, 1995

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ST 2629 10/91

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Detection of gastrointestinal cancer by elastic scattering and absorption spectroscopies with the Los Alamos Optical Biopsy System

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ABSTRACT

The Los Alamos National Laboratory has continued the development of the Optical Biopsy System (OBS) for noninvasive, real-time *in situ* diagnosis of tissue pathologies. In proceedings of earlier SPIE conferences we reported on clinical measurements in the bladder, and we report here on recent results of clinical tests in the gastrointestinal tract.

With the OBS, tissue pathologies are detected/diagnosed using spectral measurements of the elastic optical transport properties (scattering and absorption) of the tissue over a wide range of wavelengths. 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, exhibit 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 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. Additionally, the optical geometry of the OBS beneficially enhances its sensitivity for measuring absorption bands. The OBS employs a small fiber-optic probe that is amenable to use with any endoscope or catheter, or to direct surface

examination, as well as interstitial needle insertion. Data acquisition/display time is <1 second.

Keywords: optical biopsy, tissue spectroscopy, optical cancer diagnosis, noninvasive diagnostics

2. BACKGROUND

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. During the past few years significant effort has been expended toward developing optically based systems for cancer detection. The motivation is elimination of the need for surgical removal of biopsy tissue samples: rather, some form of spectral analysis of the tissue is recorded *in vivo* by an imaging system or with an optical-fiber probe placed on or near the surface of the tissue in question. A diagnosis of the tissue is then attempted based on the optical measurements. The intent of these systems is to provide diagnostic signatures, *in situ*, noninvasively and in real time, with reduced health-care costs as a consequence of eliminating histology and, in many cases, eliminating the need for the surgical environment required to take biopsy samples. Moreover, the immediacy of diagnostic information can reduce the emotional trauma to the patient waiting for an answer.

A range of spectroscopies have been investigated for optical diagnosis, all of which have one basic principle in common. The specific optical spectrum of a tissue sample contains information about the biochemical composition and/or the structure of the tissue. This basic approach is useful not only for the detection of cancer, but may also be used for detection and diagnosis of other tissue abnormalities such as atherosclerosis, endometriosis and infections.

While light scattering, fluorescence, and Raman spectroscopy have all been investigated as methods for distinguishing malignant tissue, the majority of work has utilized fluorescence spectroscopy. Fluorescence spectroscopy has been investigated with and without the aid of exogenous drugs that target malignant tissue. The fluorescence from such drugs provides a large signal, which can be helpful in the detection process[1] and may be used as a detection tool for CCD imaging of the patterns of malignancy in a given area of tissue. This, however, is not an ideal solution for routine examination, since the administration of an exogenous drug is essentially an invasive process and can result in concomitant undesirable side affects. An alternative is to use intrinsic (usually UV-induced) tissue fluorescence, or autofluorescence, as a diagnostic tool.[2,3,4] Many researchers

have pursued this approach, including, for example, Svanberg et al. who have investigated *in vivo* autofluorescence in several areas of the body including the brain, bladder and oral cavity.[5] The results from the few studies involving large sample sets demonstrate levels of reliability that range from very good (>90%, with minimal false negatives) to the unacceptable (<75%, with a significant fraction of false negatives).

Although Raman spectroscopy provides a much weaker signal than fluorescence, it has the advantage of sharp spectral features, which are more easily correlated with specific chemical components. While early studies with Raman spectroscopy invoked costly and slow instrumentation (data collection times of 15 to 30 minutes), recently, 810-nm excitation and CCD detection have been used to reduce the collection time significantly.[6] The potential for Raman spectroscopy to diagnose breast tissue and gynecological tissues has been investigated.[7,8] While initial measurements to differentiate malignant and nonmalignant tissue with Raman spectroscopy show promise, larger sample sizes will be needed for conclusive results, and instrumentation remains expensive.

