

Optical Technologies for Cervical Neoplasia: Update of an NCI Program Project Grant

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Abstract: Cervical cancer is the second most common cancer in women worldwide and the leading cause of cancer mortality in women in developing countries. In the United States, over \$6 billion is spent annually in the evaluation and treatment of low-grade lesions, many of which do not develop into full-blown cancer. In developing countries, however, the chief concern is that cervical cancer goes undetected because of the cost of testing and the lack of resources and trained personnel to screen and diagnose the disease. The goal of the National Cancer Institute Program Project Grant CA82710 is to assess the emerging technologies of fluorescence and reflectance spectroscopy and quantitative cytology and histopathology for the diagnosis of cervical neoplasia. All of these technologies should decrease mortality, morbidity, and the cost of treating cervical cancer.

Screening and detection of cervical cancer could be greatly improved by technologies that improve, automate, and decrease the cost of screening and detection. The primary goal of National Cancer Institute (NCI) Program Project Grant CA82710 is to carry out a comprehensive technology assessment of emerging optical spectroscopy and quantitative imaging technologies to improve the detection of cervical cancer and its precursors in both the developed and developing worlds. The 5 areas of new optical technologies this program project seeks to address, discussed in greater detail below, are: biologic plausibility, by examining the fluorescence/reflectance and quantitative images of cell lines, tissue cultures, and live tissue sections; technical feasibility, by conducting large screening and diagnostic trials of fluorescence and reflectance spectroscopy and quantitative cytology and histopathology; intermediate effects, by using the fluorescence and reflectance spectrometer in a randomized clinical trial; patient outcomes, by assessing patient and provider acceptability of fluorescence and reflectance spectroscopy and quantitative cytology and histopathology; and societal outcomes, by assessing the performance and cost-effectiveness of fluorescence and reflectance spectroscopy and quantitative cytology and histopathology in the screening and diagnostic setting.

The Program Project grant Optical Technologies for Cervical Neoplasia (P01CA82710) addresses technological assessment and has been funded by the NCI since 1999. This multi-institutional program project, made up of 5 research projects and 4 supportive cores, is primarily funded through The University of Texas M. D.

Anderson Cancer Center, with primary collaborators at The University of Texas at Austin and the British Columbia Cancer Center in Vancouver, British Columbia, Canada. The multidisciplinary research group on this program project consists of gynecologic oncologists, biomedical engineers, pathologists, cell biologists, behavioral scientists, and decision scientists from all 3 primary locations.

Biologic Plausibility

Fluorescence spectroscopy provides a noninvasive window to monitor the biochemical and morphologic changes that accompany the development of cervical precancer. Using fluorescence, one can probe a number of naturally occurring fluorophores present in cervical tissue, which are altered with dysplasia. For example, the pyridine nucleotides and the flavins play an important role in cellular energy metabolism.¹ Nicotinamide adenine dinucleotide is the major electron acceptor; its reduced form is NADH, and the reduced nicotinamide ring is fluorescent.¹ Flavin adenine dinucleotide is the other major electron acceptor; the oxidized form, FAD, is fluorescent, while the reduced form, FADH₂, is not.² The aromatic amino acids, tryptophan, tyrosine, and phenylalanine, contribute to protein fluorescence at ultraviolet (UV) excitation wavelengths.¹ Autofluorescence has been noted in the structural proteins, collagen,³ and elastin.⁴ Collagen fluorescence is associated with crosslinks,³ and elastin autofluorescence is also suspected to be associated with crosslinks, which are autofluorescent with an excitation maximum at 325 nm and an emission maximum at 400 nm.^{3,5}

Figure 1 shows excitation-emission matrices (EEMs) of several components of cervical tissue. An EEM is a topographical map of the fluorescence intensity as a function of the excitation wavelength and emission wavelength. The EEM of cervical epithelial cells shows contributions from tryptophan, NADH, and FAD. Several investigators have shown that the endogenous fluorescence near 500 nm when excited near the UV region is weaker in tumor tissue relative to normal surrounding tissues.^{6,7} The differences may lie in the decrease in the oxidized forms of flavins and the relative amount of NADH in malignant tissues. The EEM of collagen shows contributions from tyrosine fluorescence in the UV as well as broad peaks due to covalent crosslinks. The EEM of a multilayer cervical tissue culture, consisting of epithelial cells atop a collagen stroma (Figure 1), shows contributions of both mitochondrial fluorophores NADH and FAD, as well as tryptophan and collagen crosslinks. In intact, vascular tissue, the EEM contains contributions from these fluorophores, which are modulated by the reabsorption of fluorescence by hemoglobin in stromal blood vessels.

To assess how the fluorescence associated with these chromophores is modulated by the presence of dysplasia, we mapped the fluorescence in living organ cultures using fluorescence confocal microscopy. Cervical biopsies (2 mm × 4 mm × 1 mm) from colposcopically normal and abnormal areas were obtained, with written consent. Biopsies were immediately placed in chilled culture medium, and fresh tissue slices of 200 μm were cut perpendicular to the epithelial surface. Optical sections imaged at UV excitation (Figure 2) show that epithelial fluorescence is associated with cellular mitochondria. As the grade of dysplasia increases, the proportion of epithelial cells showing this bright mitochondrial fluorescence increases, until the whole epithelium is occupied by brightly fluorescent cells. As dysplasia develops, the collagen fluorescence in the stroma decreases in intensity, with both the fiber density and length decreasing with dysplasia. Thus, fluorescence may provide a tool to probe the increase in metabolic activity of the epithelium as well as a probe of communication between the epithelium and stroma as precancerous cells prepare to invade beneath the basement membrane.

