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TITLE: OPTICAL DIAGNOSTICS BASED ON ELASTIC SCATTERING: An Update of Clinical Demonstrations with the Optical Biopsy System

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Optical diagnostics based on elastic scattering: an update of clinical demonstrations with the 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. Our clinical studies have expanded since the last Biomedical Optics Europe conference (Budapest, September 1993), and we report here on the latest results of clinical tests in gastrointestinal tract.

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 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 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 OBS employs a small fiber-optic probe that is amenable to use with any endoscope or catheter, or to direct surface examination. 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 950 nm, and such absorption adds valuable complexity to the scattering spectral signature. More detailed discussions of the technology have appeared in earlier publications.[1,2,3]

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.

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[4] 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.[5,6,7] 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.[8] 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.[9] The potential for Raman spectroscopy to diagnose breast tissue and gynecological tissues has been investigated.[10,11] While initial measurements to differentiate malignant and nonmalignant tissue with Raman spectroscopy show promise, the small sample sizes inhibit conclusive results, and instrumentation remains expensive.

Caution:
It should be noted that researchers addressing any approach are faced with a general problem in determining the efficacy of an optical spectroscopy for tissue diagnosis. Schomaker et al.[7] 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. 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. Our approach attempts 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.

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 (~300-900 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.

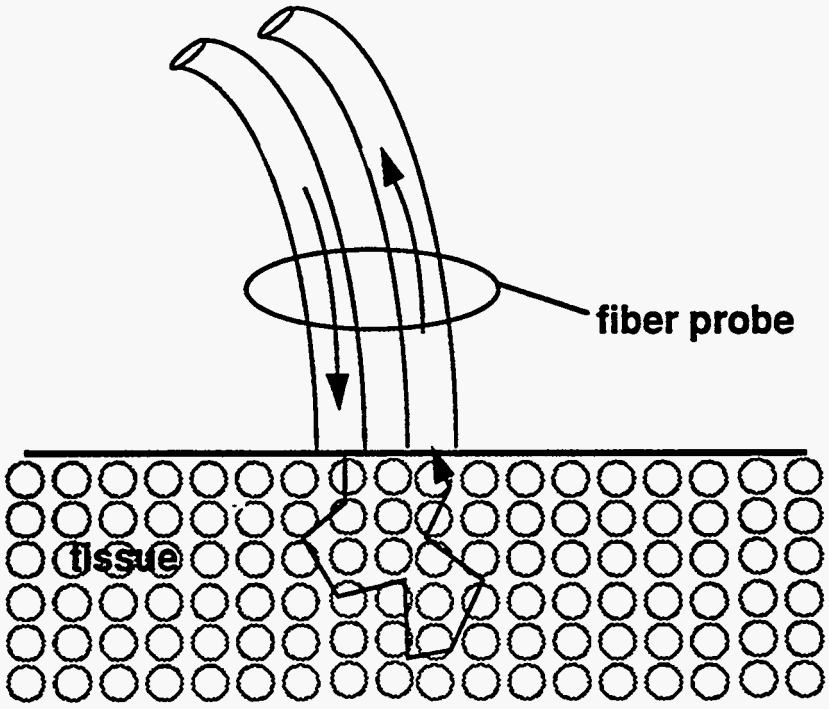

Fig. 1 Optical geometry for the probe/tissue contact area.

The details of the system concept and design of the Optical Biopsy System have been described in various earlier publications.[1,3,16] 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 2.

4. CLINICAL TESTING

Our earlier clinical studies were reported in References 3 and 16. In that work *in vivo* measurements on ten patients with suspected bladder cancer were made with the OBS. Tissue spectra over the wavelength range 280 nm to 800 nm were obtained using a fiber-optic probe, as described above, through one of the lumens of a urological cystoscope. Measurements were made on putative normal areas and areas of uncertain abnormality, as well as those suspected to be cancerous. After measurements were made with the OBS, biopsy samples were taken at the measurement sites. Comparisons of the histopathology and the optical spectra were then made. Various spectral signatures were found to correlate with pathology; specifically, variations in slopes of the spectra over the wavelength range 330 nm to 370 nm correlated excellently with the pathological diagnoses. A diagnostic algorithm for distinguishing malignant from nonmalignant tissue based on the values of the slopes had a sensitivity of 100% and specificity of 97% for the >50 biopsy samples and >100 OBS measurements made in that study.

