Novel Advancements in Colposcopy: Historical Perspectives and a Systematic Review of Future Developments

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■ **Abstract**

Objective. To describe novel innovations and techniques for the detection of high-grade dysplasia.

Materials and Methods. Studies were identified through the PubMed database, spanning the last 10 years. The key words (["computerized colposcopy" or "digital colposcopy" or "spectroscopy" or "multispectral digital colposcopy" or "dynamic spectral imaging", or "electrical impedance spectroscopy" or "confocal endomicroscopy" or "confocal microscopy" or "optical coherence tomography"] and ["cervical dysplasia" or cervical precancer" or "cervix" or "cervical"]) were used. The inclusion criteria were published articles of original research referring to noncolposcopic evaluation of the cervix for the detection of cervical dysplasia. Only English-language articles from the past 10 years were included, in which the technologies were used in vivo, and sensitivities and specificities could be calculated.

Results. The single author reviewed the articles for inclusion. Primary search of the database yielded 59 articles, and secondary cross-reference yielded 12 articles. Thirty-two articles met the inclusion criteria.

Conclusions. An instrument that globally assesses the cervix, such as computer-assisted colposcopy, optical spectroscopy, and dynamic spectral imaging, would provided the most comprehensive estimate of disease and is therefore best suited when treatment is preferred. Electrical impedance spectroscopy, confocal microscopy, and optical coherence tomography provide information at the cellular level to estimate histology and are therefore best suited when deferment of treatment is preferred. If a device is to eventually replace the colposcope, it will likely combine technologies to best meet the needs of the target population, and as such, no single instrument may prove to be universally appropriate. Analyses of false-positive rates, additional colposcopies and biopsies, cost, and absolute life-savings will be important when considering these technologies and are limited thus far. ■

Key Words: spectroscopy, dynamic spectral imaging, electrical impedance spectroscopy, confocal microscopy, optical coherence tomography

The current colposcopic examination is the technique by which all novel technology for detecting high-grade cervical intraepithelial neoplasia (CIN) is compared. The modern colposcope, in fact, still bears remarkable similarities to the original, which was invented in 1925.

Up until the mid 1990s, advancement in colposcopy was limited to the addition of a green filter, improved optics and illumination, and image acquisition. Only within the last 15 to 20 years have novel innovations and techniques been applied to colposcopy. Although not a primary study objective of the ASCUS-LSIL Triage Study (ALTS), it shed light on the limitations of colposcopy to identify high-grade dysplasia. In this article, the author reviewed novel techniques for identifying and diagnosing high-grade CIN, as well as the science and sentinel research that have lead to their development. Certain technologies are adjuncts to colposcopy and aim to improve upon it. Some represent alternatives to histology, which would allow for treatment without the need for a

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biopsy. Finally, others represent alternative applications of technologies for investigating cellular composition.

SOURCES

Studies were identified through the PubMed database, spanning January 1, 2002, through November 2012. The key words ([“computerized colposcopy” or “digital colposcopy” or “spectroscopy” or “multispectral digital colposcopy” or “dynamic spectral imaging”, or “electrical impedance spectroscopy” or “confocal endomicroscopy” or “confocal microscopy” or “optical coherence tomography”] and [“cervical dysplasia” or cervical precancer” or “cervix” or “cervical”]) were used. All references from selected articles were reviewed, and hand searches were conducted to identify the origins of specific technologies, which otherwise predated 2002. Original research leading up to the development of the aforementioned technologies was included in the text for historical perspective.

STUDY SELECTION

The inclusion criteria were (1) published articles referring to noncolposcopic evaluation of the cervix for the detection of cervical dysplasia, (2) original research conducted within the last 10 years, (3) articles in which sensitivities and specificities could be calculated, and (4) articles in the English language. The systematic review was conducted by the sole author and investigator. A single article was excluded on the basis of being non-English-language. Duplicate articles, unpublished articles, and review articles were excluded. A full-text review of every article was completed. Data elements extracted from the articles included (1) population (requirements for inclusion in the study), (2) sample size, (3) outcome (the degree of dysplasia used as the threshold for calculation of sensitivity and specificity), (4) technology, and (5) sensitivity and specificity (including whether it had been calculated on a per-patient or per-biopsy basis). All articles, with the exception of one, were prospective in nature. A single study was randomized. In some studies, concurrent colposcopy could be considered a control. In most studies, the reference population included patients with abnormal Pap smears, referred for colposcopy. Occasionally, patients had biopsy-confirmed dysplasia and were scheduled to undergo the appropriate follow-up or treatment. In 1 study, the population was predominantly positive for human immunodeficiency virus infection.

THE CURRENT COLPOSCOPIC EXAMINATION

The modern colposcope still bears remarkable similarities to the original, which was invented in 1925 by Hans Hinselmann [1]. Hinselmann, the assistant of a Viennese researcher, was assigned to the study of leukoplakia. Realizing that he would require better magnification and illumination, he mounted a Leitz binocular dissecting microscope and a light source to a stand. He used a magnification range of 3.5× to 30×, allowing him to describe and define the characteristics of both normal and cervical intraepithelial lesions. While evaluating the use of dilute acetic acid to remove cervical mucous, he recognized its potential applications in the colposcopic evaluation itself.

The basis for colposcopy as a diagnostic tool is that, with the assistance of magnification, a green filter, and the application of dilute acetic acid, the worst lesion can be visualized and biopsied, thereby estimating the degree of cervical intraepithial neoplasia present. The epithelium is colorless and acts as a filter through which the angioarchitecture of the stroma can be visualized. Incident light can pass through the epithelium to the underlying stroma or be reflected back in varying amounts. Topical application of several agents can accentuate or induce higher levels of reflection, providing information about the nature of the intracellular physiology [2].