We also developed analytic and computational models of cervical tissue fluorescence in order to analyze and deconvolve spectroscopic measurements made *in vivo*. We used a Monte Carlo method to simulate the random walk of photons in a turbid medium. As an initial step, we modeled cervical tissue fluorescence at 380 nm excitation. At this wavelength, our experiments with the organ cultures indicate that fluorescence in the epithelium is dominated by NAD(P)H, and fluorescence in the stroma is dominated by collagen; thus, the cervix was modeled as 2 infinitely wide layers, the first representing the cervical epithelium and containing NADH fluorescence and the second representing the stroma and containing collagen fluorescence. Predicted and measured spectra from normal and dysplastic low-/high-grade squamous intraepithelial lesion tissue are compared in Figure 3. The modeled normal data is scaled to the value of the measured normal data at 475 nm emission. This same scaling factor was then applied to the SIL data such that the relative magnitude of the normal and dysplastic spectra is predicted, rather than the absolute magnitude of each curve. This forward model accurately predicts both the shape and the relative intensity of fluorescence of normal and dysplastic cervix. In normal cervix at 380 nm excitation, approximately 20% of remitted tissue fluorescence is due to epithelial NAD(P)H, while the remaining 80% is due to stromal collagen. In dysplastic cervix, approximately 30–40% of remitted fluorescence is due to epithelial NAD(P)H, while 60–70% is due to stromal collagen. Thus, forward modeling can be applied broadly to data collected in the technical feasibility project to better understand the

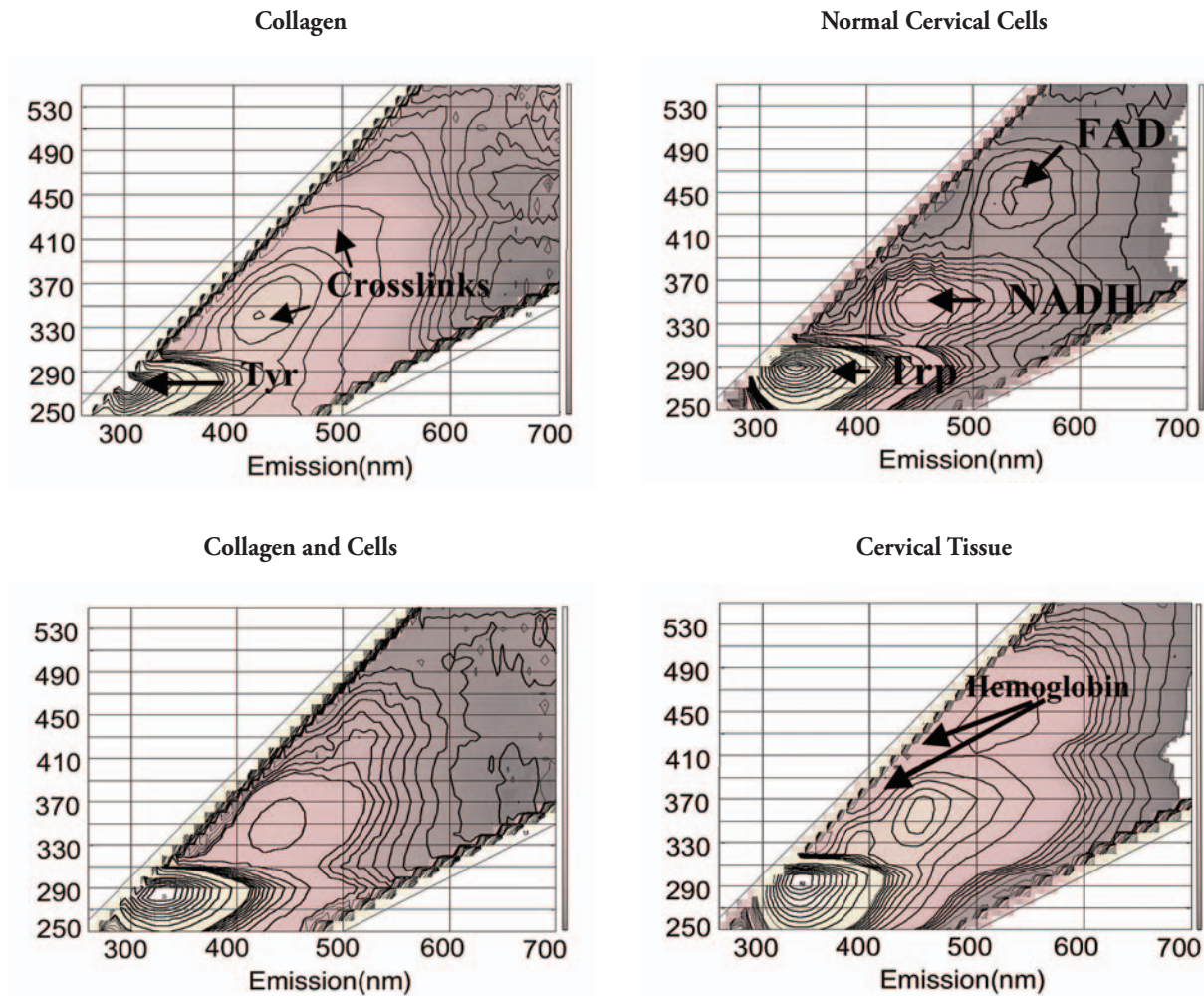


Figure 1. Excitation-emission matrices (EEMs) of several components of cervical tissue.

biologic origins of variations in fluorescence due to age and dysplasia.

Technical Feasibility

In the current system, most women with abnormal Papanicolaou (Pap) tests are referred to colposcopy for diagnosis. Although this diagnostic method is precise—providing good sensitivity and specificity for cervical preinvasive lesions—it requires directed biopsy to confirm diagnosis.⁸ This confirmation step introduces the necessity of reporting the results of the histopathologic interpretation back through the system to the patient and may require additional visits for treatment. The delayed results and reporting add significant cost to the system and anxiety for the patient; additionally, the acquisition

of the biopsy and the histopathologic interpretation steps requires substantial expertise. New, accurate real-time screening, detection, and cost-effective technologies are therefore urgently needed.

Techniques based on quantitative fluorescence and reflectance spectroscopy and imaging of cervical tissue have been investigated in pilot studies and evaluated in large trials in diagnostic and screening settings to evaluate their sensitivity, specificity, and cost effectiveness.⁹⁻¹⁹ These new technologies for early detection of cervical cancer are ultimately aimed at replacing both Pap smears and histopathologic interpretation of biopsied material by providing a real-time, immediate *in vivo* diagnosis.

