# Coherence Imaging of the Cervical Epithelium with Scanning a/LCI

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## Purpose of the Study

The purpose of this study is to develop an imaging probe that can be used to detect cervical dysplasia *in situ*. This probe will combine two imaging modalities, both types of low coherence interferometry (LCI), to examine the cervix on both the tissue and subcellular levels. The development of this probe will occur in two stages:

1. **Development of a multiplexed LCI (mLCI) endoscopic probe for mapping the epithelial types of the cervix.** This vaginal mLCI probe will obtain optical measurements in wide area scans of the cervix. These measurements can be used to create a map of the surface cells that distinguishes ectocervical epithelia, endocervical epithelia, and the squamocolumnar junction (T-zone) between them, which is the region where cervical dysplasia is most likely to occur.
2. **Development of an integrated probe that combines wide area cervical mapping with subcellular measurements of nuclear size to detect cervical dysplasia.** This combined probe will conduct wide area scans using optical coherence tomography (OCT - an updated technology similar to mLCI described above) to map the T-zone of the cervix. The mapping data will then be used to direct the locations for high resolution angle-resolved low coherence interferometry (a/LCI) nuclear morphology measurements to search for dysplasia at selected points. We have termed the combined technologies of OCT mapping and a/LCI nuclear sizing "**scanning a/LCI**." In previous studies, a/LCI has proven highly accurate in detecting dysplastic lesions in Barrett's esophagus patients via depth resolved nuclear morphology measurements. This study will adapt existing a/LCI technology to define the nuclear morphology characteristics of cervical epithelium.

The research studies described here will be preliminary studies to evaluate the performance of the LCI devices for detection of cervical dysplasia. The results from the small studies at Duke will help to optimize the function of the LCI instruments before completing larger studies with collaborators at Jacobi Medical Center. The devices will be studied in a larger subject population at Jacobi to determine how effectively they can distinguish the types of cervical epithelia and detect cervical dysplasia.

Dr. Wax, the Duke PI of this study, is the primary awardee of the NIH grant supporting this work. Dr. Wax and his team have developed the LCI research devices and will conduct small studies at Duke (described below) to assess if any adjustments to the devices are needed before they are sent to Jacobi Medical Center for the larger studies. The studies at Jacobi Medical Center will be funded as a subaward / consortium agreement of Dr. Wax's NIH grant.

Duke will recruit up to 15 women to enroll in stages 1 and 2 of the study, in order to achieve the target number of 10 subjects who complete each stage. This gives a total enrollment of up to 30 subjects at Duke to complete both stages of the study at that site.

Jacobi will enroll up to 100 women for stage 1, in order to achieve the target number of 40 women who complete stage 1. For stage 2 of the study, Jacobi will enroll up to 140 women in order to achieve the target number of 70 women who complete that stage.

Data from both sites will be analyzed at Duke. Study conduct at Duke will be under the oversight of the IRB of the Duke University Health System. Study conduct at Jacobi Medical Center will be under the oversight of the IRB of the Albert Einstein College of Medicine and Montefiore Medical Center.

## Background & Significance

**Clinical problem:** Cervical cancer, caused by persistent human papillomavirus (HPV) infection, is the second most common female malignancy worldwide. An estimated 500,000 new cases are diagnosed annually, including 12,170 new cases in the U.S in 2012, which reflects an age-adjusted incidence of 8.1 per 100,000 women [1]. Given that cervical cancer typically develops over many years, the early detection of cervical dysplasia (pre-cancers) affords a critical opportunity for treatment and thus prevention of life threatening invasive cancer. In developed countries, the availability of cervical cytology (Papanicolaou smear) for routine screening and colposcopy-directed biopsy for diagnosis has led to decreases in incidence and mortality rates [2]. However, despite their important impact, cytology and colposcopy remain very limited in their sensitivity, specificity, and interobserver reproducibility [3, 4]. The accurate identification of dysplastic areas of the epithelium is challenging and hence clinical guidelines recommend frequent patient screening schedules [5, 6] that are particularly difficult to implement in developing countries. The need for more effective evaluation tools persists.

**Potential Solution:** We propose to address this need by creating an imaging platform that uses coherence imaging to evaluate cervical tissues at two length scales. At the millimeter (tissue) scale, a multiplexed LCI (mLCI) approach will use multiple imaging channels to efficiently map the cervical epithelium to identify histological structures and red-flag suspicious regions, likely to harbor dysplasia. We have shown that mLCI can survey large areas of tissues in the female reproductive tract to obtain diagnostic information on coating by drug delivery gels [7, 8]. Once mLCI identifies suspicious areas, angle-resolved LCI (a/LCI) will obtain high resolution, depth resolved nuclear morphology measurements on the micron (subcellular) scale [9, 10]. We have shown that a/LCI can detect dysplastic lesions in vivo in the esophagus [11] and ex vivo in other types of epithelia such as colon [12] and trachea [13]. The combination of these two optical technologies, mLCI and a/LCI, will provide a unique platform for comprehensive assessment of the cervix at both tissue and subcellular scales. The novel technology will impact clinical detection of cervical dysplasia as well as support clinical and translational research on the fundamental biology of the cervical epithelium.

