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Title: NONINVASIVE SPECTROSCOPIC DIAGNOSIS OF SUPERFICIAL OCULAR LESIONS AND CORNEAL INFECTIONS

Author(s):
Judith R. Mourant, CST-5
Irving J. Bigio, CST-5
D. C. Gritz, Dept. of Ophthalmology, Univ of Texas
R. D. Glickman, Dept. of Ophthalmology, Univ of Texas
Tamara M. Johnson, CST-5
Tom Shimada, CST-5
K. Storey-Held, Dept. of Ophthalmology, Univ of Texas

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Noninvasive spectroscopic diagnosis of superficial ocular lesions and corneal infections

J. R. Mourant, I. J. Bigio, D. C. Gritz*, R. D. Glickman*,
T. Johnson, T. Shimada, and K. Storey-Held*

Laser Sciences and Applications Group
Los Alamos National Laboratory
Los Alamos, NM 87545

*Department of Ophthalmology
The University of Texas Health Science Center at San Antonio
7703 Floyd Curl, San Antonio, TX 78284-6239

ABSTRACT

The potential of a rapid noninvasive diagnostic system to detect tissue abnormalities on the surface of the eye has been investigated. The optical scatter signal from lesions and normal areas on the conjunctival sclera of the human eye were measured *in vivo*. It is possible to distinguish non-pigmented pingueculas from other lesions. The ability of the system to detect malignancies could not be tested because none of the measured and biopsied lesions were malignant. Optical scatter and fluorescence spectra of bacterial and fungal suspensions, and corneal irritations were also collected. Both scattering and fluorescence show potential for diagnosing corneal infections.

1. BACKGROUND AND MOTIVATION

Optical spectroscopy can be used to detect both physical and chemical changes in tissue. The potential exists for rapid, *in vivo*, non-invasive or minimally invasive diagnosis of tissue abnormalities such as malignancies and infections. Two possible ophthalmic applications for an optical diagnostic system are the diagnosis of ocular lesions on the sclera and the diagnosis of corneal infections. In both these instances a noninvasive, real-time diagnostic system could reduce health care costs and trauma to the patient. The current method for determining malignancy of an ocular lesion on the conjunctival sclera is to excise part of the lesion and do a histopathological diagnosis. A non-invasive system would greatly reduce risks to the patient with a concomitant reduction in medical costs. The diagnosis of bacterial infections is currently performed by culturing corneal scrapings. This takes roughly 24 hours, during which time the infection may progress. Often antibiotics are prescribed in the interim, with their own sets of risks and expenses. A non-invasive diagnostic method capable of differentiating types

of bacteria and fungi would increase the ophthalmologist's ability to treat corneal infections rapidly and effectively.

Several types of spectroscopies have been investigated for tissue diagnosis including Raman, fluorescence, and scattering spectroscopy. Both fluorescence and scattering spectroscopy were used in this study. The basic principle behind scattering spectroscopy is that changes in cell geometry are reflected in the elastic light scattering properties. Therefore, a system which measures elastic light scattering over a broad range of wavelengths should be sensitive to the same morphological changes seen by a pathologist. The principle behind fluorescence spectroscopy is that the fluorescence of a tissue depends on its chemical composition. Several researchers have attempted to diagnose cancer based on the autofluorescence spectrum with varying degrees of success.[1-4] In the typical configurations used for detecting fluorescence and scattering from tissue, the two spectroscopies do not yield orthogonal information. The fluorescence signal reaching the detector depends on the absorption and scattering properties of the tissue as well as on the intrinsic fluorescence. The scattering signal depends on the absorption as well as the scattering properties of the tissue. Therefore, both scattering and fluorescence signals depend on the structure and chemical composition of the tissue.

2. METHODS

A schematic of the optical biopsy system (OBS) is shown in Figure 1. This system can be used for either fluorescence or scattering measurements. Light from a pulsed xenon-arc lamp is imaged through a monochromator into one or more optical fibers. A probe containing the light delivery fibers and the collection fibers is placed in direct contact with the tissue being examined. Collected light is dispersed by a spectrograph and detected by an intensified diode array. For fluorescence measurements the illumination monochromator is stepped through a sequence of wavelengths. For the measurements of corneal infections, excitation wavelengths of 250 to 320 nm in 10-nm increments were used. Some of the elastic scattering measurements were also made by stepping the monochromator through 10 nm increments from 250 to 800 nm. (For example see fig. 2.) For other scattering measurements the zeroth order of the monochromator in the light delivery path was used.

