Design and validation of a multiplexed low coherence interferometry instrument for in vivo clinical measurement of microbicide gel thickness distribution

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Abstract: We present a multiplexed, Fourier-domain low coherence interferometry (mLCI) instrument for in vivo measurement of intravaginal microbicide gel coating thickness distribution over the surface of the vaginal epithelium. The mLCI instrument uses multiple delivery fibers to acquire depth resolved reflection profiles across large scanned tissue areas. Here mLCI has been adapted into an endoscopic system with a custom imaging module for simultaneous, co-registered measurements with fluorimetric scans of the same surface. The resolution, optical signal-to-noise, and cross-talk of the mLCI instrument are characterized to evaluate performance. Validation measurements of gel thickness are made using a calibration socket. Initial results from a clinical study are presented to show the in vivo capability of the dual-modality system for assessing the distribution of microbicide gel vehicles in the lower human female reproductive tract.

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1. Introduction

The HIV/AIDS epidemic continues to overwhelm current preventative measures. This is especially true in sub-Saharan Africa where women experience two-thirds of new infections, and young women are 3-5 times more likely to be infected than males of the same age. Clearly, there is a need for a discreet, female-controlled method of protection from the HIV virus that women can use proactively and independently [1].

Microbicide gels are topical products that are being developed in order to protect against sexually transmitted infections, including HIV/AIDS. Recently, the CAPRISA 004 trial of a tenofivir microbicide gel showed a 39% reduction of HIV transmission vs. control population [2]. A quality microbicide gel is one that incorporates a potent active ingredient into a robust delivery system, e.g. a gel, and achieves targeted drug delivery to target tissues and fluids. In order to evaluate the effectiveness of microbicide gels, accurate measurement of their intravaginal gel coating thickness distributions is necessary.

Previously, two optical imaging methodologies have been applied for measuring microbicide gel distribution. A fluorescence-based instrument was developed and used to measure coating thickness of fluorescein labeled gel vehicles [3–5]. There is concern, however, that the label may separate from the gel over time, thereby confounding the interpretation of the scans of local fluorescence intensity. Consequently, we have developed a multiplexed, Fourier-domain low coherence interferometry system for label free measurement of gel distribution [6,7]. This system was successfully validated in phantom studies [3,4,6,7]. We now present an endoscopic platform that integrates these two techniques—mLCI and fluorimetry—into a single instrument to enable simultaneous \textit{in vivo} imaging of gel distribution using both modalities. The combination allows validation of the mLCI system for measuring gel thickness distributions without the need of exogenous contrast agents [7]. To develop the platform, our original mLCI instrument was modified and a compact imaging module was created for integration with the fluorescence endoscope. In this study, we evaluate the clinical mLCI instrument’s performance with test measurements from a custom calibration socket, as well as demonstrate preliminary \textit{in vivo} measurements of vaginal gel coating thickness distribution in women.

2. Instrumentation and probe design

The clinical mLCI device is based on six parallel fiber optic Michelson interferometers, as shown below in Fig. 1. Light from a superluminescent diode (SLD, Superlum Diodes, Moscow, Russia, \(\lambda_o = 837.5\text{-nm} \ \Delta\lambda = 54.2\)) is split into eight channels (six are used) by a 1x8 fiber coupler (AC Photonics, Santa Clara, CA).

Six 50:50 fiber optic couplers (AC Photonics) are used to create the reference and sample arms for each interferometer. In the reference arm of each channel, light is collimated by a fiber optic collimator (AC Photonics) and reflected off of a reference mirror. Each arm includes a fiber optic shutter to enable selective gating of each reference arm. In the sample arm, the six fibers are routed together through a protective polyetheretherketone (PEEK) sheath into the fluorescence system’s endoscopic probe. This allows the probe to be mounted in its existing frame, with just the mLCI sample arm fibers interfaced with the existing fluorimetric instrument setup [3]. The optical fibers are then bonded to a silicon v-groove...
Fig. 1. System schematic of the clinical, multiplexed LCI instrument. Light from a SLD is split into 8 channels, 6 of which are used, by a 1x8 fiber optic splitter. Six 50:50 fiber optic couplers are then used to create reference and sample arms for each parallel interferometer. Fiber optic collimators are used to collimate and collect the reference light reflected off of the mirror array for each channel of the instrument. The sample arm fibers are routed through a protective sheathing, into the endoscopic probe, and secured into a custom imaging module. The reference and sample fields are recombined by the six 50:50 couplers and detected simultaneously by a six-channel spectrometer.