While further data is being collected from patients with suspected bladder cancer, our recent clinical studies have begun to address various regions of the gastrointestinal tract. We should provide the caution that all of the data that follow represent small numbers of patients, and hence are not presented on a statistical basis. Thus, we do not make any claims at this point regarding the reliability/efficacy for these areas. 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. Obviously, formal studies with a larger statistical base will be 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. Those results will be the subjects of future publications.

Colon data:

Optical biopsy measurements were made in the colons of three patients . No dysplasia (or worse) was found in any of these patients. All of the pathology reports were either quiescent colitis or chronic active ulcerative colitis. We did observe that the Soret band of Hb was shifted a few nanometers to the blue (~3 nm, from 418 nm to 415 nm) for the active ulcerative colitis. Examples of the raw spectral-signature data are shown in Figure 2.

Duodenal data

Data were taken on a number of sites with two patients with polyps in the duodenum. There appears to be a trend that the ratio of the elastic scatter signal at ~630 nm to 575 nm, which is smaller for adenomatous than for nonmalignant tissue, as can be seen in Figure 3 for one of the patients. Traces 100 and 101 we taken from putative normal 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 be noted that trace 104 in Fig. 3 was for a polyp that appeared no different to the eye from the other polyps, but returned a pathology report of "no significant pathological changes". Its spectral signature follows the same path as the normal sites in the 550-650 nm range.

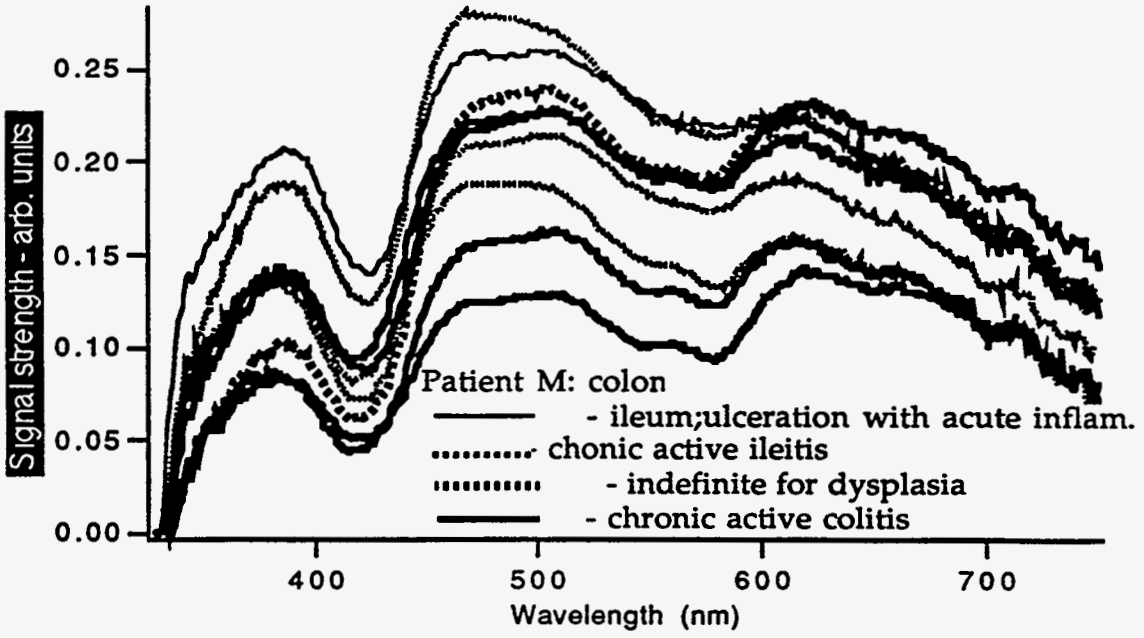

Fig. 2. OBS spectra from several sites in a patient with colitis.