In vivo measurements of lesions on the sclera were made on 11 patients and several volunteers. A topical anesthetic was used before placing the optical probe in gentle contact with the conjunctival sclera. The light scatter spectra of several pinguecula, nevi and lesions diagnosed as primary acquired melanosis were measured. A pinguecula is any lesion that shows degeneration of the collagen under histological diagnosis; these lesions often

appear yellowish in color and can frequently be diagnosed by the examining ophthalmologist. A nevus is a congenital pigmented lesion. Primary acquired melanosis refers to pigmented lesions that appeared during the patients lifetime and are not secondary to other pathologies. Histopathological diagnoses were performed on several of the lesions measured with the optical biopsy system.

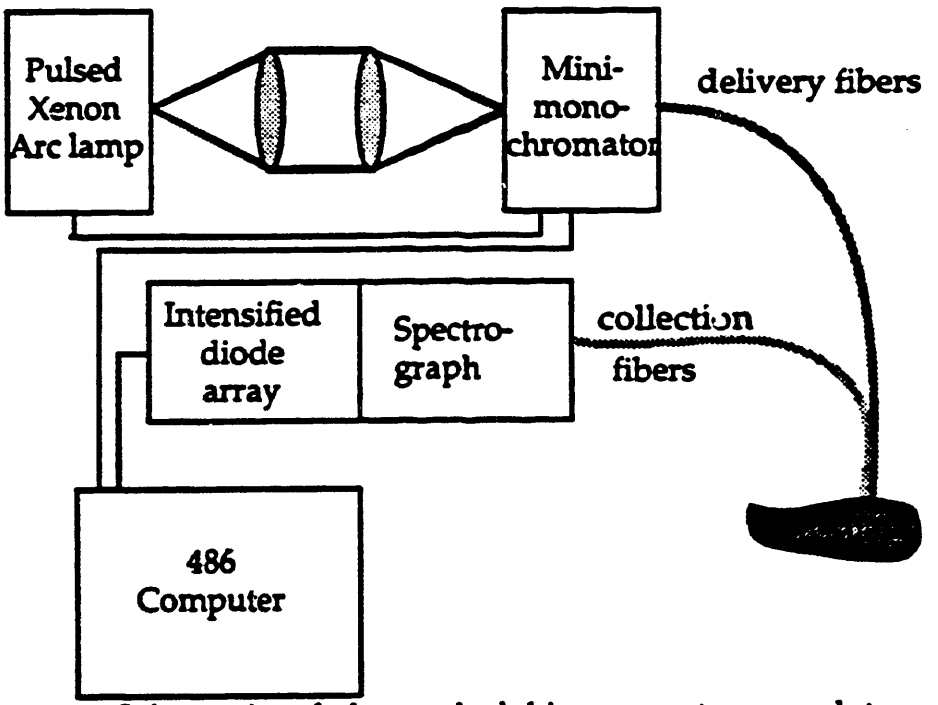

Fig. 1 Schematic of the optical biopsy system used to make scattering and fluorescence measurements.

In vitro measurements with the OBS were made on cultures of several bacteria and fungi (*Psuedomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Candida albicans and Aspergillus fumigatus)* which are common in eye infections. All measurements were made under identical conditions. A Pyrex test tube held in a black optical mount was filled to a specific level with the culture to be measured. The tip of the fiber probe was then submerged a specific distance into the solution. The probe consisted of one 400-μm delivery fiber and one 200-μm collection fiber. The fluorescence protocol was used for making the measurements.

