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Noninvasive Identification of Bladder Cancer with Sub-surface Backscattered Light

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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. Absorption bands in the tissue also add useful complexity to the spectral data collected. 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 as well as absorption. 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). We report here specifically on its potential application in the detection of bladder cancer.

2. BACKGROUND AND SIGNIFICANCE

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. Currently in the field of urology, a patient who has been diagnosed with superficial bladder cancer is evaluated by the use of office cystoscopy. Patients who are suspected of having possible bladder cancer, but as yet undiagnosed, undergo the same

procedure. If an abnormal area or an obvious tumor is identified, then the patient is scheduled for a cystoscopy with biopsy and resection of the lesion in the operating room under general or regional anesthesia. A certain percentage of these patients will have lesions that are benign and require no further treatment.

The true economic impact of such a tool would be in the office setting where the primary screening occurs. If the urologist could determine in the office setting that a lesion was benign and did not need biopsy under anesthesia, the cost saving implications are obvious. The ability to characterize the nature of a lesion in the bladder without the need for high cost intervention would not only save the cost of the diagnostic process, but would also tend to make the primary treatment more specific and effective, helping to contain the therapeutic costs as well.

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 may 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 more sophisticated method of autofluorescence diagnosis, called excitation-emission matrix spectroscopy, utilizes multiple-color illumination (sequentially) with data display that typically looks like a contour map.[7] The different excitation wavelengths might be expected to variously excite different chromophores, resulting in more complex emission patterns with more information relevant to biochemical changes, and with presumed greater likelihood of distinguishing malignancy from normal conditions.[8] . The general technique is based on earlier developments in the field of chemical engineering.[9,10]

4. ELASTIC OPTICAL TRANSPORT

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 scattering due to fluorescence. Thus, our approach 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 tested an optical biopsy system (OBS) that is fiber-optic mediate; it collects and displays data in ~1 second. (We have also built and tested a more general form of the instrument, which combines the measurement of elastic scatter/absorption with simultaneous measurement of the multiple-excitation-color fluorescence data, if desired.) 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.

It should be noted that the OBS does not image a tissue surface: rather, it provides information about the optical transport 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.

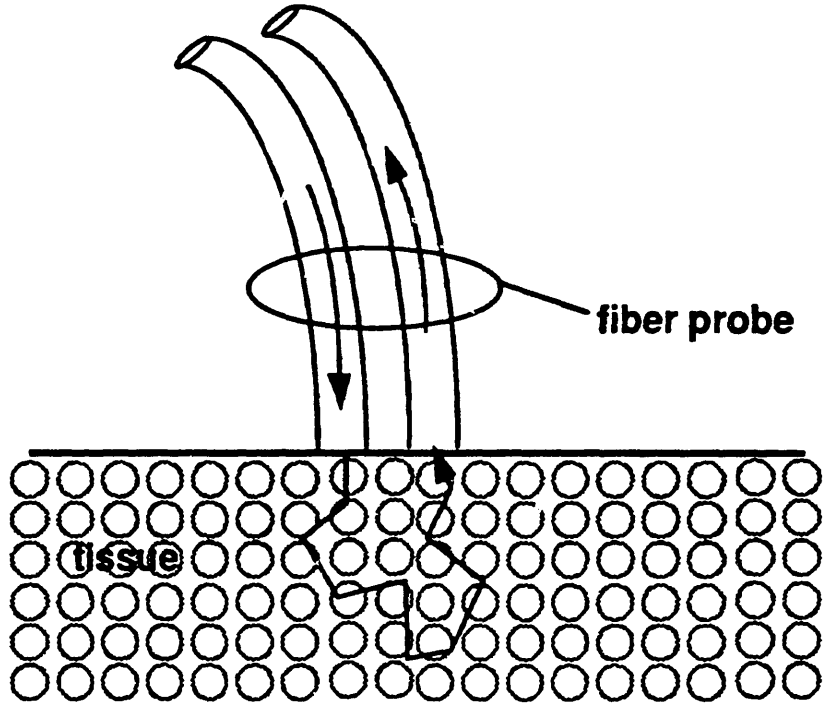


Figure 1. Light that enters the tissue from the illuminating fibers is modified by both elastic scattering and absorption processes before being collected by the fibers that lead to the spectrometer.