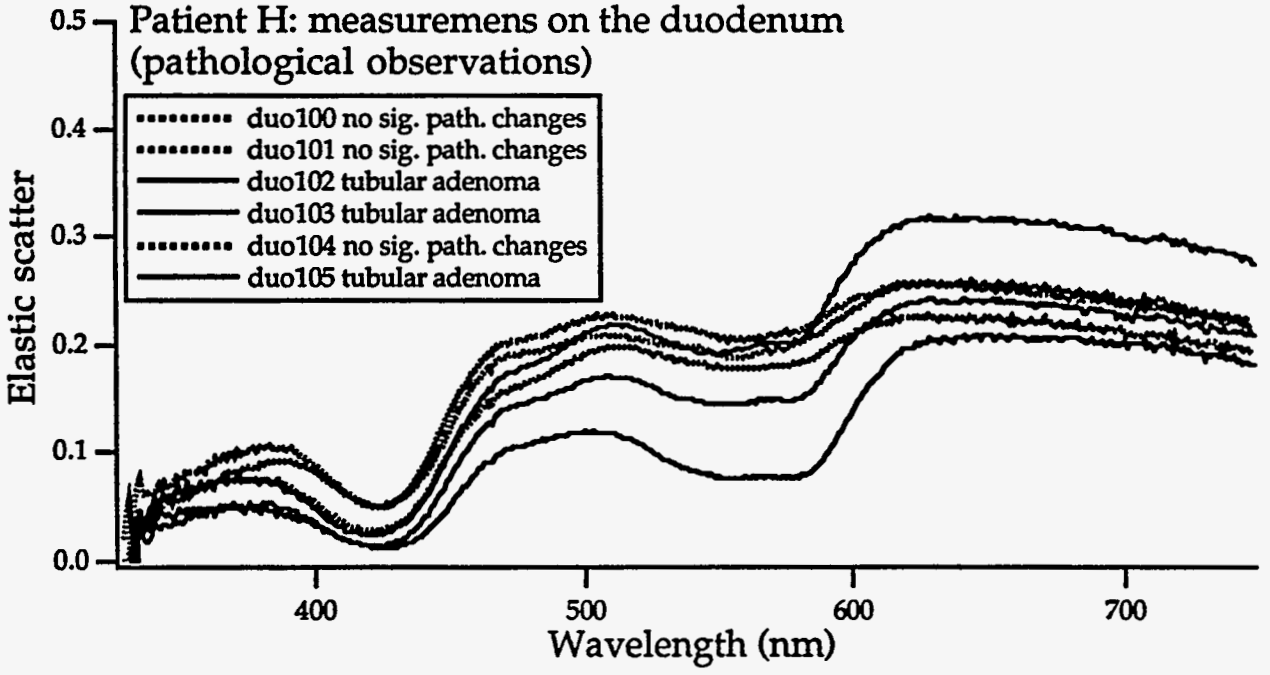

Fig. 3. OBS spectra for duodenal sites in one patient, indicated according to pathology reports.

Figure 4 shows the same data with the scale expanded to reveal more detail for the shorter wavelengths. In this case we have denoted the different traces according to whether they were normal sites or polyps. The polyps clearly have a different slope in the UV region from that of the normal sites, but the trace 104 for the nonmalignant polyp follows the trend of the malignant polyps in this region. Thus, this meager amount of data would suggest that the 550-650 spectral region may provide the more reliable correlation with malignancy.

