Detection of early colorectal cancer development in the azoxymethane rat carcinogenesis model with Fourier domain low coherence interferometry

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Abstract: Fourier domain low coherence interferometry (fLCI) is an emerging optical technique used to quantitatively assess cell nuclear morphology in tissue as a means of detecting early cancer development. In this work, we use the azoxymethane rat carcinogenesis model, a well characterized and established model for colon cancer research, to demonstrate the ability of fLCI to distinguish between normal and preneoplastic *ex-vivo* colon tissue. The results show highly statistically significant differences between the measured cell nuclear diameters of normal and azoxymethane-treated tissues, thus providing strong evidence that fLCI may be a powerful tool for non-invasive, quantitative detection of early changes associated with colorectal cancer development.

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Reference and links:

1. American Cancer Society, Colorectal Cancer Facts & Figs. 2008–2010. Atlanta:American Cancer Society, 2008.
2. A. J. Aldridge, and J. N. Simson, "Histological assessment of colorectal adenomas by size. Are polyps less than 10 mm in size clinically important?" *Eur. J. Surg.* **167**(10), 777–781 (2001).
3. A. P. Weston, and D. R. Campbell, "Diminutive colonic polyps: histopathology, spatial distribution, concomitant significant lesions, and treatment complications," *Am. J. Gastroenterol.* **90**(1), 24–28 (1995).
4. E. Jaramillo, M. Watanabe, P. Slezak, and C. Rubio, "Flat neoplastic lesions of the colon and rectum detected by high-resolution video endoscopy and chromoscopy," *Gastrointest. Endosc.* **42**(2), 114–122 (1995).
5. J. Dominitz, G. Eisen, T. Baron, J. Goldstein, W. Hirota, B. Jacobson, J. Johanson, J. Leighton, and J. Mallory; Standards of Practice Committee. American Society for Gastrointestinal Endoscopy, "Complications of colonoscopy," *Gastrointest. Endosc.* **57**(4), 441–445 (2003).
6. D. Ransohoff, and R. Sandler, "Screening for colorectal cancer," *N. Engl. J. Med.* **346**(1), 40 (2002).
7. H. Roy, A. Gomes, V. Turzhitsky, M. Goldberg, J. Rogers, S. Ruderman, K. Young, A. Kromine, R. Brand, M. Jameel, P. Vakil, N. Hasabou, and V. Backman, "Spectroscopic microvascular blood detection from the endoscopically normal colonic mucosa: biomarker for neoplasia risk," *Gastroenterology* **135**(4), 1069–1071 (2008).
8. G. Zonios, L. T. Perelman, V. Backman, R. Manoharan, M. Fitzmaurice, J. Van Dam, and M. S. Feld, "Diffuse reflectance spectroscopy of human adenomatous colon polyps in vivo," *Appl. Opt.* **38**(31), 6628–6637 (1999).
9. Y. L. Kim, V. M. Turzhitsky, Y. Liu, H. K. Roy, R. K. Wali, H. Subramanian, P. Pradhan, and V. Backman, "Low-coherence enhanced backscattering: review of principles and applications for colon cancer screening," *J. Biomed. Opt.* **11**(4), 041125 (2006).
10. N. G. Terry, Y. Zhu, M. Evoniuk, J. K. M. Thacker, J. Migaly, C. Guy, C. R. Mantyh, and A. Wax, "Detection of intestinal dysplasia using angle-resolved low coherence interferometry," *Dis. Colon Rectum* (in review).
11. R. N. Graf, F. E. Robles, X. Chen, and A. Wax, "Detecting precancerous lesions in the hamster cheek pouch using spectroscopic white-light optical coherence tomography to assess nuclear morphology via spectral oscillations," *J. Biomed. Opt.* **14**(6), 064030 (2009).

