Published in final edited form as:

J Low Genit Tract Dis. 2009 October ; 13(4): 216–223. doi:10.1097/LGT.0b013e318195d91b.

Detection of cervical intraepithelial neoplasias and cancers in cervical tissue by *in vivo* light scattering

Judith R. Mourant, PhD¹, Threse J. Bocklage, MD², Tamara M. Powers, AS¹, Heather M. Greene, BS³, Maxine H. Dorin, MD⁴, Alan G. Waxman, MD⁴, Meggan M. Zsemlye, MD⁴, and Harriet O. Smith, MD⁵

¹ Bioscience Division, Los Alamos National Laboratory, PO Box 1663, MS E535, Los Alamos, New Mexico, 87545

² Pathology Department, University of New Mexico Health Science Center, BMSB 335, 915 Camino de Salud Albuquerque, NM 87131

³ Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of New Mexico Health Sciences Center, 2211 Lomas Boulevard NE, Albuquerque, NM 87131–5286

⁴ General Gynecology Division, Department of Obstetrics and Gynecology, University of New Mexico Health Sciences Center, 2211 Lomas Boulevard NE, Albuquerque, NM 87131–5286

⁵ Department of Obstetrics and Gynecology and Women's Health, Division of Gynecologic Oncology, Albert Einstein College of Medicine and Montefiore Medical Center, 1695 Eastchester Road, Suite 601, Bronx, New York 10461

Abstract

Objective—To examine the utility of *in vivo* elastic light scattering measurements to identify cervical intraepithelial neoplasias (CIN) 2/3 and cancers in women undergoing colposcopy and to determine the effects of patient characteristics such as menstrual status on the elastic light scattering spectroscopic measurements.

Materials and Methods—A fiber optic probe was used to measure light transport in the cervical epithelium of patients undergoing colposcopy. Spectroscopic results from 151 patients were compared with histopathology of the measured and biopsied sites. A method of classifying the measured sites into two clinically relevant categories was developed and tested using five-fold cross-validation.

Results—Statistically significant effects by age at diagnosis, menopausal status, timing of the menstrual cycle, and oral contraceptive use were identified, and adjustments based upon these measurements were incorporated in the classification algorithm. A sensitivity of 77±5% and a specificity of 62±2% were obtained for separating CIN 2/3 and cancer from other pathologies and normal tissue.

Conclusions—The effects of both menstrual status and age should be taken into account in the algorithm for classifying tissue sites based on elastic light scattering spectroscopy. When this is done, elastic light scattering spectroscopy shows good potential for real-time diagnosis of cervical tissue at colposcopy. Guiding biopsy location is one potential near-term clinical application area,

corresponding author: Judith R. Mourant, MS E535, Los Alamos Nat. Lab., Los Alamos, NM 87544, (505) 665–1190 (w), (505) 672–1225 (h), jmourant@lanl.gov.

Prcis

Menstrual status was found to significantly affect the spectroscopic measurements in this study where *in vivo* light scattering showed potential for diagnosing cervical intraepithelial neoplasia.

while facilitating "see and treat" protocols is a longer term goal. Improvements in accuracy are essential.

Keywords

cervical intraepithelial neoplasia; spectroscopy; light scattering; colposcopy

1 Introduction

Screening and diagnostic strategies have greatly reduced cervical cancer incidence and mortality. However, current diagnostic strategies have many practical limitations, and are expensive and time consuming. Following referral, colposcopy, and biopsy, the patient must then wait for histopathologic review before treatment. Consequently, up to 70% of patients in inner city clinics with CIN2/3 do not complete recommended follow-up care [1]. To circumvent this, see and treat protocols in which treatment is performed at the time of colposcopy have been proposed. However, this approach leads to excessive treatment [2]. In one see and treat study, 44% of the patients had no evidence of CIN in the histological specimens taken at the time of treatment [3]

From the ALTS trials and other studies, we now know that colposcopy misses up to a third of patients with CIN2/3 [4]. In one ALTS study, in which no biopsy was taken in colposcopic exams of 538 patients, 26 (4.8%) had CIN3 or worse within 2 years [5]. In another, of 291 patients with CIN 1 or less at initial colposcopy who underwent a repeat colposcopy at 2 years, 18 (6.6%) were found to have CIN3 [6]. Furthermore, two studies have shown that when biopsies are taken in colposcopically benign cervixes ~8.6% have CIN 2/3 or higher [7,8].

