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Publication Date
1996-04-05

DOI
10.1117/12.237574

Determination of the cervical transformation zone using elastic-scattering spectroscopy

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ABSTRACT

Optical measurements of the cervical transformation zone (sometimes referred to as the transition zone) using elastic-scattering spectroscopy, demonstrate sensitivity to the epithelial cell-type differences.

keywords: optical biopsy, tissue spectroscopy, elastic scattering, noninvasive diagnostics

INTRODUCTION AND BACKGROUND

The transformation zone of the cervix, near the external os, designates the boundary area where the cervical epithelium changes from predominantly columnar cells of the interior of the uterus to predominantly squamous cells that are exposed to the vaginal cavity. The transformation zone is well studied because it is the site where most cervical cancer originates.

Under culposcopy the transformation zone can be seen in about two-thirds of women examined, but its location is not clearly discernible in the remaining one-third. Changes in the location and extent of the transformation zone are known to precede the onset of labor and to appear earlier than other indications, such as dilation or contractions. Cervical "ripening" prior to labor generally involves an increased water content of the tissue and increased microvascularization, but is generally tracked simply by palpation. A noninvasive, objective method to measure and track the changes in the condition of the tissue of the transformation zone would be of significant value in gynecological research.

0-8194-2053-0/96/$6.00

We have clinically investigated the use of elastic-scattering spectroscopy, with a fiber-optic probe from a portable instrument, for locating and characterizing tissue near the transformation zone. Tissue differences are detected/diagnosed using spectral measurements of elastically scattered light in an optical geometry that results in sensitivity to both the absorption and scattering properties of the tissue, over a wide range of wavelengths (300 - 750 nm). The intent of these preliminary studies is to determine the ability to distinguish the squamous-cell and columnar-cell epithelia in the region of the cervical transformation zone. Laser-induced-fluorescence (LIF) has been used to detect malignant and pre-malignant conditions in the cervix.[1,2] The LIF spectral signatures are expected to reflect biochemical changes in the tissue corresponding to malignant states. Our initial interest here is to distinguish different types and conditions of normal tissue, which relate to differences in cellular structure, and which may or may not be accompanied by any biochemical differences.

Measurements made on 11 patients indicate that reliable spectroscopic distinction is possible, and that the transformation in the composition of cellular structure across the zone is reflected in a transformation from the spectrum that is characteristic of columnar cells to the one that is characteristic of squamous cells. Figures 1 and 2 illustrate the epithelial structures that result in different histological appearances of squamous-cell and columnar-cell epithelia, respectively.

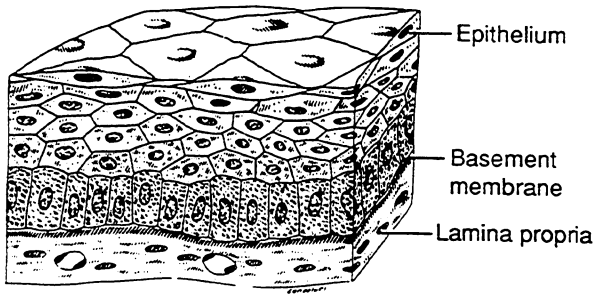

Fig. 1 Squamous-cell epithelium.

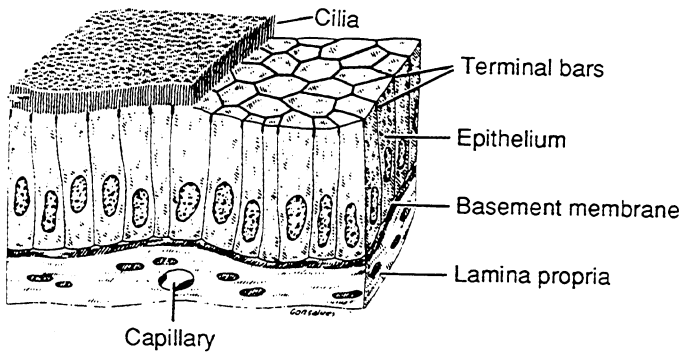

Fig. 2 Columnar-cell epithelium.

