Identification of Cervical Intraepithelial Neoplasia (CIN) Using UV-Excited Fluorescence and Diffuse-Reflectance Tissue Spectroscopy

Robert J. Nordstrom, PhD,1* Louis Burke, MD,2 Jonathan M. Niloff, MD,2 and James F. Myrtle, PhD1

1MediSpectra, Inc. Lexington, Massachusetts 02421
2Dept. of Obstetrics, Gynecology, and Reproductive Biology, Beth Israel Deaconess Medical Center, and Harvard Medical School, Boston, Massachusetts 02215

Background and Objective: The diagnostic potentials of ultraviolet-excitation fluorescence spectroscopy and diffuse-reflectance spectroscopy of tissue are assessed in a study to identify cervical intraepithelial neoplasia (CIN) in vivo. A multivariate algorithm is used to classify tissue into normal tissues, CIN I, and CIN II/III categories, based on spectral characteristics of biopsied tissue sites.

Study Design/Materials and Methods: An optical instrument with the capability of measuring fluorescence and diffuse-reflectance spectra from 120 locations uniformly distributed over the surface of the cervix is described. Using this device, these optical spectra of the cervix were measured on women referred for colposcopy due to an abnormal Pap smear.

Results: UV fluorescence differentiates CIN II/III lesions from normal squamous tissue with a sensitivity and specificity of 91 and 93%, respectively. CIN I is distinguished from normal tissue with a sensitivity of 86% and a specificity of 87%.

Conclusion: Optical spectroscopy shows promise for the detection of pre-cancerous cervical lesions in vivo. The fluorescence and reflectance methods are complementary in their ability to differentiate different tissue types, making the use of the two techniques together more diagnostic than the use of either method separately.

Key words: cervical lesions; cervix; Pap smear; tissue spectroscopy

INTRODUCTION

Early detection and treatment of cervical intraepithelial neoplasia (CIN) through Pap smear screening is recognized as the key factor in reducing the incidence of cervical cancer, and in the decrease in mortality from this disease over the past 50 years [1]. However, the Pap smear is recognized as having many inherent limitations, including specimen collection techniques, slide preparation issues, and slide interpretation errors. Accurate data on the sensitivity and specificity of the Pap smear are lacking, due to inadequate study methodology. In a recent analysis of the literature [2], McCory et al. reported a sensitivity range of 29–56% for detecting CIN and cancer. On the other hand, specificity for the condition of no presence of high-grade CIN or cancer is probably greater than 90%. A limitation of the current system is that a majority of lesions detected by Pap smears are low-grade neoplasia (CIN I), but only a few of these lesions progress to cancer.

The standard of care for patients with abnormal Pap smears is evaluation with colposcopy, a specialized visual inspection of the cervix that localizes suspicious lesions. Such lesions are confirmed by biopsy. Sites of high grade CIN are subsequently removed by excision or ablation. Unfortunately, colposcopy shares some of the shortcomings of the Pap smear. Because it is a qualitative assessment of the cervix, its accuracy is dependent on the training and experience of the physician [3]. Several studies have reported that colposcopically directed cervical punch biopsies have a false negative rate ranging from 15 to 31%. That is, CIN II and higher pathologies are missed entirely with the punch biopsy. This was established when initial punch biopsies were compared to the follow-up tissue pathology of the entire cervical specimen obtained from subsequent treatment by Loop Electrosurgical Excision Procedure (also LEEP or “loop”) or by cone excision procedure [3–6].

In inexperienced hands, colposcopic impressions have been reported to correlate with the histology of the biopsy less than 35% of the time [7]. However, even highly experienced colposcopists can vary greatly in their colposcopic interpretations. In the quality control of the colposcopy experts employed in the ongoing “ALTS” (ASCUS, Low-SIL Triage Study) trial sponsored by the National Cancer Institute, agreement between colposcopic evaluation of cervigrams and the final histopathology ranged from 47 to 75%; interobserver agreement ranged from 57 to 71%; and intraobserver agreement ranged from 68 to 80% when the images were resubmitted for evaluation [8].

Diagnostic methods based on optical spectroscopy have been shown to identify the presence of neoplasia in cervical
Accuracy of optical spectroscopy for the detection of cervical intraepithelial neoplasia: testing a device as an adjunct to colposcopy

Scott B. Cantor1, Jose-Miguel Yamal2, Martial Guillaud3, Dennis D. Cox4, E. Neely Atkinson1, John L. Benedet1, Dianne Miller5, Thomas Ehlen6, Jasenka Matisić6, Dirk van Niekerk6, Monique Bertrand3, Andrea Milbourne2, Helen Rhodes7, Anais Malpica7,8, Gregg Staerkel9, Shahla Nader-Eftekhari9, Karen Adler-Storthz10, Michael E. Scheurer11, Karen Basen-Engquist12, Eileen Shinn13, Loyd A. West7, Anne-Therese Viastos7, Xia Tao7, J. Robert Beck13, Calum MacAulay1 and Michele Follen7,8,14

1 Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX
2 Division of Biostatistics, The University of Texas School of Public Health, Houston, TX
3 Department of Cancer Imaging, British Columbia Cancer Research Centre, Vancouver, British Columbia, Canada
4 Department of Statistics, Rice University, Houston, TX
5 Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, British Columbia, Canada
6 Department of Pathology, University of British Columbia, Vancouver, British Columbia, Canada
7 Department of Gynecologic Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX
8 Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX
9 Department of Obstetrics, Gynecology, and Reproductive Sciences, The University of Texas Health Science Center at Houston, Houston, TX
10 The University of Texas Health Science Center at Houston Dental Branch, Houston, TX
11 Department of Pediatrics and Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX
12 Department of Behavioral Science, The University of Texas MD Anderson Cancer Center, Houston, TX
13 Fox Chase Cancer Center, Philadelphia, PA
14 Department of Obstetrics, Gynecology, and Reproductive Sciences, the Lyndon Baines Johnson Hospital, Houston, TX

Testing emerging technologies involves the evaluation of biologic plausibility, technical efficacy, clinical effectiveness, patient satisfaction, and cost-effectiveness. The objective of this study was to select an effective classification algorithm for optical spectroscopy as an adjunct to colposcopy and obtain preliminary estimates of its accuracy for the detection of CIN 2 or worse. We recruited 1,000 patients from screening and prevention clinics and 850 patients from colposcopy clinics at two comprehensive cancer centers and a community hospital. Optical spectroscopy was performed, and 4,864 biopsies were obtained from the sites measured, including abnormal and normal colposcopic areas. The gold standard was the histologic report of biopsies, read 2 to 3 times by histopathologists blinded to the cytologic, histopathologic, and spectroscopic results. We calculated sensitivities, specificities, receiver operating characteristic (ROC) curves, and areas under the ROC curves. We identified a cutpoint for an algorithm based on optical spectroscopy that yielded an estimated sensitivity of 1.00 [95% confidence interval (CI) = 0.92–1.00] and an estimated specificity of 0.71 [95% CI = 0.62–0.79] in a combined screening and diagnostic population. The positive and negative predictive values were 0.58 and 1.00, respectively. The area under the ROC curve was 0.85 (95% CI = 0.81–0.89). The per-patient and per-site performance were similar in the diagnostic and poorer in the screening settings. Like colposcopy, the device performs best in a diagnostic population. Alternative statistical approaches demonstrate that the analysis is robust and that spectroscopy works as well as or slightly better than colposcopy for the detection of CIN 2 to cancer.

Key words: sensitivity and specificity, diagnosis, early detection of cancer, uterine cervical neoplasms, cervical intraepithelial neoplasia

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Correspondence to: Scott B. Cantor, PhD, Department of Biostatistics—Unit 1411, The University of Texas MD Anderson Cancer Center, P. O. Box 301402, Houston, TX 77230-1402, USA, Tel.: 713-745-5984, Fax: 713-563-4243, E-mail: sbcantor@mdanderson.org

Cervical cancer remains a major cause of morbidity and mortality in the developing world, where 85% of cancers arise.1,2 Identification of cancerous and precancerous lesions at earlier stages, when interventions are more likely to be effective, is critical for effective cancer control.3–6 Recent advances in fiber-optic and semiconductor technologies have enabled the development of a new generation of inexpensive, miniature optical sensors that can probe the interaction of light with potentially cancerous tissue in real-time.7,8 Work to integrate this new technology with existing detection modalities and clinical screening efforts is ongoing.

under the covariate-adjusted ROC curves for diagnostic spectroscopy and diagnostic colposcopy were 0.77 and 0.78, respectively.

**Discussion**

We developed an algorithm for point-probe optical spectroscopy that yielded operating characteristics with reasonable performance and that has the potential for use in real time. The data show that in a diagnostic setting, research-grade point-probe devices using colposcopically-directed optical spectroscopy perform similarly to colposcopy in expert hands. The role of this technology was to be an adjunct to colposcopy so that one could avoid biopsies of inflammatory lesions and see and treat with confidence that disease would
We continue to develop a multispectral digital colposcope (MDC) for screening and for eventual combination with the probe technology. The MDC has been through two pilot trials, each of which demonstrated sensitivities of 85% and specificities of 90% in automated algorithms on few patients. We will begin testing a combined device based on this work.

In the original statistical plan of the protocol, we used a sensitivity of 84% and a specificity of 76% as parameters to calculate the sample size. These data were based on work using three wavelengths of light in 104 patients, many of whom had high-grade squamous intraepithelial lesions. We estimated that 200 patients with high-grade squamous intraepithelial lesions would allow us to study the diagnostic

Figure 5. (a) and (b). Receiver operating characteristic (ROC) test characteristics of the per-patient and per-site analysis of the data. Each graph shows the performance of the whole data set, the diagnostic trial, and the screening trial.
screening and diagnostic populations. We calculated that the development of a classifier in a screening population with a point probe would require 16,000 patients, for which funding would be a near impossible task. We also thought of the MDC as the instrument that would accompany the point probe in the screening setting. This was true of our studies of quantitative cytology, for which we also needed a large dataset to develop a classifier.

