Assessing the mechanical properties of tissue-mimicking phantoms at different depths as an approach to measure biomechanical gradient of crystalline lens

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Abstract: We demonstrate the feasibility of using the dominant frequency of the sample surface response to a mechanical stimulation as an effective indicator for sensing the depthwise distribution of elastic properties in transparent layered phantom samples simulating the cortex and nucleus of the crystalline lens. Focused ultrasound waves are used to noninvasively interrogate the sample surface. A phase-sensitive optical coherence tomography system is utilized to capture the surface dynamics over time with nanometer scale sensitivity. Spectral analysis is performed on the sample surface response to ultrasound stimulation and the dominant frequency is calculated under particular loading parameters. Pilot experiments were conducted on homogeneous and layered tissue-mimicking phantoms. Results indicate that the mechanical layers located at different depths introduce different frequencies to the sample surface response, which are correlated with the depth-dependent elasticity of the sample. The duration and the frequency of the ultrasound excitation are also investigated for their influence on this spectrum-based detection. This noninvasive method may be potentially applied for localized and rapid assessment of the depth dependence of the mechanical properties of the crystalline lens.

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1. Introduction

The increase of the stiffness in crystalline lens with age has long been considered to cause presbyopia [1]. Recent studies in mechanical modeling and destructive testing of crystalline lens have suggested that the age-related change in the mechanical property gradient may play a key role in the loss of the accommodative ability of lens which leads to presbyopia [2, 3]. Specifically, it has been reported that with age, the stiffness of the nucleus of lens increases to an order of magnitude greater than that of the cortex [4]. Thus, noninvasive assessment of the relative elasticity of cortex and nucleus can be beneficial to further understanding the mechanism of accommodation and presbyopia.

Ultrasound elastography has been well established and extensively utilized in measuring and mapping the elasticity of soft tissues [5–7], including the crystalline lens. Recently, Yoon et al. applied a high pulse repetition frequency ultrasound system for the measurement of the lens elasticity with a pulsed-laser-induced micro-bubble [8]. Young’s modulus of ex vivo animal and human lenses can be estimated, and the capability of assessing the spatially-resolved variation of elasticity has been demonstrated [9, 10]. However, the need to create bubbles in the lens makes it an invasive method. Also, the relatively low spatial resolution of ultrasonic imaging may limit the in vivo applications of this approach.

Besides acoustic imaging techniques, Brillouin optical microscopy has recently been used to evaluate the elastic properties of the crystalline lens [11, 12]. This method applies Brillouin spectroscopy to measure the frequency shift of the scattered light from the interaction between photons and acoustic phonons. Through the combination with a confocal modality, Brillouin microscopy can provide three-dimensional mapping of the hypersonic acoustic properties in the crystalline lens, which can be well related to Young’s or shear modulus [13]. The use of Brillouin microscopy for the crystalline lens elastography provides several advantages, such as high spatial resolution and noncontact measurement. Recently, in vivo application to the human crystalline lens has been demonstrated [14]. However, the low SNR
of detecting the Brillouin scattering signal and the requirement of depthwise scanning result in long data acquisition time, which may limit the clinical applicability of this technique.

Optical coherence elastography (OCE) is an emerging nondestructive technique that can provide both comparative and quantitative elasticity mapping with high spatial resolution [15–17]. OCE generally relies on a loading method to interrogate the sample and an optical coherence tomography (OCT) system to capture and measure the induced sample deformation [18]. With the recent development of phase-resolved OCT techniques, tissue displacements in the nanometer scales can be measured [19, 20], which enables the detection of the low-amplitude deformation induced in delicate soft tissues, such as the cornea [21] and crystalline lens [22]. Current applications of OCE include tumor detection [23, 24], corneal elasticity assessment [25] and atherosclerotic plaque characterization [26]. However, to the best of our knowledge, there have been no reports on the use of OCE for the detection of depth-dependent elasticity in the crystalline lens. Several special features of the crystalline lens, such as the location and the transparency, may be the factors that make it challenging to perform elastographic measurement using the current OCE methods. The crystalline lens is located within the eye globe, which makes it difficult to apply the general contact loading approach with piezoelectric transducers [27, 28]. Also, because the crystalline lens is transparent to both light and ultrasound, only surface motion is available for measurements.

