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Established and Emerging Optical Technologies for the Real-Time Detection of Cervical Neoplasia: A Review

Breana Hill[1,2], Sylvia F. Lam[3], Pierre Lane[3], Calum MacAulay[3], Leonid Fradkin[2], Michele Follen[1,2,3*]

[1]New York Medical College, Valhalla, NY, USA
[2]Department of Obstetrics and Gynecology, Brookdale Hospital and Medical Center, Brooklyn, NY, USA
[3]The British Columbia Cancer Research Centre, Vancouver, BC, Canada
Email: *michelefollen@gmail.com

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Abstract

Cervical cancer remains a critically important problem for women, especially those women in the developing world where the case-fatality rate is high. There are an estimated 528,000 cases and 266,000 deaths worldwide. Established screening and detection programs in the developed world have lowered the mortality from 40/100,000 to 2/100,000 over the last 60 years. The standard of care has been and continues to be: a screening Papanicolaou smear with or without Human Papilloma Virus (HPV) testing; followed by colposcopy and biopsies and if the smear is abnormal; and followed by treatment if the biopsies show high grade disease (cervical intraepithelial neoplasia (CIN) grades 2 and 3 and Carcinoma-*in-situ*). Low grade lesions (Pap smears with Atypical Cells of Uncertain Significance (ASCUS), Low Grade Squamous Intraepithelial Lesions (LGSIL), biopsies showing HPV changes or showing CIN 1); are usually followed for two years and then treated if persistent. Treatment can be performed with loop excision, LASER, or cryotherapy. Loop excision yields a specimen which can be reviewed to establish the diagnosis more accurately. LASER vaporizes the lesion and cryotherapy leads to tissue destruction. Under long term study; loop excision, LASER, and cryotherapy have the same rate of cure. The standard of care is expensive and takes 6 - 12 weeks for the individual patient. During the last twenty years, new technologies that can view the cervix and even image the cervix with cellular resolution have been developed. These technologies could lead to a new paradigm in which diagnosis and treatment occurs at a single visit. These technologies include fluorescence and reflectance spectroscopy (probe or wide-field, whole cervix scanning approaches) and fluorescence confocal endomicroscopy or high resolution micro-endoscopy. Both technologies have received Federal Drug Administration (FDA) and have been commer-

cialized. Research trials continue to show their remarkable performance. These technologies are reviewed and clinical trials are summarized. Emerging technologies are coming along that may compete with those already approved and include optical coherence tomography, optical coherence tomography with autofluorescence, diffuse optical microscopy, and dual mode micro-endoscopy. These technologies are also reviewed and where available, clinical data is reported. Optical technologies are ready to diffuse into clinical practice because they will save money and 3 or 4 visits in the developed world and offer the same standard of care to the developing world where more cervical cancer exists.

Keywords

Cervical Cancer Detection, Cervical Cancer Screening, Cervical Cancer Diagnosis, Optical Technologies, Real-Time Diagnosis

1. Introduction

Cervical cancer is the fourth most common cancer among women worldwide, with 528,000 new diagnoses and 266,000 deaths per year as estimated in 2012 by the World Health Organization [1]. Incidence and stage at diagnosis depend on the extent of utilization of screening programs, as evidenced by the disparate proportion of advanced disease in developing nations [2]. As an example, in Nigeria, advanced-stage disease at presentation is common (86% to 89.3% of new cases), while in the UK only 21.9% of women present with Stage II + disease [3]. With early detection, death from cervical cancer is preventable, and 5-year survival of Stage I cancers is approximately 92%. Despite the advantage of screening, such programs present a tremendous economic burden to society. According to Insigna *et al*., it is estimated that the annual cost of cervical HPV-related disease amounted to $3.4 billion in the US in 1998. A significant portion of this ($300 million) was spent on "treating" false-positive findings on Papanicolaou smears [4]. In the developing world, where fiscal burden remains a limitation to screening, it would be preferential to obtain more effective means of diagnosis and treatment (ideally simultaneously) that would decrease not only the wait time associated with diagnosis, but also the overall number of health care visits required to treat precancerous lesions of the cervix. Real-time, more affordable devices are being developed, in hopes of detecting cervical cancer in its preinvasive stages more readily. This article seeks to give a comprehensive review of the various means of optical imaging technologies developed for the early detection of cervical cancer.

2. Current Screening and Detection Programs for Cervical Neoplasia

Organized screening programs in high-resource settings have been based on the

use of the Papanicolaou smear, a cytologic sampling of the cervix. Papanicolaou smears are routinely begun at age 21, and carried on every 3 years until age 30. At age 30, the patient can opt to receive concurrent HPV testing with the Pap smear and receive testing every 5 years until age 65 [5] [6] [7]. If at any point the Pap smear is abnormal, a second visit is required for a colposcopy in which the cervix is visualized under 3.5 to 15-fold magnification. Typically, acetic acid at 5% to 6% concentration is used as a contrast agent, and the cervix is viewed using white and green light. The green light enhances the recognition of abnormal vasculature. Colposcopically-directed biopsies are then performed. After biopsies are read by a pathologist, the patient returns for a third visit for any necessary treatment [6]. The Papanicolaou smear was the subject of a meta-analysis by Fahey that used raw data and reported the sensitivity to be 61% and specificity to be 65%. Guillaud reported using tripled reviewed histopathology and the sensitivity for the detection of high grade disease was 44% and specificity 96%. It should be noted that the trial on which he reported were smears obtained before optical imaging and may have been performed more gently to avoid disrupting the imaging. Mitchell in a meta-analysis reported the Papanicoloau smear has a sensitivity of ~60% and a specificity of 90%. This low sensitivity but high specificity mean those that are referred to colposcopy most often have disease (90%) but also many with disease are missed (100% − 60% = 40%). Colposcopy, on the other hand, has a high sensitivity ~95% but a low specificity of 50% meaning some biopsies appear abnormal but are negative histopathologically when reviewed [8] [9]. Here we see colposcopy overcalls at the expense of specificity (up to 50% of the time biopsies may not show high-grade) but usually doesn't miss a lesion (correct 95% of the time). Together, they work quite well and both incidence and mortality from cervical cancer have consistently decreased seven-fold over the last 40 years.

Visual inspection with acetic acid (VIA) is a simpler screening methodology. It involves the application of acetic acid to the cervix, followed by visual examination of tissue with the naked eye and under white-light illumination for aceto-whitening. VIA is used in developing countries due to its low cost, which results from the use of inexpensive reagents, minimal equipment, and the ability to "see-and-treat" patients at that visit. Sankarananayanan reported on large and robust trials in India comparing Pap, HPV testing, and VIA. Over two publications he reports the sensitivity and specificity of: the Papaniclaou smear in 22,663 patients as 58% and 95%, HPV testing in 18,085 patients as 67% and 94%, and VIA in 54,981 patients as 77% and 86%. Because aceto-whitening requires intact cells, invasive cancers may not bind acetic acid. In those lesions, it is more valuable to observe the surface morphology, the vasculature, and/or the tendency to bleed. Unfortunately, inflammation can often appear very abnormal. More effective approaches are needed to detect cervical cancer in resource-poor settings [10] [11] [12] [13] [14].