The above results indicate that normal appearing tissues at colposcopy are not necessarily histologically normal. Sensitivity can be improved by taking more biopsies. In the ALTS trial previously referenced, in patients with a prior history of CIN3, detection rates increased from 68.3% for one biopsy to 81.8% for 2 biopsies [5]. In a study performed in China, a random biopsy was obtained at the squamocolumnar junction in a quadrant if no lesion was visible in that quadrant [9]. Of the 364 patients diagnosed with CIN 2⁺, 208 were diagnosed by colposcopically directed biopsy, and 136 were diagnosed by random biopsy. (The rest had a positive endocervical curettage only.) Obtaining extra biopsies increases detection rates, but the yield of random biopsies (5.5%) is much lower than for colposcopically directed ones (26.5%) [9]. Unfortunately, this approach of taking more biopsies is more painful, is anxiety-provoking, and increases costs.

For women with abnormal cervical cytology, the newest (2006) Consensus Guidelines emphasize the importance of balancing potential harm from over treatment against the benefit of identifying every possible lesion [4]. Risks of overtreatment include cervical stenosis, which can impair future assessment of the transformation zone, [10,11] and cervical incompetence which can result in preterm pregnancy loss and possibly infertility [12-15]. In order to circumvent excess treatment, colposcopy is discouraged in adolescents for minor abnormalities associated with HPV DNA positivity [4,14,16,17].

Our research is focused upon the development of elastic light scattering spectroscopy as a potential real-time diagnostic for the identification of high grade cervical intraepithelial neoplasia (CIN2/3) and cervical carcinoma, by detecting biochemical and morphological features unique to precancerous and cancerous conditions. The procedure is painless, and minimal additional training for the clinician evaluating the patient is required. Using this technique, estimates of hemoglobin concentration and oxygenation, and information about

morphological properties can be obtained [18-21]. Previous *in vitro* studies and a pilot study *in vivo* of cervical tissue illustrate that this technique has the potential to identify CIN2/3 and cervical cancer [19-21].

A long term goal of this work is to determine whether elastic light-scattering spectroscopy can be used in conjunction with colposcopy to facilitate one stop see-and treat programs. A second potential role is to provide immediate information regarding sites that should be biopsied, including those that are colposcopically normal. The first step, which is the focus of this report here, is to determine the accuracy of elastic-light scattering spectroscopy for identifying clinically relevant lesions within the context of other patient characteristics (menopausal status, age at diagnosis, use of oral contraceptives) that may also impact tissue morphology and/or blood flow and oxygen content within the cervical epithelium.

2 Methods

2.1 Clinical and Histopathology

Data presented here were collected from 151 consenting patients undergoing evaluation for abnormal cervical cytology at the University of New Mexico. Prior to study entry, charts were reviewed to determine eligibility. Exclusion criteria included prior treatment for CIN (LEEP, conization, or cryotherapy), but previous colposcopy was permitted. Early on, a small percentage of patients with invasive cervical cancer based upon a previous biopsy or visual inspection were also recruited. Human subjects review boards reviewed and approved this work at both the University of New Mexico and at Los Alamos National Laboratory. Each patient was consented by the study coordinator.

The method for performing colposcopy was defined by standard operating procedures. Once sites to be biopsied had been chosen, those tissue sites were all measured once with the spectroscopic system by placing a sterilized fiber optic probe in gentle contact with each tissue site. Then the spectroscopic measurements were repeated. Each measurement of each site took about two seconds. Four doctors participated in this clinical study and consequently different patients were examined by different doctors. (Only one doctor made spectroscopic measurements on each patient.) All of the clinicians involved in the study were inserviced on the use of the spectroscopic probe according to clinical protocol. Nonetheless, small systematic differences were found between the results obtained by the different doctors. As described in detail elsewhere [22], the data were corrected for these differences using methods similar to those described in Section 2.3.

Biopsies were taken after the second set of spectroscopic measurements and each biopsy was placed in a separate container. Each biopsy was characterized as normal, cervicitis, CIN 1, CIN 2/3 or cancer by the study pathologist. The study pathologist also ranked the inflammation as none, a few clusters of inflammatory cells, or many inflammatory cells. Vascularity was parameterized as normal or increased. Increased vascularity is an increased number of normal caliber vessels in the upper submucosa. The vessels comprised predominantly capillaries with a few venules. Analysis was qualitative and comprised comparison of number of vessels per 20x objective field in the abnormal area to an area of submucosa that was histologically normal. The tissue site was determined by histopathology as ectocervix, endocervix or squamous columnar junction (SCJ).