With the OBS, tissue pathologies are detected/diagnosed using spectral measurements of the elastic optical transport properties (scattering and absorption) of the tissue over a wide range of wavelengths. The probe is designed to be used in optical contact with the tissue under examination and has separate illuminating and collecting fibers. Thus, the light that is collected and transmitted to the analyzing spectrometer must first scatter through a small volume of the tissue before entering the collection fiber(s). Consequently, the system is also sensitive to the optical absorption spectrum of the tissue, over an effective operating range of 300 to 800 nm, and such absorption adds valuable complexity to the scattering spectral signature. 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. It is important to note that the OBS probe, being used in optical contact with the tissue, examines only that site and does not image the tissue surface. More detailed discussions of the technology have appeared in earlier publications.[9,10,11]

Caution:

It should be noted that researchers addressing any new approach to tissue diagnosis, including optical spectroscopy, are faced with a general problem in determining the efficacy of the new method. Schomaker et al.[4] point out that when three pathologists examined the same 91 polyp samples, a given pathologist's diagnosis was in the majority about 90% of the time. If the "gold standard" is not

perfect, it is difficult to determine the accuracy of the optical measurement. Applying a dichotomous diagnostic algorithm is also difficult. For example, the autofluorescence spectra of malignant and nonmalignant tissues tend to form a continuum between malignant and nonmalignant. The exact form of the algorithm may depend on which patients were included in a particular study.

3. ELASTIC SCATTERING SPECTROSCOPY

Efforts by other groups to utilize elastic scattering spectroscopy for tissue diagnosis have focused mainly on diffuse reflection from the surface of skin.[12,13,14,15] Their results have shown limited reliability in detecting cancer, in our judgment because of the interference of melanin and the vagaries of specular reflection from the tissue surface. The intent of our approach is to generate spectral signatures of closer relevance to the tissue parameters that a pathologists addresses. 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 process, such as fluorescence or Raman scattering. The angular distribution of scattering will have a wavelength dependence that is affected by the cellular architecture. The increased photon pathlengths in tissue, induced by multiple scattering, also accentuate the effect of spectral absorption bands. Thus, our approach to the problem is to generate a signature that comprises data from the elastic scattering and absorption - the elastic optical transport properties of the tissue.

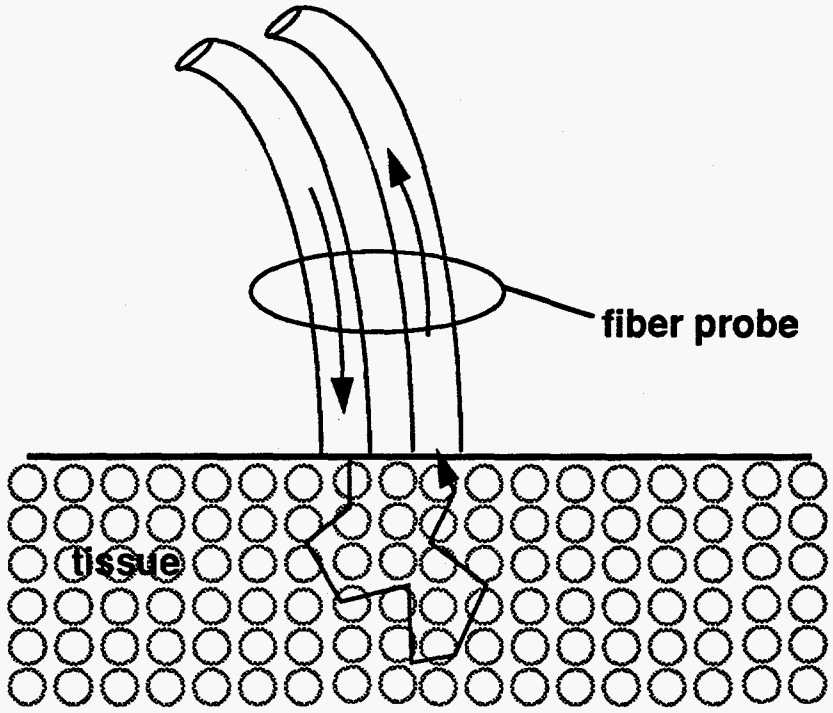

Fig. 1 Optical geometry for the probe/tissue contact area.