12. F. Robles, R. N. Graf, and A. Wax, "Dual window method for processing spectroscopic optical coherence tomography signals with simultaneously high spectral and temporal resolution," *Opt. Express* **17**(8), 6799–6812 (2009).
13. M. Wojtkowski, R. Leitgeb, A. Kowalczyk, T. Bajraszewski, and A. F. Fercher, "In vivo human retinal imaging by Fourier domain optical coherence tomography," *J. Biomed. Opt.* **7**(3), 457–463 (2002).
14. F. E. Robles, and A. Wax, "Measuring morphological features using light-scattering spectroscopy and Fourier-domain low-coherence interferometry," *Opt. Lett.* **35**(3), 360–362 (2010).
15. B. S. Reddy, "Studies with the azoxymethane-rat preclinical model for assessing colon tumor development and chemoprevention," *Environ. Mol. Mutagen.* **44**(1), 26–35 (2004).
16. E. A. McLellan, and R. P. Bird, "Aberrant crypts: potential preneoplastic lesions in the murine colon," *Cancer Res.* **48**(21), 6187–6192 (1988).
17. R. N. Graf, W. J. Brown, and A. Wax, "Parallel frequency-domain optical coherence tomography scatter-mode imaging of the hamster cheek pouch using a thermal light source," *Opt. Lett.* **33**(12), 1285–1287 (2008).
18. A. Wax, C. H. Yang, R. R. Dasari, and M. S. Feld, "Measurement of angular distributions by use of low-coherence interferometry for light-scattering spectroscopy," *Opt. Lett.* **26**(6), 322–324 (2001).
19. Y. Zhu, N. G. Terry, and A. Wax, "Scanning fiber angle-resolved low coherence interferometry," *Opt. Lett.* **34**(20), 3196–3198 (2009).
20. A. M. Zysk, S. G. Adie, J. J. Armstrong, M. S. Leigh, A. Paduch, D. D. Sampson, F. T. Nguyen, and S. A. Boppart, "Needle-based refractive index measurement using low-coherence interferometry," *Opt. Lett.* **32**(4), 385–387 (2007).
21. R. S. Gurjar, V. Backman, L. T. Perelman, I. Georgakoudi, K. Badizadegan, I. Itzkan, R. R. Dasari, and M. S. Feld, "Imaging human epithelial properties with polarized light-scattering spectroscopy," *Nat. Med.* **7**(11), 1245–1248 (2001).
22. L. Perelman, V. Backman, M. Wallace, G. Zonios, R. Manoharan, A. Nusrat, S. Shields, M. Seiler, C. Lima, T. Hamano, I. Itzkan, J. Van Dam, J. Crawford, and M. Feld, "Observation of periodic fine structure in reflectance from biological tissue: a new technique for measuring nuclear size distribution," *Phys. Rev. Lett.* **80**(3), 627–630 (1998).
23. H. C. Van de Hulst, *Light Scattering by Small Particles* (Dover, 1957).
24. W. Choi, C. Fang-Yen, K. Badizadegan, S. Oh, N. Lue, R. R. Dasari, and M. S. Feld, "Tomographic phase microscopy," *Nat. Methods* **4**(9), 717–719 (2007).
25. B. J. Braakhuis, M. P. Tabor, J. A. Kummer, C. R. Leemans, and R. H. Brakenhoff, "A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications," *Cancer Res.* **63**(8), 1727–1730 (2003).

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer death in men and women in the United States [1]. As is commonly known, the most successful practice for preventing cancer mortality is to regularly screen people at risk. This is particularly important for CRC since the disease is largely asymptomatic until it has reached an advanced stage; fortunately, if diagnosed early, the survival rate dramatically improves.

Today, the gold standard for screening CRC is conventional colonoscopy, which relies on visual inspection through an endoscope to detect polyps and adenomas. Once identified, the decision to remove these mucosal growths is based on size, where it is recommended that lesions >5 mm in diameter be removed [2]. This approach, however, suffers from serious weaknesses: 1. There is no reliable metric for determining whether lesions are adenomatous or metaplastic; hence, the decision to remove these lesions is left to the discretion of the physician. Note that approximately 90% of all cases of CRC originate through benign adenomas [2]. 2. Despite the fact that small lesions (<5 mm) are not typically removed, some studies have presented evidence that these are very likely to contain neoplasias, particularly for lesions proximal to the left colon [3]. 3. Flat adenomas, which are ten times more likely to contain malignancy compared to similarly sized polyps, appear similar to the surrounding tissue, and are consequently very difficult to detect with colonoscopy [4]. 4. Because all detected polyps are considered adenomatous [2], many unnecessary biopsies and polypectomies are performed, which increase the probability of complications [5]. Lastly, while other screening tests are available, including fecal occult blood test, sigmoidoscopy, and virtual colonoscopy, these are more limited and less effective; further, in the event that an abnormality is detected with these alternative screening tests, patients must then undergo a colonoscopy [6].

