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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Evidence from osteoarthritis (OA) studies suggests that there is a narrow time window in the early stages of the disease when cartilage can be functionally restored to reduce further degeneration. Small internal cartilage damages due to traumatic joint injuries are hard to detect with the traditional imaging technologies but pose a significant risk of inducing OA later. Our goal is to develop a non-destructive and label-free combination of optical coherence tomography (OCT) based methods for early detection of PTOA by assessment of mechanical strength, which is dependent upon both GAG and collagen, through our novel method for optical coherence elastography (OCE) based on fringe washout, and of collagen by itself through polarization-sensitive OCT. This reporting period has been the first to be uninterrupted by COVID-19-related delays. This has allowed us, for the first time to our knowledge, to track the progression of early stages of PTOA in an animal model using non-destructive optical imaging without the need for exogenous contrast agents.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

1. INTRODUCTION

Evidence from osteoarthritis (OA) studies suggests that there is a narrow time window in the early stages of the disease when cartilage can be functionally restored to reduce further degeneration. These studies collectively demonstrate the importance of early detection of OA to enhance the effectiveness of subsequent therapies. However, current technologies, including arthroscopy, X-ray radiography, and MRI, can detect OA only after significant and irreversible damage to articular cartilage has already occurred. Small internal cartilage damages due to traumatic joint injuries are hard to detect with the traditional imaging technologies but pose a significant risk of inducing OA later. Therefore, it is essential to develop tomographic imaging tools with high resolution that can provide direct assessment of intra-cartilaginous damage in individual patients at the earliest stages of PTOA. We believe that high-resolution assessment of not only the surface, but also the interior portions of cartilage will allow for detection of OA at a much earlier time point, thus providing an opportunity to prevent the progression of or even to allow for repair of cartilage damage and to guide therapies to where they are most effective for existing damage. To take advantage of this therapeutic window, our goal is to develop a non-destructive and label- free combination of optical coherence tomography (OCT) based methods for early detection of PTOA. Cartilage damage at the early stages of OA is known to heterogeneously alter local density/mechanical properties of extracellular matrix (ECM). The mechanical properties of cartilage derive from extracellular matrix components of glycosaminoglycan (GAG) and collagen, with the density and organization of both known to change early in PTOA development. Several investigators have previously established PS-OCT as a method for quantifying localized changes in collagen, but this method is not sensitive to glycosaminoglycan (GAG), which also significantly contributes to the mechanical properties of cartilage. A smaller number of separate studies have investigated the use of optical coherence elastography (OCE) to examine the overall mechanical properties of cartilage, but these techniques cannot provide volumetric quantification in real time. Our novel method for OCE takes advantage of fringe washout, an artifact related to motion during the acquisition time of spectral domain OCT systems, and can be used to rapidly scan volumes of tissue in combination with PS-OCT. Our hypothesis is that robust and sensitive detection of early PTOA can be achieved by utilizing OCE to quantify subsurface damages in GAG loss and its associated cartilage swelling when complemented by quantification of localized changes in collagen content/disorganization with PS-OCT. This project has three aims: 1. optimize fringe washout based OCE, 2. optically quantify GAG in cartilage, and 3. identify optical signatures of cartilage degeneration in early stages of a rat PTOA model. Completion of these aims will provide the first optical method capable of complete non-destructive assessment of sub-surface cartilage degeneration for PTOA diagnosis.

2. KEYWORDS

post-traumatic osteoarthritis, early detection, cartilage, glycosaminoglycan, collagen
mechanical strength, Young's modulus
optical imaging, optical coherence tomography, optical coherence elastography, polarization-sensitive

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1: optimize fringe washout based optical coherence elastography

- Major task 1: relative delay optimization
  - Milestone: autocalibration of optimized PS-OCT/OCT imaging system (month 4)
    - Progress: *100%, with improvements made to imaging protocol since last report*
- Major task 2: characterization of sensitivity and resolution
  - Milestone: optimized OCE acquisition parameters based on imaging scenario with expected sensitivity and resolution based on sample size and composition (month 7)
    - Progress: *100%, with improvements made to imaging protocol since last report*