From interim analysis of our large ongoing screening trial (1,000 subjects) and diagnostic trial (850 subjects) using a device (FastEEM) that acquires site-specific

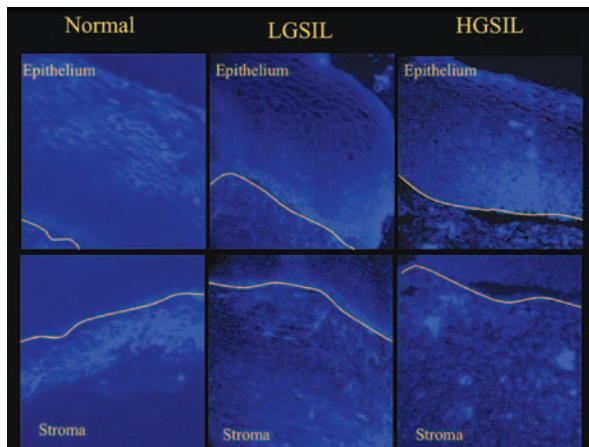


Figure 2. Normal, LGSIL, and HGSIL in the epithelium and stroma cervical tissue. LGSIL=low-grade squamous intraepithelial lesion; HGSIL=high-grade squamous intraepithelial lesion.

fluorescence EEMs and reflectance spectra for multiple probe-detector spacing when correlated with consensus histopathologic interpretation of the biopsied sites, we have found that this technology has the ability to be sensitive for the detection of HGSIL (approximately 80%) while maintaining high specificity (>85%). From these interim studies we also established that reflectance¹⁵ and fluorescence spectroscopy together are more discriminating than either alone.

One possible confounder for optical measurements in the cervix is the menstrual cycle. To address this issue we measured 10 patients with no history of an abnormal Pap smear daily throughout on average 30 consecutive days of their cycle. Fluorescence EEMs were measured from 3 cervical sites on each patient. From analysis of the fluorescence and reflectance data we found that the fluorescence emission spectra at 340–380 nm excitation appears to correlate with the cell metabolism of the cervical epithelium throughout the menstrual cycle; however, these changes do not achieve statistical significance. We concluded that the spectral data would be representative of the tissue grade throughout the menstrual cycle, but that bleeding associated with menstruation should be avoided.¹⁶⁻¹⁸

In order to further critically evaluate the colposcopically visible portion of the cervix and the endocervical canal, the data collected in the screening and diagnostic trials are being used to design a multispectral digital colposcope (MDC)²⁰ that measures fluorescence, reflectance, and differentially polarized filtered reflectance images. This device combines this image data to generate a visual representation/enhancement of the state of the cervical

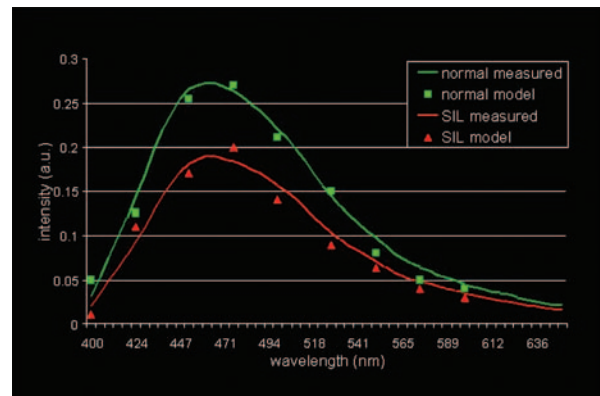


Figure 3. Predicted and measured spectra from normal and dysplastic (LGSIL/HGSIL) tissue. LGSIL=low-grade squamous intraepithelial lesion; HGSIL=high-grade squamous intraepithelial lesion.

tissue seen (Figure 4). This represents an evolution/superset-extension of the work that our group has done in fluorescence imaging for early lung cancer detection.²¹⁻²⁴ This current work advances this field by incorporating a multitude of image excitation and emission images and novel enhancements and analyses for the application to preinvasive and early invasive cervical lesion imaging. These various devices will be used in a large randomized clinical trial to demonstrate their effectiveness in a clinical setting.

Intermediate Effects

Rigorous technology assessment requires pilot, phase I, phase II, and phase III testing of new devices or medications. Many groups have tested fluorescence and reflectance spectroscopy in pilot studies, and we are near the end of phase II trials of this technology in this program project.²⁵ The randomized clinical trial provides the strongest study design for the evaluation of a diagnostic technology. We agree with Sox²⁶ that an emerging diagnostic technology should first be compared as an adjunct to the existing technology to show it adds value before comparing it alone to the existing technology. The clinical trial we are conducting is evaluating sensitivity and specificity of a spectroscopic probe with a 2-mm sampling area, with placement of the probe guided by colposcopy. The next logical step is a randomized phase III trial applying spectroscopy as an adjunct to colposcopy in the diagnostic setting. This trial requires well engineered devices for which algorithms are optimized; well planned, statistically justified trial design; and sufficient numbers of patients.

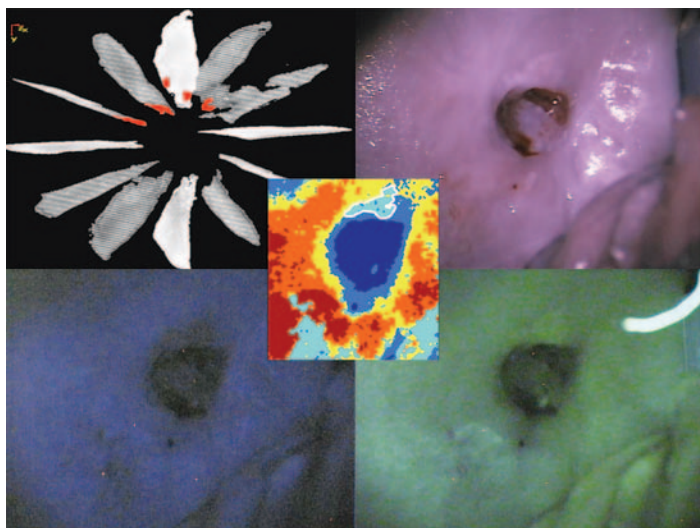


Figure 4. The multispectral digital colposcope (MDC) is initially being evaluated on LEEP patients. As part of the LEEP process, the excised tissue is cut into 12 pie sections, and sections cut from each pie piece are used for histopathological interpretation. A) The 12 sections have been arranged as they appeared in the original LEEP, and the HGSIL areas found are marked in red. B–E) are examples of images acquired from the MDC. B) Is an RGB colour image of the cervix using white light illumination. From the same patient C) is an RGB image using 345 nm illumination which generates predominately blue autofluorescence and D) is an RGB image using 440 nm illumination which generates predominately green autofluorescence. The insert pseudocolor image is a processed/analyzed combination of the central area of the MDC images in which the cluster of pixels traced in white correspond to the areas highlighted in red in Figure A.