Fig. 2. mLCo imaging module. (a) Top view of optical components: L1-3-mm cut ball lens; L2-6-mm cut ball lens. (b) Side view of module components. (c) Schematic of the mLCo imaging module mounted on the endoscope. (d) 3-D layout of the imaging module.

chip (OZ Optics, Ottawa, Ontario, CA) which acts to protect the fibers as well as enabling precise alignment. Figure 2 shows the mLCo imaging module that is adapted to permit measurement within the distal end of the endoscopic fluorescence probe.

In the imaging module, lens, L1, collimates the light from each of the fibers. A 3-mm right angle prism creates a 90-degree bend in the light path necessary for the side-firing probe design. Lens, L2, focuses the light through a 3.175 mm thick polycarbonate tube and onto the sample. The signal beam reflected by the sample is recombined with the reference field and...
detected by one channel of a 6-channel spectrometer (Avantes, Inc., Broomfield, CO) which allows simultaneous detection of all 6 interference signals. The system is operated at a speed of 48 scans/s in this application, but the spectrometer is capable of reaching speeds up to 1620 scans/s [7]. Path length between each sample and reference arm is matched by adjusting the fiber optic collimators in each reference arm relative to one another in a custom mount. Once each individual channel is matched, the entire group can be moved with the translation stage shown in Fig. 1 for gross alignment.

The imaging module shown in Fig. 2(d) is a custom mount, machined from aluminum and secured to the end of the existing endoscopic probe using machine screws. This configuration offsets the mLCI field of view by 180° azimuthally and 20 mm axially from that of the fluorescence system as shown in Fig. 2(c), but this is compensated for during data processing. The imaging module also acts to secure the v-groove chip, and allows 3-axis positioning of the v-groove chip. The 3-mm ball lens (L1) was epoxied to the face of the 3 mm right angle prism, and this assembly is secured into a well in the imaging module. The 6 mm ball lens (L2) was secured as in Fig. 2(d) to the imaging module. Four screws allow for precise alignment of this optic. The entire rigid endoscope is contained in a 27 mm diameter, polycarbonate tube (150 mm long) with a hemispherical cap, as shown in Fig. 3(c). The internal endoscope is then able to freely rotate and translate relative to the polycarbonate tube so that measurements can be made at sites that span the length and circumference of the tube. The measurement positions vary along the vagina over 360° of azimuthal angle and approximately 100 mm axially. The design in Fig. 2(c) allows increased lateral sampling and broad tissue coverage without the use of electromechanical galvanometer scanners in the probe, typical of many optical coherence tomography systems. The finished system is housed on a 12x12 in. aluminum breadboard mounted to a stainless steel utility cart for simple maneuverability, as shown in Fig. 3.

The clinical cart contains the mLCI instrument, multichannel spectrometer, SLD driver, and positional encoder interface, used to couple the mLCI instrument with the fluorimetry system. The positional encoder interface consists of a USB NI-DAQ that is used to capture the longitudinal and angular positioning coordinates of the probe from the fluorimetric
instrument, as described by Henderson, et al. [3]. These longitudinal and angular coordinates are then used to co‐register the mLCI and fluorimetric data during post‐processing.

3. System characteristics

3.1 Signal processing

The signal, $I$, captured by the detector of each channel of the spectrometer is given by

$$I = I_s^2 + I_r^2 + 2 \text{Re}[I_sI_r + \cos(2\Delta z k + \phi)]$$  \hspace{1cm} (1)

where $I_s$ and $I_r$ are the sample and reference fields, $\Delta z$ is the optical path length difference between the arms of the interferometer, $k$ is the corresponding wavenumber of the light source, and $\phi$ is a phase term. As optical path length difference increases, the frequency of spectral oscillations increases as well. Optical path length can be linearly related to physical thickness, $t$, with the simple equation $\Delta z = nt$, where $n$ is the index of refraction of the sample, which is generally a known or measurable quantity for gel vehicles.