Specific Aim 2: optically quantify GAG in cartilage

- Major task 1: bovine cartilage explant experimentation and analysis

- Milestone: calibrated determination of mechanical and biochemical properties of cartilage based on optical imaging (month 10)
  - Progress: 90%

Specific Aim 3: identify optical signatures of cartilage degeneration in early stages of a rat PTOA model

- Major task 1: early rat OA model experimentation and analysis
  - Milestone: obtain ACURO approval, obtain UCR IACUC approval of protocol amendment (month 5)
    - Progress: *100% (previously 100%) (renewal of protocol submitted and approved by UCR IACUC)*
  - Milestone: identification of early PTOA based on optical assessment of changes in cartilage (month 18)
    - Progress: 90%

What was accomplished under these goals?

Optical coherence elastography (OCE)

All methods for optical coherence elastography (OCE) rely on comparison of OCT images acquired with and without some form of mechanical perturbation. At the end of our last Annual Report, we discussed our final selection to use a cylindrical tube transducer (Figure 6 a) to generate a closed and predictable ultrasound field. Our particular transducer (Physik Instrumente, Karlsruhe, Germany) is based on a cylindrical piezo-tube with an experiment-determined resonant frequency of 2.207 MHz to sub-micron level movement within a sample. In basic principle, the magnitude of the ultrasound-induced movement is inversely related to the local Young's modulus however, the ability to accurately quantify the local modulus of tissue had previously been a problem. The textbook definition of Young's modulus is the ratio of stress to strain, where stress is governed by the applied force per unit area and strain is governed by the magnitude of the effect of that force. The magnitude of fringe washout can be directly related to strain. However, accurate assessment of the relative Young's modulus of tissue then requires reliable information about the local stress applied to tissue. This is where previous transducer geometries proved problematic; the ultrasound field was experimentally determined to be overly prone to significant enough deviations that, while we have been consistently capable of measuring strain through fringe washout, our derived quantification of the Young's modulus had been overly error prone.

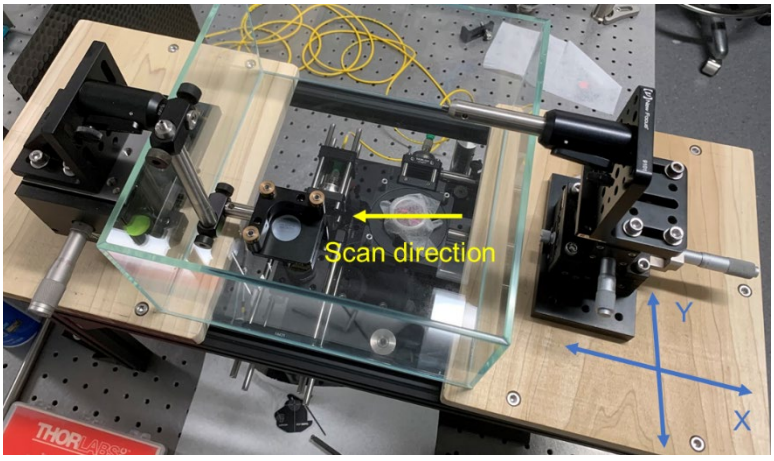

Figure 1: picture of the revised sample arm and reference arm configuration based around the use of a piezoelectric ultrasound transducer with a cylindrical geometry.

However, we have done extensive computational and experimental testing and characterization and found the area over which the ultrasound field (i.e., stress) within all of our tested samples using a cylindrical transducer have been predictable and consistent. We have modified the same 1310 nm OCT imaging engine described in previous reports with a new sample (and corresponding reference arm configuration) to allow for both OCE and PS-OCT imaging (Figure 1). The imaging beam is currently introduced from the bottom of an acrylic water tank in order to remove artifact caused by a moving water surface, and a reference beam is introduced into the same tank in order to minimize dispersion mismatch between the two arms.

