The Pocket Colposcope, a Novel Low Cost Digital Colposcope, to Improve Access to Cervical Screening in Resource Limited Settings

By

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Duke University

Date: November 5th, 2018

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Mark L. Palmeri

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John W. Schmitt

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biomedical Engineering in the Graduate School of Duke University

2018
ABSTRACT

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Abstract

Addressing disparities in the global cancer burden is a key part of the post-2015 Millennium Development Goals (MDGs). Cervical cancer and breast cancer are emblematic of that disparity, with 85% and 65% of cases and cancer-related deaths occurring in low and middle-income countries (LMICs), respectively. The American Society of Clinical Oncology (ASCO) and World Health Organization (WHO) recently released guidelines recommending that HPV be used as a screening test, followed by triage with visual inspection with acetic acid (VIA) or visual inspection with Lugol’s iodine (VILI) to confirm the presence of lesions. While this co-testing methodology may decrease overtreatment, VIA or VILI remains a poor triage test because of low sensitivity and specificity, and wide variability in interpretation due to poor quality control. Thus, there exists a need for a triage test that is low-cost, easy-to-use, and will provide reliable immediate results at the point-of-care setting.

The goal of the work presented here is to establish the design, development, and validation of a low cost portable digital microscope that has comparable image quality, functionality, and diagnostic capability when compared to standard-of-care digital colposcopes and gold-standard histopathology.

Three specific aims were proposed to address this goal. First, the conceptualization, design, and validation of this device, the Pocket Colposcope will be
presented. The Pocket Colposcope is shaped like a tampon and can be inserted and positioned such that it is 5-50 mm away from the cervix, obviating the need for high-end glass optics, high-resolution cameras, and high power illumination sources used in state of the art colposcopes, which typically operate at a working distance of 300 mm. An off the shelf miniature color complementary metal-oxide-semiconductor (CMOS) camera, with the plastic injection molded lens, and small light emitting diodes (LEDs) could be repackaged inside the compact tampon like form factor and powered directly by a smartphone, tablet, or laptop through the universal serial bus (USB). Each evolution of the Pocket Colposcope is quantitatively assessed with respect to a standard-of-care digital colposcope or predicate device using industry standard protocols and received US Food and FDA 510(k) preexisting medical device approval September 25th, 2018 (K181034).

The optical resolution for all the generations of Pocket Colposcope ranged between 10-72 line pairs per mm (lp/mm) and was substantially equivalent to the optical resolution range of 10-29 lp/mm of the pair of predicate devices. The field of view of all generations of the Pocket Colposcope ranged from 8-52 mm and was substantially equivalent to the field of view range of 12-76 mm reported by the predicate device pair. The picture-height distortion for all generations of the Pocket Colposcope ranged between -4.5 to -1.1% and were substantially equivalent to the 3-7% distortion reported by the pair of predicate devices. The depth of field range between 0.5-12 mm for all
generations of the Pocket Colposcope was substantially equivalent to 6-20 mm range reported by the predicate device pair.

The illumination intensity range of 2,800 to 20,000 lux for all generations of the Pocket Colposcope was substantially equivalent to the reported range of 3,000 to 24,000 lux of the pair of predicate devices. Similarly, the beam diameter range of 33.8 to 49.0 mm for all generations of the Pocket Colposcope was substantially equivalent to 60-62.1 mm range reported by the pair of predicate devices.

The Alpha and Beta Generation of the Pocket Colposcope was developed to incorporate the vast design experiences and user feedback from the Generation 1 through 4 devices. This penultimate iteration of the Pocket Colposcope is designed in collaboration with 3rd Stone Design. The following features are incorporated in the device: improvements in the ergonomics such as buttons on the device for image capture and LED selection, simple to use adjustable magnification mechanism, improved user comfort with an angled and tapered handle, and to incorporate design and material selection considerations for scale manufacturing. The Alpha and Beta Pocket Colposcope maintained key features such as: VIA, VILI, and GLI capability with comparable quality to the standard-of-care digital colposcope, chemical immersion cleaning compatibility, the fog resistant hydrophobic window, and enhanced portability (from Generation 1 and 4) eliminating the need for external LED driver box by combining and miniaturizing the circuit into the handle of the probe.
The second aim was to demonstrate the concordant diagnostic performance of the Pocket Colposcope when compared to reference standard-of-care digital colposcopes and gold-standard histopathology in a multi-institution clinical trial.

An image concordance study was conducted under IRB approval and written informed consent of participants. Images of the cervix collected by the Pocket Colposcope and standard-of-care device (if available) along with gold standard histopathology. These image pairs would be split, randomized, and digitally sent to blinded highly trained reviewers for clinical interpretation. These expert colposcopists are blinded to any demographic information of the patient, prior referral test results (Pap smear cytology, HPV status, HIV status, or histopathology), and each other’s interpretation of the image. A secure online questionnaire was sent to reviewers to record their responses. All data was stored securely on a Duke University Medical Center managed REDCap database server.

These interpretations would be compared between the Pocket Colposcope and standard-of-care device to demonstrate concordance performance or level agreement with the devices. The diagnostic performance of the Pocket Colposcope and standard-of-care devices are assessed by comparing the image interpretation to gold-standard histopathology with the generation of 2x2 contingency tables with entries for True Positives (TP), True Negatives (TN), False Positives (FP), and False Negatives (FN). The two binary diagnostic cut-offs are used: normal vs. LSIL/CIN1+ and normal vs.
HSIL/CIN2+. From these 2x2 contingency tables sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Lastly, the diagnostic performance are stratified by level of contrast used using Receiver Operating Characteristic (ROC) curves where the y-axis is the sensitivity, and the x-axis is 1-specificity. The resultant area under the curve (AUC) can be compared using logistic regression to determine how changing the type of contrasts used impacts overall diagnostic performance.

The performance of the Pocket Colposcope was now assessed as part of the multinational clinical study with four partner sites: Duke University Medical Center (DUMC) (Durham, NC), La Liga contra el Cancer (Lima, Peru), Kilimanjaro Christian Medical Center (KCMC) (Moshi, Tanzania), and the Centre for Infectious Disease Control Zambia (CDZIR) (Lusaka, Zambia). Pocket Colposcope Generation 1 through Beta and standard-of-care device (if available) were used to collect VIA, green light inspection (GLI), and/or VILI depending on the standard of practice at the clinical site. Gold-standard histopathology (from biopsy or post loop electrosurgical excision procedure (LEEP) specimen or post large loop excision of the transformation zone (LEETZ) specimen) are collected for eligible patients. Overall n=388 subjects have enrolled from four clinical sites, with n=129 subjects undergoing VIA with ten blinded expert reviewers, with n=92 subjects undergoing VIA+GLI with seven blinded expert reviewers, with n=127 subjects undergoing VIA+VILI with six blinded expert reviewers,
and n=40 subjects undergoing VIA+GLI+VILI with four blinded expert reviewers. For this analysis, the Pocket Colposcope versus gold-standard histopathology stratified by the types of contrasts used is the study focus.

The level of agreement between the Pocket Colposcope and standard-of-care when using the LSIL/CIN1+ vs. normal diagnostic cut-off was 77.8% with a moderate strength Cohen’s kappa coefficient (κ) of 0.53, p<0.01. When the diagnostic cut-off was changed to HSIL/CIN2+ vs. normal the level of agreement between Pocket Colposcope and standard-of-care improved to 86.0% with a strong Cohen’s (κ) of 0.64, p<0.01. The overall accuracy when using the LSIL/CIN1+ vs. normal diagnostic cut-off and comparing to gold-standard histopathology increased consistently with the type of contrasts used (VIA only, VIA+GLI, VIA+VILI, and culminating with VIA-GLI-VILI) with overall agreement improving from 53.4-70.1% with sensitivity improving from 42.3-68.1%, with specificity improving from 65.3-73.3%. The diagnostic performance when the diagnostic cut-off are set to HSIL/CIN2+ vs. normal followed the prior trend with consistent increases with the type of contrast used (VIA only to VIA+GLI+VILI) with rising accuracy 55.9-70.8%, with sensitivity increasing 41.9-52.2%, with specificity improving 78.2-88.0%. Increasing the number of contrast agents used significantly improved the diagnostic performance of the Pocket Colposcope when compared to gold-standard histopathology.
The final aim is to add fluorescent imaging capability to the Pocket Colposcope to provide an additional source of contrast targeting metabolic and structural biomarkers that will further improve the clinical performance of our system to match gold standard histology pathology and expand the application of the device. There is broad applicability in other organ sites including oral cavity, gastrointestinal tract, and skin. The Beta generation of the Pocket Colposcope was modified for fluorescent imaging by adding excitation LEDs, repurposing of the Generation 3 external LED driver circuit to drive the excitation source, a matched band-pass optical filter for emission, and reintroduction of cross-polarization, without destructive modification of the Pocket Colposcope. A proof of concept device was characterized using a similar set of procedures used to assess the Pocket Colposcope for the 510(k) regulatory approval pathway. Ex-vivo and in-vivo pilot animal studies were conducted to demonstrate successful implementation of the fluorescent imaging capability using a FITC (Fluorescein Isothiocyanate) tagged binding ligand (HS-27) with a strong affinity for the heat shock protein-90 (Hsp-90) chaperone molecule (a potential biomarker of cancer). Lastly, the fluorescent Pocket Colposcope was used in a pilot ex-vivo clinical studies in subjects undergoing breast biopsy to evaluate the diagnostic potential of HS-27 when compared to gold-standard histopathology.
Dedication

To my Grandma Le and our daily check-ins, Uncle Ben Le for your calm guidance and prayers, my parents Joseph and Sandy Lam for sacrificing so much so that my brother Jonathan and I could have an opportunity to pursue our dreams. Jonathan, I have been very lucky to have a bright, diligent, and caring younger brother. To Natalie, Sammy and I have been so fortunate to have you become part of our lives, especially during such a trying time when I was finishing my dissertation. Your passion and curiosity has been a profound comfort to me.
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<tr>
<td>MDG</td>
<td>Millennium Development Goals (MDGs).</td>
</tr>
<tr>
<td>LMIC</td>
<td>Low and Middle Income Countries</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>HPV</td>
<td>Human Papillomavirus</td>
</tr>
<tr>
<td>ASCCP</td>
<td>American Society for Colposcopy and Cervical Pathology</td>
</tr>
<tr>
<td>ASCO</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>$\mu_a$</td>
<td>Total absorption coefficient</td>
</tr>
<tr>
<td>THb</td>
<td>Total Hemoglobin Concentration</td>
</tr>
<tr>
<td>SO%</td>
<td>Oxygen Saturation</td>
</tr>
<tr>
<td>$\mu'$</td>
<td>Scattering coefficient</td>
</tr>
<tr>
<td>VIA</td>
<td>Visual Inspection with Acetic Acid</td>
</tr>
<tr>
<td>GLI</td>
<td>Green Light Inspection</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin concentration</td>
</tr>
<tr>
<td>MVD</td>
<td>Micro vessel density</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VILI</td>
<td>Visual Inspection with Lugol’s Iodine</td>
</tr>
<tr>
<td>LSIL/CIN1</td>
<td>Low Grade Squamous Intraepithelial Lesion/ Cervical Intraepithelial Neoplasia Grade 1</td>
</tr>
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HSIL/CIN2+:  High Grade Squamous Intraepithelial Lesion/ Cervical Intraepithelial Neoplasia Grade ≥2
LEEP:  Loop Electrosurgical Excision Procedure
LEETZ:  Large Loop Excision of the Transformation Zone
IARC:  International Agency for Research on Cancer
VCM:  Voice Coil Module
LEDs:  Light Emitted Diodes
IC:  Integrated Circuits
CCT:  Correlated Color Temperature
LiFePO4:  Lithium Iron Phosphate
CNC:  Computer Numerical Control
EtO:  Ethylene Oxide
PET:  Polyethylene terephthalate
ABS:  Acrylonitrile butadiene styrene
IPX:  Ingress Protection Marking
IEC:  International Electrotechnical Commission
ISO:  International Organization for Standardization
MOSFET:  Metal-Oxide Semiconductor Field-Effect Transistor
CMOS:  Complementary Metal-Oxide Semiconductor
TTL:  Transistor-transistor logic
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<th>Acronym</th>
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<td>Hsp</td>
<td>Heat Shock Protein</td>
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<td>Tetramethylrhodamine, ethyl ester</td>
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<td>2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose</td>
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Acknowledgments

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1. Introduction

1.1 Unmet Clinical Needs in the Women’s Cancer Care Cascade

In this dissertation, the global burden of women’s cancer is reviewed, with a focus on cervical cancer and then pivoting to breast cancer. Next, the current clinical paradigm for cervical cancer will be presented to identify barriers for implementation of the paradigm. The design criteria for potential solutions that address these barriers will be proposed. The development and validation of the proposed solution will be detailed and the diagnostic performance assessed with respect to the standard-of-care device and pathology. Finally, we will pivot to how molecular diagnostic imaging could improve access and treatment for breast cancer using a modified Pocket Colposcope as a fluorescent microscope.

1.1.1 The disproportionate global burden of women’s cancer in low and middle-income countries

The global burden of cervical cancer manifests at nearly 600,000 new cases and 310,000 deaths, annually (1, 2). Nearly 85% of the cervical disease burden is disproportionately distributed in Low and Middle-Income Countries (1, 3). Breast cancer like cervical cancer is a tremendous burden with over 2.1 million new cases and 630,000 deaths annually (2). Over 65% of this burden is in LMICs(2). However, these countries only have access to 5% of global health resources (4), greatly limiting the potential strategies for the prevention, screening, diagnosis, and treatment. Aims 1 and 2 will
focus on our proposed solution for cervical cancer and in Aim 3, we pivot to how our solution could impact the breast cancer care cascade through the molecular imaging diagnostics.

1.1.2 Etiology and pathogenesis of cervical cancer

The etiology of cervical cancer by Human Papilloma Virus (HPV) a double-stranded (ds)DNA virus, was first postulated by Dr. zur Hausen in 1977(5) and later confirmed by 1984 with the extraction of integrated and episomal HPV dsDNA from cervical neoplasia, carcinoma, and immortalized cervical cancer cell lines (6, 7). HPV is a family of viruses with circular dsDNA around 8kbp in length, an icosahedral nucleocapsid shell with a diameter between 45-55 nm(8-10). In cervical cancer, the target cell for infection by HPV are the basal keratinocytes bottommost layer of the cervical squamous epithelium, and are uniquely the only normally undergo cell replication. After cellular division at the basal layer, the keratinocytes begin to rise towards the surface of the cervix and undergo differentiation process that involves flattening of the cell but no additional mitosis flattening concurrent with their differentiation where when infected the virion will be duplicated up to 1000 times before being released to infect other cells as seen in Figure 1 (11).
Figure 1: Schematic life cycle of HPV infection. In normal (A) cervical epithelial cells with an increasing progression of the disease to invasive carcinoma (F). The cervical squamous epithelium consists of distinctive layers: the basal (the only layer that normally undergoes division), and three layers of differentiation: parabasal, intermediate, and superficial. The thickness of the epithelial layer is reported to be 260 to 450 µm and does not significantly change with disease severity.

HPV infection occurs in basal keratinocyte cells leading to the episomal viral presence (blue virions) in the nucleus and a low level of viral replication (<6 copies).

The HPV+ or infected cell’s (light green) internal organelles are hijacked into producing a multitude of oncoproteins that lead to uncontrolled division in the parabasal through superficial layers and vastly increased virion duplication with up to 1000 copies per infected cell in the superficial layer. These infected cells lose their ability to differentiate terminally. Detectable neovascularization occurs in HSIL/CIN2 (C) with increasing levels with progression of the disease and is also the first signs of HPV DNA integration with the host cell’s genome. Eventually, the production of HPV virion ceases, and the episomal HPV copies are lost (E), and HPV DNA integrates into the host cell’s genome (dark green) leading to the metastatic transformation of the cells. The basement membrane begins to break down and loses integrity allowing for migration of cells into the stroma and the potential spread to the lymph system and other organ sites.
These infected cells lose their ability to terminally differentiate (8). The thickness of the epithelial layer is reported to be 260 to 450 µm and does not significantly change with disease severity(9, 10, 12-17). HPV oncogene products have been shown to mediate the normal regulatory apoptosis cascade, leading to uncontrolled proliferation and lack of terminal differentiation (13, 14, 18-25). In the mid 1960’s investigations by EM (electron microscopy) revealed some curious findings: abnormal number of chromosomes in the nucleus (now believed to be episomal HPV copies), a significant increase in non-membrane associated ribosomes that aggregate together, and a significant increase in RER (rough endoplasmic reticulum) in early cervical neoplasia when compared to normal control tissue(26). These structural findings suggest viral hijacking of internal organelles of infected cells for production of oncoproteins (11), HPV virion duplication, and production of the capsid casing for the HPV virions before dispersion(26). Patients that were HPV-16+ and with biopsy-confirmed high-grade precancerous lesions (HSIL/CIN2+) had on average a 60 and 30 fold higher number of viruses per cell when compared to HPV+ women with biopsy-confirmed normal cervixes and biopsy confirmed low grade (LSIL/CIN1) cervixes, respectively (27).

1.1.3 History of cervical cancer screening and treatment

Screening techniques for cervical cancer were first developed in the early part of the 20th Century. The first tool, the colposcope, was developed in 1925 by Dr. Hinselmann allowing for the direct visual inspection of the cervix when used with the
The colposcope is a stand mounted modified stereomicroscope with an extended working distance and integrated illumination source (28). Another important screening technique utilizing loose cervical cells was first reported by Dr. Papanicolaou in 1941 and bears his name the “Papanicolaou Smear” or cytopathology (29). In 1995 detection of HPV DNA was developed as a screening method for cervical cancer (3), but have high initial capital cost and require specific sample processing to isolated HPV DNA for detection, is making their adoption in LMIC limited thus far.

### 1.1.3.1 Papanicolaou Smear (Cytology)

Loose cells and debris is collected by swab or comb tool from the cervix, placed or “smeared” across a microscope slide, the cells and debris are fixed to allow for staining with a distinctive 5-stain system consisting of: hematoxylin (for nuclei), orange G (for keratin), Eosin Y (for cytoplasm of epithelial squamous, nucleoli, cilia, and red blood cells), Light Green SF (for cytoplasmic staining of non-keratinized squamous cells), and Bismarck brown (for mucins) (29). This technique has served as the primary screening tool in developed countries but has significant difficulties in being adopted in LMIC due to the high level of equipment and expertise required (30, 31). There is a limit on the number of Pap specimens a pathologist or cytotechnologists can interpret per day according to US Federal Law and according to CLIA’88 guidelines as part of an effort to improve clinical laboratory performance (32). These highly trained individuals may
only examine up to 100 slides per 24 hours (at an average rate of 12.5 slides per hour) and less than 8-hour timespan (32).

1.1.3.2 Alternative method for screening via HPV detection

More recently, this has led to the development and introduction of a preventative vaccination for the transmission and infection by HPV, first available in 2006(33). However, even with subsidies, the multi-dose vaccination has a total cost of US$200, making it difficult for widespread adoption in LMICs. Additionally, detection of HPV infection from exfoliated cervical cells through hybrid capture (DNA/RNA hybridization) or PCR (polymerase chain reaction) has been increasingly pushed as a primary or co-primary screening technique with cytology. These techniques were first available in 1995(3), but have high initial capital cost and require specific sample processing to isolated HPV DNA for detection again making their adoption in LMICs limited thus far.

1.1.3.3 Visual Inspection Techniques and Colposcopy

In 1938, Dr. Hinselmann described the first use of colposcopy with the application vinegar solution or Visual Inspection with Acetic Acid (VIA) (34). A temporal acetowhiteness effect is observed with a differential staining pattern between cancerous and normal cervical tissue (34). The increases in proliferation activity and the cell density cause increased visible light scattering due to a temporary dehydration of
cytoplasm that leads to increased precipitation of cytokeratin by acetic acid solution (35, 36).

In 1939, Dr. Kraatz reported the first colposcope with an integrated green light filter or Green Light Inspection (GLI) to improve the visualization of underlying vascular morphology (37) and leverages the unique visible absorption spectra of hemoglobin with significantly higher absorption in blue and green visible wavelengths (38). The narrow band of illumination (±15nm) has been shown to achieve the same penetration depth of 250 µm when compared to broad green illumination (±80nm) in digestive tract mucosa (39). However, the cervical epithelium is significantly thicker between 300 to 500 µm, which would require longer wavelengths of light to penetrate adequately (10). The dimensions of the cervical stromal capillaries range from 12 to 20 µm based on corrosion casting and Scanning Electron Microscopy (40-42). Furthermore, there are seven “classical” normal vascular patterns and nine patterns associated with cervical neoplasia, first described by Dr. Koller in 1958 (Figure 2) (43). Neovascularization is one of the significant hallmark of cancer (44) and the marked increase in MVD (microvessel density) corresponding to increasing severity of cervical neoplasia has been widely reported (45-47). The prognostic utility of the in-vivo early detection of angiogenesis as a marker of cervical neoplasia development by spectroscopy as a significant increase in local tissue hemoglobin concentration [Hb] and confirmed by IHC staining of precursor signaling proteins, VEGF (48, 49).
Figure 2: Classical vessel patterns. For normal (A to G) and abnormal (H to P) for cervical neoplasia as described by in the IARC’s Colposcopy Manual, Normal patterns include (A) Network, (B) Hairpin, (C) Staghorn, (D) long parallel, (E) regular vascular network, (F) regular branching with a gradual decrease in caliber, and (G) regular branching. Abnormal patterns include (H) wide hairpin, (I) waste thread, (J) tendril, (K) bizarre branching, (L) corkscrew, (M) tree root, (N) coarse punctuation, (O) irregular branching, and (P) irregular root-like (50). Generally, increasing tortuosity would seem to correlate with abnormal cervix vascular status.

In 1928, Dr. Schiller discovered that normal cervical tissue was rich in glycogen would stain a dark maroon or mahogany color, in contrast to dysplastic and cancerous cervical tissue would stain a pale yellow (51). The use of an exogenous stain for glycogen during colposcopy is known as Visual Inspection with Lugol’s Iodine (VILI) (51).

Cryotherapy was first applied as a treatment of cervical neoplasia in 1964 by Dr. Cahan (52). Loop electrosurgical excision procedure (LEEP) was first described by Dr. Cartier in 1981 for the treatment of cervical neoplasia using a small loop (53), while Dr. Prendiville reported the large loop technique more commonly used (54). Metastatic cervical cancer is often treated with a combination of hysterectomy, chemotherapy, and radiotherapy.
1.1.4 Benefits, rationale, and challenges for current guidelines for cervical cancer screening

Screening is an essential and important tool in reducing the burden of cervical cancer. The lifetime risk of developing cervical cancer can be reduced by 20% with a single screening, 40% with two screenings, and 55% for three screenings, at any time during the lifetime of a woman (55, 56). Similarly, the introduction of screening in high-income countries has reduced the incidence and mortality of cervical cancer by 70% (31). The most recent guidelines for cervical cancer surveillance in the US, as defined by American Society for Colposcopy and Cervical Pathology, involve a multi-visit screening, diagnosis, and treatment paradigm. Generally, primary screening is suggested for women age $>30$ years of age, at a 3-year interval using cytology and HPV co-testing (57). Patients with abnormal cytology and/or high-risk HPV are referred to the second level of screening and diagnosis using colposcopy-guided biopsy. LEEP (loop electrosurgical excision procedure) or cryotherapy are referral treatment procedures (58).

However, these prevention services are not widely available in many low and middle-income countries (LMICs). Pap smear-based screening and HPV testing, which are widely available in western countries have not been feasible to implement widely in LMICs owing to their cost, lack of infrastructure and appropriately trained human resources. Randomized controlled trials conducted in India and other LMICs have
shown that visual inspection with acetic acid (VIA) is the most resource-efficient approach to screen for cervical cancer (59). VIA is just a simpler version of colposcopy which is used in western countries to diagnose cervical pre-cancer/cancer in women who have already been screened and found to have a positive Pap smear (60). During colposcopy, the cervix is exposed using a speculum and visualized at low magnification (4-7X) for subtle features on the cervix (61, 62). Whitening of the cervix from the application of acetic acid is used to determine the presence of lesions, through visualization of heterogeneity of suspicious regions (opacity, color, shape, or pattern). A green filter can be applied to the coloscope’s illumination source to aid in the visualization of vascularization, a known hallmark of severe dysplasia. The prohibitive cost of colposcopes (US$ 10,000-20,000) limits their uptake in resource-limited settings (63, 64). Therefore, VIA is performed with a simple headlamp or flashlight in most instances.

1.2 Proposed solution to improve outcomes and patient experiences while reducing health expenditures

This resource-intensive multiple visit paradigm is incompatible with the social and economic realities found in LMICs. The rate of attrition after a positive cervical cancer screening result is estimated to be almost 50% in LMICs for women, and these barriers include distance to clinic, lack of transportation, and time constraints (65, 66). Obstacles to adequate access include insufficient availability, distribution, and
portability of expensive equipment and require highly trained personnel (67). WHO recommended guidelines for resource-limiting settings suggest the implementation of a “see and treat” paradigm involving: HPV testing and cryotherapy (68). However, visual inspection with acetic acid (VIA) can be substituted, if HPV testing is not available (68).

Thus, a need exists for a low-cost portable imaging platform that can be readily implemented by community health workers via household or mobile clinic visits and/or allow self-administration, thereby empowering women in their healthcare. The “task-shifting” of cervical cancer screening would allow the reallocation of highly skilled health professionals towards treatment and management of complicated cases (69).

VIA itself has several implementation challenges that limit the scale and hence, the impact of this approach (1). Challenges include: 1) high screen positivity rates (likely due to inadequate training and technology), leading to a high volume of patients being referred for follow up and at the same time, a very high loss to follow-up (about 50%) of a large number of screen-positive women (70). If community health workers could be empowered to bring colposcopy to the primary care setting and be trained to more effectively use this technology to triage women, secondary and tertiary care facilities could focus their energies on managing priority patients who are in most urgent need of follow-up care, without being overwhelmed by women who don’t need treatment.

The implementation of the VIA/VILI screening program itself can also be a barrier from an adoption perspective. A study in rural Moshi Tanzania of 354 women
revealed that key factors for cervical cancer screening were husband approval, level of education, significant concerns about embarrassment and pain due to screening from the speculum, gender of the health provider, and distance to the screening center (71). In a survey conducted in rural Mexico by the Stanford University School of Medicine, the most frequent reason for not having a cervical exam was anxiety regarding physical privacy. Less frequent reasons were lack of knowledge and difficulty accessing health care (72). Even in the U.S., where there is greater access to health care, compliance rates with cervical screening vary, and embarrassment and fear of pain during examination have been reported as barriers to screening (73-75). The speculum itself is a cause of discomfort particularly for women with vaginismus, where there is an involuntary tightening of the vagina that is often a result of sexual abuse (76). East African countries, such as Tanzania have among the highest sexual violence rates worldwide (77, 78). There is an undeniable relationship between sexual violence and the contraction of HPV and thus it is these women who are in greatest need for frequent cervical screening.

1.3 Requirements for diagnostic screening platform and current solutions

Prior low-cost analog colposcope devices have had limited success stemming from lack of digital image capture capability, fixed magnification, limited depth of focus, and poor illumination characteristics (Figure 3) (79-83). These include a low-cost hand-held portable analog colposcope (PATH’s Aviscope) (79-83), the Magnivisualizer (84-86),
and the Family Health Ministries/Duke Portable Colposcope, which is based on surgical loupes (87). More recently, low cost digital colposcopes have come to market. The EVA (MobileODT, Tel Aviv, Israel) has a very limited range of magnification, but does have a well-integrated electronic medical record app (88). A more recent system Gynocular (Gynius AB, Goteborg, Sweden) has addressed some of these issues with the potential to have digital colposcopy capabilities, however at price point of US$2,000 (89, 90).

**Figure 3: Competing Low-Cost Analog and Digital Colposcopes**

**1.4 Cervical Cancer Screening Device Design Criteria**

The device needs to fit within a “see & treat” paradigm and allow for immediate diagnosis, have a low cost per use, conduct nondestructive surveillance of the cervix, provide comparable sensitivity and specificity to existing standard-of-care digital colposcopes. The device also needs to be compatible with logistical restraints of limited
resource settings, be durable to survive in an austere operating environment, be highly portable and non-dependent on walled electrical power, and allow for future integration of user training and quality control.

