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Relaxation time constant based optical coherence elastography (RTC-OCE)

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Abstract

This study aimed at visualizing relative relaxation time constant in soft tissue by using optical coherence elastography.

We proposed a force vibration model as a theoretical base to express relaxation time constant (RTC) using axial gradient of periodic vibration phase captured by phase sensitive optical coherence tomography (PhS-OCT). Validation of the model had been accomplished by experiments with isotropic and double layered phantoms. A fresh chicken breast sample treated with focused ultrasound was prepared to test performance of the RTC-OCE in real

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tissue. All results were cross validated with indentation test and traditional strain based elastography. This study firstly utilized RTC mapping in 2D and 3D that covers the information of both elasticity and viscosity. The generated RTC mapping revealed the same mechanical difference internal sample which is correlated with conventional strain mapping. RTC mapping is potentially to be served as new biomarker for disease diagnosis in the future.

KEYWORDS: Kelvin-Voigt fractional derivative; relaxation time constant (RTC); optical coherence tomography (OCT); optical coherence elastography (OCE)

Abbreviations: OCT, optical coherence tomography; SNR, signal to noise ratio; RH-KVFD, ramp-hold Kelvin-Voigt fractional derivative; RTC, relaxation time constant

1 INTRODUCTION

Disease, such as cancer, tends to alter tissue viscoelasticity [1, 2]. It has been reported that tissue with prostate cancer is stiffer than its surrounding normal tissue, with stiffness ratio up to 20 [3]. Chen *et al.* had discovered that an increase of viscosity is closely correlated with liver fibrosis [4]. Therefore, both elasticity and viscosity are useful biomarkers for diseases diagnosis.

Elastography is one of those methods to localize disease by mapping out elasticity distribution of soft tissue using imaging modalities. Compared with ultrasound based elastography [5] and magnetic resonance based elastography [6], optical coherence elastography (OCE) takes the advantages of high resolution of OCT (~10 μm) [7] and high sensitivity for deformation

detection and strain estimation [8, 9], attracts attentions in the application of tissue characterisation for early diseases diagnosis [10] and tissue engineering [11, 12].

Current OCE methods can be categorized into two classes for elasticity detection: wave-based methods [13-15] and strain-based (static or quasi-static compression) method. Compression OCE evaluates elasticity by strain generated by static, quasi-static or harmonic compression. The technique has been broadly applied in clinics, such as human breast [16], dermatology [17] and prostate [18]. Pre-clinical studies of compression OCE has also been investigated in mouse aorta [19], thermal reshaping of ex-vivo cartilage [20] and ex-vivo animal cornea [21]. Although promising results are shown, it has been reported that strain contrast is not only elasticity dependent but also elasticity contrast and sample geometry related [22], strain contrast will deviate from elastic contrast to an extent even up to 0.5 when elastic contrast is too high [23]. Assumptions that sample is linear elastic and stress is uniform [24, 25] are also controversial as tissue is reported to be hyperplastic [26] and usually inhomogeneous. Stress field distribution highly depends on excitation frequency, which has been reported by Adie *et al.* [27] as well as our previous work [28]. OCE can also be utilized for viscoelasticity estimation, most publications related to tissue viscosity evaluation are wave-based methods. Zvietcovich *et al.* [29], Ramier *et al.* [30] and Zhou *et al.* [31] utilized wave propagation models to characterize viscoelasticity. There are also some publications covered viscosity by measuring strain relaxation, e.g. Wijesinghe *et al.* [32] estimated viscoelasticity of sample by analysing creep deformation induced by compressional loading, where 2D relaxation time constant mapping as well as dual parameter mapping (elasticity & relaxation time constant) was utilized to quantify viscoelasticity. Adie *et al.* [27] also quantified viscoelasticity by

phase of periodic vibration which theoretically related to damping coefficient. Singh *et al.* [33] evaluated viscoelasticity by measuring air pulse induced displacement recovery. Although viscosity has been measured with accurate results, these methods are only to measure bulk viscoelasticity or with 2D results instead of 3D mapping.

In this paper, we propose a novel methodology for 2D and 3D mapping that encompasses viscoelasticity of ex-vivo biological sample by using relaxation time constant (RTC). Rational behind this method is forced vibration model for periodic vibration, vibration phase difference along the direction of vibration is proportional to RTC of sample, where RTC is defined as the ratio between viscosity and elasticity. To demonstrate accuracy of forced vibration model, axial layered phantoms with different composition were fabricated. Contrast in RTC mapping by using vibrational OCE was cross validated with the true RTC values obtained from indentation test conducted on the phantoms. In addition to tissue mimicking phantoms, ex-vivo biological sample was prepared and scanned with our vibrational OCE system, results denoted system's capability of 2D and 3D RTC mapping on soft tissue. The combination of conventional elasticity mapping and RTC mapping may provide as supplementary biomarker for disease diagnosis. Methods and algorithm in this paper for RTC mapping could also be adapted for other imaging based elastography techniques, such as ultrasound elastography, to offer a varying image field of view for RTC mapping.

