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Near-Histologic Resolution Images of Cervical Dysplasia Obtained With Gabor Domain Optical Coherence Microscopy

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Objective: Histopathology is the criterion standard for evaluating cervical squamous intraepithelial neoplasia (dysplasia). In this pilot feasibility study, we examined whether a novel 3-dimensional imaging device using Gabor-domain optical coherence microscopy (GDOCM) could distinguish features of cervical dysplasia comparable with histopathology.

Methods: A prospective observational pilot study enrolled a small sample of women undergoing loop electrosurgical excision procedure for cervical squamous intraepithelial neoplasia. Fresh ex vivo specimens were imaged with the GDOCM device. Digital images were reviewed by a pathologist who was blinded to the histopathology results. Histopathologic features were then compared with the digital observations.

Results: Standard histologic features of cervical squamous epithelium and of squamous intraepithelial neoplasia could be observed in GDOCM images. Cervical epithelium, stroma, basement membrane, and squamous papilla could all be identified. Human papillomavirus effects, such as vacuolization and cellular density, were also observed.

Conclusions: A GDOCM imaging system has the potential to obtain histologic resolution images of the cervix in the evaluation of squamous intraepithelial neoplasia. This pilot study allowed for optimizing the imaging system and paved the way for a future diagnostic accuracy study. The development of this technology could streamline the evaluation of patients at risk for cervical neoplasia.

Key Words: cervical intraepithelial neoplasia, optical coherence tomography, microscopy, histology, colposcopy, cervical cancer

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Despite the widespread use of cervical cancer screening programs and the implementation of human papillomavirus (HPV) vaccinations, cervical cancer still affects approximately 13,000 patients per year, with 4,188 deaths reported in the United States in 2016.[1] Cervical cancer is the ninth most common cancer globally, accounting for 3.2% of new cancer cases,[2] with less developed countries being affected disproportionately because of lack of access to screening and vaccination programs.[3]

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Patients with abnormal cervical cancer screening are referred to colposcopy for diagnosis and management. Colposcopy uses a binocular microscope to visualize the cervix and identify abnormal areas. Areas identified as suspicious for dysplasia are biopsied and sent for histopathologic evaluation. Colposcopy with the histologic evaluation of biopsy specimens is the criterion standard for identifying cervical intraepithelial neoplasia (CIN). Biopsy results determine the next steps in treatment, such as excision of the transformation zone by a loop electrosurgical excision procedure (LEEP). Recent changes to screening and treatment guidelines targeting patients at risk for high-grade squamous intraepithelial neoplasia (HSIL) have resulted in dramatic decreases in colposcopy volumes,[4] which can lead to a decreased provider skill. Recent studies have demonstrated low sensitivity with false-negative rates of up to 50%[5] and a single biopsy performance failing to detect 30%–50% of HSIL.[6] Wentzensen et al.[7] demonstrated that to achieve an acceptable level of sensitivity, taking 2–4 directed biopsies may be required. This raises the question: is there a role for advanced technologies such as an optical biopsy tool? The ability to image cellular structures in real time could mean decreased morbidity and cost for the patient and the health care system as a whole.

Gabor-domain optical coherence microscopy (GDOCM) is an ultrahigh-resolution optical biopsy tool that can produce microscopic images with similar features to histology.[8] The technology uses a liquid lens embedded in the optical microscope that dynamically refocuses a near-infrared light beam through the tissue with no moving parts.[9] Multiple volumes with different in-focus regions are fused together with a method called Gabor fusing.[10] The fusion produces a 3-dimensional (3D) image with a 2-μm resolution in all dimensions. When compared with other modalities, GDOCM was shown to produce invariant, isotropic cellular resolution in 3D. It was particularly shown to have ×10 better lateral resolution than optical coherence tomography (OCT) and approximately ×5 greater imaging depth with a ×4–×10 larger field of view than confocal microscopy.[11] Clinical use of OCT has been well established in ophthalmology, where it is used in clinical practice by ophthalmologists[12] to diagnose diseases of the retina.[13]