Three to five percent acetic acid is applied to the cervix, with resultant whitening of both normal and abnormal squamous epithelium. There are several potential mechanisms for this whitening, including reversible precipitation of nuclear proteins and cytokeratins, in which intracellular fluid is shifted into the extracellular matrix. With less free water present within the cells, the nuclear content is more reflective to white light. Alternatively, the acetic acid might induce a conformational change to intracellular proteins or the nuclear matrix, making them more reflective to white light [2]. In either case, the more nuclear content that is present, the less absorption of white light, and the greater the reflection. In the case of low-grade CIN, the atypical cellular morphology is limited to the inner third of the epithelium. For any visual change to develop, the acetic acid must penetrate to that depth. Therefore, acetoepithelial whitening may be less intense, delayed, and less persistent with mild disease than with severe disease.

The application of iodine to the cervix was a later addition to the colposcopic procedure but has proven useful in differentiating normal squamous epithelium and mature squamous metaplasia from all other cell types.

Original squamous epithelium and mature metaplastic squamous epithelium contain glycogen, while columnar, CIN, and invasive cancer do not. Iodine is glycophillic and is therefore taken up by cells containing glycogen. An exception to this is estrogen-deficient postmenopausal epithelium, which is minimally glycogenated. Areas of high-grade CIN and invasive cancer will often stain a mustard brown color, while areas of low-grade CIN may have a variegated "tortoise-shell" appearance, owing to randomly dispersed patches of glycogenated tissue. The application of half-strength Lugol solution can therefore help further delineate the boundaries of normal and abnormal tissue, as well as accentuate internal margins between areas of varying degrees of dysplasia.

METHODS FOR IMPROVING SENSITIVITY

Many colposcopic scoring systems have been introduced in an attempt to improve the predictive value of traditional colposcopic observations. The first such system was developed by Hinselmann, which categorized lesions as "Atypical Epithelium I and II" or "Highly Atypical Epithelium III and IV." Since that time, scoring systems have been devised by Coppleson, Stafl, Reid, Burk, and Strander, taking into account such characteristics as vascular pattern, intercapillary distance, surface pattern, color, margin, lesion size, and iodine staining [3–10]. The reproducibility of some of these scoring systems have never been assessed in prospective studies, while others report sensitivities of upward of 80% and specificities of 95% for the detection of high-grade dysplasia. It is unclear, however, if the use of a scoring system identifies lesions on colposcopic examination that would otherwise go unnoticed, therefore affecting the overall sensitivity.

Another strategy for improving the sensitivity of traditional colposcopy is to take additional biopsies. In a study by Gage et al. [11], using data from the ALTS trial, the tendency to take more biopsies varied by clinician type. Gynecologic oncologists took fewer biopsies on average than their fellows, who took fewer biopsies than general gynecologists, who took fewer biopsies than nurse practitioners. Regardless of practitioner type, the sensitivity of colposcopy tended to be greater when 2 or more biopsies were taken, as compared to 1 biopsy. Interestingly, gynecologic oncologists and oncology fellows had a higher sensitivity on the initial biopsy, but because they tended to take fewer biopsies, their overall sensitivity was no different than other practitioners. Ultimately, colposcopic performance may not be altered by experience because the sensitivity of identifying high-grade CIN seems to be better for inexperienced colposcopists, whereas the positive predictive value seems to be higher for experienced colposcopists [12].

Current data support the idea that sensitivity of colposcopy is dependent on taking more than 1 biopsy, and this seems to be true for both novice and experienced colposcopists alike, likely because the most abnormal area of the cervix may be smaller than what is easily visualized [13]. This is supported by the ALTS trial, in which the cases of CIN 3 that were missed at enrollment colposcopy were found to be very small [14]. In a large study out of China, Pretorius et al. [15] colposcopically screened the 4 quadrants separately and biopsied the worst area from each. When no abnormality was present, they performed a random biopsy of that quadrant at the squamocolumnar junction. Random biopsies diagnosed 37.1% of CIN 2 or worse. Similarly, Sellors et al. [16] found that one fifth of all CIN 2 or worse was diagnosed from quadrants in which there were no visible abnormalities.

LIMITATIONS OF CURRENT COLPOSCOPY

The ASCUS-LSIL Triage Study (ALTS) provided data on the detection and development of CIN 3 over a 2-year study period and defined an effective triage strategy for the management of ASCUS cytology. Although not a primary study objective, it also shed light on the limitations of colposcopy to identify high-grade dysplasia. All women underwent colposcopy at the conclusion of the 2-year study, and in addition to those with CIN 2 or 3, those with CIN 1 and recent cytology results of high-risk Human Papilloma Virus positive (hr-HPV+) atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial neoplasia (LGSIL) were offered loop electrosurgical excision procedure (LEEP) for persistent low-grade lesions. Within the ASCUS cytology group, 11 cases of CIN 3 were found in this manner, representing 3.6% of total CIN 3 during the 2-year study period [17]. In the LSIL cytology group, 7 cases of CIN 3 were found in this manner, representing 3.0% of total CIN during the 2-year study period. The authors concluded that, although the study colposcopists were all well trained, immediate colposcopy in the LSIL group was only 56% sensitive for cumulative CIN 3 detected in the trial. The cytologic interpretation of LSIL in the trial was associated with a 25% risk of histologic grade CIN 2 or 3 within 2 years [18]. It is unclear whether this risk correlates with the inaccuracy of the initial colposcopy or progression of the disease.

NOVEL ADVANCEMENTS ON THE HORIZON

Computerized Colposcopy

Computer-assisted colposcopy has been in development since the early 1990s [19, 20], but aside from a few promising developments, reports in the literature have been sparse. In 1995, Cristoforoni et al. [21] reported on a computer program that was capable of digitizing colposcopic images. The program could also calculate or measure an area or perimeter, a distance between 2 points, the angles of branching vessels, and the gauge of vessels. In a study of 300 women, the images of 188 were considered evaluable, and those from the first 70 participants were used to educate the software, by correlating morphologic features with known histology. The overall agreement was 85.1%, but more specifically, the concordance was 74.1%, 89.6%, and 91.2% for normal, low-grade, and high-grade lesions, respectively. The authors suggested that the technology might be most helpful at assisting the colposcopist in determining where to biopsy and thus sample the most significant area. One disadvantage of the system at the time of investigation was the cost, which ranged from $18,000 to $32,000.