In vivo measurements were made on the corneas of 15 rabbits with brown irises. The rabbits were anesthetized during the measurements to minimize eye movement. A microscope was used to ensure accurate placement of the probe over the lesion. The number of eyes injected with each occulant is shown in Tables 1 and 2. Scattering measurements on the corneas injected with fungi or bacteria were made 5-10 minutes after injection. Fluorescence

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measurements were made on the same sites a few minutes later. Scattering measurements on the corneas injected with balanced salt solution (BSS), clove oil, and *Psuedomonas aeruginosa* bacteria were made 12 to 16 hours after the injections. Fluorescence measurements were also made on the corneas injected with *Psuedomonas aeruginosa* bacteria 12 to 16 hours after the injections.

Balanced salt solution	Clove oil	*Psuedomonas aeruginosa*
5	5	5

Table 1. Distribution of injections made roughly 12 hrs before the measurements.

Staphylococcus aureus	*Streptococcus pnemoniae*	*Candida albicans*	*Aspergillus fumigatus*	Distilled water
2	2	2	2	5

Table 2. Distribution of injections made shortly before the measurements were taken. *Psuedomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumonia* are bacteria. *Candida albicans* and *Aspergillus fumigatus* are fungi.

3. RESULTS

3.1 Lesions on the conjunctival sclera

To determine if the OBS measurements are robust to changes in examiner and to determine the variability of normal sclera, measurements were made by three examiners on three people believed to have normal conjunctival scleras. Three measurements were made on each patient by each examiner. A total of 27 spectra were recorded. (One of the measurements is not plotted because the examiner moved the probe during the measurement.) The measurements are in excellent agreement with each other and show that the measurements on normal sclera are quite reproducible.

OBS measurements were made on 15 conjuncitval lesions which were to be biopsied for histopathological diagnosis. None of these lesions were found to be malignant. Figure 3 shows the scattering spectrum obtained from a non-pigmented pinguecula. There is a decrease in the signal amplitude in the UV region of the spectrum when compared with a spectrum taken at a normal site. This is typical of non-pigmented pinguecula and cysts. Pigmented lesions show a decrease in signal amplitude across the entire spectral region (Figure 3) as is expected due to melanin absorption.

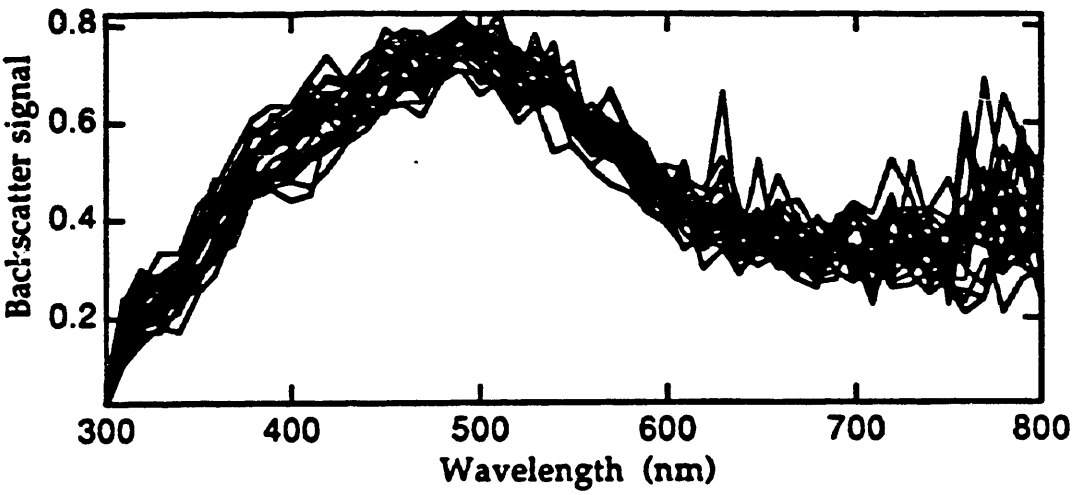

Fig. 2 Twenty-six measurements of scattering from normal conjunctival sclera. The measurements were made on three different people by three different examiners.