Previous studies have described the use of GDOCM by clinicians in the evaluation of skin cancer and corneal disease.[14–17] To our knowledge, the use of GDOCM to evaluate cervical dysplasia has not been previously studied. The objective of this study is to determine the feasibility of using GDOCM to distinguish histologic features of cervical epithelium and cervical dysplasia on ex vivo LEEP specimens. The ultimate goals are a larger diagnostic accuracy study and further development of instrumentation that can perform in vivo identification of cervical neoplasia, allowing targeted treatment.

METHODS

This study was approved by the institutional review board at the University of Rochester, and written informed consent was obtained and is on file for all subjects. After approval of the study protocol, patients were enrolled in a prospective ex vivo pilot study. Patients identified from the colposcopy clinic were already

TABLE 1. Pretreatment Clinical Diagnoses/Treatment Indications

Subject	Preceding cytology	HPV status	Preceding colposcopic biopsy	Treatment indication	Postoperative histologic diagnosis
1	HSIL	HPV 18	Negative for dysplasia	Discordant cytology and biopsy results	HSIL
2	HSIL	HPV 16	Negative for dysplasia	Discordant cytology and biopsy results	HSIL
3	ASC-H	HPV 16 HPV Other	HSIL	HSIL	HSIL
4	LSIL-H	Unknown	Negative for dysplasia	Discordant cytology and biopsy results	HSIL
5	LSIL	Unknown	HSIL	HSIL	LSIL

ASC-H indicates atypical squamous cells - cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; LSIL-H, low-grade squamous intraepithelial lesion - cannot exclude HSIL; LSIL, low-grade squamous intraepithelial lesion.

scheduled to undergo cervical conization or LEEP for the treatment of CIN. Women were prospectively enrolled and gave written informed consent at the time of enrollment. The patients enrolled in the study were not compensated, and the care that they received was the same as that of patients not enrolled in the study. Cervical specimens obtained at surgery were oriented by the surgeon with a suture designating 12 o'clock. Fresh tissue specimens were immediately imaged at several regions attempting to include scans from each clock face of the cervix using the GDOCM device. Optical coherence tomography uses ultrasound terminology when referring to scans. A 1D depth scan into a tissue sample is referred to as an A-scan. B-scan refers to the 2D depth cross-sectional image generated when scanning the light beam across the tissue in 1 direction to acquire multiple adjacent A-scans. By acquiring multiple adjacent B-scans while scanning along the orthogonal direction, a 3D image is acquired.[18] For this study, each clock face image taken represented a 3D volume of $1 \times 1 \times 0.5$ mm containing 1,000 B-scans, with each B-scan consisting of 1,000 A-scans. After imaging, the tissue was immediately placed in formalin and submitted for routine histopathologic processing and review. Ischemic times were kept to less than 1 hour to preserve tissue integrity for histology. Histology was performed on formalin-fixed, paraffin-embedded tissues using hematoxylin and eosin–stained sections. The completed cases were placed into the normal pathology workflow with review and sign-out performed by the gynecologic pathologist who was assigned to service that day.

The study pathologist completed a blinded review of the study images and corresponding histology. The image review consisted of a stack of 100 images per scan to generate a histology-like image. During review, the pathologist recorded general observations about image quality, noted any identifiable histology of the squamous epithelium, and attempted to grade the degree of dysplasia present. Qualitative histopathology was then recorded at each corresponding scan site, and GDOCM and histology were compared.

Role of the Funding Source

LighTopTech Corp provided the instrument for the study and a technician to help obtain study images and optimize the system. They did not participate in data analysis. They did review and provide editorial support for the publication.