Based on the observation that angiogenesis and neovascularization in CIN appears as mosaicism and punctuation on colposcopy, Mikhail et al. [22] used image analysis of computerized colposcopy to measure certain parameters and correlated them with the histopathologic diagnosis on cervical biopsy. The difference in mean intercapillary distance and the surface area contained within the perimeter of a mosaic was statistically significant between CIN 2 and CIN 3.

More recently, the role of diagnostic image analysis has been investigated in an effort to automate the evaluation of the cervix. Using image-processing methods, Mehlhorn et al. [23] developed a computer-assisted diagnostic device, which characterizes color, texture, and granulation of particular regions of interest. Unlike past research, which measured discrete distances and angles, the investigators sought to characterize surface structures and gain information of a more topographic nature. In a study of 198 patients, in which all dysplastic lesions were histologically confirmed, the sensitivity and specificity for the detection of high-grade cervical dysplasia was 85% and 75%, respectively (see Table 1). The study was, however, retrospective in nature, and required a trained colposcopist to identify regions of interest (ROI).

In a new approach to image acquisition, Vercellino et al. [24] used a high-definition (HD) laparoscopic camera to do "exoscopy" of the cervix, vulva, and vagina. Using only the camera in place of a colposcope, they achieved a sensitivity of 90% and a specificity of 77% for identification of high-grade cervical dysplasia. As another alternative to the relatively expensive colposcope, Cremer et al. [25] used a digital camera to acquire images of the cervix. The sensitivity and specificity of digital camera assessment of the reproductive tract (DART) on a per-biopsy diagnosis compared favorably to colposcopy (81.4% vs. 85.2% and 45.1% and 34%), suggesting a low-cost alternative, although imaging and display equipment must be truly high definition for this technology to work.

In the last 20 years, there has been immense growth in computer technology and data processing, such that the applications are what now need to be realized. This renewed interest in image analysis will likely play a future role in multiple screening and diagnostic modalities. The use of a diagnostic algorithm is already being actively applied to many of the technologies that will be discussed in this review.

Table 1. Computer-Assisted Colposcopy

Reference	Population	Sample size	Outcome	Technology	Sensitivity (%) by site	Specificity (%) by site
Cremer (2010)	Abnormal Pap smear, referred for colposcopy	207 (patients) 593 (biopsies)	Detection of ≥CIN 2	DART	97.6% (DART) 97.6% (Colpo)	23.6% (DART) 20.6% (Colpo)
Cremer (2010)/ Vercellino (2011)	Abnormal Pap smear, referred for colposcopy	207 (patients)	Detection of ≥CIN 2	DART	84.1%[a] (DART)	45.1%[a] (DART)
	Abnormal Pap smears, vulvar lesions, or biopsy report of neoplasia of the lower genital tract	593 (biopsies) 76 (54 with cervical disease, biopsy performed in 31)	Detection of ≥CIN 2	VITOM digital high-definition video exocolposcopy	85.2%[a] (Colpo) 90%[a] (for cervical disease)	34.0%[a] (Colpo) 77%[a] (for cervical disease)
Melhorn (2012)	Unremarkable or abnormal Pap smears	198	Detection of ≥CIN 2	Computer-assisted diagnostic device	85%	75%

[a]Analysis per patient.
CIN, cervical intraepithelial neoplasia; DART, digital camera assessment of the reproductive tract; Colpo, colposcope.

Table 2. Optical Spectroscopy

Reference	Population	Sample size (analyzed)	Outcome	Technology	Sensitivity (%) by site	Specificity (%) by site
Weingandt (2002)	Patients attending colposcopy clinic	68	Detection of ≥CIN 2 and CIN 3	Optical spectroscopy (intrinsic fluorescence)	Detection of high-grade CIN 88%[a]	53%[a]
					Detection of CIN 3 100%[a]	51%[a]
Parker (2002)	Abnormal Pap smear, referred for colposcopy	33	Detection of CIN 1	Optical spectroscopy (fluorescence) and neural net construction	98.2%	98.9%
Mirabal (2002)	Abnormal Pap smear, referred for colposcopy	161 (patients) 324 (sites)	Discrimination of high-grade squamous dysplasia from normal squamous and columnar epithelium	Optical spectroscopy (reflectance) and diagnostic algorithm	Normal squamous vs. high-grade 72%	81%
					Normal columnar vs. high-grade 72%	83%
Chang (2002)	Abnormal Pap smear, referred for colposcopy	146 (patients) 351 (sites)	Discrimination of normal squamous epithelium from high-grade dysplasia	Optical spectroscopy (fluorescence) and diagnostic algorithm	71%	77%
Georgakoudi (2002)	Abnormal Pap smear, referred for colposcopy	44	Detection of cervical dysplasia and separation of high-grade dysplasia from non–high-grade dysplasia	Trimodal optical spectroscopy (intrinsic fluorescence, reflectance, and light scattering) and diagnostic algorithm	Distinguishing normal epithelium from any degree of dysplasia 82%	68%
					Distinguishing high-grade dysplasia from non–high-grade 79%	78%
Huh (2004)	Abnormal Pap smear or biopsy, referred for colposcopy or LEEP	604	Detection of ≥CIN 2	ODS (fluorescence and backscattered white light) and classification algorithm	Colposcopy 67%[a]	54%[a]
					ODS with classification algorithm 92%[a]	50%[a]
Chang (2005)	Abnormal Pap smear, referred for colposcopy	146 (patients) 351 (sites)	Discrimination of normal squamous epithelium from HSIL	Optical spectroscopy (fluorescence and reflectance) and diagnostic algorithm	83%[a]	80%[a]
Mourant (2007)	Patients undergoing colposcopy	29 (patients) 88 (sites)	Discrimination of HSIL from all other lesions	Optical spectroscopy (light scattering) and diagnostic algorithm	100% 100%[a]	80% 55%[a]
DeSantis (2007)	Abnormal Pap smear, referred for colposcopy, or follow-up of cervical disease	572	Detection of ≥CIN 2	Optical spectroscopy (fluorescence and reflectance) and diagnostic algorithm	95.1%	55.2%
Park (2008)	Biopsy-confirmed high-grade lesion, scheduled to undergo LEEP	29	Correct identification of ≥CIN 2 on LEEP	Multispectral digital colposcope and diagnostic image analysis	79%[a]	88%[a]
Weber (2008)	Not stated	330 (patients) 614 (sites)	Detection of ≥CIN 2	Optical spectroscopy (reflectance and fluorescence) and diagnostic algorithm	85% 85%[a]	51% 53%[a]
Mo (2009)	Abnormal Pap smear, referred for colposcopy	46 (patients) 92 (sites)	Discrimination of normal from any degree of dysplasia	Raman spectroscopy (near-infrared and vibrational) and diagnostic algorithm	93.5%	97.8%
Cantor (2011)	No history of abnormal Pap smears or abnormal Pap smear, referred for colposcopy	1,442 (patients) 3,463 (sites)	Detection of ≥CIN 2	Optical spectroscopy (reflectance and fluorescence) and diagnostic algorithm that included colposcopic impression	100%[a]	71%[a]
Duraipandian (2011)	Abnormal Pap smear, referred for colposcopy	29 (patients) 57 (sites)	Discrimination of dysplasia from normal cervical tissue	Raman spectroscopy (near-infrared and vibrational) and diagnostic algorithm	72.5%	89.2%
Yamal (2012)	No history of abnormal Pap smears or abnormal Pap smear, referred for colposcopy	1,442	Detection of ≥CIN 2	Optical spectroscopy (reflectance and fluorescence) and diagnostic algorithm that excluded colposcopic impression	98%[a]	62%[a]
Duraipandian (2012)	Abnormal Pap smear, referred for colposcopy	44 (patients) 120 (sites)	Discrimination of dysplasia from normal cervical tissue	Raman spectroscopy (near-infrared and vibrational [fingerprint and high wavenumber]) and diagnostic algorithm	85%	81.7%