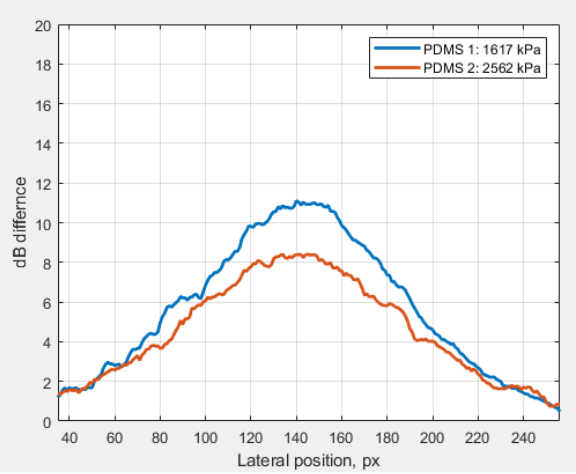

Figure 2: fringe washout as functions of lateral position for two homogeneous PDMS phantoms with different Young's moduli.

Figure 2 shows examples of characterization of the ultrasound-induced stress profile. By imaging homogenous samples (i.e., constant Young's modulus), our derived images of strain based on cross-sectional images of fringe washout provide us with characterization of the stress field. We characterized this for homogeneous samples of differing Young's moduli (Figure 2) in order to characterize the lateral area (150 μm diameter) over which we experimentally confirmed

no significant asymmetries of the stress field based on sample position within the enclosed transducer geometry (i.e., consistent stress profile).

We then developed the following optimized imaging protocol for fringe washout-based OCE. The OCE images are acquired with a lab-developed spectral-domain PS-OCT system with a center wavelength of 1310 nm (the same OCT system as used for polarization-sensitive image acquisition). A 2.2 MHz cylindrical ultrasonic transducer is incorporated into the OCT system to provide synchronized external mechanical excitation for OCE imaging. An acquisition line rate of 4.435 kHz with camera exposure time of 221.5 μs was selected based on the characteristics of the transducer in response to electrical driving signal. During OCE imaging, a group of four A-scans is acquired at the same lateral scan location governed by a MEMS mirror scanner, and a burst trigger is sent to the ultrasound-driving system in the beginning of the A-scan group to generate a 450 μs sinusoidal ultrasound pulse (which covers the acquisition time of the first two A-scans in the group). A B-scan of cross-sectional image is achieved by acquiring 256 four-A-scan groups at successive lateral locations.

To obtain the fringe washout OCE information, the B-scan image is divided into four subsets of images based on the A-scan indices within each group. The subset with the highest amount of fringe washout is used as the image with ultrasound excitation while the subset with no ultrasound-induced fringe washout is used as a reference image that indicates the stationary state of the sample. An averaging filter with a kernel size of 32 by 8 (width by height) is applied to each subset image to reduce the effect of speckle noise. The OCE image can then be processed by subtracting the decibel-scale subset with fringe washout (lower intensity) from the one without (higher intensity).

The ultrasound transducer provides an ultrasonic focus with a FWHM of ~150 μm as previously described. Scanning a region that covers the width of ultrasound focus can only yield sample mechanical properties information within a narrow lateral range because only the region within the focus receives sufficient and consistent magnitude of excitation. To achieve a cross-sectional OCE image that contains mechanical properties distribution in a wider range, a series of imaging sessions are performed by carefully moving the sample laterally with a step size of 100 μm between two consecutive sessions. There are 50 repeated B-scans in each session for averaging to improve OCE measurement accuracy, and a full view of fringe washout OCE image can be generated by stitching the image section at ultrasound focus across different imaging sessions. To correct the lateral non-uniformity of fringe washout OCE image, the distribution of relative ultrasound stress