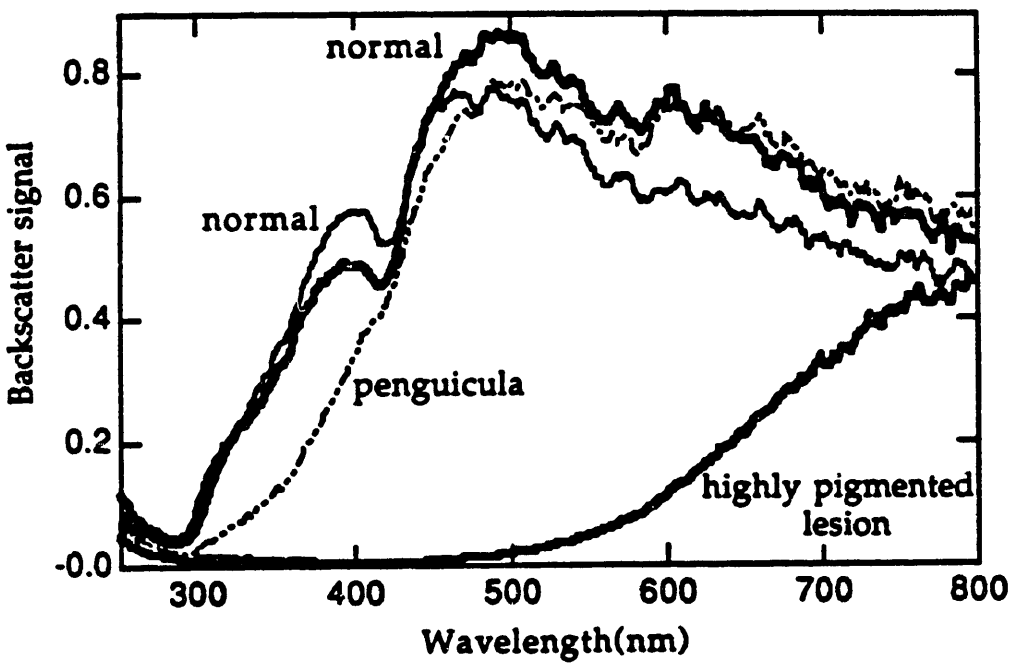

Fig. 3 Optical scattering spectra of scleras of various ocular surface conditions. The two thick traces are spectra taken on the same eye. One is of a normal region. The other is of a highly pigmented lesion. The two thin lines are again spectra taken on the same eye. One is from a region expected to be normal and the other is over an area with a pathological diagnosis of 'subconjunctival degenerative nodule', referred to clinically as penguicula.

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3.2 Bacterial and fungal suspensions

The response of bacterial and fungal suspensions to excitation at 280 nm is shown in figure 4. The shape of the responses for *P. aeruginosa*, *A. fumigatus* and *C. albicans* are very distinctive. The signal obtained for *S. pneumoniae* and *S. aureus* are similar in shape, but differ in amplitude.