In discussion of cervical cancer screening, it is important to note the basis for additional Human Papilloma Virus (HPV) testing. The HPV test is extremely sen-

sitive with sensitivities of 95+ % and specificities also high at 90+ % [15]. HPV testing is an excellent adjunct test to the Papanicolaou smear and may someday replace it. HPV infection is the major risk factor for cervical cancers, especially with the high-risk strains HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Although the vaccine against high-risk HPV strains became routine in 2006, it is approximated that only about a third of teenage girls in the US have been fully vaccinated as of 2013 [16]. The vaccines have proven effective, however, as the prevalence of vaccine-type strains of HPV has decreased from 11.5% to 5.1% in females aged 9 - 14, a decrease of 56% [16]. The effectiveness of the HPV vaccine remains limited by accessibility, cost, and willingness of parents to allow their children to partake in the vaccine schedule.

Due to the high prevalence rate in women under 30, HPV testing remains a valuable tool for early detection of cervical neoplasia in adults over the age of 30. Researchers found several weaknesses with cytology (Papanicolaou smear) alone: results are dependent on the high quality of the sample being collected; the test requires subjective interpretation of morphological changes within cells, and the prevalence of false negatives. HPV DNA is present in almost all cervical cancers, the tests are readily available and easily performed, and have been found to demonstrate higher sensitivity for high grade CIN (CIN2+) than that achieved by cytology (96.1% vs. 53.0%) [17]. These tests are limited in being used as the primary screening method for cervical cancer because of the lack of specificity. Many patients are identified with infections but both the Papanicolaou smear sampling at colposcopy is negative. Furthermore, because of the high prevalence of HPV DNA before age 30, HPV co-testing is not recommended for women ages 21 to 29. Over the age of 30, past the peak of HPV infections, HPV testing has a higher positive predictive value.

Another limitation of HPV testing, especially in developing countries, is its cost. According to Mandelblatt *et al*., the Pap smear costs a mere $10 for the laboratory fee compared to an HPV test at an average laboratory fee of $30 for the hybrid capture II or $77 for PCR [18]. One must also understand that a positive HPV test requires a second visit for either a repeat Pap smear or further diagnosis with colposcopy. Many patients are identified that are HPV positive but their Papanicolaou smear and colposcopically-directed biopsies are negative. The aims of optical technology are to reduce these costs and to provide the patient with a diagnosis and treatment in the same visit.

Figure 1 shows the current paradigm for the standard of care and a suggested alternative using the established and emerging technologies in this review.

There are three important and predictable changes in tissue that form the foundation for the use of real-time, automated optical technologies. These are changes in cell turnover affecting the biochemistry of the tissue, the development of abnormal blood vessels to fuel growth, and changes in nucleus of the cells that reflects abnormal duplication and growth. These three changes form the biological basis for the adoption of optical technologies. Fluorescence spectroscopy measures the biochemical changes in tissue. Reflectance spectroscopy

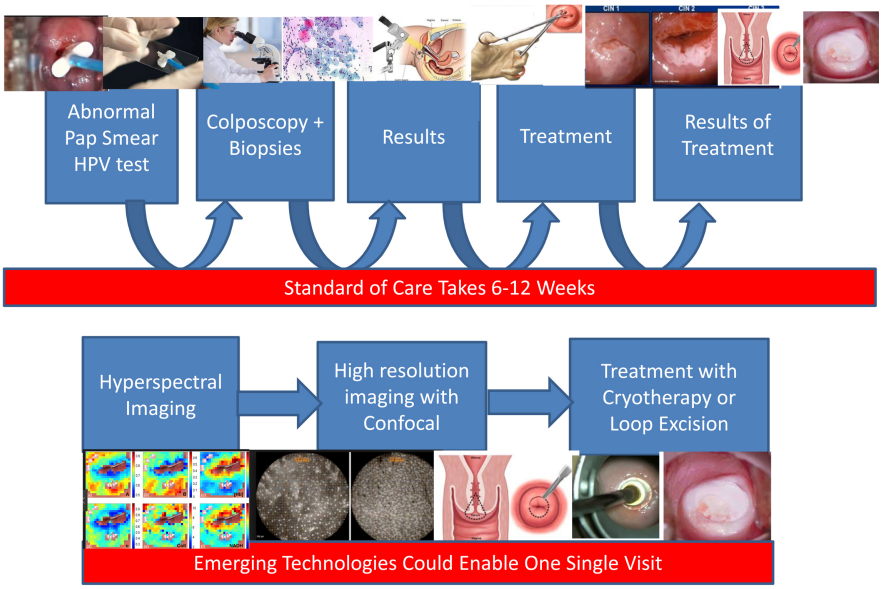

Figure 1. Current standard of care versus possible new path with FDA approved technologies.

visualizes the changes in chromatin in the cells. Confocal Micro-endoscopy has the resolution to detect cell nuclei. These three changes form the basis for the biological basis for the adoption on new technologies. Table 1 shows endoscope-compatible optical technologies and their role in cancer screening and detection in all organ sites [19].

We know that as intraepithelial neoplasia develops, it secretes growth factors that increase the vasculature to supply nutrients to the lesion. The nuclear-to-cytoplasmic ratio increases within cells as less cytoplasm is produced and the amount of DNA within the nucleus increases. Additionally, the stroma underlying

Table 1. Established optical technologies for cancer detection.

Modality	Biological Measure	Resolution	Cost in US$	Clinical Development
White light endoscopy	Surface Morphology and Vasculature	1 mm	4 - 13,000	Standard of Care
Fluorescence and Reflectance Spectroscopy	Redox Ratio, Collagen and Elastin Remodelling	1 mm	30,000	Additive to Oral Exam, Bronchoscopy, Esophagoscopy, Colonoscopy
Narrow Band Imaging	Neovascularization	1 mm	13,000	Additive to Colonoscopy
Optical Coherence Tomography	Mucosal Stratification and Subsurface Tissue Morphology	5 - 15 microns	40 - 100,000	Clinical Approved in Ophthalmology, Clinical Trials in Other Organ Sites
Confocal Endomicroscopy	Subsurface Tissue and Cell Morphology	1 micron	70,000	Being Evaluated in Multiple Organ Sites

even preinvasive lesions can show an influx of inflammatory cells as well as changes in collagen type, collagen cross-linking and elastin modifications to prepare for further growth of the lesion. Advances in magnification and resolution could increase the sensitivity of the endoscope by allowing clinicians to see microvasculature and surface changes in epithelium. With this foundation, we can begin to understand the utility of optical technologies in cancer detection. Table 2 shows the subjects of this review that pertain to the cervical detection [20]-[105].

3. Methods

We searched PubMed and Google scholar from 2007 to 2017 for: fluorescence spectroscopy and screening/detection of cervical neoplasia, and human trials; reflectance spectroscopy and screening/detection of cervical neoplasia, and human trials; fluorescence and reflectance spectroscopy, screening/detection of cervical neoplasia, and human trials; confocal endomicroscopy, fluorescence confocal endomicroscopy, and high resolution Micro Endoscopy, screening/detection of cervical neoplasia, and human trials; optical coherence tomography and screening/detection of cervical neoplasia, and human trials; diffuse optical microscopy and screening/detection of cervical neoplasia, and human trials; and dual mode endomicroscopy and screening/detection of cervical neoplasia, and human

Table 2. Established and emerging technologies for the detection of cervical neoplasia.