Although we expected the per-site analysis to yield a higher AUC and higher sensitivities and specificities than the per-patient analysis, it did not. This may be because the spectroscopy assesses the epithelial/stromal interaction. Thus, one would expect that if any area of the cervix has high-grade dysplasia, there is a field effect such that the entire cervical epithelial-stromal interface is different from that of a patient with no disease. Biomarker data supports that normal areas of a diseased cervix are not as genetically stable as normal areas in a normal cervix. We are actively investigating the stromal biology of these types of lesions.

How do our results compare to the literature? Table 4 shows the spectroscopic approach and modality as well as the sensitivity and specificity obtained in the trials. Our results compare favorably with those of other investigators.

The main strengths of this study are that (1) each patient had several biopsies that underwent multiple blinded reviews and thus provided an excellent gold standard on which to judge all the technologies under study, (2) few registration problems occurred with the biopsied tissues, (3) robust analysis of the multiple algorithms yielded similar results using different approaches for both data reduction and data analysis and (4) attention was paid to all aspects of technology assessment. Previous trials by Alvarez and DeSantis used multispectral technologies to view the whole cervix and compared the multispectral readings to both biopsies and areas of loop electrosurgical excision procedure specimens from the cervix. However, registration, or linking, of the optical image to the area of histopathologic reading was difficult in their study designs. In our studies, each 2 mm area that was measured was biopsied, thus registration was not an issue. Further, because we had spectroscopic measurements from both colposcopically positive (if any were present) and colposcopically negative sites on all patients, we were able to find false positive and false negative colposcopic lesions, eliminating the problem of verification bias and something that was not done in other studies.

The weaknesses of the study are (1) the number of scrapes to the cervix before measurement, (2) the probe placement possibly not being precisely over the area biopsied, (3) the discarding of approximately 30% of spectrographs and (4) the use of a cutoff point for the classifier. The scrapes may have affected the epithelium, but we believe we sampled the epithelial stromal interface; however, in future studies of the probe we will not take any scrapes. Also, the probe placement may never be precisely over the area imaged. However, the microenvironment in a region may be similar over the microns that are measured. Discarding spectrographs comes with the territory of studying emerging technologies, as does the development of first- and second-generation devices. We resisted changes to the devices but instead quantified how the devices performed differently for our own understanding of how changes might impact the data. Finally, choosing a cut point is a complicated process. We have not focused on this in this paper, but the cut point changes the trade-off between the number of false negatives and the number of false positives. This will be the subject of a future cost-effectiveness analysis. Because we subjected the algorithmic analyses to a robust number of methods of data reduction and then analysis using the cut point of CIN 2 and above, we are certain the results reflect what is contained in the data set.

The endocervical canal presents challenges for two reasons: first, there may be squamous lesions high in the canal, and second, there is an increasing incidence of adenocarcinoma in situ and adenocarcinoma arising in the columnar epithelium. In general, patients referred for colposcopy with abnormal Papanicolaou smears (diagnostic patients) usually receive an endocervical curettage (scraping of the endocervix) along with their cervical biopsies, whereas those patients with a history of normal Papanicolaou smears (screening patients) receive only a cytologic evaluation of the endocervical canal. Many gynecologic oncologists have found an invasive cancer or lesions of adenocarcinoma in situ or adenocarcinoma in a patient for whom the only abnormality was a positive endocervical curettage or suspicious endocervical cytology. This is a particular concern in older patients, as the squamocolumnar junction moves farther up the endocervical canal with age and endocervical lesions could be missed without a vigilant approach. At present, we would still recommend an evaluation of the endocervical canal apart from or in addition to optical spectroscopy. This is a major limitation of this spectroscopy, but also of visual inspection of the cervix with acetic acid and other existing screening devices. We are researching ways to use spectroscopy to detect abnormalities in the canal and perhaps will have other solutions in the future. For now, the Papanicolaou smear and/or endocervical curettage remain critically important in the clinical evaluation of patients to diagnose all cervical cancers.

The device developed in this study would be an adjunct to colposcopy and probably would not be commercially viable by itself. As we found, a point probe cannot be used for screening; for that purpose we are developing the MDC, a device that sees the whole cervix. The combined MDC and point probe would be made for the developed world, where it is important to save health care dollars by eliminating unnecessary biopsies and treating only those patients with CIN 2 to cancer. For the developing world, the lack of sufficient electricity led us to develop a portable battery-powered device that we hope to test with our collaborators in developing nations.
Effective cervical neoplasia detection with a novel optical detection system: A randomized trial

Ronald D. Alvarez a, *, Thomas C. Wright b, *
Optical Detection Group 1

a University of Alabama at Birmingham, Birmingham, AL, USA
b Columbia University, New York, NY, USA

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Abstract

Objective. To assess whether the use of a novel optical detection system (ODS) as an adjunct to colposcopy increases the detection of biopsy-confirmed CIN 2,3.

Methods. This is a multicenter two-arm randomized trial comparing colposcopy alone with colposcopy plus a pre-commercial ODS system that utilizes fluorescence, white light tissue reflectance, and cervical video imaging. Patients were recruited from 13 colposcopy clinics in a variety of practice settings. 2299 women referred for the evaluation of an abnormal cervical cytology were randomized with stratification by cytology; subsequently 113 women were excluded for a variety of reasons. The main study outcomes were differences in true-positive rates (CIN 2,3 and cancer identified) and false-positive rates between the study arms.

Results. The true-positive (TP) rates were 14.4% vs. 11.4% (p = 0.035, one-sided) for the combined colposcopy and ODS arm compared to colposcopy-only arm, respectively, in women with either an atypical squamous cell (ASC) or low-grade squamous intraepithelial lesion (LSIL) cytology result. TP rates were similar between the two arms among women referred for the evaluation of HSIL. The 26.5% gain in true-positives observed with the use of ODS and colposcopy among women referred for an ASC or LSIL cytology was achieved with only a fractional increase in number of biopsies obtained per patient (0.30) and a modest increase in false-positive rate (4%). In the combined colposcopy and ODS arm among women with ASC or LSIL, the PPV of biopsies indicated by ODS was 15.0% and the PPV of biopsies indicated by colposcopy was 15.2%. Joint hypothesis testing indicates that ODS and colposcopy provides benefit compared to colposcopy alone among women with ASC or LSIL.

Conclusions. Combining ODS with colposcopy provides a clinically meaningful increase in the detection of CIN 2,3 in women referred for the evaluation of mildly abnormal cytology results.

Keywords: Cervical intraepithelial neoplasia; Optical spectroscopy; Colposcopy

Introduction

For the last three decades, colposcopic examination with cervical biopsy has been considered the standard of care for evaluating women with abnormal cervical cytology. However, two recent studies have shown that this approach has a much lower sensitivity than previously recognized. In the U.S. multicenter ASCUS/LSIL Triage Study (ALTS), a single colposcopic examination failed to detect 33% to 36% of women who were subsequently identified as having biopsy-confirmed high-grade cervical intraepithelial neoplasia (CIN 2,3) [1,2]. Similarly, in a study from China colposcopy failed to detect 40% of CIN 2,3 lesions [3].

Quantitative optical spectroscopy and imaging offer considerable promise as an approach to improving the performance of colposcopy [4]. The optical methods currently being developed include intrinsic tissue fluorescence, white light backscatter, and video imaging techniques [4]. These methods exploit the biochemical and structural changes that occur within tissue during the pathogenesis of neoplasia to produce a probabilistic prediction of tissue type. Several studies that have...
Spectroscopic techniques examine the interactions of light with biologic tissue and provide information about the biochemical and structural tissue composition. Techniques that use near-UV and visible light are easily implemented in vivo in a clinical setting and can provide feedback with respect to the properties that they assay in real time. Thus, they could serve as a useful guide in the detection of biochemical and/or morphologic changes that reflect the presence of precancerous and early cancerous lesions. In addition, the capability to monitor such changes in vivo could enhance our understanding of some of the fundamental events that are involved in neoplastic progression.

Colposcopy and biopsy are the standard methods of diagnosing precancerous lesions in the uterine cervix. Although colposcopy is quite sensitive in detecting tissue abnormalities, it has very low specificity, even when performed by an experienced gynecologist. As a result, a significant number of abnormal colposcopic examinations result in a normal biopsy, needlessly consuming health care resources. Techniques that provide a more precise assessment of underlying preinvasive disease could reduce the frequency of unnecessary biopsy and, potentially, permit a single-visit triage strategy that bypassed biopsy.

Spectroscopic modalities have been under development as a method for diagnosing disease for a number of years. Fluorescence spectroscopy has been studied extensively in several tissues. Its success as a technique for the detection of neoplastic changes is based on the hypothesis that the development of neoplasia is accompanied by modification in the biochemical composition of tissue. The latter can be assessed by the fluorescence spectral signatures of fluorescing biochemicals. Promising results have been acquired for several organs, demonstrating the potential of this technique as a clinical tool. Several studies of in vivo fluorescence measurements of normal and precancerous cervical tissues indicate that spectroscopy could be a useful guide for the detection of cervical lesions during colposcopy. Analysis of the...
Multimodal Hyperspectral Imaging for the Noninvasive Diagnosis of Cervical Neoplasia

Daron G. Ferris, MD,* Raymond A. Lawhead, MD,‡
Eileen D. Dickman, PhD, MBA,* Nina Holtzapple, MD,†
Jill A. Miller, MD,* Stephanie Grogan, MD,‡ Shabbir Bambot, PhD,§
Anant Agrawal, MS,§ Mark L. Faupel, PhD§

*The Departments of Family Medicine and Obstetrics and Gynecology, the Medical College of Georgia, Augusta;
‡Atlanta Medical Center, Atlanta, and §SpectRx, Inc., Norcross, Georgia

Abstract

Objective. To determine the ability of Multimodal Hyperspectral Imaging (MHI) to noninvasively detect, localize and diagnose cervical neoplasia.

Materials and Methods. The cervical epithelium was interrogated by MHI using tissue fluorescence and reflectance measurements after the probe was placed on the ectocervix. A Papanicolaou smear was taken, and a colposcopic examination was performed and cervical histologic specimens were collected, when indicated. MHI and Pap smear sensitivity and specificity data were compared with colposcopic and histologic results.