The weaknesses of colonoscopy, as described above, highlight the need for technologies that assess tissue health quantitatively and in a minimally invasive manner. To this end, biomedical optics has emerged as a promising field, in which various techniques have been developed to probe different biomarkers accessible via optical absorption and/or scattering measurements. For example, 4-dimensional elastically scattered light fingerprinting (4D ELF) [7] and diffuse reflectance spectroscopy [8] have been able to quantify tissue hemoglobin concentration as a surrogate biomarker for malignancy. Further, low-coherence enhanced backscattering spectroscopy (LEBS) [9] and angle-resolved low coherence interferometry [10] have retrieved information regarding nano- and micro- tissue morphology, thus providing insight to precancerous states.

In this paper, we present an application of an emerging optical technique, namely Fourier domain low coherence interferometry (fLCI), to measure early CRC changes using an analysis of *ex-vivo* tissues drawn from the azoxymethane (AOM) rat carcinogenesis model. fLCI measures oscillatory features in depth resolved spectra, also known as local oscillations, which result from coherent fields induced by the scattering by the front and back surfaces of cell nuclei in tissue [11]. Thus, fLCI uses nuclear morphology as a biomarker of disease, making it sensitive to the earliest stages of precancerous development. To achieve depth resolved spectroscopic analysis, we use the dual window (DW) method, which obtains simultaneously high spectral and depth resolution, and yields access to the local oscillations [12]. Further, fLCI signals can be processed to yield cross sectional images of samples, as in Fourier domain optical coherence tomography (FD-OCT) [13], thereby enabling co-registration of the structural information with the spectroscopic analysis. The capabilities of fLCI using the DW method have been demonstrated using scattering phantoms [14] and *ex-vivo* samples from a hamster cheek pouch model [11]. Here, we use fLCI to provide a spatially resolved, functional analysis of *ex-vivo* tissue samples at three depths and along two different sections of the left colon to demonstrate fLCI's ability to detect early CRC development.

2. Materials and methods

2.1 Animal model

This study used the AOM rat carcinogenesis model, a well characterized and established model for colon cancer research and drug development [15]. The cancerous progression of this model is similar to that seen in humans and is a good surrogate for human colon cancer development. In addition, the short induction period and high incidence of aberrant crypt foci (ACF), which are preneoplastic lesions [16], make this model a practical choice for testing the ability of fLCI to detect precancerous development in the colon.

All animal experimental protocols were approved by Institutional Animal Care and Use Committee of The Hamner Institute and Duke University. Forty F344 rats (six-week old, male; Charles River Laboratories Inc., Kingston, NY) were housed in The Hamner's animal facility for a 10-day acclimation period prior to any testing. All the animals were provided with a regular National Institutes of Health-07 diet (Ziegler Brothers, Gardners, PA) for the first 4 days of acclimation. Thereafter, the diet was switched to the pellet form of American Institute of Nutrition (AIN)-76A (Dyets Inc., Bethlehem, PA) and continued for the rest of study period. Two animals per cage were housed in polycarbonate, solid-bottom cages with Alpha-dry bedding in an animal room with a 12-hr light/dark cycle. Cages were changed twice a week. Pelleted, semipurified AIN-76A diet and water were available *ad libitum*. Weekly body weights were collected during the whole study period, and clinical observations were performed to monitor the health of the animals.

After 10 days of acclimation, the 40 rats were randomized into groups of 10. Thirty animals received intraperitoneal (IP) injections of AOM >90% pure with a molar concentration of 13.4 M (Sigma, St. Louis MO) at a dose level of 15 mg/kg body weight, once

per week, for 2 consecutive weeks (2 doses per animal). The remaining ten animals received saline by IP and served as the control group. At 4, 8, and 12 weeks after the completion of the dosing regimen, the animals (10 AOM-treated and 3 or 4 saline-treated rats per time point) were sacrificed by CO₂ asphyxiation. The colon tissues were harvested, opened longitudinally, and washed with saline. Then, the tissues were split into 4-5 different segments, each with a length of 3-4 cm. Only the two most distal segments of the colon were analyzed for these experiments: the distal left colon (LC) and proximal LC. Then, the samples were placed on a cover glass for examination with the parallel frequency domain OCT system (see Section 2.2). Finally, the tissue samples were fixed in formalin and stained with methylene blue in order to be scored based on the number of ACF, which are defined as foci containing more than two aberrant crypts. Figure 1 shows an image of a stained tissue sample, 4 weeks post treatment with three ACF that contain 2, 3, and 4 aberrant crypts.