The major elements of the Optical Biopsy System:

- A broadband light source (various types of point arc lamps have been used) 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. (When fluorescence from the tissue is to be measured, the illumination source is imaged through a filtering mini-monochromator, and then onto the illumination fibers.)

- 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 spectral signature on the computer's monitor.

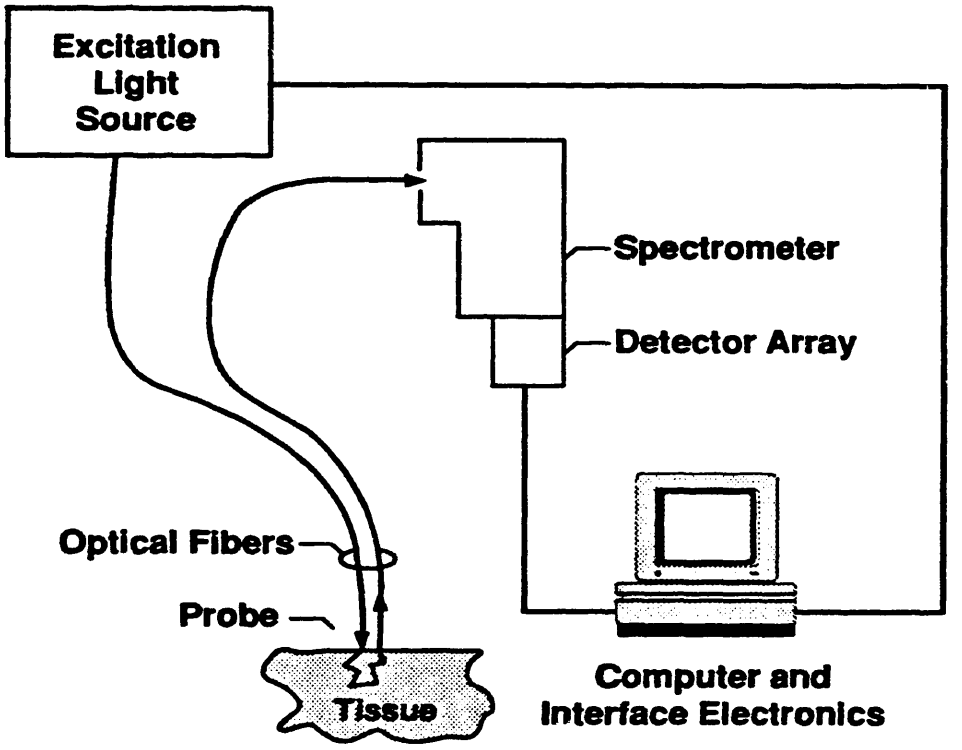


Figure 2. A schematic of the major elements of the OBS system.

6. PRELIMINARY RESULTS

Clinical testing with bladder cancer:

Initially with the first two patients, both white-light backscatter and fluorescence measurements were made. In the case of fluorescence measurements, the illumination light source was filtered with a rapidly stepped mini-monochromator to provide a sequence of narrowband light pulses in 10-nm increments, from 250 to 750 nm, and the full band from 250 to 800 nm was recorded for each illumination color. For each wavelength the data

from 10 lamp pulses were averaged to reduce the pulse-to-pulse variations. (Under these conditions data acquisition time was lengthened to ~5 seconds.) When compared with the white-light scattering measurements, the fluorescence data did not provide any improvement in spectral signature distinction between normal and malignant sites. Therefore, only scattering data were collected on subsequent patients, and only the scattering data for all patients are presented here.