1.5 Similar barriers associated with breast cancer screening and treatment paradigm in limited resource settings

Current guidelines for breast cancer screening and treatment involve a multiple visit paradigm similar to the one for cervical cancer with some additional levels of therapy beginning with mammography at age 40 every 1-2 years for women of “average” risk. Positive results are referred for ultrasound guided biopsy, positive women are then referred to neo-adjuvant therapy in conjunction with breast conserving surgery. Surgery therapy can require re-incision for further tissue removal due to inadequate tumor margins and can also include further adjuvant therapy. But there are issues with this current paradigm being applied in LMICs, these include the frequent number of visits required and potential for overtreatment (91, 92). Recommendations and viable options are much less clear in resource limited settings when compared to cervical cancer. Mammography is still the preferred option but not readily available in LMICs thus clinical breast exam with ultrasound has been suggested but not yet universally accepted despite becoming more affordable and portable (91, 92). All the current recommended therapeutic options are quite resource intensive including: surgery and systemic therapy. The WHO also recommends devoting some resources to
establishing palliative care (91, 92). Thus there exists a need for a platform that could both diagnose and provide therapy, a theranostic approach for breast cancer for LMICs.

1.6 Breast Cancer Theranostic Device Design Criteria

First, an agent that exploits differential expression of tumor and normal cells will be identified, thereby allowing for targeted therapeutic effects without side effects commonly associated with chemotherapy. Next, the agent should target a marker with ubiquitous expression in cancer subtypes and to ameliorate the burden on pathology. The system should have both wide-field and high resolution real time surveillance? While there exists a plethora of molecular diagnostics one could consider for this application, we chose to leverage one that is already being used for breast cancer treatment, a Heat shock protein-90 inhibitor. Hsp-90 is a chaperone protein that increases in response to stress and is highly expressed on the surface of different breast cancer subtypes. By tethering the Hsp-90 inhibitor with a fluorescent we can transform it into a molecular diagnostic and theranostic modality with the modified Pocket Microscope.

1.7 Specific Aims

The goal of this work is to develop a low-cost portable digital imaging device that can be used to noninvasively detect pre-cancerous and cancerous lesions with comparable clinical performance to a gold standard clinical wide-field imaging system
in limited resource settings. We will first evaluate our system for cervical cancer screening and then pivot to an application in breast cancer. There is broad applicability in other organ sites including oral cavity, gastrointestinal tract, and skin.

Our first aim is to determine if the quantitative and qualitative imaging performance between our low-cost portable Pocket Colposcope is comparable to a reference predicate digital colposcope system using industry standard imaging targets and benchmark metrics.

Our second aim is demonstrating the clinical equivalence in diagnostic performance of our device to a predicate reference system and determine if increasing the levels of contrast will improve the diagnostic performance of Pocket Colposcope to better match gold-standard histopathology.

In the final aim, we pivot to an application of the Pocket Colposcope for breast cancer. The Pocket Microscope is employed to conduct molecular imaging diagnostics to impact the breast cancer care paradigm through adding fluorescent imaging capability to the Pocket Colposcope. Here a fluorescent agent was selected that has differential expression in tumor versus normal cells, has ubiquitous expression across breast tumors sub-types, has a targeted therapeutic effect, and could be imaged at both wide-field and high-resolutions modes to address the shortage of pathologists in resource limited settings. Furthermore, in the future additional sources of contrast targeting metabolic and/or structural biomarkers could be interrogated to improve the
clinical performance of our system to match gold standard histology pathology.
2. Aim 1: Conceptualization, Design, and Validation of the Pocket Colposcope

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2.1 Preface

This first aim establishes the conceptualization, design, development, and validation of Pocket Colposcope using industry standardized testing protocols and was selected in anticipation of US FDA 510(k) application, which requires equivalence be shown with a predicate device or existing approved device(93, 94). On September 21st, 2018 the Pocket Colposcope received US FDA regulatory approval for the Pocket Colposcope K181034 through the 510(k) pathway (94). Advancements in rapid prototyping techniques and proliferation of smartphone/tablets with miniaturized detectors, optics, and illumination components have enabled the evolution of the Pocket
Colposcope with comparable capabilities as standard-of-care digital colposcopes in a much more portable, low cost, and durable package.

2.2 Introduction

We have developed a novel, low-cost point of care digital Colposcope (Pocket Colposcope) to address many of the limitations described above for low-cost colposcopes. The Pocket Colposcope is shaped like a tampon and can be inserted and positioned such that it is 5-50 mm away from the cervix, obviating the need for high-end optics and high-resolution cameras used in state of the art colposcopes, which need to have a working distance of 300 mm. The Pocket Colposcope leverages consumer grade light sources and cameras used in smartphones, and 3D printing is used to create the tampon like form factor handle. Although the use and emphasis on low-cost technology might suggest a limited potential, our novel approach of reducing the working distance by a 10-fold factor (30 vs. 300 mm) with respect to the traditional colposcopes. Thereby allowing the Pocket Colposcope to deliver the clinical diagnostic capabilities of the standard-of-care digital colposcope costing US$20,000 and weighing nearly 200 pounds; into our device that can be carried in one’s jacket pocket at 100-fold price reduction.
2.3 Materials and Methods

2.3.1 Conceptualization and Core Component Selection

2.3.1.1 Detector and Optics

First, we set out to review and understand the optical design of a traditional colposcope, in order to determine the potential limitations one might encounter during the effort to miniaturize into a more compact form factor. Generally, colposcopes are stereomicroscopes with an extended working distance of between 250-350 mm. Working distance is defined as the length from the last optical element (outermost) to the target object. Colposcopes have five glass lens elements: common objective, zoom system (two elements), erecting beam splitter, and eyepieces (see Figure 4A) (95-97). The mechanically compensated zoom lens systems allow for the working distance to remain constant when changing between magnifications, these two elements move independently (98, 99). A digital camera can be added with the use of a beam splitter between the rightmost zoom element and eyepiece. The colposcope lens elements are comprised of different glass materials of different refractive indices (crown and flint), with anti-reflective coating, and between 25 to 50 mm in diameter. Miniaturization the colposcope optical train and zoom element translation system to at least 1/10th the size of current systems make cost prohibitive.

The increasing ubiquity of smartphone and tablets have led to an abundance of low-cost digital camera systems that can produce near equivalent magnifications with
injection molded aspheric plastic lenses that are fused to form a single lens element or Prime lens system. The movement of the prime lens is relative to the detector and allows for a wide range of magnifications and working distances without the need for complex focusing mechanism.

Figure 4: Comparison of typical colposcope optical lens system and proposed prime element solution. (A) Colposcopes use a mechanically compensating zoom mechanism that allows for a constant working distance but relies on internal and independent movement of zoom element lenses. This system is difficult to miniaturize due to the complex mechanical system and size of the glass-based optical
elements. (B) A potential solution is to use a Prime element zoom system, which necessitates the use of different working distances and movement of the whole lens element (which are fused) with respect to the detector to achieve the varying magnification.

The original concept for the Pocket Colposcope was inspired by the form factor of a tampon applicator and Universal Serial Bus (USB) spy pen camera as a way to miniaturize the essential components from the standard-of-care digital colposcope: a color detector, a light source, and a lens to focus the image of the cervix onto the detector. All these components must fit within the confines of a traditional speculum, the maximum outer diameter of 25 mm and length of approximately 140-60 mm.

2.3.1.2 Generation 1 Pocket Colposcope – Proof of Concept

Thus, a proof of concept prototype (Generation 1 Pocket Colposcope) was developed using an off the shelf 2MP color CMOS USB inspection camera (Vividia Technologies UM07, Greenville, SC) with an integrated concentric white LEDs ring and manual focus capability(100). The color CMOS detector was based off the Omnivision OV2685 with 1.75 μm pitch and pixel density of 1600 x 1200 with a four element plastic injection molded prime lens system with a back focal length of between 3 to 4 mm. The systems f/# is between 2 and 3, with an emphasis on wide angle imaging (101-103). A protective probe shell and adjustment knob were rapidly prototyped using a medical grade Acrylonitrile butadiene styrene (ABS) plastic extrusion printer (Dimension 1200es Stratasys Inc., Eden Prairie MN)(100). Rotation of the magnification adjustment knob causes linear translation of the detector with respect to a fixed lens box. The camera lens
and LEDs are protected from any biological fluid infiltration and dust by an impact and scratch resistance Gorilla Glass® clear optical window (Edmund Optics #83-361, Barrington, NJ) mated to tip of the protective probe shell and sealed with ISO-10993 certified medical grade cyanoacrylate adhesive (Loctite Prism 4011, Dusseldorf Germany). The color CMOS camera and white LEDs were powered completely by the host device’s USB port (5V DC), and image capture was controlled by off the shelf web camera software on the host device.

Figure 5: An exploded schematic, and function diagram for the Generation 1 Pocket Colposcope, (A) USB Cable provides power to the 2MP color CMOS detector and concentric White LEDs. A rotational magnification adjustment knob moves the camera module with respect to a fixed injection molded lens and allows for manual
focusing of the image. The camera module is housed in a protective 3D printed ABS plastic probe housing with an insertion diameter of 18 mm and a clear optical window. (B) The Pocket Colposcope’s functional diagram begins with a control and display device, e.g. (laptop, tablet, smartphone) which powers and provides two-way communication between the microcontroller unit (MCU). The MCU controls the intensity of illumination through pulse-width modulation by the LED driver circuit to the concentric White LED ring and also interfaces with the color CMOS camera.

The Generation 1 Pocket Colposcope was cleaned with a Super Sani-Cloth (PDI Inc., Parsippany, NJ) germicidal wipe and then placed in a permeable container for Ethylene Oxide (ETO) gas sterilization prior the next patient use. This reprocessing technique entailed a 24-hour turnaround time between each patient, so collecting data from multiple patients each day required multiple probes be ready.

The proof-of-concept Generation 1 Pocket Colposcope was successful in capturing images of the cervix when stained with acetic acid (VIA), improve imaging contrast was desired due to higher amounts of specular reflection when compared to standard-of-care digital colposcope and lacked the ability for green light inspection (GLI) (100). Also, the 2MP camera had only manual focus, and the prototype often lost focus to accident bumping of the adjustment knob. Thus, the next generation of the Pocket Colposcope needed to have increased illumination output, green light capability, and automatic focus capability.

2.3.1.3 Generation 2 Pocket Colposcope – Improved optics and illumination

We first identified a 5 MP (megapixel) color CMOS camera with a 4 to 5 element prime lens configuration, and a back focal length of between 3 to 4 mm as the basis for
the Generation 2 through Beta Pocket Colposcope (100, 104). The optical system has an 
\( f/\# \) is between 2 and 3, with an emphasis for wide angle imaging (101-103). This imager 
is comparable to the camera modules used in Apple iPhone 4S and Motorola Droid 
Bionic and is chosen as the basis of our system camera (Supereyes Co. A005+, Shenzhen, 
China) and based on the OmniVision OV5640 Color CMOS chipset with a pixel size of 
1.4 micron and a pixel density of 2592 x 1944 (100, 104). A voice coil module (VCM) 
focusing mechanisms allows for automated movement of the lens box relative to the 
camera module and controlled by the MCU. Instead of allowing for continuous user 
adjustment of the focus, by rotating the rear half of the camera module, it was set at the 
most often used working distance of 35 mm to prevent accidental shifting of the focus by 
the user. The magnification was fixed by imaging the Pocket Colposcope at the 35 mm 
working distance against a USAF1951 resolution target and rotating the rear handle of 
the camera module until the image came into focus on the screen. The position was then 
temporarily fixed with a strip of electrical tape to prevent the rotation of the rear handle 
joining the proximal tip of the camera. More permanent fixation is described later in this 
document.

The Generation 2 Pocket Colposcope has a custom fabrication concentric 
illumination ring with an alternating sequence of equally spaced white and green LEDs 
to mimic the functionality of traditional colposcopes with green illumination capability. 
For the illumination sources, the Luxeon Z series of LEDs (Lumileds, Amsterdam
Netherlands) were chosen as for broad range of spectra (eight different white Correlated Color Temperature (CCT): 2700K to 6500K and over 20 single color options: UV-A to Deep Red), a compact footprint (2.2 mm²), broad viewing angle (120-150°), and ability to be driven up to 1,000 milliamps. The 5000K CCT white LED (Luxeon LXZ1-5070), and lime green LED (LXZ1-PX01) to better mimic the reference standard-of-care colposcope 5000K CCT white LED and broader filtered green spectra (Figure 6)(100).

![Figure 6: Comparison of hemoglobin absorption spectra and vascular illumination spectra of colposcopes. (A) Absorption spectra for oxygenated (blue) Hemoglobin and deoxygenated (pink) Hemoglobin versus wavelength (nm). (B) Optical power weighted (µw) spectra for the Leisegang Optik 2 (red), Pocket Colposcope narrow green LEDs (blue), and Pocket Colposcope lime LEDs (green).](image)

Furthermore, the Generation 2 Pocket Colposcope incorporated cross-polarization to minimize specular reflection and improve contrast from the moist cervix tissue surface (105-108). Cross-polarization is accomplished with a linear glass polarizer
placed (#43-783; Edmund Optics, Barrington NJ) in-line and parallel to the optical imaging axis. A second linear plastic film polarizer (#86-178; Edmund Optics, Barrington NJ) was laser cut with a donut hole shape configuration such that it was placed over the illumination source (LEDs) at an orthogonal orientation to the imaging axis polarizer but not obfuscate the preexisting linear polarizer (100, 104).

The custom LED ring, and both polarizers are held in place by a custom (computerized numerical control) CNC milled 6061 aluminum round stock. A longer full-length protective probe shell was rapidly prototyped using a medical grade Acrylonitrile butadiene styrene (ABS) plastic extrusion printer (Dimension 1200es Stratasys Inc., Eden Prairie MN). This probe handle or casing is then covered by a medical grade Medical USP Class VI and ISO-10993 compliant radiation cross-linked acrylate olefin shrink tubing (Insultab HS-714, Woburn, MA) were used to finish off the Generation 2 Pocket assembly and permanently fix the magnification previously fixed (100, 104). These components fixed together with ISO-10993 certified medical grade cyanoacrylate adhesive (Loctite Prism 4011, Dusseldorf Germany).
Figure 7: Generation 2 and 3 Pocket Colposcope Summary
- Exploded View, Functional Block Diagram, and Representative Image of Prototypes
  (A) a USB Cable and LED power cable (1) provides power and communication to the
  5MP color CMOS camera with autofocus lens module (3), a 3D printed ABS plastic
  clamshell handle (2) with a maximum diameter of 22 mm, (4) linear glass polarizer for
  the camera, (5) a hydrophobic window (Generation 3 only), (6) aluminum probe tip,
  (7) white and green concentric LED ring, and (8) orthogonally oriented film linear
  polarizer over the LEDs. (B) When compared to the Generation 1 Pocket Colposcope,
  note the addition of an external LED Driver circuit and green LEDs, a secondary
  MCU, rechargeable power pack, and autofocus mechanism controlled by primary
  MCU. (C) Representative images of Generation 2 (a single Ethernet cable connector)
and Generation 3 with custom USB and LED power cable and waterproofing techniques applied.

In order to compensate for the reduced illumination and collection signal from the cross-polarization technique (~75% loss), higher power LEDs were used, which supersede the USB power supply capability (5V DC, 500 ma). Thus, a custom design external LED driver circuit, with a second microcontroller unit (MCU), and LiFePO4 rechargeable battery supply. Each same color LEDs are connected in series (4 in a row) with a common anode (positive), the cathode side is connected to a constant current regulator integrated circuit (IC). The LED driver circuit is composed of constant current ICs (Supertex Inc. CL520 or CL525, Sunnyvale, CA) are selectively grounded with an 8 channel transistor-transistor logic (TTL) compatible Darlington transistor (ULN2803) when the secondary microcontroller unit (MCU) sends a “high” signal to the input side of the Darlington. These Supertex constant current regulator ICs are chosen as they incorporate onboard temperature compensation and minimal dropout voltage of 1.0V instead of resistors, which would provide fluctuating current with temperature. The driving current for white LEDs is 100 mA and 80 mA for green LEDs. The Darlington transistor (ULN2803) was chosen as our low side switch, to allow for higher current control by the MCU and integrates internal protection diodes. These constant current ICs are connected in parallel to allow for 4 level discrete stepping of LED intensity. The MCU reads the voltage across a rotary 10k potentiometer to control the intensity of the LEDs and the state of an on/off toggle switch is used to control LED color. A custom
cable was created by splicing the USB cable and custom LED ring power wires to a CAT6e Ethernet cord that connected into a female Ethernet jack that was panel mounted on an off the shelf external aluminum case housing the LED Driver Circuit, Secondary MCU, rechargeable LiFePO4 battery, and USB hub.

The Generation 2 Pocket Colposcope like its predecessor was cleaned with a Super Sani-Cloth (PDI Inc., Parsippany, NJ) germicidal wipe and then placed in a permeable container for Ethylene Oxide (EtO) gas sterilization prior the next patient use. However, future planned partner site studies are situated in resource-limited settings, where this form of sterilization was not readily available. Thus immersion sterilization with a chemical agent would be the main method for reprocessing the device between patient uses, in accordance with AAMI TIR12-2010 (109) and FDA Guidance document UCM437347(110) for a high level of disinfection and/or sterilization. The Generation 2 Pocket Colposcope device was successful in capturing images of the cervix when stained with acetic acid (VIA) and Lugol’s Iodine (VILI), had improved imaging contrast with cross polarization, and added green light inspection (GLI). Although, the system still required the use of anti-fogging wipes to prevent fogging of the tip when placed in the moist body cavity and would not survive reprocessing with liquid chemical immersion cleaning after each patient use.
2.3.1.4 Generation 3 Pocket Colposcope – Refinement of the LED driving circuit and device waterproofing

The Generation 3 Pocket Colposcope incorporates important lessons learned from the pilot clinical study at Duke University Medical Center (DUMC) of the Generation 1-2 device (100). Minor revisions were made to improve LED driver circuit, communication and LED power cable. Major revisions included the incorporation of waterproofing and hydrophobic window to eliminate the need for antifogging wipes, in preparation for multisite international clinical studies (104).

The LED driver circuit was further refined with a transistor-transitory logic (TTL) compatible multichannel metal-oxide semiconductor field-effect transistor (MOSFET), TPL7407 an almost pin for pin more efficient replacement for the ULN2803 Darlington transistor employed in the prior generation circuit. The TPL7407 has a lower voltage drop (0.2 V vs. 1.0 V at 100 mA output drain current) and improved response time (104). We also experienced periodic connectivity issues with the camera module when using the Ethernet cord to transmit the USB video information and LED power. After careful research, we identified that USB specifications require an impedance of 90 ohms for the data communication lines (111) which was not matched by Ethernet’s cable impedance of 50 ohms(112). This impedance is achieved by a specified twist rate for the data line cables and incorporates two layers of EMI shielding in the cable (111). If this requirement is not met the USB video signal would be compromised. The Generation 3 Pocket Colposcope addresses this issue with a a hybrid cord with a dedicated USB 2.0
cable and detected shielded stranded three wire cable for power the LEDs. The two cables are fed together into a medical grade heat shrink and expanded Polyethylene Terephthalate (PET) mesh jacketing (Techflex’s Flexo PET Sparta, NJ) and bifurcated at the end to allow for the USB to connect directly to the control device and the LED power cable to the box. A dedicated ruggedized USB 2.0 type A connector and specialized LED power connector was used to replace the fragile Ethernet interface with a more robust industrial connector rated for >10,000 mating cycles (Minicon MSCM12 and MRF12, Neutrik Inc., Schaan, Liechtenstein).

Thus, the Generation 3 Pocket Colposcope using a protective medical grade heat shrink outer casing sealed with ISO 10933 certified medical grade epoxy (Epotek’s MED-320LV and MED-301, Billerica, MA)(113), internal secondary protection medical grade and moisture wrap heat shrink to meet the IEC 60529’s IPX-7 waterproof rating standard (ingress protection marking) (114). Each device after fabrication was tested by immersion in a 2.0 liter graduated cylinder and immersed at a depth of 1 meter for 30 minutes. The Pocket Colposcopes would need to survive ten cycles of disinfection of at 20° C for 2.0% hydrogen peroxide at 8 minutes of immersion duration, 660-675 ppm active chloride (bleach) at 10 minutes of immersion duration, and Cidex OPA (0.55% ortho-phthalaldehyde) at 12 minutes of immersion duration. The first two reagents are the most commonly available solutions at our future field sites (109, 110). The device are tested for successful image capture with both illumination modes immediately after
removal and 30 minutes’ post-immersion. We then performed drop testing in accordance for IEC 62262 standard for mechanical impact to simulate a fall from examination table (height of 1 meter to concrete flooring) at six differential device geometric orientations for the target IK06 rating (115). The devices were tested immediately after each drop to ensure full functionality (successful image capture with LED illumination by both colors).

In order to remove the need for antifogging wipes before each patient use, we incorporated a protective, antireflection coated, hydrophobic window (#88-356; Edmund Optics, Barrington NJ) for the Generation 3 through Beta Pocket Colposcope. The hydrophobic window are placed into a custom computer numerical control (CNC) machine milled polycarbonate (1/16” thick) LED diffuser window, which has a lip machined out to hold the window in place and channels for each LED in the Gen. 4 Pocket Colposcope. The Generation 3 Pocket Colposcope maintained the prior generations crossed linear polarizers into the optical imaging pathway as previously described (100). This waterproof optical imaging barrier eliminated the need to use anti-fog wipes before each procedure (116).

The imaging performance of the hydrophobic optical window are evaluated in a simulated moisture rich environment by comparing it to a bare uncoated protective glass protective window (#83-359; Edmund Optics, Barrington NJ) and a protective glass window pre-treated with commercial anti-fog wipe (Bausch & Lomb’s Fogshield XP,
Rochester, NY). Three working prototypes were constructed with the different window configurations and misted with a dark green food coloring dye (FD&C Green No. 3). They were used to image a cervix phantom and cleaned with 70% isopropyl solution and lens wipe, and this is repeated three times. The commercial anti-fog wipe is reapplied after each cleaning as required by the manufacturer. These images were randomized and qualitatively scored by a blinded highly trained colposcopist from a scale of 0 to 10, where best image quality = “10” and the poorest image quality = “0”. A control set of images (no misting) are also included. The mean ± standard deviation of the image quality score for each system are calculated. Statistical differences between groups were assessed using one-way analysis of variance (ANOVA) (Stata 13.1 MP; STATA Corp, College Station TX).

The Generation 3 Pocket Colposcope addresses several shortcomings of the prior generation with the addition of the hydrophobic window and waterproofing strategies while retaining the improved contrast from cross-polarization and green light inspection (GLI) capabilities (104). Providers from the international clinical study sites disliked stiffness of the custom cable and reduced portability due to the required external LED driver circuit box (117). Thus, the next generation Pocket Colposcope would need to retain the waterproofing and antifogging components but would need to return to a more portable package. However, an alternative strategy to maintain the improved
imaging contrast without the cross-polarizers and external LED driver box would need to be developed.

2.3.1.5 Generation 4 Pocket Colposcope – ergonomic refinement and alternative strategies for increasing illumination

The Generation 4 Pocket Colposcope was redesigned to maximize the efficiency of the onboard LEDs without the need of cross-polarizers and external LED driver box using a specialized reflector surface incorporated into the device’s tip. However, this version of the Pocket Colposcope lost the ability for GLI (green light inspection).

A reflective surface incorporated into the Pocket Colposcope’s tip was designed with an optimized (height and angle) to provide consistent beam uniformity and increased optical power of the integrated white LEDs. SolidWorks 3D computer-aided drafting (CAD) software (Dassault Systèmes SolidWorks Corporation, Waltham, MA) was used to create virtual polished aluminum reflectors with various heights ranging from 0 to 4.82 mm and angles of reflection ranging from 15° to 75° in 15° increments. Next Zemax (Zemax LLC., Kirkland, Washington), a Monte Carlo, based ray tracing program (118) and to simulate the performance of different external polished aluminum reflectors (with angles of reflection 15° to 75° at 15° steps). Our simulation parameters were using 499,000 rays per LED (4 total) at the same geometry used in our working prototypes, with 50 mm x 50 mm detectors at 5, 30, and 50 mm working distances. We are interested in the reflector angle that provides the highest total optical power and beam pattern homogeneity at the three working distances of interests versus our original
collar design. These simulations will be confirmed by empirical testing using methods describe (briefly, beam pattern will be captured when projected onto a diffuser plate by a large format CMOS sensor). Of note, we will be revising our simulated detector density to match our empirical test set up with 60 pixels per mm versus the 1000 pixels per mm used in the preliminary testing, which would lead to significant undersampling if not corrected.

Figure 8: Schematic of Reflector Optimization Ray Tracing Experiments, this figure shows the layout of our computer-aided optimization of the angle and height of the reflective surface with the probe tip facing the cervix to the right. The probe (left to right) contains the camera detector, lens, light emitting diode (LED) ring, LED diffuser, and reflector cone (orange). The geometric position and optical illumination properties of our LEDs are taken from manufacturer provided data files. A clear polycarbonate diffuser was modeled and placed over the concentric LED ring. Plate beam detectors are placed in the simulation at working distances from 5, 30, and 50 mm (yellow). These working distances are representative of the range of highest,
most commonly used, and lowest magnifications of our system. Three-dimensional models of each reflector design are placed into the ray-tracing simulation. The reflector angles ($\Theta_i$) ranged from 0 to 75 ° degrees in 15° increments (orange). An outer probe diameter limit of 18 mm limited the range of reflector heights ($Z_i$) from 0 - 4.82 mm. The effect of the reflector height alone is also investigated, where the reflector angle ($\Theta_i$) are fixed at 90°, and the reflector heights ($Z_i$) are varied from 0 - 4.82 mm.

For each reflector design, simulations of light delivery were implemented using Zemax Optic Studio (Zemax LLC; Kirkland, WA). The manufacturer provided LED beam patterns and ray databases are used in our simulations and matched the exact make and model used by our prototypes. The simulation parameters are set using 499,000 rays per LED (4 total) with light beam detectors spaced at intervals of 5, 30, and 50 mm away from the tip of the probe’s camera parallel to the optical imaging axis. These working distances were selected to demonstrate the range of possible magnifications achievable with the Pocket Colposcope. The light intensity patterns on these detectors illustrates the cross-sectional beam shape as a function of working distance. The beam shapes are plotted with the x- and y-axis as the dimensions of the detector, and the z-axis is plotted as a heat map color-coded for optical power. Horizontal line scans through the center of beam shapes were used to characterize the homogeneity of the beam pattern at the various working distances.

The reflector angle that provided the highest total optical power and beam pattern homogeneity at the three working distances was rapidly prototyped using a medical grade Acrylonitrile butadiene styrene (ABS) plastic extrusion printer (Dimension 1200es Stratasys Inc., Eden Prairie MN). The inner reflector is polished with
sandpaper, and silver metallic paint is applied to the smooth inner surface. The Generation 4 Pocket Colposcope was waterproofed using a similar method as the Generation 3 device described previously (104).

Figure 9: Generation 4 Pocket Colposcope Summary - Exploded View, Functional Block Diagram, and Representative Image of Prototype. The exploded view (A) the USB cable provides power to the camera and integrated white LEDs, (2) 3D ABS plastic strain relief, (3) 3D printed ABS plastic rear handle, (4) silicone O-ring, (5) 5MP color CMOS camera with autofocus lens box, (6) concentric White LED ring, (7) polycarbonate LED diffuser, (8) hydrophobic window, (9) 3D printed ABS plastic probe tip with integrated reflector surface.

Although, Generation 4 Pocket Colposcope device was successful in capturing images of the cervix when stained with acetic acid (VIA) and Lugol’s Iodine (VILI), had improved imaging contrast comparable to cross-polarization, eliminated the need for the use of anti-fogging wipes with the hydrophobic window, and maintained the chemical
immersion sterilization compatibility from Generation 3 system (104). However, the lack of magnification adjustment capability, lack of green light inspection (GLI) capability for enhanced vessel interrogation, and important ergonomic feedback to incorporate image capture and illumination mode selection are requested by pilot study site clinical users and key informants (117, 119). The lack of green illumination capability for improved vessel imaging, a higher frequency of blurred images when compared to a standard-of-care colposcope, and the higher prevalence of specular reflection were key limitations reported when using an unmodified smartphone for cervical imaging (120, 121). A penultimate version of the Pocket Colposcope should incorporate the vast design experiences and user feedback from the Generation 1 through 4 devices (100, 104, 117, 122).