LEEP=loop electrosurgical excision procedure; HGSIL=high-grade squamous intraepithelial lesion.

This type of clinical trial is also ideally suited to evaluate the outcomes of patient satisfaction and cost-effectiveness, and these tests will be conducted concurrently with this clinical trial.

The phase III randomized trial we will conduct is a multicenter trial of fluorescence and reflectance spectroscopy with 4 specific aims: (1) to conduct a randomized clinical trial of fluorescence and reflectance spectroscopy, comparing colposcopy alone with colposcopy plus fluorescence and reflectance spectroscopy in the diagnostic setting, using the spectroscopy information clinically; (2) to compare the number of unnecessary procedures performed with colposcopy alone and colposcopy plus spectroscopy; (3) to measure patient satisfaction with fluorescence and reflectance spectroscopy in this trial using patient interviews; and (4) to measure the cost-effectiveness of fluorescence and reflectance spectroscopy in this trial using patient interviews. We have several hypotheses that these specific aims will test: (1) fluorescence and reflectance spectroscopy in combination with colposcopy will have superior efficacy to that of colposcopy alone; (2) fluorescence and reflectance spectroscopy in combination with colposcopy will result in fewer unnecessary procedures than colposcopy alone; (3) patients will be more satisfied with spectroscopy in combination with colposcopy than colposcopy alone; and (4) fluorescence and reflectance spectroscopy will prove to be a cost-effective addition to colposcopy.

There are 2 potential benefits that can be achieved with optical spectroscopy in the treatment of cervical neoplasia. The first is that it can enable combined diagno-

sis and therapy in one visit, because it makes a diagnosis in real time. The second is that it can reduce the number of unnecessary biopsies by virtue of its increased specificity. This randomized clinical trial will have 2 components, 1 to evaluate each of these potential benefits. Patients referred to a colposcopy clinic because of an abnormal Pap smear will be eligible.

Patient Outcomes

Literature on technology assessment strongly emphasizes the need for evaluating the effect of new medical technology on patient outcomes, such as improved physical, functional, or emotional well-being.²⁷⁻³⁰ The consumer's perspective on the acceptability of the technological innovation must be taken into account during the development of new technologies. Despite the importance of studying technology from the consumer's perspective, research on screening and diagnostic technologies generally emphasizes diagnostic accuracy outcomes such as the sensitivity and specificity of the test or device. There is a significant need for more research on how the use of a screening or diagnostic technology affects patient well-being and receptivity of healthcare providers. Technology diffused into healthcare practice without assessment of patient and provider outcomes can result in increasing health care costs without improving health and may have unanticipated negative results, such as lowering rates of screening rates or adherence. Researching patient outcomes during the technology development process is rarely done³¹ but can identify potential problems with the technology so

they can be remedied prior to technology dissemination.

The goal of our project is to assess patient outcomes (distress, satisfaction, preferences) resulting from the use of emerging technologies for screening and early detection of cervical cancer while these technologies are still in developmental phases, so that information about the effect of the technologies on patient well-being and behavior can be used in their refinement.

We have analyzed data from the first 314 patients enrolled in the clinical trial to test optical spectroscopy in the screening setting. The 3 primary ethnic groups represented in the sample are white (51%), Hispanic (29%), and African American (16%) patients. Most women were married or living with a partner (64%), and over three fourths of the participants had at least some post-high school education (83%). Participant age ranged from 18 to 80 years, with a mean of 44 years.

Patients reported significantly less pain and anxiety during optical spectroscopy than during Pap smear, colposcopy, and biopsy (adjusted $P < .001$ for all comparisons with spectroscopy). Similarly, patients were less likely to make distress vocalizations during optical spectroscopy than during colposcopy ($P < .0001$), but there were no significant differences in the likelihood of vocalizations during spectroscopy and Pap smear or biopsy. Participant age was not significantly related to pain and anxiety (pain: $P = .74$; anxiety: $P = .06$) or the probability of distress vocalizations ($P = .47$). Ethnicity was not associated with any of the distress indicators.

Responses to certain satisfaction questions demonstrated high satisfaction across Pap smear, biopsy, and spectroscopy procedures. Nearly all participants responded “yes, a lot” when asked whether the provider explained the procedure well, answered questions, was gentle, and knew what he or she was doing. There were also no significant differences among the 3 procedures in the patients’ ratings of whether the instruments used for the test were frightening, whether there were too many people in the examination room, and satisfaction with the room temperature. Patients preferred the lighting in the exam room during spectroscopy (when the lights were off) to the lighting during Pap smear and biopsy (when the lights were on). Participants responded that they were more uncomfortable and experienced more pain during Pap smear and biopsy than during spectroscopy. They also thought that the biopsy was more frightening than spectroscopy. However, they viewed the biopsy as more accurate in terms of both sensitivity and specificity than either Pap smear or spectroscopy. Spectroscopy was rated as more accurate than Pap smear. However, participants were more likely to respond that spectroscopy took too long, compared to the other 2 procedures.

These results indicate that optical spectroscopy has

the potential to be well accepted by patients, although there are certain aspects of the technology, such as the amount of time taken for the measurements, that should be remedied to maximize patient receptivity.

Societal Outcomes

We are developing mathematical models for the effectiveness and cost-effectiveness of strategies of optical spectroscopy and quantitative cytohistopathology for the screening and diagnosis of cervical precancer. Only preliminary results are currently available for this project (Figure 5), because the results of other projects are used as inputs to the mathematical models being developed. We have several aims in the development process of mathematical models of cost-effectiveness.

The first major aim is to compare the discriminative ability, using receiver operating characteristic (ROC) curve analysis of several emerging and existing technologies for the screening and diagnosis of cervical precancer. We will evaluate optical spectroscopy in the diagnosis setting by performing ROC curve analysis based on the data from 850 patients in the diagnostic arm of the technical feasibility clinical trial. In addition, we will evaluate optical spectroscopy in the screening setting by performing ROC curve analysis based on the data from 1,000 patients in the screening arm of the technical feasibility clinical trial. Also, the algorithm for optical spectroscopy in the diagnostic setting will be validated by the additional data collected in the randomized clinical trial conducted in the study of the intermediate effects of this technology. We will also evaluate clinical and quantitative histopathology in the diagnostic setting and clinical and quantitative cytology in the screening setting by performing ROC curve analyses for the collected data from the diagnostic and screening arms of the technical feasibility clinical trial.