**Rationale for collaborative project:** The Duke team has had significant success in developing endoscopic imaging modalities for detection of precancerous tissues. Ongoing collaboration between the group in Duke BME and the Department of Ob/Gyn at Duke University Medical Center (DUMC) has produced important studies of the pharmacology of anti-HIV microbicides delivered to the lower female reproductive tract [7, 8, 14-17]. Here we seek to forge a broader collaboration to bring together the established team at Duke with a leading group in clinical research at Jacobi Medical Center. Jacobi serves a predominately minority population, and due to the largely immigrant status of women over age 26, HPV vaccination rates are low. Therefore, it has a large population of women with HPV-associated cervical dysplasia and a significant number of women with cervical cancer.

This collaboration will create a powerful combination of technology development and translation at the leading edge of gynecological biomedicine. This project will develop and test new instruments for cervical imaging at Duke and field test these technologies in the large, diverse patient population available at Jacobi Medical Center. The anticipated outcome is the creation of a diagnostic imaging tool that can screen women for cervical precancers and cancers and/or triage precancerous lesions in women who have had a positive cytology test.

**INNOVATION**

Novel multimodal imaging platform that combines mLCI and a/LCI: The mLCI technology is unique in its ability for depth resolved imaging of wide areas of tissues. While LCI can obtain high resolution tomographic images at micron scales, as is done in optical coherence tomography (OCT), that approach is limited by often cumbersome scanning mechanisms and the large volume of data generated. Instead, mLCI uses sparse sampling and multiple parallel channels to more efficiently scan wider tissue areas. We will exploit this ability to create a new modality for quickly mapping the cervical epithelium, analogous to the function of colposcopy, but with improved performance. This is a particular challenge due to the complex combination of cervical epithelial types that are often in transitional states (columnar to metaplastic to squamous). As a counterpart to the mLCI, the a/LCI modality will combine the depth resolution and penetration of OCT with the sensitivity to subcellular structure that is obtained via light scattering, providing a function analogous to biopsy. Our group has recently validated a/LCI as an in vivo method for identifying dysplastic esophageal tissues [11]. Although prior research has applied OCT and light scattering to cervical dysplasia [18, 19] these approaches have not been clinically feasible. The unique capabilities of a/LCI, which enable high accuracy in detecting dysplasia, offer a significant potential for clinical translation. By combining mLCI and a/LCI into a single integrated platform, we will capitalize on recent trends in multimodal imaging. As identified by clinicians and researchers in multiple fields [20, 21], optimal screening methods can be realized by using a wide area scan to survey for suspicious regions in tandem with a high resolution modality to further diagnose those regions. Thus, we will implement this paradigm and create a hybrid imaging modality that integrates tissue and cellular imaging data. The unique power of this multimodal LCI device will lie in its ability to accurately identify dysplastic and cancerous tissues against the background of the complex tissue types that comprise the cervical epithelium.

**Innovation in clinical care of cervical disease:** In the clinic, the clear advantage of the multimodal LCI platform will be its ability to support clinical decision-making without relying on gross clinical colposcopic impression, the traditional but somewhat subjective diagnostic method. Further, the potential to provide reliable "real-time" information to the clinician would allow immediate treatment of epithelial abnormalities. We expect this device to be uniquely applicable to women of all ages in contrast to the recent implementation of screening biomarkers such as HPV DNA testing which are restricted to older women. Our field trials will access the diverse patient population found at Jacobi OB/GYN. This population represents the full spectrum of cervical health, including healthy young women and those with incident HPV infection, persistent HPV infection, benign cervical lesions, cervical precancers, and cancer. Our proposal to develop an accurate and efficient tool for cervical cancer screening will rely on its ability to detect disease in the setting of complex tissue compositions inherent to the cervix—the downfall of many prior optical technology attempts. Innovation is inherently risky; however our combined expertise in coherence imaging and the clinical study of the cervical epithelium will support the success of the proposal.

## Design & Procedures

***STAGE 1: mLCI device development***

**mLCI instrument description**

The purpose of this instrument is to obtain optical measurements from the cervix via a vaginal probe to create a map of the surface cells that distinguishes the ectocervical and endocervical epithelia. The boundary between the two cell types is known as the squamocolumnar junction, or "t-zone", which is the transitional region at which cervical dysplasia is most likely to occur.

The instrument delivers infrared light to 36 locations on the cervix, and measures the depth-profile of back-scattered light from each location using low coherence interferometry (LCI). This technique is thus termed multiplexed LCI (mLCI). The LCI profile data is stored by a computer for subsequent analysis to identify cell type.

The components of the mLCI instrument are grouped in the following modules: 1) Optical probe, 2) mLCI interferometer, 3) white light imaging module, 4) computer, and 5) physical enclosure.

***Optical Probe***

The optical probe is primarily cylindrical in form, with a diameter of approximately 25 mm and a rigid length of approximately 270 mm. The probe housing is fabricated by a 3D printing process known as fused deposition modeling (FDM). The material is MED-610 (Stratasys Inc., Eden Prairie, MN), a transparent polymer certified as biocompatible. The adhesives used on the external seams of the probe are Silastic Medical Adhesive Type A (Dow Corning, Midland, MI), a soft silicone adhesive. A 36-channel single-mode fiber bundle is contained within the probe housing, as well as several glass optical elements for imaging. The rounded tip of the probe is intended to contact the cervical surface, and the focus of mLCI imaging is located on the plane of this tip.

The probe also contains fiber optics that deliver white LED illumination to the region of imaging and collects wide-field imaging information through a fiber bundle to provide real-time video endoscopic guidance for probe placement.