Fig. 1. Stained tissue sample, 4 weeks post treatment with three ACF containing 2, 3, and 4 aberrant crypts.

2.2 Detection system

The system used is a parallel frequency domain OCT (pfdOCT) system [17], which consists of a Michelson interferometer geometry with the addition of four lenses that form a 4-F interferometer [18]. Using lenses L2 and L3 as seen in Fig. 2, the multimode fiber-coupled light from a Xe-arc lamp (150 W, Newport Oriel, Stratford, Connecticut) is collimated onto a sample. The samples are placed atop a #0 cover glass, which is tilted slightly to avoid saturation from specular reflection by the glass-air interface and thus allowing detection of only the scattered light. This is known as *scatter mode imaging*. For the *ex-vivo* colon tissue, the lumen side was placed facing down (against the cover glass), since the light illuminates from below the sample as seen in the inset of Fig. 2. Then, using L3 and L5, light scattered from a sample is imaged onto the entrance slit of an imaging spectrograph (SP2156, Princeton Instruments, Trenton, NJ). The reference arm follows a similar optical path, with lenses L2 and L4, and L4 and L5. After light is dispersed into its wavelength components by the spectrograph, the interference between the sample and reference fields is recorded using a CCD camera (Pixis 400, Princeton Instruments, Trenton, NJ). Detection is centered about 600 nm with a bandwidth of 240 nm. This configuration allows for 201 interferograms to be collected simultaneously (limited by the beam width), yielding B-mode OCT images from a single exposure.

For this particular configuration, the system underwent slight modifications compared to previous system implementations reported in [11,14,17]. First, a 2X magnification of the sample field at the spectrometer slit was achieved by setting the focal length of L3 and L4 equal to 50 mm, and that of L2 and L5 equal to 100 mm; with a pixel size of 20 μ m, this resulted in a lateral resolution of 10 μ m. The use of shorter focal length lenses also allowed for the total footprint of the system to be reduced, ultimately allowing the system to be made portable. Portability is achieved by placing the system inside a 8"X18"X24" custom made

aluminum alloy box atop a heavy-duty stainless steel utility cart for transportation to on-site analysis of tissue samples.

Fig. 2. Parallel frequency domain OCT system operating in *scatter mode*.

2.3 Data processing

The fLCI process for assessing cell nuclei diameter involves multiple steps. The first step is to obtain OCT images of the samples. Next, spatially resolved spectra are calculated using the DW method. Then, the spatial information provided by the OCT images is used to co-register the spectroscopic information; this allows for the spectra to be consistently analyzed at specific tissue depths. Finally, spectra from specific regions within the tissues are averaged to yield spectral oscillations that reveal cell nuclear diameters. In this section we provide a detailed procedure of these steps.

To obtain OCT images, the initial step is to digitally remove the DC background from the interferometric signal using separate acquisitions of the sample arm, reference arm, and dark signal. Then, the interferometric data are normalized by the reference arm intensity to remove any spectral dependence originating from the source and detector efficiency. The interferograms are then resampled from wavelength to a linear wavenumber vector ($k = 2\pi/\lambda$), and digitally corrected for chromatic dispersion [19]. Subsequently, the signals are Fourier transformed to obtain OCT images with an axial resolution of $\sim 1.10 \mu\text{m}$ (experimental). A refractive index (RI) of $n = 1.38$ is used to convert the optical path length to physical axial distance in tissue [20]. Figure 3 illustrates a representative image of an *ex-vivo* rat colon sample.

Fig. 3. pfdOCT image of an *ex-vivo* rat colon sample. The red line delineates an example region that is averaged across to determine the nuclear diameter.

To obtain depth-resolved spectroscopic information, the DW method is used [12]. As illustrated in Fig. 4, the method consists of multiplying two STFTs that operate on each interferogram. A STFT is implemented by sweeping a window across the interferometric data

while simultaneously taking a Fourier transform at each step, thus giving a map of the spectral content confined within a spatial (or axial) region. These maps are known as time-frequency distributions (TFDs). However, TFDs obtained using a single STFT suffer from an inherent trade-off between the resulting spectral and spatial resolutions. The DW method, on the other hand, utilizes the high spectral resolution of a STFT using a narrow window, and the high spatial resolution of a STFT using a wide window to avoid the deleterious effects of the time-frequency trade-off [12]. Here, Gaussian windows were used with standard deviations $w_1 = 0.029 \mu\text{m}^{-1}$ and $w_2 = 0.804 \mu\text{m}^{-1}$, resulting in TFDs with an axial resolution of $3.45 \mu\text{m}$ and spectral resolution of 1.66 nm . Note that this process is conducted for each A-scan, thus giving a spectrum for each point in an OCT image.