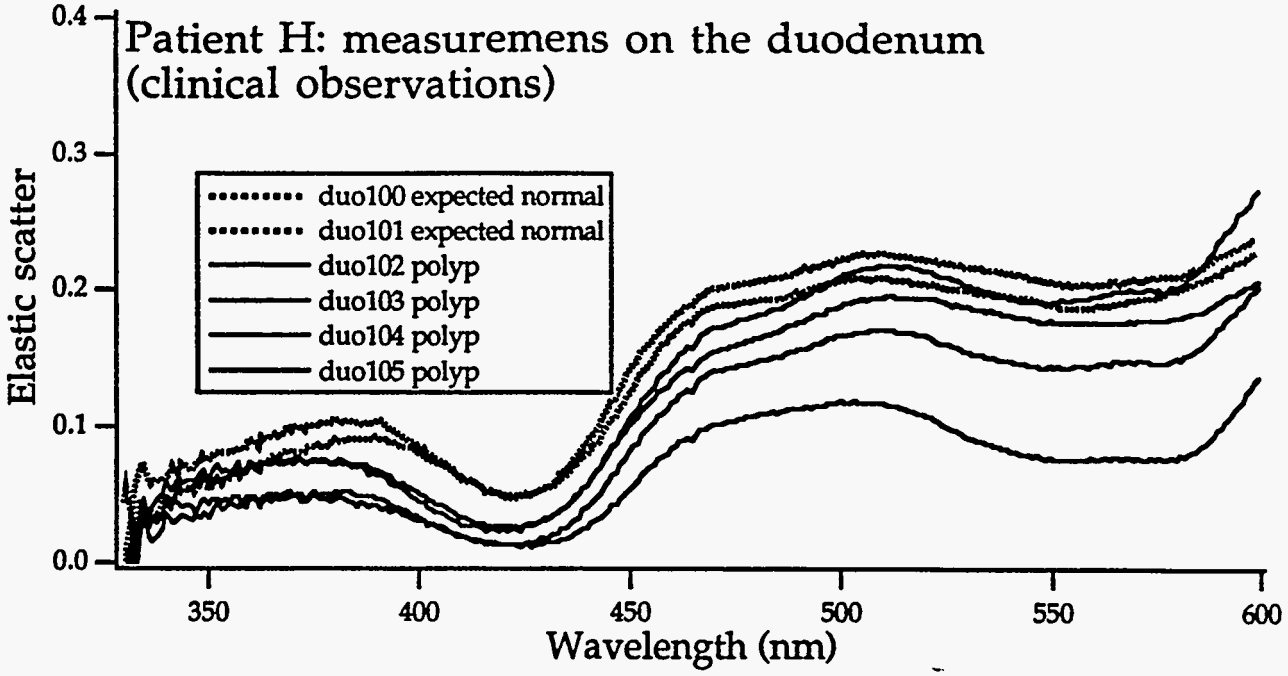

Fig. 4. Expanded spectrum showing slope differences in the UV between polyps (regardless of malignant state) and putative normal duodenal mucosa.

Figure 5 shows similar measurements from another patient with suspected duodenal cancer. Despite instrumental problems encountered on that day (an unstable light source), the same trend is seen in the 630-to-580 nm ratio for malignant vs. nonmalignant sites.

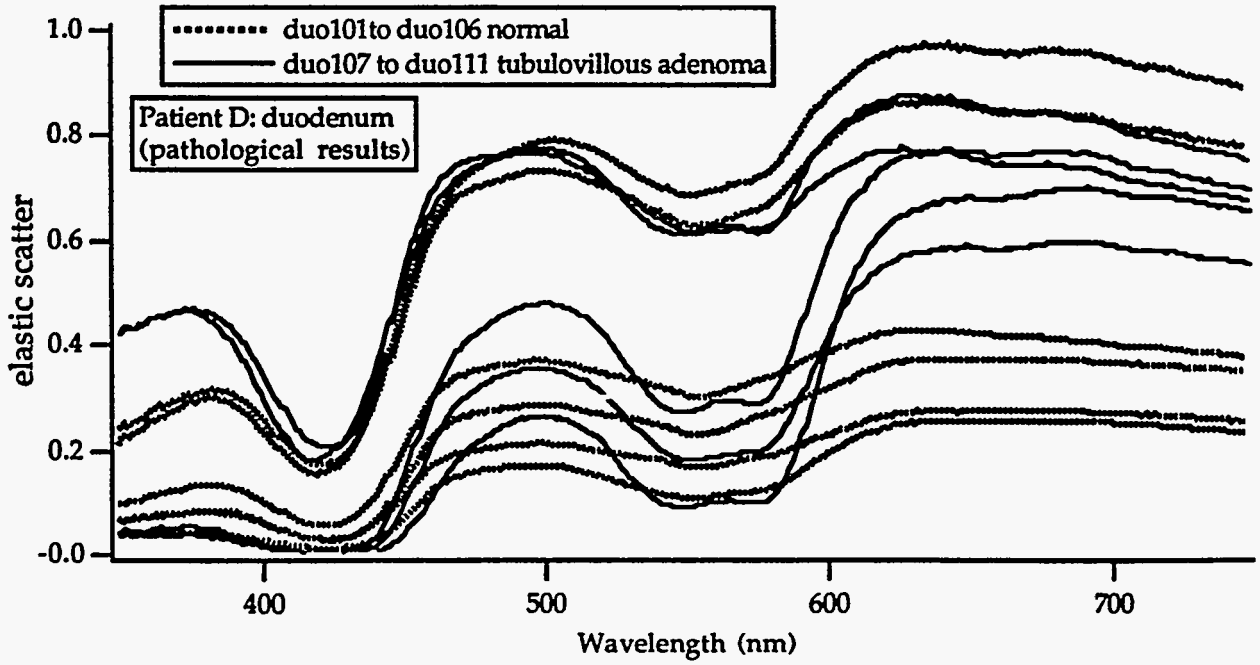

Fig. 5. OBS data for duodenal sites on another patient.