A flexible tether containing the 36-fiber bundle and white light optical fibers protrudes from the proximal end of the probe and is connected to the mLCI interferometer and white light imaging modules, respectively.

The full length of the probe is not intended for vaginal insertion. A demarcation line approximately 220 mm from the probe tip indicates the line beyond which the probe should not be inserted. High-level disinfection procedures as described in this document will be performed up to the line.

The probe contains no electrical components or wires.

***mLCI interferometer***

The centerpiece of LCI instrumentation is an interferometer, in which light from a known reference distance is combined with light returning from the probe. In our multiplexed design, 6 channels are simultaneously illuminated and interfered with each of 6 independent references. The mLCI thus contains 6 complete fiber optic interferometers, and fiber-optic switches redirect the optical inputs and outputs to progress through the 36 channels in a sequence of 6 acquisitions.

The 6 interferometers in the mLCI system are connected individually to each of 6 parallel fiber-optic spectrometers (Avantes, Broomfield, CO), which interface with the computer via one common USB connection.

The light source from the mLCI system is a superluminescent diode (Superlum, Moscow, Russia) emitting infrared light at wavelengths between 815 and 845 nm. The total output power of the SLD at its output

aperture is less than 25 mW, but the amount of power delivered by the probe is far less. The power at each of the individual 36 foci at the probe output is less than 500 µW (0.5 mW).

The maximum permissible exposure (MPE) for exposures greater than 10 seconds on skin is 3.6 $mW/mm^2$ at our principal wavelength of 830 nm (ANSI Z136.1). In our system, only 6 channels are simultaneously illuminated. Our imaging region in the tissue is 16 mm (defined by the length of our row of 6 spots) x 3.5 mm (defined by the minimum limiting aperture given in ANSI Z136.1) = 56 $mm^2$. Our total permissible power is thus 3.6 $mW/mm^2$ x 56 $mm^2$ = 201 mW. The totality of our 6 foci = 6 x 0.5 mW = 3 mW, which is far short of the ANSI MPE.

***White light imaging module***

A white light imaging module is configured to provide video endoscopy guidance for the probe placement on the cervix. This module consists of a white light LED optically coupled to an illumination fiber that is enclosed in the probe, as well as an imaging camera and relay optics to digitize the endoscopic image from the probe's white light fiber bundle.

***Computer***

A laptop computer (Lenovo, Beijing, China) performs the data acquisition and control through a custom application in Labview (National Instruments, Austin, TX). Real-time display of the white-light endoscopic images is provided, as well as synchronized acquisition of LCI data from spectrometers. Through a customized microcontroller module, this software also implements control of reference mirror positions by stepper motor and control of fiber-optic switch positions.

***Physical enclosure***

The mLCI interferometer, white light imaging module, and computer are situated upon a wheeled steel cart. The fiber-optic interferometer components are protected by a metal enclosure. Power to all components is provided through standard 120V mains routed through an uninterruptible power supply (UPS), which allows temporary disconnection of AC power for system transportation or due to accidental action without interrupting the operating state of the mLCI system.

**Stage 1 Study Procedures for subjects at Duke**

Each subject will attend 1 study visit that will last between 1 – 1.5 hours and include the following procedures.

- Read consent form
- Ask / answer questions
- Sign consent form
- Complete brief medical questionnaire to determine eligibility
- Urine pregnancy test – must be negative before imaging can occur
- mLCI examination of the cervix (without speculum)
- mLCI examination of the cervix (with speculum)

We will obtain written informed consent and confirm eligibility criteria. Data collection will include a brief medical history and pregnancy testing. Participants will be interviewed one-on-one to complete the questionnaire regarding medical and reproductive history. The nurse or doctor will then perform the mLCI study measurements.

The nurse or doctor will gently place the mLCI probe against the cervix. Lubricant will be placed on the outside of the probe for subject comfort. White light camera visualization, incorporated into the mLCI probe, will guide the probe positioning by the clinician to confirm contact with the cervical epithelium. Up to 4 minutes will be allotted for probe manipulation for optimal contact. The probe will then be held in place for mLCI data acquisition for up to 1 minute. When data collection is completed, or permitted time is elapsed, the mLCI probe will be removed and placed on a custom holder that prevents the contact portions of the probe from coming into contact with contaminating surfaces.

The nurse or doctor will then insert a speculum in order to directly visualize the cervix. The mLCI probe will again be placed against the cervix with 4 minutes allotted for probe manipulation and 1 minute for mLCI measurements.

If manipulation of the probe with the aid of a speculum does not allow satisfactory placement on the cervix for mLCI imaging within the permitted time, an additional attempt may be made with an alternate speculum, such as a Pedersen Open-Sided speculum, if the medical practitioner performing the procedure believes the alternate speculum to be more likely to result in correct probe placement. If the alternate speculum is used, then an additional 4 minutes will be allotted for probe manipulation with the alternate speculum, and 1 minute for mLCI measurements. The timing of each phase of the procedure is summarized in the following table.