The second major aim of this project is to construct decision analysis models to evaluate the economic costs and health benefits of the emerging and existing technologies for the screening and diagnosis of cervical precancer. The results of the analysis from the data collection of the technical feasibility clinical trial and the analysis performed to complete the first specific aim of this societal outcomes project will be the primary input for these models. From this modeling, we can determine the effectiveness and cost-effectiveness of the various technologies, including optical spectroscopy and quantitative cytohistopathology. Also, with additional data collection, new technologies still under development, such as the multispectral optical wand, and the use of these technologies in developing nations can be evaluated.

We have made substantial progress regarding the development of the decision-analytic model. We have cre-

ated the capability to determine the expected economic costs and clinical benefits for several diagnostic strategies, including colposcopy (ie, usual care), optical spectroscopy, see-and-treat colposcopy, and see-and-treat spectroscopy. We can also vary the costs of individual technologies, as well as incorporate the possibilities of quality of life to calculate not only life expectancy, but also quality-adjusted life expectancy of the various clinical strategies. Thus, mathematical modeling has the flexibility to consider many scenarios.³²

Biostatistics and Informatics

Any project evaluating a new technology on the scale of this project needs personnel responsible for the study design, analysis, and presentation of data, and maintenance of databases. Our statisticians, Drs. Atkinson and Cox, have been integral to all areas of optical technology development in this project. They were instrumental in writing the initial proposal, computing sample sizes for the diagnostic and screening trials to evaluate the technical feasibility of this optical technology and the randomized clinical trial that evaluate the technology's intermediate effects. We will briefly discuss the analyses of one of the technical feasibility studies below. The statisticians have continuously helped design pilot studies to determine the biologic plausibility of various optical technologies and

have assisted the behavioral researchers in developing their questionnaires. Also, they have worked on performing meta-analyses to estimate the transition probabilities between various health states as part of the modeling effort for the societal outcomes portion of the program project. They have worked with the instrumentation personnel on numerous fronts, including design and implementation of a quality assurance system for the EEM and with the pathologists to analyze data and design a quality assurance system for the Cytosavant Imaging System, which performs quantitative pathological analyses.

An important initial undertaking of the biostatistics and informatics group was to design and implement the database. This has been an ongoing effort as new data types have been introduced (eg, the image data produced by the multispectral digital colposcope) and, of course, computational equipment has continuously evolved. Our goal is to provide easy access by all investigators and other personnel, such as research nurses who must input basic patient data. This has been a significant challenge, given the geographic separation of the main sites (Houston, Austin, and Vancouver). A further issue that requires vigilance is the integrity and security of the database, including patient confidentiality.

One of the studies conducted during technical feasibility was to assess any possible effect of multiple measurements at the same site in the cervix, essentially to test for

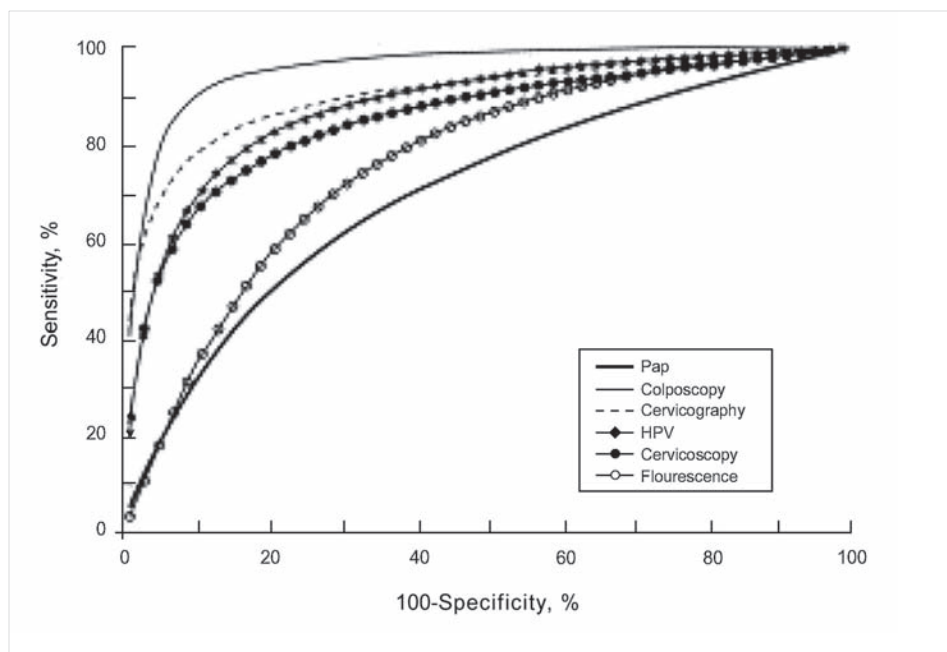


Figure 5. Superimposed receiver operating characteristic curves for all six screening techniques studied. HPV=human papillomavirus testing; Pap=Papanicolaou screening.