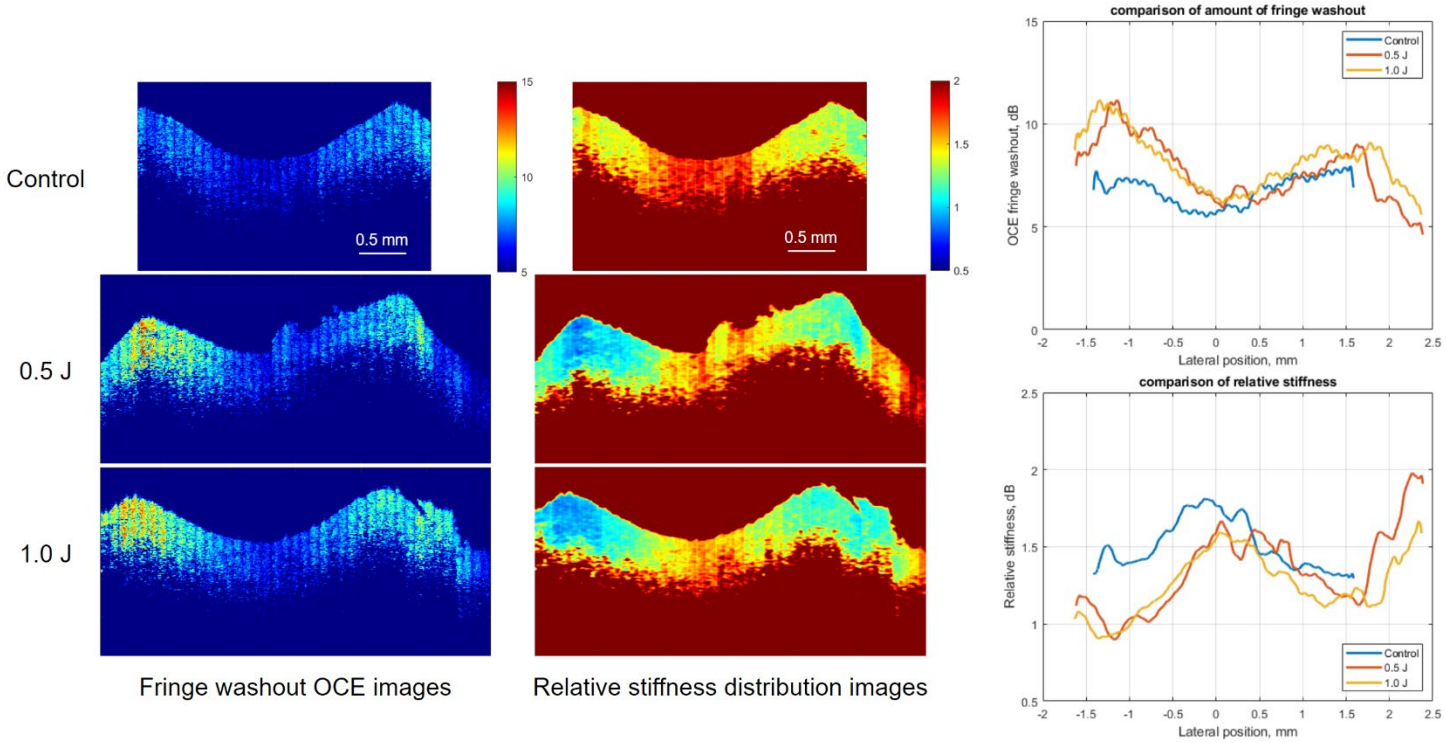

Figure 3: panel of OCE images and graphs from the patella groove of the femur from an animal model for PTOA. (left column): stitched cross-sectional images of the magnitude of fringe washout for a control sample (top row), from a rat subjected to a 0.5 J impact to the knee (middle row), and of a 1.0 J impact (bottom row). The impacted joint was excised 24 hour following impact. (middle column): corresponding cross-sectional images of the relative Young's modulus. (right column): quantification of fringe washout magnitude (top) and relative Young's modulus (bottom) for the three samples.