2.3.1.6 Penultimate Alpha and Beta Pocket Colposcope

Through a National Institutes of Health (NIH) Academic-Industry Partnership (AIP) funding opportunity R01-CA193380, Third Stone Design (San Rafael, California) an engineering consulting firm partnered with Duke University towards translation of the Pocket Colposcope into a commercial device. First, the penultimate iteration of the Pocket Colposcope was designed in collaboration with Third Stone Design and incorporated the following: improvements in the ergonomics such as buttons on the device for image capture and LED selection, and a simpler to use adjustable magnification mechanism, and incorporating design and material selection
considerations for scale manufacturing. Also this iteration of the Pocket Colposcope should maintain prior key features such as: GLI (green light inspection) capability (100), chemical immersion cleaning compatibility (104), the fog resistant hydrophobic window (104), and enhanced portability (Generation 1 and 4) without the need for external LED driver box (100, 104).

Briefly, the core components of the Pocket Colposcope were unchanged in specification and imaging performance from Generation 2 through Beta, with the identical CMOS camera, auto-focus lens module, and higher power White and Green LEDs, and hydrophobic window retained. The long straight tubular shape of prior generations of Pocket Colposcope are redesigned with regards for improved user comfort with an angled handle and ease of scale production and assembly. Key informants (clinicians who had used the Pocket Colposcope as part of clinical trials or review images as part of the concordance study) are presented with looks like prototypes made from foam. The most highly rated design was then 3D drafted and alignment of all the electrical and optical components is create to ensure fitment by engineers at 3rd Stone Design. Injection molding with medical grade plastic are used instead of 3D printed ABS plastic to reduce the per-unit cost at volume when at scale production. Instead of multiple layers of medical grade heat shrink, epoxy, and superglue, the components would be ultrasonically welded wherever possible for enhanced durability. The camera’s MCU was redesigned by 3rd Stone Design to allow
for integration of the high power white and green LED drivers and still be powered off
the USB port (eliminating the need for the external control box). Two capacitive buttons
are also added in front and behind of the slider for user control of image capture and
illumination selection. A custom designed flexible PCB ribbon is used connect the MCU
to the camera module and autofocus lens module to allow for the slider mechanism for
magnification selection. This novel implementation by 3rd Stone Design eliminated the
rotational twist motion previously required to move the detector with respect to the
fixed lens module.

An initial pilot manufacturing run of four Alpha Pocket Colposcopes was used to
evaluate the magnification adjustment slider and check if imaging performance matched
that of prior generations of Pocket Colposcopes. When tested there was the issue of
image vignetting or clipping (where the outer portions of the field of view are obscured
by an intermediate structure) when at the low magnification setting. We identified that
the light baffle’s narrow pipe-like shape (blue outline) Figure 10C was blocking the full
viewing angle of the lens. We redesigned the baffle with a conical shape (Figure 10D)
that matched the viewing angle to eliminate the vignetting issue. The light baffle is used
to prevent any stray environmental light or illumination light into the optical pathway
of the camera which could lead to ghosting or light bloom on the detector. In prior
generations a thin layer of light absorbing medical grade epoxy was careful applied to
the interior of the clamshell and around the circumference of the LED diffuser (Epotek
3rd Stone Design built a larger preproduction batch of 30 Beta Pocket Colposcopes and tested by Duke University to quantify the imaging and illumination performance against a predicate digital colposcope and allow for testing as part of an on-going multisite clinical study.
Figure 10: Penultimate Pocket Colposcope Exploded View, Functional Block Diagram, and Alpha vs. Beta changes. (A) the USB cord enters the back half of the Pocket Colposcope through a molded strain relief (1) and connects to the redesigned MCU (3), the user can select from 3 to 52X magnification slider mechanism (2, 4, 5, 6) translates linear motion to the CMOS camera (7) with respect to the fixed autofocus lens module (8), an opaque light baffle (9) holds the hydrophobic window in place.
prevents stray light from entering the lens and sits tightly with the white and green LED ring (10), a polycarbonate LED diffuser (11) mates with the baffle and probe tip (12) to prevent liquid or dust infiltration into the device. The light baffle is redesigned from the narrow cut-off of the Alpha (C) to the conical opening of the Beta (D) due to image vignetting issues caused by the previous shape of the baffle.

The Beta generation of the Pocket Colposcope was also tested for electrical safety and essential performance (IEC 60601-1), electromagnetic compatibility (IEC 60601-1-2, IEC 61000-4-2 through 11, and CISPR 11 / EN 55011), and biocompatibility (ISO 10993-1), by several independent accredited safety laboratories as part of the US FDA’s 510(k) submission. The 510(k) pathway is a premarket submission made to US’S FDA to demonstrate that the device to be marketed is at least as safe and effective, that is, substantially equivalent, to a legally marketed device (21 CFR 807.92(a)(3)) that is not subject to premarket approval (PMA). On September 21st, 2018 the Pocket Colposcope received US FDA regulatory approval for the Beta Pocket Colposcope K181034(94).

2.3.5 Imaging Performance Characterization

The following tests are conducted on the Pocket Colposcope based on a review of prior predicate digital colposcope 510(k) submissions: Optical Resolution, Field of View, Distortion, and Depth of Field. These tests were chosen to demonstrate a comparable level of essential imaging functionality when compared to these reference predicate devices (colposcopes, 21CFR884.1630). Colposcopes are considered Class II devices that permit direct viewing of the tissues of the vagina and cervix.
2.3.5.1 Image Quality Equipment and Test Fixture

An image quality testing platform was constructed using commercial off the shelf components consisting of sliding dovetail rails for coarse adjustment, XYZ translational stage with Vernier adjusters allowing for 10-100 µm fine adjustments in all three axes (Figure 11). These optical components were mounted on an optical table with active auto leveling (#T46HK Thorlab Inc., Newton NJ) to provide continuous protection from vibration and maintaining test fixture alignment. An off-the-shelf illumination system was adapted using White (D50/5000K) LED sources with custom 3D printed mounts for the 120 grit ground glass diffusers were used to improve light uniformity. Testing is conducted in a specially constructed imaging chamber with the matte black light absorbing coating. Working distance is measured with a Bosch DLR130 Laser Distance Tool, which has a range of 50 to 39,624 mm, with accuracy of ±1.6 mm, and with precision of ±1.6 mm and a Mitutoyo Digimatic 500 series digital caliper with range 0 to 150 mm, with accuracy ±0.01 mm, and with precision ±0.01 mm. A BK Precisions 615 light meter, which has a flat detector surface, was used to capture incident light (lux) on the imaging targets for use in the image quality calculations and to ensure uniform target illumination.
Figure 11: Imaging target testing fixture, (1) Imaging target, (2) Thorlab XYFM1 target translation stage, (3) Newport 433 Z-axis target translation stage, (4) Collimated White LED sources with diffuser, (5) Tested Device, (6) V-mount clamp, (7) dovetail sliding rail for coarse z-axis adjustment. (B) the 5000 K LED white light sources were positioned between 20° and 40° from the horizontal of the imaging target to provide uniform lighting and to minimize specular reflection from the imaging target, per ISO 12233 guidelines

2.3.5.2 General Image Quality Testing Procedure

The testing schedule is outlined as follows: at least six sets of images per target were captured and analyzed for each system, the mean and standard deviations were calculated where possible in Microsoft Excel. The device was placed coarsely into position, and the using of precision target translation stage was used to set the working distances specified. The digital caliper was used to record the working distance before each image capture. The probe was coarsely moved by at least 1 mm after each image capture and reset to the specified working distance with the translation of the precision
stage and verified with digital calipers. The illumination lux was measured before each image capture and verified to be > 500 lux. Objective image quality parameters is calculated with these targets using the ImageJ, Imatest, and Matlab software packages (123).

The probe is placed and secured into v-clamp shown previously in Figure 11A. The proximal (imaging end) of the probe are cleaned with Edmund Optic’s Eclipse High Purity Len’s Cleaning Fluid (Edmund Optics, Barrington, NJ, #59-638) and lint free lens paper. The imaging target was cleaned similarly to remove smudges or dust. Unless otherwise stated and according to ISO 8600-3 Section 3.4 which permits the use of either the internal integrated white lights of the endoscope or the use of external white light source, but does not specify the orientation of the illumination (124). The orientation of lights was selected to follow ISO-12233:2007 recommended illumination set up with two sources positioned between 20° and 40° from the horizontal of the imaging target to provide uniform lighting and to minimize specular reflection from the imaging target as seen in Figure 11 (125). A white light illumination source with a minimum intensity of 500 lux shall be provided, using either the integrated illumination of the endoscope or an external source (124, 126). Generally, for all the imaging tests, the minimum accuracy of the test method used to measure the various imaging targets shall be ±5° for rigid endoscopes, which most closely matches our device configuration (126). Our optical
testing hardware and measurement tools are described above (Figure 6) and meet the ±5° error margin.

2.3.5.3 Field of View and Direction of View

The purpose of this test was to provide objective evidence documenting the field of view and direction of view of the Pocket Colposcope is comparable to predicate devices, according to ISO 8600-3:1997 (Amendment 1:2003), “Optical and photonics – Medical endoscopes and endotherapy devices – Part 3: Determination of field of view and direction of view of endoscopes with optics” (124) and ISO 12233: 2017, “Photography -- Electronic still picture imaging -- Resolution and spatial frequency responses” (125).

The Field of View (FOV) is defined in ISO 8600-3 standard, as the view of an endoscope with optics (or similar imaging device), expressed as the vertex angles (in degrees) of the cone whose vertex is at the distal window surface of the imaging device (124). The direction of view is defined in ISO 8600-3 standard, as the angular positions in degrees of the protractor relative to the perpendicular imaging axis of the endoscope(124). For the Pocket Colposcope and predicate standard-of-care devices, the viewing angle is 0°. Per ISO 8600-3 the following components were used during testing: 1) An optical bench that will support the optical device being tested and allowing for adjustment of the central axis of the field of view to the center of the target to measure the field of view at a set working distance in millimeters (WD). This working distance
(WD) is often set at 50 mm between the distal window surface of the optical device and the center of imaging target, 2) an imaging target have circles showing the field of view (β) in degrees with major divisions marked at every 10° with the corresponding number in degrees and four finer marks indicating every 2° between each major division. The field of view can be calculated from equation (1) when using the ISO 8600-3 circular ring target with the formula described below:

\[ \text{Equation 1: } T = 100 \times \tan \left( \frac{\beta}{WD} \right) \]

The working distance (WD) is set at 50 mm, and the working distance defines as the distance from the most distal optical window of the device and the imaging target. Per ISO 8600-3, Section 4.4, the angular field of view (β) is determined as the largest visible circle, and for a non-circular image, only segments of the largest circle may be visible. The angular field of view (β) can be determined using equation (2), where the radius (r) of the largest visible circle on the target with units of mm and the working distance, WD, also in a unit of mm. The direction of view in angular position, in degrees, is made with a protractor relative to the perpendicular axis of the imaging device per ISO 8600-3 Section 4.5(124).

\[ \text{Equation 2: } \beta = 2 \times \tan^{-1} \left( \frac{r}{WD} \right) \]

Per the 2003 Amendment 1 to the ISO 8600-3 standard, Page 2, subclause 4.2, we will test and report at designed working distances (WD) of 5, 35, and 50 mm in lieu of the single working distance of 50 mm as our device is capable of a continuous range of
working distances from 5 to 50 mm (124). Per Amendment 1 from 2003 to the ISO 8600-3 standard, Page 2, subclause 3.3, we will substitute the specified ISO 8600-3 concentric circular ring target with the ISO-12233:2007 compliant imaging test target, SFRplus, (see Figure 12) which allows us to measure at a range of working distances that our device is capable of and not the singular 50 mm working distance as prescribed in the ISO 8600-3 standard (124, 125).

![ISO 8600-3 FOV and Imatest SFRplus](image)

**Figure 12**: ISO 8600-3:1997 target (left) and the substitute target for FOV determination ISO 12233:2007 compliant SFRplus target (right)

The working distance of 35 mm, were chosen to calculate the angular field of view ($\beta$) as it is the one that provides the full view of the majority of cervixes and minimizes the background structures such as the speculum or other retractors. The range of working distances was also tested for our device at 5 and 50 mm for further characterization as they represent the maximum and minimum magnification ranges of
our Pocket Colposcope. The field of view parameter are calculated with these images of these SFRplus targets and the Imatest (Boulder, CO) software packages (123).

The field of view can be calculated by Equation 3 using the known active area linear measurement (LAAM) which could consist of the height, width, or diagonal of the SFRplus imaging target, in units of mm, and the definition of a tangent (opposite over adjacent) for right triangles. The angular field of view is equal to 2 times the inverse tangent of ratio of the length of the opposing leg (½ the linear active area measure (height, width, or diagonal)) to the length of the adjacent leg of the right triangle (working distance from the probe tip to the target), which is equivalent to Equation (2), where the ½ the diagonal linear value is concordant with the radius of the largest visible inscribed circle if one were using the ISO 8600-3 circular target.

Equation (3): Field of View (°) = \(2 \times \tan^{-1}\left(\frac{\frac{1}{2} \text{LAAM}}{\text{WD}}\right)\)

2.3.5.4 Optical and Angular Resolution

Per ISO 8600-5 an imaging target having adequately graduate resolution test patterns arranged at least in two directions, preferably in the horizontal and vertical direction in the range of 1-line pair per mm (lp/mm) to 100 lp/mm(126). The optical and angular resolution will be measured at the center of the image axis (on-axis) and four congruent quadrants at 70% of the total image height (off-axis)(126).
The optical resolution ($r(d)$) is defined in ISO 8600-5 Section 2.2 as the maximum number of line pairs per millimeter which can be just perceived at a given working distance ($WD$) of the device(126). A line scan is performed across the horizontal and the vertical bars to confirm three distinct peaks can be observed to confirm the “just perceived” line pair measurement.

**Figure 13:** Schematic for on-axis and off-axis resolution testing, schematic for the five imaging positions test for on-axis characteristics at position A and off-axis characteristics at position B1 to B4 for ISO 8600-5 and ISO 12233 testing. The Maximum Image Height (MIH) was defined as the diameter of the largest inscribed circle. Positions B1 to B4 were located at 70% of the MIH in 4 congruent quadrants.

The working distance of 35 mm, were chosen to calculate the angular field of view ($\beta$) as it is the one that provides the full view of the majority of cervixes and
minimizes background structures such as the speculum or other retractors. The range of working distances was also tested for our device at 5 and 50 mm for further characterization as they represent the maximum and minimum magnification ranges of our Pocket Colposcope. The optical and angular resolutions are calculated with the USAF 1951 target using the open source FIJI image processing package (127). The sampling sites are chosen as indicated in Figure 13 for the 35 and 50 mm working distances. At the 5 mm working distance, a single slanted square are translated through the five positions (A, B1, B2, B3, and B4).

2.3.5.5 Magnification

The purpose of this test was to provide objective evidence documenting the magnification of the Pocket Colposcope is comparable to predicate devices using ISO 18221:2016, “Microscopes- Microscopes with digital imaging displays –information provided to the user regarding imaging performance” (128). We used in the magnification calculations data collected according to ISO 8600-3:1997 (Amendment 1:2003), “Optical and photonics – Medical endoscopes and endotherapy devices – Part 3: Determination of field of view and direction of view of endoscopes with optics” (124), ISO 8600-5:2005, “Optical and photonics – Medical endoscopes and endotherapy devices – Part 5: Determination of optical resolution of rigid endoscopes with optics” (126) and ISO 12233: 2017 “Photography - Electronic still picture imaging - Resolution and spatial frequency responses” (125).
The optical magnification, \((M_{TOT\ PROJ})\) is defined in ISO 18221’s Section 5.1, as the lateral magnification at the image projected onto the image sensor and should be expressed in the proportional form, e.g., 10:1 (128). Display magnification, \((M_{DIS})\) is the defined in ISO 18221’s Section 5.2, as the lateral magnification at the digital image presented on the digital display or monitor and should be expressed in the proportional form, e.g., 100:1 (128). Visual display magnification, \((M_{DIS\ VIS})\) is the defined in ISO 18221’s Section 5.3, as the lateral display magnification by observing the digital image presented on the digital display and should be expressed in numerical form with the multiplication sign, e.g., 50x (128).

The visual display magnification is given by equation (3): 

\[
M_{DIS\ VIS} = \frac{M_{DIS} \times 250\ mm}{d_{view}},
\]

where \(M_{DIS\ VIS}\) is the visual display magnification, \(M_{DIS}\) is the display lateral magnification, 250 is the reference viewing distance in mm, and \(d_{view}\) is the actual viewing distance in mm, which we will set at 300 mm, typical of prior predicate colposcopes. It is important to note that ISO 18221’s Section 5.3 states that \(M_{DIS\ VIS}\) is comparable with the value of \(M_{TOT\ VIS}\) of analog light microscopes with eyepieces. We will report general information and imaging performance data (magnification) for our system according to guidelines provided in Annex A of ISO 18221(128).
Table 1: Summary of Pocket Colposcope’s Optical System Specifications

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td>Lenses</td>
<td>5 element (Prime)</td>
</tr>
<tr>
<td>ii.</td>
<td>Fine Focus (auto)</td>
<td>Voice Coil Module</td>
</tr>
<tr>
<td>iii.</td>
<td>Coarse Focus (manual)</td>
<td>Physical Slider</td>
</tr>
</tbody>
</table>

Table 2: Summary of Pocket Colposcope’s Detector Specifications

<table>
<thead>
<tr>
<th>Component</th>
<th>Description / Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td>Sensor Type</td>
<td>RGB CMOS</td>
</tr>
<tr>
<td>ii.</td>
<td>Sensor</td>
<td>5.04 megapixels</td>
</tr>
<tr>
<td>iii.</td>
<td>Pixel Pitch ($PP_{sen}$)</td>
<td>1.4 microns</td>
</tr>
<tr>
<td>iv.</td>
<td># of pixels in x-direction ($N_x^{SEN}$)</td>
<td>2592 N/A</td>
</tr>
<tr>
<td>v.</td>
<td># of pixels in y-direction ($N_y^{SEN}$)</td>
<td>1944 N/A</td>
</tr>
<tr>
<td>vi.</td>
<td>Standard imaging mode</td>
<td>Full frame no binning</td>
</tr>
<tr>
<td>vii.</td>
<td>Digital Zoom</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 3: Representative Digital display Specifications paired with the Pocket Colposcope

<table>
<thead>
<tr>
<th>Display</th>
<th>Component</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td>Lenovo ThinkPad Helix 2 Tablet/Laptop</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>ii.</td>
<td>Pixel Pitch ($PP_{DIS}$)</td>
<td>0.3401 mm</td>
<td></td>
</tr>
<tr>
<td>iii.</td>
<td>Aspect Ratio</td>
<td>16:9</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Full screen imaging mode with a 16:9 aspect ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv.</td>
<td># of pixels in x-direction ($N_x^{DIS}$)</td>
<td>1920 N/A</td>
<td></td>
</tr>
<tr>
<td>v.</td>
<td># of pixels in the y-direction ($N_y^{DIS}$)</td>
<td>1080 N/A</td>
<td></td>
</tr>
<tr>
<td>vi.</td>
<td>Lenovo ThinkPad 13 2 Laptop</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>vii.</td>
<td>Pixel Pitch ($PP_{DIS}$)</td>
<td>0.3897 mm</td>
<td></td>
</tr>
<tr>
<td>viii.</td>
<td>Aspect Ratio</td>
<td>16.9</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Full-screen imaging mode with a 16:9 aspect ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ix.</td>
<td># of pixels in x-direction ($N_x^{DIS}$)</td>
<td>1920 N/A</td>
<td></td>
</tr>
<tr>
<td>x.</td>
<td># of pixels in the y-direction ($N_y^{DIS}$)</td>
<td>1080 N/A</td>
<td></td>
</tr>
</tbody>
</table>
2.3.5.6 Determination of Distortion

Per ISO 9039 (Annex B) we will test and report distortion at working distances (WD) of 5, 35, and 50 mm instead of the single working distance of 50 mm as our device is capable of a continuous range of working distances from 5 to 50 mm. An ISO 9039 and ISO 12233 compliant imaging test target, SFRplus was used for testing (Figure 14) (129). The ISO 9039 standard does not require measurements at on-axis and off-axis positions, but rather uses the full field of view.

The picture-height distortion (PHD) can be calculated from equation (4) when using the ISO 9039 compliant target with a rectangular shape below (129):

\[
\text{Equation 4: } PHD = \frac{100\Delta H - H}{H}, \text{ and } \Delta H = \frac{A_2 - A_1}{2},
\]

where \( A_1 \) and \( A_2 \) are the outer side lengths of a rectangle and \( H \) is the distance between the midpoints of the sides of the rectangle \( A_1 \) and \( A_2 \). The PHD value is positive if the corner of the image format boundary is shifted away from the center of the format (pincushion distortion) and is negative if it is shifted towards the center (barrel distortion).

The following polynomial equation is used to complement equation (4) to determine the distortion beyond the active field of view.

\[
\text{Equation (5): } r_u = r_d + h_1 r_d^3 + h_2 r_d^5,
\]

where \( r_u \) is the undistorted radius and \( r_d \) is the distorted radius are both normalized to the center-to-center distance. If \( h_1 \) & \( h_2 \) are both equal to 0, there is no distortion, if \( h_1 \) & \( h_2 \) are < 0 there is pincushion distortion, and \( h_1 \) & \( h_2 \) are > 0 there is barrel distortion (Figure 14).
Figure 14: Representative Picture Height Distortion (PHD) Target, (A) ISO 9039 sample calculation with (1) inscribed distortion rectangle, (2) actual detector format, (B) Imatest SFRplus target used to measure distortion, representative effects of distortion with (C) no distortion, with (D) negative distortion value or barrel (D), and (E) positive distortion or pincushion (126, 129, 130)

2.3.5.7 Depth of Field

The optical resolution threshold we selected is five lp/mm (based on target availability) and no prior predicates reporting their respective threshold value. We also will be measuring at the center of the image axis (on-axis) using FIJI open source software package (127) at working distances of 5, 35, and 50 mm. The depth of field target (#DA035, Max Levy Autograph Inc., Philadelphia, PA) with horizontal and
vertical Ronchi rulings at five lp/mm. Between the ruling groups are accurate linear scales with a range of 50 mm in length with 0.05 mm divisions. The target made of high contrast film on an aluminum block with a 45° orientation with respect to the imaging axis (Figure 15).

![Figure 15: Depth of Focus target, high contrast film on aluminum block with 45° and planar (°) imaging testing with 5 lp/mm in the horizontal and vertical orientation. There is also a linear scale 50 mm in length with 0.05 mm graduations.](image)

2.3.5.8 Qualitative Image Quality – Simulated Cervix phantom

In-vitro imaging of simulated high grade and normal cervixes in a life scale educational mannequin (Gaumard Zoe S504.100 (Gaumard Scientific, Miami FL)) was
performed using the Pocket Colposcope’s system and other digital colposcopy and cervicography systems. Images captured from this testing help confirm that the Pocket Colposcope could function in enclosed space at the designed working distance and compatibility with a speculum and provide standardized a method for assessing techniques for improving contrast. Weber’s contrast are measured with Equation (6):

\[ C_w = \frac{I_B - I_F}{I_B} \]

where, \( I_B \) = Background Pixel Intensity and \( I_F \) = Foreground Pixel Intensity.

2.3.6 Illumination Performance Characterization and Safety

2.3.6.1 Illumination Characterization

Surface light intensity measurement per section 1.2.10 of ANSI/NEMA FL-1 and it is defined as surface illuminance and is measured in units of lux(131). Per ANSI/NEMA FL-1 Section required equipment include a light measuring device is a commercially available device that is used to measure the amount of light striking a surface from a given source. The light measuring device should be calibrated to the CIE eye response curve and report in values of lux. A BK Precision 615 Light Meter (B&K Precision Instruments (Yorba Linda, CA)) met these requirements and are used for these illumination characterization measurements (131). Per section 1.3.1 of the standard, the minimal sample size is 3 (individual devices)(131).

Testing will be conducted at lab conditions per 2.1.1 of ANSI/NEMA FL-1, a dark location where ambient conditions determine to be less than 1 lux in the entire test area prior to the start of the test and controlled temperature of 22±3 °C, relative humidity of
50% nominal and 80% maximum (131). Permitted deviations from ANSI/NEMA FL-1, the standard is for flashlights and uses distance of 2, 10, or 30 meters per the typical usage of such devices, however for our device our working distances are between 5 and 50 mm, thus we use the working distances of 5, 35, and 50 mm to take measurements. We also included 300 mm working distance to allow for direct comparison to similar colposcope systems. Beam diameter are measured as the distance in the lateral or vertical translation of the device with respect to the central axis midpoint of the detector, where the surface light intensity falls to 50% of the (full width half maximum) with the working distance held fixed. Note: To our knowledge, there doesn’t exist a standard for this parameter, but remains an oft-reported item, as identified by other similar devices.

The testing schedule is outlined as follows: at least 2 sets of measurements per device, with 3 probes devices tested, the mean and standard deviations are calculated where possible. The device was placed coarsely into position, and the using of precision target translation stage was used to set the working distances specified (Figure 16). The digital caliper was used to record the working distance before surface light intensity measurement. Once the central surface intensity are captured 3 times per device, the light intensity probe was coarsely adjusted first in the lateral (x-axis) in 1 mm increments away from the centered aligned axis until the lux value fell to 50% of the peak value. This distance would be recorded as the beam radius, and then the light intensity probe is re-centered at the origin (central imaging axis) and translated in the vertical (y-axis) in 1
mm increments away from the centered aligned axis, again until the lux value fell to 50% of the peak value. These measurements are repeated two more times in the opposing directions for y and x-axis, respectively. The illumination range (beam diameter) is determined as two times the mean of these radius measurements.

Figure 16: Representative test set up an image of the Pocket Colposcope in the testing apparatus. (A) the probe (1) is secured and aligned at a set working distance (5, 35, or 50 mm) in the v-clamp block (3). The lux meter (2) is connected by a wire to the lux sensor (5) which is held by 3-way axis target holder (4) that allows for fine adjustment to precisely align the lux meter probe with the center imaging axis of the probe, see (B) the origin is defined as the central imaging axis with respect to the center of the probe (green arrow) and allows for precise lateral (x-axis, orange) and vertical (y-axis, purple). Thus, translation to capture the beam diameter at each fixed working distance. If the lateral translation was greater than the range of the target translator, the probe and v-clamp assembly are moved to a parallel dovetail rail (hidden) to allow for full range detection.

2.3.6.2 Photobiological Safety

Labsphere integrating sphere coated with Spectralon 99% diffuse reflective coating was used to assess the illumination source optical power and spectra, with custom 3D printed port adapters for a collimating lens to SMA fiber for spectra capture and an OP-VIS2 power meter sensor (Figure 17). Integrating sphere is used to eliminate illumination hotspots and normalize power measurements across systems. The Optical
Power (mW) and Irradiance (mW/cm²) of each system were captured five times with a Coherent Fieldmax II-TO meter (Coherent Inc., Santa Clara CA), with the mean and standard deviation calculated for each parameter. The spectra of each system are characterized using an Ocean Optics USB4000 spectrometer coupled to a 1.0mm diameter fiber attached to the integrating sphere. Five sets of spectral measurements with dark correction are captured for each system, with an integration time of 100 ms. In order to prevent oversaturation, varying 1” diameter neutral density filters were placed in the optical pathway to attenuate the light signal using an Ocean Optics FHS-UV in-line filter holder.
Figure 17: Illumination Characterization and Photobiologic Safety Test Fixture

(A)(1) Spectral sampling port with triplet collimating lens and SMA terminated housing, (2) Optical Power Sensor port, (3) Spectralon coated Integrating Sphere, (4) V-Clamp Probe Holder and dovetail rail for coarse probe alignment and adjustment, (5) Probe being tested, (6) micrometer equipped linear translation stage for fine positional adjustment. (B) The simplified schematic layout of a light path from tested devices within the integrating sphere. The baffle prevents direct illumination of optical power sensor and spectrometer to prevent oversaturation.