Modality	Biological Measure	Resolution	Clinical Development
Established Technologies			
Colposcopy Using White and Green/Blue Light	Surface Morphology and Vasculature	1 mm	Standard of Care
Multispectral IMAGING	Cell Turnover and Elastin/Collagen Remodelling	1 mm	LUMA and DYsis (FDA Approved) and LuViva (Undergoing FDA Approval)
Confocal Endomicroscopy or High Resolution Micro Endoscopy	Cellluar Architecture	1 micron	Mauna Kea Cellvizio (FDA Approved)
Emerging Technologies			
Optical Coherence Tomography	Mucosa and Stroma	5 - 15 microns	Research
Diffuse Optical Microscopy	Depth Resolved Cellular Architecture	1 micron	Research
Dual Mode Endomicroscopy	Depth Resolved Cellular Architecture	1 micron	Research

trials; Google Scholar brought up over 5000 articles in these searches. We reviewed the first 1000; 762 were rejected, 20 could not be obtained, and 218 were cited (of those rejected: 296 other organ site, 56 patents, 75 review articles, 3 case reports, 31 tissue/blood studies, 5 animal studies, 145 other technologies, 53 phantom studies or instrumentation only, 41 contrast agents, and 57 photodynamic therapy). We rejected articles that were studies of cells or animal studies and those performed on *in vitro* specimens only without a human trial. We rejected patents, instrumentation papers, and probe design articles. We rejected case studies of one patient. We rejected articles from other organ sites: head and neck, gastrointestinal tract, pancreas, brain, bladder, ovary, and skin. The remainder of the rejections were clinical trials in humans of the cervix, but were not the technologies of interest for this review. We excluded Raman spectroscopy, electric impedance, vibrational spectroscopy, photoacoustic devices, digital time-delayed colposcopy, infrared spectroscopy, and photodynamic technologies. We have performed extensive reviews of fluorescence and reflectance and other optical technologies in 2003, 2005, and 2007.

4. Overview of the Established Devices

Some technologies, like colposcopy, have been used since 1940. The big advance in its use was the addition of acetic acid as a contrast agent. Multispectral imaging devices were/are still being tested and several have been commercialized and have/waiting for Federal Drug Administration (FDA) approval. Confocal endomicroscopy (also called fluorescence confocal endomicroscopy and high resolution microendoscopy) has/is being studied and one company has received FDA approval and commercialized its use in other organ sites. Richards-Kortum *et al.* have designed a device of this kind that fits in a briefcase and can be used in the resource-poor setting globally [20]-[108].

4.1. Colposcopy

The colposcopy is a mounted microscope that can enlarge the cervix from 3.5 to 15 fold. White light is used before and after the placement of acetic acid. Acetic acid binds to the DNA in the cellular nucleus in keratinized cells. The blue or green light is used to make vessels more prominent. As vascular abnormalities progress with the advancement of dysplastic lesions to neoplastic lesions, the vessels are an important part of the exam. The vessels have been found to change in predictable formations as lesions advance to cancer. The cervix is made up of two parts: the ectocervix and endocervix. Either can harbor a cancer. Colposcopically-directed biopsies are taken of the ectocervix as well as a sampling of the endocervical canal. Treatment can be decided after these results are obtained. It should be noted that entire process of obtaining a Papanicolaou smear, its reading, the return visit at which the patient may or may not undergo colposcopy, the colposcopy, the histopathological review, the treatment, and a follow up visit take 6 - 8 weeks in the US and Canada [109] [110].

4.2. Fluorescence and Reflectance Spectroscopy

4.2.1. Probe

Fluorescence spectroscopy is one of the most widely used techniques for studying the structure and function of macromolecules in biology and chemistry. Fluorescence reveals ligand-induced conformational changes in proteins and subtle environmental changes of chromophores. When the sample is autofluoresced, there is an excitation of intrinsic fluorophores that causes these molecules to emit their photons on return to ground state. It has been reported that an increase in NADH fluorescence and a decrease in collagen fluorescence provide clinically significant differences between normal and dysplastic tissues and could be used as quantitative fluorescence biomarkers for *in vivo* detection of dysplasia. That spectral output is analyzed using an algorithm and can report the likelihood of high grade dysplasia [111]-[133].

4.2.2. Multi-Spectral Digital Colposcopy

The multispectral digital colposcope views the cervix in its entirety. Multispectral imaging systems are a modification of the standard colposcope with a video camera adapter that measures reflectance and fluorescence images of the cervix using a color video camera. With two-dimensional imaging, the entire surface of the cervix, including the endocervix, can be visualized, possibly reducing the chance of sampling error. **Figure 2** shows commercialized devices and their interface with the cervix and/or output [134]-[171].

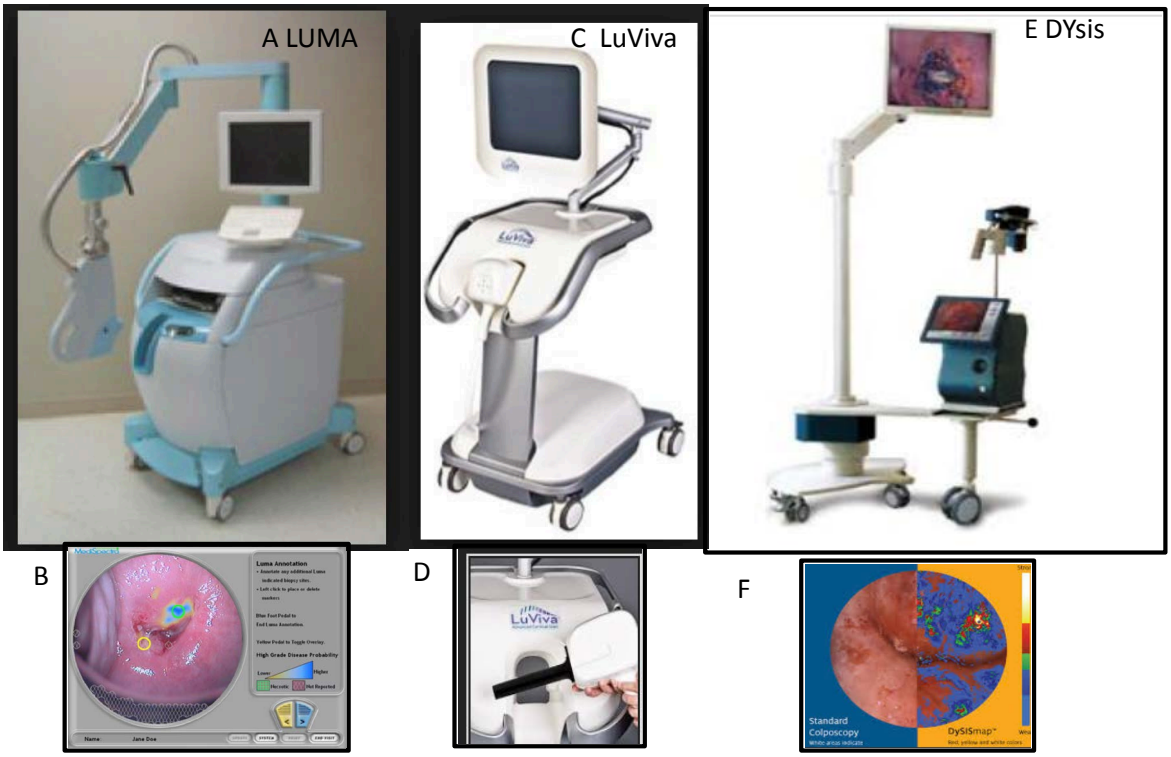

Figure 2. (A) LUMA device; (B) View from LUMA to clinician; (C) LuViva device; (D) Interface of LuViva with the cervix; (E) Dysis device; (F) Cervical digitized images ready for automated analysis in real-time.