The optical geometry of our fiber-optic probe is relation to the tissue is shown in Figure 1. It is critical to note that the fiber-optic probe is used in optical contact with the tissue. Thus, surface reflection is purposefully avoided, and all light reaching the collection fiber(s) has undergone multiple scattering through a distance of tissue. Each of the fibers is typically 200-400 microns in diameter, and the entire probe, including jacket, can be less than 1 mm diameter. Broadband "white" light (~300-800 nm) enters the tissue from the illumination fiber(s). The wavelength dependence of the light that manages to reach the collection fiber will be a function of both the elastic scattering and absorption properties of the tissue, and such spectral variations form the basis of the signatures used for detection/diagnosis by the OBS. As illustrated in the figure, the effective pathlength (through tissue) of photons entering the collection fiber can be many times greater than the simple distance between the emitting and collecting fibers. Thus, the sensitivity of the system to spectral absorption bands is enhanced.

The details of the system concept and design of the Optical Biopsy System have been described in various earlier publications.[9,11,16] For the parameters relevant to mammalian tissue, the physics of the scattering interactions are best described by

Mie scattering theory. The use of this theory in Monte Carlo numerical simulations of the photon density patterns has been described in Reference 10.

4. CLINICAL TESTING

Our earlier clinical studies (on bladder cancer) were reported in References 11 and 16. Certain spectral features were found to correlate well with the pathological diagnoses. Our recent clinical studies have begun to address various regions of the gastrointestinal tract. About 25 patients were examined at two clinical sites. Since instrument changes were made in the Optical Biopsy System between use at the two sites, and because of possible systematic differences in the pathology analysis at the two institutions, spectra from the two sites are not directly compared. Even with two clinical sites all of the data that follow represent small numbers of patients for each type of pathology, and hence are not presented on a statistical basis. Thus, we do not make any claims at this point regarding the reliability/efficacy for GI pathologies, as more rigorous FDA trials are required. The main intent is to show the potential for OBS spectral signature identification in these new regions, which are of great importance to medical practice. In general, separate studies with a larger statistical base are required to demonstrate reliable efficacy in the detection and diagnosis of pathologies for each new organ area of interest and for each type of pathology. FONET, Inc., a company in Clearwater, Florida, has licensed the patented technology of the OBS system from the Los Alamos National Laboratory, and will soon proceed in gathering much larger statistical bases of data for FDA approval.

Duodenal data

Data were taken at a number of sites of duodenal polyps with two patients. Figure 2 shows data from one of the patients. There is a notable trend, that the ratio of the elastic scatter signal at ~630 nm to that at 575 nm is smaller for adenomatous than for nonmalignant tissue. Traces 100 and 101 were taken from putative normal mucosa sites, while 102 through 105 were frank polyps. This metric is an interesting one in that the dips in the spectra at ~542 and 579 nm are due to hemoglobin absorption. One might conclude that there is simply more Hb in the malignant tissue. However, the dip at ~420 nm is also due to Hb and this does not vary significantly. It should also be noted that trace 104 in Fig. 2 was for a polyp that appeared no different clinically (to the eye) than the other polyps, but was evidently non-malignant. Its spectral signature follows the same path as malignant polyps in the UV region, but follows the normal sites in the 550-650 nm range. Thus, this meager amount of data would suggest that the 550-650 spectral region may provide the more reliable correlation with malignancy.