Results. Nineteen patients had CIN2 or higher, 30 had CIN1, 34 had benign cellular changes or metaplasia, and 28 were normal by both Pap smear and colposcopic examination. At equal specificity (70%) for both tests, the sensitivity of MHI was 97%, compared to 72% for the Pap smear.

Conclusion. MHI detected cervical cancer precursors at a rate greater than that obtained by a simultaneously collected Pap smear.

Key Words: cervical neoplasia, noninvasive diagnosis, Multimodal Hyperspectral Imaging, Papanicolaou smear

Health care professionals encounter multiple dilemmas with the surveillance, diagnosis, and management of women with cervical neoplasia. An imperfect screening test, a lengthy Papanicolaou (Pap) smear collection to notification interval, and a substantial patient noncompliance rate with recommended practices may all adversely impact the screening process [1–8]. Cervical neoplasia is suspected from an abnormal screening Pap smear or positive triage test for oncogenic human papillomavirus (HPV) DNA that is localized and detected by specific epithelial features noted during colposcopic examination of the lower genital tract. The diagnosis is confirmed by histologic results obtained from sampling the ectocervix and endocervical canal, when deemed necessary [9]. When results from these evaluations are considered collectively, optimal management ensues. Most of the tests involve a subjective appraisal that varies considerably and depends on specimen or anatomic variation, as well as evaluator expertise. Thus, diagnoses may be influenced adversely by technical issues and are not made consistently at an expert level; the diagnoses also are not always reproducible.
Detecting high-grade squamous intraepithelial lesions in the cervix with quantitative spectroscopy and per-patient normalization

Jelena Mirkovic,1,2* Condon Lau,1 Sasha McGee,1 Christopher Crum,2 Kamran Badizadegan,3 Michael Feld,1 and Elizabeth Stier4

1George R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02179, USA
2Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA
3Department of Pathology, Massachusetts General Hospital, Boston, MA 02114, USA
4Department of Obstetrics and Gynecology, Boston Medical Center, 85 East Concord Street, Boston, MA 02118, USA
*jedza@alum.mit.edu

Abstract: This study develops a spectroscopic algorithm for detection of cervical high grade squamous intraepithelial lesions (HSILs). We collected reflectance and fluorescence spectra with the quantitative spectroscopy probe to measure nine spectroscopic parameters from 43 patients undergoing standard colposcopy with directed biopsy. We found that there is improved accuracy for distinguishing HSIL from non-HSIL (low grade SIL and normal tissue) when we “normalized” spectroscopy parameters by dividing the values extracted from each clinically determined suspicious site by the corresponding value extracted from a clinically normal squamous site from the same patient. The “normalized” scattering parameter (A) at 700nm, best distinguished HSIL from non-HSIL with sensitivity and specificity of 89% and 79%, suggesting that a simple, monochromatic instrument measuring only A may accurately detect HSIL.

OCIS codes: (170.0170) Medical optics and biotechnology; (170.6510) Spectroscopy, tissue diagnostics

References and links
1. Introduction

The main target of the clinical management of women with suspected squamous intraepithelial lesions (SIL) is the accurate diagnosis of precancerous changes, specifically high grade SIL (HSIL). The current clinical standard for diagnosis of HSIL is colposcopy, a procedure that involves visual inspection and biopsy of at-risk tissue, followed by histopathological diagnosis. The diagnostic accuracy of colposcopy greatly depends on the physician’s expertise and even when conducted by experts, is subject to significant diagnostic variability [1]. Spectroscopy is a technique that may reduce the interobserver disagreement of colposcopy and improve its diagnostic accuracy by diagnosing HSIL in an objective manner. The effectiveness of spectroscopic techniques, specifically reflectance and fluorescence, for in vivo diagnosis of HSIL has been extensively evaluated [2–16] These studies demonstrate the potential of spectroscopy to improve the effectiveness of disease detection [17].

The spectroscopic diagnosis of cervical dysplasia is based on the contrast in tissue spectra caused by loss of differentiation of the epithelial cells [18], degradation and reorganization of stromal collagen by matrix metalloproteinase activity [19,20], increased metabolic activity, and angiogenesis [21]. Tissue spectroscopy is not only affected by disease, but also by age [15,22–26], menopausal status [15,23–27], time after the application of acetic acid [12], and normal variations in cervical anatomy [6,23,28,29].

Historically, spectroscopic studies have included clinically normal squamous sites, either non-biopsied or histopathologically confirmed, in the validation set for diagnosing HSILs [5,6,8,10,28]. Mourant et al. [11,14] and Georgakoudi et al. [9] noted an apparent increase in diagnostic power when clinically normal tissues were included in the validation set. Similarly, Freeberg et al. [23] observed that tissue type influences both reflectance and fluorescence measurements.

In a recent study by our laboratory, we demonstrated that underlying differences in tissue anatomy can have a confounding effect on diagnostic spectral algorithms [29]. Normal transformation zone of the cervix, the area where the vast majority of HSILs are found [30], is anatomically, histologically, and spectroscopically different from the normal squamous mucosa. As the vast majority of the HSILs are found in the transformation zone, the spectral differences between normal squamous mucosa and HSIL are largely due to normal anatomical differences. Based on the findings of this study, a common practice of including clinically normal squamous sites into the data set which is used to develop or evaluate the performance of the algorithm for detection of HSIL is a confounding artifact that artificially increases performance values with respect to the key differentiation to be made, namely distinguishing HSILs from clinically suspicious non-HSILs. The data in this study demonstrated the confounding influence of including clinically normal squamous sites not only affects the performance levels but also the number of specific spectroscopic parameters that can be used in the diagnostic algorithm. The affected parameters included those describing the scattering, absorption, and fluorescence properties of tissue. In order to properly evaluate the accuracy of clinical disease detection, spectroscopic data must be analyzed within the appropriate anatomical context.

The aim of the present study was to develop an algorithm for detection of HSILs free of the confounding effect of cervical anatomy. We studied patients undergoing colposcopic examination and used reflectance and fluorescence spectroscopy to differentiate HSILs from non-HSILs among abnormal sites identified by the clinician as needing biopsy. Physical models were used to fit the spectra and extract parameters related to tissue morphology and biochemistry. We investigated the effect of per-patient parameter normalization on diagnostic performance as well as the effect of normal anatomical variation within the transformation zone, the glandular content, on the extracted spectroscopy parameters. The spectroscopy parameters were then used to develop a spectroscopic diagnostic algorithm to distinguish HSILs from non-HSILs.
4. Comment

Both disease and normal variations in microscopic anatomy are significant sources of spectroscopic contrast in clinically obtained tissue spectra. In order to properly evaluate the accuracy of disease detection, spectroscopic data must therefore be analyzed within the appropriate anatomical context. This study specifically focuses on differentiating HSILs from non-HSILs among clinically suspicious sites, the majority of which are found within the transformation zone. We find that after normalization by an internal standard, the $A$ parameter alone provided the best diagnostic performance in differentiating HSIL from non-HSIL sites. AUC, sensitivity and specificity of 0.84, 89% and 79%, respectively, were achieved. Positive and negative predictive values were 48% and 97%, respectively. The high negative predictive value suggests that this technique may be useful for reducing the number of unnecessary biopsies. Even though the spectroscopy parameters including $A$ parameter are affected by glandular content, the $A$ parameter was the most diagnostic parameter in the discrimination of HSIL from both GS and non-GS sites.

In our study HSIL sites exhibit significantly lower values of $A$ parameter relative to the non-HSIL sites. This result is in agreement with results from similar studies in the literature. In a study of 161 patients, Mirabal et al. [10] found that there is a gradual decrease in mean reflectance intensity as the severity of dysplasia increases. In studies by Nordstrom et al. [5], and Huh et al. [8], reflectance was found to differentiate between HSIL and normal sites in the transformation zone (squamous metaplasia), while fluorescence did not yield significant differences. For both studies it is not known whether the differences in reflectance spectra were due to a higher hemoglobin concentration, lower scattering, or a combination of the two effects. Furthermore, our study cannot be directly compared to these two studies, as our study compares HSIL to all clinically suspicious non-HSIL sites, including the LSIL sites, while their studies report LSIL and squamous metaplasia sites separately. Georgakoudi et al. [9] also report lower reduced scattering coefficient (similar to $A$ parameter) for distinguishing SILs from biopsied non-SILs. However, we cannot directly compare their findings to those of this study as they did not discriminate HSIL from non-HSIL. Finally, the findings of the follow-up clinical in vivo study conducted with the Quantitative Spectroscopy Imaging system (manuscript in preparation) were consistent with the findings of our study. This study also observed that after normalization by an internal standard, the $A$ parameter alone provided the best diagnostic performance in differentiating HSIL from non-HSIL sites [33].

The lower value of the $A$ parameter for HSIL sites compared to non-HSIL sites is also physically justified. Arifler et al. [34] used Monte Carlo modeling of cervical tissue to show that the smaller stromal reduced scattering coefficient is the major cause for decreased reflectance intensity of HSIL compared to normal squamous tissue. Degradation of stromal collagen by matrix metalloproteinase activity [19,20] is the likely explanation for the lower value of $A$ parameter for HSIL sites compared to non-HSIL sites. We also observe higher hemoglobin concentration of HSIL sites relative to the non-HSIL sites. Higher hemoglobin concentration in HSIL sites compared to other tissue types has been noted by Chang et al. [4] Additionally, Marin et al. [2] report that hemoglobin features of the reflectance tissue spectra are more prominent in abnormal tissue compared to normal squamous tissue. However, studies that considered diagnosing HSIL within clinically suspicious sites, such as Georgakoudi et al. [9], as well as Mourant et al. [11] reported no significant change in the hemoglobin concentration between HSIL and non-HSILs.

Our finding of higher hemoglobin oxygenation for HSIL sites compared to non-HSIL within the clinically suspicious sites is consistent with the results of a study by Mourant et al. [11] which also looked at the difference between HSIL and clinically suspicious non-HSIL sites, 90% of which were found in the transformation zone. The source of higher hemoglobin oxygenation for HSIL sites is not well understood and needs further investigation.