The Pocket Colposcope and other digital colposcopy systems use broadband white light sources, so there is little potential for eye and tissue damage. Maximum permissible emissions (MPE) were measured and calculated using IEC 62452(132). The Pocket Colposcope illumination sources are in the visible with little to know ultraviolet or infrared spectra due to the use of LEDs. The two primary safety concerns being Blue Light Corneal Hazard, $B(\lambda)$ and Retinal Thermal Hazard, $R(\lambda)$. The Optical Power...
(mW) and Irradiance (mW/cm²) of each system are captured with a Coherent Fieldmax II-TO meter (Coherent Inc., Santa Clara CA) and OP-VIS2 and OP-IR2 detectors. The photochemical blue light hazard metric is spectrally weighted for radiance, equation (7):

\[ L_B = \sum_{300\,\text{nm}}^{700\,\text{nm}} L_\lambda \cdot B(\lambda) \cdot \Delta\lambda. \]

\( L_B \) is weighted radiance in W*cm²*sr, \( L_\lambda \) is the spectral radiance with units W*cm²*nm⁻¹, \( \Delta\lambda \) is the calculation interval in nm, and \( B(\lambda) \), is a weighted function of blue light hazard from 300 nm to 700 nm.(133).

The retinal thermal hazard metric is spectrally weighted for irradiance, equation (8):

\[ L_R = \sum_{400\,\text{nm}}^{1400\,\text{nm}} L_\lambda \cdot R(\lambda) \cdot \Delta\lambda. \]

\( L_R \) is the weighted irradiance in W/cm², \( L_\lambda \) is the spectral radiance in W*cm²*nm⁻¹, \( \Delta\lambda \) is the calculation interval in nm, and \( R(\lambda) \), is a weighted function of burn hazard from 400 to 1400 nm (133). The risk group for exemption is 10 W/cm²*sr for Blue Light Hazard \( L_B \) at 10,000-second exposure timeframe and Retinal Thermal \( L_R \) is \( 2.8/\alpha \) W/cm²*sr or 25.45 W/cm²*sr for a 10-second exposure timeframe (133). Here \( \alpha \) is the angular subtense of the source in radian defined by 50% peak radiance points, \( L \) as the beam diameter and the working distance \( R \), and the subtended angle \( \alpha = L / R \) (radians). However, from IEC 62471 guidelines if the angle \( \alpha > 0.11 \) radians, 0.11 radian is used and if \( \alpha < 0.011 \) radian, 0.011 is used in the calculation for the MPE limits(132). The results from the MPE calculations would provide the optical safety of each system’s illumination source and if beyond the “Exempt” limit need to have precautions taken to prevent patient and operator eye
damage including the appropriate PPE (personal protective equipment) and device labeling.

2.3.7 Control Software Evolution

Frontline health workers trained to conduct a visual inspection of the cervix should be able to deploy the Pocket Colposcope and obtain colposcopy images that will duplicate what is achievable with state of the art colposcopy. Once images are captured, they can then be transmitted to tertiary centers and reviewed by expert physicians so that referrals are made for only those women who require treatment. The images along with the health worker and physician diagnosis and when available, a confirmatory biopsy can be uploaded to a database that can eventually be utilized as a virtual training tool for dynamic improvements in quality control and improve the quality of training of future health workers. Data can also be archived for retrospective or longitudinal studies. For control and communication with the Window O/S based devices, a custom design GUI created in Matlab 2013b was developed by a fellow graduate student Mercy Asiedu to allow for image capture control, labeling, the storage location of image files (Figure 18) (104). The following support libraries or software toolboxes are required: ArduinoIO, Image Acquisition Toolbox, Image Processing Toolbox (134, 135). The microcontroller is programmed in the open source Arduino language (136). We use TeamViewer Enterprise secure remote access applications to allow us to troubleshoot systems and deploy updated software to systems deployed in the field (i.e., TeamViewer
Enterprise). Every 12 months remotely deployed systems need to connect to the Duke network via VPN in order to renew the Matlab license. On-going work to allow for a compiled version that could run independently of a Matlab license was pursued and not successful due to an issue with the Image Acquisition Toolbox not being available for stand-alone use.

Figure 18: Representative screenshots of the Matlab based graphical user interface and off the shelf Android app. (A) The image acquisition control Matlab Graphical User Interface (GUI) developed by fellow graduate student Mercy Asiedu. (Top left) Users enter the numeric patient ID number, select from procedure type from the dropdown bar, (far left) different types of image capture modes allows for burst image capture (3-5 images) that are auto-named and saved to the patient folder, (lower left) last image capture review, (center) real-time image preview. (B) Deployed version of our GUI on a Dell Venue 8 Pro Tablet (Windows 8.1 and Matlab 2013b) connected via USB 2.0 port to Pocket Colposcope, (C) Google Nexus 5 smartphone using commercial off the shelf camera control app (USB web camera Pro) connected via micro-USB to OTG (on-the-go) adapter to Pocket Colposcope.
2.4 Results

2.4.1 Design Summary for the Evolution of Generation 1 through Beta Pocket Colposcope

The Pocket Colposcope is shaped like a tampon and can be inserted and positioned such that it is 5-50 mm away from the cervix, obviating the need for high-end glass optics, high-resolution cameras, and high power illumination sources used in state of the art colposcopes, which typically operate at a working distance of 300 mm. An off the shelf miniature color complementary metal-oxide-semiconductor (CMOS) camera, with plastic injection molded lens, and small light emitting diodes (LEDs) could be repackaged inside the compact tampon like form factor and powered directly by a smartphone, tablet, or laptop through the universal serial bus (USB). The evolution of the Pocket Colposcope is highlighted in Figure 19 and Table 4, with changes in improvements in the ergonomic form factor and the introduction of key components to improving the performance of the system.
Figure 19: Ergonomic Evolution of the Pocket Colposcope, the iterative development of the system from Generation 1 Proof of Concept (A), Generation 2 (B) which added an external power box for increased illumination power, cross polarizers to reduce specular reflection, Generation 3 (C) which incorporated a hydrophobic window to reduce fogging and added waterproofing, Generation 4 (D) which eliminated the external box by using a reflector collar, Alpha (E) added improved ergonomics, slider focus mechanism, and buttons for illumination and image capture control, Beta (F) with production level ultrasonic welds for waterproofing and finalized material.

Table 4: Key distinguishing characteristics of the Pocket Colposcope

<table>
<thead>
<tr>
<th>Key Features</th>
<th>Gen. 1</th>
<th>Gen. 2</th>
<th>Gen. 3</th>
<th>Gen. 4</th>
<th>Alpha</th>
<th>Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector</td>
<td>2MP</td>
<td>1600x1200</td>
<td>5MP</td>
<td>2592x1922</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autofocus</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selectable Magnification</td>
<td>No</td>
<td>Yes</td>
<td>No, Fixed Focus at 35 mm</td>
<td>Yes, Slider mechanism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High power White LEDs</td>
<td>No</td>
<td>Yes, Req. External Box</td>
<td>No</td>
<td>Yes, USB powered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green LEDs</td>
<td>N/A</td>
<td>Cross-Polarization</td>
<td>Reflector</td>
<td>Optical baffle and light guide LED diffuser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast Enhancement</td>
<td>Anti-Fog Wipes</td>
<td>Cross-Polarization</td>
<td>Reflector</td>
<td>Optical baffle and light guide LED diffuser</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-fog Strategy</td>
<td>Gas ETO</td>
<td>Chemical Immersion, Gas ETO</td>
<td>Chemical Immersion, Gas ETO</td>
<td>Chemical Immersion, Gas ETO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterilization</td>
<td>No</td>
<td>Yes,</td>
<td>Yes,</td>
<td>Yes,</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The proof of concept first generation Pocket Colposcope was successful in capturing VIA and VILI images of the cervix but suffered from issues that are addressed in subsequent generations. These issues included: frequent lens fogging, which required the use of anti-fogging wipes prior to each patient use, the need for EtO gas sterilization between patient uses, a difficulty maintaining image focus due to lack of automated mechanism, and a reduced image quality due to specular reflection from the moist cervical tissue when compared to standard-of-care digital colposcope.

In order to minimize the amount of specular reflection and maximize image contrast during, the Generation 2 Pocket Colposcope employed the technique of cross-polarization as glare or specular reflection can obfuscate proper diagnosis of precancerous and cervical lesions. However, the cross-polarization technique for improving contrast strategy reduced the collected signal intensity by ~75%. A more powerful set of concentric White and Green LEDs ring are paired with an external LED driving circuit, and the rechargeable battery pack was employed to overcome the loss in signal. The additional features enabled the Pocket Colposcope to capture VIA, VILI, and now green light inspection (GLI) for enhanced vessel contrast comparable to the standard-of-care digital colposcope.
The Generation 3 Pocket Colposcope further refined the prior designs by eliminating the need for the use of antifogging wipes with the implementation of a protective hydrophobic optical window, improved the LED driving circuit with more efficient components. The refinement of the probe cable for improved reliability and durability and incorporation of medical grade heat shrink and epoxy for liquid ingress protection now made the Pocket Colposcope compatibility with harsh chemical immersion cleaning used as these partner sites. The Pocket Colposcope downtime between patient uses for cleaning was also significantly reduced from 12-24 hours (the typical turnaround time for EtO gas sterilization) to 20-30 minutes.

### 2.4.1.1 Hydrophobic window

The ability of a hydrophobic window and single-use anti-fogging wipes to prevent potential condensation, or liquid film formation is assessed. Both approaches are compared to uncoated glass and a non-misted Pocket Colposcope serving as a control. The mean Weber’s contrast for all three lesions in the uncoated glass group was significantly depressed when compared to that of the control group (Figure 20). The Weber’s contrast values for the anti-fog wipe treated window, and hydrophobic window are not significantly lower than that of the control group (Figure 20E). There was a significant difference in the perceived image quality score between the misted uncoated glass and control groups (Figure 20F).
Figure 20: Representative cervical mannequin images misted with green food dye with varying treatments, with qualitative image quality assessed by three blinded clinicians, and quantitative image quality by computing Weber’s contrast. The cervix mannequin has aceto-whitened lesions at 12, 3, and 7 o’clock positions and a cyst at the 4 o’clock position. Control untreated glass optical window (A), dye misted untreated glass optical window (B), dye misted anti-fog wipe treated glass window (C), and dye misted hydrophobic window (D). (E) Weber’s contrast values are calculated for each lesion (n=3), and the mean and standard deviation are determined from 3 repeated images of the same cervix. The uncoated glass (red) performed significantly worse than the control (white) at all three lesion positions (p<0.02 for all using 2-sample t-test and one-way ANOVA). The anti-fog wipe (green) and hydrophobic window (light blue) were not significantly different from the control group (p>0.1). (F) Qualitative assessment by three blinded clinicians of each treatment and control group noted a significant degradation in image quality for the uncoated glass that was misted with dye when compared to control (p<0.005) (104)
The Generation 4 Pocket Colposcope eliminated the need for the external LED driver box with the use of a reflector at the tip of the probe, while maintaining the improved imaging contrast without cross-polarization, maintained the chemical immersion sterilization compatibility and antifogging benefits of the hydrophobic window from the Generation 3 system. The Generation 4 device was able to still able to capture VIA and VILI images comparable to standard-of-care digital colposcope but had now lost GLI capability for enhanced vessel interrogation. Lastly, key informants and clinical trial users were now requesting the ability to change the magnification to interrogate suspicious lesions better.

The strategies for improving contrast using cross-polarization or the reflector trip are compared between the Generation 3, Generation 4 Pocket Colposcope, and standard-of-care digital colposcope.

2.4.1.2 Alternative strategies for improved contrast

Simulations were carried out for six different tip designs (Figure 21 A-F) with one design for the Generation 3 Pocket Colposcope and five different designs for the Generation 4 Pocket Colposcope. The Generation 4 Pocket Colposcope with reflector surface at a 75° angle and reflector height of 4.82 mm with respect to the vertical axis (which is perpendicular to the optical imaging axis) (Figure 21F) provided the greatest recovery of optical power and the most homogenous beam pattern across our target working distances of 5, 30, and 50 mm. Increasing the angle with respect to the imaging
axis, generally improved beam uniformity and total optical power across all working distances (Figure 21H) (104). Note the gradual improvement in “tightness” of the beam cutoff at the 5 mm working distance across the different designs (Figure 21KLM). We also observed that the angled reflector, when compared to the matched height straight reflector, netted a 20-25% increase in surface area (Figure 21G). A comparison between the Generation 3 and 4 Pocket Colposcopes showed a widespread scattering of illumination rays at the 30 and 50 mm working distances of the Generation 3 Pocket Colposcope when compared to the Generation 4 device. These beam patterns revealed a more consistent and uniform beam profile across working distances with the Generation 4 Pocket Colposcope, as noted with a sharper beam profile and lack of illumination hot spots (red or white speckling) especially apparent at the 5 mm working distance (Figure 21IJ vs. Figure 21KL) (104).
Figure 21: Representative graphical simulations of differences in light ray path, beam shape, and beam intensity between reflector designs with varying sidewall angles using the integrated white LEDs, Zemax ray tracing and Solidworks 3D digital drafting programs were used to simulate the effect if any of different integrated reflector cone designs with the Gen. 4 Pocket Colposcope. Simplified diagrams of simulation set up are shown for (A) Generation 3, (B) Bare LEDs, (C) Diffuser Window, (D) Reflector at 15° from the indicated axis (perpendicular to the
imaging axis) (E) Reflector at 45° from indicated axis, and (F) Reflector at 75° from indicated axis. The surface area of the reflectors is shown as a function of height and angle and the total optical power for a working distance of 5, 30 and 50 mm (G). Angling the reflector (light blue) netted a gain of 20-30% surface area when compared to the matched height straight reflector (orange). Similarly, (H) we noted that the 75° angled reflector performed the best out of all angled (solid blues) or straight (hatched blues) reflectors, at all working distances. The simulation (I, M, Q) and experimental (J, N, R) beam patterns for the Gen. 3 system were well matched across the working distances of 5 (IJ), 30 (MN), and 50 (QR) mm. The simulation (K, O, S) and experimental (L, P, T) beam patterns for the Gen. 4 system with the 75° angled reflector were well matched across the working distances of 5 (KL), 30 (OP), and 50 (ST) mm. Note, the absence of extreme illumination hotspots (red or white speckling) in the beam patterns of Generation 4 (KL) system when compared to the beam patterns of Generation 3 (KJ) system, at the 5 mm working distance. For both Pocket Colposcopes, the expected field of view is roughly equivalent to the respective working distance(104).

Furthermore, a simulated high-grade cervical lesion mannequin are imaged with the Generation 3 and 4 Pocket Colposcopes and standard-of-care digital colposcope. Note, the mean Weber’s contrasts were significantly higher than the standard-of-care colposcope for both generations of the Pocket Colposcope with p<0.0001, by 2-sample t-test and one-way ANOVA. However, there was not a significant difference between the generations of the Pocket Colposcope. The percent specular reflection are calculated from 5 repeated image captures and the mean and standard deviation with no significant difference in specular reflection between the Pocket Colposcope and standard-of-care digital colposcope (Figure 22H).
Figure 22: Improved contrast is observed with the Generation 4 and Generation 3 Pocket Colposcopes when compared to standard-of-care digital colposcope high-grade cervical simulated mannequin lesion. Representative images are taken of a high-grade mannequin cervical lesion in a custom dark chamber with Generation 4 Pocket (A), Generation 3 Pocket (B), and standard-of-care digital colposcope (C). The scale bars are 10 mm (ABC). A representative horizontal line scan is shown to assess the level of contrast of the simulated lesions across systems (DEF). These horizontal scans were repeated five times on five repeated image captures to calculate the mean Weber’s contrast ratio and standard deviation, which are shown in (G).

The Alpha and Beta Generation of the Pocket Colposcope was developed to incorporate the vast design experiences and user feedback from the Generation 1
through 4 devices. This penultimate iteration of the Pocket Colposcope was designed in collaboration with 3rd Stone Design and incorporated the following features:

improvements in the ergonomics such as buttons on the device for image capture and LED selection, a simple to use adjustable magnification mechanism, improved user comfort with an angled handle, and incorporating design and material selection considerations for scale manufacturing. The Alpha and Beta Pocket Colposcope maintained key features such as: VIA, VILI, and GLI capability with comparable quality to the standard-of-care digital colposcope, chemical immersion cleaning compatibility, the fog resistant hydrophobic window, and enhanced portability (from Generation 1 and 4) eliminating the need for external LED driver box by combining and miniaturizing the circuit into the handle of the probe.

2.4.2 Summary of Performance Characteristics of Generation 1 through Beta Pocket Colposcope

Representative images captured by the Generation 1 through Beta Pocket Colposcope showed comparable quality for optical resolution using the USAF1951 resolution target, the field of view and distortion SFRplus target, and depth of field target are shown in Figure 23. The key imaging (Table 5) and illumination (Table 6) parameters are conserved throughout the ergonomic evolution of the Pocket Colposcope.

The optical resolution for all the generations of Pocket Colposcope ranged between 10-72 line pairs per mm (lp/mm) and was substantially equivalent to the optical
resolution range of 10-29 lp/mm of the pair of predicate devices. The field of view of all
generations of the Pocket Colposcope ranged from 8-52 mm and was substantially
equivalent to the field of view range of 12-76 mm reported by the predicate device pair.
The picture-height distortion for all generations of the Pocket Colposcope ranged
between -4.5 to -1.1% and were substantially equivalent to the 3-7% distortion reported
by the pair of predicate devices. The depth of field range between 0.5-12 mm for all
generations of the Pocket Colposcope are substantially equivalent to 6-20 mm range
reported by the predicate device pair.

The illumination intensity range of 2,800 to 20,000 lux for all generations of the
Pocket Colposcope was substantially equivalent to the reported range of 3,000 to 24,000
lux of the pair of predicate devices. Similarly, the beam diameter range of 33.8 to 49.0
mm for all generations of the Pocket Colposcope was substantially equivalent to 60-62.1
mm range reported by the pair of predicate devices.
Figure 23: Representative Target Images from Generation 1 Through Beta Pocket Colposcope for Optical Resolution, Field of View, Distortion, and Depth of Focus. Images were captured at 3X magnification for the Pocket Colposcopes at a working distance of 35 mm and standard-of-care was captured at 3.75X magnification and 300 mm working distance. Row (A) are USAF 1951 resolution target, Row (B) are checkerboard or SFRplus distortion targets, and Row (C) is the depth of field target (100, 104).

Table 5: Summary of Imaging Characteristics from Generation 1 through Beta Pocket Colposcope and compared to two standard-of-care predicate digital colposcopes, Leisegang Optik 2 (LO2) and Edan C3/C6

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Gen. 1</th>
<th>Gen. 2</th>
<th>Gen. 3</th>
<th>Gen. 4</th>
<th>Beta</th>
<th>SOC 1</th>
<th>SOC 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical resolution</td>
<td>lp/mm</td>
<td>18.0 (10-64)</td>
<td>20.2 (10-72)</td>
<td>22.1 (10-72)</td>
<td>24.8 (10-72)</td>
<td>17 (13-121)</td>
<td>18 (14-29)</td>
<td>≥10</td>
</tr>
<tr>
<td>Field of View</td>
<td>mm</td>
<td>30.2 (8-51)</td>
<td>30.7 (8-52)</td>
<td>35.7 (8-52)</td>
<td>30.7 (8-52)</td>
<td>38.2 (6-54)</td>
<td>38 (19-76)</td>
<td>35 (12-80)</td>
</tr>
<tr>
<td>Picture-Height Distortion</td>
<td>%</td>
<td>-4.5</td>
<td>-1.1</td>
<td>-1.8</td>
<td>-1.2</td>
<td>-1.9</td>
<td>&lt;7</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Depth of Field</td>
<td>mm</td>
<td>5 (0.5-7)</td>
<td>8.5 (1-10)</td>
<td>9.5 (1-10)</td>
<td>8.5 (1-11)</td>
<td>10.1 (2-12)</td>
<td>10 (6-20)</td>
<td>≥6</td>
</tr>
</tbody>
</table>
Table 6: Summary of Illumination Characteristics from Generation 1 to Beta Pocket Colposcope

<table>
<thead>
<tr>
<th>Device Generation</th>
<th>Parameters</th>
<th>Units</th>
<th>Gen. 1</th>
<th>Gen. 2</th>
<th>Gen. 3</th>
<th>Gen. 4</th>
<th>Beta</th>
<th>SOC #1</th>
<th>SOC #2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IlluminationIntensity</td>
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<td>8,000</td>
<td>8,000</td>
<td>4,000</td>
<td>20,000</td>
<td>24,000</td>
<td>3,000</td>
</tr>
<tr>
<td></td>
<td>Beam Diameter</td>
<td>mm</td>
<td>42.8</td>
<td>40.2</td>
<td>40.2</td>
<td>33.8</td>
<td>49.0</td>
<td>62.1</td>
<td>≥60</td>
</tr>
</tbody>
</table>

2.4.3 Detailed Quantification of Imaging Characteristics of the Beta Pocket Colposcope

2.4.3.1 Optical and Angular Resolution

The on-axis optical resolution was 121.02±7.65 lp/mm at the 5 mm working distance (highest magnification) and 13.51±1.24 lp/mm at 50 mm working distance (lowest magnification). The on-axis angular resolution was 0.095±0.006 ° at the 5 mm working distance (highest magnification) and 0.085±0.009 ° at the 50 mm working distance (lowest magnification), Table 7. The spatial frequency response MTF₅₀ was 73.8±23.1 lp/mm at 5 mm working distance (highest magnification) and 78.7±24.3 lp/mm at the 50 mm working distance (lowest magnification). The off-axis values did not vary significantly across the range of magnifications for both optical resolution, angular resolution, and spatial frequency response, Table 7. Representative on and four corner off-axis measurements for the 5 mm working distance in Figure 24. Representative images from 5, 35, and 50 mm working distance is shown in Figure 25.
Table 7: On-axis and off-axis optical resolution and angular resolution of the Pocket Colposcope. Optical resolution (line pairs per mm), angular resolution (°), and spatial frequency response at MTF50 (line pairs per mm), from n=6 repeated measures at 5 mm working distance (maximum magnification), 35 mm working distance (typical use), and 50 mm working distance (minimum magnification).

<table>
<thead>
<tr>
<th>Working Distance</th>
<th>Incident Illumination</th>
<th>Optical Resolution</th>
<th>Angular Resolution</th>
<th>Optical Resolution</th>
<th>Angular Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>units</td>
<td>mm</td>
<td>Lux</td>
<td>lp/mm</td>
<td>(°) degree</td>
<td>lp/mm</td>
</tr>
<tr>
<td>DMax</td>
<td>5.00±0.02</td>
<td>837</td>
<td>±1</td>
<td>121.02</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±7.65</td>
<td>±0.06</td>
<td>±5.50</td>
</tr>
<tr>
<td>DTyp</td>
<td>35.0 ±0.1</td>
<td>1614</td>
<td>±18</td>
<td>17.31</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±1.01</td>
<td>±0.006</td>
<td>±0.91</td>
</tr>
<tr>
<td>DMin</td>
<td>50.1 ±0.1</td>
<td>1615</td>
<td>±36</td>
<td>13.51</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>±1.24</td>
<td>±0.009</td>
<td>±1.85</td>
</tr>
</tbody>
</table>

Figure 24: On-axis and off-axis resolution target representative images at 5 mm working distance. Representative images of USAF 1951 target captured by the Pocket Colposcope at the working distances of 5 mm at the central A-center (AFK), top right B1 (BGL), bottom right B2 (CHM), bottom left B3 (DIN), and top left B4 (EJO). The top row (A to E) are the full field of view and middle row (F to J) are the corresponding cropped and digitally zoomed region of interest (ROI). The bottom row (K to O) are the corresponding line scans (yellow line) to assess smallest discernable line pairs (which were Group 6 Element 6) across the sample with pixel intensity y-axis and distance (pixels) the x-axis.
2.4.3.2 Determination of Field of View and Distortion

The angular field of view was 57.0±0.8°, and the direction of view was 0.0±0.0° at the 35 mm working distance Table 8. The angular field of view varies slightly across the magnification range with 60.8±0.5° at 5 mm working distance (highest magnification) and 56.8±0.3° at the 50 mm working distance (lowest magnification). The field of view of diameter varies from 5.875±0.084 to 54.2±0.4 mm at the 5 and 50 mm working distances, respectively. A representative image from each working distance is shown in Figure 26.

Table 8: Summary of diagonal field of view (mm), field of view (°), and direction of view (°), the mean and standard deviation of the Pocket Colposcope’s from n=6 repeated measures during on-axis testing at 5 mm working distance (maximum magnification), 35 mm working distance (typical use), and 50 mm working distance (minimum magnification).

<table>
<thead>
<tr>
<th>Units</th>
<th>Working Distance</th>
<th>Field of View Diameter</th>
<th>Angular Field of View</th>
<th>Direction of View</th>
<th>Incident Illumination</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD&lt;sub&gt;Max&lt;/sub&gt;</td>
<td>5.00±0.02</td>
<td>5.875±0.084</td>
<td>60.8±0.5°</td>
<td>0.0±0.0</td>
<td>837±1</td>
</tr>
<tr>
<td>WD&lt;sub&gt;Typ&lt;/sub&gt;</td>
<td>35.0±0.1</td>
<td>38.2±0.4</td>
<td>57.0±0.8</td>
<td>0.0±0.0</td>
<td>1614±18</td>
</tr>
<tr>
<td>WD&lt;sub&gt;Min&lt;/sub&gt;</td>
<td>50.1±0.1</td>
<td>54.2±0.4</td>
<td>56.8±0.3</td>
<td>0.0±0.0</td>
<td>1615±36</td>
</tr>
</tbody>
</table>
The typical distortion was -1.942±0.101% at the 35 mm working distance, Table 9. The distortion ranged from -3.235±1.045% to 0.271±0.363 at the 5 and 50 mm working distances, respectively. A representative image from each working distance shown in Figure 26.

Figure 26: Distortion target representative images of SFRplus field of view target captured by the Pocket Colposcope at the working distances of 5 mm (A), 35 mm (B), and 50 mm (C). The scale bar is 1 mm for the 5 mm working distance and is 10 mm for the working distance of 35 and 50 mm, respectively.

Table 9: On-Axis Distortion for the Pocket Colposcope Summary of the mean and standard deviation of the Pocket Colposcope’s on-axis distortion, from n=6 repeated measures during on-axis testing at 5 mm working distance (maximum magnification), 35 mm working distance (typical use), and 50 mm working distance (minimum magnification).
2.4.3.4 Determination of Magnification

The Pocket Colposcope has a range of magnification of between 3 to 52 x using three representative display systems (tablet, tablet/laptop convertible, and laptop) see Table 10. We show our calculations per ISO 18221:2016 Sections 5.1 to 5.3 and using 3 representative display systems we have used during our development of the Pocket Colposcope, a 7” tablet (Google Nexus 7, referred to as Display 1), an 11.6” convertible laptop/tablet hybrid (Lenovo ThinkPad Helix 2, referred to as Display 2), and 13” traditional laptop (Lenovo ThinkPad 13 2). Sample calculations are in Appendix A.

Table 10: Summary of the Beta Pocket Colposcope’s magnification against working distance

<table>
<thead>
<tr>
<th>Units</th>
<th>Working Distance</th>
<th>Optical Zoom</th>
<th>Digital Zoom</th>
<th>Display Magnification</th>
<th>Visual Display Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td></td>
<td></td>
<td></td>
<td>M&lt;sub&gt;DIS&lt;/sub&gt;</td>
<td>M&lt;sub&gt;DIS VIS&lt;/sub&gt;</td>
</tr>
<tr>
<td>5</td>
<td>1x</td>
<td>1x</td>
<td></td>
<td>32-62 x</td>
<td>27-52 x</td>
</tr>
<tr>
<td>35</td>
<td>1x</td>
<td>1x</td>
<td></td>
<td>5-10 x</td>
<td>4-8 x</td>
</tr>
<tr>
<td>50</td>
<td>1x</td>
<td>1x</td>
<td></td>
<td>3-7 x</td>
<td>3-6 x</td>
</tr>
</tbody>
</table>

2.4.3.5 Determination of Depth of Field

The on-axis depth of field was 1.86±0.29 mm at the 5 mm working distance (highest magnification) and 11.71±0.31 mm at the 50 mm working distance (lowest magnification), see Table 11. Representative images from 5 and 50 mm working distance are shown in Figure 27.
Figure 27: Representative Depth of Field target images captured by the Pocket Colposcope at the working distances of 5 mm (A), 35 mm (B), and 50 mm (C). The bottom row (DEF) are matched line scans (yellow bar) for the horizontal Ronchi rulings with pixel intensity for the y-axis and depth of focus distance (mm) for the x-axis.