[a]Analysis per patient.
CIN, cervical intraepithelial neoplasia; ODS, optical detection spectroscopy; HSIL, high-grade squamous intraepithelial lesion.

Optical Spectroscopy

When light strikes tissue, it is absorbed and can then be re-emitted or scattered. The wavelength characteristics of the re-emitted light are based on the biochemical and structural features of the tissue. This is the basis for spectroscopy, which is a noninvasive method for investigating cellular composition. Tissue fluorescence seems to vary with age, menopausal status, and changes in specific tissue components, including nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), and collagen [26–30]. Dysplastic tissue undergoes changes in both biochemical and structural features, with changes in fluorophore concentrations observed in preneoplastic and neoplastic tissues, accounting for differences in autoflorescence [31–34].

An early meta-analysis by Mitchell et al. [35] compared the receiver operating characteristic curves for fluorescence spectroscopy, colposcopy, Pap smears, and HPV testing. Receiver operating characteristic curves are useful for comparing performance and essentially plot the false-positive rate against the true-positive rate of a particular test. The area under the curve (AUC) for the net neural analysis of fluoroscopy was 0.87 as compared to 0.84 for diagnostic colposcopy, 0.76 for Pap smear, and 0.75 for HPV testing. An AUC of 1 represents a perfect test; therefore, the authors concluded that fluorescence outperformed the other tests and compared favorably with colposcopy.

Huh et al. [36] tested an optical detection system (ODS), which collected intrinsic fluorescence and white light backscatter measurements (see Table 2). The measurements were taken at 499 distinct locations in each of more than 500 subjects to develop a diagnostic algorithm for detection of high-grade cervical neoplasia. Using histopathologic data from directed biopsies and LEEP specimens, an algorithm for predicting high-grade dysplasia was developed and demonstrated a sensitivity of approximately 90% [37]. In a multicenter 2-arm randomized trial, colposcopy was compared with colposcopy plus ODS to evaluate the differences in true-positive rates of CIN 2 or worse. A 26.5% gain in true-positive rates was observed with the use of ODS in combination with colposcopy, with only a small increase in the number of biopsies obtained per patient (0.3) and a modest increase in the false-positive rate (4%) [37].

A multisite research team has developed computational tools to predict the scattering of light in normal and dysplastic epithelial cells, as described above. The optimal excitation wavelengths have been determined for measuring fluorescence and reflectance, such that excitation emission matrices can be plotted for any given tissue sample, at a variety of wavelengths. In this way, healthy tissue can be identified as being different from premalignant disease. In a cost-effectiveness analysis, Cantor et al. [38] determined that if a spectroscopic method for detecting high-grade dysplasia achieved a sensitivity of 84% and a specificity of 76% then the biopsies avoided would result in a large savings of health care dollars, and appropriate patients could undergo a loop electrosurgical excision procedure in a "see-and-treat" paradigm. With this in mind, Cantor et al., in association with the National Cancer Institute–funded Program Project, developed a point probe for the collection of spectroscopic data.

The specific point probe that was developed [39] measures 5.1 mm in diameter and is used in direct contact with the surface epithelium. The fiber-optic spectrometer is able to measure fluorescence and reflectance at 16 excitation wavelengths and interrogate a 2×2-mm surface area. In a study involving 1,850 patients, and a total of 4,864 biopsies, an optical spectral algorithm yielded a sensitivity of 100% and a specificity of 71% [40].

A multispectral digital colposcope (MDC), capable of collecting data from the entire cervix, was described in an update of status and long-term goals of the Program Project [41]. A small study yielded a sensitivity of 79% and a specificity of 88% for differentiating between high-grade disease and all other tissue types [42]. A low-powered portable version of the MDC is being developed. The Diagnostic Imaging Aid is intended for use in developing countries.