The last step to obtaining spectral information from specific tissue depths (i.e., local oscillations) is to co-register the OCT images with the DW TFDs. This process involves using the images to identify the contour of the tissue surfaces and calibrate the analysis relative to this 'zero' depth. Note that if a surface is not clearly discernable at any particular A-scan, no further analysis is conducted there. With this information, the DW TFDs can be properly aligned and thus consistently provide spectral information from specific tissue depths.

Fig. 4. DW processing method to obtain TFDs with simultaneously high spectral and spatial resolution. Two STFTs, one obtained with a narrow window and another with a wide window, are multiplied together to obtain the DW TFD. Gaussian windows were used with standard deviations $w_1 = 0.029 \mu\text{m}^{-1}$ and $w_2 = 0.804 \mu\text{m}^{-1}$, resulting in TFDs with an axial resolution of $3.45 \mu\text{m}$ and spectral resolution of 1.66 nm .

Once the spectra are properly aligned, regions of interest, both laterally and axially, are identified and averaged in order to provide sufficient signal-to-noise ratio for the spectral analysis that follows. In the lateral direction, twenty DW TFDs are averaged to yield 10 different lateral segments in each OCT image. Note that in previous studies all TFDs in an image were averaged [11]; thus, the analysis provided here produces a 10-fold increase of the spatial information. In the axial direction, we calculate the spectral averages of $25 \mu\text{m}$ depth segments from three different sections: at the surface (surface section $0\text{--}25 \mu\text{m}$), centered about $35 \mu\text{m}$ in depth (mid section, $22.5\text{--}47.5 \mu\text{m}$), and centered about $50 \mu\text{m}$ in depth (low section $37.5\text{--}62.5 \mu\text{m}$). The area inside the red dotted line in Fig. 3 gives an example of a resulting mid section from which the spectra are averaged to determine the nuclear diameter.

The spectra from the averaged regions contain two components. The first component is the low frequency oscillations that have been associated with the periodic fine structures induced by spherical scatterers, which have been analyzed previously using the van de Hulst approximation in light scattering spectroscopy (LSS) [14,21–23]. The approximation gives an analytical solution to the scattering cross section of spherical scatterers, which tells us that the periodicity of the spectral oscillations depends on size, as well as on the ratio between the RI of the scatterer and surrounding medium [23]. This ultimately results in relatively low frequency oscillations. However, we have found that due to the lack of knowledge of the

precise RI of the scatterer and the surrounding medium [24], the amount of useful information that can be extracted from the LSS method is limited. Therefore, the low frequency oscillations are isolated using a smoothing function in MATLAB (Mathworks, Natick, Massachusetts) and subsequently removed from the spectra. This process isolates the second component: the high frequency oscillations of the spectra, which correspond to the local oscillations resulting from coherent fields induced by the cell nuclei in the averaged region. Unlike the periodic fine structures in LSS, the local oscillations only depend on the size and RI of the scatterer, thus resulting in higher frequencies. Specifically, the periodicity of the local oscillations is given by the sample field's round trip optical path length (ΔOPL) through the scatterer, and is related to the scatter size (in this case, d_c) by $d_c = \Delta OPL / (2n_n)$, where n_n is the RI of the cell nuclei. Figure 5(a) illustrates the average spectrum (solid blue line) along with the isolated low frequency component (dotted black line) for the averaged region shown in Fig. 3. Figure 5(b) shows the resulting local oscillations.