2.2 Spectroscopic Instrumentation

The experimental measurement system and probe are described in detail elsewhere [22]. Briefly, the optical probe was about 3 mm in diameter and the end that is placed in contact with tissue is covered in medical grade epoxy. One of the probes used in this study is shown in Fig. 1. Light scattering spectroscopy is performed in a backscattering geometry using both

polarized and unpolarized light in succession. The intensity of detected light depends on light scattering and absorption properties and is a function of wavelength. Hemoglobin is the only significant absorber in cervical tissue. At wavelengths longer than 600 nm, the intensity of detected light depends only upon light scattering properties. When unpolarized light is incident and detected, the slope of the unpolarized collected light intensity as a function of wavelength is dependent upon the proliferative status of cells [23]. The instrument also delivers linearly polarized light to the tissue, and measures the intensity of both co-polarized and cross-polarized light that has returned to the surface. These measurements provide information on the size and density of the light scattering centers [18]. Finally, by angling the collection fibers towards the delivery fibers, the penetration of the collected light is reduced so that more of the scattered light analyzed is from the surface epithelium rather than the stroma. Using this technique, six parameters are measured: total hemoglobin (Hb) concentration; fraction of Hb that is oxygenated; slope of the unpolarized light scattering signal; I1/I3 (a ratio of co-polarized to cross-polarized collected light which is a measure of the amount of scattering); I1/I4 (a ratio of co-polarized to co-polarized collected light from different locations which is a measure of the size of scattering centers); and water concentration [21].

Data for this study were acquired with two very similar but not identical probes. A Student's t test was used to compare data from the two probes within each pathology classification. For I1/I4 and water concentration, significant differences were observed for every pathology classification and measurements made with the second probe were multiplied by correction ratios. Total Hb was also corrected because differences were found for some pathology classifications and because the physical differences in the probes that affected water concentration are expected to also affect hemoglobin concentration. The correction ratio for a given variable was determined as follows. For each pathology classification, the average for probe 1 divided by the average of probe 2 was calculated. The average of these ratios was the correction ratio. No significant differences were found after the data were corrected [22].

2.3 Identifying and correcting for dependencies on patient characteristics

The effects of the clinical characteristics of interest on the spectroscopic data were determined as follows. To examine the effects of cycle day/menopausal status, the patients were first grouped into four categories; 1) premenopausal and cyclic, 2) no menstrual cycle because of birth control, 3) pregnant or post partum and 4) post menopausal. The Student's t-test was then used to determine if there were significant differences in the mean values of the spectroscopic variables between the different groups. A p-value of 0.1 was used to reduce type II error. Category 1 contained the largest number of measurements. Therefore, when differences were found between Category 1 and another category, the values in the other category were multiplied by the appropriate factor to make the average for that category the same as for Category 1. After these corrections were made, there were no significant differences between categories. Category 1 was then divided into 3 subcategories; menstrual cycle days 1–6, days 7 – 20, and all other cycle days. These categories were compared using the Student's t-test and corrections were made when significant differences were found. The corrections were done in a manner that left the average for the original category of 'premenopausal and cyclic' unchanged.

Subsequent to corrections for cycle day/menopausal status, the effects of age were determined. To examine the effects of age, linear regressions of each spectroscopic variable (for each pathology classification) versus age were performed. If the slopes determined from linear regression were non-zero with a 95% or greater confidence, then the effect was considered significant. If significance was found for multiple pathology classifications and

all linear regression lines sloped in the same direction, the data for that spectroscopic variable were corrected so that the slope of all data versus age was zero.

The Student's t-test was used to determine if there were any significant differences in the spectroscopic measurements between sites in patients who had or had not delivered a baby vaginally. The comparisons were done within each diagnostic category. No significant differences were found.