Table 11: On-axis depth of field performance of the Pocket Colposcope

Summary of the mean and standard deviation of the Pocket from n=6 repeated measures at 5 mm working distance (maximum magnification), 35 mm working distance (typical use), and 50 mm working distance (minimum magnification).

<table>
<thead>
<tr>
<th>Working Distance</th>
<th>Incident Illumination</th>
<th>On-axis Depth of Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Units</td>
<td>mm</td>
<td>Lux</td>
</tr>
<tr>
<td>$D_{\text{Max}}$</td>
<td>5.00±0.02</td>
<td>837±1</td>
</tr>
<tr>
<td>$D_{\text{Typ}}$</td>
<td>35.0±0.1</td>
<td>1614±18</td>
</tr>
<tr>
<td>$D_{\text{Min}}$</td>
<td>50.1±0.1</td>
<td>1615±36</td>
</tr>
</tbody>
</table>

2.4.3.6 Illumination characteristics and photobiological safety

The Pocket Colposcope’s illumination modes generally fall well below the thresholds for the exempt risk for emission limits. However, one parameter Actinic UV risk (row 1) for the High White illumination setting, see Table 12, is slightly
(0.0006 W/m²²) above the exempt group limit of 0.001 and well below the threshold for the low-risk group of 0.003. Thus, our device should be classified as a low-risk (Risk Group 1) device.

Table 12: Summary of the Beta Pocket Colposcope weighted emission for low white, high white, and green illumination modes with the exempt risk group limit shown.

<table>
<thead>
<tr>
<th>Risk</th>
<th>Action Spectrum</th>
<th>Symbol</th>
<th>Emission Limits: Exempt</th>
<th>Units</th>
<th>Low White</th>
<th>High White</th>
<th>Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinic UV</td>
<td>S_UV(λ)</td>
<td>E_s</td>
<td>0.001</td>
<td>W/m²</td>
<td>1.017*10⁻⁴</td>
<td>0.0016</td>
<td>1.56*10⁻⁴</td>
</tr>
<tr>
<td>Near UV</td>
<td>N/A</td>
<td>E_UVA</td>
<td>10</td>
<td>W/m²</td>
<td>2.58*10⁻⁴</td>
<td>0.0031</td>
<td>3.02*10⁻⁴</td>
</tr>
<tr>
<td>Blue Light</td>
<td>B(λ)</td>
<td>L_b</td>
<td>100</td>
<td>W/m² sr</td>
<td>0.0039</td>
<td>0.0194</td>
<td>4.16*10⁻⁴</td>
</tr>
<tr>
<td>Blue Light, small source</td>
<td>B(λ)</td>
<td>E_b</td>
<td>1.0</td>
<td>W/m²</td>
<td>0.0017</td>
<td>0.0081</td>
<td>1.68*10⁻⁴</td>
</tr>
<tr>
<td>Retinal thermal</td>
<td>R(λ)</td>
<td>L_R</td>
<td>28,000/α</td>
<td>W/m² sr</td>
<td>0.057</td>
<td>0.27</td>
<td>0.018</td>
</tr>
<tr>
<td>Retinal thermal, weak visual stimulus</td>
<td>R(λ)</td>
<td>L_R</td>
<td>6000/α</td>
<td>W/m² sr</td>
<td>0.0063</td>
<td>0.0048</td>
<td>0.0054</td>
</tr>
<tr>
<td>IR eye</td>
<td>N/A</td>
<td>E_IR</td>
<td>100</td>
<td>W/m²</td>
<td>0.012</td>
<td>0.0780</td>
<td>0.0080</td>
</tr>
</tbody>
</table>

The Pocket Colposcope has white light illumination of between >20,000 lux to 462lux at working distances of 5 to 50 mm, respectively. The illumination range (beam diameter) of the Pocket Colposcope was 20.2 mm to 74.5mm at a working distance of 5 to 50, respectively see Table 13.

Table 13: Summary of the Pocket Colposcope’s illumination range as a function of working distance from n=3 devices with two repeated measures per device and illumination modes. *Comparable device measurements were only made at 200 mm, but that system’s working distance ranges from 200-300 mm (most similar devices, report illumination at the 300 mm range).
<table>
<thead>
<tr>
<th>Units</th>
<th>mm</th>
<th>lux</th>
<th>lux</th>
<th>lux</th>
<th>mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10228±1</td>
<td>20000±8</td>
<td>18190±1</td>
<td>20.2±1.9</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>964±2</td>
<td>7745±18</td>
<td>1608±1</td>
<td>49.0±3.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>462±1</td>
<td>3707±8</td>
<td>769±1</td>
<td>74.5±3.1</td>
</tr>
<tr>
<td></td>
<td>200*</td>
<td>34±1</td>
<td>238±3</td>
<td>56±2</td>
<td>250.8±7.3</td>
</tr>
<tr>
<td></td>
<td>300*</td>
<td>22±1</td>
<td>135±2</td>
<td>34±1</td>
<td>367±5.8</td>
</tr>
</tbody>
</table>

### 2.5 Discussion

In this aim we established that the imaging and illumination performances of the Pocket Colposcope are conserved across each generation with only the form factor evolving. The non-inferiority of the Pocket Colposcope was demonstrated to the standard-of-care digital colposcope and approved by the US FDA 510(k) approval process on September 21st, 2018 (K181034)(137).

Each evolution of the Pocket Colposcope was an important step in building a robust low cost portable digital colposcope for use in limited resource settings. The use of computer-aided three-dimensional modeling and ray tracing simulations (138) allows us to perform virtual design optimization of the illumination technique reducing the amount of time and resources required for iterative prototyping and experimental validation. The elimination of the cross-polarizers and removed the need for an external control box for the Beta generation of the Pocket Colposcope (100), lowering the required electrical power budget. The waterproofing strategy is vastly improved through collaboration with 3rd Stone Design with ultrasonic welding the probe handle clamshells together to remove the prior strategy of medical grade heat shrink and epoxy.
The hydrophobic window design allows us to develop a more robust probe that is compatible with chemical immersion sterilization most often used in limited resource settings. The new design is also better suited for implementation in the limited resource setting due to the reduced consumable cost of anti-fog wipes when compared to the Generation 1 and 2 Pocket Colposcopes.

When compared to the predicate colposcope systems, the Edan C3A/C6A digital colposcope (FDA 510(k) K151878), and our system has a comparable range of optical resolution, the field of view range and can adequately capture a broad range of cervix sizes. While the Pocket Colposcope had a larger and more consistent angular field of view of between 56.8° to 60.8° when compared to the Edan C3A/C6A Digital Colposcope which reported a range of the angular field of views of 16.5° at highest magnification and ≥2.5°at the lowest magnification. This difference is likely due to the different lenses and detector used in each system, but this difference in the angular field of view does not impact functional concordance. As the range of the field of view diameter of the Pocket Colposcope (5.875 to 54.2 mm) is very comparable to the Edan reported range of the field of view diameter (12 to 80 mm). This broad range of the field of views allows for both devices to provide wide field surveillance of the cervix in single image capture and a magnified view of suspicious regions. Both systems had a direction of view of 0° as neither has a flexible neck for articulation or angled lens for oblique viewing. The Pocket Colposcope had a comparable picture height distortion range of -3.2% to +0.3% at
the highest and lowest magnifications, respectively, when compared to the 3% distortion reported by the Edan C3A/C6A Digital Colposcope. The Pocket Colposcope’s depth of field ranges between 1.86 to 11.71 mm at the highest to lowest magnification, when compared to the Edan C3A/C6A Digital Colposcope which reports a broader range of ≥6 mm to ≥120 mm. The differing range could be due to the type of imaging target used by the Edan device (which is not reported) and if a lower lp/mm threshold or target was used could explain the broader range reported by the Edan device. It would be beneficial for a standard to be developed for depth of field testing to allow for easier comparison across optical devices. The Pocket Colposcope’s magnification ranges from 3x to 52x (depending on the display size) as determined according to ISO 18221:2016 guidelines. The reference predicate the Edan C3A Digital Colposcope reported 1-28x magnification and the Edan C6A Digital Colposcope reported 1-36x magnification ranges, respectively. Our device has sufficient magnification range to allow for both wide field and selective region of interest high-resolution imaging of the cervix.

Pocket Colposcope system has a comparable illumination lux and illumination range. The Pocket Colposcope’s white light illumination that ranges 20,000 lux to 462 lux at the working distances of 5 and 50 mm, respectively. Which is similar to the reported illumination values of the referenced Edan C3A Digital Colposcope of 1600 lux at a working distance of 300 mm and the Edan C6A Digital Colposcope reported 3000 lux at a working distance of 300 mm, respectively. They do not report the illumination at the
200 mm working distance. The Pocket Colposcope’s illumination range (beam diameter) was 20.2 mm to 74.5mm at a working distance of 5 to 50 mm, respectively and comparable to the Edan system. Other similar reference devices report greater than 60 mm diameter for the illumination range at the working distance of 200 mm and don’t report it for the 300 mm working distance. Of note, the comparative digital colscopes do not report the standards they used to measure the illumination and illumination range. Thus we used the most appropriate available standard which was the ANSI/NEMA FL-1:2009 for our methodology.

The Pocket Colposcope system has a comparable level of photobiological safety of low-risk. The Pocket Colposcope based on IEC/EN 62471:2008 guidelines, is classed as low risk (Risk Group 1) due to marginal elevated Actinic UV hazard (0.006 W/m²), which still safely allows for 10,000 seconds of continuous direct viewing before damage may occur. There is no evidence that the referenced comparative device reported photobiological safety testing according to the IEC/EN 62471:2008 guidelines. The Pocket Colposcope emits several fold less light radiation when compared to the referenced device with a maximum illumination of 135 lux at the same working distance of 300 mm, which is 12-22 fold lesser than the referenced Edan C3A/C6A Digital Colposcope’s reported illumination of 1600 to 3000 lux at a working distance of 300 mm, respectively.
Future work should pivot towards the implementation for a speculum free approach to colposcopy as being currently pursued by my labmate Mercy Asiedu and team (140), as prior studies have suggested the use of the speculum is a large hurdle for even countries like the US, where there is greater access to health care. The compliance rates with cervical screening vary due to embarrassment and fear of pain during examination have been reported as significant barriers to cervical cancer screening (73-75). The speculum is a cause of discomfort particularly for women with vaginismus (an involuntary tightening of the vagina) that is often a result of sexual abuse (76). In a survey conducted in rural Mexico, the most frequent reason for not having a cervical exam was anxiety regarding physical privacy. Less frequent reasons were lack of knowledge and difficulty accessing health care (72). Furthermore, the implementation of the screening program itself can also be a barrier from an adoption perspective. A study in rural Tanzania revealed that key factors for cervical cancer screening; were significant concerns about embarrassment and pain due to screening from the speculum, spousal approval, level of education, the gender of the health provider, and distance to the screening center (71).

Furthermore, automated algorithm to in clinician diagnosis of lesions should be pursued given the ability of the Pocket Colposcope to capture, VIA, GLI, and VILI images (140-143) as being currently pursued by my labmate Mercy Asiedu and team.
3. Aim 2: Demonstrate the non-inferiority of the Pocket Colposcope’s diagnostic capability with respect to the standard-of-care digital colposcopes and gold-standard histopathology

This work was published with assistance from authors Christopher Lam, Jenna Mueller, Mercy Asiedu, Betsy Asma, Denali Dahl, Jenna Peters, Max Kellish, Rhea Chitalia, Marlee Krieger, Al Erkani, Allison Hall, Yenny Bellido-Fuentes, Ernesto Ortiz, Jennifer Gallagher, Lisa Muasher, Peyton Taylor, Roopa Hariprasad, J.S. Malliga, Bariki Mchome, Olola Onoko, Gino Venegas, Anthony Wanyoro, Ravi Mehrotra, John Schmitt, and Nirmala Ramanujam.

3.1 Preface

Aim 2 demonstrates the concordant diagnostic performance of the Pocket Colposcope when compared to reference standard-of-care digital colposcopes and gold-standard histopathology in a multi-institution clinical trial. Increasing the number of contrast agents used significantly improved the diagnostic performance of the Pocket Colposcope when compared to gold-standard histopathology.

3.2 Introduction

The Pocket Colposcope has been determined by the FDA to be equivalent to predicate digital colposcope based on bench side quantitative and qualitative testing according to international standards through the 510(k) pathway (94). Characterizing
the clinical diagnostic performance was not required for this approval pathway. However, we set out to demonstrate that the Pocket Colposcope can deliver comparable diagnostic capability to existing digital colposcopes in a much more affordable, durable, and portable package.

### 3.3 Semi-quantification of visual inspection – Reid Index

Visual inspection with acetic acid (VIA), Green Light Inspection (GLI), and Visual inspection with Lugol’s Iodine (VILI) are used widely today, however not all 3 are used concurrently due to the limitation of resources or capabilities (i.e., lack of green light or difficulty procuring Lugol’s Iodine). These historical hallmarks have been used by clinicians since 1925 as part of a diagnostic scoring technique known as modified Colposcopic Reid index (144, 145), summarized in Table 14. However, this implementation has relied on qualitative interpretation from direct visual inspection of the cervix leading to significant inter-observer variability, poor specificity, and potential over-treatment. The visual inspection of the cervix with the modified Colposcopic Reid index system, which is the most often used method for the semi-quantitative scoring of visual indicators of cervical neoplasia using exogenous staining agents (144, 145), has been shown via meta-analysis to have a high rate of false positives of nearly ~30% (146) leading to the significant overtreatment (Table 15) (147-150). Thus, techniques are needed for the improvement in clinical performance to better match pathology, but in noninvasive and nondestructive manner.
Table 14: Summary of the modified Reid Colposcopic Index used as a semi-qualitative aid in the clinical assessment of cervical cancer. Aided by application of exogenous staining agents, key hallmarks of cervical neoplasia can be visualized such as the differential temporary acetowhitenening uptake provides potential boundaries between normal and abnormal tissues, the observation of the irregular pattern of vessels, and poor staining of glycogen by Lugol’s Iodine is indicative of potentially abnormal sites. Scores of ≥5 are indicative of high-grade lesions and likely to progress to cervical cancer unless treated. Early score elevations are indicative HPV induced increasing cell density, angiogenesis, and altered metabolism. Low-grade squamous intraepithelial lesion or cervical intraepithelial lesion grade 1(LSIL/CIN1), High-grade squamous intraepithelial lesion or cervical intraepithelial lesion grade ≥2 (HSIL/CIN2+), the Total absorption coefficient (µₐ), Total Hemoglobin Concentration [THb], Oxygen Saturation (SO%), Scattering coefficient (µₛ')

<table>
<thead>
<tr>
<th>Colposcopic Signs</th>
<th>Stain/Technology</th>
<th>Biological Mechanism</th>
<th>Scoring Scale (pts)</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface Shine, Opaque, Color</td>
<td>5% Acetic Acid</td>
<td>Temporary precipitation of cytoplasmic proteins due to dehydration</td>
<td></td>
<td>Intense shine</td>
<td>Trans-parent/-lucent Snow / Pure White</td>
<td>Shiny</td>
</tr>
<tr>
<td>Lesion Margin Surface Configuration</td>
<td>Red Free or Green Illumination</td>
<td>Increasing duration of AW associated with increasing severity of disease</td>
<td></td>
<td>Feathered/Scalloped /Indistinct Flat</td>
<td>Smooth/Strait Symmetrical</td>
<td>Rolled/Peeked</td>
</tr>
<tr>
<td>Vascullature</td>
<td>Lugo's Iodine</td>
<td>Hemoglobin absorption spectra</td>
<td></td>
<td>Fine</td>
<td>Absent</td>
<td>Coarse Punctuation Mosaicism Random</td>
</tr>
<tr>
<td>Iodine Staining</td>
<td>Lugo's Iodine</td>
<td>Glycogen staining</td>
<td></td>
<td>Positive Mahogany/Brown</td>
<td>Partial Uptake Speckle</td>
<td>Negative Pale Yellow</td>
</tr>
</tbody>
</table>

Quantitative Optical Property: µₐ, Increased scattering due to higher density of cells and nuclear content
Quantitative Optical Property: µₐ, [THb]. SO% Angiogenesis/increased microvessel density Shift to aerobic glycolysis: local hypoxia
Quantitative Optical Property: µₛ, Higher metabolism and immature cells

<table>
<thead>
<tr>
<th>RCI Score</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>Normal</td>
</tr>
<tr>
<td>3-4</td>
<td>LSIL/CIN1</td>
</tr>
<tr>
<td>≥5</td>
<td>HSIL/CIN2+</td>
</tr>
</tbody>
</table>

Table 15: Summary of Pooled Sensitivity and Specificity for Cervical Cancer Screening Procedures using an HSIL/CIN2+ cutoff and histopathology confirmation (147-150)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap Smear (Cytology)</td>
<td>42.6</td>
<td>26.5-58.6</td>
<td>99.3</td>
<td>98.8-99.7</td>
<td>24,900</td>
</tr>
<tr>
<td>HPV-Hybrid Capture</td>
<td>61.9</td>
<td>56.2-67.7</td>
<td>93.6</td>
<td>92.4-94.8</td>
<td>22,737</td>
</tr>
<tr>
<td>Visual Inspection</td>
<td>61.3</td>
<td>50.0-72.6</td>
<td>87.0</td>
<td>81.4-92.7</td>
<td>114,186</td>
</tr>
</tbody>
</table>
3.4 Summary of Aim 2

In this aim, the Pocket Colposcope is shown to have strong concordance with standard-of-care digital colposcopes when assessing the cervix for women undergoing colposcopy or follow-up therapy for previously diagnosed lesions in multisite international clinical trials. The Pocket Colposcope could successfully capture images comparable to a reference digital colposcope in a resource-limited setting. The Pocket Colposcope’s diagnostic performance parameters (including sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) all significantly improved with increasing levels of contrast from visual inspection with acetic acid (VIA), green light inspection (GLI), and visual inspection with Lugol’s Iodine (VILI).

3.5 Material and Methods

3.5.1 Pilot Clinical Study – Pocket Colposcope vs. Reference Digital Colposcope

A pilot exploratory study in human subjects was conducted to demonstrate feasibility of the Generation 1 and 2 Pocket Colposcope could be used to successfully capture images of comparable to a standard-of-care digital colposcope (Leisegang Optik 2, Cooper Surgical Trumbull, CT) under an institutional IRB approved protocol.
(Pro00008173) and ClinicalTrials.gov (NCT02477124) at the Duke University Medical Center (DUMC) in Durham, NC, USA.

3.5.1.1 Patient Population

Adult subjects (n=45) between the ages of 18-65 years who were referred for routine colposcopy and/or Loop Excision Electrosurgical (LEEP) treatment provided written informed consent using an institutional IRB approved consent form for concurrent imaging with the Pocket Colposcope and standard-of-care digital colposcope followed by standard-of-care biopsy or excised pathological specimen analysis. Participating patients are also provided a copy of their signed consent form. The original consent form is kept in a secured research binder that also is also used to document the consent process and procedure followed the approved protocol in a locked cabinet residing in a locked office that is limited in access to those on the IRB protocol.

3.5.1.2 Study Procedure

As part of the standard-of-care procedure at Duke University Medical Center (DUMC), a speculum are placed, and any blood or mucous was removed from the cervix using a cotton fox swab. A 5% acetic acid solution is then applied followed by image capture with the standard-of-care digital colposcope at 3.75X magnification under white light mode for visual inspection with acetic acid (VIA) and green light inspection (GLI) for enhanced vessel inspection. The standard-of-care digital colposcope is moved out of
position, and the Pocket Colposcope set at ~6X magnification (35 mm working distance) was placed into the speculum to capture white with 3-5 images of the cervix were captured with the standard-of-care and Pocket Colposcope. Acetic acid was reapplied when necessary after the initial application before the second set of image captures. This process added between 1-2 minutes to the overall procedure time. All clinical decisions are made with the standard-of-care colposcope, including guiding the biopsy. The procedure continued with biopsy sample collection and/or LEEP treatment as part of the standard protocol. Cervix specimens were processed, immuno-stained for Ki-67(a nuclear protein marker for cellular proliferation) and p16 (a cyclin-dependent kinase-4 inhibitor that is a surrogate marker for the presence of HPV infection), and read by blinded (to colposcopic impression) institutional pathologists as the gold-standard diagnostic reference (151). Pathological diagnosis are grouped as negative (normal, cervicitis, condyloma) versus pre-cancer or cancer (CIN1-3, cancer).

3.5.2 International Clinical Study – Pocket Colposcope vs. Reference Digital Colposcope in resource limited setting

We sought to determine if the Pocket Colposcope could successfully capture images comparable to a reference digital colposcope in a resource-limited setting. Thus an international pilot study site was established in collaboration with La Liga Contra el Cáncer in Lima, Peru. The Generation 3 and Generation 4 Pocket Colposcope are employed for the duration of this study site.
3.5.2.1 Patient Population

Peruvian women (n=129) who had been referred for colposcopy or loop large excision transformation zone (LLETZ) due to abnormal cytology and/or a positive HPV test are recruited for this study (117). Informed written consent is obtained from each participating patient. Images were collected with both the Pocket Colposcope and standard-of-care digital colposcope (Goldway SLC-2000B, Ronkonkoma, NY) at La Liga Contra el Cáncer under Duke University Medical IRB approved protocol (Pro00052865) and registered to ClinicalTrials.gov (NCT02477124).

3.5.2.2 Study Procedure

The speculum is placed in the vaginal canal, acetic acid (VIA) is applied to the cervix for one minute, and then 3-5 images of the cervix were captured with the standard-of-care and Pocket Colposcope. The time delay between standard-of-care digital colposcope and Pocket Colposcope was typically 1-3 minutes. Next, Lugol’s Iodine (VILI) is applied to the cervix, and then 3-5 images were captured with the Pocket Colposcope and then with the standard-of-care digital colposcope (117). All clinical decisions are made with the standard-of-care colposcope, including guiding the biopsy.

3.5.3 Multinational Effect of Contrast Agents Clinical Study – Pocket Colposcope vs. gold standard histopathology

In order to bolster the number of enrolled patients to reach statistical power targets to allow for assessment for the Pocket Colposcope vs. gold standard histopathology and to evaluate the effects of level of contrast used for imaging the
cervix, two additional partnering sites are added to the clinical study. Kilimanjaro Christine Medical Center (KCMC) in Moshi, Tanzania and Centre for Infectious Disease Control Zambia (CDZIR) in Lusaka, Zambia. These sites operated under partner site and DUMC’s Medical IRB approved protocol (Pro00052865) and registered to ClinicalTrials.gov (NCT02477124). Informed written consent are obtained from each participating patient. The two pre-existing sites continued enrollment and employed a combination of Generation 3, Generation 4, alpha, and beta generations of the Pocket Colposcope.

3.5.3.1 Patient Population

North American women (n=142) between the ages of 18-65 years who were referred for routine colposcopy and/or Loop Excision Electrosurgical (LEEP) treatment provided written informed consent enrolled in the study at Duke University Medical Center (Durham, NC). Imaging with the Pocket Colposcope and standard-of-care digital colposcope (Leisegang Optik 2, Trumbull, CT) was followed by standard-of-care biopsy or excised pathological specimen analysis.

Peruvian women (n=126) who had been referred for colposcopy or loop large excision transformation zone (LLETZ) due to abnormal cytology and/or a positive HPV test is recruited for this study, at La Liga Contra el Cáncer. Informed written consent is obtained from each participating patient. Images are collected with both the Pocket Colposcope and standard-of-care digital colposcope (Goldway SLC-2000B,
Ronkonkoma, NY) followed by standard-of-care biopsy or excised pathological specimen analysis.

Tanzanian women (n=71) who were visiting for colposcopy, cryotherapy, or loop excision electrosurgical procedure (LEEP) are enrolled for this study at Kilimanjaro Christian Medical Center (KCMC) located in Moshi, Tanzania. Images are collected with both the Pocket Colposcope and digital zoom camera (Canon SX40HS, Tokyo, Japan), followed by standard-of-care biopsy or excised pathological specimen analysis.

Zambian women (n=18) who were visiting for cervical cancer screening (colposcopy) or therapy with loop excision electrosurgical procedure (LEEP) are enrolled for this study at Centre for Infectious Disease Control Zambia (CIDRZ) located in Lusaka, Zambia. Images are collected with the Pocket Colposcope and naked eye evaluations (no digital standard-of-care available at this site), followed by standard-of-care biopsy or excised pathological specimen analysis.

3.5.3.2 Study Procedure

The speculum are placed in the vaginal canal, acetic acid (VIA) are applied to the cervix for one minute, and then 3-5 images of the cervix were captured with the standard-of-care (if available) and then the Pocket Colposcope at all four sites. The time delay between standard-of-care digital colposcope and Pocket Colposcope was typically 1-3 minutes. Next, 3-5 images are captured with the green light inspection (GLI) at KCMC, Duke, and CIDRZ with the Pocket Colposcope and standard-of-care system (if
available). Lastly, at DUMC, KCMC, and CIDRZ Lugol’s Iodine (VILI) was applied to the cervix, and then 3-5 images were captured with the Pocket Colposcope and then standard-of-care (if available). All clinical decisions are made with the standard-of-care (digital colposcope, digital camera, or naked eye visual inspection), including guiding biopsy.

Images with only visual inspection with acetic acid (VIA) had 129 subjects reviewed by ten blinded clinicians. Visual inspection with acetic acid and green light inspection (VIA+GLI) had 92 subjects reviewed by seven blinded clinicians. Visual inspection with acetic acid and Lugol’s Iodine (VIA+VILI) had 127 subjects reviewed by six blinded clinicians. Visual inspection with acetic acid, green light inspection, and Lugol’s Iodine (VIA+GLI+VILI) had 40 subjects reviewed by four blinded clinicians.

3.5.4 Data Management

3.5.4.1 REDCap Encrypted Database

The data from all study sites including: demographic information, cervical images captured by the Pocket Colposcope, cervical images captured by the standard-of-care digital colposcope or digital camera (if available), and pathology results before being stored on an IRB approved, HIPAA compliant, encrypted and password protected database server, Research Electronic Data Capture Platform (REDCap), maintained by the Duke Translational Medicine Institute (152). A new study randomized identification code are generated for each enrolled patient, and all data was de-identified of all
Protected Health Information (PHI). Team members based at each study site would access the REDCap database through a password-protected online portal to upload data.

3.5.4.2 Image Quality Review

Cases were excluded from subsequent analysis if: 1) images and pathology were missing because statistics could not be calculated if a case was incomplete, or 2) images were unreadable due to low image quality because statistics could be confounded by user error that occurred during image acquisition. In order to evaluate image quality, a blinded reviewer scored each image as having low, medium, or high image quality. A high quality image is in focus and all 4 quadrants of the cervix are visible, a medium quality image is slightly out of focus and a majority of the 4 quadrants of the cervix are visible, and the low quality image is not in focus and a majority of the 4 quadrants of the cervix are not visible. Discrepancies between the two reviewers were resolved by consensus review. Images with medium or higher image quality were included in the analysis and sent out to participating clinicians.