**Table 1**: Maximum allotted times of mLCI probe insertion

| Insertion method | Criteria | Time for probe placement | Time for mLCI acquisition | Maximum total time |
|---|---|---|---|---|
| **Without speculum** | All patients | 4 min | 1 min | 5 min |
| **With standard speculum** | All patients | 4 min | 1 min | 5 min |
| **With alternate speculum** | Optional (only if standard speculum not satisfactory AND practitioner believes alternate may achieve better result) | 4 min | 1 min | 5 min |

Comparing measurements made by inserting the mLCI device with and without a speculum will allow us to determine if the white light visualization by the camera is sufficient to correctly place the probe over the cervix without having to use a speculum in future studies. The ability to use an alternate in the event that a conventional speculum does not permit adequate mLCI probe placement will also inform our selection of the type of speculum, if any, that is most suited for subsequent studies.

**Stage 1 Study Procedures for subjects at Jacobi**

Subjects will be informed of the study when they present to the clinic for a regular clinical care visit. If interested in participating, subjects will complete the following study-related procedures in addition to the standard of care visit procedures.

- Read consent form
- Ask / answer questions
- Sign consent form
- Urine pregnancy test – must be negative before imaging can occur
- Colpophotography of cervix to compare with mLCI data
- mLCI examination of the cervix (with speculum)

We will obtain written informed consent and confirm eligibility criteria. Data collection will include a brief medical history and pregnancy testing.

The doctor will then insert a speculum in order to directly visualize the cervix. Colpophotographs will be taken to compare to mLCI data during data analysis. The doctor will then gently place the mLCI probe against the cervix. White light camera visualization, incorporated into the mLCI probe, will guide the probe positioning by the clinician to confirm contact with the cervical epithelium. The probe will then be held in place for mLCI data acquisition for up to 1 minute. When data collection is completed, the mLCI probe will be removed and placed on a custom holder that prevents the contact portions of the probe from coming into contact with contaminating surfaces.

The doctor will then continue with the rest of the patient's standard of care visit procedures.

At some visits, the physician may choose to make an additional measurement with the mLCI device, by inserting it without a speculum and using the white light camera (incorporated into the mLCI probe) for visual guidance to position the probe. Comparing measurements made by inserting the mLCI device with and without a speculum will allow us to determine if the white light visualization by the camera is sufficient to correctly place the probe over the cervix without having to use a speculum in future studies.

***STAGE 2: integrated scanning a/LCI device development***

**Scanning a/LCI instrument description**

The purpose of this instrument is to obtain optical measurements from the cervix via a vaginal probe to detect the presence of epithelial dysplasia. Measurements are performed in two modes: a mapping mode, followed by a sizing mode.

In the mapping mode, the instrument creates a map of the surface cells that distinguishes the ectocervical and endocervical epithelia. The boundary between the two cell types is known as the squamocolumnar junction, or "t-zone", which is the transitional region at which cervical dysplasia is most likely to occur. Motorized components within the handle of the probe cause a beam of infrared light to be scanned on the cervix, and the device measures the depth-profile of back-scattered light from each location using low-coherence interferometry (LCI). This form of imaging is known as optical coherence tomography (OCT).

Next, a sizing mode is engaged that targets a second set of infrared light scattering measurements designed to measure the size of nuclei in the cervical epithelium. The technology used for this component is angle-resolved low-coherence interferometry (a/LCI). These measurements are directed to locations determined based on the mapping data generated during the mapping mode. These locations may be manually specified by the user or automatically determined by an algorithm.

The components of the scanning a/LCI instrument are grouped in the following modules: 1) Optical probe, 2) a/LCI module, 3) OCT module, 4) electronic control module, 5) computer, and 6) physical enclosure.

**Optical Probe**
The optical probe is subdivided into two main components: 1) cervical probe, and 2) scanning handle. During operation, the two components are mechanically linked as a single unit.

***Cervical probe*** The optical probe is primarily cylindrical in form, with a diameter of approximately 23 mm diameter and a rigid length of approximately 20 cm. The probe housing is fabricated by a 3D printing process known as fused deposition modeling (FDM). The material is MED-610 (Stratasys Inc., Eden Prairie, MN), a transparent polymer certified as biocompatible. The adhesives used on the external seams of the probe are Silastic Medical Adhesive Type A (Dow Corning, Midland, MI), a soft silicone adhesive. The probe contains an endoscopic video camera, light-emitting diode (LED), as well as several glass optical elements for imaging. The rounded tip of the probe is intended to contact the cervical surface, and the focus of LCI imaging is located on the surface of this tip.

The endoscopic camera consists of a cylindrical camera module (Misumi Corp., Taiwan) with electrical conductors enclosed within a polymer sheath. The LED (various manufacturers under consideration) is placed near the camera module to provide illumination; the electrical power is provided through the camera's circuitry. The electrical components in the handle are powered by a low voltage (5 V) and draw less than 100 mA current.

The cervical probe can be disconnected from the scanning handle for disinfection and servicing. Disinfection is typically accomplished using a peroxide-based high-level disinfection device, such as a Trophon, similar to ultrasound transducers.

***Scanning handle*** The handle of the optical probe serves two purposes: to allow the cervical probe to be manipulated, and to house the moving parts needed to provide scanning of the infrared beam. The infrared beam is provided by an optical fiber enclosed in a polymer sheath, which we term the a/LCI catheter and is part of the a/LCI module (see section below for a more detailed description).

The scanning handle contains a stepper motor, a scanning mirror, control electronics, lenses, and mirrors. The stepper motor rotates a sub-assembly of lenses and mirrors that cause the image of the illumination light to be rotated. The scanning mirror allows that beam to be directed laterally to a desired position on the cervix (when relayed through the cervical probe). Both the stepper motor and scanning mirror require control electronics that are placed in proximity within the handle. These electronic boards also communicate via electrical wires with control electronics located within the physical enclosure that are too bulky to include in the handle.