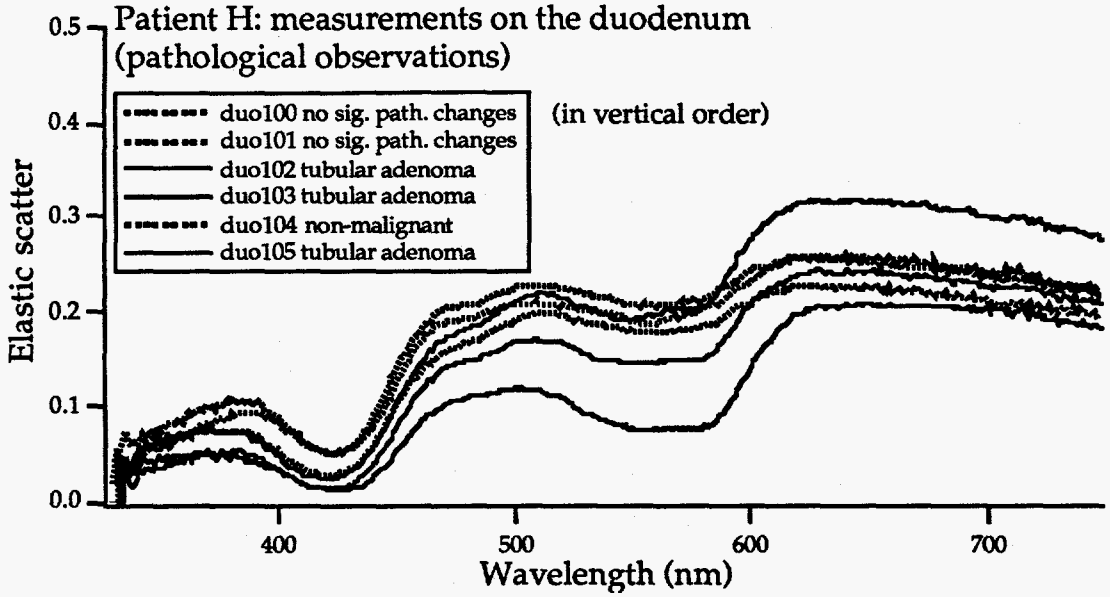

Fig. 2. OBS spectra for duodenal sites in one patient, indicated according to pathology reports.

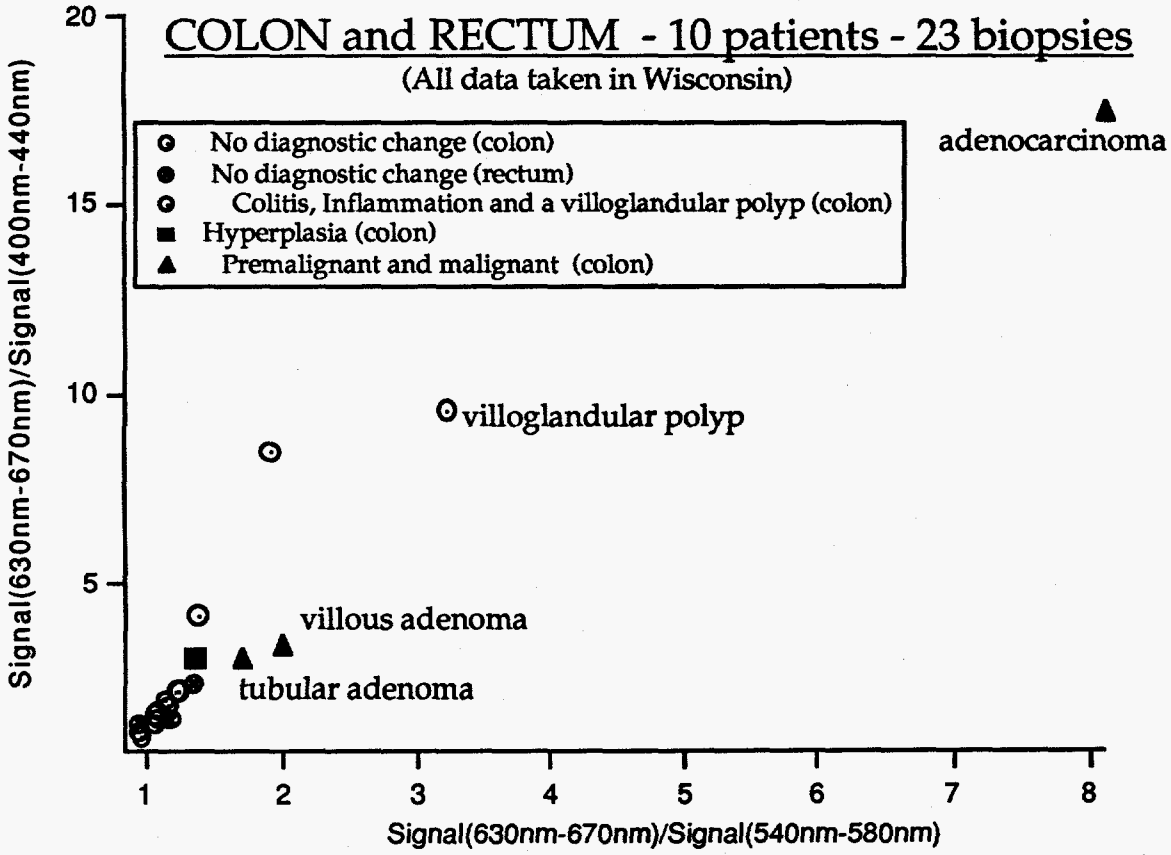

Fig. 3 Results of applying a simple metric to spectra taken of colon and rectal tissue. For clarity two data points have been left off of this graph. Both are for a diagnosis of colitis and have y values greater than 20 and x values less than 7.