3.5.4.3 Image Interpretation

In order to remove bias, images that passed image quality review were cropped to remove the view of the speculum and vaginal side walls, thereby blinding the physician to which colposcope was used to acquire the image. Then these images were split up (if Pocket Colposcope and matched standard-of-care were both captured), randomized, labeled with a random identifier, and placed into different documents that
were sent electronically to clinicians at Duke University Medical Center (Durham NC, USA), La Liga Contra el Cáncer (Lima, Peru), Kilimanjaro Christian Medical Center (Moshi, Tanzania), Kenyatta University (Nairobi, Kenya), Institute of Cytology and Preventative Oncology (New Delhi, India), and WIA Cancer Institute (Chennai, India). If multiple contrast methods are captured, these were paired together in a single panel (e.g., VIA, VIA+GLI, VIA+VILI, or VIA+GLI+VILI). No indicators were visible to identify which colposcope device captured each image. Clinicians were sent a web portal to a HIPAA compliant secure encrypted REDCap (152) database portal to enter their clinical interpretation of each randomized image set using a standardized questionnaire based on the modified Reid Index (122, 153). The survey includes the randomized identifier code, technical questions about the image quality, clinical questions evaluating the properties of the cervix necessary to make a colposcopic diagnosis, and ends with an overall diagnosis of the cervical image.

The physicians provided a diagnosis of normal, cervical intraepithelial neoplasia (CIN) 1, CIN2, CIN3, or cancer for each cervix image. The level of agreement between the diagnosis from the images collected with the Pocket and standard-of-care colposcope is calculated by grouping the diagnoses into normal versus abnormal (CIN1-3, cancer) categories. The physician diagnoses of the cervical images are compared to the pathology confirmed diagnosis given as normal, cervicitis, condyloma, no biopsy, CIN1,
CIN2, CIN3, or invasive cancer. Pathological diagnosis is grouped as negative (normal, cervicitis, condyloma) versus pre-cancer or cancer (CIN1-3, cancer).

3.5.1 Statistical Analysis

3.5.1.1 Baseline Demographic Analysis

STATA/MP 13.1 (StataCorp LLC, College Station) statistical package was used to assess our dataset (154). A significance level of p≤0.05 was considered to reject the null hypothesis for all analyses. Continuous variables will have an analysis of variance (ANOVA) with Tukey’s post hoc analysis for pairwise comparisons using STATA’s “anova” and “tukeyhsd” functions(155). For categorical variables, Fisher’s exact test is used if any parameter had < 5 entries and Chi-square test otherwise.

3.5.5.2 Primary Outcome Measures – Level of Agreement and Cohen’s kappa coefficient (κ)

Our primary outcome measures include the level of agreement (%) and Cohen’s kappa coefficient (κ) a measure of inter-rater agreement using 2x2 contingency tables to compare between systems and/or gold standard histopathology (Table 16, Equation 6a-c). The binary classification used as the diagnostic cut-off for our analysis include low-grade lesion and higher (LSIL/CIN1+) vs. normal or high-grade lesion (HSIL/CIN2+). The measures are tabulated using STATA’s function “kappa” and “kappaetc” (156, 157).

Table 16: Representative 2x2 Contingency Table used to the calculated level of agreement and Cohen’s kappa coefficient (κ) for assessing different diagnostic techniques
<table>
<thead>
<tr>
<th>Diagnostic System Y</th>
<th>Disease (+)</th>
<th>Disease (-)</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease (+)</td>
<td>a</td>
<td>b</td>
<td>a+b=m₁</td>
</tr>
<tr>
<td>Disease (-)</td>
<td>c</td>
<td>d</td>
<td>c+d=m₀</td>
</tr>
<tr>
<td>Column Total</td>
<td>a+c=n₁</td>
<td>b+d=n₀</td>
<td>m₁+m₀=n₀+n₁</td>
</tr>
</tbody>
</table>

Eqn. 6(a): Observed Agreement, \( P_0 = \left( \frac{a + d}{n_T} \right) \)

Eqn. 6(b): Expected Agreement, \( P_E = \left[ \left( \frac{n_{11} \times m_1}{n_T \times n_T} \right) + \left( \frac{n_{00} \times m_0}{n_T \times n_T} \right) \right] \)

Eqn. 6(c): Kappa, \( \kappa = \left( \frac{P_0 - P_E}{1 - P_E} \right) \)

### 3.5.5.3 Secondary Outcome Measures – Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value

Our secondary analysis included the following diagnostic performance measures: sensitivity (true positive rate), specificity (true negative rate), Positive Predictive Value (PPV), and Negative Predictive Value (NPV). These parameters are also tabulated using 2x2 contingency tables (Table 18 and Equations 7a-c) using STATA’s “diagt” function (158). The binary classification used as the diagnostic cut-off for our analysis include low-grade lesion and higher (LSIL/CIN1+) vs. normal or high-grade lesion (HSIL/CIN2+) using gold standard histopathology as the reference group.

Table 17: Representative 2x2 Table Used to Calculate Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV). True Positive (TP), False Negative (TN), False Positive (FP), True Negative (TN)
<table>
<thead>
<tr>
<th>System X</th>
<th>Disease (+)</th>
<th>Disease (-)</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold Standard Reference</td>
<td>Disease (+)</td>
<td>TP</td>
<td>FN</td>
</tr>
<tr>
<td>Disease (-)</td>
<td>FP</td>
<td>TN</td>
<td>FP+TN</td>
</tr>
<tr>
<td>Column Total</td>
<td>TP+FP</td>
<td>FN+TN</td>
<td>TP+FP+TN+FN</td>
</tr>
</tbody>
</table>

Eqn. 7(a): Sensitivity, \( S_E = \left( \frac{TP}{TP + FP} \right) \)

Eqn. 7(b): Specificity, \( S_P = \left( \frac{TN}{FN + TN} \right) \)

Eqn. 7(c): Positive Predictive Value (PPV), \( PPV = \left( \frac{TP}{TP + FP} \right) \)

Eqn. 7(d): Negative Predictive Value (PPV), \( NPV = \left( \frac{TN}{TP + FP} \right) \)

3.5.5.4 Confirming diagnostic performance with Receiver Operating Curves and logistic regression

In order to confirm our secondary analysis, Receiver Operating Curves (ROC) will be generated by plotting sensitivity on the y-axis and 1-specificity of the Pocket Colposcope with respect to gold-standard histopathology using the previously stated binary diagnostic cut-offs (159). The Area Under Curve (AUC) of these ROC plots stratified by type of contrast used will be compared using logistic regression modeling (159, 160).

3.5.5.5 Power Calculations

Our main power calculation will focus on Cohen’s kappa coefficient (\( \kappa \)), a measure of the concordance or agreement between diagnostic tests. We estimated \( n = \)
379 interpretations would be required, with $\beta = 0.1$ and $\alpha = 0.05$. The STATA function “sskdlg” (156) to perform the power calculation based on the asymptotic variance equation presented by Fleiss et al. (161). For this power calculation we need our expected $\kappa$, the proportion that should be identified as (normal vs. LSIL/CIN1+) by each system, the statistical power (1-$\beta$) and confidence interval ($\alpha$) statistical coefficients.

For the expected $\kappa$ values we choose 0.41, as $\kappa \geq 0.41$ is the threshold for moderate agreement and where higher $\kappa$ values are indicating stronger concordance (162). Based on our study population recruitment and enrollment at Duke, we expect prevalence of 33% high-grade lesions (HSIL/CIN2+) based on historical data, which is much higher than the population at whole.

Our secondary power calculation to detect a 5% difference in sensitivity and specificity with $\beta = 0.1$ and $\alpha = 0.05$, would be at least 1404 image interpretations with gold-standard histopathology. The STATA function “pss” (163) was used to perform the power calculations we would expect to need for estimated sensitivity values of 75% and 70% and estimated specificity values of 80% and 75% based on prior literature (147-150).
3.6 Results

3.6.1 Pilot Study- Pocket Colposcope Non-inferiority to the standard-of-care digital colposcope

To establish the Pocket Colposcope is non-inferior to the standard-of-care digital colposcope we conducted a pilot clinical study where both systems captured cervical images using acetic acid (VIA). These 44 image pairs were then randomized and assessed by n=8 blinded clinicians and compared to gold-standard histopathology (122).

3.6.1.1 Patient Demographics

The prevalence of HPV+ was 62%, and abnormal cytology was 77%. The distribution of pathology was 48% normal, 22% low grade (LSIL/CIN1), and 30% high grade (HSIL/CIN2+) (122).

Table 18: DUMC VIA Study Population Demographics

<table>
<thead>
<tr>
<th>n=44</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD Range</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.0±8</td>
</tr>
<tr>
<td></td>
<td>18-56</td>
</tr>
<tr>
<td>HPV</td>
<td>Positive (+)</td>
</tr>
<tr>
<td></td>
<td>27 (62%)</td>
</tr>
<tr>
<td></td>
<td>Negative (-)</td>
</tr>
<tr>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td></td>
<td>Not Available</td>
</tr>
<tr>
<td></td>
<td>16 (36%)</td>
</tr>
<tr>
<td>Cytology (Pap Smear)</td>
<td>Unknown Normal Abnormal</td>
</tr>
<tr>
<td></td>
<td>3 (17%)</td>
</tr>
<tr>
<td></td>
<td>4 (9%)</td>
</tr>
<tr>
<td></td>
<td>34 (77%)</td>
</tr>
<tr>
<td>Pathology</td>
<td>Normal LSIL/CIN1 HSIL/CIN2+</td>
</tr>
<tr>
<td></td>
<td>21 (48%)</td>
</tr>
<tr>
<td></td>
<td>10 (22%)</td>
</tr>
<tr>
<td></td>
<td>13 (30%)</td>
</tr>
</tbody>
</table>

3.6.1.2 Concordance with Standard-of-Care Colposcope

Note the comparable field of view and qualitative image quality for representative cervixes captured with acetic acid with the standard-of-care digital colposcope.
colposcope and Pocket Colposcope stratified by gold-standard histopathology Figure 29 (122).

<table>
<thead>
<tr>
<th>Normal</th>
<th>LSIL/CIN1</th>
<th>HSIL/CIN2+</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure 28: Representative visual inspection with acetic acid (VIA) images captured with the standard-of-care and Pocket Colposcope, the standard-of-care images (ABC) were taken at working distance of 300 mm, and the Pocket Colposcope images (DEF) were taken at 35 mm working distance. The scale bar is 10 mm, and yellow arrows indicate suspicious lesion sites biopsied. (AD) show biopsy confirmed normal cervix tissue, (BE) show LSIL/CIN 1 at 5-6 o’clock and (CF) show HSIL/CIN2+ confirmed pathology at 5-11 o’clock positions (122)

The level of agreement or overall accuracy between our Pocket Colposcope and the standard-of-care digital colposcope was 77.8% with a moderate κ value of 0.53 for the normal vs. LSIL/CIN1+ diagnostic cut-off and 86.0% with a moderate κ value of 0.64, p <0.001, Table 19 (122).
Table 19: Concordance between the Pocket Colposcope and Standard-of-Care reference digital colposcope using VIA for normal vs. LSIL/CIN1+ and normal vs. HSIL/CIN2+ diagnostic cut-offs, respectively.

<table>
<thead>
<tr>
<th>Standard-of-Care</th>
<th>Pocket Colposcope</th>
<th>Accuracy</th>
<th>$\kappa$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. LSIL/CIN1+ (n=44)</td>
<td>77.8±4.3</td>
<td>0.53</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Normal vs. HSIL/CIN2+ (n=34)</td>
<td>86.0±6.2</td>
<td>0.64</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

3.6.1.3 Similar Diagnostic Performance Against Gold Standard Histopathology

The overall accuracy or agreement for the early generation Pocket Colposcope and standard-of-care to gold-standard histopathology using the LSIL/CIN1+ diagnostic cut-off was between 56.1% and 63.9%, Table 20 (122). The level agreement of both systems approved when the diagnostic cut-off is changed to HSIL/CIN2+ with agreements of between 68.9% and 74.7% with improvement $\Delta 0.14$ in the Cohen’s kappa coefficient ($\kappa$). The sensitivity ranged from 39.1% to 56.0%, and the specificity ranged from 72.2% to 73.9% for both systems respectively when using the LSIL/CIN1+ diagnostic cut-off. The sensitivity remained stable while the specificity improved to 86.1% to 86.4% for both systems when using the HSIL/CIN2+ diagnostic cut-off, Table 20 (122).

Table 20: Pocket Colposcope and Standard-of-Care Diagnostic Performance using VIA (acetic acid) only

<table>
<thead>
<tr>
<th>Device</th>
<th>Accuracy</th>
<th>$\kappa$</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. LSIL/CIN1+ Pocket Colposcope</td>
<td>56.1±5.2</td>
<td>0.13</td>
<td>39.1±7.5</td>
<td>73.9±6.3</td>
<td>61.0±8.9</td>
<td>53.7±6.4</td>
</tr>
<tr>
<td>Normal vs. LSIL/CIN1+ Standard-of-Care</td>
<td>63.9±5.0</td>
<td>0.28</td>
<td>56.0±7.3</td>
<td>72.2±6.4</td>
<td>67.8±7.3</td>
<td>61.1±6.6</td>
</tr>
</tbody>
</table>
3.6.2. Pocket Colposcope Non-inferiority to Standard-of-Care Digital Colposcope in a Resource-Limited Setting

3.6.2.1 Patient Demographics

The prevalence of abnormal cytology was 18% but was not available for 73% of enrolled patients. The distribution of pathology was 53% normal, 30% low grade (LSIL/CIN1), and 17% high grade or cancer (HSIL/CIN2+) in Table 21 (117).

Table 21: Lima, Peru VIA+VILI Population Demographics

<table>
<thead>
<tr>
<th></th>
<th>n=129</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>37.1</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20-67</td>
<td></td>
</tr>
<tr>
<td><strong>Cytology (Pap Smear)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>68 (53%)</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>39 (30%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>15 (12%)</td>
<td></td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>68 (53%)</td>
<td></td>
</tr>
<tr>
<td>LSIL</td>
<td>39 (30%)</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>15 (12%)</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>7 (5%)</td>
<td></td>
</tr>
</tbody>
</table>

3.6.1.2 Concordance with Standard-of-Care Colposcope

Note the comparable field of view and qualitative image quality for representative cervixes captured with acetic acid with the standard-of-care digital colposcope and Pocket Colposcope stratified by gold-standard histopathology, Figure 29, with lesions in LSIL/CIN1 (from 2 to 7 o’clock), HSIL/CIN2+ (from 3 to 12 o’clock), and invasive cancer (from 7 to 3 o’clock) by both systems (117). VIA lesions were
confirmed and somewhat enhanced with VILI, with potential lesions having a mustard yellow appearance due to poor uptake of Lugol’s Iodine in contrast to a dark mahogany characteristic of uptake (117).

Figure 29: Representative visual inspection with acetic acid (VIA) and Lugol’s Iodine images (VILI) captured with the standard-of-care and Pocket Colposcope, with acetic acid images (C to J) and Lugol’s Iodine images (K to R) captured by Pocket Colposcope (G to J & O to R) and Standard-of-Care (A to D & K to N), with lesions in LSIL/CIN1 (DHLP) with lesions at 2 to 7 o’clock, HSIL/CIN2+ (EIMQ) with lesion at 3 to 12 o’clock, and invasive cancer (FJNR) at 7 to 3 o’clock. The scale bar is 10 mm. VIA lesions were confirmed and somewhat enhanced with VILI, with potential lesions
having a mustard yellow appearance due to poor uptake of Lugol’s Iodine in contrast to a dark mahogany characteristic of uptake. The overall accuracy or agreement for the Generation 3 and 4 Pocket Colposcope and standard-of-care to gold-standard histopathology using the LSIL/CIN1+ diagnostic cut-off was between 63.9% and 67.6%, Table 17 (117). The level agreement of both systems approved when the diagnostic cut-off is changed to HSIL/CIN2+ with was 63.1% for both with no significant change in the fair agreement Cohen’s kappa coefficient (κ). The sensitivity ranged from 71.2% to 79.8%, and the specificity ranged from 57.7% to 56.6% for both systems respectively when using the LSIL/CIN1+ diagnostic cut-off (117). The sensitivity improved slightly to 80.7% to 82.2%, while the specificity stays unchanged 57.5% to 56.6% for both systems when using the HSIL/CIN2+ diagnostic cut-off, Table 22 (117).

Table 22: Pocket Colposcope and Standard-of-Care Diagnostic Performance using VIA and VILI

<table>
<thead>
<tr>
<th>Device</th>
<th>Accuracy</th>
<th>κ</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. LSIL/CIN1+ (n=129)</td>
<td>Pocket Colposcope Standard-of-Care</td>
<td>63.9±6.8</td>
<td>0.29</td>
<td>71.2±21.4</td>
<td>57.5±26.2</td>
<td>62.3±7.5</td>
</tr>
<tr>
<td></td>
<td>Standard-of-Care</td>
<td>67.6±6.0</td>
<td>0.46</td>
<td>79.8±15.6</td>
<td>56.6±18.7</td>
<td>63.4±6.2</td>
</tr>
<tr>
<td>Normal vs. HSIL/CIN2+ (n=90)</td>
<td>Pocket Colposcope Standard-of-care</td>
<td>63.1±16.9</td>
<td>0.31</td>
<td>80.7±15.5</td>
<td>57.5±26.2</td>
<td>41.4±10.0</td>
</tr>
<tr>
<td></td>
<td>Standard-of-care</td>
<td>63.1±11.2</td>
<td>0.32</td>
<td>82.2±16.0</td>
<td>56.6±18.7</td>
<td>39.8±6.1</td>
</tr>
</tbody>
</table>
3.6.3. Increasing the number of contrast agents improves the diagnostic performance of the Pocket Colposcope

3.6.3.1 Patient Demographics

The prevalence of high-risk HPV infection was 21.4%, for all sites. However, 73.4% did not have this information available. Colposcopy accounted for 77% of the procedures that patient was participating in, and the remaining 23% were LEEP or LEETZ. The distribution of pathology was 47.6% normal, 26.4% low grade (LSIL/CIN1), and the remaining 26% high grade or cancer (HSIL/CIN2+), Table 23.

Table 23: Multisite Study Population Demographics stratified by Level of Contrast

<table>
<thead>
<tr>
<th>Classification</th>
<th>Overall</th>
<th>VIA</th>
<th>VIA+GLI</th>
<th>VIA+VILI</th>
<th>VIA+GLI+VILI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td>n=388</td>
<td>n=129</td>
<td>n=92</td>
<td>n=127</td>
</tr>
<tr>
<td>HPV+</td>
<td>Unknown</td>
<td>73.4%</td>
<td>26.8%</td>
<td>9.1%</td>
<td>33.1%</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>5.2%</td>
<td>0.7%</td>
<td>0.7%</td>
<td>0.7%</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>21.4%</td>
<td>14.8%</td>
<td>3.6%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Procedure Type</td>
<td>Colposcopy</td>
<td>77.0%</td>
<td>32.4%</td>
<td>5.5%</td>
<td>36.0%</td>
</tr>
<tr>
<td></td>
<td>LEEP/LEETZ</td>
<td>23.0%</td>
<td>11.6%</td>
<td>8.0%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Pathology</td>
<td>Normal</td>
<td>47.6%</td>
<td>21.2%</td>
<td>3.9%</td>
<td>20.2%</td>
</tr>
<tr>
<td></td>
<td>LSIL</td>
<td>26.4%</td>
<td>12.3%</td>
<td>2.2%</td>
<td>10.4%</td>
</tr>
<tr>
<td></td>
<td>HSIL</td>
<td>23.2%</td>
<td>10.1%</td>
<td>6.9%</td>
<td>4.3%</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>2.8%</td>
<td>0.36%</td>
<td>0.36%</td>
<td>0.36%</td>
</tr>
</tbody>
</table>

3.6.3.2 Diagnostic Performance against Gold Standard Histopathology

Note the comparable field of view and qualitative image quality for representative cervixes captured with acetic acid, green light inspection, and Lugol’s Iodine, for the Pocket Colposcope stratified by gold-standard histopathology Figure 30, with lesions in LSIL/CIN1 (from 1 to 3 o’clock), HSIL/CIN2+ (from 2 to 10 o’clock).
Figure 30: Representative images captured with the Pocket Colposcope for all 3 contrasts, stratified by histopathological classification. Normal (ADG), LSIL/CIN1 (BEH), HSIL/CIN2+ (CFI), with acetic acid (VIA) stained cervix (ABC), green light inspection (GLI) shown in second row (DEF) and Lugol’s Iodine (VILI) stained cervix (GHI) in the bottom row. The scale bar is 10 mm.
The overall accuracy or agreement for the Colposcope and gold-standard histopathology using the LSIL/CIN1+ diagnostic cut-off progressively increased from 53.4% to 70.1%, as the number of contrast agents increased from acetic acid only (VIA) to all three contrast agents (VIA+GLI+VILI), Table 24. The trend is mirrored when the diagnostic cut-off is changed to HSIL/CIN2+, and the overall accuracy ranged from 55.9% to 70.8%, as contrast agents increased from VIA to all three contrast agents (VIA+GLI+VILI). The Pocket Colposcope’s Cohen’s kappa coefficient (κ) a measure of agreement with gold-standard histopathology also improved from for both diagnostic cut-offs from slight to a moderate agreement as the number of contrast agents increased (0.2 to 0.41), Table 24. The Pocket Colposcope’s sensitivity improved from 42.3% to 68.1% and the specificity generally improved from 56.7% to 73.3%, when using the LSIL/CIN1+ diagnostic cut-off. When the HSIL/CIN2+ diagnostic cut-off is used, the Pocket Colposcope’s sensitivity was comparable previous diagnostic cut-off ranging from 41.9% to 73.9% improving as a function of increasing the number of contrast agents used, while the specificity improved 67.5% to 88.0%, Table 24.

Table 24: Summary of Level of Contrast stratified Diagnostic Characteristics for Normal vs. LSIL/CIN1+ and Normal vs. HSIL/CIN2+ cut-offs. The number of patient images (n), Accuracy, kappa statistic (κ) Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) are summarized. Mean ± 95% Confidence Interval is reported when applicable.

<table>
<thead>
<tr>
<th>Diagnostic Cut-Off</th>
<th>Level of Contrast</th>
<th>n</th>
<th>Accuracy ± CI</th>
<th>κ</th>
<th>Sensitivity ± CI</th>
<th>Specificity ± CI</th>
<th>PPV ± CI</th>
<th>NPV ± CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. LSIL/CIN1+</td>
<td>VIA</td>
<td>129</td>
<td>53.4±2.7</td>
<td>0.08</td>
<td>42.3±5.5</td>
<td>65.3±5.8</td>
<td>56.7±7.5</td>
<td>51.4±5.3</td>
</tr>
<tr>
<td></td>
<td>VIA+GLI</td>
<td>92</td>
<td>55.4±5.3</td>
<td>0.11</td>
<td>46.4±9.4</td>
<td>61.6±13.1</td>
<td>72.2±6.7</td>
<td>34.8±9.7</td>
</tr>
<tr>
<td></td>
<td>VIA+VILI</td>
<td>127</td>
<td>61.5±3.1</td>
<td>0.25</td>
<td>67.4±6.6</td>
<td>56.7±6.1</td>
<td>56.4±6.1</td>
<td>67.7±4.0</td>
</tr>
<tr>
<td></td>
<td>VIA+GLI+VILI</td>
<td>40</td>
<td>70.1±8.2</td>
<td>0.40</td>
<td>68.1±15.2</td>
<td>73.3±14.4</td>
<td>80.0±10.9</td>
<td>59.5±12.0</td>
</tr>
<tr>
<td>Normal vs. HSIL/CIN2+</td>
<td>VIA</td>
<td>93</td>
<td>55.9±7.6</td>
<td>0.21</td>
<td>41.9±9.2</td>
<td>78.2±5.0</td>
<td>49.5±9.2</td>
<td>72.5±5.3</td>
</tr>
<tr>
<td>-----------------------</td>
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<td>------</td>
<td>----------</td>
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<td>----------</td>
</tr>
<tr>
<td>VIA+GLI</td>
<td>77</td>
<td>58.7±13.2</td>
<td>0.23</td>
<td>48.1±11.4</td>
<td>80.0±10.9</td>
<td>83.0±9.4</td>
<td>43.2±12.1</td>
<td></td>
</tr>
<tr>
<td>VIA+VILI</td>
<td>91</td>
<td>68.9±8.5</td>
<td>0.32</td>
<td>73.9±9.8</td>
<td>67.4±6.0</td>
<td>40.2±9.0</td>
<td>89.7±4.1</td>
<td></td>
</tr>
<tr>
<td>VIA+GLI+VILI</td>
<td>30</td>
<td>70.8±21.4</td>
<td>0.41</td>
<td>52.2±21.0</td>
<td>88.0±9.5</td>
<td>80.0±15.7</td>
<td>66.7±15.3</td>
<td></td>
</tr>
</tbody>
</table>

In order to compare differences in diagnostic performance of the Pocket Colposcope when stratifying by level of contrast used, we created Receiver Operating Characteristics (ROC) Curves which plot the pooled sensitivity in the y-axis and 1-specificity in the x-axis. The overall diagnostic performance per type of contrast agent can be quantified as the area under the curve (AUC), using logistic regression we can compare the differing AUCs stratified by level of contrast. The Pocket Colposcope AUC was significantly higher when using all three contrast agents (VIA+GLI+VILI) and two contrast agents (VIA+VILI) when compared to VIA only using the LSIL/CIN1+ diagnostic cut-off (Table 25, Figure 31A). When the diagnostic cut-off is changed to HSIL/CIN2+ the Pocket Colposcope’s AUC using all three contrast agents (VIA+GLI+VILI) and two contrast agents (VIA+VILI) was significantly larger when compared to VIA only (Table 20, Figure 31B).
Figure 31: Receiver Operating Characteristic (ROC) Curves Stratified by Sources of Contrast with (diagnostic cut-off of (A) LSIL/CIN1+ and (B) HSIL/CIN2+ (B) vs. normal are shown with the pooled Sensitivity plotted on the y-axis and pooled 1-Specificity plotted on the x-axis. The level of contrast is indicated with: random chance (dashed line), VIA (gray triangle), VIA+GLI (green diamond), VIA+VILI (purple square), and all three contrasts (blue circle). Each clinicians’ sensitivity and 1-specificity are scatter plotted. For both diagnostics cut-offs, there is a statistically significant increase in the AUC with increasing levels of contrasts used.

Table 25: Summary of Logistic Regression comparison of Receiver Operating Characteristic (ROC) Curves by the level of contrast for the diagnostic cut-offs LSIL/CIN1+ vs. normal and HSIL/CIN2+ vs. normal. There are statistically significant increases in the Area Under the Curve (AUC) as a function of increased leveling number of contrasts used for both diagnostic cut-offs. The logistic regression uses the Visual Inspection with Acetic Acid (VIA) as the reference group for comparison to combinations with Green Light Inspection (GLI) and Visualization with Lugol’s Iodine (VILI).

<table>
<thead>
<tr>
<th>LSIL/CIN1+ vs. Normal</th>
<th>HSIL/CIN2+ vs. Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contrast</strong></td>
<td><strong>AUC</strong></td>
</tr>
<tr>
<td>VIA</td>
<td>0.5380</td>
</tr>
<tr>
<td>VIA+GILI</td>
<td>0.5655</td>
</tr>
<tr>
<td>VIA+VILI</td>
<td>0.6204</td>
</tr>
</tbody>
</table>
3.7 Discussion

The purpose of Aim 2 was to demonstrate the concordant diagnostic performance of the Pocket Colposcope when compared to reference standard-of-care digital colposcopes and gold-standard histopathology in a multi-institution clinical trial. The overall accuracy when using the LSIL/CIN1+ vs. normal diagnostic cut-off and comparing to gold-standard histopathology increased consistently with the type of contrasts used (VIA only, VIA+GLI, VIA+VILI, and culminating with VIA-GLI-VILI) with overall agreement improving from 53.4-70.1% with sensitivity improving from 42.3-68.1%, with specificity improving from 65.3-73.3%. The diagnostic performance when the diagnostic cut-off is set to HSIL/CIN2+ vs. normal followed the prior trend with consistent increases with the type of contrast used (VIA only to VIA+GLI+VILI) with rising accuracy 55.9-70.8%, with sensitivity increasing 41.9-52.2%, with specificity improving 78.2-88.0%. Increasing the number of contrast agents used significantly improved the diagnostic performance of the Pocket Colposcope when compared to gold-standard histopathology.