The beam, following rotation and scanning by the components of the handle, is relayed to the cervix by the cervical probe component. Scattered light returning from the cervix travels along the illumination path in reverse and is collected by a combination of optical fibers contained within the a/LCI catheter.

**A/LCI Module**

The centerpiece of LCI instrumentation is an interferometer, in which light from a known reference distance is combined with light returning from the probe. For the mapping and sizing modes in our

system, two separate interferometers are provided. The interferometer used for the sizing mode is the a/LCI module described in this section. The a/LCI module is a fully integrated prototype a/LCI system. The module includes a fiber-optic probe encased in a polymer sheath, which we refer to as the a/LCI catheter.

The a/LCI module contains a light source, which is a superluminescent diode (Superlum, Moscow, Russia) emitting infrared light at wavelengths between 815 and 845 nm. The total power of the SLD at its output is approximately 25 mW, but the amount of power delivered by the a/LCI catheter is reduced. The power at the output of the a/LCI catheter is limited to less than 4 mW.

The tip of the a/LCI catheter is fixed within the scanning handle. The illumination and detection beams provided by the catheter are scanned as described in the optical probe section.

The a/LCI module also contains a spectrometer, a device that measures the interference signal by wavelength. The spectrometer output is communicated by USB to a host computer. Additionally, the module contains circuitry that controls and synchronizes the behavior of the spectrometer, illumination shutter, and reference stepper motor (for altering the optical path length of the reference arm).

A metal housing encloses the a/LCI module. A single power and USB connection are provided on a rear panel that serves all enclosed devices. A front panel power button allows the entire module to be powered on and off simultaneously.

Several optical fibers pass from the a/LCI module to the OCT module. These fibers carry illumination and signal light to and from the a/LCI light source, the optical probe, and the OCT module spectrometer.

**OCT Module**

The OCT module contains the interferometer to produce OCT images during the mapping mode of a scanning a/LCI procedure. The OCT module does not contain an independent light source. A fiber from the a/LCI module delivers light into the OCT module, where illumination light passes through an optical circulator and is returned to the a/LCI module to be inserted into the a/LCI catheter. Light backscattered by the sample returns via the a/LCI catheter along the same fiber, and returns to the OCT module circulator. The circulator redirects the backscattered light to a fiber coupler within the OCT module, where it combines with light from a reference arm (also within the OCT module) and is detected by an OCT spectrometer. The spectrometer is served by its own power supply and USB connection to the host computer.

**Electronic Control Module**

Several circuit boards and devices are mounted within the physical enclosure for the purpose of controlling and supplying power to the components within the scanning handle. A controller for the stepper motor receives power from its own power supply and communicates via USB to the host computer. Several insulated wires connect the stepper motor controller to the scanning handle. Another device controls and powers the scanning mirror in the scanning handle. This unit also receives its own power and USB connection to the host computer, and is connected to the scanning mirror in the handle via a multi-conductor cable.

**Computer**

A small form factor computer (VivoMini, ASUS, Taipei, Taiwan) performs the data acquisition and control through a custom application in Labview (National Instruments, Austin, TX). Real-time display of the white-light endoscopic images is provided, as well as synchronized acquisition of OCT and a/LCI data from spectrometers. The data from both mapping modes and sizing modes are stored on the encrypted hard drive.

**Physical enclosure**

The a/LCI module, OCT module, electronic control module, and computer are situated upon a wheeled steel cart. Power to all components is provided through standard 120V mains routed through a medical-grade isolation transformer.

**Stage 2 Study Procedures for subjects at Duke**
Each subject will attend 1 study visit that will last about 1 hour and include the following procedures.

- Read consent form
- Ask / answer questions
- Sign consent form
- Complete brief medical questionnaire to determine eligibility
- Urine pregnancy test – must be negative before imaging can occur
- Scanning a/LCI examination of the cervix (without speculum)
- Scanning a/LCI examination of the cervix (with speculum) – only if necessary

We will obtain written informed consent and confirm eligibility criteria. Data collection will include a brief medical history and pregnancy testing. Participants will be interviewed one-on-one to complete the questionnaire regarding medical and reproductive history. The nurse or doctor will then perform the LCI study measurements.

The nurse or doctor will gently place the scanning a/LCI probe against the cervix. Lubricant will be placed on the outside of the probe for subject comfort. White light camera visualization, incorporated into the scanning a/LCI probe, will guide the probe positioning by the clinician to confirm contact with the cervical epithelium. Up to 4 minutes will be allotted for probe manipulation for optimal contact. The probe will then be held in place for scanning a/LCI data acquisition for up to 2 minutes. When data collection is completed, or permitted time is elapsed, the scanning a/LCI probe will be removed and placed on a custom holder that prevents the contact portions of the probe from coming into contact with contaminating surfaces.

If the nurse or doctor could not obtain a good view of the cervix using the integrated white light camera, they may choose to insert a speculum for direct visualization of the cervix. The scanning a/LCI probe will again be placed against the cervix with 4 minutes allotted for probe manipulation and 2 minutes for scanning a/LCI measurements.