Colon data

Optical biopsy measurements were made in the colons of 10 patients. Of the 25 biopsy sites three were malignant. Fig. 3 shows that it may be possible to separate malignant from the nonmalignant data by using multiple metrics. The x-axis is the integrated signal from 630 to 670 nm divided by the integrated signal from 540 to 580 nm. The y-axis is the integrated signal from 630 to 670nm divided by the integrated signal from 400 to 440 nm. Both of these ratios involve hemoglobin absorption bands. In general it is expected both ratios would increase together. What is interesting about Figure 3, however, is that as the value of the ratio involving the Soret band, for a given value of the ratio involving the alpha and beta bands, is not as great for the malignant as for the nonmalignant data. Other metrics are being investigated, and, again, continuing studies will benefit from more data.

Rectal polyps

Fig. 4 shows spectra from the rectum of one patient in Wisconsin. Operator error with the recording computer caused the loss of data in the spectral range of 440 to 540 nm. Nonetheless, the salvaged data on either side show repeated signature differences between pathology reports indicating malignancy vs. nonmalignant sites. Note the similarity in the 550 -650 nm range with the duodenal data.

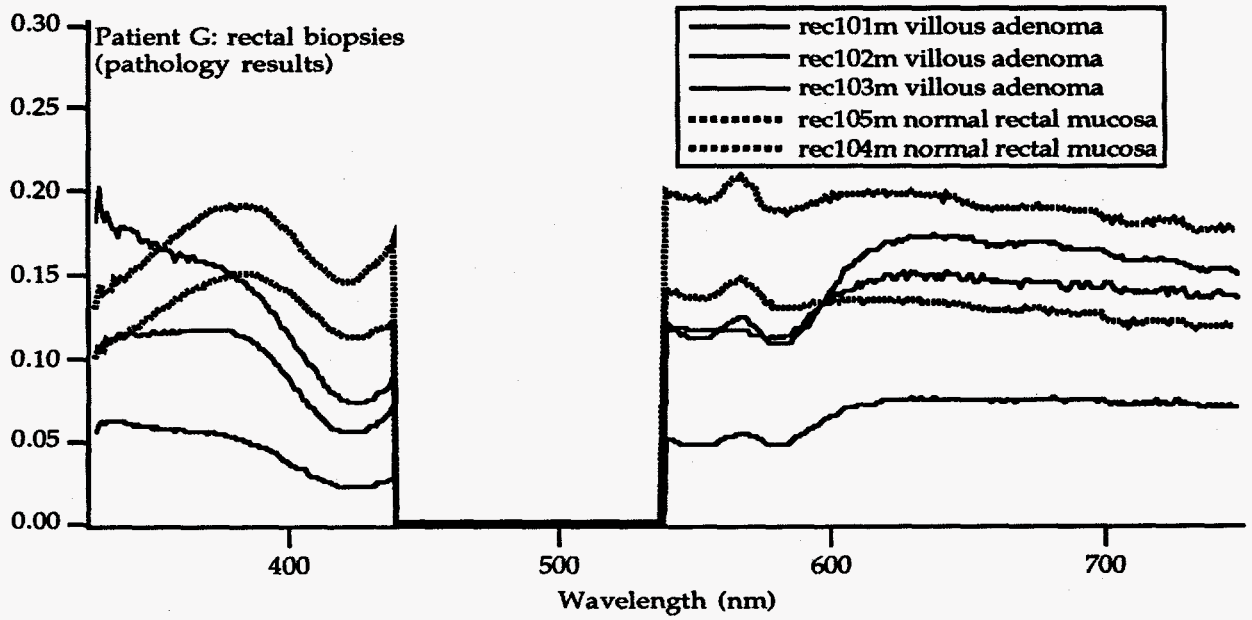

Fig. 4. OBS spectra from rectal polyps and normal rectal sites on one patient

Barret's esophagus

OBS measurements were made on several patients with Barret's esophagus. From this data there is some indication that we might be able to separate Barret's with dysplasia from Barret's without dysplasia. Fig. 5 shows the results of one particular metric applied to the data taken in Oklahoma. The