The performance of the Pocket Colposcope was in line with prior studies with remote image review. Interestingly, remote review of cervical images has some potential limitations, the NCI’s ALTS (ASCUS LSIL Triage Study) reported sensitivities of 37.3% and specificities of 89.7% from 4 expert colposcopists reviewing 3549 individual digital
cervical images captured using high-end colposcopy system(164). The expected level of agreement is between around 84-89% based on NCI’s ALTS (ASCUS LSIL Triage Study) from 3 expert colposcopists interpreting 3,639 different subject cervical images captured from the same high-end colposcopy system(165).

The concerns with VIA with magnification (VIAM) as a primary screening tool are the potentially poor specificity (too many false positives) and resultant overtreatment of healthy women (166). Studies on VIAM have indicated very large inter-observer variability that would be indicative of a need for improving training due to the subjective nature of the test (63). Barriers for widespread implementation of VIAM include lack of funds for training (166), staff shortages that prevent in-service training (166), “brain drain” loss of skilled workers to other countries, leading to a shortage of nearly 1,000,000 skilled healthcare workers in 2006 (167) and in some cases lack of required equipment(167). In Tanzania, the physician to population ratio is 1 per 23,000, one of the lowest globally, and it only has 15 pathologists in the country (1 per 2.5 million people) (168). One way to address this quality control issues would be to incorporate training into the control software of the device. There are no standardized teaching or training programs in low-resource settings(169). For example, an intensive five-day education program for VIA is implemented on a cohort of health care workers from Uganda and El Salvador and pre-test accuracy was 57% and post-training markedly improved to 80% with sustained retention at six months post-training(170).
We plan to incorporate the curriculum utilized into A/V interactive module such that instead of requiring five days of lost in-service training, modules could be done in smaller portions in the distance based learning model. Whereas the prior paper utilized traditional methods and require the transportation and housing to a central location for training, distance learning could be readily implemented and online quizzes could be used for periodic post-training assessment and provide an avenue for continuing medical education via updates to the mobile app.

Further improvements in the diagnostic performance of the Pocket Colposcope could also include an automated algorithm to aid with clinician diagnosis of lesions by using machine learning and the training on the repository of previously collected digital images(142). CDSS is a computerized clinical decision support system for cervical cancer screening that can interpret Pap reports, a pilot design was modeled from 49,293 Pap reports and successfully guided the optimal treatment recommendation for 73/74 against gold standard experts classification (171). This system could be adapted for Reid Index (145) using and weighing different clinical observations of the VIAM exam and helping frontline health workers in properly identifying high-risk patients needing treatment. Lastly, we are beginning implementation studies to determine the feasibility and cost benefit of using the Pocket Colposcope as part of a “see and treat” paradigm with a low cost Liger Thermocoagulator (172).
4. Aim 3: Design and integration of fluorescent imaging capability to the Pocket Microscope to allow the interrogation of an additional source of contrasts and expanded applications outside the cervix

We shift our focus now in the final aim to breast cancer and how the fluorescent Pocket Colposcope can impact the clinical care paradigm. In this aim our goal is to add fluorescent imaging capability to the Pocket Microscope to provide an additional source of contrast targeting metabolic and/or structural biomarkers that will further improve the clinical performance of our system to match gold standard histology pathology and expand the application of the device. There is broad applicability in other organ sites including cervix, oral cavity, gastrointestinal tract, and skin.

4.1 Preface

This work was published with assistance from authors Christopher Lam, Brian Crouch, Jennifer Gallagher, Robert Morhard, Corrine Nief, Megan Madonna, Jenna Mueller, Riley Deutsch, Roujia Wang, Allison Hall, Mary Scott Soo, Philip Hughes, Timothy Haystead, and Nirmala Ramanujam.

4.2 Introduction

4.2.1 Motivation

Breast cancer like cervical cancer is a tremendous burden with over 2.1 million new cases and 630,000 deaths annually (2). Over 65% of this burden is in LMICs(2). Current guidelines for breast cancer screening and treatment involve a multiple visit
paradigm similar to the one for cervical cancer with some additional levels of therapy beginning with mammography at age 40 every 1-2 years for women of “average” risk. Positive results are referred for ultrasound guided biopsy, positive women are then referred to neo-adjuvant therapy in conjunction with breast conserving surgery. Surgery therapy can require re-incision for further tissue removal due to inadequate tumor margins and can also include further adjuvant therapy. But there are issues with this current paradigm being applied in LMICs, these include the frequent number of visits required and potential for overtreatment (91, 92). Recommendations and viable options are much less clear in resource limited settings when compared to cervical cancer. Mammography is still the preferred option but not readily available in LMICs thus clinical breast exam with ultrasound has been suggested but not yet universally accepted despite becoming more affordable and portable (91, 92). All the current recommended therapeutic options are quite resource intensive including: surgery and systemic therapy. The WHO also recommends devoting some resources to establishing palliative care (91, 92).

4.2.1.1 Potential fluorescent targets

What criteria is needed for the theranostic platform? First we want an agent that exploits differential expression of tumor and normal cells, thereby allowing for targeted therapeutic effects without side effects commonly associated with chemotherapy, we also want ubiquitous expression in cancer subtypes, and to ameliorate the burden on
pathology. Could we have a system that allows for both wide-field and high resolution real time surveillance? While there exists a plethora of molecular diagnostics one could consider for this application, we chose to leverage one that is already being used for breast cancer treatment, a Heat shock protein-90 inhibitor. Hsp-90 is a chaperone protein that increases in response to stress and is highly expressed on the surface of different breast cancer subtypes. By tethering the Hsp-90 inhibitor with a fluorescent we can transform it into a molecular diagnostic. Our group has a key collaborator with a FITC-labeled Hsp-90 inhibitor (HS-27) which we could use to demonstrate as proof of concept capability of the Fluorescent Pocket Colposcope (173-175). Hsp-90 overexpression has been linked with breast cancer malignancy and is a profound target for therapy(176, 177). Our group and others have demonstrated that Hs-27 binds to all receptor subtypes of breast cancer in-vitro(176, 177).

4.3 Material and Methods

4.3.1 Design and integration of fluorescent imaging capability with Pocket Colposcope

The Pocket Colposcope will serve as the basis for the fluorescent imaging device, which contains a 5 MP color CMOS camera with both manual and automatic focus capabilities that allows adjusts in magnification from 3-52X (137). An exploded schematic view of the prototype Pocket Colposcope with fluorescent imaging capability shown in Figure 32. A modular probe tip CNC milled from 6061 aluminum acts as a
heatsink and holds the excitation light source, a concentric ring of blue LEDs (470±15 nm, FWHM Luxeon Z) to excite fluorophores that share FITC’s excitation and emission profile (such as HS-27), a band-pass emission filter (535±25nm) located in front of the CMOS detector to block LED excitation light and collect fluorescence. Cross-polarization is accomplished with a linear glass polarizer placed (#43-783; Edmund Optics, Barrington NJ) in-line and parallel to the optical imaging axis. A second linear plastic film polarizer (#86-178; Edmund Optics, Barrington NJ) was laser cut with a donut hole shape configuration such that was placed over the illumination source (LEDs) at an orthogonal orientation to the imaging axis polarizer but not obfuscate the preexisting linear polarizer (100, 104).

Figure 32: Exploded view of the fluorescent Pocket Colposcope, with (1) linear film polarizer in the parallel configuration, (2) blue LED ring, (3) aluminum LED ring heat sink and optic mount, (4) band-pass emission filter, (5) linear film polarizer in the orthogonal orientation, and (6) Pocket colposcope base device with 5-megapixel CMOS camera.
The Beta generation of the Pocket Colposcope was modified for fluorescent imaging by adding excitation LEDs, repurposing of the Generation 3 external LED driver circuit to drive the excitation source, a matched band-pass optical filter for emission. The fluorescent Pocket Colposcope also reintroduces cross-polarization to improve the fluorescent signal to noise, and a modular tip that houses the LED ring and optical components without destructive modification of the Pocket Colposcope.

4.3.2 Bench tests for Fluorescent Colposcope

A proof of concept device was imaging and illumination performance characteristics previously used to assess the Pocket Colposcope for the 510(k) regulatory approval pathway presented in Aim 1 Section 2.3.5. A phantom consisted of fluorescence spheres (Polysciences, Fluoresbrite YG Microspheres) and TiO2 (Sigma, T8141) in a polydimethylsiloxane (PDMS) sample (Dow Corning, Slygard 184)(178). The phantoms were constructed in a petri dish with a cover glass window on the bottom (Mattek, P35G-0-14-(C))(178). The phantoms used a layer of 10-μm diameter fluorescent spheres dried on the cover glass to generate an optically thin layer of fluorescence (simulating the Hsp-90 expression as bound to the HS-27). A 1 cm layer of PDMS was added behind the fluorescent layer with variable concentrations of TiO2 to create three separate phantoms, each with different scattering levels where the reduced scattering coefficients were $\mu_s'=10 \text{ cm}^{-1}$. A quantity of 2.25 milligrams of TiO2 per gram of uncured PDMS was added to the PDMS for each respective scattering level, calculated according to a previously published procedure(178). The TiO2 was thoroughly mixed with the
PDMS prior to curing. Finally, the phantom was placed in a vacuum chamber to draw out all residual air bubbles from the mixing process and also to effectively cure the PDMS.

4.3.3 Animal Studies

All animal experiments are performed in accordance with protocols approved by the Duke University Institution for Animal Care and Use Committee. Animals were housed on-site with continual access to food and water under normal 12-hour light/dark cycles.

4.3.3.1 Ex vivo imaging of excised tumor

In-vivo and ex-vivo pilot animal studies were conducted to demonstrate successful implementation of the fluorescent imaging capability using a FITC (Fluorescein Isothiocyanate) tagged binding ligand (HS-27) with a strong affinity for the heat shock protein-90 (Hsp-90) chaperone molecule (a potential biomarker of cancer)(173, 174). A metastatic murine breast cancer cell line (4T1) (179) were used in the pre-clinical study and were acquired from the American Type Culture (ATC) collection and cultured under standard conditions at 37°C and 5% CO₂. Cells are maintained in RPMI-1640 (L-glutamine) medium supplemented with 10% FBS and 1% penicillin-streptomycin. These cultured cells are used within one month of the first passage. The 4T1 tumors are grown in the flank of n=6 nude/nude mice for optimizing ex vivo imaging parameters. Specifically, 10⁶ 4T1 cells were re-suspended in 100 µL serum-
free medium and injected into the right flank to establish tumors\(^{(180)}\). Tumors were allowed to grow to a target volume of \(1 \text{ cm}^3\) to form a mass similar in size to those evaluated in clinical radiology. We compared the performance of our device to that of a high resolution micro endoscope developed by a collaborating lab at Rice University \(^{(181, 182)}\).

### 4.3.4 Pilot Clinical Ex-Vivo Study

We performed a preliminary clinical study in a diagnostic biopsy setting to get a variety of receptor subtypes as well as non-tumor tissues. First, we imaged ultrasound guided biopsy specimens from women being referred for breast conserving surgery using the Fluorescent Pocket Microscope and HS-27 agent. All clinical imaging is performed in accordance with Duke IRB approved protocol number Pro00008003. After giving informed consent, a pilot investigation of \(n=9\) adult patients undergoing standard of care ultrasound-guided core needle biopsy (USGCNB) are enrolled. Each biopsy was received within 5-minutes of tissue excision and had 100 \(\mu\text{M}\) HS-27 topically applied to the biopsy for 1-minute before thorough rinsing with PBS\(^{(176)}\). A trained pathologist reviewed the biopsies which were inked with three colors to allow for co-registration with the fluorescent Pocket Colposcope captured images. The pathology reports also include the percent tumor area (PTA), tumor cellularity, and the percent benign tissue area.
4.4 Results
4.4.1 Fluorescent Pocket Colposcope Bench Testing

The modifications to the fluorescent Pocket Colposcope did not diminish the quality of the images captured, however, due to the physical constraints (height in the imaging axis) the introduction of image vignetting or clipping around the circumference of the detector is seen. This reduced the usable field of view by 15-20% across and limited the maximum possible magnification (as the minimum work distance is now between 10-12 mm versus the unmodified Pocket Colposcope which used at the 5 mm working distance and allows for the 72-lp/mm resolving capability (~7-micron feature size).

Table 26: Summary of Imaging Characteristics Beta and Fluorescent Pocket Colposcope

<table>
<thead>
<tr>
<th>Device Generation Parameters</th>
<th>Beta</th>
<th>Fluorescent Pocket</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical resolution</td>
<td>24.8 (10-72)</td>
<td>24.6 (10-54)</td>
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<tr>
<td>Field of View</td>
<td>30.7 (8-52)</td>
<td>25.2 (6-43)</td>
</tr>
<tr>
<td>Picture-Height Distortion</td>
<td>-1.2</td>
<td>-1.4</td>
</tr>
<tr>
<td>Depth of Field</td>
<td>8.5 (1-11)</td>
<td>8.4 (2-11)</td>
</tr>
<tr>
<td>Excitation LED</td>
<td>N/A</td>
<td>470±20nm</td>
</tr>
<tr>
<td>Emission Filter</td>
<td>N/A</td>
<td>535±26nm</td>
</tr>
</tbody>
</table>
4.4.2 Application of *Ex vivo* Clinical Breast Biopsies

Clinical *ex-vivo* detection of HS-27 was confirmed with *ex-vivo* breast needle biopsy with the Pocket Colposcope. Representative Pocket mammoscope fluorescence images (left) and histology images (right) of a Her2+, ER+, and benign biopsy (Figure 36), shown in with heat map color coding, where red indicates high fluorescent signal and blue with low or no signal. This finding corresponded to the coregistered pathology of cancer for the brightest staining region. Wide field fluorescent image of an ER+ tumor needle biopsy with 100 uM HS-27 for 1-minute demonstrating and high resolution image imaging (Figure 37). Cumulative Density Functions comparing pathology stratified pixel intensity between benign (black) and tumor (red). (C) mean fluorescence calculated from Pocket’s fluorescence images demonstrate increased fluorescence in
tumor relative to benign biopsies (Figure 38). These CDFs were compared using Kolmogorov-Smirnov testing. Mean fluorescence was compared with a Wilcoxon Rank Sum test. This data demonstrates the potential feasibility for HS-27 to be used as a diagnostic tool in the clinical setting. The modified Pocket Microscope can capture the whole field of view of the specimen (about 15-25 mm in length) in a single capture.

Table 27: Patient demographics for ultrasound guided needle biopsy study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td></td>
</tr>
<tr>
<td>Ultrasound biopsy</td>
<td>9</td>
</tr>
<tr>
<td>Patient Demographics</td>
<td></td>
</tr>
<tr>
<td>Average Age (range)</td>
<td>48 (27-73)</td>
</tr>
<tr>
<td>Average BMI (range)</td>
<td>27 (18-44.7)</td>
</tr>
<tr>
<td>Pathology Breakdown</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>5 (56%)</td>
</tr>
<tr>
<td>Benign</td>
<td>4 (44%)</td>
</tr>
<tr>
<td>Receptor Status (malignant only)</td>
<td></td>
</tr>
<tr>
<td>ER +,-</td>
<td>5 (100%), 0 (0%)</td>
</tr>
<tr>
<td>PR +,-</td>
<td>5 (100%), 0 (0%)</td>
</tr>
<tr>
<td>Her2 +,-</td>
<td>1 (20%), 4 (80%)</td>
</tr>
<tr>
<td>TNBC</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Menopausal Status</td>
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<tr>
<td>Pre-menopause</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Peri-menopause</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Post-menopause</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Breast Density</td>
<td></td>
</tr>
<tr>
<td>Fatty</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Scattered Fibroglandular</td>
<td>2 (27%)</td>
</tr>
<tr>
<td>Heterogeneous Density</td>
<td>3 (47%)</td>
</tr>
<tr>
<td>Extremely Dense</td>
<td>1 (13%)</td>
</tr>
</tbody>
</table>

Figure 34: Comparison of the FOV and dynamic range of the Pocket Mammoscope and fiber system, 4T1 biopsies were treated with either 100 µM HS-27 or HS-217 for 1-minute prior to washing and imaging with both the fiber system and Pocket mammoscope. (A) Co-registered overlay of an image taken with the fiber system on top of a Pocket Microscope image taken from the same HS-27 stained biopsy. The fiber system image is highlighted by the white box. (B) CDFs of the ratio of HS-27 to HS-217 fluorescence demonstrate comparable specificity ratios between the two systems. Sample size – n = 4 for both the fiber system and Pocket mammoscope. (C) Fiber system and Pocket Mammoscope (PM) fluorescence images taken of an ER+ biopsy stained with 100 uM HS-27 for 1 minute. (B) Comparison of CDFs created from the fiber system and Pocket mammoscope images of the ER+ biopsy in (C).
Figure 35: Representative images with corresponding histology, Her2+ having the greatest HS-27 fluorescence (all significant vs normal). Representative Pocket mammoscope fluorescence images of a Her2+, ER+, and benign biopsy. False color with red intensity indicating peak HS-27 fluorescence corresponding to the elevation of Hsp-90 expression, with 1 mm scale bars.
Figure 36: Ex-vivo HS-27 detection with the Fluorescent Pocket Colposcope in Human Breast Needle Biopsy Sample with (top) full field of view and (bottom) higher resolution mode. False color with red intensity indicating peak HS-27 fluorescence corresponding to the elevation of Hsp-90 expression, with 1 mm scale bars.
Figure 37: Increased fluorescent in tumors when compared to benign specimens. (B) Cumulative Density Functions comparing pathology stratified pixel intensity between benign (black) and tumor (red). (C) mean fluorescence calculated from Pocket’s fluorescence images demonstrate increased fluorescence in tumor relative to benign biopsies. Sample sizes – n = 5 tumor biopsies, n = 4 benign biopsies. These CDFs were compared using Kolmogorov-Smirnov testing. Mean fluorescence was compared with a Wilcoxon Rank Sum test.

4.5 Discussion

The use of HS-27 a potent ligand for Hsp-90 is a particular interest for breast cancer as both a therapeutic and diagnostic agent (173, 174). We have previously demonstrated in Aim 2, that increasing the sources of contrast improves the diagnostic performance of the Pocket Colposcope when compared to gold-standard histopathology. These heat shock proteins could be potent targets for combined diagnostic and therapeutic intervention when coupled with ethanol ethyl-cellulose injection therapy for the cervix. Another potential target would be overexpressed biomarkers in cervical pre-cancer and cancer, such as heat shock proteins including Hsp-
40, Hsp-60, and Hsp-70 (183). These heat shock proteins could be potent targets for combined diagnostic and therapeutic intervention when coupled with ethanol ethylcellulose injection therapy for the cervix. Hsp-70 has been shown as a potential antiddengue virus activity and it would be interesting to see if its antiviral activity would also encompass HPV (184).

The metabolic activity could be readily assessed in the fluorescent Pocket Colposcope were modified to detect TMRE (Tetramethylrhodamine, ethyl ester) a probe for mitochondrial potential and 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose) a fluorescent glucose analog to allow for detailed surveillance of tumor metabolic state (185). Tracking metabolism could be used to discriminate between normal and abnormal tissue and improve the diagnostic performance of the Pocket Microscope for cervical, breast, and other cancer types.

Our group has extensively used the fluorescent probes TMRE and 2-NBDG as a technique to observe tumor metabolism (185). Tetramethylrhodamine, Ethyl Ester, Perchlorate (TMRE) is a probe for mitochondria potential which can be imaged using a Cy3 (cyanine 3) or TRITC (Tetramethylrhodamine) fluorescent profile (186), and 2-NBDG is a fluorescent glucose analog tagged with a FITC-like profile (185).

Concordant with the replication of the virus and controlled cellular replication, there is a dramatic increase in energy consumption by the HPV infected cervical cells. Upregulation of glucose (glucose transporter, GLUT-1) (187-189) and lactate
(monocarboxylate transporters, MCT-1 and MCT-4) transporters (190-194) has been widely reported in cervical pre-cancers and cancers with increasing expression highly correlated with increasing severity of the disease. Increased activity of the first two enzymes in the pentose pathway, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase has been reported in cultured samples from patients with biopsy-confirmed cervical intraepithelial neoplasia(195) and confirmed with studies in HPV+ cervical carcinoma cell lines(196), indicating the Warburg effect on the metabolism towards aerobic glycolysis. HPV-16 E7 oncoproteins increased glycolysis and glutaminolysis through upregulation of pyruvate kinase type M2(197). Studies in human keratinocytes cell lines infected by HPV demonstrated a profound increase in energy consumption when compared to non-infected control cells. This increased demand in energy was concordant with an increased protein translation and virion duplication, followed by contraction of cell size and shift to IRES (internal ribosome entry site) dependent translation for production of proteins (198). Furthermore, primary cervical cells immortalized by HPV infection demonstrated markedly higher rates of estrogen (16α-hydroxylate estradiol) (199, 200) and cholesterol metabolism (201) when compared to normal control cell lines, potentially indicative of additional virally induced metabolic adaptations. More recently, the mechanism for this metabolic adaptation has been further elucidate with the discovery that the E2 oncoproteins encoded by high-risk HPV-16 and -18, but not low-risk HPV-6 is shown to co-localize
with the host cell’s mitochondria and induce distinct cristae morphology changes concordant with a significant increase in mitochondrial reactive oxygen species (ROS) production without the induction of apoptosis (202). Furthermore, the E1^E4 oncoproteins encoded by high-risk HPV-16 has been shown to the first bind to and initiate the breakdown of the cytokeratin support network of the infected cell and then is translocated to the mitochondria (203). The mitochondria bound to E1^E4 begin to migrate and aggregate around the nucleus before depolarizing and initiation apoptosis (203). This is the hypothesized mechanism for the release of the duplicated HPV virion once the cervical epithelial cell reaches to the outermost epithelial surface to infect other cells (203). Furthermore, there is a profound and significant association for the increasing number of mitochondrial DNA mutations with the severity of cervical pre-cancer and cancer grade (204-208). Increases in mitochondrial DNA copy number have been associated with higher risk of other cancers including breast, head and neck, endometrial, ovarian, among others (209, 210). Although, it is unknown if these mutations occurred before HPV infection or as a result of HPV infection’s increased mitochondrial ROS production. Each mitochondrion is estimated to contain between two to ten copies of mitochondrial (mt)DNA, which is also a circular dsDNA like HPV (211). There are approximately 1000 total copies of mtDNA per normal human cell (212).
5. Conclusion

5.1 Summary

Addressing disparities in the global cancer burden is a key part of the post-2015 Millennium Development Goals (MDGs). Cervical cancer is emblematic of that disparity, with 85% of cases and cancer-related deaths occurring in low and middle-income countries (LMICs). Many LMICs lack the healthcare infrastructure and highly trained personnel required for cytology-based screening and subsequent referral colposcopy based diagnosis, which have dramatically reduced the disease burden in wealthier countries. The World Health Organization (WHO) recommends the adoption of alternative protocols that employ low-cost and simple-to-use screening technologies and treat all women who are positive based on these tests. Even those who manage to receive a proper diagnostic test are all too frequently lost to follow up care, leading to a disproportionately high burden of cervical cancer mortality in LMICs. One strategy, highly sensitive human papillomavirus (HPV) testing has been shown to reduce the incidence and mortality from cervical cancer when coupled directly with outpatient treatment for women with HPV-positive results. Although, recent guidelines have moved back from this “screen & treated” approach, given concerns about overtreatment. The American Society of Clinical Oncology (ASCO) and World Health Organization (WHO) recently released guidelines recommending that HPV be used as a screening test, followed by triage with visual inspection with acetic acid (VIA) or visual inspection
with Lugol’s iodine (VILI) to confirm the presence of lesions. Uptake of HPV testing requires a significant capital investment in the automated device and samples are often banked and run in batches to reduce waste of reagents. While this co-testing methodology may decrease overtreatment, VIA or VILI remains a poor triage test because of low sensitivity and specificity, and wide variability in interpretation due to poor quality control. Thus, there exists a need for a triage test that is low-cost, easy-to-use, and will provide reliable immediate results at the point-of-care setting.

The goal of the work presented here is to establish the design, development, and validation of a low cost portable digital colposcope that has comparable image quality, functionality, and diagnostic capability when compared to standard-of-care digital colposcopes and gold-standard histopathology.

Three specific aims were proposed to address this goal. First, the conceptualization, design, and validation of this device, the Pocket Colposcope will be presented. The Pocket Colposcope is shaped like a tampon and can be inserted and positioned such that it is 5-50 mm away from the cervix, obviating the need for high-end glass optics, high-resolution cameras, and high power illumination sources used in state of the art colposcopes, which typically operate at a working distance of 300 mm. An off the shelf miniature color complementary metal-oxide-semiconductor (CMOS) camera, with the plastic injection molded lens, and small light emitting diodes (LEDs) could be
repackaged inside the compact tampon like form factor and powered directly by a smartphone, tablet, or laptop through the universal serial bus (USB).

Each evolution of the Pocket Colposcope is quantitatively assessed with respect to a standard-of-care digital colposcope or predicate device using industry standard protocols and in preparation for the application to the US Food and Drug Administration (FDA) 510(k) preexisting medical device approval pathway. The Edan C3A/C6A digital colposcope (K151878) served as the predicate reference device within the Pocket Colposcope’s 510(k) application and formally approved on September 25th, 2018 (K181034). We also characterized and compared the Pocket Colposcope to the standard-of-care digital colposcope at Duke University Medical Center (DUMC) the Leisegang Optik 2 (K140754), as part of a multisite international clinical trial evaluating the diagnostic performance of Pocket Colposcope discussed in Aim 2. The essential imaging parameters are characterized with industry standards: ISO 8600-5 used for optical resolution, ISO 8600-3 used for the field of view, ISO 9039 and ISO 12233 used for distortion, depth of field, and ISO 18221 used for magnification. The essential illumination characteristics are characterized by ANSI FL-1 for both beam diameter and illumination intensity.

The optical resolution for all the generations of Pocket Colposcope ranged between 10-72 line pairs per mm (lp/mm) and was substantially equivalent to the optical resolution range of 10-29 lp/mm of the pair of predicate devices. The field of view of all
generations of the Pocket Colposcope ranged from 8-52 mm and was substantially equivalent to the field of view range of 12-76 mm reported by the predicate device pair. The picture-height distortion for all generations of the Pocket Colposcope ranged between -4.5 to -1.1% and were substantially equivalent to the 3-7% distortion reported by the pair of predicate devices. The depth of field range between 0.5-12 mm for all generations of the Pocket Colposcope was substantially equivalent to 6-20 mm range reported by the predicate device pair.

The illumination intensity range of 2,800 to 20,000 lux for all generations of the Pocket Colposcope was substantially equivalent to the reported range of 3,000 to 24,000 lux of the pair of predicate devices. Similarly, the beam diameter range of 33.8 to 49.0 mm for all generations of the Pocket Colposcope was substantially equivalent to 60-62.1 mm range reported by the pair of predicate devices.

The Alpha and Beta Generation of the Pocket Colposcope was developed to incorporate the vast design experiences and user feedback from the Generation 1 through 4 devices. This penultimate iteration of the Pocket Colposcope is designed in collaboration with 3rd Stone Design. The following features are incorporated in the device: improvements in the ergonomics such as buttons on the device for image capture and LED selection, simple to use adjustable magnification mechanism, improved user comfort with an angled and tapered handle, and to incorporate design and material selection considerations for scale manufacturing. The Alpha and Beta
Pocket Colposcope maintained key features such as: VIA, VILI, and GLI capability with comparable quality to the standard-of-care digital colposcope, chemical immersion cleaning compatibility, the fog resistant hydrophobic window, and enhanced portability (from Generation 1 and 4) eliminating the need for external LED driver box by combining and miniaturizing the circuit into the handle of the probe.

The second aim was to demonstrate the concordant diagnostic performance of the Pocket Colposcope when compared to reference standard-of-care digital colposcopes and gold-standard histopathology in a multi-institution clinical trial.

An image concordance study was conducted under IRB approval and written informed consent of participants. Images of the cervix collected by the Pocket Colposcope and standard-of-care device (if available) along with gold standard histopathology. These image pairs would be split, randomized, and digitally sent to blinded highly trained reviewers for clinical interpretation. These expert colposcopists are blinded to any demographic information of the patient, prior referral test results (Pap smear cytology, HPV status, HIV status, or histopathology), and each other’s interpretation of the image. A secure online questionnaire was sent to reviewers to record their responses. All data was stored securely on a Duke University Medical Center managed REDCap database server.