A number of in vivo studies have evaluated the use of optical spectroscopy for the detection of high-grade CIN, using combinations of fluorescence, reflectance, and backscatter [36, 40, 43–52]. For real-time interpretation, they all used some form of diagnostic algorithm, which yielded a sensitivity of 71% to 100% and a specificity of 50% to 81% for detection of high-grade cervical dysplasia. Several investigators have evaluated Raman spectroscopy, which is a vibrational spectroscopic technique, in conjunction with near-infrared excitation. The results have been promising, yielding sensitivities and specificities in the range of 75.2% to 100% and 81.7% to 97.8%, respectively [53–55], although the threshold typically included any degree of dysplasia. The application of Raman spectroscopy to in vivo detection of cervical dysplasia has been relatively recent, and future studies will likely use a stricter threshold of CIN 2 or worse.

Visible light-based optical spectroscopy may also have applications in deriving total hemoglobin concentration and saturation. The development of blood vessels within the epithelium has long been observed in the development of dysplasia. Development of new vasculature, observed through the colposcope as punctuation or mosaicism, is associated with the upregulation of vascular endothelial growth factor and matrix metalloproteinases [56–60]. In a study by Chang et al. [61], the authors established a significant increase in total hemoglobin concentration in vivo, as cervical tissue progresses from normal to dysplastic. In a subsequent study, they quantitatively compared optical measurements of total hemoglobin concentration to microvessel density, measured by immunohistochemistry. While a statistically significant increase in microvessel density was observed in CIN 2+ versus normal epithelium and CIN 1, no statistically significant difference was observed for total vessel or large vessel densities. Preexisting large vessels as part of normal cervical anatomy were found to be deeper in the stroma, emphasizing the importance of the probe geometry to measure total hemoglobin concentration at the appropriate level [62].

Optical spectroscopy holds great promise in the development of tools for a "see-and-treat" paradigm of colposcopy that would not require a biopsy for histology before therapy. Multispectral digital colposcopes that are capable of surveying an entire cervix would be most useful in areas where colposcopic experience is limited.

Dynamic Spectral Imaging

In 2001, Balas et al. [63] published an article on a novel optical imaging method for the early detection of cancerous and precancerous lesions of the cervix, based on the kinetic characteristics of acetic acid–induced changes. Before the application of acetic acid, the appearance of the cervix is determined by the reflected and backscattered photons from the underlying vascular network. After the application of acetic acid, however, abnormal epithelium becomes opaque and scatters incident wavelengths, altering the intensity and spectral characteristics of backscattered light. Balas et al. [63] recognized that the intensity of the backscattered light (IBSL) could be recorded as a function of time and wavelength at any given point on the cervix. Data were captured at each spectral band, both before and after the application of acetic acid. The IBSL was then plotted against time and wavelength in a 3-dimensional graph. Measurements captured before the application of acetic acid were subtracted from those captured at the IBSL peak time, generating a map, which illustrated the magnitude of maximum alteration in tissue light scattering at each point on the cervix, with differences represented in a color scale. The maximum IBSL value was greater in CIN 3 than it was in CIN 2 and was sustained for a longer period, supporting the theory that the differences in kinetic characteristics observed with the application of acetic acid are correlated with neoplasia grade. This technology thus exploits the phenomenon that has long been observed with the application of acetic acid and quantifies those observations to differentiate between normal and varying grades of CIN.

Using the dynamic spectral imaging (DSI) technique, Soutter et al. [64] reported on the use of an automated device that maps every image pixel of the cervix, where each pixel approximately corresponds to the area occupied by a single cell (see Table 3). Changes in reflectance over time were calculated, and a diffuse reflectance-versus-time integral was calculated through curve modeling and fitting. The integral value was termed the CB parameter and formed the basis for CB

Table 3. Dynamic Spectral Imaging

Reference	Population	Sample size	Outcome	Technology	Sensitivity (%) by site	Specificity (%) by site
Soutter (2009)	Abnormal Pap smear or symptoms suggestive of possible cervical neoplasia, referred for colposcopy	308	Detection of ≥CIN 2 on biopsy	DSI with CB unit cutoff of 553	79%[a]	Not reported
Louwers (2010)	Abnormal Pap smear, referred for colposcopy, or follow-up of CIN 1/2	239	Detection of ≥CIN 2 on biopsy	DSI + colposcopy	88%[a]	69%[a]
Zaal (2012)	Abnormal Pap smear, referred for colposcopy	177 (133 positive for any HPV, 123 positive for hr-HPV)	Detection of ≥CIN 2 in women HPV-16 (+) and non–hr-HPV-16 (+)	DSI with HPV testing	97% HPV-16 (+) 74% non-hr-HPV-16 (+)	100% HPV-16 (+) 67% non-hr-HPV-16 (+)

[a]Analysis per patient.
CIN, cervical intraepithelial neoplasia; DSI, dynamic spectral imaging; HPV, human papillomavirus.

units. The maximum change in reflectance over time was recorded in CB units and was displayed as a pseudo color map over the image of the cervix.

The DSI colposcope was paired with and compared to traditional colposcopy by Louwers et al. [65] in a prospective multicenter trial. Using predetermined threshold values, the map provides a prediction for the presence and grade of dysplasia, indicating which sites are likely to harbor the worst disease. The sensitivity of conventional colposcopy, DSI colposcopy, and combined DSI and conventional colposcopy was 55%, 79%, and 88%, respectively.

Dynamic spectral imaging offers several advantages over colposcopy alone, including increased sensitivity for detecting high-grade lesions, while limiting the number of biopsies required. In the 2010 study by Louwers et al. [65], hr-HPV testing was collected before colposcopy. The high-grade lesions that were missed by DSI colposcopy were more often hr-HPV-16 negative, likely owing to the fact that hr-HPV-16–positive lesions are more densely acetowhite. In a follow-up study, Zaal et al. [66] collected cervical smears for HPV testing, followed by conventional and DSI colposcopy. The sensitivity and specificity for lesions identified on DSI colposcopy of hr-HPV-16+ patients was 97% and 100%, respectively, compared to that of non–hr-HPV-16+ patients, in which the sensitivity and specificity was 74% and 67%, respectively. Interestingly, there was no statistically significant difference in sensitivity between hr-HPV-16+ and non–hr-HPV-16+ patients on conventional colposcopy. The sensitivity of DSI colposcopy for the entire population was 80%, whereas conventional colposcopy yielded a sensitivity of 55%.