two spectra of malignant and dysplastic tissue have unusually high values of this metric as compared to the rest of the data, and almost all of the 17 individual measurements made on normal mucosa and 18 measurements made on areas of inflammation have lower values than that for adenoma. Again, more data will be needed to determine if this metric can reliably distinguish malignant and dysplastic tissues.

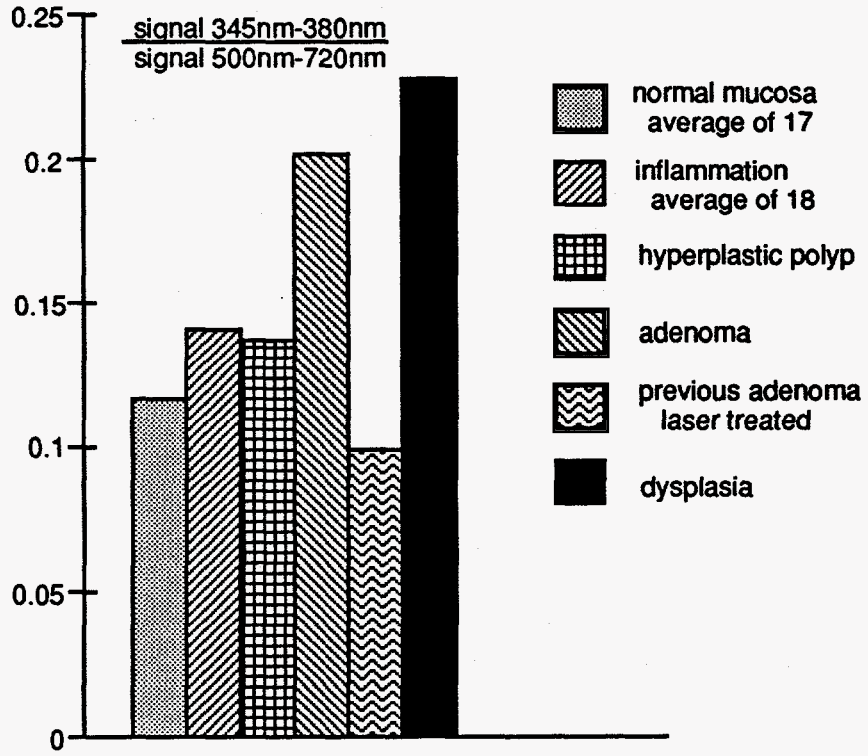

Fig. 5. Barret's esophagus: values of one specific spectral metric

5. CONCLUSIONS

An initial set of studies have been carried out on various regions of the gastrointestinal track. The main purpose of this initial round of measurements was to provide guidance for expanded studies of the efficacy of the Optical Biopsy System in this area. Nonetheless, these initial studies indicate promise for this

convenient and low-cost diagnostic technology. Formal FDA testing is expected to begin in February of 1995.

"Teething pains" encountered with this new-generation, low-cost version of the OBS system are being corrected. These include the spectral illumination-lamp stability, computer operating software, and an appropriate method for keeping the tip of the fiber probe clean once it is inside the GI tract. Commercial development of the instrument will speed the engineering of system details and user friendliness.

6. REFERENCES

1. D. Kessel, "Tumor localization and photosensitization by derivatives of hematoporphyrin: a review", IEEE J. Quantum Electron. Vol. 23, p. 1718, 1987.

2. R.R. Alfano, D.B. Tata, J. Cordero, P. Tomashefsky, F.W. Longo, M.A. Alfano, "Laser induced fluorescence spectroscopy from native cancerous and normal tissue", IEEE J. Quantum Electron., Vol 20, p.1507, 1984.