These interpretations would be compared between the Pocket Colposcope and standard-of-care device to demonstrate concordance performance or level agreement.
with the devices. The diagnostic performance of the Pocket Colposcope and standard-of-care devices are assessed by comparing the image interpretation to gold-standard histopathology with the generation of 2x2 contingency tables with entries for True Positives (TP), True Negatives (TN), False Positives (FP), and False Negatives (FN). The two binary diagnostic cut-offs are used: normal vs. LSIL/CIN1+ and normal vs. HSIL/CIN2+. From these 2x2 contingency tables sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Lastly, the diagnostic performance are stratified by level of contrast used using Receiver Operating Characteristic (ROC) curves where the y-axis is the sensitivity, and the x-axis is 1-specificity. The resultant area under the curve (AUC) can be compared using logistic regression to determine how changing the type of contrasts used impacts overall diagnostic performance.

A pilot clinical study (n=45 eligible subjects and read by 10 blinded highly trained clinicians) at Duke University Medical Center (Durham, NC) using the Generation 1 and 2 Pocket Colposcope, a standard-of-care digital colposcope (Leisegang Optik 2), using the contrast technique of visual inspection with acetic acid (VIA), and with gold-standard histopathology (from biopsy or post loop electrosurgical excision procedure (LEEP) specimen). The level of agreement between the Pocket Colposcope and standard-of-care when using the LSIL/CIN1+ vs. normal diagnostic cut-off was 77.8% with a moderate strength Cohen’s kappa coefficient (κ) of 0.53, p<0.01. When the
diagnostic cut-off was changed to HSIL/CIN2+ vs. normal the level of agreement between Pocket Colposcope and standard-of-care improved to 86.0\% with a strong Cohen’s (κ) of 0.64, p<0.01. The Pocket Colposcope and standard-of-care diagnostic performance when compared to the gold-standard histopathology is similar. The overall accuracy when using the LSIL/CIN1+ vs. normal diagnostic cut-off and comparing to gold-standard histopathology was between 56.1-63.9\%, with sensitivity between 39.1-56.0\%, with specificity between 73.9-72.7\%, for the Pocket Colposcope and standard-of-care, respectively. The diagnostic performance improved for both devices when the diagnostic cut-off are set to HSIL/CIN2+ vs. normal with accuracy climbing to 68.9-74.7\%, with sensitivity unchanged 40.9-56.4\%, with specificity improving by ~14\% for both systems to 86.1 to 86.4\%, with respect to the Pocket Colposcope vs. standard-of-care.

The performance of the Pocket Colposcope are now assessed in a resource-limited setting with a clinical study (n=129 eligible subjects and read by 4 blinded highly trained clinicians) at La Liga contra el Cáncer (Lima, Peru). The Generation 3 and 4 Pocket Colposcope, a standard-of-care digital colposcope (Goldway SLC-2000B), using the contrast techniques of visual inspection with acetic acid (VIA) and visual inspection with Lugol’s iodine (VILI), and with gold-standard histopathology (from biopsy or post loop electrosurgical excision procedure (LEEP) specimen). The level of agreement between the Pocket Colposcope and standard-of-care when using the LSIL/CIN1+ vs.
normal diagnostic cut-off was 83.6 % with a strong Cohen’s kappa coefficient (κ) of 0.67, p<0.01. When the diagnostic cut-off was changed to HSIL/CIN2+ vs. normal the level of agreement between Pocket Colposcope and standard-of-care improved to 86.2% with a strong Cohen’s (κ) of 0.66, p<0.01. The Pocket Colposcope and standard-of-care are comparable with respect to their diagnostic performance to the gold-standard histopathology. The overall accuracy when using the LSIL/CIN1+ vs. normal diagnostic cut-off and comparing to gold-standard histopathology was between 63.9-67.6%, with sensitivity between 71.2-79.8%, with specificity between 57.5-56.6%, for the Pocket Colposcope and standard-of-care, respectively. The diagnostic performance was unchanged for both devices when the diagnostic cut-off is set to HSIL/CIN2+ vs. normal with an accuracy of 63.1% for both systems, with sensitivity increasing slightly 80.7-82.2%, with specificity unchanged to 57.5-56.6%, with respect to Pocket Colposcope vs. standard-of-care.

The performance of the Pocket Colposcope was now assessed as part of the multinational clinical study with two additional partner sites joining the two prior sites: Kilimanjaro Christian Medical Center (KCMC) (Moshi, Tanzania) which used a digital camera (Canon SX40HS) as the standard-of-care device for part of the study, however the device broke and thus some patients are missing matched standard-of-care images). The second additional site, the Centre for Infectious Disease Control Zambia (CDZIR) (Lusaka, Zambia) which used naked eye inspection and did not employ a standard-of-
care device for this study. Pocket Colposcope Generation 1 through Beta and standard-of-care device (if available) were used to collect VIA, green light inspection (GLI), and/or VILI depending on the standard of practice at the clinical site. Gold-standard histopathology (from biopsy or post loop electrosurgical excision procedure (LEEP) specimen or post large loop excision of the transformation zone (LEETZ) specimen) are collected for eligible patients. Overall n=388 subjects have enrolled from four clinical sites, with n=129 subjects undergoing VIA with ten blinded expert reviewers, with n=92 subjects undergoing VIA+GLI with seven blinded expert reviewers, with n=127 subjects undergoing VIA+VILI with six blinded expert reviewers, and n=40 subjects undergoing VIA+GLI+VILI with four blinded expert reviewers. For this analysis, the Pocket Colposcope versus gold-standard histopathology stratified by the types of contrasts used is the study focus. The overall accuracy when using the LSIL/CIN1+ vs. normal diagnostic cut-off and comparing to gold-standard histopathology increased consistently with the type of contrasts used (VIA only, VIA+GLI, VIA+VILI, and culminating with VIA-GLI-VILI) with overall agreement improving from 53.4-70.1% with sensitivity improving from 42.3-68.1%, with specificity improving from 65.3-73.3%. The diagnostic performance when the diagnostic cut-off are set to HSIL/CIN2+ vs. normal followed the prior trend with consistent increases with the type of contrast used (VIA only to VIA+GLI+VILI) with rising accuracy 55.9-70.8%, with sensitivity increasing 41.9-52.2%, with specificity improving 78.2-88.0%. Increasing the number of contrast
agents used significantly improved the diagnostic performance of the Pocket Colposcope when compared to gold-standard histopathology.

The final aim is to add fluorescent imaging capability to the Pocket Colposcope to provide an additional source of contrast targeting metabolic and structural biomarkers that will further improve the clinical performance of our system to match gold standard histology pathology and expand the application of the device. There is broad applicability in other organ sites including oral cavity, gastrointestinal tract, and skin. The Beta generation of the Pocket Colposcope was modified for fluorescent imaging by adding excitation LEDs, repurposing of the Generation 3 external LED driver circuit to drive the excitation source, a matched band-pass optical filter for emission, and reintroduction of cross-polarization, without destructive modification of the Pocket Colposcope. A proof of concept device was characterized using a similar set of procedures used to assess the Pocket Colposcope for the 510(k) regulatory approval pathway. Ex-vivo and in-vivo pilot animal studies were conducted to demonstrate successful implementation of the fluorescent imaging capability using a FITC (Fluorescein Isothiocyanate) tagged binding ligand (HS-27) with a strong affinity for the heat shock protein-90 (Hsp-90) chaperone molecule (a potential biomarker of cancer). The real-time visual tracking of diffusion of ethanol from an ethyl-cellulose gel to surrounding tissue by using solvent properties of ethanol for FITC when compared to water (20 mg per mL vs. 0.1 mg per mL). This ethyl-cellulose gel is being investigated as
a potentially low-cost technique for treating cancer with an extended release of ethanol over time to localize treatment. Lastly, the fluorescent Pocket Colposcope was used in a pilot ex-vivo clinical studies in subjects undergoing breast biopsy to evaluate the diagnostic potential of HS-27 when compared to gold-standard histopathology.

Future work is proposed to continue refining the Pocket Colposcope to improve the devices integration into clinical workflow by adding the exact magnification without the need to image a resolution target and reducing the reprocessing downtime between patients with a sterile disposable sleeve. Lastly, the internal integration of the fluorescent imaging capability to the Pocket Colposcope with multiple sequential fluorophore imaging without the need to switch physically switch probe tips and eliminate the external LED driver circuit.

The ease of use of the magnification mechanism could be improved with the addition of magnetic linear potentiometer to the camera sled that will measure the position of the camera detector with respect to the fixed lens box. The distance is proportional to the magnification of the Pocket Colposcope due to the prime lens system employed. Currently, the user can calculate the exact magnification of an image if and only if they have imaged a resolution target before or immediately after capturing the image of the cervix and not move the position slider. Partnering with 3rd Stone Design an optical clear disposable sheath will be created as a sterile barrier between the patient and the device and reduce the current 20 to 30-minute device reprocessing time between
patient usage to less than two minutes. Preliminary testing revealed that minimizing the air gap between the outermost optical element of the Pocket Colposcope (hydrophobic window) and the protective sleeve is critical to preserving image quality. Thus, we propose a physical snap in place solution for a low-cost disposable injection molded optically clear sleeve with specialized geometry to preserve image quality. Lastly, to better integrate the fluorescent imaging capability to the Pocket Colposcope a miniature motorized emission filter wheel with band-pass optical filters and an open slot (to enable normal reflectance imaging) will be placed in between the detector and lens module to allow for user-controlled selection of imaging modes. The current two colors (white and green) concentric LED ring will be expanded to include multiple narrowband LEDs. The excitation wavelength and filter wheel will be synchronized and optimized for a specific set of fluorophores based on user needs. The LED driving circuits will be miniaturized and placed inside the handle with a redesign MCU to replace the external box currently being implemented for the proof-of-concept prototype.

5.2 Future Directions

Refinement of the Pocket Colposcope to improve integration into clinical workflow by adding the exact magnification without the need to image a resolution target, reduce the downtime between patients due to device sterilization, and internal integration of the fluorescent imaging capability to the Pocket Colposcope with multiple
sequential fluorophore imaging without the need to switch physically switch probe tips and eliminate the external LED driver circuit.

5.2.1 Improving the zoom mechanism with digital readout of exact magnification without the need for pre/post calibration image capture

Improving the ease of use of the magnification mechanism by adding a magnetic linear potentiometer to the camera sled that will measure the position of the camera detector with respect to the fixed lens box. The distance is proportional to the magnification of the Pocket Colposcope due to the prime lens system employed.

Currently, the user can calculate the exact magnification of an image if and only if they have imaged a resolution target before or immediately after capturing the image of the cervix and having not moved the position slider.

This allows the Pocket colposcope to provide a broad range of the field of views of 6-54 mm and a resolution of 4-40 µm, and at working distances of 5-50 mm, respectively, without the need for multiple expensive objective lenses. The user controls magnification by sliding a toggle that is connected to a camera sled mechanism, where linear translation of the detector can achieve optical zoom with respect to the fixed prime lens system for continuous magnification between 3 and 52X. The slider mechanism is watertight with low actuation force achieved by ultralow friction PTFE films compressing a rubber gasket held under compression with a constant force wave spring. The innovation will be magnification selection will be monitored with a novel noncontact magnetic digital linear potentiometer, where the displacement of the camera
sled with respect to the fixed lens module, will also translate an embedded miniature magnet across the potentiometer causing a linear change in voltage (Figure 38). The microcontroller unit (MCU) in the probe handle will read the voltage and compare to a pre-calibrated look-up table to output a magnification value to the image file and/or display on the image capture software.

Figure 38: Optical zoom tracking system, a zoomed digital rendering of our coarse focus adjustment mechanism, which allows for the user to select from 3 to 52X magnification by linear translation of the slide button (1), which is translated (2) by the slider connector to the (3) camera sled which is connected to the (6) CMOS color camera detector with respect to a fixed 5 element prime lens module (not shown off to
the right). The magnification read by a linear digital potentiometer (4) will translate
the displacement of the embedded magnet (5) in the sled assembly as a change in
voltage. An autofocus mechanism for fine adjustment will be made by a VCM (voice
coil module) and controlled by the (7) onboard microcontroller (MCU).

5.2.2 Reducing Pocket Colposcope Down Time - Sterile Disposable
Sleeve

Preliminary testing revealed that minimizing the air gap between the outermost
optical element of the Pocket Colposcope (hydrophobic window) and the protective
sleeve is critical to preserving image quality. Thus, we propose a physical snap in place
solution for a low-cost disposable injection molded optically clear sleeve with
specialized geometry to preserve image quality. Commercially available sleeves are not
suitable for use with the Pocket Colposcope, as they are not optically clear, induce
specular reflection, and may fog in the vaginal canal (Figure 39). There is a profound
loss of image quality integrity when an external “optically” clear barrier is placed over
the tip of the Pocket Colposcope. Thus, a specially designed sleeve needs to be created to
preserve image quality while also being biocompatible and providing a durable sterile
barrier between the device and the patient.
Figure 39: Preliminary evaluation of common commercial sterile “optically” clear barriers when placed over the tip of the Pocket colposcope. (A) no barrier, (B) LDPE (low density polyethylene), (C) PP (polypropylene), (D) Polyurethane, (E) PVC (polyvinyl chloride), (F) PET (Polyethylene terephthalate), (G) 3M Tegaderm (acrylic), and (H) Polyisoprene.

We propose a single use device that will snap into place to the tip of the Pocket colposcope (Figure 40) and consist of several key components: an antireflection and hydrophobic coated window, a window holder and baffle, concentric LED ring diffuser and light pipe (which improves illumination efficiency through physically coupling the LED to the light pipe and helps provide uniform illumination with a fine grit (1500) diffuser treated surface). Based on our prior work, an antireflection and hydrophobic coated optical window is an important element for maintaining high image quality when imaging in a humid environment such as the vaginal cavity and helps eliminate the need for an external device warmer (often used for laparoscopes and endoscopes) or single use chemical based anti-fog wipes. In order to combat fogging and preserve image quality, the optically clear anti-reflection, the hydrophobic window needs to be
molded in the tip of the sleeve to maximize light transmission. Furthermore, to eliminate the stray light (specular reflection) from the illumination sources, a baffle or light-absorbing barrier is needed to preserve image quality. The baffle also needs to be incorporated to isolate the window from the LEDs. The baffle snap fits into the tip of the probe and also helps align the eight light pipe extensions with the concentric LED ring. Concentric to the window and baffle will be a polycarbonate clear optical diffuser and light pipe that interfaces with each LED emitter and combines them for a more uniform illumination profile allowing for enhanced visual contrast for the user. Lastly, the outer rim of the light pipe and diffuser will be mated to a flexible clear gasket that is ultrasonically welded to a thin polyethylene (PET) sleeve that covers the remainder of the probe providing a sterile barrier between the patient and the device. This sleeve will be single-use disposable. The fabrication process is widely used for ultrasound and dental probe sterile sheaths. Note, most endoscopes and laparoscopes typically do not use a sterile sleeve but rather depend on chemical liquid immersion and physical agitation complementary cleaning devices to achieve successful reprocessing and thus are not relevant here. If the polycarbonate and PET (polyethylene) sheath material is not found to be biocompatible an alternative USP Type IV flexible plastic will be selected such as FEP (Fluorinated Ethylene Propylene) or PVDF (polyvinylidene difluoride).
Figure 40: Schematic view of our proposed biocompatible sterile sleeve (1). Note the USP Type IV polycarbonate unique light pipe (2) and LED diffuser (3) with a fringed gasket (4) mated to a thin film (few mils in thickness) USP Type IV polyethylene sleeve (5). In the center is an anti-reflection and hydrophobic optical window (6) molded into a baffle (7) that eliminates stray light from the environmental and total internal specular reflection from the illumination LEDs. An embedded RFID chip (8) is sensed and checked by the colposcope handpiece when assembled.

5.2.3 Refinement and improved integration of fluorescent imaging capability to the Pocket Colposcope

Lastly, to better integrate the fluorescent imaging capability to the Pocket Colposcope a miniature motorized emission filter wheel with band-pass optical filters and an open slot (to enable normal reflectance imaging) will be placed in between the detector and lens module to allow for user-controlled selection of imaging modes. The current two colors (white and green) concentric LED ring will be expanded to include multiple narrowband LEDs. The excitation wavelength and filter wheel will be
synchronized and optimized for a specific set of fluorophores based on user needs. The LED driving circuits will be miniaturized and placed inside the handle with a redesign MCU to replace the external box currently being implemented for the proof-of-concept prototype.

Furthermore, recent advances in illumination technology enable our design to use efficient, compact (2.2 mm² surface area), high powered LEDs in a concentric ring design. This orientation enables for uniform reflectance and excitation illumination through a novel alternating sequence of single color LEDs radially spaced at 30° intervals around the perimeter of the entrance aperture (Figure 41B) without the need for a cumbersome and slow excitation filter wheel or expensive electronic liquid crystal tunable filter. The fluorescent Pocket Colposcope will use blue LEDs (470 nm, 80 mW) for 2-NBDG excitation, and green LEDs (545 nm, 118 mW) for TMRE excitation. Enhanced reflectance vasculature imaging will make use of the three LEDs in the visible spectral range including the two for fluorescence excitation. The three LEDs will be selected to fulfill the wavelength needs for the radiometric algorithm to compute oxygen saturation and two will also be selected to be within the absorption bands of 2-NBDG and TMRE.

Our imaging pathway will start with the CMOS camera followed by autofocus lens module (Figure 41C label 1,3). Bandpass filters matched to the emission peaks of 2-NBDG (540 nm) and TMRE (580 nm) will be used for fluorescence detection with a
motorized micro filter wheel (Figure 41C label 2,4) which are rotated into the imaging axis pathway by a micro-stepper motor in the handle which changes the filter wheel circular orientation to the desired filter as controlled by the MCU. A clear or open filter slot is allocated for filter-free reflectance imaging. We also propose a novel design on the geometry of the light baffle which acts as a physical entrance aperture with an opaque light absorbing coating to prevent image vignetting and eliminate environmental or stray illumination light from the concentric LED ring (Figure 41C label 7,8). Specular reflection (glare) rejection will be addressed using cross-polarization which is achieved by placing a thin film linear polarizer over the excitation LEDs that is orthogonally oriented to a high-contrast glass linear polarizer placed in front of the lens module (Figure 41C 6,9). A hydrophobic window, to reduce fogging and protect the optical components, is mated to the center of the polycarbonate LED light guide via a press fit locking mechanism and reinforced by medical grade epoxy to prevent liquid and particle infiltration (Figure 41C label 10,11). The light guide acts as a light pipe conduit for the LEDs to the reflector tip and protects the LEDs from environmental exposure. A reflector tip (Figure 41C label 12) will be designed to structurally supports the concentric LED PCB ring and maximize uniform illumination onto the tissue or sample surface based on our prior work (104).
Figure 41: Exploded Schematic View of Internally Integrated Fluorescent Pocket Colposcope (A) Exploded view of the focus mechanism, user input motion from the (1) slider button moves the (2) connector rail and attached (3) sled with securely bedded (4) CMOS camera for smooth easy to use optical zooming user control. (B) Schematic of proposed LED illumination layout, with alternating radial symmetric single color LEDs along the perimeter of the entrance aperture. (C) Exploded view of the key optomechanical components proving contrast enhancement via cross-polarization (6) and (9), a motorized internal filter wheel (2 to 5), light baffle (7), and reflector (12).
Appendix A

Table 28: Calculations for Pocket Colposcope Magnification at 5 mm working distance

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<thead>
<tr>
<th>Component</th>
<th>Formula</th>
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<td>ii. Reference viewing distance (standard)</td>
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<td>mm</td>
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Table 29: The Pocket Colposcope’s magnification ranges for the 35 mm working distance

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Working Distance 2</td>
<td>WD$_2$</td>
<td>35.00</td>
<td>mm</td>
</tr>
<tr>
<td>ii. Reference viewing distance</td>
<td>RVD</td>
<td>250.0</td>
<td>mm</td>
</tr>
<tr>
<td>iii. Actual viewing distance</td>
<td>d$_{view}$</td>
<td>300.0</td>
<td>mm</td>
</tr>
<tr>
<td>iv. Actual Object Size (</td>
<td>Obj$_{hor}$</td>
<td>30.6</td>
<td>mm</td>
</tr>
<tr>
<td>(Horizontal Field of View)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>v. Sensor Horizontal Size</td>
<td>Sen$<em>{Hor}$ = PP$</em>{sen}$ * N$_{sen}$ SEN</td>
<td>3.629</td>
<td>mm</td>
</tr>
<tr>
<td>via. Display 1 Nexus 7 Horizontal</td>
<td>Dis$<em>{1</em>{Hor}}$ = PP$<em>{DIS1}$ * N$</em>{DIS1}$ DIS1</td>
<td>150.8</td>
<td>mm</td>
</tr>
<tr>
<td>size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>viib. Display 2 Helix 2 Horizontal</td>
<td>Dis$<em>{2</em>{Hor}}$ = PP$<em>{DIS2}$ * N$</em>{DIS2}$ DIS2</td>
<td>256.9</td>
<td>mm</td>
</tr>
<tr>
<td>size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vic. Display 3 Thinkpad 13</td>
<td>Dis$<em>{3</em>{Hor}}$ = PP$<em>{DIS3}$ * N$</em>{DIS3}$ DIS3</td>
<td>294.5</td>
<td>mm</td>
</tr>
<tr>
<td>Horizontal size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>viiia. Display 1 Nexus 7 Magnification</td>
<td>M$<em>{DIS1</em>{VIS}}$ = PP$<em>{DIS1}$ * N$</em>{DIS1}$ DIS1</td>
<td>4</td>
<td>x</td>
</tr>
<tr>
<td>viib. Display 2 Helix 2 Magnification</td>
<td>M$<em>{DIS2</em>{VIS}}$ = PP$<em>{DIS2}$ * N$</em>{DIS2}$ DIS2</td>
<td>7</td>
<td>x</td>
</tr>
<tr>
<td>viic. Display 3 Thinkpad 13</td>
<td>M$<em>{DIS3</em>{VIS}}$ = PP$<em>{DIS3}$ * N$</em>{DIS3}$ DIS3</td>
<td>8</td>
<td>x</td>
</tr>
</tbody>
</table>
Table 30: The Pocket Colposcope’s magnification ranges for the 50 mm working distance

<table>
<thead>
<tr>
<th>Component</th>
<th>Formula</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Working Distance 2</td>
<td>WD&lt;sub&gt;i&lt;/sub&gt;</td>
<td>50.00</td>
<td>mm</td>
</tr>
<tr>
<td>ii. Reference viewing distance (standard)</td>
<td>RVD</td>
<td>250.0</td>
<td>mm</td>
</tr>
<tr>
<td>iii. Actual viewing distance (colposcope)</td>
<td>d&lt;sub&gt;view&lt;/sub&gt;</td>
<td>300.0</td>
<td>mm</td>
</tr>
<tr>
<td>iv. Actual Object Size (Horizontal Field of View)</td>
<td>Obj&lt;sub&gt;hor&lt;/sub&gt;</td>
<td>43.3</td>
<td>mm</td>
</tr>
<tr>
<td>v. Sensor Horizontal Size</td>
<td>Sen&lt;sub&gt;hor&lt;/sub&gt; = PP&lt;sub&gt;sen&lt;/sub&gt; * N&lt;sub&gt;x&lt;/sub&gt;SEN</td>
<td>3.629</td>
<td>mm</td>
</tr>
<tr>
<td>vi. Display 1&lt;sub&gt;Nexus 7&lt;/sub&gt; Horizontal Size</td>
<td>Dis1&lt;sub&gt;hor&lt;/sub&gt; = PP&lt;sub&gt;DIS1&lt;/sub&gt; * N&lt;sub&gt;x&lt;/sub&gt;DIS1</td>
<td>150.8</td>
<td>mm</td>
</tr>
<tr>
<td>vii. Display 2&lt;sub&gt;Helix 2&lt;/sub&gt; Horizontal Size</td>
<td>Dis2&lt;sub&gt;hor&lt;/sub&gt; = PP&lt;sub&gt;DIS2&lt;/sub&gt; * N&lt;sub&gt;x&lt;/sub&gt;DIS2</td>
<td>256.9</td>
<td>mm</td>
</tr>
<tr>
<td>vici. Display 3&lt;sub&gt;Thinkpad 13&lt;/sub&gt; Horizontal Size</td>
<td>Dis3&lt;sub&gt;hor&lt;/sub&gt; = PP&lt;sub&gt;DIS3&lt;/sub&gt; * N&lt;sub&gt;x&lt;/sub&gt;DIS3</td>
<td>294.5</td>
<td>mm</td>
</tr>
<tr>
<td>viiia. Optical Magnification</td>
<td>M&lt;sub&gt;TOT PROJ&lt;/sub&gt; = PP&lt;sub&gt;SEN&lt;/sub&gt; / Obj&lt;sub&gt;hor&lt;/sub&gt;</td>
<td>0.084</td>
<td>:1</td>
</tr>
<tr>
<td>viiib. Display 1&lt;sub&gt;Nexus 7&lt;/sub&gt; Magnification</td>
<td>M&lt;sub&gt;DIS1&lt;/sub&gt; = Dis1&lt;sub&gt;hor&lt;/sub&gt; / Obj&lt;sub&gt;hor&lt;/sub&gt;</td>
<td>3</td>
<td>:1</td>
</tr>
<tr>
<td>viiic. Display 2&lt;sub&gt;Helix 2&lt;/sub&gt; Magnification</td>
<td>M&lt;sub&gt;DIS2&lt;/sub&gt; = Dis2&lt;sub&gt;hor&lt;/sub&gt; / Obj&lt;sub&gt;hor&lt;/sub&gt;</td>
<td>6</td>
<td>:1</td>
</tr>
<tr>
<td>viiic. Display 3&lt;sub&gt;Thinkpad 13&lt;/sub&gt; Magnification</td>
<td>M&lt;sub&gt;DIS3&lt;/sub&gt; = Dis3&lt;sub&gt;hor&lt;/sub&gt; / Obj&lt;sub&gt;hor&lt;/sub&gt;</td>
<td>7</td>
<td>:1</td>
</tr>
<tr>
<td>viiib. Visual Display 1&lt;sub&gt;Nexus 7&lt;/sub&gt; Magnification</td>
<td>M&lt;sub&gt;DIS1 VIS&lt;/sub&gt; = D&lt;sub&gt;DIS1&lt;/sub&gt; * RVD / d&lt;sub&gt;view&lt;/sub&gt;</td>
<td>3</td>
<td>x</td>
</tr>
<tr>
<td>viiib. Visual Display 2&lt;sub&gt;Helix 2&lt;/sub&gt; Magnification</td>
<td>M&lt;sub&gt;DIS2 VIS&lt;/sub&gt; = D&lt;sub&gt;DIS2&lt;/sub&gt; * RVD / d&lt;sub&gt;view&lt;/sub&gt;</td>
<td>5</td>
<td>x</td>
</tr>
<tr>
<td>viiib. Visual Display 3&lt;sub&gt;Thinkpad 13&lt;/sub&gt; Magnification</td>
<td>M&lt;sub&gt;DIS3 VIS&lt;/sub&gt; = D&lt;sub&gt;DIS3&lt;/sub&gt; * RVD / d&lt;sub&gt;view&lt;/sub&gt;</td>
<td>6</td>
<td>x</td>
</tr>
</tbody>
</table>
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Biography

Christopher Thy Lam graduated Cum Laude with a Bachelor of Science in Biomedical Engineering from the University of Cincinnati in May 2007. In May 2012, Chris received his Master of Science Global Health from Duke University. In August 2012, Chris started working for the Center for Global Women’s Health Technologies and Tissue Optics and Spectroscopy Laboratory at Duke University under guidance and mentorships of Dr. Nimmi Ramanujam. He graduated in May 2019 with his Ph.D. in Biomedical Engineering and a Certificate in Global Health.

Publications:
* denotes authors contributed equally


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Honors and Awards:
Doctoral Scholar Duke Global Health Institute 2016-2018

Education Scholarship SPIE Optics and Photonics 2014-2015

Second Year Graduate Fellowship BME Honorable mention 2013-2014

John T. Chambers Fellow First year fellowship for entering Ph.D. students 2012-2013

Merit Scholarship Duke Global Health Institute 2010-2012