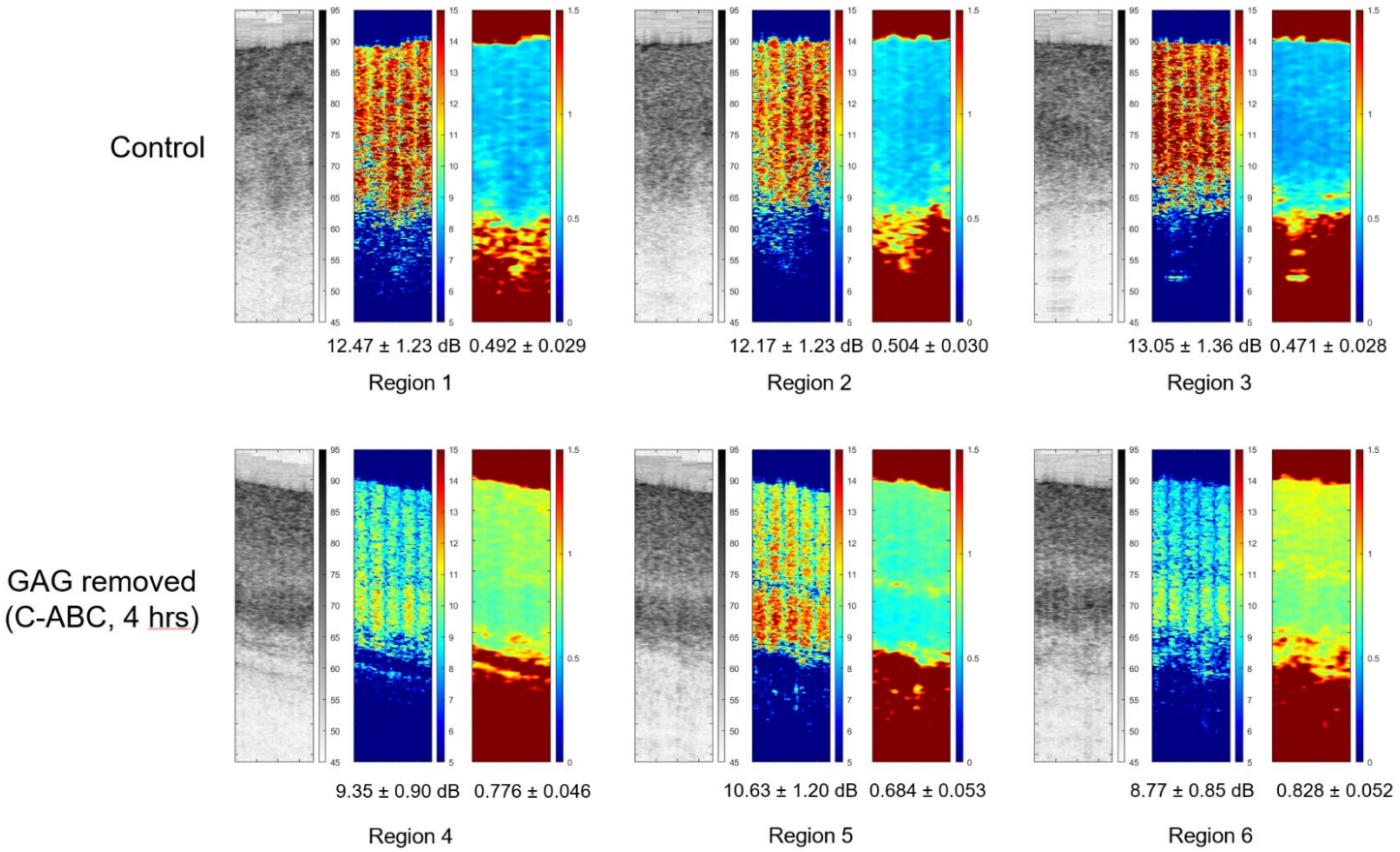

Figure 4: panels of OCE images for bovine cartilage samples. Each set is composed of cross-sectional intensity (left), fringe washout magnitude (middle), and relative mechanical strength (right). The upper row of data are from untreated bovine cartilage samples, and the lower row is of samples treated with C-ABC for 4 hours to remove GAG.

curve is pre-determined by performing OCE scanning on a piece of homogeneous elastic phantom with the same ultrasound and imaging settings. The map that shows relative sample stiffness distribution can then be calculated by dividing the relative stress curve by the stitched fringe washout OCE image. A 32 by 8 (width by height) averaging filter is applied to yield the final cross-sectional mechanical property map of the sample.