4.2.3. Confocal Endomicroscopy, Fluorescence Confocal Endomicroscopy, and High Resolution Micro Endoscopy

Fluorescence confocal endomicroscopy can provide clinicians with the ability to assess tissue grade by generating real-time cellular-level images of morphological features, providing the patient with immediate diagnosis and treatment options simultaneously. Figure 3 shows a research grade confocal endomicroscopy device and its output.

In fluorescence microscopy, exogenous DNA-staining fluorophore molecules (acriflavine hydrochloride used in the study by Schlosser *et al.*) are topically administered onto the cervical mucosa. Fluorescent light is emitted from these fluorophores and because a dichroic mirror is added to the set-up, efficient separation of excitation and emission light is achieved, producing a high-quality image. Imaging depth is limited to about 50 μm due to the finite permeation of the applied fluorophore dye.

Acriflavine temporarily intercalates nucleic acids, accumulating predominantly in the nucleus and the fluorescent light passes through the dichroic beam splitter and detection pinhole. This scanned fluorescence emission is captured by an avalanche photodiode. A frame grabber amplifies and digitizes this detected light and generates images by use of synchronization signals from the resonant scanner. The imaging device is built into a handheld wand, which is controlled using custom software that allows for changing the focal depth, recording video and images, basic image corrections, and contrast enhancement. Figure 4 and Figure 5 show how well the device mimics histopathologic sections [172]-[191].

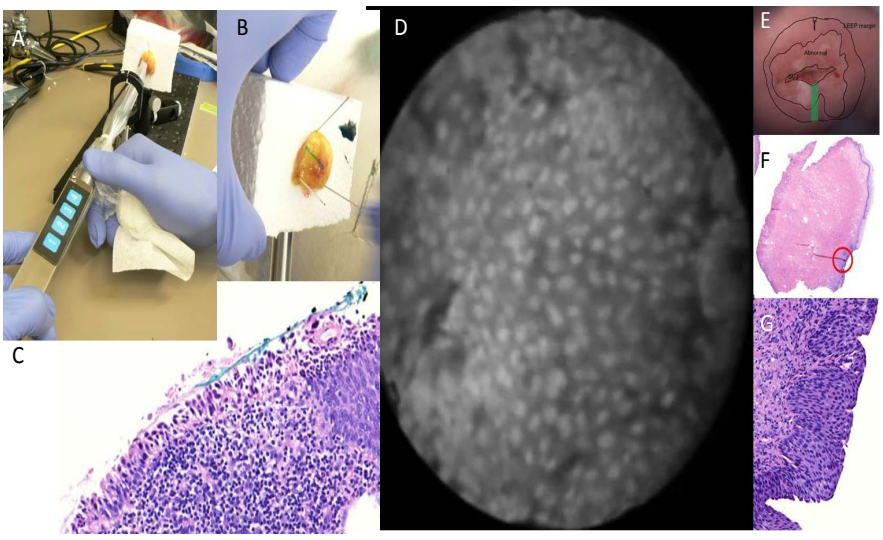

Figure 3. The confocal endomicroscopy device showing the setup for (A) Measurement of the cervical specimen taken by loop excision; (B) Excised and inked cervical specimen mounted for measurement; (C) Inked section of high grade dysplasia histopathologic marked in (B) and in (E); (D) Screenshot image of inked section taken with the confocal endomicroscopy device; (E) Cervix *in situ* in patient before excision; (F) Section of tissue under low power; and (G) Another view of tissue section showing carcinoma-*in-situ* under high power.

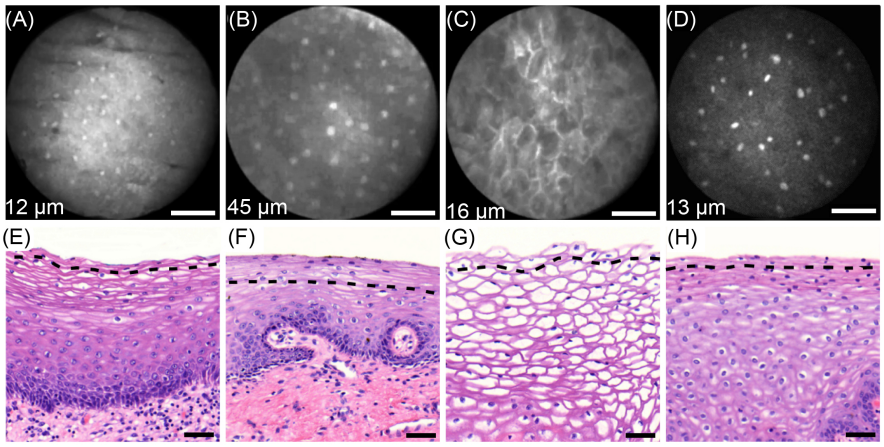

Figure 4. Normal squamous epithelium as imaged by the confocal endomicroscopy device (A)-(D) along with co-registered histological sections (E)-(H). Scale bars measure 50 µm. Dashed lines indicate the corresponding imaging depth as quantified in (A)-(D). Image (C) and section (G) depict glycogen rich epithelium. Image (D) was captured *in vivo*.

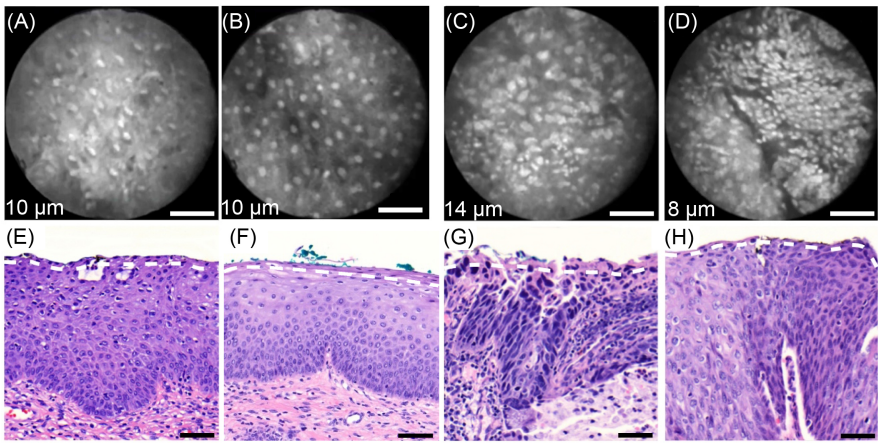

Figure 5. Confocal endomicroscopy of high grade squamous intra-epithelial lesions as captured as captured at superficial depths (A)-(D) and stained histology sections (E)-(H) corresponding to the same imaging locations. Scale bars measure 50 µm. (A) (B) (E) (F) is classified as CIN 2, while (C) (D) (G) (H) contain CIN 3 tissue.