We find that the effective blood vessel radius is increased for HSIL sites compared to non-HSIL sites. This may be due to the presence of dilated atypical blood vessels associated with precancerous and cancerous changes [35].
Finally, we observe decreased Coll for HSIL sites compared to non-HSIL sites. This observation is consistent with decreased collagen fluorescence due to degradation of collagen matrix, increased NADH fluorescence due to changes in cellular metabolism, or a combination of both. The increase in epithelial thickness is not a source of this feature, since there was no significant difference in measured epithelial thickness between HSIL and non-HSIL sites in our study. Increased NADH contribution of SILs compared to non-SILs within the transformation zone has been observed by Georgakoudi et al. [9] Chang et al. [4] report a decreased stromal collagen contribution; however, their study included clinically normal squamous sites. Studies by Nordstrom [5], and Huh [8], which utilize 340 nm fluorescence, reported no significant differences between HSIL and normal sites in the transformation zone (squamous metaplasia). A study of Ramanujam et al. reported that 340 nm excitation HSIL sites could not be differentiated from non-HSIL sites in the transformation zone (cervical intraepithelial neoplasia 2/3 (equivalent to HSIL) vs. squamous metaplasia).

We found that normalization of the spectroscopy parameters enhanced the contrast between HSIL and non-HSIL sites. Normalization may reduce spectroscopic variations due to intrinsic patient-to-patient variations associated with age, hormonal contraception, and menopausal status. Furthermore, it may account for differences caused by the time-dependent effect of acetic acid on tissue scattering and absorption. The vasoconstrictive and light scattering effects of acetic acid [35,36] may affect the extracted hemoglobin concentration, effective blood vessel radius, and also the scattering parameters. Recent work in our laboratory, which uses the same data set described in this paper, showed that without parameter normalization, HSIL sites could be differentiated from non-HSIL with AUC, sensitivity, and specificity of only 0.68, 78% and 67%, respectively [29]. In the present study, we found that parameter normalization resulted in substantial improvements performance metrics, as reported above. However, we point out that per-patient normalization of Coll parameter has decreased the ability of this parameter to differentiate between non-HSIL and HSIL sites. Further investigation is required to determine the best strategy for fluorescence per-patient normalization.

The finding that only the A parameter has diagnostic importance suggests that a significantly simpler, faster, and less expensive instrument which measures tissue scattering using one wavelength may be all that is required to reliably detect cervical disease. The limitation of our study is a relatively small number of patients. If the results of our study are further confirmed in the ongoing larger imaging clinical study, our laboratory will design a simplified instrument for detecting cervical dysplasia and investigate how effective this approach is in improving the accuracy of cervical dysplasia detection. In developed countries, this simple instrument could be used as an adjunct to colposcopy to reduce the number of unnecessary biopsies. However, the greatest impact of this simple and relatively inexpensive instrument would be on cervical cancer diagnosis in developing countries, where the lack of medical infrastructure precludes cytology-based cervical cancer screening program. In these settings, the common mode of cervical cancer screening is by visual inspection after application of acetic acid (VIA) followed by immediate treatment of suspicious lesions. VIA has a significant false positive rate. The simple spectroscopic instrument could be used as an adjunct to VIA to improve accuracy in the diagnosis of cervical cancer and its precursors.

Acknowledgments

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Spectroscopic diagnosis and imaging of invisible pre-cancer

Kamran Badizadegan,a Vadim Backman,b Charles W. Boone,c Christopher P. Crum,d Ramachandra R. Dasari,e Irene Georgakoudi,e Kristin Keefe,e Karl Munger,f Stanley M. Shapshay,g Ellen E. Sheetse and Michael S. Feld*ec

a Department of Pathology, Children’s Hospital, Boston, MA, USA
b Biomedical Engineering Department, Northwestern University, Evanston, IL, USA
c MIT Laser Biomedical Research Center, GR Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
E-mail: msfeld@mit.edu
d Department of Pathology, Brigham and Women’s Hospital, Boston, MA, USA
e Division of Gynecologic Oncology, Brigham and Women’s Hospital, Boston, MA, USA
f Department of Pathology, Harvard Medical School, Boston, MA, USA
g Department of Otolaryngology, Boston University School of Medicine, Boston, MA, USA

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The theme of this paper is the use of optical spectroscopy to diagnose invisible pre-cancer in patients undergoing endoscopy and similar medical procedures. We describe three techniques that provide diagnostic information and two instruments to implement them, the FastEEM for studying small regions of tissue and the LSS (light scattering spectroscopy) imaging system for wide-area surveillance. The FastEEM is an optical fiber clinical device that collects spectra of reflected light and fluorescence at multiple excitation wavelengths from the tissue, all in a fraction of a second. Quantitative information is obtained in real time, without removing the tissue and without the need for staining and fixation. Three types of spectral information are extracted—intrinsic fluorescence, diffuse reflectance and elastic light scattering. Each of the three analyses is based on a biophysical model, and each provides complementary quantitative physical and chemical information about cellular/tissue structures. This information is used to make a combined spectral diagnosis, a method we call tri-modal spectroscopy (TMS). Promising clinical studies are being carried out on patients undergoing routine pre-cancer surveillance in the oral cavity, the uterine cervix and the gastrointestinal tract. The LSS imaging system provides wide-area spectroscopic images of the epithelium, typically 2 cm in each dimension, depicting the size distribution and chromatin content of the cell nuclei, which are key parameters in diagnosing pre-cancer. This instrument is in preclinical stages of development, although a laboratory prototype has been used to create diagnostic images in resected colon polyp samples. The combination of the TMS/FastEEM and LSS imaging instrument will constitute a powerful new diagnostic tool, with LSS imaging to provide wide area surveillance and the TMS probe to provide detailed information on suspect tissue sites.

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thus be used prospectively to follow patients during and post-therapy to detect very early recurrence of neoplastic lesions.

II. Optical spectroscopy as a diagnostic tool

Optical spectroscopy is a potentially important tool for tissue diagnosis. Spectroscopic measurements can be implemented in vivo, thus providing information about tissue in its native state, free of artifacts introduced by tissue excision and processing. Different spectroscopic techniques can be used to provide a variety of information about tissue morphology and biochemistry. The capability to obtain in vivo information about specific biochemical/morphological changes that take place during the development or regression of neoplasia can provide a rich source of diagnostic information, and can further the understanding of the biological processes involved, as well.

Light propagating in biological tissue can undergo a variety of interactions. Elastic scattering (i.e., scattering without wavelength change) is the predominant mechanism, and light impinging on the tissue can be scattered once (single scattering) or multiple times (diffusive scattering) before returning to the surface to be detected. Light can also be absorbed by chromophores such as hemoglobin without being re-emitted (absorption), or by fluorophores such as NAD(P)H and re-emitted at longer wavelengths (fluorescence) before being detected. In addition, inelastic processes that shift wavelength (such as Raman scattering) can occur. The physical processes relevant to the work described below are fluorescence, diffuse scattering and single scattering.

Fluorescence

Fluorescence can provide information about the biochemical state of the tissue and the changes that occur during disease development. Promising in vivo results to diagnose cancer have been reported in a number of tissues, including the cervix, the lung, the gastrointestinal tract, the oral cavity, the skin and the bladder. These results have employed statistical or empirical analyses of the observed tissue fluorescence, the spectral features of which can be significantly distorted by the interplay of absorption and diffusive scattering, which is ubiquitous in biological tissue, limiting the ability to extract quantitative biochemical information. We have developed the technique of intrinsic fluorescence spectroscopy to disentangle these artifacts and extract the undistorted (intrinsic) tissue fluorescence.

Diffuse scattering

Light propagating in tissue is rapidly diffused due to multiple scattering. Diffusely reflected (or transmitted) light can be used to detect changes in the scattering and absorption properties of tissue. Diagnostic applications study the spectrum of diffusive back-scattered CW white light delivered and collected by means of a small (∼1 mm diameter) optical fiber probe. Since this source–detector configuration samples the tissue to a depth of ∼1 mm or less, such techniques predominantly probe the subepithelial region of tissue, and are well suited for studying stromal changes in epithelial lesions. Promising clinical results have been reported for distinguishing normal and pre-cancerous tissues in the skin, the breast, the gastrointestinal tract and the bladder. The above studies have employed empirical methods of spectral analysis, correlating features in the diffuse reflectance spectrum with disease state. We have developed an analytical method for analyzing diffuse reflectance spectra, based on diffusion theory, in which information about the tissue scatterers and absorbers is extracted from the diffuse reflectance spectrum. This method, diffuse reflectance spectroscopy (DRS), provides quantitative information about tissue composition, such as hemoglobin concentration, collagen matrix composition, etc., from which diagnostic information is obtained.

Single scattering

As shown by our group, the back-scattered light also contains spectral information about single scattering from the epithelium, which we study via light scattering spectroscopy. This spectral
Optical Imaging of the Cervix

Recent advances in fiber optics, sources and detectors, imaging, and computer-controlled instrumentation have stimulated a period of unprecedented growth in the development of photonics technologies for a wide variety of diagnostic and therapeutic clinical applications. These include the application of quantitative optical spectroscopy and imaging for the detection of precancerous lesions in the uterine cervix, a topic of interest at the Second International Conference on Cervical Cancer, which was held April 11–14, 2002. Investigators have applied the Littenberg method of emerging technology assessment to new optical methods used to detect cervical neoplasia. Currently, such technologies as fluorescence spectroscopy (the combination of fluorescence and diffuse reflectance spectroscopy), tri-modal spectroscopy, and light-scattering spectroscopy that probe the spectral characteristics of tissue are being investigated. Optical technologies that create images of subcellular structure without biopsy subsequent to pathology that currently are under investigation include in vivo confocal imaging and optical coherence tomography. Numerous small studies have demonstrated the potential of these optical technologies. What remains to be elucidated are the fundamental biophysical origins of variations in remitted optical signals between normal and dysplastic tissue. Large multicenter randomized controlled trials are needed to confirm the detection and imaging capabilities of optical technology. Furthermore, the development of contrast agents that could boost detection with these technologies is needed, and basic biologic characterization of signals should be pursued. Applying the Littenberg assessment will help ensure that superior, not simply alternative, technologies are implemented. Cancer 2003;98(9 Suppl):2015–27. © 2003 American Cancer Society.