Finally, a Fourier transform of the local spectral oscillations is taken to produce a correlation function, where we attribute the peak in this function to indicate the average cell nuclear diameter in the region of analysis [11]. Other scatterers, such as other cellular organelles and nuclear content, may also produce peaks in this function, but due to their random orientation, size, and spacing with one another, the resulting signal is unlikely to produce a peak greater in magnitude than that of the average cell nuclear diameter. The correlation function for the local oscillations in Fig. 5(b) is shown in Fig. 5(c), where the correlation distance (d_c) has been properly scaled to account for the round trip optical path length and the RI of the cell nuclei. We assumed a constant nuclear RI of $n_n = 1.395$ for this analysis [20]. As a last step, the peak detection process is automated to enable analysis of large data sets. To achieve this, the correlation function is subject to further processing, where the $1/f$ noise is removed using a smoothing function. Then, only maxima that are 3.5 standard deviations above the mean of the correlation function are considered to be clear peaks. If this criterion is not met at any particular region, the measurement is discarded.

Fig. 5. Average spectrum (blue solid line) from the delineated region in Fig. 3, along with low frequency component (black dotted line) (a). The low frequency component is subtracted from the averaged spectrum to obtain the local oscillations (b). A Fourier transform yields a correlation function (c), where the peak corresponds to an average cell nuclear diameter of 7.88 μm in the region of analysis.

3. Results

3.1 Depth sections

The nuclear diameters from the three different tissue depth sections and for all time points are summarized in Fig. 6 and Table 1. We note that the control group measurements of all the time points were combined, since no statistically significant differences were found between them. Statistical tests were conducted using a two-sided student t-test.

Fig. 6. Nuclear diameter by depth sections. The mid section (35 μm in depth) provided the most significant results, with $p\text{-values} < 10^{-4}$ ** for the treated samples at all time points when compared to the control group. No statistical significance was found at the surface, and mildly significant differences ($p\text{-values} < 0.05$ *) were found at the low (50 μm) section.

As shown in Fig. 6, the mid section (35 μm depth) provided the most significant results, where the treated groups at all three time points yielded $p\text{-values} < 10^{-4}$ ** when compared to the control group. The fLCI measurement for the control group at the mid section yielded an average cell nuclear diameter of 5.15 ± 0.05 μm , while for the treated groups it was found to be 5.91 ± 0.15 μm , 6.02 ± 0.18 μm , and 6.49 ± 0.49 μm at 4, 8, and 12 weeks after treatment, respectively. For the deepest (low, 50 μm depth) section, mildly statistically significant results were observed, with $p\text{-values} < 0.05$ *. At the surface, however, no statistical significance was found.

Table 1. Measured cell nuclear diameter by depth sections

	Surface (mean+/-SEM)	Mid (mean+/- SEM)	Low (mean+/- SEM)
Control	5.88+/- 0.20	5.15+/-0.05	5.25+/-0.05
Week 4	5.96 +/- 0.18	5.91+/-0.15**	5.84+/-0.16*
Week 8	6.36 +/- 0.21	6.02+/-0.18**	5.97+/-0.18*
Week 12	5.48 +/- 0.33	6.49+/-0.49**	5.95+/-0.47*
All measurements in μm ; $p\text{-values} < 10^{-4}$ **, $p\text{-values} < 0.05$ *, $N = 10$.			

3.2 Length segments

The two tissue segments (proximal and distal left colon) were further analyzed separately for the mid depth section. The measured cell nuclear diameters and number of ACF are summarized in Table 2. We found that for all the time points, and for both segments, the measured nuclear diameters for the treated groups were significantly different from the control group ($p\text{-values} < 10^{-4}$ **).

The results are also summarized in Figs. 7(a) and 7(b). Note that significant differences were observed for both segments after only 4 weeks post treatment. The measured increase in the nuclear diameter, however, remained relatively constant thereafter, with the exception of the last time point in the proximal LC. Here, the nuclear diameter increased dramatically from ~ 6.0 μm to ~ 7.2 μm . To investigate this further, Fig. 7(c) plots the nuclear diameter as a function of the average number of ACF, which are preneoplastic lesions. For clarity, we also identify each point with its corresponding time period. Note that the formation of ACF was faster in the proximal LC compared to the distal LC, and that the plot shows a region of little nuclear morphological change after the initial formation of ACF. This plateau region is present in both sections and is initially independent of the number of ACF. However, once the number of ACF increased to the maximum value observed in this study (~ 70), the measured

increase of the nuclear diameter was specific to the region manifesting more advanced neoplastic development, in contrast to the ubiquitous and relatively constant cell nuclear diameter measurements of the plateau region.