The data reported here are from measurements made on the bladder walls of ten patients.[11] Except for a few sites, noted later, tissue biopsy samples were taken from all areas measured with the optical biopsy system. Pathological diagnosis of these biopsy samples revealed 21 malignant sites (20 of papillary cell carcinoma and one of carcinoma *in situ*) and 28 nonmalignant sites. In some cases a single tissue sample taken for pathological diagnoses corresponded to several closely-located OBS measurement sites. This allowed us to learn about the sensitivity to variations in physician technique in making the optical measurements. In total, 50 optical measurements were made on malignant sites and 32 optical measurements were made on normal or nonmalignant sites. (In a very few cases multiple measurements were made in the exact same location, to verify the reproducibility of the system. These measurements showed very small variations; they were averaged and counted as one measurement.)

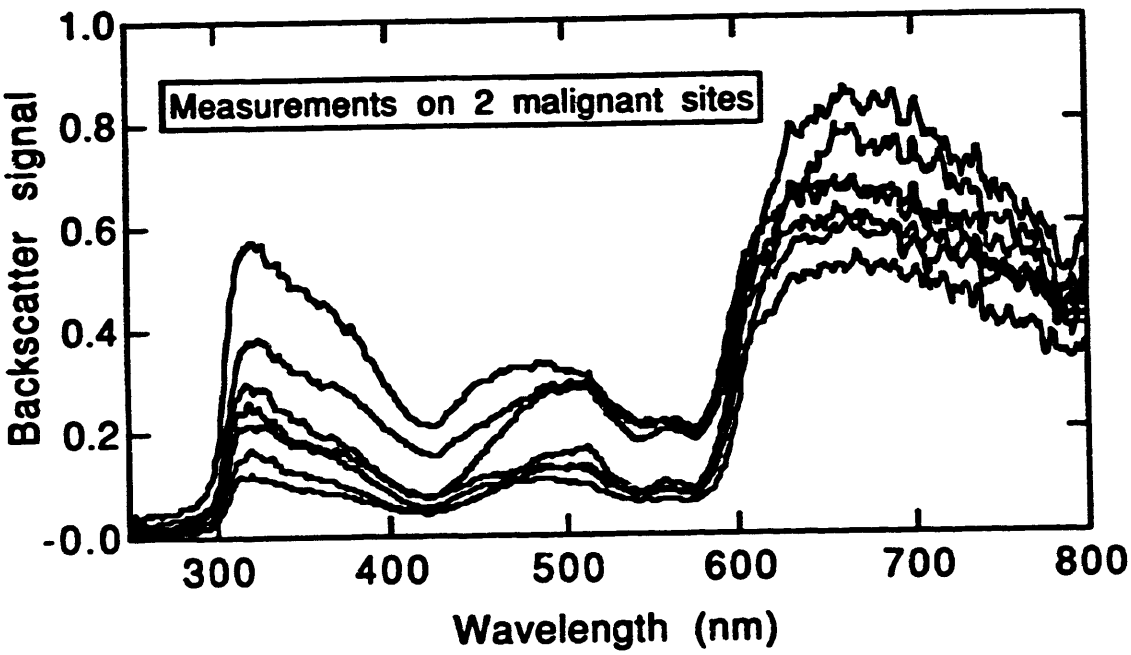
The wavelength calibration of the system was checked (and corrected if necessary) each time the system was transported from Los Alamos to the operating room. This was done using either the peaks in the xenon arc-lamp spectra or the spectra of a mercury lamp. (The more robust design of a next generation system will eliminate the need for recallibration.) A diffuse-reflectance reference spectrum was also taken each time a different fiber probe was attached to the system. The diffuse reflectance of the thermoplastic resin used (Spectralon, Labsphere, Inc.) is > 99% in the wavelength range 400-900 nm and > 95% in the wavelength range 250 - 400 nm.