APPARATUS AND METHODS

The general principles for our method of elastic-scattering spectroscopy (ESS), and the operating features of our apparatus have been described in several earlier publications.[3,4,5] A broadband light source is used to cover the spectral range of 300-750 nm. Figure 3 depicts the optical geometry of our fiber-optic probe and the tissue being examined.

The ESS probe is placed 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 undergo multiple scattering through a small volume of the tissue before entering the collection fiber(s). (No light is collected from surface reflection, specular or diffuse, and we submit that ESS is a more accurate name for our method than "reflectance" spectroscopy.) The fiber-core diameters range from 200 to 400 μm, with center-to-center separations of 400 μm typically.

The collected spectrum is sensitive to both the absorption features and the wavelength-dependence of the scattering properties of the tissue. The resulting effective path length of the collected photons is generally several times greater than the actual separation of the fiber tips. Consequently, the system has good sensitivity to the optical absorption bands of the tissue components, over its effective operating range of 300 to >750 nm, and such absorption features add valuable complexity to the scattering spectral signature. For the short source-to-detector fiber separations used in this geometry, the wavelength dependence of the scattering properties of the tissue are sensitive to the average size and refractive index of the scattering centers. It is important to note that the fiber probe, being used in optical contact with the tissue, examines only that site and does not image the tissue surface.

The clinical measurements reported here were taken in the presence of ambient and culposcope illumination (either white of blue), without background subtraction, which may have contributed modestly to variations in the measurements. While our intent with these measurements was proof-of-concept, we plan to modify the system for future measurements so that a background measurement is made sequentially for each tissue measurement, allowing subtraction of any contribution from the external illumination sources.

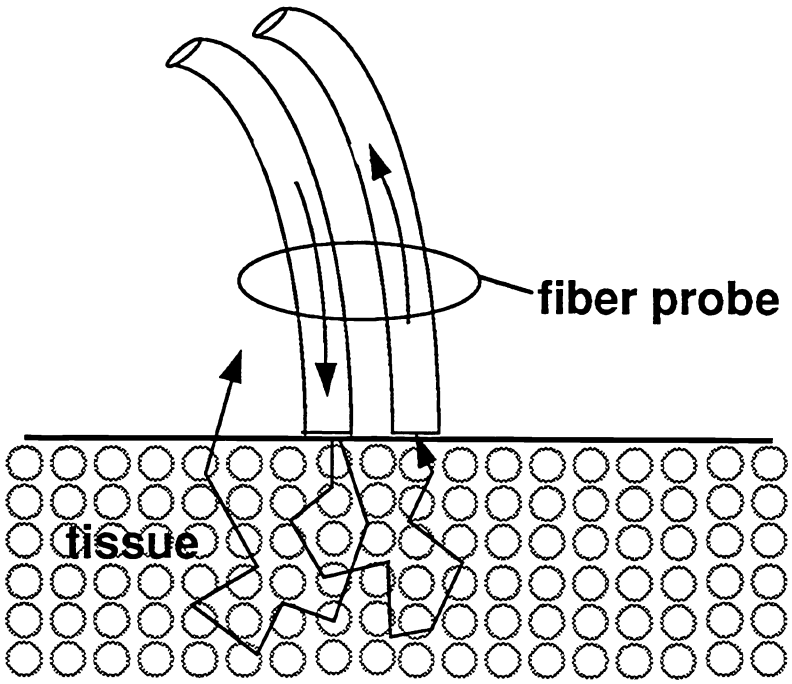

Fig. 3. The optical geometry of the fiber probe in optical contact with the tissue surface.

CLINICAL MEASUREMENTS

Measurements were made on 11 patients at two clinical sites: the UC-Irvine Medical Center and the Orange County Healthcare Center. All measurements were made during culposcopy, and in most cases corresponding biopsies were taken for histological examination. Some of the optical measurements were made on tissue sites that were not biopsied, but for which the attending physician was able to unambiguously identify as being normal, and as being clearly located with respect to the transformation zone. Data were not included if a biopsy was not performed and the transformation zone was not clearly observable. The typical spectra for squamous-cell and columnar-cell epithelia are shown in Figure 4.