Table 2. Measured cell nuclear diameter (fLCI measurement) and number of ACF by length segments

	Proximal LC (mean+/-SEM)		Distal LC (mean+/-SEM)	
	fLCI [μm]	ACF	fLCI [μm]	ACF
Control	5.15+/-0.05	0	5.15+/-0.05	0
Week 4	5.87+/-0.19	13.4+/-3.64	5.98+/-0.26	5.9+/-1.33
Week 8	5.88+/-0.27	55.1+/-5.60	6.14+/-0.24	16+/-1.71
Week 12	7.23+/-1.21	68.6+/-4.74	6.10+/-0.39	33.3+/-5.95

all p -values $< 10^{-4}$ **, $N = 10$.

Fig. 7. Results by colon length segments. Highly statistical differences (p -value $< 10^{-4}$ **) were observed between the control group and treated groups for the proximal LC (a) and distal LC (b). (c) Plots the measured cell nuclear diameter as a function of the number of ACF. For clarity, the time of measurement is noted next to each point (wk = week).

4. Discussion

The results, presented in Section 3.1, highlight the importance of obtaining spatially resolved information for assessing tissue health. Other optical methods have also demonstrated the need for depth selectivity, but the specific depth that provides the most diagnostic information has varied. Using LEBS, which assesses changes in tissue nano-architecture, it was found that a penetration depth of 70 μm yielded the most significant results [9]; whereas using 4D ELF to measure hemoglobin concentrations, a penetration depth of 100 μm was found to yield significant results [7]. With these optical methods, however, useful information is obtained by integrating to a particular depth, rather than sampling specific locations, which may explain the differences. In contrast, fLCI is an interferometric technique that uses a broadband source, and thus enables the coherence gating imaging capabilities of OCT and allows sampling of specific points in three-dimensional space. Image guidance was vital in this study in order to identify the tissue surface and probe specific tissue depths.

Along with the imaging capabilities of fLCI, the DW method is an equally important feature to enable this study. The DW method avoids the spectral and spatial resolution trade-off that has hindered quantification using STFTs or continuous wavelet transforms. Acquisition of the local oscillations necessitates high resolution in both dimensions, otherwise the phenomenon of fringe washout, resulting from phase and frequency differences from

different scattering nuclei, would obscure the local oscillations from which the cell nuclear diameter is assessed.

In Section 3.2, the results were analyzed by segments along the length of the colon. Here, fLCI detected significant changes in segments and at time points that presented early evidence of preneoplastic development, underscoring the sensitivity of the method. Further, the measured early nuclear morphological change was observed in both segments and independently of the number of ACF, which suggests a ubiquitous micromorphological change of the colon. This, however, was not the case when neoplastic development became more advanced (demarcated by the high number ACF); at which point, the nuclear diameter increase was specific to the affected region. These sets of results present significant findings: First, these results suggest that fLCI may be able to detect the “field effect” of carcinogenesis. This phenomenon describes observations that neoplastic development in one part of the colon distorts nano- and micro- tissue morphology, as well as tissue function, along the entire organ. This has been a subject of much interest since it indicates that adequate screening may be achieved by only probing certain (and more readily accessible) sections of the colon [7,9,25]. These results also indicate that fLCI can identify specific regions where more advanced neoplastic development has occurred, which is paramount for detecting CRC development and initiating a localized therapy.

While the results presented here are very promising, there are certain limitations that still need to be explored in order to take advantage of all the information provided by the method. As described in Section 2.3, the procedure for obtaining fLCI measurements assumes a constant RI value for the cell nuclei, and a different constant value for the bulk tissue; however, it is known that the RI can vary depending on tissue type and tissue health. Thus, these variations, which are currently not assessed with our method, may be introducing an additional degree of uncertainty in the calculated nuclear diameters. Further, these variations have hindered our ability to use the low frequency oscillations with LSS, as we have done previously using tissue phantoms [14]. However, we believe that a more rigorous treatment of the LSS fitting algorithm may provide insight to the variations of the RI in future analyses.

5. Conclusion

In this study, we have used an AOM-treated rat model to demonstrate the ability of fLCI to quantitatively distinguish between *ex-vivo* colon tissue that is normal and that which exhibits early precancerous development. The results show highly statistically significant differences between the AOM-treated and control group samples. Further, the results suggest that fLCI may be able to detect changes due to the field effect of carcinogenesis, in addition to identifying areas where more advanced neoplastic development has occurred. Future work will be directed towards developing an optical fiber based pfdOCT system to demonstrate non-invasive, *in-vivo* early CRC detection.

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