Figure 3 and 4 illustrate the results of OCE imaging from excised femurs harvested 24 hours following induction of an animal model for PTOA using a drop tower (as described in the application) and of bovine cartilage treated to remove GAG (also as described in the application). It can be readily observed that we can observe local maps of mechanical strength through up to ~500 μm beneath the surface of the ends of these femurs. Further, a loss of mechanical strength can be observed at the left and right peaks of the imaged portion at the end of the femurs in which damage was induced in comparison to the control sample. It can also be seen that the same degree of mechanical loss is not seen in the groove between these peaks. This is consistent with the initial increase in swelling due to hyper GAG secretion from chondrocytes in response to acute injury that is expected in this early phase after injury. OCE data obtained from bovine cartilage samples, some of which were treated with C-ABC to remove GAG, is shown in Figure 4. The results from this data is consistent with expectation; removal of GAG results in an increase in modulus. These sets of results demonstrate that our novel method for OCE is capable of detecting changes in modulus of cartilage known to occur in acute (a decrease due hyper GAG secretion and water swelling) and early stage (subsequent increase to GAG degradation) PTOA.

Polarization-sensitive OCT

We reported a modified PS-OCT imaging protocol to achieve reduced speckle artifact in the last report. This was essential due to the limited meaningful depth range for PS-OCT imaging of the cartilage is reporting period, we improved our PS-OCT image processing pipeline to reduce the effect of speckle which will enable us to localize and quantify any changes in cartilage due to PTOA in terms of both the minimum size and degree of change required for detection.

Figure 5 illustrates PS-OCT results acquired from the same control and 1 J samples from Figure 3. The cross-sectional images were processed with MATLAB to generate structural intensity and phase retardation

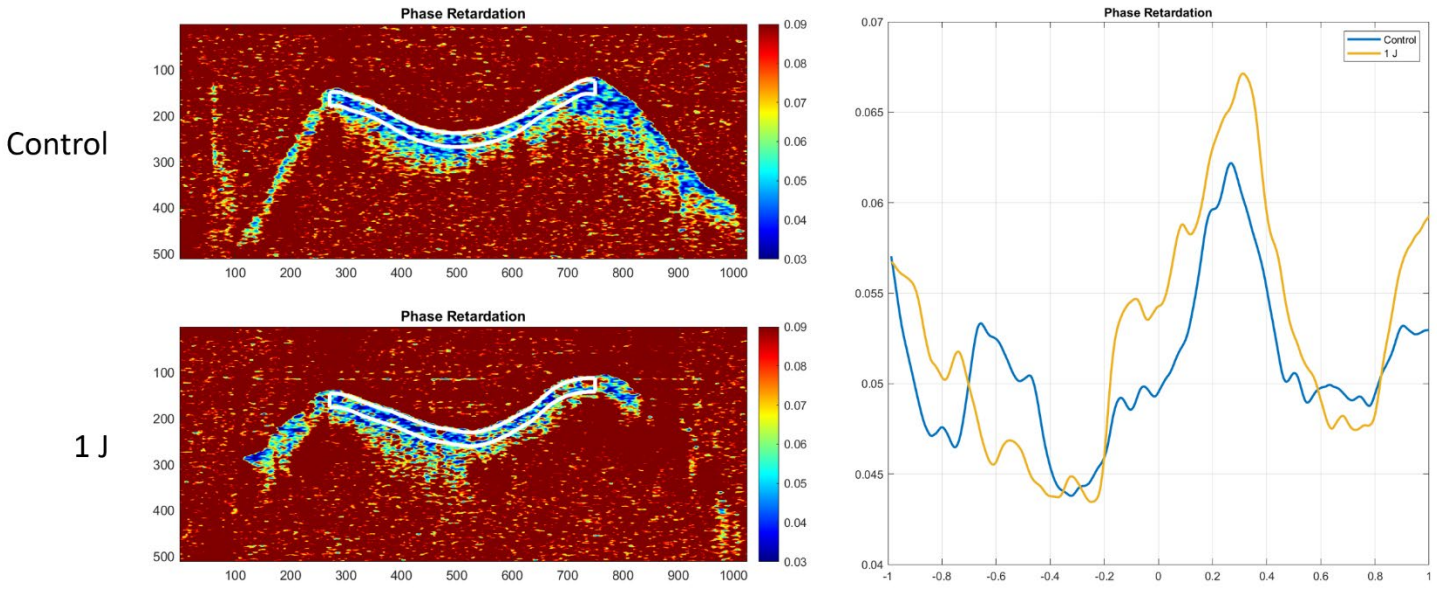

Figure 5

images. The structural intensity images were generated with standard Fourier domain processing method. The depth-resolved phase retardation images were generated through spectral binning. An averaging filter of size 3 × 8 pixels (height × width; corresponds with the system resolution) was used for reducing speckle. For quantitative comparison, the phase retardation at different lateral location between the two peaks were measured by averaging the local phase retardation of 30 axial pixels under the surface at each A-line.