Table 1. Randomization Schema Used on the “Excitation/Emission Wavelength Pairs Data” to Assess the Effect of Variation

Real Data

	Site 1	Site 2
Patient 1	Soft	Medium
Patient 2	Medium	Hard
Patient 3	–	–
Patient 4	–	–
Patient 17	–	–
Patient 18	Medium	Soft

Randomized Data

	Site 1	Site 2
Patient 1	Medium	Hard
Patient 2	Medium	Soft
Patient 3	–	–
Patient 4	–	–
Patient 17	–	–
Patient 18	Soft	Medium

The randomized dataset was created by permuting the soft, medium, and hard labels. In order to do this, we first randomly selected site 1 or 2 for the medium pressure label. The remaining sites were then randomly selected for soft or hard as they were in the Study Data Set. Eighteen sites were medium, 9 soft, and 9 hard.

repeatability. As probe pressure was considered a possible confounding effect, a pressure sensitive fluorescence probe was calibrated at light, medium, and firm levels. The effect of different probe pressures at the same site had already been considered in a previous study.³³ Measurements were made 3 times at each of 2 sites in the cervixes of 18 subjects for a total of 108 measured EEMs. At each site, the probe pressure was fixed at 1 of the 3 levels. One of the sites was measured at medium pressure, and the other at either medium or firm. Whether the first or second site was medium or not, and whether the other site was light or firm pressure, was all determined from a randomized list provided by the statisticians. Spectroscopic data were preprocessed and analyzed to compare order of pressure and intensity variability as a function of pressure on measurements. Four EEMs were deleted from the analysis after quality assurance inspection. Randomization-based methods were used both to generate *P* values uncorrected for multiple comparisons using a “reference set” of randomized measurements. A “correction set” of randomized measurements was also generated to correct the initial *P* values for multiple comparisons using the method of Westfall and Young.³⁴ To test for an effect from measurement order, we used the correlation coefficient between measurement order and intensity at each of 1,957 excitation-emission wavelength pairs. This would detect either an increasing or decreasing trend in the order of measurement. The randomizations were performed by randomly reassigning the order within each site in a patient (so that any effect specific to a patient or site within patient was preserved). Some sites had to be omitted because they did not have 3 measurements. After corrections for multiple comparisons, we found no significant trend in fluorescence intensity from order of measurement, suggesting that there is no effect from making a second or third measurement at the same site. To assess any possible effect of pressure on variability of intensity, we computed the variance of intensities at each of the 1,957 excitation emission wavelengths. The randomization in this case was between the 2 sites within each patient so that any effect on variability specific to a patient would be maintained. This randomization is depicted in Table 1. The *P* values from this analysis both before and after correction from multiple comparisons are shown plotted in Figure 6. We see that even before the multiple comparisons correction, there was no significant effect on fluorescence intensity variability from probe pressure.

Instrumentation

The role of instrumentation personnel in a technology evaluation project is to provide critical support for clinical trials, support existing instrumentation, and develop new

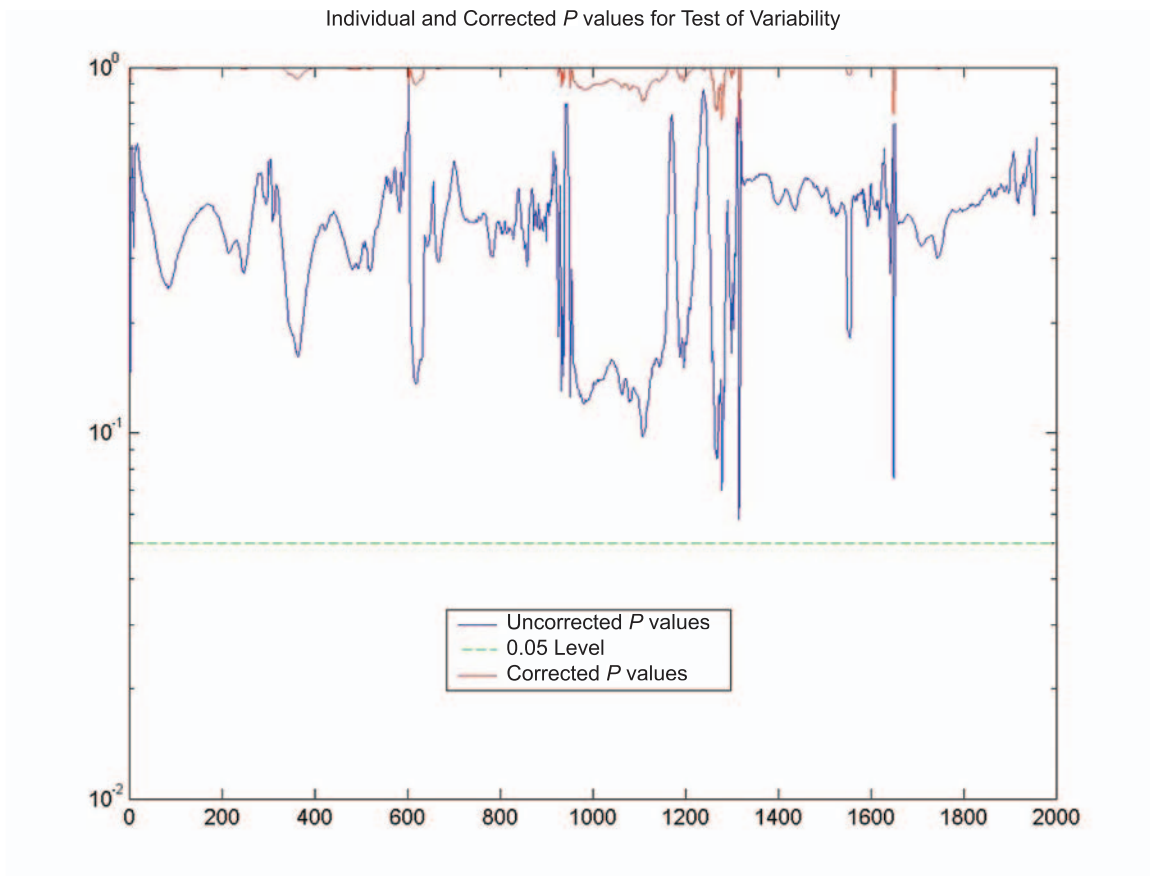


Figure 6. Uncorrected and corrected P values at all excitation/emission wavelength pairs for the test of an effect on variation from probe pressure. Note that, after correction, no P values are significant.

instrumentation in support of the first 3 projects of this program project. The instrumentation core works with the biostatisticians and the technical feasibility personnel to develop algorithms based on the analysis of EEM data and to realize these in clinically suitable instrumentation. Close cooperation and feedback between these groups is required to develop algorithms that are realizable in instrumentation and insensitive to subject, device, procedural, or operator-sourced artifacts.

The data analyzed from the biologic plausibility and technical feasibility projects research and trials is being used to determine optimized diagnostic and screening algorithms that will define design parameters for 2 new optical devices: an imaging device optimized for sensitivity, the MDC, and a point measurement device optimized for specificity, a multispectral optical wand.³⁵

These devices will incorporate feedback from the patient outcomes project in their design and will be

tested for clinical performance both independently and in combination as part of the technical feasibility project. Instrumentation personnel are also furthering the biologic plausibility aims of this project by building a hyperspectral-imaging microscope from tissue optical properties studies.