2.4 Classification of the measured sites

A slightly modified 2 out of 3 voting method is used for classification. For each variable there is a cut-off value. If the measured value for a site is on one side of the cut-off then the vote is positive, i.e. for CIN 2/3 or cancer. If it is on the other side of the cut-off, it is for the negative category. A vote is cast "by" each of three variables I1/I4, slope, and I1/I3. Initial classification is a two out of three vote. In addition, if totalHb is very high, an initially negative classification is changed into a positive classification if one of the three variables, I1/I4, slope, and I1/I3, had a positive vote. The cut-off values for I1/I4, slope, and I1/I3 were optimized for the largest sum of sensitivity and specificity with a sensitivity greater than or equal to 80% [22]. The criteria of a high sensitivity was used in order to limit the number of false negatives, since one goal of developing this optical technique is to increase detection rates. The cut-off value for total Hb was fixed at a large value. Stratified five-fold cross-validation was used as a validation method for the classification algorithms [24,25].

3 Results

3.1 Pathologies, epithelial type, inflammation and vascularity

Table 1 summarizes the pathology of the measured sites as well as the epithelial type, inflammation, and vascularity of the biopsied sites. 362 sites were measured, half of which were biopsied sites and half of which were normal via colposcopic examination (and not biopsied). The vast majority of biopsied sites were of the squamous-columnar junction. Eleven sites were endocervix, while 24 sites were ectocervix. Half of the ectocervical sites were normal. Inflammation was generally greater for cervicitis and CIN 2/3 than for normals or CIN 1. A similar trend is seen for vascularity.

3.2 Dependence of spectroscopic parameters on patient characteristics

All of the spectroscopic variables had some dependence on at least one patient characteristic. Below we describe the dependencies for the four spectroscopic variables, I1/I4, I1/I3, slope and totalHb, that were found to be useful for classification.

3.2.1 Menopausal status and cycle day—The values of I1/I4 and slope did not vary significantly between the previously described categories, 1 through 4 (Section 2.3). However, the average value of I1/I3 was significantly less for patients who were menopausal than for any other category. Additionally, the average value of I1/I3 was significantly less for patients with a regular cycle than for pregnant patients. Significant differences in the values of totalHb were also found between some of the categories. The average value of totalHb was significantly less for patients who had a regular cycle and who were menopausal than for patients who had no cycle because of birth control or were pregnant or post partum. The data were corrected for all differences as described in the Methods section. Then category 1 was divided into 3 subcategories: days 1–6; days 7 – 20; and all other days. The average of I1/I4 was significantly less for the subgroup 'days 1–6' than for the other subcategories. The average value of slope was greater for the 'days 7–10' subgroup than for the 'all others days' subcategory. The average totalHb value was greater for the 'all others days' subcategory than for 'days 7–10' subcategory. There were no

significant differences between subgroups for I1/I3. The data were corrected for these differences as described in the Methods section.

3.2.2 Age—Linear regression of I1/I3 versus age showed that I1/I3 decreases with age (95% confidence). This decrease indicates that cervical tissue becomes less scattering with age. All data were corrected for the I1/I3 age dependence. None of the other spectroscopic variables had any significant dependence on age.

3.2.3 Vaginal delivery status—No significant differences were found in the values of spectroscopic variables depending on vaginal delivery status.

3.2.4 Dependence of spectroscopic parameters on the clinician—This study was performed by four clinicians and a few differences were found between doctors. Doctor 4 had significantly higher values of I1/I3 than doctors 2 or 3. Doctor 1 had higher values of slope (i.e. less steep) than doctors 3 or 4. Doctors 1 and 2 had a lower value of total Hb than doctor 4. Corrections to the data were made as described in detail elsewhere [22].

3.3 Diagnosing CIN 2/3 and cancer

A goal of this work is to determine the accuracy of elastic light scattering spectroscopy for identifying CIN 2/3 and cancer. None of the measured parameters were adequately diagnostic by themselves. Consequently, a combination of several measured parameters was used to classify biopsy sites (see Section 2.4). The inputs to the voting classification method are measured values for I1/I4, slope, I1/I3, and total hemoglobin. The results are shown in Table 2. When the positive category is CIN 2/3 or cancer and the negative category is CIN 1 or non-neoplastic, a sensitivity of $77\pm5\%$ is obtained with a specificity of $62\pm2\%$. When the colposcopically normal sites are not included, a sensitivity of $77\pm5\%$ and a specificity of $44\pm3\%$ are obtained. If the negative category does not include CIN 1 (while the positive category stays the same), then the results improve slightly as shown in the bottom row of Table 2.