KEYWORDS: photonics, optical technology, detection, precancerous lesions, uterine cervix.

Recent advances in fiber optics, sources and detectors, imaging, and computer-controlled instrumentation have stimulated a period of unprecedented growth in the development of photonics technologies for a wide variety of diagnostic and therapeutic clinical applications. The use of noninvasive optical techniques for the early detection of precancerous conditions is one rapidly emerging area within the field of biophotonics. Quantitative optical spectroscopy and imaging can improve the current clinical strategies for the screening and diagnosis of epithelial precancerous lesions in a variety of organ sites, including the uterine cervix. Detection of cervical precancerous lesions is a particularly important clinical application of emerging optical technologies because of the potential for improvement in the current standard of care both in the U.S. and abroad. Greater than $6 billion is spent annually in the U.S. in managing low-grade cervical lesions, and significant monetary resources are consumed in monitoring and treating lesions unlikely to progress to malignancy (findings such as abnormal squamous cells of unknown significance and low-grade...
OCCUPATIONAL EXPOSURE TO HUMAN RIGORUS MAJOR AND ITS BIOLOGICAL EFFECTS

Squamous intraepithelial lesions [LGSILs]). By eliminating unnecessary biopsies and treatments, optical technologies could have a significant impact on care in the U.S. through cost reduction. In developing countries, cervical cancer often goes undetected because of insufficient personnel and resources to perform adequate screening and diagnosis. Optical technologies could greatly improve care worldwide by providing automated, machine-read diagnosis at a screening visit. Automation would permit use of the technology by health care workers with less clinical training than that of physicians and nurse practitioners.

Both industrial and academic research groups believe that the screening and detection of cervical cancer precursors could be improved significantly by optical technologies that automate and decrease the cost of screening and detection while improving accuracy. In the current study, we summarize the current research in developing emerging optical technologies for the detection of cervical precancerous lesions based on such spectroscopic approaches as fluorescence spectroscopy, diffuse reflectance spectroscopy, or trichromal spectroscopy and on direct high-resolution imaging methods, including confocal reflectance microscopy and optical coherence tomography. After a review of current research using each of these optical modalities, we discuss potential future research directions for the optical assessment of cervical neoplasia.

OPPORTUNITIES FOR EMERGING TECHNOLOGIES

Although the introduction of organized screening and detection programs has decreased cervical cancer mortality and morbidity, significant gaps in care persist. Because the Papanicolaou (Pap) smear has a reported average sensitivity of 58% and a specificity of 69%, many lesions are missed or overcalled. Thus, false-negative cytology findings, the test must be repeated annually, resulting in both considerable anxiety and economic cost.

Expert colposcopy has an average sensitivity of 96% (average colposcopy has a specificity reportedly estimated as low as 79% by some) and a specificity of 48%. Small, 1-quadrant lesions are missed in 30% of cases, and in 1 study, 58% of microinvasive tumors were not detected by expert colposcopy. Thus, multiple biopsies are required to confirm diagnosis. This low sensitivity allows for the detection of most cancers, but the specificity suggests that many lesions are overcalled, leading to many unnecessary biopsies. Those patients with accurately identified high-grade lesions may wait 2 weeks for confirmation and may be lost to follow-up. Until recently, considerable controversy existed regarding the evaluation and treatment of low-grade disease because of the difficulty of determining which lesions will progress to cancer or regress to normal. Consensus guidelines were promulgated in 2002 in an effort to resolve the issue.

Emerging technologies have the potential to address these gaps. Optical spectroscopy and imaging provides a tool with which to examine the entire epithelial volume in situ and provides an objective assessment of the biochemical and morphologic status. Used for screening and diagnosis, these technologies have the potential to provide immediate and accurate diagnostic information, potentially reducing both the costs associated with unnecessary biopsies and treatment delays.

Although these emerging techniques appear promising, all emerging technologies should be evaluated in a systematic way that allows them to be optimized during development. Technology assessment provides an explicit methodology with which to achieve this goal. In 1992, Littenberg proposed five levels of technology assessment: biologic plausibility, technical feasibility, intermediate effects, patient outcomes, and societal outcomes.8 Biologic plausibility refers to whether current understanding of the biology and pathology of the disease in question can support the technology. Technical feasibility refers to the level of assessment in which physicians can safely and reliably deliver the technology to the intended patients. Intermediate effects assess the sensitivity and specificity in a relevant population. Patient outcomes assess whether the technology improves the patients’ health and societal outcomes assess the cost and ethical implications of a technology. Wortman and Saxe described the Fineberg proposal for a hierarchy of evaluation of diagnostic technologies: technical capacity (whether the device performs reliably and delivers accurate information), diagnostic accuracy (whether the test result improves it), diagnostic impact (whether the test result influences the pattern of testing or subsequent testing or replaces other tests) therapeutic impact (whether the test result influences the selection and delivery of therapy), and patient outcome (whether test performance contributes to the improved health of the patient).9 The Littenberg classification can be applied to emerging or existing technologies, whereas that described by Wortman and Saxe is best suited to existing technologies.

The next section focuses on the emerging technology of fluorescence spectroscopy. Following the Littenberg model of technology assessment, we examine the biologic basis of the technology first.
contributor to cost is the false-positive rate of colposcopy. Furthermore, confocal images can provide point-of-care diagnostics, allowing combined diagnosis and therapy in a single see-and-treat office visit. This can further reduce health care costs and reduce the number of patients lost to follow-up.

OPTICAL COHERENCE TOMOGRAPHY

To image at depths within cervical tissue beyond the range of a confocal microscope, researchers must employ a different imaging approach known as optical coherence tomography (OCT). Although confocal microscopy can be used only to image tissue within a few hundred microns of the surface, OCT can yield backscattering data for depths of several millimeters. OCT has been shown to achieve resolutions in the cellular and subcellular range (range, 2–10 μm) and could improve the diagnostic capabilities of many clinical imaging procedures, including colposcopy. To evaluate OCT for the detection of those microstructural changes associated with cervical neoplasia in vivo, an integrated OCT colposcope was constructed by Pitriss, who described this work at the conference discussed in this supplement to Cancer. This instrument permits the simultaneous en face viewing of structural features and allows precise registration of the OCT scan plane without interfering with normal medical procedures. The first clinical feasibility study resulted in the successful identification of neoplastic and microstructural changes. The use of image processing techniques can be a powerful method for analyzing OCT image data, especially when large numbers of patients are involved. Image processing allows complex image formation to be reduced to quantitative variables that can be statistically analyzed, quantified, interpreted, and used to predict the presence of disease. In addition, it enables faster and better coverage and visualization of large areas and large volumes of data.

An example of such quantitative information from segmented OCT images is the extraction of statistical properties that describe tissue intensity. Preliminary results from Dr. Pitriss appear to correlate regions of high intensities with areas of the most advanced neoplasia, and the application of OCT could be extended to large volumes of data.

Grading neoplasia most likely will require more information than the microstructural features resolved by standard resolution OCT. Cellular and even subcellular characteristics may be critical for the accurate determination of neoplastic grade. A very broad bandwidth Ti:Al2O3 laser was used for the acquisition of ultrahigh-resolution OCT images. The system was comprised of a specially balanced and compensated interferometer and achromatic optics. Ex vivo ultrahigh-resolution OCT images of the normal cervix to our knowledge demonstrated for the first time the ability of OCT to delineate the presence of cells in human tissue. The squamous cells of the cervical epithelium, most likely with the presence of koilocytosis, are clearly evident in the images obtained. They range in size from approximately 10–30 μm. This finding implies that diagnostically useful cellular information can be extracted from human tissue using a high-resolution OCT system. Potential disadvantages of OCT include limitations in backscattering contrast between normal and dysplastic tissue, limited field of view, and limitations in imaging depth.

FUTURE DIRECTIONS

The current study summarizes current research aimed at developing effective optical technologies for detecting cervical neoplasia. A variety of technologies including both spectroscopic approaches (fluorescence spectroscopy, reflectance spectroscopy, and TMS) and direct imaging methods (confocal microscopy and OCT) currently are under development. These technologies are designed to address the detection and diagnosis of cervical precancerous lesions in the developing and developed world. In developing countries, critical needs include low-cost technology, low-maintenance equipment, and real-time diagnosis. In developed countries, cost reduction is a priority that can be achieved by avoiding overtreatment and identifying patient cohorts for see-and-treat strategies. Although numerous studies published to date have demonstrated the potential of optical technologies in small pilot studies, in order for these technologies to be translated successfully into clinical practice we need results from large multicenter trials, based on validated endpoints and conducted with validated equipment. Although preliminary studies have demonstrated the potential of optical spectroscopy and imaging to deliver sensitive and specific detection of precancerous conditions, to our knowledge the fundamental biophysical origins of variations in remitted optical signals between normal and neoplastic tissue have not been elucidated fully. We believe increased research efforts in this area are critical to exploit the potential of emerging optical technologies fully.

Finally, although optical technologies promise high-resolution, noninvasive functional imaging of tissue at competitive cost, to our knowledge, optical technologies currently probe only a limited number of endogenous chromophores. The combination of emerging optical technologies with the development of novel exogenous contrast agents, designed to probe the molecular specific signatures of cancer, would dramatically improve the detection limits and clinical
The performance of fluorescence and reflectance spectroscopy for the in vivo diagnosis of cervical neoplasia; point probe versus multispectral approaches

J. Adrian Freeberg, J.L. Benedet, Calum MacAulay, Loyd A. West, Michele Follen

Abstract

Objective. This review evaluates the diagnostic efficacy of fluorescence spectroscopy, reflectance spectroscopy, and their combination that use both point probe and multispectral imaging approaches in diagnosing cervical neoplasia in vivo.

Methods. Articles were selected for this review from a literature search which report the performance of fluorescence and reflectance spectroscopy devices in diagnosing cervical neoplasia in vivo. This analysis focused on the comparison of point probe versus multispectral approaches; the use of fluorescence, reflectance, and their combination; and finally the types of populations that have been studied for in vivo diagnosis of squamous intraepithelial lesions (SIL).