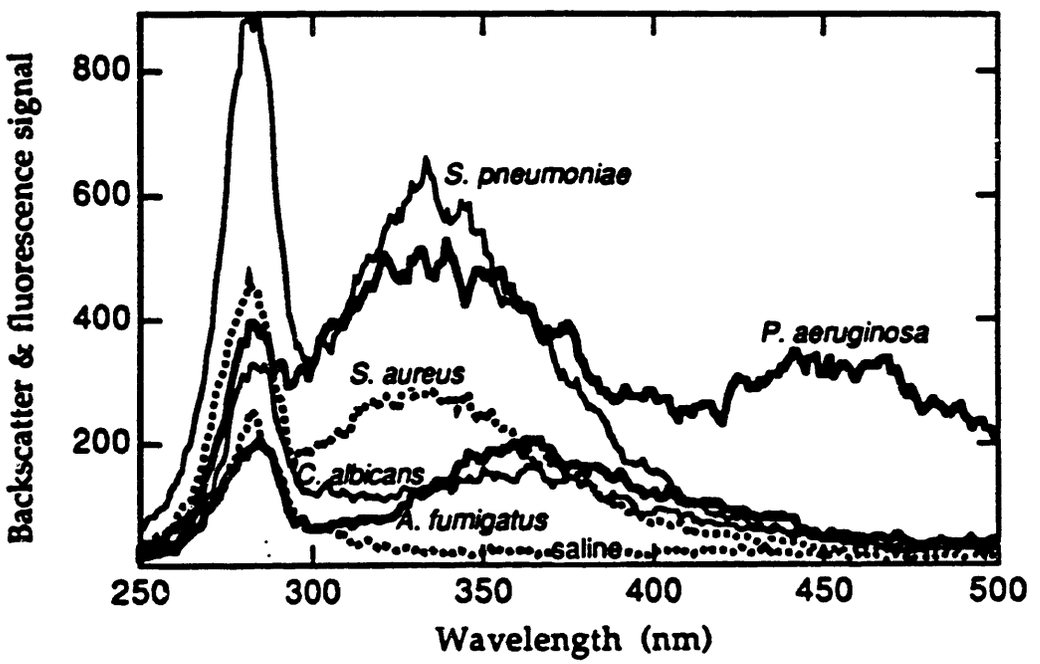

Fig. 4 Response of bacterial and fungal suspensions (in saline) to 280 nm excitation.

3.3 Corneal inflammations and infections

The purpose of the clove oil, BSS and distilled water injections was to cause a noninfectious irritation. It was hoped that scattering signals from infected corneas would be distinguishable from those of irritations. However, a large variability was found in the measurements of BSS, clove oil and distilled water and it is not possible to distinguish the measurements of bacterial or fungal infections from those of noninfectious irritations.

Nonetheless, when only the spectra of bacterial infections are examined, the different bacteria have distinctive signatures, as shown in fig. 5. However, there is some variability seen in measurements of corneas injected with the same bacteria and more measurements are needed to determine the reliability of the bacterial signatures.

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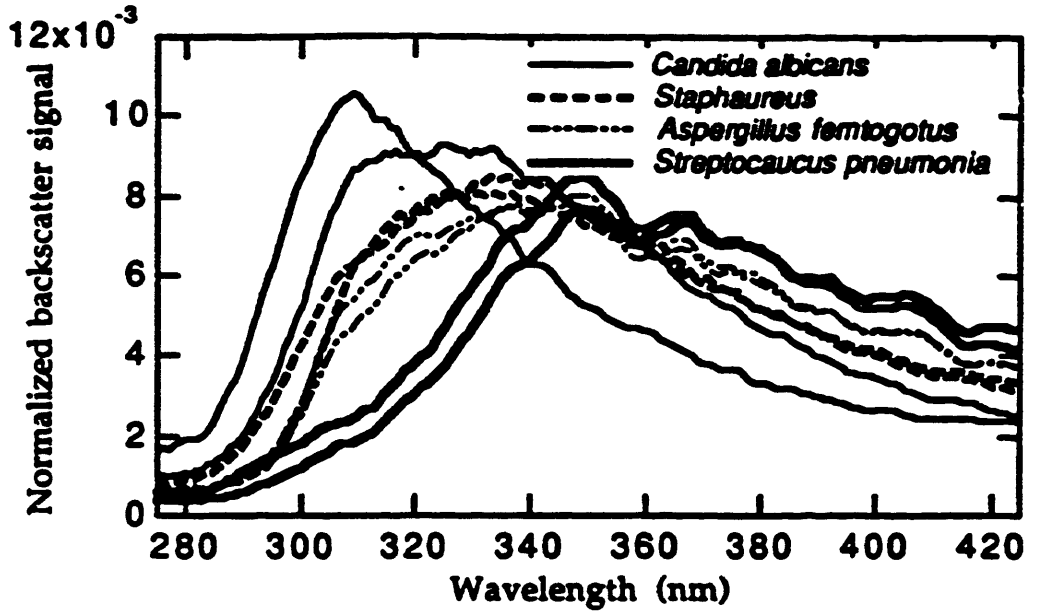

Fig. 5 Backscatter spectra of corneas injected with *Candida albicans, Staphylococcus aureus, Aspergillus fumigatus* and *Streptococcus pneumonia*. Each spectrum shown is from a different rabbit and is the average of three spectra. The spectra were normalized to have equal areas under the curve in the wavelength range 250 to 500 nm.