Prior to instrument application, it is necessary to perform a background measurement to enable subtraction of noninterferometric and common path interference signals from the captured spectra, $I$. Before the endoscope is placed into the polycarbonate tube, the signal $I_R$ is captured. The endoscope assembly is then placed into the polycarbonate tube and the signal $I_D$ is obtained. At each measurement point, computer controlled mechanical fiber optic shutters are used to capture $I_s$ immediately prior to data acquisition, to minimize fluctuations in the subtracted signal. The background subtracted spectra is then given by

$$I = I_{\text{raw}} - (I_s + I_r - I_D)$$  \hspace{1cm} (2)

The interference spectrum is then converted from wavelength, $\lambda$, to wavenumber, $k$, by the transformation, $k = 2\pi/\lambda$, and resampled using a spline interpolation to restore linear spacing. The wavenumber interference spectra is then digitally corrected for chromatic dispersion effects and Fourier transformed to reveal the spatial cross correlation between the reference field and sample reflectors.

3.2 Instrument performance

Optical signal‐to‐noise (OSNR) was determined for each channel of the system by using a single reflector in place of the sample. OSNR can then be calculated as

$$\text{OSNR}(dB) = 20(\log\left(\frac{E_s}{E_N}\right) + ND),$$  \hspace{1cm} (3)

where $E_s$ is the amplitude of the processed interference spectrum from the mirror surface, $E_N$ is the standard deviation of the shot noise, and $ND$ is the attenuation of a neutral density optical filter which is used to prevent detector saturation due to the relatively strong signal from the mirror reflector. OSNR values range from 87 to 113 dB, and are summarized in Table 1. These results show marked improvement over the first generation multiplexed LCI instrument [7].

Axial resolution was determined for each channel of the mLCl system. Again, a mirror was used as the sample, and the full width at half maximum (FWHM) of the resultant reflection profile was determined. The FWHM results, defining the axial resolution for each channel, are shown in Table 1. While these results are consistent with the first generation system, they do not reach the theoretical resolution, 5.7 $\mu$m, as given by

$$l_c = \frac{2\ln(2)}{\pi} \frac{\lambda^2}{\Delta \lambda}$$  \hspace{1cm} (4)
where $\lambda$ is the central wavelength of the light source and $\Delta\lambda$ is the bandwidth of the source [7,8]. The use of fiber optics and differences in optical components between the sample and reference arms of the mLCI system lead to chromatic dispersion in the system, and thus decreased resolution and variations in OSNR from channel to channel. Off axis focusing of the light from the multiple channels in the imaging module causes the relatively small differences in axial resolution between the channels as seen in Table 1. As in the previous generation system described by Drake et al., chromatic dispersion in the mLCI system is digitally corrected, improving resolution and approaching the theoretical limit [7].

Table 1. Measured optical signal-to-noise (OSNR) and axial resolution

| Channel | 1    | 2    | 3    | 4    | 5    | 6    |
|---------|------|------|------|------|------|------|
| OSNR (dB) | 90.83 | 87.55 | 102.50 | 92.58 | 110.90 | 113.56 |
| Axial Resolution (µm) | 9.6 | 9.2 | 8.3 | 6.8 | 7.0 | 8.7 |

Cross-talk between the mLCI channels was also examined, in order to characterize the performance of the clinical instrument. A single channel was connected to the SLD light source, and optical power was measured at the detector of the adjacent channels. Cross-talk is then given by

$$P_{AS} = 10\log\left(\frac{P_{A}}{P_{S}}\right)$$

where $P_{AS}$ is the cross-talk from an adjacent fiber, $P_{A}$, to the signal fiber, $P_{S}$ [9]. Cross-talk is negligible in the clinical mLCI system as all values were found to be less than −53dB.