3.3.1 Characteristics of the incorrectly classified sites—The classification performed on the data set containing only biopsied sites was analyzed to determine if the misclassified sites were of a particular epithelial type, had more or less inflammation, or had more or less vascularity. Neither the false positives nor the false negatives had any dependence on vascularity or tissue type. Sites with mild inflammation were slightly over represented in the false positives as compared to the true negatives. All results were obtained using a paired Student's t-test, $p < 0.05$.

The data set containing only biopsied site was also analyzed to determine how frequently the categories of normal, cervicitis and CIN 1 were being classified as false positives. 53% of the biopsied sites that were found by pathology to be normal were false positives. 48% of the cervicitis sites were false positives and 67% of the CIN 1 samples were false positives.

4 Discussion

4.1 Comparison to other studies

Several *in vivo* studies of the efficacy of fluorescence and/or light scattering for diagnosing cervical lesions have been reported in the literature [26-32]. Examination of the literature reveals several trends relevant to our study. The use of expected normal sites (ENS) increases the reported accuracy of the technique [27]. Secondly, the reported accuracy appears to be greater for smaller studies. For example, our pilot clinical study indicated

better results than was found in this larger study. Finally, studies using a similar number of patients with a similar fraction of ENS had results similar to those reported in this paper.

One of the long term goals of our work is to increase detection rates of CIN 2⁺. An optical imaging system that performs both fluorescence and reflectance has been reported to increase detection rates of CIN 2⁺ [33,34]. However, it is also possible that the increase in detection rates was simply due to the increase in the number of biopsies that occurred when the imaging system was used.

4.2 Effects of parameters other than neoplasia on the spectroscopic measurements

The values of the spectroscopic variables were found to correlate with patient physiological characteristics including menopausal status, cycle day, and age at diagnosis. There is a growing body of evidence that these hormonally regulated factors can affect the morphology of the cervical epithelium. We found that I1/I3, which is roughly proportional to the amount of light scattering [18], is less in patients that are menopausal. Possibly relevant to this observation that I1/I3 is decreased in menopausal women is the fact that the reduction in estrogen levels after menopause causes the volume of cytoplasm to decrease, and the epithelium becomes atrophic, consisting only of a few layers of cells. The nuclear–cytoplasmic ratio is also enlarged [35]. Changes in cervical tissue during the menstrual cycle also occur and could be related to changes in light scattering properties we observed during the menstrual cycle. For example, the mean number of nuclei per cm² in the ectocervix is about a factor of 4 greater in ewes in the midluteal phase as compared to ewes at estrus [36].

Effects of age, menopausal status, and menstrual cycle on fluorescence and reflectance measurements have been noted by the Richards-Kortum/Follen group. They concluded that while menstrual cycle does cause intra-patient variability, the inter-patient variability is much larger [37]. Later this group reported that fluorescence intensity is greater in older and menopausal women [38,39]. Their data also indicate the reflectance intensity may be decreased for post menopausal women [39] which is consistent with the decrease in I1/I3 seen in our data. In contrast to our results and the results of the Richards-Kortum/Follen group, age was found not to affect the spectroscopic results in a previously published study of an optical imaging method by Ferris *et al.* [32].

In our study, the values of the spectroscopic variables also depended slightly on the doctor making the measurement and we corrected for these effects. Since this variability can potentially influence sensitivity/specificity and in practice, can not be adjusted for, we are attempting to modify our technique to minimize these differences.

4.3 Clinical utility

In its present configuration, our system has a PPV of ~52% and a NPV of ~78% for separating CIN 2/3 from non-dysplastic locations. (These values were calculated using only biopsied sites, and ignoring all CIN1 sites.) Potentially, the system could be used to assist the colposcopist in deciding where to take a biopsy. In addition to taking biopsies based on colposcopic examination, biopsies could be taken where the spectroscopic system gives positive readings.

Improvement in the accuracy of the system is essential for clinical utility. To make these improvements, it will be important to elucidate factors that lead to false positive and false negative results. We know that patient characteristics such as menopausal status and age are important and since these properties can be routinely obtained at colposcopic examination, these factors can be used in the classification algorithms. The role of HPV subtype should also be elucidated and in some cases this information may be used in the classification algorithm. Identifying the factors involved is critical to further refine our techniques. Other

factors that lead to false positive and false negative results may involve histological characteristics of the human tissue involved, e.g. inflammation may be important. These properties can not be used in the algorithm, instead measurement methods should be modified and/or additional parameters used in the classification algorithm. Other possible factors that can be addressed by modifications to the methods or analysis include physical differences in the amount of pressure applied to the probe, and the timing of measurement after application of acetic acid.