Results. Twenty-six studies were included and their heterogeneity precluded formal meta-analysis. Though point probes were expected to have greater specificity and multispectral approaches greater sensitivity, there was considerable overlap in the performance of point probe and multispectral devices. There were few studies that studied fluorescence spectroscopy alone and reflectance spectroscopy alone. Combined fluorescence and reflectance approaches showed considerable overlap among point probe and multispectral devices. The overlap of performance suggests that fluorescence and reflectance may have similar performance. Currently the paucity of data precludes definitive conclusions regarding the additive effect of both approaches. Only two of twenty-six trials have recruited patients with no history of an abnormal Papanicolaou smear (screening populations) and twenty-four trials include patients with a range of cervical abnormalities from atypia to cancer (diagnostic populations).

Discussion. Optical spectroscopy using a point probe and multispectral approaches appears to overlap in performance. Fluorescence and reflectance spectroscopies examine different aspects of epithelial–stromal biology and appear to yield similar diagnostic performance. While intuitively appealing, their combination may or may not be additive. There have been few studies of these technologies in screening populations. Better definitions of device trial design and reporting requirements would facilitate combining analyses to formally examine performance.

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Keywords: Cervical intraepithelial neoplasia (CIN); Squamous intraepithelial lesion (SIL); High-grade SIL (HG-SIL); In vivo cervical diagnosis; Real-time diagnosis; Optical spectroscopy; Fluorescence spectroscopy; Reflectance spectroscopy; Combined fluorescence and reflectance spectroscopy; Point probe spectroscopy; Multispectral spectroscopy or imaging, sensitivity, and specificity of cervical diagnosis
performance alone (A), reflectance spectroscopic performance alone (B), combined fluorescence and reflectance approaches (C), and the summary of the three of the aforementioned (D). Again, the studies are not sufficiently similar to allow a precise comparison, but these data suggest that both approaches yield fairly similar performance. While all these figures show considerable overlap, panel A suggests that multispectral approaches may perform better than point probe devices. This result may be confounded by trial design; that is pilot/Phase I versus Phase II/III design. The reflectance approach in panel B has few points and it is difficult to draw any conclusions. Similarly, the combined approach of fluorescence and reflectance in C shows only two probe trials. The performance of these two probe trials clearly falls in the middle of the multispectral trials, but probably on the same imaginary ROC curve. The combined approach does not appear to perform better than either approach alone, at least in these crude analyses. Panel D, that shows all three approaches, is suggestive of overlap. Further work will need to establish whether both fluorescence and reflectance are necessary or in which populations their use is preferable. These data are important for device design and for cost-effectiveness of the device in the population in which it is intended to be used.

Finally in Fig. 4, those studies aimed at diagnostic populations are contrasted to those carried out in screening populations. Diagnostic populations are those referred with an abnormal Papanicolaou smear to a colposcopy clinic, while screening populations are those in whom there is no history of an abnormal Papanicolaou smear and the patient is screened. Only two studies concern screening populations and while both use fluorescence spectroscopic point probes, their performance is quite different in terms of sample size, history of previous screening, economic level, race, and specificity. They are sufficiently diverse that they preclude any combination of data or any summary of performance.

Discussion

The FDA has described four possible uses for optical technologies in the diagnosis of cervical neoplasia (40): 1) to localize biopsies as an adjunct to colposcopy, 2) to triage patients after an atypical Papanicolaou smear, 3) as an adjunct to cervical or vaginal cytology, and 4) as a primary screen in the place of cervical or vaginal cytology. Clearly these four uses require different clinical trial designs for their evaluation [36].

One might intuitively think that a point probe could be most useful as an adjunct to colposcopy, while a multispectral approach would be more suitable for screening. This logic would imply that the point probe might be more suitable for evaluation of an adjunct to colposcopy and possibly for triage of atypical Papanicolaou smears, while a multispectral approach might be more logical for an adjunct to or replacement of cytology. What has been studied and reported is quite different than this logic.

The Polartechnics point probe has been suggested as an adjunct to the Papanicolaou smear for screening [19,20]. The investigators suggested using the probe in a circular fashion to measure the entire cervical surface area. They show diagrams of measuring the cervix in circular fashion 20–30 times to cover the entire cervical surface. Their reports contain ROC curves that demonstrate a possible replacement for the Papanicolaou strategy despite the study of a diagnostic population rather than a screening population. It is unclear if one can conclude how a device replaces screening when the population under study is a diagnostic population with a high prevalence of disease.

Not unlike the Polartechnics probe studies, both Mitchell and Belinson report the only studies that focus on screening populations and both use fluorescence point probe devices in their clinical trials [12,14]. There are no reports of multispectral imaging devices or devices that use both multispectral and point probe approaches in screening populations, at least at the present time. With such different performance results in these two trials, it is unclear what the performance of these devices could be and how they could be optimized in a screening population.

Alternatively, both Medispectra and SpectRx are using multispectral imaging. Both companies suggest that their devices could be an adjunct to colposcopy by showing an area on a cervix-like image that might additionally be abnormal to that observed in colposcopy. Medispectra has reported Phase III randomized clinical trials, while SpectRx has reported Phase II clinical trials [26–32]. In each case, there is an Independent Device Exemption from the FDA, the spectroscopic site must be evaluated by the clinician and the clinician must decide if the spectroscopically abnormal area is different or the same as that identified by colposcopy in order to decide to take the additional biopsy.

Clinical trial design will be the subject of a future review. Thus far, the FDA has favored study designs that compare colposcopy in one arm to colposcopy plus spectroscopy in the other arm. This trial design is most suitable for the evaluation of adjunct to colposcopy and does not address the other three possible uses of optical technologies.

Only one such Phase III trial has been completed, that of Alvarez [32]. There was no statistically significant difference in
Introduction

The present study was designed to assess the potential impact on cervical disease management that spectroscopic imaging would have if employed as a pre-colposcopy imaging tool. Study endpoints included sensitivity to detect biopsy proven cervical dysplasia, especially moderate and severe dysplasia (CIN2+), as well as specificity to rule out benign cervical conditions that also had been scheduled for colposcopy and biopsy. The study population included women with a history of abnormal cervical cytology or other risk factors, such as previously documented histopathology of cervical dysplasia in need of follow-up.

Objective

The objective of the study was to evaluate the potential safety and effectiveness of tissue spectroscopy for the diagnosis of cervical cancer in a prospective multicenter, IRB approved study of women scheduled for colposcopy on the basis of an abnormal Pap test or other risk factors.

Cervical Neoplasia Detection System (CENDS)

The device system (Guided Therapeutics, Inc., Norcross, GA, USA) used in the study is a noninvasive risk device by FDA standards that noninvasively and automatically scans the ectocervix and distal endocervix for disease related changes in fluorescence and reflectance spectra (see Figure 1). Alterations in fluorescence spectra are a function of disease related changes associated with neoplasia, while alterations in reflectance and scattering are indicative of structural changes associated with neoplasia, such as epithelial thickening, nuclear size and content and angiogenesis.2,25

A plurality of equally spaced points over a one-inch diameter area of the cervix was automatically scanned during a four-minute period using a filtered xenon arc lamp as an illumination source. For cervical tissue fluorescence measurements, broadband spectral output ranging from about 350 to 900 nm was directed to the cervix for tissue excitation and a fiber optic cable was used to control the cervix using the same xenon arc lamp. The resultant reflected light from the biopsy cervical tissue was imaged onto the CCD camera and stored for processing. For cervical tissue reflectance measurements, light from the arc lamp was band pass filtered to limit exposure of the cervix to bands within the 500 to 600 nm range. These spectral bands are known to excite fluorophores associated with neoplastic processes as described above. Each of the fluorescence wavelengths were applied automatically under software control in a predetermined order and scan pattern. The resultant fluorescent spectral output of the cervical tissue was imaged onto a charge coupled device and stored for processing and analysis.

The system consists of two main physical components, the hand held unit and the base unit (Figure 1). The hand held unit is connected to the base unit via fiber optic cables for transmission of light to and from the base unit, which contains the xenon arc lamp, optical processing elements (e.g., filters and lenses) and the CCD camera on a rolling cart (CENDS Device). The main object of the CENDS is a computer for control and data processing. This includes the capability for a diagnostic algorithm based on spectroscopic information measured from the cervix, calibration data and other patient data, such as Pap results or patient demographic data.

Methods

Subjects meeting the inclusion criteria underwent spectroscopy of the cervix during their colposcopy visit. Spectroscopic measurements taken over a four minute scan period were applied to a color scale validated by 30 regulated pathologists and compared with expert panel resolved histopathology to yield sensitivity and specificity of cervical spectroscopy. After the spectroscopy measurements were taken, a Pap test was taken, followed by biopsy and histopathology if indicated.

Histopathology Quality Control

Each clinical site fixed tissue per current clinical practice. An additional slide adjacent to the diagnostic slide was prepared and reviewed. If the clinical site and the expert (EW) agreed with the diagnosis, the slide was sent to the clinical pathology lab of one of the authors (EW). If EW disagreed with the diagnosis of the clinical site pathologist, the slide was then sent to a third pathologist (SR). A case was considered non-evaluable when either EW agreed or disagreed with one of the three diagnoses agreed (i.e., either benign, CIN1, CIN2+) using the most severe disease grade for each case. A case was considered non-evaluable when all three pathologists disagreed.

Summary and Conclusions

P

Spectroscopic Imaging as a Triage Test for a Prospective Multicenter Clinical Trial

Timothy DeSantis M.D.1,Nahida Chakhtoura M.D.1,Leo Twiggs M.D.1,Daron Ferris M.D.2,Manocher Lashgari M.D.3,Lisa Flowers M.D.4, Mark Faupel Ph.D.5, Shabbir Bambot Ph.D.5,Steven Raab M.D.6, Edward Wilkinson M.D.7

1University of Miami, 2Medical College of Georgia, 3St. Francis Hospital—University of Connecticut, 4Emory University, 5SpectRx, Inc. 6University of Pittsburgh, 7University of Florida

A total of 488 consecutive women from the four clinical sites met inclusion criteria; the consent form were therefore eligible to participate in the study. Data could not be collected from four patients because they withdrew before the study could complete. Median age of the 587 participants was 27.7 (range 18-75) years and under the age of 30 at the time of the study. Three hundred forty-five characterized themselves as Hispanic, 141 as Caucasian and 4 as Asian American or another race. Demographic data are summarized in Figure 2 and Table 1. No adverse events were reported.