Instrumentation personnel develop and maintain the equipment used in the biologic plausibility and technical feasibility projects. The biomathematics core then analyzes the data to develop diagnostic and screening algorithms. The instrumentation group and the biomathematics group modify the algorithms, taking into account engineering limitations of optical and electronic systems, to make the algorithms realizable in instrumentation.

This instrumentation takes the form of an optical wand system for point measurements and a multispectral imaging system, the MDC, for viewing the entire cervix. The optical wand system will be designed to maximize specificity and the MDC imaging system to maximize

sensitivity. These devices will be tested independently and together in the clinical outcomes project. Initially the wand system will use the FastEEM device programmed to perform the reduced set of optical measurements required by the algorithms developed above. As the sensitivity and specificity of the algorithm is validated, the low-cost wand system will be introduced and tested.

Developing instrumentation in a technology-assessment process is very different from the typical industry approach of trying to develop a single effective instrument or diagnostic algorithm. Our goals are to explore aspects of the technology that go beyond specific instrumentation and understand factors that affect this entire class of technologies. We have incorporated a large number of positive and negative standards measurements into our trials and have been able to apply iterative engineering analysis and statistical analysis to these measurements.³⁶ This has been invaluable in identifying and providing correction for a number of unanticipated instrument artifacts and calibration dependencies that we believe affect most existing optical point measurement and imaging systems. Adoption of these techniques should assist industry in improving sensitivity and specificity of optical technologies, especially fluorescence-based systems currently in clinical trials.

The design of the MDC and the optical wand system will incorporate the feedback from the patient/provider acceptance studies and the patient outcomes and the cost/benefit studies in other areas of this optical technology development. These will feed into the physical design of the systems and the design of the calibration and maintenance procedures and software, as well as the way in which the device communicates diagnostic and screening information to the providers.

Pathology

The primary responsibilities of the pathologists in evaluating and developing these new optical technologies are: (1) to archive and store all histopathologic specimens and biopsy material (eg, embedded blocks, frozen tissue, tissue sections) related to biologic plausibility, technical feasibility, and intermediate effects; (2) to prepare pathology slides for routine and special histopathologic examinations required for the conduct of clinical projects; (3) to provide a blinded reference diagnosis and consensus review of all clinical and quantitative cytology and histology specimens, to coordinate and correlate subsequent interpretations with the clinical histopathology and cytopathologic diagnoses, and to identify diagnostic areas on histologic slides to be imaged; (4) to provide guidance to the optical engineers, physicians, behavioral scientists, decision scientists, and biomathematicians involved in

this optical technology development project to ensure the clinical and biologic relevance of the work and interpretations, and to provide assistance and advice for construction and evaluation of model of cytologic patterns and other biologic parameters, which will be investigated in a quantitative manner; (5) to store and transfer specimens for human papillomavirus (HPV) testing; (6) to start and transfer plasma specimens from all the clinical trials to the laboratory for hormones assays; (7) to provide quantitative cytohistology data related to biologic plausibility, technical feasibility, and intermediate effects; (8) to provide help and support in communicating quantitative cytohistopathology results to patients; and (9) to assist in determining the cost-effectiveness of quantitative cytohistopathology.

Quantitative cytohistopathology has the potential to reduce the need for highly skilled cytohistopathologists in developing countries. In developed countries, quantitative cytohistopathology can reduce sampling error, improve inter- and intra-observer agreement, and be used as an emerging biomarker of carcinogenesis. The role of the pathologists in this optical technology development project is to coordinate and provide professional and technical services for proper handling and processing of all histologic and cytologic specimens to be examined in this trial, to provide reference cytopathologic and histopathologic diagnoses, to conduct consensus review of these diagnoses, to store and transfer specimens for HPV typing, and to store and transfer plasma for estradiol, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormones (LH) assays. The pathologists also provide quantitative cytohistopathology data to biostatisticians to assist in algorithm development. As many investigators know, agreement among cytologists and pathologists can sometimes be difficult to obtain for cervical cytologic and histopathologic specimens. The motivation for the development of the field of quantitative cytology and pathology was to objectify, as much as possible, the art and science of cytology and pathology.

To this end, pathology personnel have focused on quantifying cytologic and pathologic data obtained in conjunction with data from the optical devices. As charge-coupled device (CCD) technology improved, microscopes were attached to scientific-grade digital cameras, which captured images that could be processed by sophisticated algorithms. The stoichiometric Feulgen stain was developed for use in quantitative measurement. Optical density increases linearly as the nuclear DNA content increases. Flow-cytometric and computer-assisted image analyses have demonstrated consistent increases in nuclear DNA as lesions progress from intraepithelial neoplasia to cancer. The cervix is an organ site for which the phenomenon of progression is well established both