4. Device performance using a calibration socket

A custom machined calibration socket was used to verify the accuracy and linearity of the mLCI instrument. The test socket is used for calibration of the fluorimetric system and it was used as an accurate model to test the linearity and accuracy of the mLCI instrument, independently verifying both modalities against the same standard [3,4]. The test socket consisted of an aluminum half cylinder with five grooves of varying depth, from 150 µm to 500 µm, as shown in Fig. 4(a). These depths are representative of those previously measured

![Fig. 4. Calibration socket validation: (a) photograph of the calibration socket with a placebo gel (dyed pink), (b) plot of mLCI measurements versus those taken with digital calipers, (c) b-scan of the three wells of the socket where the red lines illustrate socket depth (from digital caliper measurement) and the blue lines illustrates the point where a single a-scan of the 310 µm well in (d) was captured.](image)

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in vivo, and predicted to provide prophylaxis against HIV/AIDS [10,11]. A test gel was placed into the socket and measured with the clinical mLCI instrument. This gel was the commercial vaginal moisturizer Replens (LDS Consumer Products, Cedar Rapids, IA). Fluorimetric imaging studies of the vaginal coating of this gel have previously been performed [3,4]. Measurements were taken in each of the five grooves, the measurements from all six channels were averaged, and these were compared to those from digital calipers. Figure 4(b) shows excellent agreement between the two methods, with a correlation coefficient of 0.9947, and a slope of 1.0082. The y-axis error bars are determined from the axial resolution values given in Table 1, and the x-axis error is given by the uncertainty of the digital calipers, or \( \pm 5 \mu m \). These results are comparable to those of previous generation systems, such as Braun et al. and Drake et al. [2,7].

By translating the calibration socket and capturing an A-scan, or axial depth scan, at every 3.5-mm of lateral position using a single, central channel (channel 3), the tomographic image in Fig. 4(c) was created. Three of the five wells are clearly seen in the image, and the depths again correlate with those of the digital calipers. Figure 4(d) shows a single A-scan from the image (blue line in Fig. 4(c)), and the peak at approximately 310 \( \mu m \) reveals the expected depth of the well.

5. Human in vivo feasibility measurement

With the incorporation of mLCI imaging optics into the fluorimetric endoscopic probe, simultaneous in vivo measurements of gel coating are now possible. Device performance shown in this pilot study results from in vivo measurements of the test gel, Replens (n = 1.34), in a single human subject. This gel is considered a useful biophysical model of current microbicidal gel products [3,4,12].

5.1 Clinical data acquisition

Initially, the polycarbonate tube is lubricated with a thin coating of non-labeled Replens for insertion. The physician then inserts the lubricated tube assembly into the subject’s vagina and adjusts an opaque flange to eliminate ambient light. The tube is then zero-positioned, with 0° oriented vertically, and the endoscope advanced entirely towards the fornix defined as 0-mm. The tube is then locked in place, so that it remains stationary during the course of the measurements. The physician then manually advances the endoscope inside the polycarbonate tube distally towards the introitus, capturing mLCI measurements at 1-mm increments and fluorescence measurements at 5-mm increments. This sampling method is performed to achieve equal coverage of the two modalities in the axial direction, since the fluorimetric system has a larger field of view than the mLCI instrument [3,7]. Once an axial scan is completed, the physician then rotates the endoscope 45° and continues the scan towards the fornix. Once advanced entirely to 0°, the endoscope is rotated another 45° and this sequence continues until an entire 360° mapping of the vagina is completed, and the probe is removed.

Once this background scan is finished, 3.5 mL of fluorescein-labeled Replens is inserted into the subject’s vagina for the test scan. There is then a waiting period, during which the participant executes a defined protocol of activity in private. In the study here, after gel insertion, the participant remained seated for 1 minute, stood for 1 minute, and then was seated for 1 minute; she then assumed a supine position for 7 minutes. This protocol is typical of those used to capture a woman’s posture after application of a microbicidal gel and prior to sexual activity. That is, this protocol captures that period during which microbicidal drug delivery by the gel can lead to a prophylactic concentration of drug—which is governed by the vaginal surface coating measured here—prior to exposure to HIV. The physician then reinserts the entire probe assembly into the subject’s vagina, to the same axial depth as the background scan. The scan procedure is repeated, until the entire 360° mapping of the surface of the vagina is completed. In total, approximately 750 regions are sampled with mLCI for the background and final scans, each region comprising 4 A-scans yielding a total of 3000 A-
scans per patient. The entire procedure averaged 49 minutes per patient (maximum = 1 hour, 1 minute; minimum = 36 minutes).