**Stage 2 Study Procedures for subjects at Jacobi**
Subjects will be women presenting to the clinic for evaluation after an abnormal cytology result. Subjects will be informed of the study when they present to the clinic. If interested in participating, subjects will complete the following study-related procedures in addition to the standard of care visit procedures.

- Read consent form
- Ask / answer questions
- Sign consent form
- Urine pregnancy test – must be negative before imaging can occur
- Scanning a/LCI examination of the cervix (with speculum)

We will obtain written informed consent and confirm eligibility criteria. Data collection will include a brief medical history and pregnancy testing.

The doctor will then insert a speculum in order to directly visualize the cervix. The cervix may or may not be wiped clear to aid visibility if necessary. The doctor will then gently place the scanning a/LCI probe against the cervix. White light camera visualization, incorporated into the scanning a/LCI probe, will guide the probe positioning by the clinician to confirm contact with the cervical epithelium. The probe will then be held in place for scanning a/LCI data acquisition for up to 2 minutes. When data collection is completed, the scanning a/LCI probe will be removed and placed on a custom holder that prevents the contact portions of the probe from coming into contact with contaminating surfaces.

The doctor will then continue with the standard of care portion of the visit (including collection of cervical biopsies).

## Selection of Subjects

This project will recruit healthy women who:

- are able to provide informed consent
- are at least 21 years old
- are willing to abstain from sexual intercourse for at least 24 hours before the study visit

Participants meeting any of the following exclusion criteria will be excluded from taking part in this study. Women must not:

- be pregnant
- have a current gynecologic infection or discharge
- have had any cervical surgery
- have had medical or cosmetic surgery involving the reproductive organs or genitals within the past 6 months
- be currently enrolled in any research studies involving the application of vaginal formulations
- be employed or supervised by the study investigators
- have any other condition that, in the opinion of the study clinician, would contraindicate participation in the study

In addition, to participate in stage 1 (mLCI device development) at the Duke site, women must not:

- have given birth to a baby
- be using an IUD

To participate in Stage 2 (scanning a/LCI device development) at the Jacobi site: subjects will be recruited from women who present to the clinic for evaluation for abnormal cytology. The study will recruit women with no prior history of cervical surgical procedures.

## Subject Recruitment

**Subject Recruitment at Duke**

Duke will recruit up to 15 women to enroll in stages 1 and 2 of the study, in order to achieve the target number of 10 subjects who complete each stage. This gives a total enrollment of up to 30 subjects at Duke to complete both stages of the study at that site.

The study will be advertised on the ResearchMatch website, the DukeList website, and/or flyers posted around campus. Recruiting will be managed by our study coordinator. The inclusion of minorities in the group of women recruited will reflect the local distribution in the Durham area.

After learning about the study, prospective subjects will call the study coordinator for more information. During this telephone call, the study coordinator will state the study requirements plus a brief description of the procedures. If the prospective subject is interested in participating in the study, a study visit will be scheduled. The prospective subject's contact information will be kept in a locked desk or file cabinet or password protected file in the study coordinator's office. If the prospective subject states that she does not qualify or is not interested in participating, the contact information will be destroyed.

**Subject Recruitment at Jacobi**

Potential subjects will be informed of the study when they present to the clinic for a regular clinical care visit.

Jacobi will enroll up to 100 women for stage 1, in order to achieve the target number of 40 women who complete stage 1. For stage 2 of the study, up to 140 women will be recruited in order to enroll 70 participants in this cross-sectional study (50 HIV-negative participants and 20 HIV-positive participants). The goal distribution of participants in this study is approximately 14 women with normal histology, 21 women with CIN-1, 14 women with CIN-2 or CIN-2/3, 14 women with CIN-3, and 7 women with invasive cancer.

## Consent Process

The study coordinator will conduct the consent process. The consent process will be conducted at a meeting in a private office or other suitable private location (consult room, conference room). To ensure privacy during the meeting, the door will be closed. Thirty minutes will be allocated for conducting the initial consent discussion, allowing time for the prospective subject to read the consent form and ask any questions she may have. The prospective subject can choose to sign the consent form at that time, or take more time to decide if she wishes. The prospective subject will have as much time as she needs

to decide whether or not to participate. However, enrollment will continue, and if all study slots are full by the time the prospective subject decides to participate, she may not be enrolled in the study. The prospective subject may ask questions at any time by contacting the study coordinator or PI.

To minimize the possibility of coercion or undue influence, study personnel will inform subjects of their rights as a research participant, including their right to refuse to participate or to withdraw from the study at any time without penalty. Duke students and employees under direct supervision of the study staff will not be allowed to participate in this study.

## Subject's Capacity to Give Legally Effective Consent

Subjects who do not have the capacity to give legally effective consent will not be included in this study.

## Risk / Benefit Assessment

**Risk/Benefit Assessment of the study in general**

***Potential Risks***

Potential risks include:

1. breach of confidentiality
2. coercion
3. discomfort with the nature of the research
4. side effect from study procedures and/or devices

***Potential Benefits***

There is no direct benefit to subjects participating in this research study. However, the novel LCI devices to be developed in this proposal would potentially advance the management of cervical dysplasia by assisting clinical decision-making without relying on gross colposcopic impression. The current proposal is to conduct the early feasibility studies necessary for technology development. Data will also be collected about epithelial characteristics as visualized by LCI, which could improve our understanding about the basic biology of the cervical epithelium. The long-term goal is to develop devices that could be incorporated into routine clinical care. The LCI device could provide real-time information to the clinician and reduce the number of clinical visits needed.