Figures 3a,3b show the white-light backscatter signals obtained from one patient. The measured spectra, $I(\lambda)$, were calculated according to eqn. 1. I_{tissue} is the raw spectrum of the tissue being examined. $I_{background}$ is a detector background spectrum taken with the excitation lamp off. To a very good approximation the intensity of this spectrum is only a function of the dark noise of the detector. (Since this spectrum is a function only of the detector it must only be taken once when the system is first assembled.) I_{ref} is the diffuse-reflectance reference spectrum.

$$I(\lambda) = \frac{I_{tissue} - I_{background}}{I_{ref} - I_{background}} \qquad \text{eqn. 1}$$

The traces in Fig. 3a are seven OBS measurements of a papillary tumor, four of which were made in the middle of the tumor and three of which were made on the stalk. Separate biopsies were taken of the stalk and the middle of the tumor. Both pathological diagnoses were "papillary transitional cell carcinoma, grade I with no vascular invasion". The solid traces in Fig. 3b are seven measurements from other sites for which the pathological diagnoses were "negative for tumor". The dashed lines are spectra of blood vessels. The probe was intentionally placed on blood vessels in an area expected to be normal. No biopsies were taken at these sites.

a)



b)

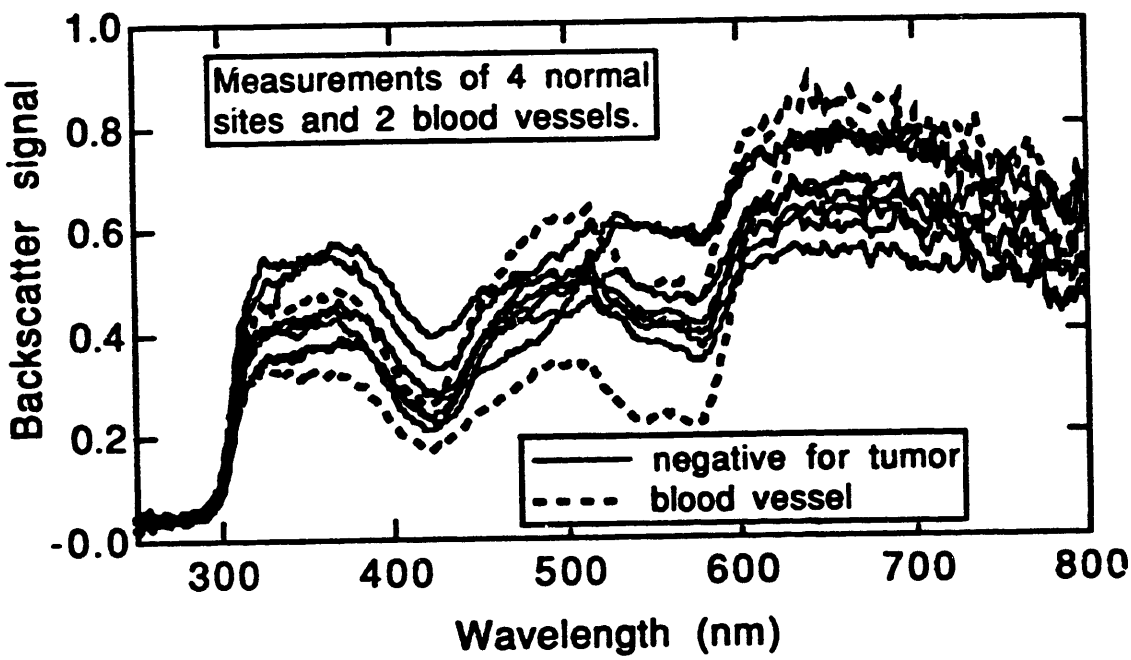


Figure. 3 All of the measurements made on the fourth patient with suspected bladder cancer. a) Measurements corresponding to areas of papillary transitional cell carcinoma. b) measurements corresponding to nonmalignant areas. The two dashed curves are measurements made on blood vessels in areas expected to be normal.