Finally, we note that light scattering is a strong signal (e.g. stronger than fluorescence) and relatively easy to measure. There is tremendous potential to develop a relatively inexpensive instrument. The next generation spectroscopic system will be modified with the goal of minimizing intraobserver variability.

5 Conclusions

Elastic light scattering spectroscopy is being developed as a non-intrusive technique for real-time diagnosis of cervical tissue during colposcopy. It has the potential to increase the detection rate of CIN 2/3 at colposcopy and to facilitate "see and treat" methods. Age at diagnosis, menopausal status, timing of the menstrual cycle, and oral contraceptive use all can effect the spectroscopic measurements and the developed algorithms took these effects into account. When only colposcopically abnormal sites are included in the analysis, a sensitivity of 79±4% and a specificity of 47±4% are obtained for separating CIN 2/3 and cancer from non-neoplastic sites. Particularly for use during "see and treat" procedures, improvements in accuracy are essential, and will require fundamental research as well as close collaboration between pathologists, clinicians and researchers.

Acknowledgments

This work was funded by NIH grant CA71898.

funding: NIH CA71898

References

1. Spitzer M, Cheryns AE, Seltzer VL. The use of large loop excision of the transformation zone in an inner-city population. *Obstet Gynecol* 1993;82:731–5. [PubMed: 8414317]
2. Dainty LA, Elkas JC, Rose GS, Zahn CM. Controversial topics in abnormal cervical cytology: "See and Treat". *Clinical Obstetrics and Gynecology* 2005;48:193–201.
3. Sankaranarayanan R, Rajkummar R, Esmy PO, Fayette JM, Shanthakumary S, Frappart L, Thara S, Cherian J. Effectiveness, safety and acceptability of 'see and treat' with cryotherapy by nurses in a cervical screening study in India. *Br. J. Cancer* 2007;96:738–43. [PubMed: 17311015]
4. Wright TC Jr, Massad LS, Dunton CJ, et al. 2006 consensus guidelines for the management of women with abnormal cervical screening tests. *J Low Genit Tract Dis* 2007;11:201–22. [PubMed: 17917566]
5. Gage JC, Hanson VW, Abbey K, Dippery S, Gardner S, Kubota J, Schiffman M, Solomon D, Jeronimo J, The ASCUS LSIL Triage Study (ALTS) Group. Number of Cervical Biopsies and Sensitivity of Colposcopy. *Obstetrics & Gynecology* 2006;108:264–72. [PubMed: 16880294]
6. Cox JT, Schiffman M, Solomon D. Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy". *Am J Obstet Gynecol* 2003;188:1401–12. [PubMed: 12824969]
7. Benedet JL, Matisie JP, Bertrand MA. An analysis of 84,244 patients from the British Columbia cytology-colposcopy program. *Gyn Onc* 2004;92:127–34.