5. Overview of Emerging Technologies

5.1. Optical Coherence Tomography (OCT) with or without Autofluorescence Imaging (AFI)

OCT provides high-resolution structural information from below the tissue surface. Analogous to ultrasound but with higher resolution and lower penetration, it allows the clinician to visualize and quantify the thickness of epithelia and detect micro invasion through the basement membrane.

Some investigators are combining the measurement of autofluorescence imaging (AFI) with OCT which shows the functional contrast of structural proteins, and enables visualization of vascular networks, fibrosis, and nodules. It has been used clinically for the early detection of lung and oral cancer and has shown potential in multi-center trials for the detection of cervical cancer. **Figure 6** shows

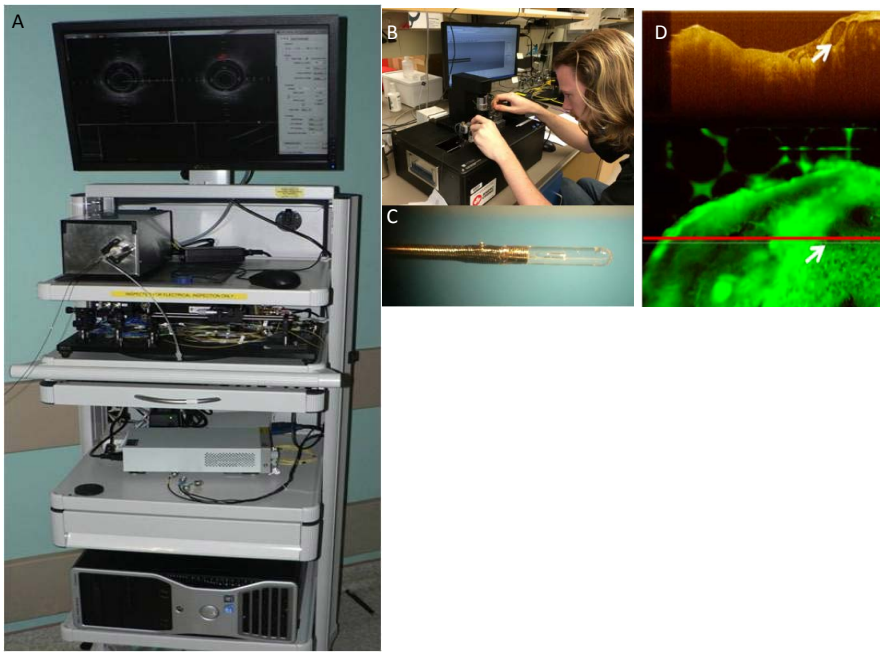

Figure 6. Optical coherence tomography with auto-fluorescence (A) Device; (B) Post-doctoral fellow working on device; (C) Photo of catheter tip; and (D) Co-registered OCT-AF image of a freshly excised cervical loop specimen imaged using the bench-top instrument featured in image.

a bench-top OCT device with autofluorescence [192]-[205].

5.2. Diffuse Optical Microscopy (DOM)

Another way of measuring tissue microstructure is to study the tissue's light-scattering properties. Experimental studies suggest that the microscopic and sub-microscopic refractive index variations within the cell are the dominant sources of cellular scattering. Owing to the high optical density of the nucleus, cellular light backscattering is mostly influenced by the size of the nucleus and its chromatin texture, allowing detection of dysplasia or cancer-related morphological changes through quantification of tissue light backscattering. In DOM, multiple laterally structured sinusoidal projection patterns are employed to quantify tissue light backscattering. **Figure 7** shows the DOM [206].

5.3. Dual Mode Endomicroscopy (DME)

This imaging modality combines fluorescence endomicroscopy (FE) with diffuse optical microscopy (DOM). Combining these two modalities affords complementary sensitivity to the structural changes found in precancerous development. FE images nuclear structure of superficial epithelial cell layers by using a nuclei-staining extrinsic fluorophore dye that reveals dysplasia via increased nuclear size and density. The use of FE alone, however, is limited by its inability to detect moderate dysplasia where structural changes are limited to deeper cell layers. **Figure 8** shows the device and images. When FE is combined with DOM, we gain the ability to

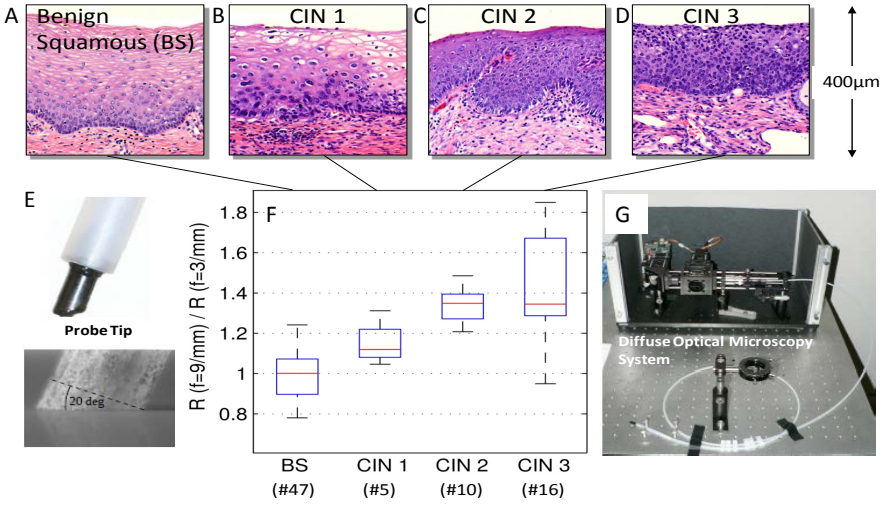

Figure 7. The diffuse optical microscopy device (DOM). Cervical tissue showing (A) benign normal epithelium; (B) Cervical intraepithelial neoplasia (CIN) grade 1; (C) CIN 2; and (D) CIN 3; (E) DOM probe top; (F) Graph showing comparison of measurements from tissue by diagnosis; and (G) Actual diffuse optical microscopy device used for this study.