RESULTS

Five subjects were prospectively enrolled in the study. The median age was 28 years (range = 26–45 y). The median time from colposcopic biopsy to cervical excision procedure was 9 weeks

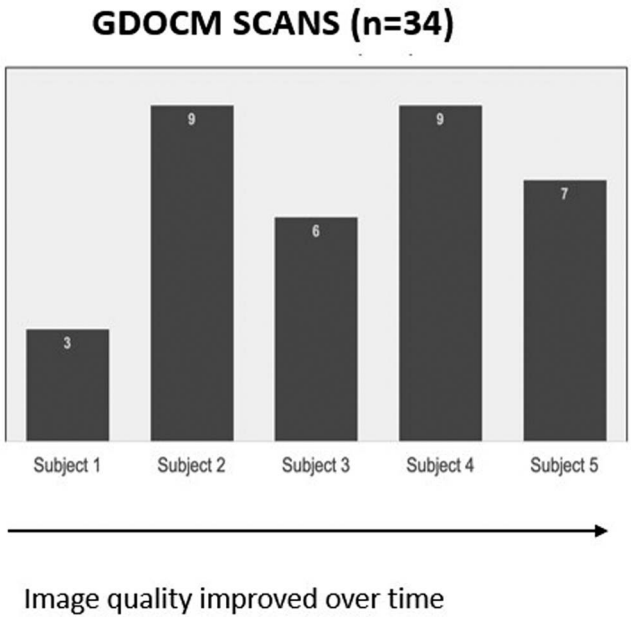

FIGURE 1. Experience was gained using the GDOCM device the number of successful scans completed in the allotted time increased.

TABLE 2. Qualitative Image Findings

Qualitative findings	*n* (*n*=34)
Significant artifact	3/34 (9%)
Epithelial-stromal junction identified	27/34 (79%)
Individual epithelial cells	28/34 (82%)
Hyperkeratosis	21/34 (62%)
Papillary structures	15/34 (44%)
Cervical stromal vessels	17/34 (50%)

(range = 3–27 wk). Preoperative histologic and cytologic diagnoses as well as HPV status and treatment indications are listed in Table 1. There were a total of 34 scans obtained during the course of the study. Three to 9 scans were obtained per subject in the allotted time of 30 minutes (to allow for transport and total ischemic time not to exceed 60 min). Each stacked scan reviewed was roughly equivalent to the size of a typical targeted biopsy taken during colposcopy. Image sharpness without the presence of artifact and scanning capability improved over time (see Figure 1) as GDOCM parameters were optimized and experience was gained. Only 3 scans could be acquired on the first subject, but by the last 2 subjects, 7–9 quality scans were successfully obtained in the allotted time.

Qualitative features identified by the pathologist included significant artifact obscuring image evaluation, identification of the epithelial-stromal junction (basal layer), identification of individual epithelial cells, hyperkeratosis, papillary structures, and cervical stromal vessels (see Table 2). Significant artifact was observed in the early subjects and improved with image optimization over time (see Figure 2A). The epithelial-stromal junction and basement membrane were identified in 27 (79%) of 34 scans (see Figure 2B), individual epithelial cells could be observed in 28 (82%) of 34 scans (see Figure 2C), hyperkeratosis was identified in 21 (62%) of 34 scans (see Figure 2D), papillary structures such as dermal papillae or surface papillae associated with HPV changes were identified in 15 (44%) of 34 scans (see Figure 2E),