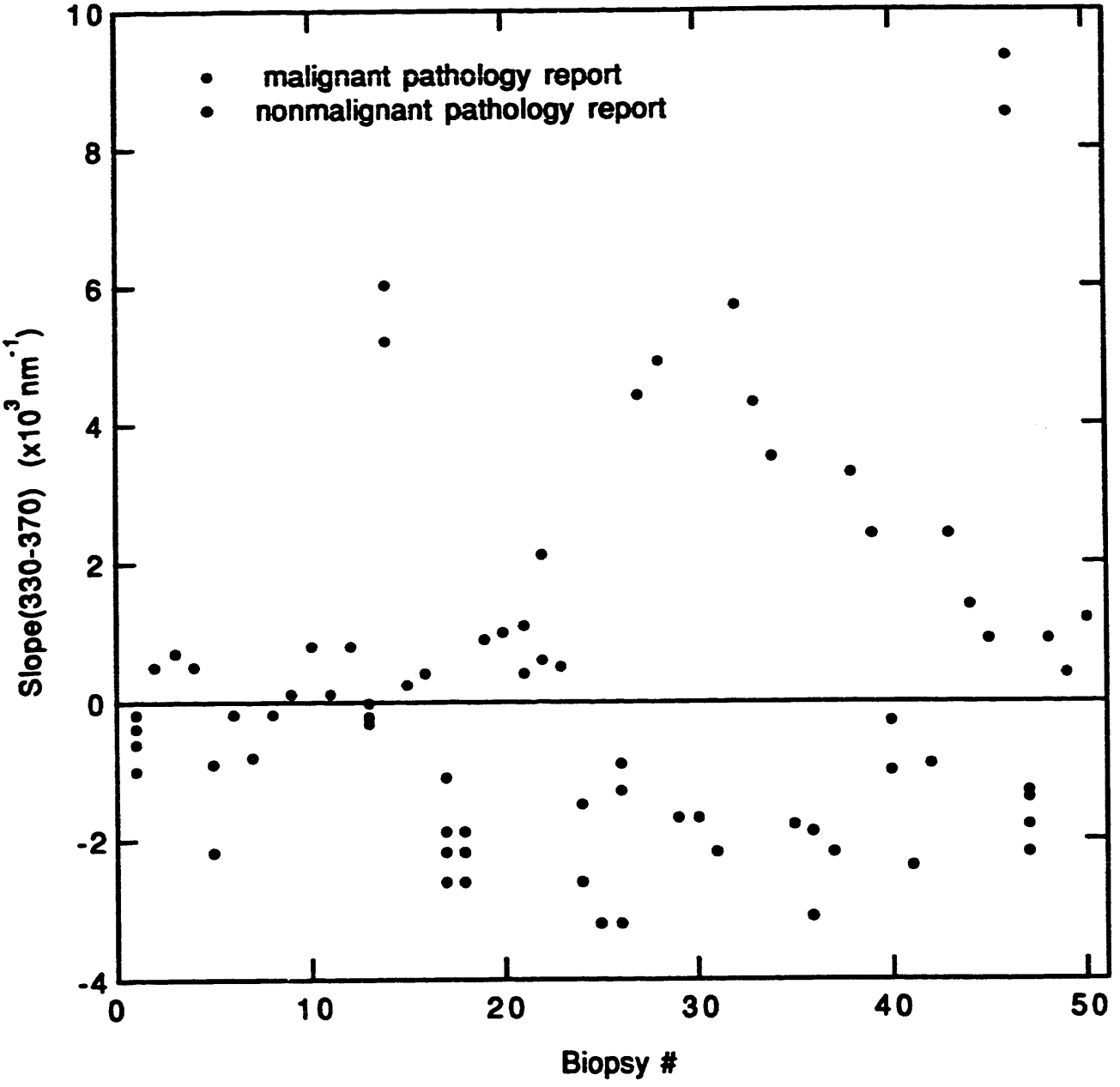


Fig. 4 Normalized slopes in the region 330 - 370 nm for all OBS data with corresponding pathology reports. Open symbols relate to pathology reports of nonmalignant tissue, while solid symbols relate to malignant tissue. In several cases OBS measurements were made on more than one part of a lesion, corresponding to a single biopsy pathology report. These are shown as separate points vertically aligned for the same biopsy #.