***Alternative Treatments***

There are no alternative treatments to participating in this research. It is not intended to be therapeutic, but to acquire basic scientific knowledge.

**Risk Assessment and Risk Minimization for Study Activities**

***Potential Risks***

Potential risks include:

1. breach of confidentiality
2. coercion
3. discomfort with the nature of the research
4. side effects from study procedures and/or devices

***Breach of Confidentiality***

Subjects will provide personal information, including sexual history and health information. Breach of confidentiality will be avoided by training research staff in the protection of human subjects and by coding and limiting access to data. Each participant will be assigned a research subject number. The only documents linking the subject's identity with her subject number will be the consent form and the enrollment log. The contact information list and payment request forms will contain the subject's identity only. The medical questionnaire, pregnancy test result, and data spreadsheets will reference the subject number only. The subject's social security number, required on the payment request form, will be removed once payment has been processed. Once the study is complete, all contact information will be deleted, unless the participant requests to be contacted for future studies conducted by Dr. Wax or his colleagues. All of the participant's study documents will be kept in a locked file cabinet in the study coordinator's office. Databases containing PHI will be maintained on a password-protected computer with full disk encryption.

***Coercion***

Coercion occurs when potential participants feel compelled to participate in research for reasons such as perceived demand or the availability of large sums of reimbursement. This can be particularly true when there is little benefit to the individual for their participation. In this study, there is minimal risk of coercion as personnel will inform subjects of their rights as a research participant, including their right to refuse to participate or to withdraw from the study at any time without penalty. Reimbursement is not believed to be coercive, based on the time required and the nature of the research. The amount of reimbursement is comparable to similar studies.

***Discomfort with the Nature of the Research***

Participants may feel discomfort with the personal nature of this research. Before the participant is enrolled in the study, detailed study procedures will be explained so she is aware of what the research entails. Subjects will provide personal information, such as sexual health and history. Some study procedures will be conducted with the physician/nurse and research assistant/chaperone in the room, including insertion of the LCI devices into the vagina by the physician/nurse (similar to a pelvic exam) and making measurements with the devices. Participating in these research procedures and providing personal information may be uncomfortable for some women. It is unlikely this discomfort will result in any serious adverse events. Should participants report discomfort with the research, they will be reminded of their rights to not participate in the study or to withdraw from the study at any time. Subjects may contact the principal investigator or one of the study physicians at any time to discuss questions or concerns about the study.

***Side Effects from Study Procedures and/or Devices***

Risks of the LCI Devices: The LCI devices will be handled gently for placement against cervical tissue. We expect very minimal risk for tissue abrasion or irritation as the devices will have a smooth plastic and silicone surface and commercial gynecological lubricant will be applied before cervical contact. The devices will be cleaned in full accordance with high level disinfection clinical procedures at DUHS. Specifically, probes will be enclosed in a Trophon EPR high-level disinfection system to disinfect all external surfaces. Study staff will complete training and competency reviews per institutional procedures. For the LCI technique, a fiber optic bundle transmits near infrared light onto the epithelia to image the cervix. The light source has been either certified for medical use or tested to demonstrate that it complies with medical safety standards.

## Costs to the Subject

There will be no costs to the subject as a result of participating in this study.

## Data Analysis & Statistical Considerations

**Data Analysis & Statistical Considerations**

***STAGE 1: mLCI device development***

***Data analysis for Duke site***

The proposed study is designed as a pilot study, intended to provide feasibility and usability data to inform future studies on a larger scale. The planned pilot study will include 10 subjects, which will yield success/failure metrics of the probe design with 10% precision. Elements of the probe performance that will be evaluated include ease of use by the medical practitioner, patient comfort, ability to access the relevant anatomy, and quality of mLCI imaging data. Because the goal of the pilot study is the evaluation of general feasibility, no subject data will be excluded if no predetermined exclusion criteria are met.

***Data analysis for Jacobi site***

Construction of epithelium maps by mLCI: Maps of the cervix delineating the T-zone will be created from each subject's mLCI data. The extent of columnar and squamous epithelia will be reported as a percentage of the image of the epithelium. Each scan in the 36 point field will initially be reviewed manually to determine the epithelium type based on the depth-resolved reflection profile for that point. The presence of clear histological layers in indicative of squamous epithelium while a thinner layer with less structure indicates columnar epithelium [22]. As we compile a database of mLCI maps of the cervix, we will develop automated algorithms for assessing epithelial types and transitions by using cluster analysis to discriminate scans. The mLCI maps will be compared to digital colpophotography analysis to assess accuracy.

Measurement of epithelial areas by colpophotography: Digital colpophotographs will be viewed in Adobe Photoshop for quantitative computerized planimetry as previously published [23, 24]. We will manually outline all areas of columnar, metaplastic and squamous epithelia and the total visible cervical face. Total pixel counts of the various epithelial types will be calculated and ectopy expressed as percentage of the total cervix.

Correlation of mLCI images to colpophotographs and detailed written maps: The mLCI epithelial maps will be visually compared to colpophotographs to achieve co-registration. This is feasible based on the relatively modest number of patients in the study. The written map and white light imaging via the probe will aid in coregistration. We will correlate mLCI readings with calculated areas of epithelial types (pixel counts and percentage). Biostatistical analyses will address covariates including age, infections, cytology, menstrual cycle, and sexual behaviors.