To address these issues, we have developed a system combining ultrasound excitation and OCT measurement to noninvasively assess the dominant frequency of the sample surface dynamics for rapid detection of the depth-dependent elasticity that is suitable for measurements of the crystalline lens. The loading method with focused ultrasound waves induces low-amplitude deformation localized on the sample surface. Phase-sensitive OCT (PhS-OCT) is applied to capture the surface displacement with high sensitivity in both spatial and temporal aspects. Spectral analysis performed on the recovery process of the surface response provides the natural frequency from the sample under the particular loading parameters. Our experiments on homogeneous and layered phantoms indicate that the dominant frequency is sensitive to the depthwise distribution of the elasticity in the samples. The effects of varying the excitation duration and ultrasound frequency on the surface response in the frequency domain are also investigated in this study. The proposed method does not require OCT measurement inside the sample and the single-point depth-related assessment can be noninvasively conducted in real time. We believe that this method can be further developed as a technique for nondestructive detection of the mechanical property gradient in the crystalline lens.

2. Materials and methods

2.1 Tissue-mimicking phantoms

Our pilot experiments were conducted on gelatin phantoms (that optically and mechanically simulate the properties of the crystalline lens). Four types of tissue-mimicking phantoms were prepared. The homogeneous cylindrical samples were prepared with 6% and 18% gelatin concentrations (weight by weight), and with 35 mm diameter by 17 mm height. The layered phantoms were composed of 6% for the top layer and 18% for the bottom layer, and had a top layer thickness of either 2 mm or 5 mm. The cylindrical samples were tested uniaxially using an In-Spec 2200 benchtop portable tester (Instron, Inc., MA). For each sample, the tests were repeated five times to evaluate the accuracy and reproducibility of the measurements. Based on the uniaxial load-displacement measurements, the gelatin samples with 6% and 18% concentration had Young’s moduli of 8 kPa and 48 kPa, with the standard deviation of 0.3 kPa and 2.5 kPa, respectively. The layered phantoms were made from the same gelatin solutions, and were stored in the same conditions as the samples to minimize any possible differences in elastic properties. All gelatin phantoms were optically transparent.
2.2 Combined system

The schematic of the combined system is shown in Fig. 1. The detailed description of the PhS-OCT system can be found in our previous work [29, 30]. Briefly, this spectral-domain system employs a superluminescent diode (Superlum Diodes, Ltd., Ireland) as the laser source which provides a central wavelength of 840 nm and a bandwidth of ~49 nm. The interference of the light from the reference and the sample arms is based on a Michelson interferometer. The fringes of interference are spatially resolved through a home-built high-resolution spectrometer with a CCD line scan camera (Basler, Inc., Germany). The PhS-OCT system provides a temporal resolution of 40 µsec for the A-line acquisition. The Gaussian beam at the imaging focal plane has a FWHM of ~8 µm. The measured phase stability of the system is ~0.03 radians at maximal SNR (from a mirror placed at the sample arm) and ~0.11 radians at SNR of 30 dB (from the phantom placed inside a water bath). The latter corresponds to a sensitivity of ~7 nm for the measurement of phantom surface displacement.

A pulsed ultrasound system was used to interrogate the top surface of the gelatin phantom. Two focused single-element transducers were used in the experiments: 3.7 MHz transducer with 12.7 mm diameter, and a focal length of 19 mm; and 25 MHz transducer with 6.35 mm diameter, and a focal length of 25.4 mm. Gated sinusoidal signals were generated to drive the transducers through the amplifier (55 dB power, A150, ENI, Rochester, NY), and the duration of the ultrasound excitation was varied from ~27 µsec to ~13 msec. The driving signals had the same frequencies as the resonant frequencies of the transducers with a rectangular pulse envelope and the amplitudes were 5-30 mV. For each loading parameter, at least three measurements were conducted for statistical purposes. During the experiments, the transducers were adjusted to be co-focused with the OCT beam on the sample surface. The phantom was fixed on a sample holder which is connected to a two-dimensional translation stage for sample positioning. The ultrasound waves can reach the phantom unobstructedly through the hole at the bottom of the sample holder. The whole sample setup was kept within a water bath, as shown in Fig. 1. M-mode imaging was utilized to capture the sample surface response over time. The synchronization of the two systems is achieved through sharing of a transistor–transistor logic signal (from the DAC shown in Fig. 1) for triggering the OCT frame (B-scan) acquisition (through channel 2 of the ADC in Fig. 1) and the ultrasound excitation (through the function generator in Fig. 1).
2.3 Sensing mechanism