Although DSI colposcopy outperforms conventional colposcopy, a lower sensitivity in the hr-HPV-16+ population represents the major disadvantage of the technology, where the proportion of hr-HPV-16–negative lesions is expected to increase in postvaccination populations.

Electrical Impedance Spectroscopy

In a *Lancet Early Report*, Brown et al. [67] described the relationship between tissue structures and imposed electrical current in cervical neoplasia. It was previously recognized that tissue impedance, representing the total opposition to electrical current, is dependent on both the cellular structure and arrangement. The beta dispersion region is the low-frequency range where cellular arrangement is the main determinant of tissue impedance. At these frequencies, the current flows around cells in the extracellular space. At high frequencies, the current can penetrate the cell membranes, passing through both intracellular and extracellular spaces. Nuclear size and intracellular volume are the elements that most contribute to tissue impedance. Loss of epithelial stratification and increase in the nuclear-to-cytoplasmic ratio, which are observed in the progression of cervical dysplasia, alter the impedance at both low and high frequencies. Tissue impedance at low frequencies is thus affected by loss of stratification and increase in the overall size of the extracellular space. Tissue impedance at high frequencies is thus affected by the loss of intracellular space and the increase in nuclear size. Typical changes in the cervical squamous epithelium that are associated with the progression towards CIN are thus also associated with changes in tissue impedance. Brown et al. [67] recorded impedance spectra using 8 different frequencies and then fitted them to a Cole equation. They were able to solve for 3 variables: *R*, which is the impedance at very low frequencies; *S*, which is the impedance at very high frequencies; and *C*, which is related to the cell membrane capacitance. Because *R* decreases, and *S* increases with progression from normal epithelium, the lowest value of *R*/*S* (*R*/*S* minimum) was used as a single indicator of disease state (see Table 4). This laid the groundwork for developing a finite element modeling tool to predict the electrical properties of biological tissues.

Finite element analysis is a method that is routinely used in the solution of physics field problems to calculate an approximate solution for the potential distribution within a volume partitioned into elements such as cervical squamous epithelium. Walker et al. [68] constructed a finite element model of the normal cervical epithelium by taking into account cell type at different depths, including an underlying basement membrane and an overlying layer of cervical mucous.

The next step was to model the pathologic epithelium to predict a pattern of current flow and to further refine electrical impedance spectroscopy. This was accomplished by Walker et al. [69]; they estimated the parameters of cell size, shape, distribution, N/C ratio, and volume of extracellular space of varying degrees of dysplasia.

In a prospective study, Brown et al. [70] used a pencil probe to measure electrical impedance spectra, which were correlated with histopathology results from cervical biopsies. As in previous studies, the lowest value of the *R*/*S* ratio (*R*/*S* minimum) was determined to be the

Table 4. Electrical Impedance Spectroscopy

Reference	Population	Sample size (analyzed)	Outcome	Technology	Sensitivity (%) by site	Specificity (%) by site
Brown (2005)	Abnormal Pap smear (moderate or severe dysplasia), referred for colposcopy	82	Detection of any CIN and ≥CIN 2	Electrical impedance spectroscopy, pencil probe	75%[a] (any CIN) 70%[a] (≥CIN 2) 80%–90% (≥CIN 2)	71%[a] (any CIN) 70%[a] (≥CIN 2) 80%–90% (≥CIN 2)
Abdul (2006)	Abnormal Pap smear, referred for colposcopy	159	Discrimination between normal epithelium and CIN 2+, and detection of ≥CIN 2	Electrical impedance spectroscopy, impedance probe (MKIII)	Discrimination of CIN 2+ from CIN 1, normal squamous, columnar, and mature metaplastic epithelium: 74%[a]	53%[a] Unable to discriminate CIN 2+ from immature metaplastic epithelium
Balasubramani (2009)	Abnormal Pap smear or clinical indication, referred for colposcopy	104 (women) 22 (biopsies)	Differentiation of normal/CIN 1 and ≥CIN 2	Electrical impedance spectroscopy, pencil probe (epitheliometer)	Impedance spectra compared to colposcopic impression: 85.9%[a] (CIN vs. all other tissue) 78.8%[a] (normal/CIN 1 vs. ≥CIN 2) Impedance spectra compared to histology: 100%	62.6%[a] (CIN vs. all other tissue) 73%[a] (normal/CIN 1 vs. ≥CIN 2) 100%

[a]Analysis per patient.
CIN, cervical intraepithelial neoplasia.

most sensitive for identifying the greatest abnormality. In a larger prospective follow-up study, impedance measurements were taken over a wider range of frequencies. In a per-women analysis, the sensitivity and specificity for discrimination of CIN 2+ from CIN 1, normal squamous, columnar, and mature metaplastic epithelium was 74% and 53%, respectively. Separation of CIN 2+ from immature metaplastic epithelium could not be achieved [71].

Balasubramani et al. [72] tested a refined version of the pencil probed, termed the *epitheliometer*, in a prospective study. Impedance spectra were then compared with the finite element model of cervical epithelium and were used to categorize the measurements as normal squamous or columnar epithelium, immature metaplasia, or high-grade CIN. Biopsies were correlated with impedance measurements, and although only 22 biopsies were available for analysis, there was a 100% agreement between impedance prediction and histology. Differentiation of high-grade CIN from mature metaplasia remained problematic, although there was a slight improvement in the ability to discriminate between those tissue types. They also demonstrated that impedance was not significantly altered by the application of acetic acid.