Potential problems, alternative strategies, and benchmarks for success: The mLCI approach will be viewed as successful if there is less than average absolute difference of 10% in the area estimate compared with manual analysis.

***STAGE 2: scanning a/LCI device development***

***Data analysis for Duke site***

The proposed study is designed as a pilot study, intended to provide feasibility and usability data to inform future studies on a larger scale. The planned pilot study will include 10 subjects, which will yield

success/failure metrics of the probe design with 10% precision. Elements of the probe performance that will be evaluated include ease of use by the medical practitioner, patient comfort, ability to access the relevant anatomy, and quality of scanning a/LCI imaging data. Because the goal of the pilot study is the evaluation of general feasibility, no subject data will be excluded if no predetermined exclusion criteria are met.

***Data analysis for Jacobi site***

Data collected from the scanning a/LCI device will include nuclear size and density as a function of depth into the tissue at each "sizing mode" measurement location selected during "mapping mode". All biopsy samples will be read by Jacobi Pathology per standard clinical care procedure.

To determine sensitivity and specificity for diagnosis of cervical dysplasia, an ROC curve will be created based on the a/LCI data and histological classification. Repeated measures logistic regression models will be used to assess the association of histology with nuclear morphology characteristics after adjusting for other relevant factors. The outcome variable will be dichotomized as "CIN-2 or worse" vs. CIN-1/normal. The predictor will be the morphological nuclear characteristics (nuclear density and diameter at selected depths beneath the tissue surface). Potential confounders will be adjusted for, including age, type of epithelium (i.e. metaplastic, squamous), infections if found, menstrual cycle timing, and sexual behaviors. A compound symmetric correlation matrix will be used to account for the repeated measures within subjects. P-values associated with type I error rates of less than 0.05 will be considered statistically significant in this study. Analyses will be accomplished using JMP Version 9 software (SAS Inst., Cary NC).

One potential problem may be co-registration of a/LCI scan points with biopsy. We will mitigate this risk by using acetowhitening both prior to and after probe application to first guide a/LCI scans to suspicious regions and to return to those points afterwards, as well as using the white light visualization built into the probe for guidance. Another potential problem may arise with co-locating and registering the two optical modalities (OCT and a/LCI). The approach of using the same fiber source as illumination for both modalities will minimize this risk. The benchmark for success will be successful operation of both modalities in sequence without loss of performance in assessing the T-zone (less than 10% error vs. colpophotography) or detecting dysplasia (90% sensitivity / 90% specificity vs. histopathology).

## Data & Safety Monitoring

***Reporting of Adverse Events***

An adverse event is defined as any untoward medical occurrence in a clinical research subject participating in the clinical study of the LCI devices. Any AE that occurs between the time a study participant signs the informed consent form and the time she departs the study at the end of the imaging session will be captured and recorded. Study participants will be instructed to contact the study site staff to report any AEs they may experience after completion of their participation. AE resolution will consist of evaluating instrument design and application to assess the potential source and implementing design changes to mitigate the risk of further AE. If necessary, AEs will be reported to the IRB according to IRB guidelines.

***Safety Monitoring***

During the proposed trials of the LCI devices, weekly status reports will be submitted to the Duke PI from the Jacobi site. These reports will include all adverse events reported for the study, determined to be

related or unrelated to the study protocol. The study team will meet, as needed, throughout the period of study to review any reported adverse events and address any potential safety concerns.

***Data accuracy and protocol compliance***

Data acquired during the clinical study will be reviewed on a weekly basis by Duke lab personnel to assess if the data appear to be consistent with expected operating characteristics of the device. In the event that data do not appear satisfactory, a review of protocol compliance will be conducted. If the protocol has been followed, a technical inspection of the device by Duke lab personnel will be scheduled and further study will be suspended until instrument function has been verified.

## Privacy, Data Storage & Confidentiality

Due to the personal nature of this research, all subject interviews will take place in a private office and all study visits will take place in a private clinic room. All study documents (including lab results) will be kept in a locked filing cabinet in the study coordinator's office. Identifiers will be removed from any data being analyzed. Some study procedures will be conducted with the physician/nurse and research assistant/chaperone in the room, including insertion of the LCI device into the vagina by the physician/nurse (similar to a pelvic exam) and making measurements with the device. The subject will be draped with a sheet during these study procedures to provide as much privacy as possible.

Subjects will provide personal information, including sexual history and health information. All study personnel will complete training in the conduct of human subjects research in order to protect against possible breach of confidentiality. Identifiers will be removed from any data being analyzed. Each participant will be assigned a research subject number. The only documents linking the subject's identity with her subject number will be the consent form and the enrollment log. The contact information list and payment request form will contain the subject's identity only. The medical questionnaire, pregnancy test result, and data spreadsheets will reference the subject number only. The subject's social security number, required on the payment request form, will be blacked out once payment has been processed. Once the study is complete, all contact information will be deleted or destroyed, unless the participant requests to be contacted for future studies conducted by Dr. Wax or his colleagues. All of the study documents will be kept in a locked file cabinet in the study coordinator's office. Databases will be maintained on a password-protected computer with full disk encryption in the study coordinator's office.

To protect against the possibility of loss of research records, database files will be periodically backed up to a DUHS network drive. Hard copies of documents (consent forms, medical questionnaires, lab results, payment request forms) will be scanned and backed up in this same manner.

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