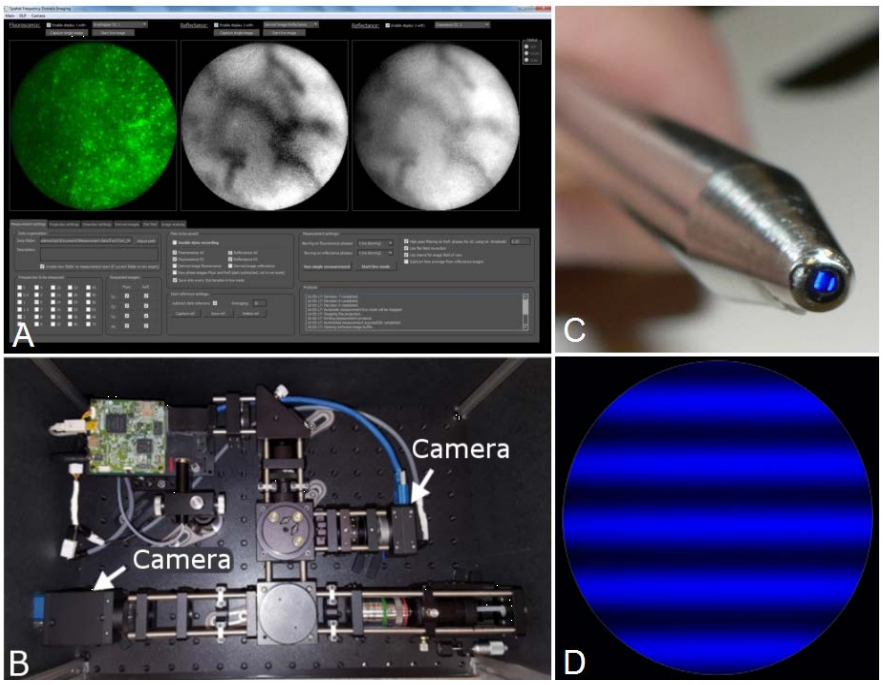

Figure 8. (A) The clinical dual mode endomicroscopy system software interface; (B) The optical setup; (C) A close up of the imaging probe containing the fiber bundle for light projection and detection; (D) A close up of a structured light pattern emanating from the tip of the probe.

penetrate tissue to diagnostically meaningful depths within the epithelium. DOM has been described above.

With DME, blue excitation light encounters three major interaction processes relevant to the device: fluorescence, backscattering, and absorption. Acriflavine

hydrochloride is topically applied to the tissue surface and fluoresces with a spectrum centered around wavelength of 500 nm. In the setup, wavelengths above 460 nm are transmitted through a dichroic mirror and focused onto a digital camera. A second portion of the light is backscattered while retaining wavelength, and if directed back into the optical setup, it is partly guided to a second digital camera. Light absorption by intrinsic tissue molecules is the third interaction process which affects DOM, with hemoglobin being the dominant absorber at the 445-nm excitation wavelength being used. This allows for observation of vascular patterns in the images [207].

6. Results of Pilot Studies, Clinical Trials, and Randomized Clinical Trials for Established Technologies

Table 3 summarizes the trials reported in this review by author, trial design, number of patients, sensitivities and specificities. Pilot studies are unplanned observational trials without a calculated sample size that explore the technology in the desired population. Clinical trials are planned and statistically justified trials that account for confounders. Randomized clinical trials are planned experiments that balance confounders in both arms through the randomization process. Sensitivities and specificities can be reported by patient or by site in the case of a probe study. In our analyses of our own trials, the per patient and per site analyses did not differ. If both were provided the report in the table is a per patient sensitivity and specificity. If per patient was not provided, we reported the per site number.

6.1. Colposcopy

Mitchell *et al.* performed a meta-analysis of colposcopy in 1998 and included the largest clinical trials in which patients were referred for abnormal Papanicolaou smears and who presented raw data that could be analyzed. The studies were published in English but came from around the world from recognized coloposcopists. There were 5378 patients for whom raw data was available. The study included the calculation of sensitivities, specificities, positive and negative predictive values, likelihood ratios, and area under the Receiver Operating Curve. In consideration of separating high-grade disease from all other, the weighted sensitivity of colposcopy was 96% and the weighted specificity was 48%.

The ASCUS/LSIL Triage Study for Cervical Cancer (ALTS) trial reported sensitivities for the trial but not specificities. The sensitivity of colposcopy in the Immediate Colposcopy Group was 54% and in the Standard of Care Group with a preceding abnormal Papanicolaou smear was 55%. In subsequent publications, they found the sensitivity varied by the number of cervical biopsies with 68% for one biopsy, 82% for two biopsies, and 83% for three biopsies. There were many study sites with different levels of attendings and residents participating. The findings differ from the meta-analyses mentioned earlier. Those may suffer from publication bias as those that publish are probably more interested in colposcopy

Table 3. Clinical studies of established and emerging technologies for cervical detection.

Technology	Clinical Trial Design	Investigator Year	Threshold	Number of Patients	Sensitivity	Specificity
Fluorescence Spectroscopy						
Probe						
	Pilot	Ramanujam 1994	SIL	28	87	73
	Pilot	Ramanujam 1996	SIL	64	91	76
	Pilot	Ramanujam 1996	SIL	92	88	70
	Pilot	Mitchell 1999	SIL	92	88	70
	Pilot	Mitchell 1999	SIL	54	76	81
	Pilot	Weingandt 2002	HG	68	88	53
	Clinical Trial	Belinson 2001	HG	1997	94	9
	Clinical Trial	Chang 2002	HG	146	71	70
Multispectral						
	Pilot	Wright 1999	SIL	19	99	95
	Pilot	Dattamajurndar 2001	SIL	52	84	93
	Pilot	Parker 2000	HG	35	80	81
	Pilot	Parker 2002	LG	17	98	99
Reflectance Spectroscopy						
Probe						
	Clinical Trial	Coppleson 1994	HG	163	90	90
	Clinical Trial	Mirabal 2002	HG	161	72	83
	Clinical Trial	Singer 2003	HG	651	70	77
	Pilot	Mourant 2007	HG	36	100	80
Multispectral						
	Clinical Trial	Orfaoudaki 2005	SIL	134	91	100
	Clinical Trial	Soutter 2009	HG	296	53	86
	Clinical Trial	Louwers 2010	HG	239	65	70
	Clinical Trial	Zaal 2012	HG	177	80	77
	Pilot	Prabitha 2014	HG	41	100	100
	Clinical Trial	Coronado 2016	HG	150	88	86
	Clinical Trial	DeNardis 2017	HG	365	75	47
Combined Fluorescence and Reflectance Spectroscopy						
Probe						
	Pilot	Geogeakoudi 2005	SIL	44	92	71
	Clinical Trial	Chang 2005	HG	161	83	80
	Clinical Trial	Cantor 2010	HG	1850	100	71
	Clinical Trial	Yamal 2012	HG	1850	98	62
	Clinical Trial	NCT01094132 2018	HG	552	Pending	Pending