3 R.M. Cothren, R. Richards-Kortum, M.V. Sivak, M. Fitzmaurice, R.P. Rava, G.A.Boyce, M. Doxtader, R. Blackman, T.B. Ivanc, G.B. Hayes, M.S. Feld, R.E. Petras, "Gastrointestinal tissue diagnosis by laser induced fluorescence spectroscopy at endoscopy", Gastrointest. Endosc. Vol. 36, p. 105, 1990.

4 K.T. Schomacker, J.K. Frisoli, C.C. Compton, T.J. Flotte, J.M. Richter, N.S. Nishioka, T Deutsch, "Ultraviolet laser-induced fluorescence of colonic tissue", Lasers in Surg. and Med. Vol.12, p.63, 1992.

5. S. Andersson-Engels, J. Johansson, K. Svanberg, S. Svanberg, "Laser-induced fluorescence in medical diagnostics", SPIE Vol.1203, p. 76, 1990.

6 J.J. Baraga, M.S. Feld, R.P. Rava, "Rapid near-infrared Raman spectroscopy of human tissue with a spectrograph and CCD detector", Appl. Spectrosc. Vol.42, p. 187, 1992.

7 D.C.B. Redd, Z.C. Feng, K.T. Yue, T.S. Gansler, "Raman spectroscopic characterizatio of human breast tissues: implications for breast cancer diagnosis", Appl. Spectrosc., p. 787, 1993.

8 C.H. Liu, B.B. Das, W.L. Sha Glassman, G.C. Tang, K.M. Yoo, H.R. Zhu, D.L.Akins, S.S. Lubicz, J. Cleary, R. Prudente, E. Celmer, A. Caron, R.R. Alfano, "Raman, fluorescence, and time-resolved light scattering as optical diagnostic techniques to separate

diseased and normal biomedical media", J. Photochem. Photobiol. B: Biol Vol.16, p.187, 1992.

9 Irving J. Bigio, Thomas R. Loree, Judith Mourant, Tsutomu Shimada, K. Story-Held, R.D. Glickman and Richard Conn, "Optical diagnostics based on elastic scattering: recent clinical demonstrations with the Los Alamos Optical Biopsy System", *Proc. Optical Biopsy*, R. Cebeddu, S. Svanberg and H. van den Bergh, eds., SPIE Vol. 2081, p.174, Budapest 1993.

10 James Boyer, Judith R. Mourant and Irving J. Bigio, "Theoretical and experimental investigations of elastic scattering spectroscopy as a potential diagnostic for tissue pathologies", *Proc. Advances in Optical Imaging and Photon Migration*, R.R. Alfano, ed., Orlando, 1994.

11 Irving J. Bigio, Judith R. Mourant, James Boyer, Tamara Johnson and Tsutomu Shimada, "Noninvasivve identification of bladder cancer with sub-surface backscattered light", *Proc. Advances in Laser and Light spectroscopy to Diagnose Cancer and Other Diseases*, SPIE Vol. 2135, p.26, Los Angeles, 1994.

12 M. Osawa, S. Niwa, "A portable diffuse reflectance spectrophotometer for rapid and automatic measurement of tissue", Meas. Sci. Technol., p.666, 1993.

13 M. Hajizadeh-Saffar, J.W. Feather, J.B. Dawson, "An investigation of factors affecting the accuracy of *in vivo* measurements of skin pigments by reflectance spectrophotometry", Phys. Med. Biol. Vol. 9, p. 1301, 1990.

14 H. Zeng, C. MacAulay, G. Palcic, D.I. McLean, "A computerized autofluorescence and diffuse reflectance spectroanalyser system for *in vivo* skin studies", Phys. Med. Biol. Vol. 8, p. 231, 1993.

15 R. Marchesini, M. Brambilla, C. Clemente, M. Maniezzo, A.E. Sichirolli, A. Testori, D.R. Venturoli, N. Cascinelli, "*In vivo* spectrophotometric evaluation of neoplastica and non-neoplastic skin pigmented lesions- I. Reflectance measurement", Photochem. Photobiol. Vol. 53, p. 77, 1991.

16 J.R. Mourant, I.J. Bigio, J. Boyer, R.L. Conn, T. Johnson and T. Shimada, "Spectroscopic diagnosis of bladder cancer with elastic light scattering", Lasers in Surgery and Medicine (1994, in press).