All women underwent colposcopic examination and all 542 had a Pap test result and valid histopathology available. Of the 587 subjects that had valid histopathology and/or colposcopy results available, 15 cases could not be analyzed because of a device or operator error. Thus sensitivity and specificity of the test was calculated for the remaining 572 evaluable subjects (see Table 2).

Sensitivity of cervical spectroscopy for CIN2+ lesions (n= 142) was 95.1% with a corresponding specificity of 56.6%. Sensitivity of cervical spectroscopy for CIN1 lesions (n= 29) was 119% with a corresponding specificity of 56.6%. Sensitivity of cervical spectroscopy for CIN2+ lesions (n= 24) was 88.9% with a corresponding specificity of 41.7%. See Table 2. A plurality of equally spaced points over a one-inch diameter area of the cervix was automatically scanned during a four-minute period using a filtered xenon arc lamp as an illumination source.

For cervical tissue fluorescence measurements, broadband spectral output ranging from about 350 to 900 nm was directed to the cervix for tissue excitation and a fiber optic cable was used to control the cervix using the same xenon arc lamp. The resultant reflected light from the biopsy cervical tissue was imaged onto the CCD camera and stored for processing. For cervical tissue reflectance measurements, light from the arc lamp was band pass filtered to limit exposure of the cervix to bands within the 500 to 600 nm range. These spectral bands are known to excite fluorophores associated with neoplastic processes as described above. Each of the fluorescence wavelengths were applied automatically under software control in a predetermined order and scan pattern. The resultant fluorescent spectral output of the cervical tissue was imaged onto a charge coupled device and stored for processing and analysis.

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Methods

Subjects meeting the inclusion criteria underwent spectroscopy of the cervix during their colposcopy visit. Spectroscopic measurements taken over a four minute scan period were applied to a color scale validated by 30 regulated pathologists and compared with expert panel resolved histopathology to yield sensitivity and specificity of cervical spectroscopy. After the spectroscopy measurements were taken, a Pap test was taken, followed by biopsy and histopathology if indicated. A plurality of equally spaced points over a one-inch diameter area of the cervix was automatically scanned during a four-minute period using a filtered xenon arc lamp as an illumination source. For cervical tissue fluorescence measurements, broadband spectral output ranging from about 350 to 900 nm was directed to the cervix for tissue excitation and a fiber optic cable was used to control the cervix using the same xenon arc lamp. The resultant reflected light from the biopsy cervical tissue was imaged onto the CCD camera and stored for processing.

For cervical tissue reflectance measurements, light from the arc lamp was band pass filtered to limit exposure of the cervix to bands within the 500 to 600 nm range. These spectral bands are known to excite fluorophores associated with neoplastic processes as described above. Each of the fluorescence wavelengths were applied automatically under software control in a predetermined order and scan pattern. The resultant fluorescent spectral output of the cervical tissue was imaged onto a charge coupled device and stored for processing and analysis.
The clinical effectiveness of optical spectroscopy for the \textit{in vivo} diagnosis of cervical intraepithelial neoplasia: Where are we?

Marylou Cardenas-Turanzas\textsuperscript{a}, J. Adrian Freeberg\textsuperscript{b}, J.L. Benedet\textsuperscript{c}, E. Neely Atkinson\textsuperscript{a}, Dennis D. Cox\textsuperscript{d}, Rebecca Richards-Kortum\textsuperscript{e}, Calum MacAulay\textsuperscript{c}, Michele Follen\textsuperscript{a,f,}\textsuperscript{*}, Scott B. Cantor\textsuperscript{a}

\textsuperscript{a} Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 447, Houston, TX 77030-4009, USA
\textsuperscript{b} Center for Biomedical Engineering, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 193, Houston, TX 77030-4009, USA
\textsuperscript{c} The British Columbia Research Centre, Department of Cancer Imaging, Vancouver, British Columbia, Canada V5Z-1L3
\textsuperscript{d} Department of Statistics, Rice University, 6100 South Main St., Houston, TX 77005, USA
\textsuperscript{e} Department of Bioengineering, Rice University, 6100 South Main St., Houston, TX 77005, USA
\textsuperscript{f} Department of Obstetrics, Gynecology and Reproductive Sciences, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA

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Abstract

\textbf{Objective.} In this review, we evaluate the diagnostic efficacy of optical spectroscopy technologies (fluorescence and reflectance spectroscopy) for the \textit{in vivo} diagnosis of cervical neoplasia using both point probe and multispectral imaging approaches.

\textbf{Methods.} We searched electronic databases using the following terms: cervical cancer, cervical intraepithelial neoplasia, squamous intraepithelial lesion, and spectroscopy, fluorescence spectroscopy, or reflectance spectroscopy. We included studies that evaluated fluorescence and reflectance spectroscopy devices for \textit{in vivo} diagnosis, compared those results with biopsy results, and reported on the sensitivity and specificity of the devices tested.

\textbf{Results.} Twenty-six studies, including seven phase II trials and one randomized clinical trial, met our acceptability criteria. We found several important differences across the studies including device approach (multispectral versus point probe), study population, disease classification system, and disease threshold. This heterogeneity prevented formal combination of sensitivity and specificity results.

\textbf{Conclusion.} Optical spectroscopy has similar performance to colposcopy and may help localize lesions and therefore be an effective adjunct to colposcopy. Reports on the diagnostic accuracy of these devices should use common thresholds for the construction of receiver operating characteristic curves to enable comparisons with standard technologies and facilitate their adoption. Optical spectroscopy has also been identified for possible use as ASCUS triage and primary screening, yet neither has been sufficiently evaluated to warrant a conclusion as to their suitability in this role.

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Keywords: Cervical intraepithelial neoplasia (CIN); Squamous intraepithelial lesion (SIL); High-grade SIL (HG-SIL); \textit{in vivo} cervical diagnosis; Real-time diagnosis; Optical spectroscopy; Fluorescence spectroscopy; Reflectance spectroscopy; Multispectral imaging; Point probe imaging; Sensitivity and specificity

Introduction

Optical technologies could have an important impact on the real-time diagnosis of epithelial neoplasia across several organ sites, including the oral cavity, lungs, esophagus, stomach, colon, bladder, ovary, cervix, and skin [1]. This review focuses on the use of optical spectroscopy to diagnose cervical neoplasia \textit{in vivo}. Two optical spectroscopy device approaches have been developed, those using a point probe to interrogate...
Another important source of potential bias among these studies was the use or disuse of colposcopic impression to guide biopsy sites. Some studies used colposcopy to identify all biopsy sites; some used colposcopy to identify normal areas that were not biopsied and to identify abnormal areas to biopsy; some used random biopsies; and some used colposcopy to identify normal and abnormal sites and biopsied both of these. Few studies have reported the histological diagnosis of cervical sites which appear colposcopically normal.

Discussion

Despite the heterogeneity of the studies, there are several conclusions that can be drawn from this review. Optical technologies for screening and diagnosing cervical intraepithelial neoplasia have advanced from pilot studies to one reported randomized clinical trial. Researchers have also addressed various aspects of technology assessment [38], including the biologic plausibility [39–44], patient and provider acceptance [45,46], and cost-effectiveness [47] of these technologies in separate studies. As the trials progressed, the sample sizes increased and the study designs grew in complexity.

Twenty-four of the 26 trials identified were performed in diagnostic populations that included patients with abnormal Papanicolaou smears not addressing the FDA recommendation regarding the use of optical spectroscopy for cervical screening and diagnosis.

The clinical effectiveness of optical spectroscopy as an adjunct to colposcopy has been studied. Key to the analysis of the clinical effectiveness of optical spectroscopy is the performance of colposcopy itself. As shown in Table 2, the performance of colposcopy varies considerably when the reports by Mitchell and Alvarez are compared. Mitchell reports a sensitivity of 96% and Alvarez report 53% per patient; Mitchell reports a specificity of 48% and Alvarez a specificity of 89% per patient. The ALTS trial results report sensitivities of ~50% for colposcopy either immediately or as part of the standard of care, called conservative management [54]. Gage showed that the number of biopsies performed can affect the sensitivity [55]. Since their results report the outcome of two years of follow-up, they are likely to be accurate but difficult to compare to other studies.

Georgakoudi and Chang report the use of both fluorescence and reflectance spectroscopy using a point probe with sensitivities of 92% and 83% and specificities of 71% and 80%, respectively. Thus a point probe might be used as a clinically effective adjunct to localize lesions in patients with abnormal Papanicolaou smears.

Multispectral imaging has also been shown to have similar performance to colposcopy; like colposcopy, the performance is at both ends of the spectrum. Both multispectral devices currently perform very differently, with Alvarez/Medispectra reporting a sensitivity of 52% and specificity 90% and DeSantis/SpectRx reporting a sensitivity of 95% and specificity of 55%. Both studies use a multispectral approach and neither study addresses registration issues in intimate detail; that is neither study performs three-dimensional histopathology so that the exact area of spectroscopic abnormality can be mapped to the histopathologic area of abnormality.

Clearly, the performance results of colposcopy and optical spectroscopy at both extremes would fall along a receiver operating characteristic curve that is similar. One could conclude that optical spectroscopy is comparable to colposcopy. The point probe results lie between the two extremes, suggesting that investigators have set the operating characteristics to maximize sensitivity and specificity. The performance measures of the multispectral devices reported are at opposite extremes of the receiver operating characteristic curve. Algorithms that allow the performance to be in between extremes would improve the implementation of multispectral imaging as an adjunct to colposcopy.