Continued

Multispectral					
Pilot	Burke 1996	SIL	36	96	71
Pilot	Burke 1999	SIL	46	93	94
Pilot	Burke 1999	SIL	36	89	93
Clinical Trial	Nordstrom 2001	SIL	41	82	67
Clinical Trial	Nordstrom 2001	SIL	41	91	93
Clinical Trial	Ferris 2001	HG	111	95	83
Clinical Trial	Desantis 2003	HG	572	83	80
Pilot	Benevides 2003	HG	29	82	68
Clinical Trial	Huh 2004	HG	604	92	50
Pilot	Milbourne 2005	HG	46	80	70
Clinical Trial	Werner 2007	HG	113	95	66
Randomized Clinical Trial	Alvarez 2007	HG	Colpo only 55	53	89
			Colpo+ device 56	52	90
Pilot	Park 2007	HG	29	79	88
Clinical Trial	Twiggs 2013	HG	1607	91	39
Clinical Trial	NCT01094132 2018	HG	552	Pending	Pending
Clinical Trial	NCT00602368 2018	HG	219	Pending	Pending
Confocal Microendoscopy or High Resolution Microendoscopy					
Pilot	Tan	HG	25	97	93
Pilot	Quinn	HG	26	86	87
Pilot	Pierce 2012	HG	63	100	67
Clinical Trial	NCT02574442	HG	pending	pending	pending
Clinical Trial	NCT02420665	HG	pending	pending	pending
Optical Coherence Tomography					
Clinical Trial	Escobar 2006	HG	212	56	59
Clinical Trial	Wulan 2010	HG	183	61	80
Clinical Trial	Liu 2010	HG	299	24	96
Clinical Trial	Gallwas 2010	HG	60	95	46
Clinical Trial	Gallwas 2011	HG	120	64	60
Clinical Trial	Kang 2011	HG	74	51	92
Clinical Trial	NCT02272075	HG	pending	pending	pending
Clinical Trial	NCT01766284	HG	pending	pending	pending

and usually have many years of clinical experience. The ALTS trial was trying to look at what happened in the real world, not in centers that specialized in colposcopy.

Cantor *et al.* reported on a phase II trial in which 1000 screening patients and 850 diagnostic patients with abnormal Papanicolaou smears underwent colposcopy and colposcopically directed biopsies along with fluorescence and reflectance spectroscopy. The sensitivity of colposcopy for the diagnosis of high grade was 98% and the specificity was 45% in the diagnostic population. Colposcopy in the screening population, in which there were few high-grade lesions, showed a sensitivity of 29% and the specificity was 88%. Most diagnostic tests perform poorly when the prevalence of disease is low [109] [110].

6.2. Fluorescence and Reflectance Spectroscopy

6.2.1. Probe

Probe-based fluorescence and reflectance spectroscopy has been studied by 30 investigators, some with industrial funding and others with peer-reviewed funding from the National Institutes of Health. The potential for use of fluorescence spectroscopy in cervical cancer diagnosis has been investigated extensively. Cardenas-Turanzas, Freeberg, and Freeberg have performed extensive reviews and have examined many aspects of the trials designs that were used and the endpoints of interest. The largest trial which was reported by Cantor and colleagues showed a sensitivity of 100% and a specificity of 71%. In this single study the large data set was divided into training, validation, and testing sets. The code was not broken on the test set until the algorithm was optimized on the training set. The algorithm in this analysis included colposocopic impression. In another analysis when colposcopic impression was eliminated in the algorithm, the sensitivity was 98% and specificity 62%. This study showed that point probe fluorescence and reflectance spectroscopy could be additive to colposcopy by increasing the specificity. That group planned a randomized trial comparing colposcopy to spectroscopy but the study didn't accrue and had to be closed [111]-[133].

Across all studies, the fluorescence intensity of precancerous lesions is lower than normal squamous tissues, and peak emission wavelength of precancers is shifted to longer emission wavelengths relative to normal tissue (attributed to increased hemoglobin absorption and increased mitochondrial fluorescence in precancers). The performance, however, of optical algorithms is limited by the challenge of discriminating precancers at the junction between squamous and columnar epithelium, where cervical cancers frequently develop. Despite the good performance of this modality, it has not been commercialized.

6.2.2. Multispectral Digital Colposcopy (MDC) Using Fluorescence and Reflectance Spectroscopy

Benavides, Park and Milbourne have all reported on research-grade MDCs and across all three studies the sensitivity and specificity for the diagnosis of high grade is ~80% and ~80%. More important to the commercialization, however, are the trials conducted by Medispectra Inc. and by SpectraRx Inc. now Guided Therapeutics. In 2007 SpectraRx bought Medispectra.

SpectraRx published several trials that were clinical trials with calculated sam-

ple sizes. Desantis reported on 648 patients with sensitivities of 95% and specificities of 55% for the diagnosis of high grade. Twiggs reported on the same trial with more patients (1607) accrued and reported a sensitivity for the diagnosis of high grade as 91% and a specificity of 39%. The LuViva device is undergoing FDA approval.

The strongest study is a randomized clinical trial. One has been conducted and reported by Alvarez. The trial evaluated colposcopy versus colposcopy plus the Medispectra LUMA device. The device in the trial showed a sensitivity for the diagnosis of high grade of 56% and a specificity for the diagnosis of High Grade of 87% while for colposcopy the sensitivity was 55% and the specificity was 85%. The device was not additive but was the same as their colposcopy. While the device did not perform with their previously reported high sensitivities (91%, 92%), their colposcopy performance differs from that in the literature. If their colposcopy performance had a sensitivity of 95% then the additional specificity provided by the device would be additive and helpful to decrease unnecessary biopsies [134]-[171]. The LUMA device was approved by the FDA.

6.2.3. Fluorescence Confocal Endomicroscopy

High-resolution techniques such as the confocal can show tissue changes in epithelial cell morphology and architecture without the need for biopsy. Video-rate reflectance confocal microscopy yields images of intact epithelial tissue with 1 - 2 micron spatial resolution and with acetic acid, can determine the nuclear to cytoplasmic ratio. Collier showed that the Nuclear to Cytoplasmic (N:C) ratio measured by confocal microscopy could be used to separate high-grade cervical precancers with a sensitivity and specificity greater than 90%.

Confocal endomicroscopy provides high resolution imaging of tissue. Thekkek and Richards-Kortum describe an overview of optical technologies and describe the use of a portable confocal endomicroscopy, appropriate for the developing world in 2008. Cervical confocal microscopy has the potential to provide clinicians with real-time cellular-level images, allowing it to be used as an adjunct to colposcopic examination in facilitating proper biopsy-site selection. The clinical benefit of the tool could be greatly enhanced if its use could be extended from biopsy guidance to an independent tool for instant and accurate diagnosis. Furthermore, although it was limited in reliably differentiating all four tissue categories, additional use of Lugol's iodine to demarcate squamous and columnar epithelium could make it possible to reach an unambiguous diagnosis of HSIL in a majority of cases.

Schlosser *et al.* investigated fluorescence confocal endomicroscopy and its ability to differentiate variability between four cervical tissue types: normal columnar, normal and precancerous squamous epithelium, and stromal tissue. **Figure 4** and **Figure 5** represent the endomicroscopy and histological results of normal squamous epithelium and high-grade squamous intraepithelial lesions respectively. The high nuclear density of the latter clearly distinguishes the precancerous lesions from the normal epithelium. However, the limitation was in the observers' ability to dis-

tinguish HSIL from normal columnar; 38% of the 150 cases associated with the HSIL group had all five observers selecting either "HSIL" or "normal columnar" as their diagnosis.