The focused acoustic waves generated by an ultrasound transducer provide a continuous radiation force applied to the surface of the sample. The force lasts for the duration of the ultrasound pulse with constant magnitude. In response to this mechanical loading, the sample surface is deformed to a certain displacement within the excitation duration, and recovers back to the original position after removing the radiation force. Temporal dynamic of the self-recovering process depends on the biomechanical properties of the sample. During the sample self-recovery process, the deeper layer in the sample with different elastic properties can generate damped echo vibrations, which can also contribute to the surface response with distinct frequency components. Thus, through analyzing the spectral response of the sample surface, the mechanical properties of the sample at different depths may be assessed.

2.4 Data processing

The optical phase is computed based on the complex OCT signal generated from the inverse Fourier transform on the interference fringes with linear $k$-space [31, 32]. Using the M-mode OCT intensity image, the surface of the phantom can be easily found due to the high intensity value resulting from light scattering at the air-phantom interface [20]. The phase signal at the sample surface is obtained and unwrapped for the profile of the surface temporal response. Figure 2 shows the typical surface response obtained from a 6% homogeneous gelatin phantom with focused ultrasound excitation. The deformation and the recovery process of the sample surface can be clearly visualized, as called out in Fig. 2. Spectral analysis is performed on the recovery process (indicated with red dashed square in Fig. 2) to study the frequency characteristics of the surface relaxation. The dominant frequency (defined as the position of the major peak from the amplitude spectrum) of the sample surface response is obtained using a fast Fourier transform with ~3 Hz spectral resolution. Our approach is based on a frequency analysis of the sample surface dynamics rather than an analysis of the deformation amplitude. Thus, the temporal deformation signals are normalized to their maximum values before the Fourier transforms are conducted.

![Fig. 2. Typical surface response from a 6% gelatin phantom with focused ultrasound excitation. The deformation and the recovery processes of the sample surface are called out. The squared part with dashed lines indicates the recovery process selected for the spectral analysis.](image)

3. Results

Our pilot experiments focus on demonstrating the feasibility of using the dominant frequency of the surface response as an effective indicator for the depth-dependent elasticity of a
sample. We also investigate the influence of different loading parameters including the excitation duration and the ultrasound frequency.

The typical recovery processes from the surface dynamics of homogeneous and layered phantoms are presented in Fig. 3(a). The ultrasound excitations have a duration of \(~8\) msec and a frequency of \(3.7\) MHz. For the homogeneous samples, a faster recovery can be observed with the \(18\)% concentration. Also, unlike the homogeneous phantoms, the inhomogeneity in the layered samples generates damped vibrations after the surface returns to its original position. Figure 3(b) shows the corresponding amplitude information of the frequency spectra from the temporal responses in Fig. 3(a). The result indicates that the homogeneous sample with \(6\)% concentration has more low-frequency components in its surface dynamics compared with the \(18\)% gelatin phantom. This suggests a higher natural frequency coming from the sample with larger elastic modulus, which is in accordance with previous studies [33]. For the layered phantoms, as the thickness of the top \((6\)% layer increases, the dominant frequency of the surface response moves to the lower region, which indicates that this value of dominant frequency reflects the mechanical properties of both layers. Thicker the top layer, the smaller the effect from the bottom layer on the surface spectral response. The quantitative results (Fig. 4) show that with excitation duration of \(~8\) msec and ultrasound frequency of \(3.7\) MHz, the dominant frequencies detected from the layered phantoms with 2 mm top layer and 5 mm top layer are approximately 401 Hz and 156 Hz, respectively. These results demonstrate that the dominant frequency from the surface recovery process is sensitive to the depth-dependent elasticity in tissue-mimicking phantoms, and layered samples with different top layer thickness can be well differentiated by using the dominant frequency as an indicator.
Fig. 3. (a) Typical recovery processes from the surface dynamics of homogeneous and layered phantoms. (b) The amplitude spectra of the surface responses corresponding to (a), showing the frequency characteristics of the homogeneous and layered phantoms. Partial magnifications are presented with black and red borders for (a) and (b), respectively.