Several characteristics of the spectra can be explained by specific absorptions. All of the spectra show a dip at about 415 nm, which is due to hemoglobin absorption. Oxygenated hemoglobin has an absorption maximum at 415 nm. (The absorption maximum of deoxygenated hemoglobin is at 430 nm.) The dips in the backscatter signal at roughly 540 nm and 569 nm are due to the a and b absorption bands of hemoglobin.

None of the spectra show a significant signal below 300 nm. Both proteins and nucleic acids absorb strongly in this region. The amino acids tryptophan and tyrosine have strong absorption bands at 280 nm. Nucleic acids are derivatives of the aromatic rings pyrimidine and purine and consequently absorb strongly in the UV.

All of the backscatter spectra from malignant tissue in Fig. 3 have a negative slope in the region 330 to 370 nm, while all of the spectra of nonmalignant tissue have a positive slope. We found this simple "figure-of-merit" (FOM) to be an almost universal trend with all optical measurements on all patients. It is encouraging to note that, in addition to frank papillary tumors (this identifier also holds for superficial bladder lesions for which clinical observation does not provide any indication of the likely diagnosis (e.g., nonmalignant inflammation versus *carcinoma in situ*). Figure 4 shows a scatter plot of the slope values in the region 330 to 370 nm of the spectra, from all patients, for which there is a corresponding pathology report. Spectra from all locations on papillary tumors always have negative slopes as do the spectra of *carcinoma in situ*. All but one point representing data taken in areas that were identified as nonmalignant by pathological diagnosis have positive slopes. Thus, this simplistic FOM yields no "false negatives" (in terms of malignancy diagnosis) and only one "false positive" out of 73 OBS measurements. All measurements made on putative normal sites (for which biopsy was not taken, and consequently no pathology report) also yielded negative slopes, but are not included in this plot.

7. DISCUSSION AND CONCLUSIONS

Many of the bladder sites examined in this study were frank papillary ("cauliflower") tumors that the urologist could clinically identify as likely to be malignant. Similarly, visually unblemished sites, far from any lesions, are essentially always normal. However, there were several questionable lesions, whose diagnoses were not clinically obvious to the urologist, for which the OBS was correctly able to distinguish between malignant and nonmalignant diagnoses. These included superficial lesions that were reported histologically as being *carcinoma in situ* , papillary transitional cell *carcinoma*, inflammation, edema, etc. These are the types of cases where the cost, risk and discomfort of surgical biopsy could be avoided with an optical diagnostic like the OBS.

The underlying specific biochemical and structural changes in the tissue that result in different OBS signatures for malignant and nonmalignant tissues have not yet been identified. However, one of the most prominent cellular changes in malignant transitional-cell epithelium, compared with normal transitional-cell epithelium, of the bladder wall is the increase in the nucleus/cytoplasm ratio. Further investigation of the specifics of the scattering and absorption in the tissue is warranted.

8. CURRENT AND POTENTIAL FUTURE DEVELOPMENTS

A simplified low-cost and portable version of the OBS, which measures the elastic optical transport over a broad spectral range but does not measure fluorescence, is being developed. This version differs in that the white-light source 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.

A second round of measurements on bladder cancer, with an additional 20 patients and additional physicians, will begin soon at the Lovelace Medical Center.

Experiments are being initiated to determine the usefulness of the OBS in other organ areas: initially, colon, stomach, gynecological, and ocular. Preliminary tests of the OBS on superficial ocular lesions are reported in another paper in these proceedings.[12]

We believe there are numerous other potential medical applications of the OBS. Recently we conducted tests of 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[13]. 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 (see ref. 12). Among gynecological applications, we speculate that diagnosis of endometriosis could be made quickly with laproscopic mediation. (Although this is not cancer, cellular architecture differences are the key to diagnosis.) Finally, the diagnosis of periodontal gum disease may yield to OBS technology.

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