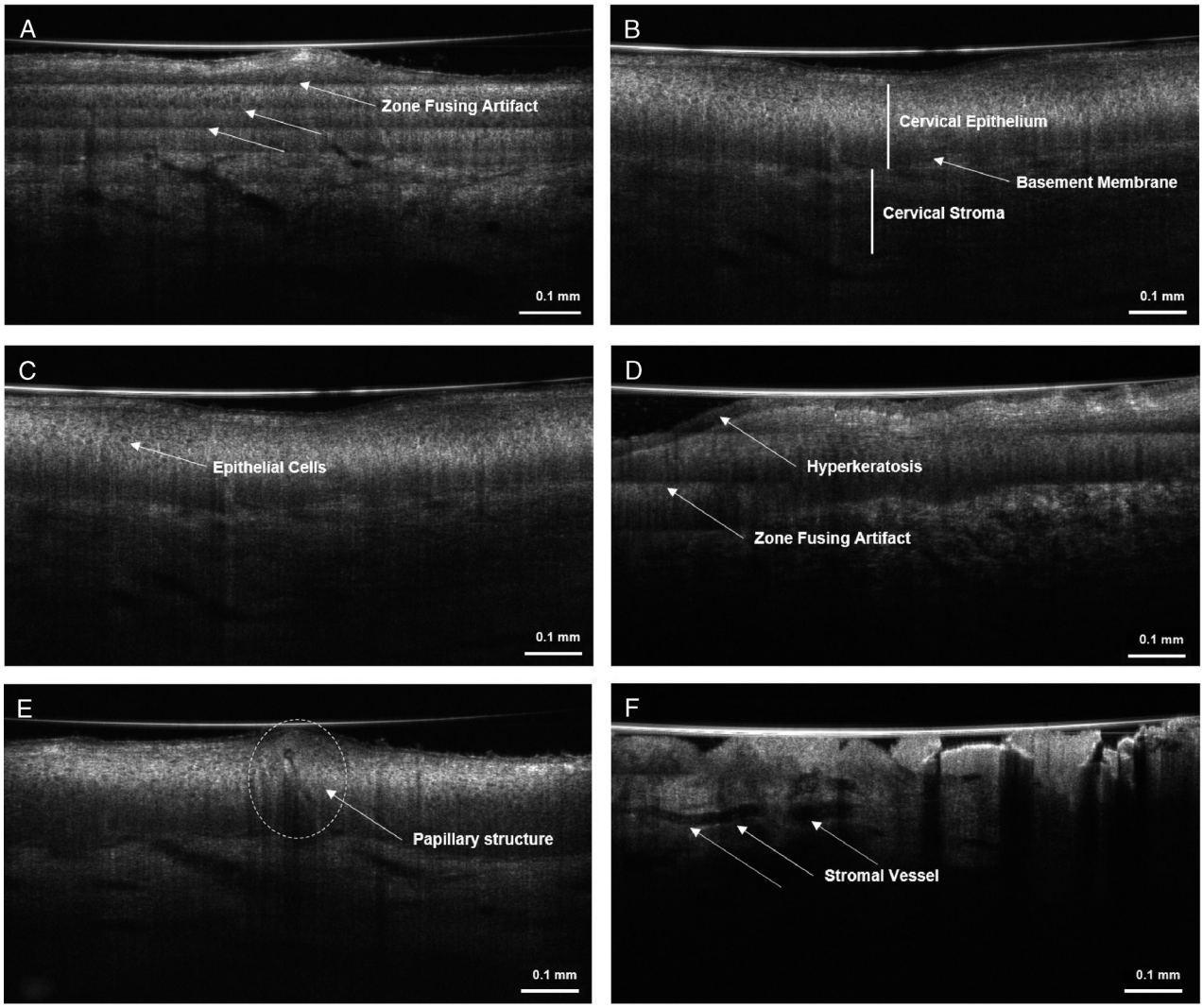

FIGURE 2. A, An example of significant artifact that was observed early on in the series and obscured image evaluation. The artifact observed is called zone fusing artifact and results from the way the GDOCM instrument refocuses at various depths throughout the tissue. B, A scanned image of the epithelial-stromal junction and basement membrane, which are key points of orientation for the evaluation of squamous intraepithelial neoplasia. Individual epithelial cells can be observed in Figure 2C. Hyperkeratosis was identified in 2D along with another example of zone fusing artifact. Papillary structures such as dermal papillae or surface papillary changes associated with HPV infection can be identified in Figure 2E. Cervical stromal vessels can be visualized in Figure 2F. Scale bars are 0.1 mm.