Only one trial has addressed the issue of spectroscopy-based triage after ASCUS, and this trial addressed the issue in post hoc analysis. Alvarez showed no difference in the detection of HGSIL in the randomized trial of colposcopy (sensitivity 53%, specificity 89%) versus colposcopy plus spectroscopy (sensitivity 52%, specificity 90%). There were 225 patients with HGSIL in the colposcopy only arm and 214 in the colposcopy plus spectroscopy arm. Although a sample size was calculated for the trial, there was no analysis of the power in the trial. The discussion suggests that the HG lesions yielded heterogeneous spectroscopic results.

A post hoc analysis was performed of patients referred to the Alvarez trial with Papanicolaou smears showing ASCUS and LGSIL. All patients in the trial were referred with abnormal Papanicolaou smears ranging from ASCUS to
Optimal Excitation Wavelengths for Discrimination of Cervical Neoplasia

Sung K. Chang, Michele Follen, Anais Malpica, Urs Utzinger, Gregg Staerkel, Dennis Cox, E. Neely Atkinson, Calum MacAulay, and Rebecca Richards-Kortum*

Abstract—Fluorescence spectroscopy has shown promise for the in vivo, real-time detection of cervical neoplasia. However, selection of excitation wavelength has in the past been based on in vitro studies and the availability of light sources. The goal of this study was to determine optimal excitation wavelengths for in vivo detection of cervical neoplasia. Fluorescence excitation-emission matrices (EEMs) were measured in vivo from 351 sites in 146 patients. Data were analyzed in pairs of diagnostic classes to determine which combination of excitation wavelengths yields classification algorithms with the greatest sensitivity and specificity. We find that 330–340-, 350–380-, and 400–450-nm excitation yield the best performance. The sensitivity and specificity for discrimination of squamous normal tissue and high-grade squamous intraepithelial lesion (HGSIL) were 71% and 77% on cross validation using three excitation wavelengths. These results are comparable with those found in earlier in vivo studies; however, in this study we find that the proportion of samples which are HGSIL influences performance. Furthermore stratification of samples within low-grade squamous intraepithelial lesion and HGSIL also appears to influence diagnostic performance. Future diagnostic studies should be carried out at these excitation wavelengths in larger groups so that data can be stratified by diagnostic subcategory, age and menopausal status. Similarly, large studies should be done in screening populations.

Index Terms—Algorithm, cancer diagnosis, fluorescence spectroscopy.

NOMENCLATURE

SN Squamous normal tissue.
CN Columnar normal tissue.
HPV Human papilloma virus.
CIN 1 Grade-1 cervical intraepithelial neoplasia.
CIN 2 Grade-2 cervical intraepithelial neoplasia.
CIN 3 Grade-3 cervical intraepithelial neoplasia.
CIS Carcinoma in situ.
SIL Squamous intraepithelial lesion.
LGSIL Low-grade squamous intraepithelial lesion.
HGSIL High-grade squamous intraepithelial lesion.
EEM Excitation emission matrix.
ESL Eigenvalue significance level.

PAPANICOLOAU smear, in which a small sample of cells collected from the cervical epithelium are diagnosed under the microscope by an expert, is at present the most comprehensive means of screening and detecting cervical cancer. Although the Papanicolaou smear has been effective in reducing the mortality due to cervical cancer [1], [2], it is highly dependent on the skill of the investigator. In fact, the mean sensitivity and specificity in screening using Papanicolaou smear are 73% and 63%, respectively [3].

An abnormal Papanicolaou smear is followed by colposcopy, where a mounted magnifying lens is used to view the cervix. The sensitivity of colposcopy is excellent (96%) but the specificity is poor (48%) [4]. The cervix is covered by two types of epithelial tissue; the ectocervix has a stratified squamous epithelium and the endocervix has a columnar epithelium. The junction between these two is known as the transformation zone. Cervical precancers usually originate on the squamous side of the transformation zone and can be recognized based on their characteristic colposcopic appearance. Typically, it is relatively easy to discern colposcopically the mature squamous epithelium of the ectocervix from the columnar epithelium of the endocervix. However, within the transformation zone the colposcopic features of the squamous metaplastic/neoplastic and columnar epithelium are sometimes not very distinct and contribute to the low specificity of colposcopy.

Fluorescence spectroscopy has been investigated as an effective and noninvasive method for screening and detecting cervical cancer. Fluorescence spectroscopy of the tissue is affected by various optical interactions. Changes in index of refraction in the tissue and scatterers such as the cell nuclei causes scattering of light. Hemoglobin molecules are significant light absorbers at certain wavelengths. Light is also absorbed by chromophores, which then emit fluorescent light. Biological chromophores such as NADH and flavins are closely related to cellular metabolism. Scattering, absorption and fluorescence properties convey significant morphologic, cytologic and histo-pathologic information of the tissue under investigation.
A number of clinical trials have shown that fluorescence spectroscopy has promise for in vivo, real-time detection of cervical neoplasia [5]–[10]. Typically in these trials, fluorescence emission spectra are measured at one to three excitation wavelengths and diagnostic algorithms are developed retrospectively based on features of these spectra. Ramanujam reports a sensitivity and specificity of 92% and 90%, respectively, using one excitation wavelength at 337 nm for detecting CIN 1 and above [5]. In a separate study, Ramanujam reports sensitivity and specificity of 82% and 68%, respectively, when three excitation wavelengths at 337, 380, and 460 nm were used to differentiate HPV and above from normal tissue [6]. Burke also reports a sensitivity and specificity of 93% and 94%, respectively, at 337-nm excitation for discriminating CIN against benign (including normal, inflammation and metaplasia) [8]. Based on the fluorescence spectroscopy algorithm developed in [6], LifeSpex Inc. has developed a system to image fluorescence from cervical epithelium at multiple excitation emission wavelength pairs. Over 100 patients were evaluated with this device; the initial data from the study show that the device discriminates precancerous cervical lesion from normal tissue with a sensitivity and specificity of 98% and 95.4% [9]. Recently, a similar device, which incorporates the ability to measure both reflectance and fluorescence was used to measure the colposcopically visible cervical epithelium [10]. 136 patients were measured in the colposcopy setting, of which 111 patients were included for analysis. An algorithm was derived to recognize cervices with grade-2 cervical intraepithelial neoplasia (CIN 2) or greater from CIN 1 and normal tissue. Encouraging sensitivities and specificities were reported (97% and 70% respectively). However in both studies [9], [10], algorithm results are reported from the same data set used to derive the algorithm; thus, estimates of sensitivity and specificity may be high due to over-training bias.

An important limitation of past studies is that the selection of excitation wavelength was based either on availability of a light source [8] or on the basis of small, in vitro studies surveying many different excitation wavelengths [11]. It is well known that the optical properties of epithelial tissue differ in vitro, implying that different excitation wavelengths may be optimal for in vivo studies [12], [13].

Recently, several groups have developed spectroscopic systems which enable measurement of fluorescence emission spectra at many excitation wavelengths in vivo [14]–[16]. These emission spectra can be assembled into an EEM, which contains the fluorescence intensity as a function of both excitation and emission wavelength. These systems provide a convenient way to characterize the autofluorescence properties of epithelial tissue over the entire UV-visible spectrum. While these research level systems enable clinical trials to determine the optimal excitation wavelengths for diagnostic purposes, they are not suited for office-based diagnosis. Cost-effective devices, using a smaller number of optimized excitation wavelengths will be required to allow the technology to enter wide scale clinical practice [17].

The goal of this study was to carry out in vivo measurements of fluorescence EEMs and analyze these data to determine the optimal excitation wavelengths for diagnosis of cervical neoplasia and to estimate the sensitivity and specificity at this combination of excitation wavelengths.

II. METHODS

A. Materials

The study protocol was reviewed and approved by the Institutional Review Boards at the University of Texas M.D. Anderson Cancer Center and the University of Texas at Austin. Eligible patients included those over the age of 18 who were not pregnant and who were referred to the Colposcopy Clinic at the UT M.D. Anderson Cancer Center with an abnormal Pap test. After signing informed consent, all patients underwent a demographic interview, risk-factor questionnaire, complete history, and physical exam and pan-colposcopy of the vulva, vagina, and cervix. Initially, each patient underwent a urine pregnancy test, chlamydia and gonorrhea cultures and a Pap test. Additionally, patients underwent Virapap testing (DiGene, Bethesda, MD) as well as HPV, DNA, and mRNA sampling. Each patient had blood drawn for follicle-stimulating hormone (FSH), estradiol, and progesterone levels. The last menstrual period and menstrual history were asked of each patient.

During colposcopy, two colposcopically normal sites and one colposcopically abnormal site were chosen by the physician (MF) or nurse colposcopist and fluorescence EEMs were measured from these three sites. It was noted whether these sites corresponded to squamous or columnar epithelium or the transformation zone.

Following fluorescence measurement, each site was biopsied and submitted for histopathologic diagnosis. Each Papanicolaou smear was read by the cyto-pathologist assigned to the case that day and was subsequently reviewed by the study cytopathologist (GS). Discrepant cases were reviewed a third time for consensus diagnosis by the study cytopathologist (GS). Each biopsy was read by the pathologist assigned to the case that day and was subsequently reviewed by the study histopathologist (AM). Again, discrepant cases were reviewed a third time for consensus diagnosis by the study histopathologist (AM). Standard diagnostic criteria were used [18] and consensus diagnostic categories included: normal SN, normal CN, HPV, CIN 1, CIN 2, and CIN 3. For initial analysis, HPV infection and CIN 1 were grouped together as LGSIL and CIN 2 and CIN 3 were grouped together as HGSIL.

B. Instrumentation

The spectroscopic system used to measure fluorescence EEMs has been described in detail previously [19], [20]. Briefly, the system measures fluorescence emission spectra at 16 excitation wavelengths, ranging from 330 nm to 480 nm in 10-nm increments with a spectral resolution of 5 nm. The system incorporates a fiber-optic probe, a Xenon arc lamp coupled to a monochromator to provide excitation light and a polychromator and thermo-electrically cooled charge-coupled device camera to record fluorescence intensity as a function of emission wavelength. The fiber-optic probe consists of 25 excitation fibers and 12 collection fibers, arranged randomly on a 2-mm-diameter quartz fiber at the tip.