At present, tissue boundaries between tissues of differing resistance are problematic for electrical impedance spectroscopy and could result in the false-positive detection of high-grade CIN. The ability to detect a potential difference in impedance is based on the presumption that the tissue is homogenous and conforms to one of the predictable impedance curves. When moving forward with this technology, it will be important for the probe to be able to reliably identify tissue boundaries. Another limitation is that cervical epithelial atrophy, which results from hypoestrogenism, and increased vasculature, which results from pregnancy, are associated with alterations in the expected electrical impedance. For this reason, postmenopausal and pregnant women have typically been excluded from the study populations.

Confocal Endomicroscopy

Confocal microscopy is an optical imaging technique that can be used to reconstruct 3-dimensional images by collecting multiple images at varying focal planes. The high-resolution images have sufficient contrast to visualize individual cells and nuclei. Before its use in the evaluation of the cervix, confocal microscopy was used in vivo in the fields of dermatology, ophthalmology, and gastroenterology.

In a study by Carlson et al. [73], confocal reflectance imaging was able to demonstrate an increase in the nuclear-to-cytoplasmic ratio with progression of dysplasia. In addition, the usual pattern of progressive increase in the nuclear-to-cytoplasmic ratio when approaching the basal layer was not observed in areas of severe dysplasia, where the nuclear-to-cytoplasmic ratio remained relatively constant.

Fluorescence confocal microscopy can detect changes in the concentration of NADH and FAD, similar to fluorescence spectroscopy. In the normal cervical epithelium,

cytoplasmic fluorescence due to NADH and FAD is observed in basal cells, with peripheral fluorescence around the nuclei of intermediate and superficial cells. Cytoplasmic fluorescence can be observed in increasing thickness of the epithelium with progression of dysplasia. A pilot study of ex vivo cervical biopsies previously demonstrated a sensitivity of 100% and a specificity of 91% for detecting dysplasia using confocal reflectance microscopy [74], whereas in a larger study by the same authors, the sensitivity and specificity in vivo was 91% and 62%, respectively [73].

In a pilot study by Tan et al. [75], a miniaturized fiber-optic confocal microscope was evaluated for detecting CIN (see Table 5). A fluorescent contrast agent was applied to the cervix to stain the nuclei of the squamous and columnar epithelium. Images were correlated with histologic diagnosis and were used to derive a pattern classification to predict histologic results. As expected, cell nuclei of uniform size and shape were observed in the normal cervical epithelium, whereas increased nuclear atypia and increased staining of nuclei was observed relative to worsening CIN. Untrained observers were asked to score images, using example images and brief descriptions of morphological changes associated with different grades of CIN. The sensitivity for detecting any CIN was 97%, whereas the specificity for predicting high-grade CIN was 80%.

In a pilot study of 26 women referred to a colposcopy clinic in Botswana, a high-resolution microendoscope (HRME) was used to acquire images from 52 sites. Two expert observers reviewed the images, and the nuclear-to-cytoplasmic ratio was calculated using image analysis. The observer sensitivity was 86% to 93%, and the specificity was 70% to 73%. The sensitivity of N/C ratio was 86%, and the specificity was 87%. The authors suggested that HRME imaging could potentially be used in conjunction with visual inspection with acetic acid (VIA) or visual inspection with Lugol iodine as a screening modality in low-resource settings [76].

Optical Coherence Tomography

Optical coherence tomography (OCT) is a noninvasive technology that allows for cross-sectional imaging. It produces a 2-dimensional image of optical scattering from tissue microstructures, similar to ultrasound, but using light rather than sound. Small fluctuations in the refractive index of tissue produce reflections that can be detected by OCT [77]. Much like other imaging techniques discussed in this review, its application in the detection of CIN is being recognized after demonstrating clinical usefulness in the fields of ophthalmology, dermatology, and gastroenterology.

In a pilot study by Zuluaga et al. [77], patients were recruited from 3 colposcopy clinics, where OCT images were captured and correlated with histopathology from either cervical biopsies or LEEP specimens. Using a fiber-optic probe, they were able to record depth-resolved intensities of reflected light, resulting in a 2-dimensional image. In normal cervical tissue, OCT images typically show a layered structure, with gradual changes in backscattered light. The average epithelial brightness increases, however, as dysplasia develops. They found that the OCT system could be used in vivo to produce real-time images of cervical tissue and that the average epithelial intensities of normal and abnormal cervical tissue is statistically different.

In a study by Gallwas et al. [78], OCT images were taken from both normal and suspicious appearing cervical epithelium, followed by biopsy. A 2.7-mm fiber-optic probe was used in direct contact with the tissue. Images of healthy cervical tissue were characterized by a 3-layered architecture, with a basement membrane dividing the epithelium and stroma. Images of CIN 2 and 3

Table 5. Confocal Microscopy

Reference	Population	Sample size	Outcome	Technology	Sensitivity (%) by site	Specificity (%) by site
Tan (2009)	Patients undergoing treatment for CIN 1–3 (colposcopically directed biopsies, followed by LEEP)	15 (biopsies)	Detection of any CIN, differentiation between grade of CIN	Confocal endomicroscopy (imaging probe)	97% (detection of any CIN)	80% (prediction of normal to CIN 1) 93% (prediction of CIN 2–3)
Quinn (2012)	Abnormal Pap smear, referred for colposcopy	25 (patients)	Differentiation of non-neoplastic cervical tissue and ≥CIN 2	High-resolution microendoscope	86%–93% (observers)	70%–73% (observers)
	A majority were HIV positive	44 (biopsies)			86% (N/C ratio)	87% (N/C ratio)

CIN, cervical intraepithelial neoplasia; HIV, human immunodeficiency virus.

showed a much less organized architecture. In the cases of invasive cancer, there was no apparent layered architecture. When detection of CIN 1 was used as the cutoff, a sensitivity and specificity of 95% and 46%, respectively, were observed. In a larger study by the same authors, the sensitivity for detecting CIN 2 or worse was 84% to 86%, and the specificity was 60% to 64%. There were a large number of false positives, contributing to the low specificity. The authors theorize that this may be a result of ascertainment bias because the patients were all referred to colposcopy, and the percent of histologically normal findings was only 13% [79].