Fig. 4. Box plots of the obtained dominant frequencies from the surface recovery processes on the layered phantoms. $N = 3$ for both measurements. The solid dots represent the mean values, and the whiskers represent the standard deviations. The measured values are identical for the dominant frequency from the layered phantom with 5 mm top layer.

The dominant frequencies obtained from the surface dynamics of the layered phantoms under different durations of excitation are presented in Fig. 5. The variation of the frequency
is fitted with exponential functions. It can be seen that for both the layered phantoms, with an increase of the excitation duration, the dominant frequency of the surface response increases. When the loading time is long enough for the surface of the sample to reach the equilibrium position [34], the dominant frequency stays relatively constant. This indicates that longer durations of excitation can be used to probe the mechanical properties from regions that are located deeper inside the sample. The excitation duration that corresponds to the steady state of the sample surface enables the detection with the deepest level. When using short duration of excitation, for example, ~27 µsec, the recovery process of the surface response from the layered phantom with 5 mm top layer appears to be identical to the one from the 6% homogeneous phantom (Fig. 6). This shows that the very short excitation duration probes the superficial layer only and is insensitive to the biomechanical properties of the stiffer bottom layer. Comparing Fig. 5(a) and 5(b) shows that when the excitation duration changes, the increase of the dominant frequency of the layered phantom is faster in the phantom with 2 mm top layer thickness than in the phantom with 5 mm top layer thickness.

Fig. 5. Plots of the dominant frequencies with respect to the duration of excitation for the layered phantoms with (a) 2 mm top layer and (b) 5 mm top layer. Data are fitted with exponential functions. N = 3 for all measurements.
With two different ultrasound excitation frequencies which generate distinct focused beam diameters, the surface recovery process of the layered phantom with 2 mm top layer shows different dominant frequencies. As shown with the box plots in Fig. 7, with excitation duration of ~8 msec, the dominant frequencies are 400.8 ± 3.5 Hz and 384.5 ± 5.3 Hz for excitation with ultrasound frequencies of 3.7 MHz and 25 MHz, respectively. This indicates that with low ultrasound excitation frequency, the surface spectral response is more affected by the mechanical properties from deeper inside the sample, while the detection depth is reduced when applying higher ultrasound excitation frequency. These results show the influence of different loading parameters on the dominant frequency. They also provide the possible approaches for optimizing the depth-related detection when using the dominant frequency from the surface response as an indicator for the depth-dependent elasticity.
configuration is an ideal fit for the proposed method and will be applied for our future \emph{in situ} experiments on the crystalline lens.

The phase-resolved OCT technique relies on extracting the optical phase information from the interference fringes. The phase stabilization of the system determines the sensitivity of detecting the sample surface displacement. The high sensitivity of the PhS-OCT system at the level of nanometer scale makes it possible to apply low-intensity ultrasound pulses to the sample surface, which is critically important for the use of the acoustic radiation force in clinical research and practice [36]. In addition, for the crystalline lens located in the eye globe, large amplitude of excitation from the ultrasound waves may induce bulk movement of the lens, preventing accurate frequency measurements from the lens surface dynamics.