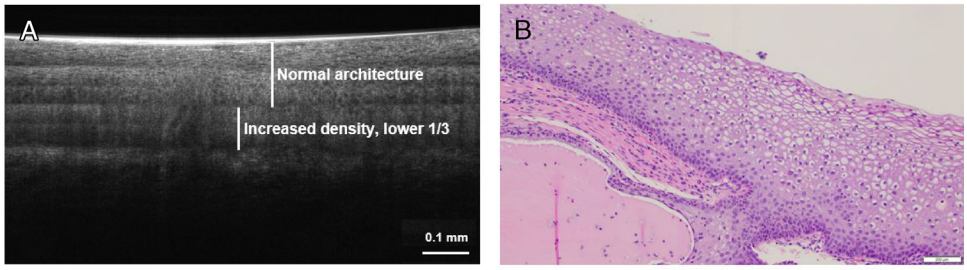

FIGURE 3. A and B, Histologic and corresponding GDOCM images of CIN 1. Scale bars are 0.1 mm for GDOCM and 0.2 mm for histology.

and cervical stromal vessels were identified in 17 (50%) of 34 scans (see Figure 2F).

The study pathologist was also able to correlate the histologic findings from different grades of dysplasia with findings in scanned images. In our GDOCM images, dysplastic cells were seen as a loss of normal epithelial architecture and increased density. Figures 3A and B show histologic and scanned images of CIN 1. Figures 4A and B show histologic and scanned images of CIN 3, where there is increased density observed over the entire epithelial thickness correlating with the histologic findings of loss of maturation throughout the whole epithelial thickness.

DISCUSSION

The use of OCT for the management of cervical and vulvar neoplasia has been previously described in the literature.[19–25] Optical coherence tomography is an optical image generating technique that analyzes optical scattering from internal tissue microstructure to create a cross-sectional image of tissue structure in vivo using a fiber-optic probe to transmit information to an optoelectronic processor and a computer to generate a 2D or 3D map of backscattered intensity. In OCT, the optical scattering pattern can be analyzed in the histologically normal and neoplastic cervical epithelium. This analysis can then be used to define criteria for the differentiation of normal and neoplastic epithelium.

Gabor-domain optical coherence microscopy can produce a 3D image with a 2-μm resolution in all dimensions. This optical biopsy tool can produce microscopic images with improved lateral and axial resolution while still maintaining an appropriate depth of view. Compared with the previously published work using OCT, with GDOCM, there is an improvement of image resolution that approaches the level of cellular detail seen with tissue biopsy and examination of histologic preparations with traditional microscopy.

The purpose of this study was to determine if the use of GDOCM technology to evaluate the uterine cervix for neoplasia was achievable and potentially practical for clinical application. As this technology had not been used previously to evaluate cervical neoplasia, the system's initial optimization was necessary. Over the course of the study, as experience was gained in using the system with this type of tissue specimen, image quality and efficiency improved. In this small pilot study, we could visualize typical histologic features of cervical epithelium, including the epithelial-stromal junction (basal layer), individual epithelial cells, stromal vessels, hyperkeratosis, and papillary structures. In addition, features of dysplastic cervical epithelium were observed, and the grade of dysplasia was correlated with density changes over the epithelial layer. Optical coherence tomography has primarily been described as an adjunct to colposcopy.[26] Our small ex vivo pilot study was able to generate images of the cervix that demonstrate histologic features of both normal and dysplastic cervical epithelium. A potential limitation of the technology noted in the study is that not all histologic features were visualized in every image reviewed. However, the same is true for a histologic image reviewed on a slide. The histologic image ultimately reviewed by the pathologist is never perfect and is at the mercy of sample size, orientation on the slide, processing artifact, and staining quality. If the image quality is not adequate with histologic slide review, the only option is to make the patient return for a repeat examination and biopsies. With this technology, 1,000 scans are obtained at each imaging site and can generate a stacked scan image for review, which does not involve invasive tissue sampling. Another potential limitation could be the time and technology required to virtually biopsy the entire cervix transformation zone. However, this is unnecessary as these virtual biopsies can be targeted using acetic acid and Lugol iodine application, just as is done in colposcopy. In addition, as the technology is developed with probes that can scan more surface area in vivo, the