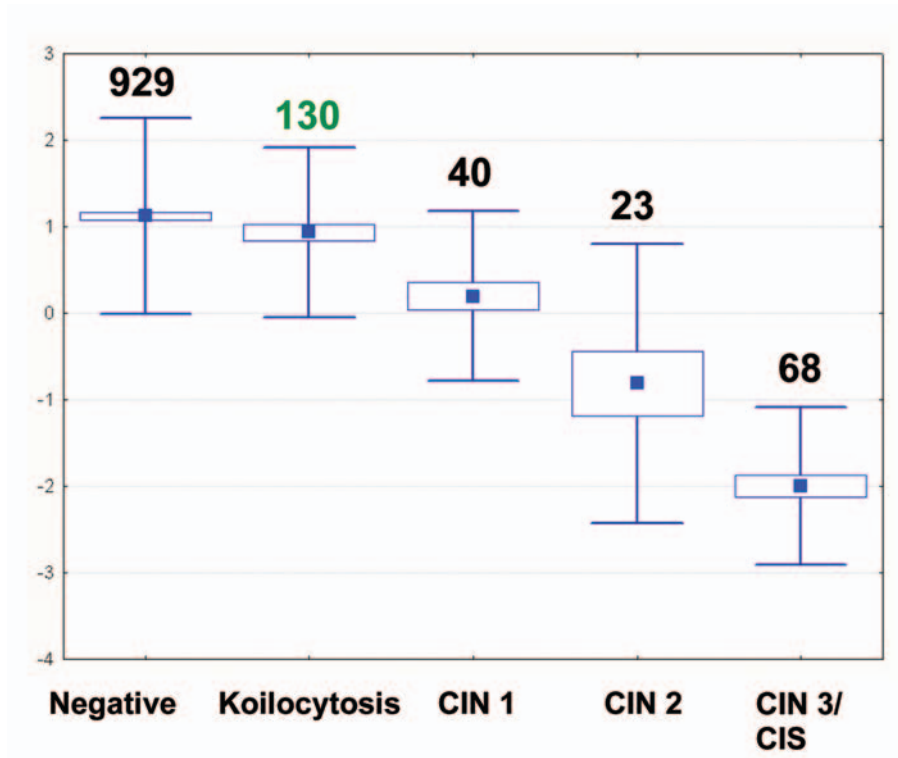


Figure 7. Morphometric score.

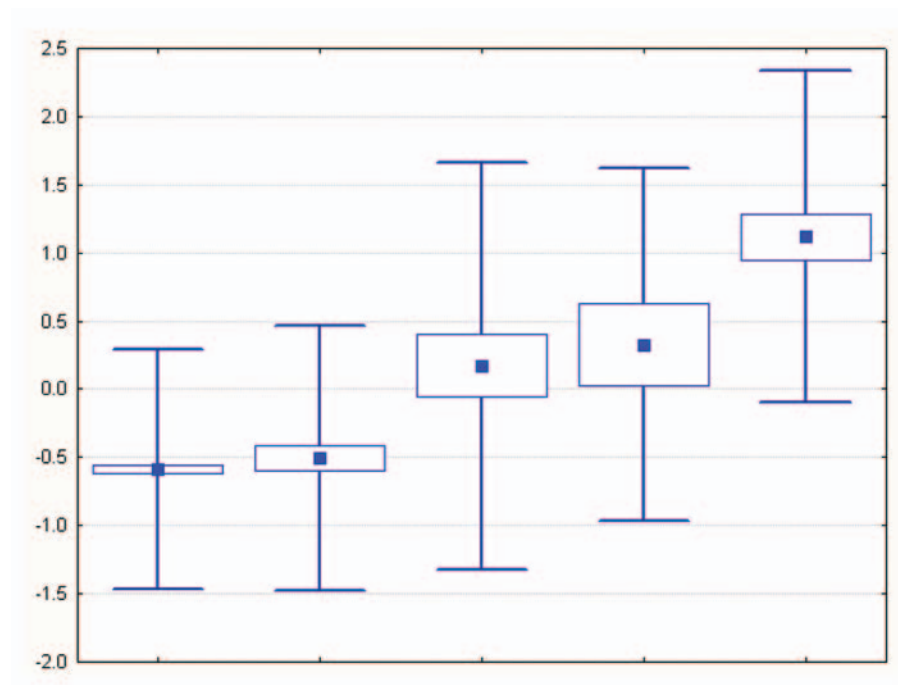


Figure 8. Discrete texture score.

clinically and quantitatively. Despite this well established continuum, qualitative pathology cannot predict which lesions will progress. Quantitative cytohistopathology has demonstrated that high DNA content, high nuclear density, and several textural features are predictive of aneuploidy. As in other tissues, aneuploidy has been demonstrated to predict progression of cervical neoplasia. Thus, quantitative cytohistopathology may provide an objective tool to predict progression and regression of cervical lesions.

Two of the measured values are shown in Figures 7 and 8. Figure 7 shows a plot of a morphometric score as a function of histopathology interpretation, generated from a linear discriminant analysis using 3 shape features: variance of the maximum radius, mean sphericity, and mean elongation. Figure 8 shows a plot of a discrete texture score as a function of histopathology interpretation, generated from a linear discriminant analysis using 3 discrete texture features, variance across the cells in the sample of the medium-high density average distance, DNA index, variance of the cell's ODVAR (variance of the cells' optical density) and the mean of the cell's ODMAX (mean of the cells' maximum optical density), the variance of the low-density center mass and the mean of the cell's low-density object number. In Figures 7 and 8 the mean is represented by a black square, the standard deviation by a vertical line with horizontal bars, and the standard error by an open rectangle.

Our group is correlating optical signatures with quantitative pathology measurements. We expect these data to generate many new hypotheses regarding the pathobiology of cervical neoplasia and neoplasia in general.

Conclusion

This program project grant has greatly facilitated research into optical technologies for the treatment of cervical neoplasia. The organization of this project provides a useful model for other researchers interested in technology development and evaluation for early detection of gynecologic malignancy—or many other technology development projects. Each area of the project has generated a great deal of quality information to further this area of research. Our biologic plausibility personnel have completed forward and inverse modeling for fluorescence in cervical tissue and have made important discoveries about the binding of tissue fluorescence. The technical feasibility group has determined that the FastEEM device is a very robust technology: we are able to consistently detect fluorescence throughout the menstrual cycle except during menstruation. Also, the technical feasibility group has developed 2 working versions of the FastEEM system and are in the process of designing a MDC using the data

collected in the screening and diagnostic trials of this project. Our group will be submitting an Investigational Device Exemption to the Food and Drug Administration for the FastEEM. In addition to determining patient comfort with all aspects of the spectroscopy exam, behavioral science personnel are developing models for the most effective methods of communicating information to patients. The societal outcomes group has already published preliminary ROC curve analysis of fluorescence spectroscopy. The decision-analytic model will allow us to determine the expected economic costs and clinical benefits for several diagnostic strategies for cervical neoplasia. Our biostatisticians have developed an integrated collaborative database that is accessible to everyone involved with this project and has made it possible to input and analyze data with great efficiency. Instrumentation personnel have built and maintained the FastEEM devices and are in the process of developing the MDC and a multispectral optical wand. In addition, they are developing new quality assurance procedures for the FastEEM devices. As well as making significant advances in quantitative pathology, the pathologists are implementing a new quality assurance procedure for reviewing all clinical and quantitative cytology and histology specimens and plan to focus on achieving hypothesis-driven results. We are pleased with the results this program project has generated over the last 5 years and are eager to begin the randomized clinical trials at the end of this year.

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