This feasibility study relies on tissue-mimicking gelatin phantoms to simulate the optical and mechanical properties of the crystalline lens. The layered phantoms can be produced with known distributions of elastic property. These phantoms are well suited for our pilot proof-of-principle experiments and can be used to experimentally confirm the predictions of our theoretical model [37]. Our numerical simulations with a multi-layer phantom show that low-frequency vibrations are more sensitive to the deeper layers, while high-frequency responses involve information only from the upper layers [37]. This indicates that the depth-dependence of the mechanical properties can be resolved by relying on spectral analysis. In the current study we have used simple discrete multiplayer model of the crystalline lens-type media with layers corresponding to the cortex and nucleus of the lens (as often utilized in finite-element models of the lens). In our future studies we will extend this model for continuous mechanical gradient analysis of the depth-dependent mechanical properties of \emph{in situ} crystalline lenses. Specifically, a three-dimensional OCT image of the crystalline lens and its internal structure will be used to develop a more advanced model that will take into account the lens geometry and structure. We expect that the combination of an inverse model based on our numerical simulations, with the structural information from the three-dimensional OCT and the noninvasive measurement of dominant frequency will allow depth-resolved assessment of the relative elasticity distribution of the nucleus and cortex using this technique.

As a sensing technique for depth-dependent elasticity, the sensitivity of the depth-related change of mechanical property is an important factor. We utilize the dominant frequency value from the surface response as the indicator for the depthwise distribution of the elastic properties. The spectral resolution of the fast Fourier transform and the repeatability of the dominant frequency measurement determine the ability of resolving the smallest change of the top layer thickness of the sample. As the bin resolution ($\Delta f$) of the resulting spectrum is determined by the time duration ($t_{\Delta}$) of the temporal signal ($\Delta f = 1/t_{\Delta}$), to acquire and process a longer-duration signal can help to improve the resolution in the frequency domain, however, with increasing the total time of detection. The repeatability of the dominant frequency measurement can be characterized using the standard deviation divided by the mean value, which, for our experiments on the tissue-mimicking phantoms, is \~2.6\%. This parameter can be affected by the random frequency components of the background noise during the phase-resolved measurement, and the relevant characterization will be one of our major focuses when applying the technique to the \emph{in situ} crystalline lens.

Previous studies have assessed depth-dependent elasticity based on the measurement of sample surface deformation, with application in skin elastography [28, 38]. The surface acoustic wave OCE technique developed by Li et al. is capable of providing depth-resolved mapping of the sample elasticity by monitoring the phase velocity of the wave propagation on the sample surface. It has the advantage of producing quantitative measurement [16, 39]. However, compared with our proposed method, the requirements of performing at least two points of measurement and conducting phase velocity calculation based on the wave dispersion reduces the detection and data processing speed.
The use of frequency information from the tissue temporal response for the assessment of tissue elasticity has been previously reported by Crecea et al. for the development of an optical rheological method [33]. In their study, magnetomotive nanoparticles need to be added into the sample as the loading transducers and the relaxation frequency is obtained through fitting with an underdamped oscillator model, which makes the technique difficult to be utilized for \textit{in vivo} applications and rapid detection. Besides, the demonstration of this natural-frequency-based approach is limited to homogeneous tissue samples. Recently, Qi et al. have developed a resonant OCE technique that uses the mechanical resonant frequency to map the tissue elastic properties [40]. The depthwise distribution of the sample elasticity is achieved through detecting the vibrational amplitudes from the scattering parts inside the sample at varying driving frequencies. However, this approach requires reliable OCT signals with sufficient SNR from within the sample for an accurate detection, which might not be applicable for the crystalline lens due to its transparency. On the contrary, our proposed method relies on the OCT signal from the surface of the sample only and does not require any additional (natural or artificial) targets inside the sample. Also, our approach is based on fast Fourier transform which enables relatively high detection speed. To the best of our knowledge, this study is the first to demonstrate that the dominant frequency of the sample surface dynamics can be utilized as an effective indicator for noninvasive detection of the depth-dependent mechanical properties in transparent samples.

Current OCT-based elastography techniques rely on several major approaches: mapping the strain or displacement related parameters based on the extensive deformation of the whole tissue [15, 23, 27]; quantifying the elastic modulus based on the velocity measurement of the shear or surface waves propagating in the tissues [39, 41, 42]; analyzing the tissue temporal movement in the area of force applications [17, 33, 43]. The special features of the proposed method such as surface monitoring, localized detection and rapid depthwise assessment make it possible to act as a complementary approach for general tissue elastography with OCT, and also allow it to be further developed as a major technique to rapidly assess the mechanical property gradient of the crystalline lens.

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