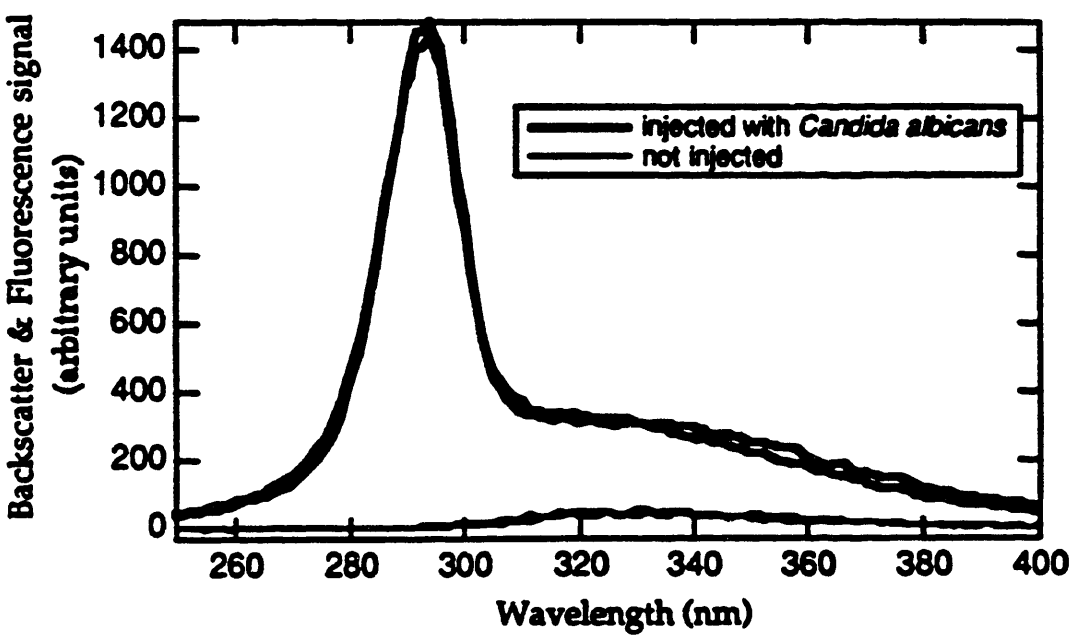

Fig. 6 Backscatter and fluorescence from corneas injected with *Candida albicans* and from corneas which were not injected. Excitation at 290 nm.

Fluorescence measurements were made on a few corneas before injection of bacteria or fungi as well as on injected corneas. The corneas which were not injected have a weaker fluorescence signal. Fig. 6 shows the fluorescence signal of two corneas before injection and of two corneas injected with *Candida albicans*. The fluorescence signal can be used to distinguish *Candida albicans* and *Streptococcus pneumoniae* from *Psuedomonas aeruginosa, Aspergillus fumigatus,* and *Staphylococcus aureus* as shown in Table 1. The ratio of the peak amplitude of the fluorescence signal due to excitation at 280 nm to the backscatter at 280 nm is less for *Candida albicans* and *Streptococcus pneumoniae* than for the other types of bacteria and fungi. *Candida albicans* and *Streptococcus pneumoniae* may be separated from each other by the peak position of the fluorescence signal, which for the two cases of *Streptococcus pneumoniae* infections we measured was red shifted compared to *Candida albicans*.