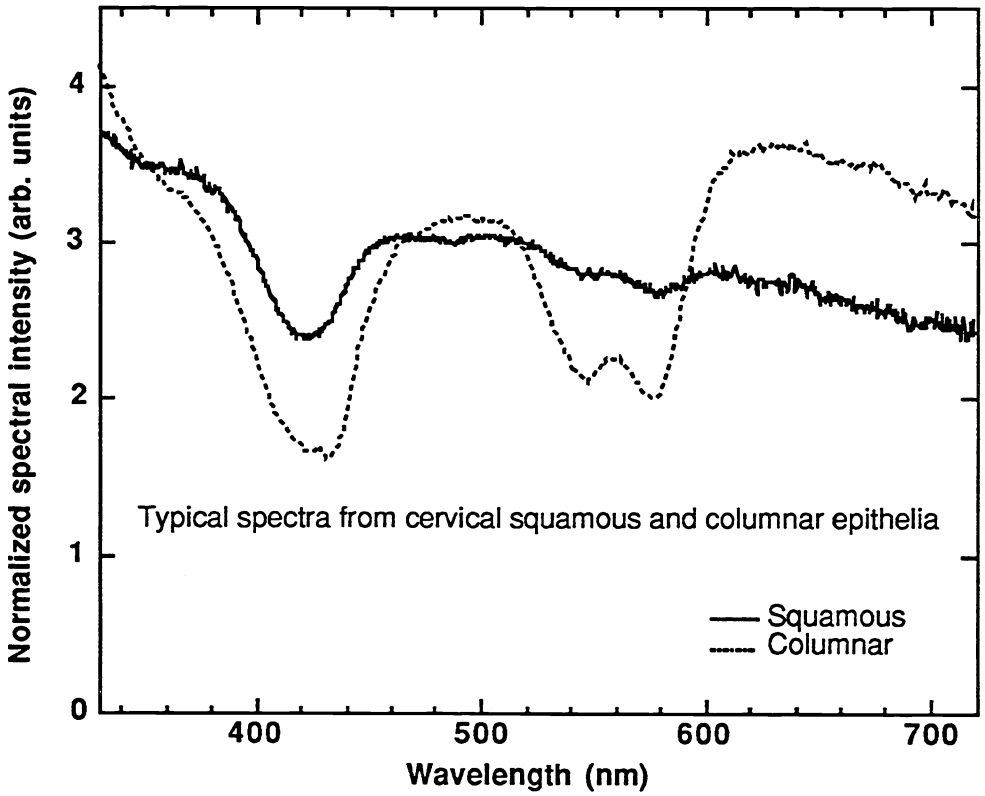

Fig. 4. Normalized spectra representative of typical data for squamous-cell and columnar-cell epithelia.

These specific spectra are representative of the averages of spectra for each type of epithelium. Both traces are normalized to have the same total area under the curve. The absorption from the hemoglobin Soret and Q bands are clearly stronger for the columnar-cell epithelium, and this feature provides useful information about the nature of the tissue, although it was not the only spectral signature that appears to be useful for separating the epithelium types.

Figure 5 shows the data from 22 optical measurements for a spectral signature that relates to the relative strength of hemoglobin (Hb) absorption, grouped by tissue type. (In this plot the Hb bands have been divided by a spectral region that has relatively low Hb absorption, so that what is plotted is a unitless parameter, inversely proportional to the relative hemoglobin absorption.) In Fig. 4 it can also be seen that the overall slants of the spectra from UV to NIR are different for the two tissue types. We believe this is due to the differences in the wavelength dependence of the scattering efficiency for larger scattering angles, due to differences in the typical scattering centers for the two different tissue structures. Moreover, the unnormalized raw spectra reveal that the squamous epithelium has a larger total scattering efficiency than the columnar epithelium. This is consistent with the intuitive conclusion that one would draw from the diagrams in Figs. 1 and 2, where one can see that squamous epithelium has a layered structure of more tightly packed cells, with a commensurately larger concentration of nuclei.