Phase retardation is a measure of the cumulative path length difference incurred due to the birefringence (light polarization dependent differences in index of refraction), which, in biological tissue, stems from the density and organization of fibrous structures. The primary source of birefringence in cartilage is collagen, and so the PS-OCT shows that little difference in articular birefringence between the control and injured samples. This is consistent with our expectation at this early stage of PTOA, as a loss of collagen normally occurs at later stages of PTOA (after GAG loss). Further investigation will be performed to determine if the slight differences in birefringence observed here (and in the other samples that have yet to be fully analyzed) are found to be meaningful optical biomarkers of early stage changes in collagen even before widespread loss.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

During the last reporting period in which we finally had no COVID-19 related interruptions, we have been able to successfully (i.e., artifact-free) perform both PS-OCT and OCE imaging on both bovine and rat samples. During these last few months of the project period, we plan to complete submission of peer-reviewed manuscripts describing our work, which will necessitate completion of post-processing and analysis of imaging data obtained from the rest of our samples (increased samples and other time points during progress of PTOA) to determine statistical significance of the promising preliminary findings described here, histopathological analysis of our rat samples and more detailed correlation of histologically-derived parameters with those obtained through optical imaging.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
While a number of other groups have previously demonstrated the ability to separately perform PS-OCT or OCE imaging, we have successfully demonstrated, for the first time to our knowledge, the ability to obtain both modalities using the same imaging system in an animal model for PTOA. Moreover, most OCE methods are capable of assessing the mechanical properties of soft tissues but cannot do the same in cartilage and bone due to the difference of shear wave propagation between these tissue types; we have demonstrated the ability to overcome this hurdle by exploiting fringe washout instead of phase-based tracking. We further believe our unique combination of PS-OCT and OCE to more fully localize and quantify changes during early stages of PTOA will prove to have a great impact on the study of PTOA, as it will allow for non-destructive and wide-field assessment and early detection of PTOA onset and progression.

What was the impact on other disciplines?
Nothing to report.

What was the impact of technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES / PROBLEMS

Changes in approach and reasons for change
No changes to report.

Actual or anticipated problems or delays and actions or plans to resolve them
As previously mentioned, this was the first reporting period with no COVID-19-related interruptions and delays. We do not anticipate further interruptions or delays for the remainder of the project period.

Changes that had a significant impact on expenditures
No changes to report.

Significant changes in use or care of human subjects
N/A.
Significant changes in use or care of vertebrate animals
No changes to report.

Significant changes in use or biohazards and/or select agents
N/A.

6. PRODUCTS

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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Project Role:	PI
Research Identifier:	https://orcid.org/0000-0002-0282-7162
Nearest person month worked:	3
Contribution to project:	Supervision of overall project progress through weekly meetings

Name:	Jin Nam
Project Role:	PI
Research Identifier:	https://orcid.org/0000-0001-5117-8958
Nearest person month worked:	3
Contribution to project:	Supervision of overall project progress through weekly meetings

Name:	Junze Liu
Project Role:	Graduate student
Research Identifier:	https://orcid.org/0000-0001-7946-4833
Nearest person month worked:	12
Contribution to project:	Design and construction of OCE system; optimization of processing method to visualize tissue mechanical properties

Name:	Youyi Tai
Project Role:	Graduate student
Research Identifier:	https://orcid.org/0000-0002-2530-4225
Nearest person month worked:	12
Contribution to project:	Preparation of bovine explant samples, perform drop tower injuries, preparation of animal models

Name:	Thamidul Islam
Project Role:	Graduate student
Research Identifier:	https://orcid.org/0000-0002-8430-1104
Nearest person month worked:	12
Contribution to project:	Polarization-sensitive OCT data acquisition and analysis

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

There have been no changes to the active other support for the PD/PI(s).

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

N/A.

9. APPENDICES

N/A.