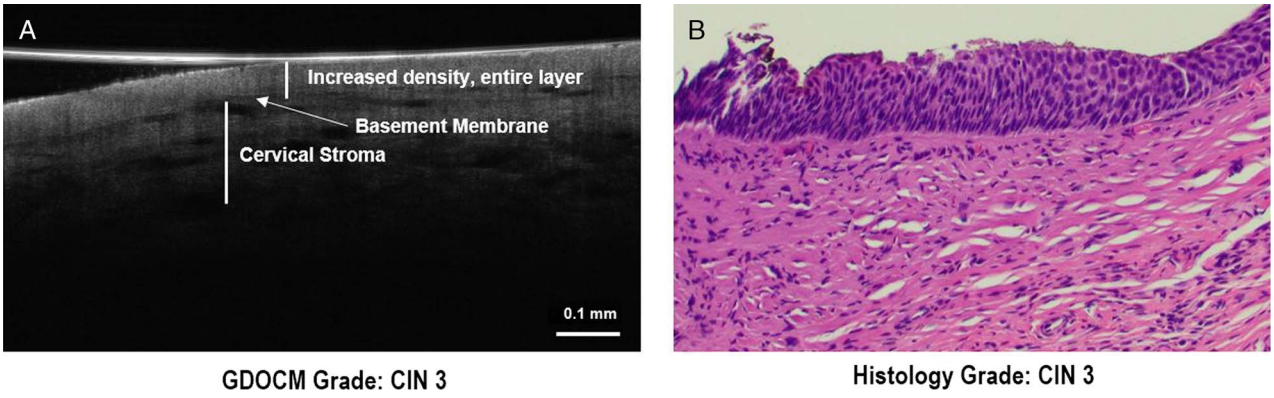

FIGURE 4. A and B, Histologic and corresponding scanned images of CIN 3. Scale bar for the GDOCM image is 0.1 mm.

transformation zone could be imaged in real time and the targeted treatment applied, eliminating the extra step of biopsy acquisition and review before treatment. The next steps include the development of a translational device with a vaginal probe that can be used in vivo to perform a larger validation study powered to determine sensitivity, specificity, and interobserver agreement. Optical coherence tomography has well-established clinical applications in ophthalmology, dermatology, and invasive cardiology.[12,27,28] Gabor-domain optical coherence microscopy generates histologic quality images and has the potential to function as a virtual biopsy tool instead of an adjunct to traditional biopsy. This improved technology is scalable and already has clinical applications in dermatology and opthalmology,[11,14,15] where the providers in those specialties use it. The development of a GDOCM device with a probe for use in vivo has the potential to replace colposcopy and deliver cervical cancer screening and treatment in 1 visit. Further development of this technology for the treatment of cervical neoplasia is necessary to determine whether it would be feasible to train gynecologists to visualize areas of neoplasia or whether it would be more efficient to involve a pathologist as they would require limited additional training. This approach has been advocated for the management of cervical neoplasia in resource-poor settings by the World Health Organization[29] and The American College of Obstetrics and Gynecology.[30] The continued development of this technology for the diagnosis and management of cervical dysplasia has the potential to decrease patient morbidity and health care costs by improving image sensitivity and specificity and reducing the number of visits for evaluation and treatment. In resource-poor settings where access to care is limited, further development of this technology for in vivo use has the potential to decrease mortality from cervical cancer and improve access to care.

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