8. Massad LS, Collins YS. Strength of correlations between colposcopic impression and biopsy histology. *Gyn Onc* 2003;89:424–28.
9. Pretorius RG, Zhang W-H, Belinson JL, Huang M-N, Wu L-Y, Zhang X, Qiao Y-L. Colposcopically directed biopsy, random cervical biopsy and endocervical curettage in the diagnosis of cervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. *Am. J. of Obst. and Gyn* 2004;191:430–4.
10. Chen RJ, Chang DY, Yen ML, et al. Loop electrosurgical excision procedure for conization of the uterine cervix. *J Formos Med Assoc* 1994;93:196–9. [PubMed: 7920057]
11. Dunn TS, Landry E, Ring C, et al. Absent endocervical cells on Pap smears after loop electrosurgical excision procedure. *J Low Genit Tract Dis* 2007;11:138–40. [PubMed: 17596757]
12. Mathevet P, Chemali E, Roy M, et al. Long-term outcome of a randomized study comparing three techniques of conization: cold knife, laser, and LEEP. *Eur J Obstet Gynecol Reprod Biol* 2003;106:214–8. [PubMed: 12551795]
13. Acharya G, Kjeldberg I, Hansen SM, et al. Pregnancy outcome after loop electrosurgical excision procedure for the management of cervical intraepithelial neoplasia. *Arch Gynecol Obstet* 2005;272:109–12. [PubMed: 15912414]
14. Case AS, Rocconi RP, Straughn JM Jr. et al. Cervical intraepithelial neoplasia in adolescent women: incidence and treatment outcomes. *Obstet Gynecol* 2006;108:1369–74. [PubMed: 17138768]
15. Chirenje ZM, Rusakaniko S, Akino V, et al. A randomised clinical trial of loop electrosurgical excision procedure (LEEP) versus cryotherapy in the treatment of cervical intraepithelial neoplasia. *J Obstet Gynaecol* 2001;21:617–21. [PubMed: 12521783]
16. Perlman SE, Lubianca JN, Kahn JA. Characteristics of a group of adolescents undergoing loop electrical excision procedure (LEEP). *J Pediatr Adolesc Gynecol* 2003;16:15–20. [PubMed: 12604140]
17. Sayed K, Korourian S, Ellison DA, et al. Diagnosing cervical biopsies in adolescents: the use of p16 immunohistochemistry to improve reliability and reproducibility. *J Low Genit Tract Dis* 2007;11:141–6. [PubMed: 17596758]
18. Mourant JR, Johnson TM, Freyer JP. Characterizing mammalian cells and cell phantoms by polarized backscattering fiber-optic measurements. *Appl. Opt* 2001;40:5114–23. [PubMed: 18364793]
19. Mourant JR, Hielscher AH, Eick AA, Johnson TM, Freyer JP. Evidence of intrinsic differences in the light scattering properties of tumorigenic and nontumorigenic cells. *Cancer Cytopath* 1998;84:366–74.
20. Ramachandran J, Powers TM, Carpenter S, Garcia-Lopez A, Freyer JP, Mourant JR. Light scattering and microarchitectural differences between tumorigenic and nontumorigenic cell models of tissue. *Optics Express* 2007;15:4039–53. [PubMed: 19532648]
21. Mourant JR, Bocklage TJ, Powers TM, Greene TM, Bullock KL, Marr-Lyon LR, Dorin MH, Waxman AG, Zsemlye MM, Smith HO. *In vivo* light scattering measurements for detection of precancerous conditions of the cervix. *Gynecol Oncol* 2007;105:439–445. [PubMed: 17303229]
22. Mourant JR.; Powers, TM.; Bocklage, TJ.; Greene, TM.; Dorin, MH.; Waxman, AG.; Zsemlye, MM.; Smith, HO. *Applied Optics*. 2008. *In vivo* light scattering for the detection of cancerous and precancerous lesions of the cervix.. Accepted in
23. Mourant JR, Canpolat M, Brocker C, Esponda-Ramos O, Johnson TM, Matanock A, Stetter K, Freyer JP. Light scattering from cells: the contribution of the nucleus and the effects of proliferative status. *JBO* 2000;5:131–7.
24. Kohavi R. A study of cross-validation and bootstrap for accuracy estimation and model selection. *IJCAI* 1995;2:1137–43.
25. Breiman L, Spector P. Submodel selection and evaluation in regression - the X-random case. *International Statistical Review* 1992;60:291–319.
26. Ramanujam N, Mitchell MF, Mahadevan Jansen A, Thomsen SL, Staerkel G, Malpica A, Wright T, Atkinson N, Richards-Kortum R. Cervical precancer detection using a multivariate statistical algorithm based on laser-induced fluorescence spectra at multiple excitation wavelengths. *Photochemistry and Photobiology* 1996;64:720–35. [PubMed: 8863480]