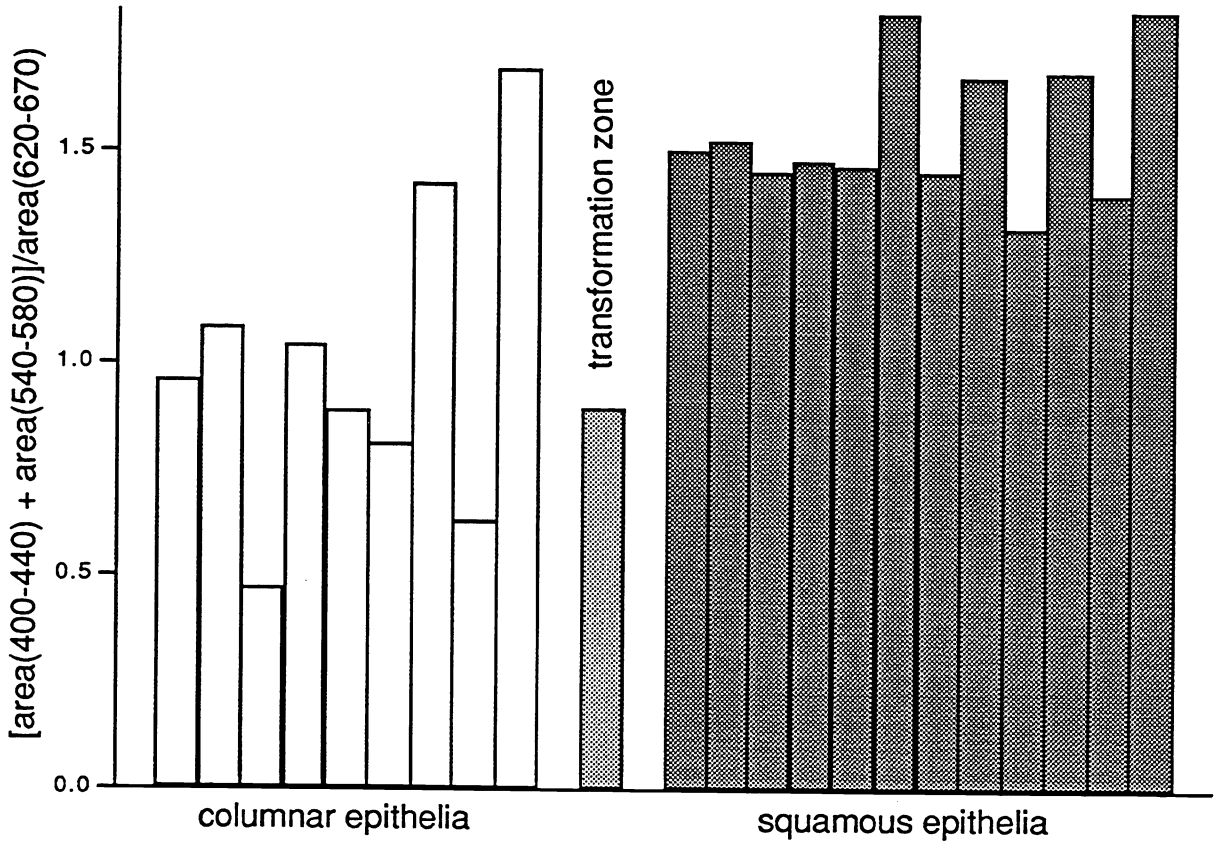

Fig. 5 Spectral signature based on the signal in the Hb absorption bands, normalized by the signal in the red region of 620-670 nm.

Figure 6 shows the values for a different spectral signature: the locally-normalized slope of the spectrum between 330 and 370 nm. While the oxy/deoxy Hb ratio of can affect the slope

in this region, the spectra in the regions of the Soret and Q bands indicate that the ratio does not change between the two epithelium types. Other, more minor biochemical constituents can also contribute to a change in the slope in this region, but we believe that the major differences are due to variations in the angular-probability distribution for scattering, caused by the structural differences discussed above. The reasoning underlying this expectation has been discussed in Reference 4.