5.2 Clinical data processing

The mLCI measurements must be properly reconciled with the fluorimetric data, since mLCI data are sampled 5-times more frequently and offset from the fluorimetric data due to the endoscope design. First, the mLCI data set is rotated by 180° and shifted ~20 mm to compensate for the field-of-view offset. Then, for each fluorimetric measurement point, five consecutive axial mLCI scans are averaged to compensate for the larger field of view of the fluorimetric system. The background scan and test scan are both processed in this manner. Finally, a point-to-point subtraction of the background scan from the test scan is performed in order to remove any effects from the non-labeled gel used for probe insertion.

5.3 Clinical data results

Figure 5 shows the coating thickness distributions obtained from our human in vivo data, presented as a topological plot, with gel thickness represented by the colorbar. Zero degrees is aligned with vertical orientation, and 0 mm is closest to the fornix (deepest portions of the vagina). The 20 mm axial offset between the modalities is clearly seen in Fig. 5, as the mLCI data extends further towards the fornix.

![Fig. 5. Human in vivo microbicide gel coating measurements: (a) Fluorimetric system and (b) mLCI instrument. The colorbar shows coating depth in μm.](image)

The fraction of scanned vaginal surface with detectable coating was calculated for each modality: the mLCI instrument was found to have a value of 0.4500 while the fluorimetric system had a value of 0.4453, for an absolute difference of 0.0047, or 1.06%. The fraction of scanned vaginal surface with coating thickness > 50 μm was also calculated since this is a putative threshold of minimum coating thickness needed for a microbicide gel at any position over the vaginal epithelium. The clinical mLCI instrument produced a value of 0.3265 and the fluorimetric method had a value of 0.2891, with an absolute difference of 0.0374, or 12.9%. We believe that this difference is not biologically significant, based on our studies of HIV and drug transport through distributed gel coatings [10,11].

Figure 6 shows the contour lines of the fluorimetric coating data superimposed onto the topological plot from the mLCI thickness distribution data. Qualitatively, the coating distributions appear to correlate spatially, as the areas with coating overlap and are distributed within approximately 50 mm of the fornix. Again, the 20 mm axial offset between the data sets is seen as the fluorimetric contour lines begin at 20 mm axial distance. It should be noted that at thicker distributions of gel coating, greater than approximately 600 μm, the mLCI
system yields lower thickness measurements than the fluorescence system because the in vivo mLCI system lacks penetration depths at such thick gel layers. However, since we are investigating coatings that exceed a minimum thickness, this upper threshold does not alter our analysis.

![Image](image.jpg)

**Fig. 6.** Contour lines of the fluorimetric coating data overlaid on a topological plot of the mLCI coating data.

### 6. Summary

In this paper, we have demonstrated a multiplexed, Fourier-domain mLCI system that has been adapted to a fluorimetric endoscope system for simultaneous, multimodal optical imaging of microbicide gel coating distribution over the vaginal epithelial surface in women in vivo. We showed that the mLCI system obtained comparable results to the previously validated fluorimetric system, but without the need for extraneous contrast agents. The mLCI system also achieved broad area scanning over large tissue area without the need of electromechanical scanners typical of many optical coherence tomography systems. The resolution, optical signal-to-noise, and cross-talk of the multiplexed system were characterized, and an in vitro validation experiment was performed on a custom calibration socket. In vivo measurements from a clinical study of a placebo microbicide gel have been presented to demonstrate the ability of the clinical mLCI system to obtain vaginal gel coating distributions. Studies have shown that a coating layer with a thickness in excess of 50 µm may be sufficient enough to neutralize HIV before it can contact epithelium tissue and result in an infection, and the mLCI instrument demonstrated the ability to measure distributions at this scale [11]. These pilot results are comparable to the previously validated fluorimetric system. They demonstrate that this new system offers significant promise for measuring vaginal gel coating thickness distributions in vivo, in clinical studies of anti-HIV microbicide gels and also other gel therapeutic and prophylactic products. Based on the validation presented here, we expect that future studies will be performed solely with the mLCI device, eliminating the need for exogenous contrast agents for imaging and allowing for extended time studies. Gel distributions from large time intervals (6–12 hours) between gel insertion and imaging cannot currently be studied with the fluorimetry system because of diffusion of the fluorophore from the gel to the tissue. The mLCI device offers promise to perform such studies, allowing us to gain a better understanding of in vivo gel distributions as they would appear with actual use of a microbicide gel product.

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