Rectal polyps

Measurements were made on one patient with rectal polyps. Operator error with the recording computer caused the loss of data in the spectral range of 440 to 540 nm. Nonetheless, as shown in Figure 6, the salvaged data on either side show repeated signature differences between pathology reports indicating malignancy vs. nonmalignant sites, with some spectral similarities to those of other GI locations.

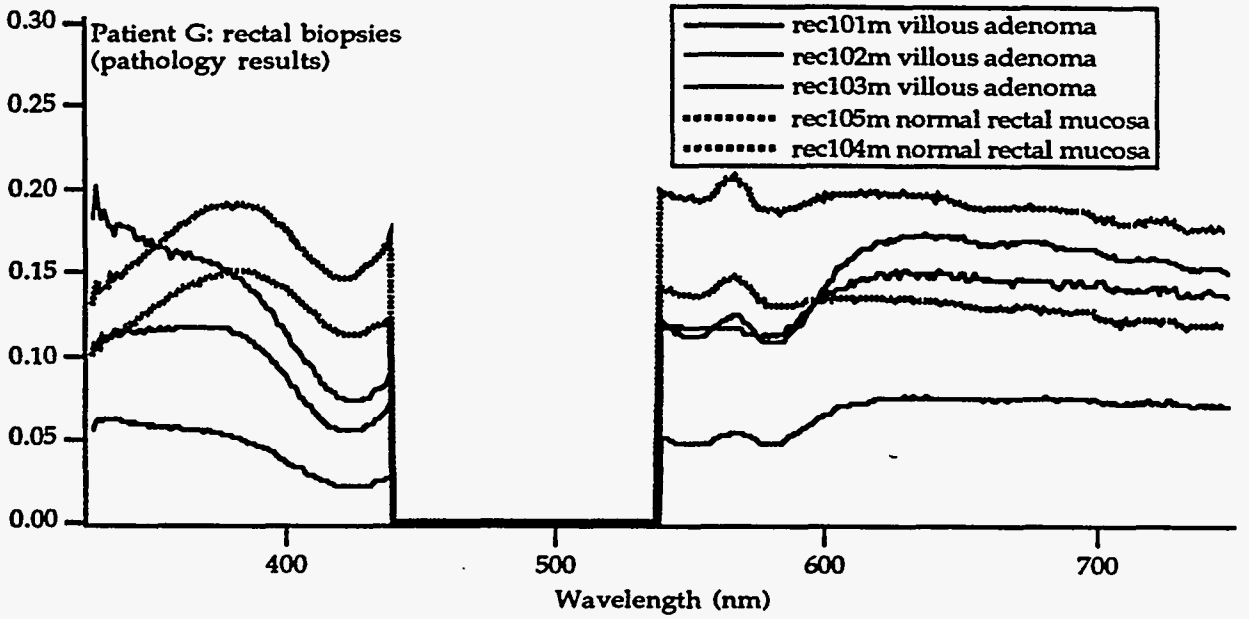

Fig. 6. OBS spectra from rectal polyps and normal rectal sites on one patient.

Barret's esophagus

OBS measurements were made on four 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. However, the data is not conclusive. All four patients exhibited some "mild dysplasia" at some sites, and nondysplastic Barret's condition at other sites. Although a general trend was seen in the OBS data between Barret's with mild dysplasia and nondysplastic Barret's, the spectral differences did not repeat for all cases. Since different pathologists were involved in the biopsy reports, we are again concerned that a more consistent set of criteria be used for the pathology to be able to serve as the standard against which the OBS data are compared.

5. CONCLUSIONS

An initial set of studies have been carried out on various regions of the gastrointestinal tract. 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 the GI tract. Nonetheless, these initial studies indicate promise for this convenient and low-cost diagnostic technology. The expanded studies have begun as of August 1994.

"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.

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