27. Georgakoudi I, Sheets EE, Muller MG, Backman V, Crum CP, Badizadegan K, Dasari RR, Feld MS. Trimodal spectroscopy for the detection and characterization of cervical precancers in vivo. *Am J Obstet Gynecol* 2002;186:374–82. [PubMed: 11904594]
28. Chang SK, Mirabal YN, Atkinson EN, Cox D, Malpica A, Follen M, Richards-Kortum R. Combined reflectance and fluorescence spectroscopy for in vivo detection of cervical pre-cancer. *JBO* 2005;10:024031-1–024031-11.
29. Mirabal YN, Chang SK, Atkinson EN, Malpica A, Follen M, Richards-Kortum R. Reflectance spectroscopy for in vivo detection of cervical precancer. *JBO* 2002;7:587–94.
30. Nordstrom RJ, Burke L, Niloff JM, Myrtle JF. Identification of cervical neoplasia (CIN) using UV-excited fluorescence and diffuse-reflectance tissue spectroscopy. *Lasers Surg. Med* 2001;29:118–27. [PubMed: 11553898]
31. Huh WK, Cestero RM, Garcia FA, Gold MA, Guido RS, McIntyre-Seltman K, Harper D, Burmke L, Sum ST, Flewelling RF, Alvarez RD. Optical detection of high-grade cervical intraepithelial neoplasia in vivo: results of 604 patient study. *Am. J Obst Gyn* 2004;190:1249–57.
32. Ferris DG, Lawhead RA, Dickman ED, Holtzaple N, Miller JA, Grogan S, Bambot S, Agrawal A, Faupel MA. Multimodal hyperspectral imaging for the noninvasive diagnosis of cervical neoplasia. *J. Lower Gen. Tract Disease* 2001;5:65–72.
33. Alvarez RD, Wright TC. Effective cervical neoplasia detection with a novel optical detection system: A randomized trial. *Gyn Onc* 2007;104:281–9.
34. Alvarez RD, Wright TC. Increased detection of high-grade cervical intraepithelial neoplasia utilizing an optical detection system as an adjunct to colposcopy. *Gyn Onc* 2007;106:23–8.
35. Walker DC, Brown BH, Blackett AD, Tidy J, Smallwood RH. A study of the morphological parameters of cervical squamous epithelium. *Physiol. Meas* 2003;24:121–35. [PubMed: 12636191]
36. Bott EM, Young IR, Jenkin G, McLaren WJ. Detection of morphological changes of the ovine cervix in response to sex steroids using a fluorescence confocal endomicroscope. *Am. J. of Obst Gyn* 2006;194:105–12.
37. Chang SK, Dawood MY, Staerckel G, Utzinger U, Atkinson EN, Richards-Kortum R, Follen M. Fluorescence spectroscopy for cervical precancer detection: Is there variance across the menstrual cycle? *JBO* 2002;7:595–602.
38. Brookner C, Utzinger U, Follen M, Richards-Kortum R, Cox D, Atkinson N. Effects of biographical variables on cervical fluorescence emission spectra. *JBO* 2003;8:479–83.
39. Freeberg JA, McKinnon N, Price R, Atkinson EN, Cox DD, MacAulay C, Richards-Kortum R, Follen M. Fluorescence and reflectance device variability throughout the progression of a phase II clinical trial to detect and screen for cervical neoplasia using a fiber optic probe. *JBO* 2007;12:034015.

Figure 1.

A picture of one of the probes used in this study. The thin polarizers and medical grade epoxy on the end of the probe can be seen where the light used to take the picture reflects off of them.

Table 1

Characteristics of the measured sites. Column 1 is the histopathology results, except that “normal” are non biopsied sites assumed to be normal by the colposcopist. The tissue locations, inflammation, and vascularity are also based on histopathology of the biopsies, and were not possible to determine for a few sites. ecto = ectocervix, endo = endocervix, SCJ = squamous columnar junction

Pathology	sites	tissue location		inflammation			vascularity	
		ecto	endo	SCJ	none	a little	a lot	normal
“normal”	181	—	—	—	—	—	—	—
Normal	36	12	2	20	19	9	5	33
Cervicitis	44	2	5	35	0	10	33	29
CIN 1	43	6	1	36	10	12	17	31
CIN 2/3	56	4	2	50	7	16	29	35
Cancer	2	0	1	0	0	0	2	0
total	362	24	11	141	26	47	86	128
								34

Table 2

The ability to detect CIN 2/3 and cancer. The errors presented are standard errors of the mean. Abbreviations: Sens., sensitivity; Spec., specificity.

	all measured sites				colposcopically abnormal sites			
	training sets		testing sets		training sets		testing sets	
	Sens. %	Spec. %	Sens. %	Spec. %	Sens. %	Spec. %	Sens. %	Spec. %
CIN2/3 & cancer vs CIN1 & non-neoplastic	82.8	60.9	77.1±4.5	62.2±1.7	82.3	43.1	77.1±4.5	43.8±3.3
CIN2/3 & cancer vs non-neoplastic	82.3	66.1	77.1±4.5	67.8±2.0	81.9	49.7	78.9±4.4	47.3±3.7