In a similar study, using CIN 2 as the cutoff, Liu et al. [80] observed a sensitivity of 29% and a specificity of 93% when colposcopy was used to direct OCT measurements (see Table 6). Colposcopy alone was associated with a sensitivity of 60% and a specificity of 83%. It is worth noting that a rigorous biopsy protocol was used, as described in the Shanxi Province Cervical Cancer Screening Study [81]. The same study group investigated the use of VIA-directed OCT and observed a per-site sensitivity and specificity of 62% and 80%, respectively. Visual inspection with acetic acid alone was observed to have a sensitivity and specificity of 43% and 95%, respectively. The authors hypothesized that the use of such a rigorous biopsy protocol is more likely to result in the detection of microscopic disease that is not visible by VIA or colposcopy, contributing to the lower sensitivity seen in the investigative modalities [82].

In an attempt to replace subjective image interpretation with statistical analysis, Gallwas et al. [83] performed ex vivo OCT imaging of LEEP and hysterectomy specimens. Although they observed an improvement in specificity, the method could not be used to reliably distinguish between grades of dysplasia. A similar study of computer-aided diagnosis (CADx) was undertaken by Kang et al. [84], in which an automated algorithm was developed to extract OCT image features. Based on the observation that high-grade CIN usually lacks a distinct border between the epithelium and the stroma, they developed a CADx algorithm to differentiate between normal, inflammation, CIN 1, and CIN 2+. There were a significant number of misclassified images that appeared to have irregular contact of the probe to the tissue, resulting in a low sensitivity. They argue that a more sophisticated edge detection algorithm and improved image quality would result in improved image analysis. One of the major limitations to the technology is that the geometry of the probe does not allow for imaging of the endocervix.

RESULTS

The results of the systematic review are summarized in Tables 1 to 6.

Table 6. Optical Coherence Tomography

Reference	Population	Sample size (analyzed)	Outcome	Technology	Sensitivity (%) by site	Specificity (%) by site
Liu (2010)	Abnormal Pap smear or hr-HPV+	299	Detection of ≥CIN 2	OCT with colposcopy	Colposcopy	
					60%	83%
					Colposcopically directed OCT	
					29%	93%
Wulan (2010)	Abnormal Pap smear or HPV+	182	Detection of ≥CIN 2	OCT with VIA	VIA	
					43%	96%
					33%[a]	93%[a]
					VIA-directed OCT	
					62%	80%
					59%[a]	65%[a]
Gallwas (2010)	Referred for colposcopy	60 (patients) 97 (biopsies)	Detection of ≥CIN 1	OCT with colposcopy	95%–96%	29%–46%
Kang (2011)	Abnormal Pap smear or suspicious lesion by VIA, colposcopy, or both	74 (patients) 152 (biopsies)	Detection of ≥CIN 2	OCT with VIA and/or colposcopy, and computer-aided diagnosis	51%	92%
Gallwas (2011)	Abnormal Pap smear, referred for colposcopy	120 (patients) 210 (biopsies)	Detection of CIN	OCT with colposcopy	96%–98% (CIN 1 threshold) 84%–86% (CIN 2 threshold) 85%–87% (CIN 3 threshold)	39%–41% (CIN 1 threshold) 60%–64% (CIN 2 threshold) 78%–81% (CIN 3 threshold)

HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; OCT, optical coherence tomography; VIA, visual inspection with acetic acid; HIV, human immunodeficiency virus.

DISCUSSION

When evaluating these novel approaches to the detection of dysplasia, one must first establish the target population and, in so doing, determine whether the goal is to "see and treat" or to be conservative. When only a single interaction can be guaranteed, screening, diagnosis, and treatment must rapidly follow one another. In such populations, histologic confirmation is not feasible or economical. An instrument that globally assesses the cervix, such as computer-assisted colposcopy, optical spectroscopy, and dynamic spectral imaging, would provide the most comprehensive estimate of disease and is therefore best suited when immediate treatment is preferred. The better the specificity, the lower the chances of overtreatment, which is the major risk in a "see and treat" paradigm. Of the aforementioned technologies, optical and Raman spectroscopy seem to offer the most promise at identifying high-grade disease among dysplasia arising from all HPV types.

When the goal is to be conservative, as the 2012 ASCCP Consensus Guidelines would suggest, the ideal instrument is one that noninvasively interrogates the most concerning lesions to exclude high-grade disease. Electrical impedance spectroscopy, confocal microscopy, and OCT provide information at the cellular level to estimate histology and thus the grade of the disease. Because the goal is to defer treatment and allow for spontaneous regression, sensitivity must be sufficiently high to detect those lesions that are unlikely to regress. Confocal microscopy seems to offer the best promise of excluding disease as well as interrogating the endocervix.

One serious limitation in the evaluation and comparison of these technologies is the absence of data beyond sensitivity and specificity. Only one of the studies examined in this review did an analysis of gain in true-positive rates compared to the expected increase in the number of biopsies obtained per patient [37]. Analyses of cost and absolute life-saving are similarly absent in the literature. In addition, some studies correlated their data with histology from a biopsy site, while others used LEEP specimens. If a cone or LEEP specimen is considered the gold standard for evaluating the presence and grading of cervical disease, then anything less would have inherent inaccuracies and be subject to selection bias.

CONCLUSIONS

It is unlikely that these technologies will continue to develop in parallel. If a device is to eventually replace the colposcope, it will likely combine technologies to best meet the needs of the target population. As such, no single instrument may prove to be universally appropriate. None of the modalities discussed in this review are currently in a position to replace standard colposcopy, and until such time as a device is proven to be both superior and economical, a simple way to improve diagnostic accuracy on colposcopy may be to take additional biopsies. This too, however, is not without its own risk or cost.

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