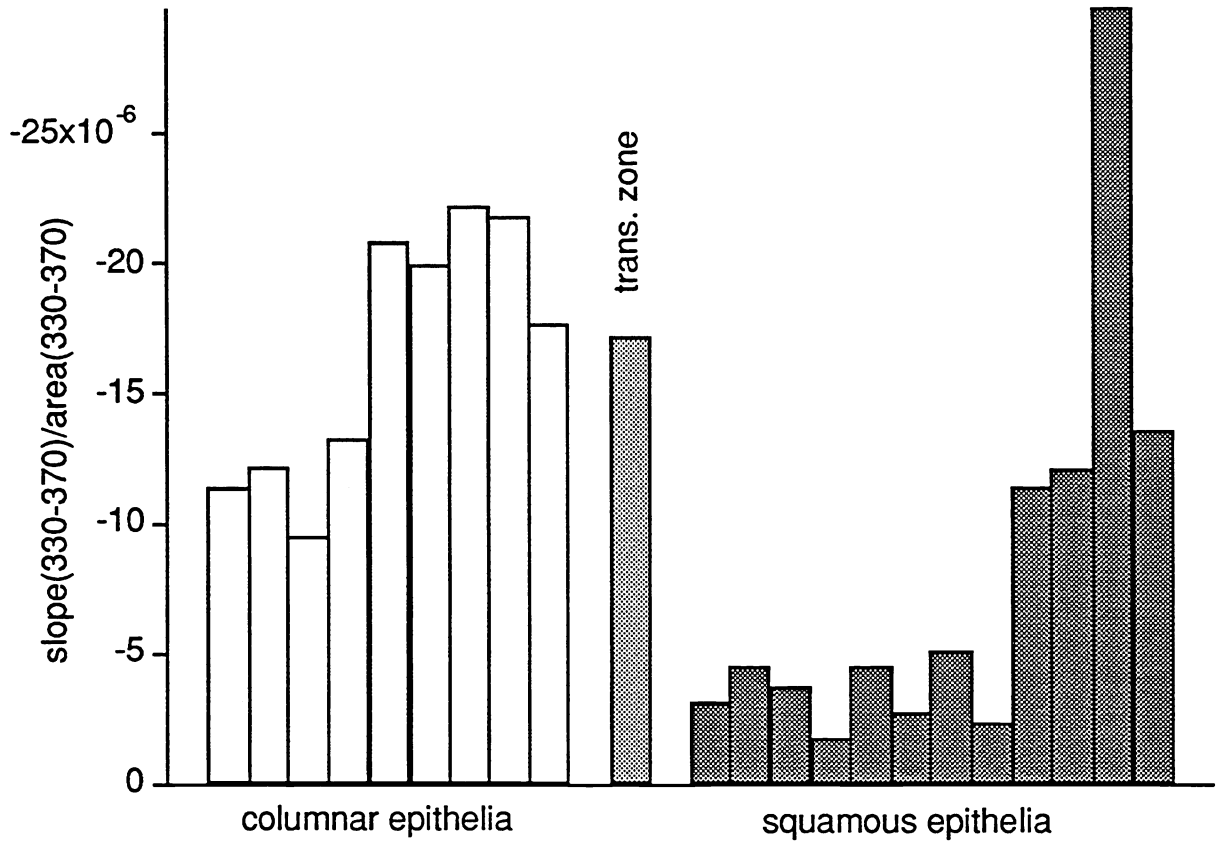

Fig. 6 Spectral signature based on the slope of the spectrum in the near-UV region.

During our clinical study, several optical measurements were made directly on the transformation zone of several patients. However, only one such data point appears in Figures 5 and 6. This is because the biopsy reports for the excluded points revealed dysplastic or malignant conditions. Since the purpose of this preliminary study was to characterize different normal tissue types and conditions, and other pathological conditions could alter the spectra, these were omitted for this study. The ability to identify dysplastic conditions may be the object of a future study.

CONCLUSIONS AND FUTURE PLANS

Preliminary optical measurements on women undergoing follow-up culposcopy indicate that elastic-scattering spectroscopy may provide a valuable tool as a noninvasive method for characterizing the condition of the cervical tissue and for identifying the location of the transformation zone in cases where it is not readily visualized under culposcopy. Future measurements will be conducted with an improved instrument that performs a real-time background subtraction. This would eliminate any effects of the culposcope illuminator or the room lights. Also, a larger study, with rigorous correlation to biopsy reports, will determine the sensitivity to dysplastic conditions.

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