Schlosser describes the challenges, like those of endogenous fluorescence or with tissue type delineation, will arise with *in vivo* pathology and these will need to be overcome in the future. Confocal endomicroscopy yields videos and stacks at different depths. In a pilot trial ex vivo, 60 patients with high grade yielded 500 videos and 352 stacks. While the high grade disease was easily seen, the columnar epithelium appeared abnormal when it wasn't. The addition of contrast or combinations with other techniques with increases in penetration depth may add further value to cervical endomicroscopy [172]-[191].

7. Results of Bench Studies, Pilot Studies, and Clinical Trials for Emerging Technologies

7.1. Optical Coherence Tomography (OCT)

There are seven published works on OCT in the cervix. Fujimoto described technology and its potential. Zuluaga presented a pilot study and Escobar recruited 50 patients and studied attenuation but did not report sensitivities and specificities.

Wulan and Escobar each reported the use of OCT following the Visual Imaging with Acetic Acid. We acknowledge this is a potential use of the technology that could place it in resource poor settings. This makes the study preselected in a way that is different from those studies in which the only entry is an abnormal Papanicolaou smear.

Liu reported on a large trial in China. There were 1237 participants but this report focuses on the first 299 women for the whom the sensitivity of OCT for the diagnosis of high grade was 24% and the specificity was 96%. Gallwas has two reports, the first from 2010 and the second from 2011. In the first she reports on 60 patients and the sensitivity for the diagnosis of high grade was 95% and the specificity 46%. In the second trial, she reports on 120 women and found that the sensitivity for the diagnosis of high grade was 64% with a specificity of 60%. This is not unusual in the early reporting of a technology.

Future improvements in resolution and the development of new light sources and optics may improve the specificity as well as differentiation of cervical dysplasia. OCT is also being studied for practical use in other gynecologic cancers, including those of the ovaries and fallopian tubes. Our group is combing OCT with autofluorescence imaging [192]-[205].

7.2. Diffuse Optical Microscopy (DOM)

Bodenschatz *et al.* demonstrated that reflectance intensities increase with the progression from benign squamous toward high-grade dysplasia at frequencies of 9 mm^{-1} and 15 mm^{-1}. The increase in high-frequency reflectance for HSIL is expected due to the low cellular differentiation and high N:C ratio. They also noted that the addition of acetic acid amplified backscattering by >70% for spatial fre-

quencies of 9 and 15 mm^{-1}. In contrast to common endomicroscopic modalities that rely on maximized resolution, DOM makes use of microscopic information encoded in backscattering signals to visualize dysplasia.

The pilot study performed by Bodenschatz *et al.* suggests that backscattering can be quantified using structured illumination. He studied 18 cervical specimens in which the entire cervical specimen was intact and was measured 30 - 90 minutes after excision. The tissue was processed normal and sectioned and read by a gynecologic pathologist. Sensitivities and specificities were not reported.

Low overall costs of the optical setup may make the system ideal for developing countries with the highest incidence of cervical cancer. Also, given its imaging speed and potential for a much larger field of view, DOM may be well suited for large-area tissues screening or for the assessment of resection and tumor margins.

However, the heterogeneity of the cervical epithelium presents a challenge in interpretation of imaging data. Larger imaging fields of views enabled by a wider image guide or a potential noncontact colposcopic or endoscopic implementation may help with the overall differentiation. Image quality may also be improved in the future with enhanced suppression of internal specular reflections using a microscope objective designed for epi-illumination as well as a camera with higher dynamic range. Further studies are needed to confirm the sensitivity of DOM to high nuclear density in cancer [206].

7.3. Dual Mode Endomicroscopy (DME)

We have developed a technology that will soon move to clinical trials in the cervix. Bodenschatz's pilot study with the Dual Mode Endomicroscopy on cervical lesions indicates a strong shift in depth-dependent epithelial backscattering related to the development of cervical precancer. Given the relatively low cost of the DME system, the hope is that after more comprehensive patient studies and acquisition of data, the DME can be used for biopsy guidance and *in vivo* diagnosis, and possibly also for tumor margin assessment.

Bodenschatz *et al.* conducted a pilot study on 21 patients with oral premalignant lesions or squamous cell carcinoma. The dual mode endomicroscopy was used to capture imaging data from known high-risk anatomical sites of the oral cavity, including the lateral tongue, ventral tongue and floor of the mouth. The team observed reflectance images to appear more heterogeneous in comparison to corresponding images of normal epithelium. In more than 50 percent of measured sites, nuclei were not visible in fluorescence, likely attributed to enhanced tissue fluorescence from surface keratin. However, they did reveal a significant increase in depth-dependent epithelial backscattering for almost all imaged dysplastic tissue with a biopsy-confirmed diagnosis; there is an increasing backscattering ratio when the degree of pathology increases. The study was too small to make any conclusions about the sensitivity and specificity values for dual mode endomicroscopy in the oral cavity, but it does demonstrate complementary contrast of two imaging modalities with the anticipation that it will give a high diagnostic yield.

Schlosser *et al.* investigated fluorescence confocal endomicroscopy and its ability to differentiate variability between four cervical tissue types: normal columnar, normal and precancerous squamous epithelium, and stromal tissue. Figure 4 and Figure 5 below represent the endomicroscopy and histological results of normal squamous epithelium and high-grade squamous intraepithelial lesions respectively. The high nuclear density of the latter clearly distinguishes the precancerous lesions from the normal epithelium. However, the limitation was in the observers' ability to distinguish HSIL from normal columnar; 38% of the 150 cases associated with the HSIL group had all five observers selecting either "HSIL" or "normal columnar" as their diagnosis [207].

8. Conclusions

Here we report on established and emerging technologies relevant to cervical detection. Note that the standard of care paradigm takes not only 6 - 12 weeks in the US and Canada but also the full treatment of high grade disease including loop excision pathology bills for ~$5000. The emerging technology arm could be performed in one visit and would cost ~$2000.

It may be hard to make the case for change in the developed world. Rogers writes eloquently about diffusion of technologies and the average length for diffusion is 17 years. It has been 18 years since fluorescence and reflectance spectroscopy probe data showed promise, 14 years since multi/hyperspectral colposcopy was successfully reported, and 10 years since a hyperspectral imaging device was FDA approved. It has been 14 years since confocal microendoscopy was reported and 9 since Richards-Kortum put it in a briefcase for the developing world. It's harder to make the case not to use technology in the developing world where resources are not available. We should be ready for translation of both technologies which complement each other nicely in both parts of the world.

OCT has promise and is in clinical trials. OCT has good tissue information but lacks biochemical information. OCT may have more promise if combined measurements of autofluorescence are included. The DOM could complement confocal microendoscopy in the future and maybe less expensive than the current systems under use. Whether it can be placed in a briefcase is left to be seen.

Our hope is that the more established technologies be used and that the emerging ones be tested in large trials to examine clinical effectiveness. Once demonstrated to be effective, they can be scaled to work without electricity in places where cervical cancer runs rampant.

In summary, real-time technologies have the capacity to change screening and diagnosis considerably. The use of these technologies has already been shown to be cost-effective. The impact will be greater, and may extend to the developing world, as the technologies become less expensive to build and simpler to implement [208]-[216].

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