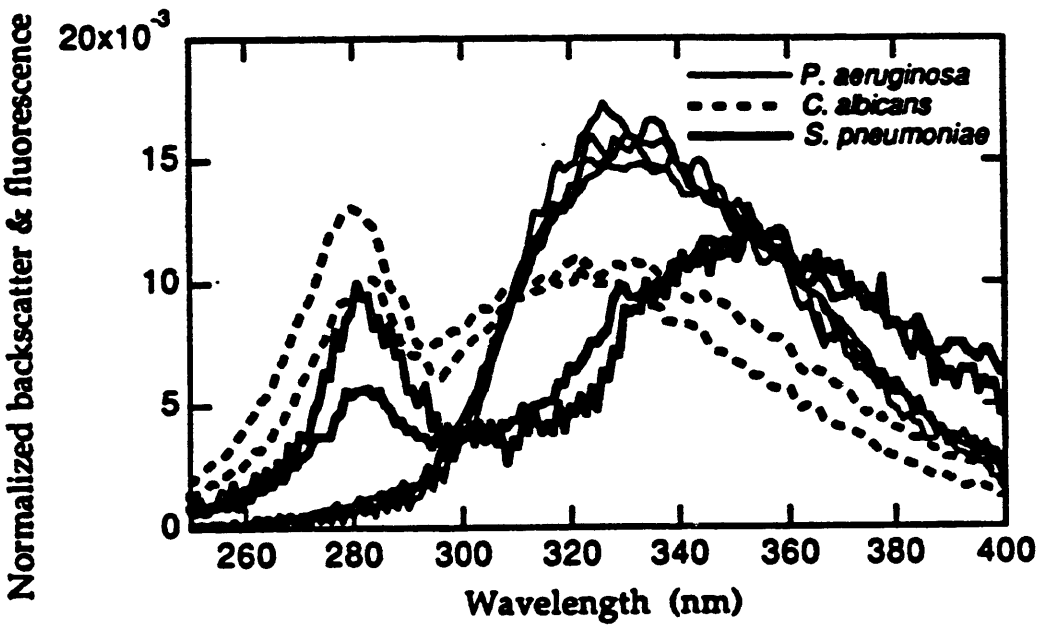

Fig. 7 Fluorescence and scatter signals from *P. aeruginosa, C. Albicans* and *S. pneumoniae*. Excitation was at 280 nm. The spectra were normalized from 250 to 400 nm and each spectra is the average of three spectra taken in the same location.

S. aureus	*P. aeruginosa*	*C. albicans*	*S. pneumoniae*	*A. fumigatus*
14.3	16.7	0.8	1.7	5.2
7.1	32.0	1.5	1.3	52.3
	41.3			
	29.0			

Table 2. Ratio of the fluorescence peak to the backscattered signal at the excitation wavelength for 280 nm excitation.

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There was a large degree of variability in the spectra of *Staphylococcus aureus* infections and it is not possible to separate the spectra of *Staphylococcus aureus* from those of *Psuedomonas aeruginosa* and *Aspergillus fumigatus*. It is, however, possible to separate the spectra of the four lesions injected with *Psuedomonas aeruginosa* from the two lesions injected with *Aspergillus fumigatus*. The amplitude of the fluorescence from *Psuedomonas aeruginosa* is always greater than the fluorescence from *Aspergillus fumigatus*.

In the above discussion the signal at a red shifted wavelength relative to the excitation signal was referred to as the fluorescence signal. It is important to note that the amplitude and apparent shape of this signal depends strongly on the scattering properties of this signal as well as on the fluorescence. Recently algorythms for extracting the intrinsic fluorescence from fluorescence and scattering measurements when some of the tissue optical properties are known have been developed.[5,6]

4.0 CONCLUSIONS AND DISCUSSION

A non-invasive, real time, optical diagnostic system capable of diagnosing abnormalities on the surface of the eye could make a significant contribution to ophthalmology. Preliminary measurements of the scattering signal of lesions on the conjunctiva and cornea of the eye showed that different lesions have different optical signatures. Non-pigmented pinguecula can be distinguished from normal tissue and pigmented lesions. The presence of melanin in many of the lesions makes the differentiation of pigmented lesions difficult because the melanin absorption may mask other tissue changes. The detection of lesion margins may, however, be a potential application for the OBS.

The fluorescence signals of bacterial and fungal suspensions common in eye infections were measured. The fluorescence spectra for *P. aeruginosa*, *A. fumigatus* and *C. albicans* are very distinctive. The signals obtained for *S. pneumoniae* and *S. aureus* are similar in shape, but differ in amplitude. Scattering and fluorescence measurements of rabbit corneas injected with bacteria, fungi and other irritating agents were made. Both the scattering and fluorescence measurements of corneas injected with bacteria or fungi can potentially differentiate types of bacteria and fungi. However, more data are needed, and the causes of the variability must be determined.

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