2 THEORETICAL BACKGROUND

Forced vibration model is introduced to analyse periodic vibration of sample with isotropic viscoelasticity. Model is constructed under normal situations with 3 assumptions: first, region

been scanned is far from boundary of sample that boundary effects is not considered.; second, sample is thick enough that is larger than the wavelength of generated wave induced by excitation; standing wave is a particular situation where wavelength and thickness of sample matches, which is not taken into account in this study. Sample is divided into infinitesimal elements with equal mass, denoted as m , which are connected by elastic spring and viscous damper in parallel. Figure 1 shows the aligned elements that are located along the axial direction, which is parallel to the direction of mechanical excitation. $x_1(t)$ $x_2(t)$ and $y(t)$ are sinusoidal displacements for each element. Force and deformation in shear direction are out of consideration to simplify the mathematical model. Position of the aligned elements are far from boundary where oscillations of particles are in the same phase.

Figure 1 Model of forced vibration with viscous damping. Connection between elastic spring and viscous damper is parallel, k represent elastic modulus and c stands for damping viscosity. m is the mass, $x_1(t)$ $x_2(t)$ and $y(t)$ are periodic vibrations for each element.

Forced vibration phase difference between adjacent elements is shown as:

$$d\theta = \text{angle} \{ y(t) * \text{conj}[x_1(t)] \} = \frac{c\omega}{m\omega_n^2 - m\omega^2} = \frac{c}{m} * \frac{\omega}{\omega_n^2 - \omega^2} = \frac{cf}{m\pi(f_n^2 - f^2)} \quad (1)$$

Where $d\theta$ is the vibration phase difference between adjacent elements, ω is frequency of forced vibration of elements that accords to applied excitation, c is viscosity and k is

elasticity, ω_n and f_n are natural frequency in angular and SI expression which depends on k and m .

If frequency of external stimulation is much smaller than natural frequency of sample, Equation 1 could be simplified as:

$$d\theta \approx \frac{c}{m} * \frac{\omega}{\omega_n^2} = \frac{c\omega}{k} = \omega \tau_{abs} \quad (When \omega_n \gg \omega) \quad (2)$$

τ_{abs} is relaxation time constant (RTC) that equals to c/k , subscript *abs* is utilized to differentiate τ from ramp hold Kelvin-Voigt fraction derivative model. Equation 2 represents vibration phase shift between adjacent elements is majorly determined by RTC of sample and vibration frequency. Axial phase slope detected by phase sensitive OCT (PhS-OCT) in greater scale should be proportional to $d\theta$.

For sample with double layers in axial direction, the ratio between phase slopes of each layer is expressed as:

$$\frac{d\theta_1}{d\theta_2} = \frac{\omega \tau_{abs1}}{\omega \tau_{abs2}} = \frac{\tau_{abs1}}{\tau_{abs2}} \quad (When \omega_{n1} \gg \omega \quad \omega_{n2} \gg \omega) \quad (3)$$

Equation 3 explains theoretical base of the vibrational OCE to construct 2D and 3D RTC mapping by visualizing RTC contrast. Please refer appendix for more detail.

3 SAMPLE PREPARITION & SYSTEM SETUP

3.1 Tissue Mimicking Sample

Gelatin and agar (Agar-Agar, Pure Granular Powder, Fisher Chemical, Pittsburgh, USA) mixed phantoms were fabricated by mixing gelatin & agar powders and deionized water.

Mechanical stir was administrated at 80 °C for 1 h to speed up dissolving, one percent titanium dioxide (TiO₂) water solution was added in the end of stirring process to serve as optical scattering. Solution was then poured into Petri dish to cure for 2 h before using. The top layer was constructed by pouring liquid phantom solution onto cured bottom layer. The thickness of top layer is monitored and controlled by OCT real-time structural imaging. The overall thickness of sample is around 5 mm. Viscoelasticity variance was controlled by varying gelatin and agar concentration, quantification of viscoelasticity for samples are illustrated in section 4.2.1. Several double and single layered phantoms were fabricated, detail of those layered phantoms is shown in Table 1.

Table 1 Detail of layered phantom

group number #	top layer concentration (%) gelatin	bottom agar/gelatin mixture concentration (%)	top layer thickness (mm)
1	20	0/15	1.08
2	15	0/20	0.97
3	10	0/15	0.89
4	10	0/20	0.93
5	15	0.5/5	0.95
6	10	0.5/5	1.01

3.2 Indentation Test for Tissue Mimicking Phantoms

To measure RTC of isotropic tissue mimicking phantoms, ramp hold relaxation test was implemented using Tinius Olsen H5KS Benchtop Tester (Tinius Olsen Ltd., U.K.), where the displacement data were fitted to ramp hold Kelvin-Voigt fractional derivative (RH-KVFD) model [34-36]. A 10 N load cell was applied to measure the force. Spherical tipped indenter

made of aluminum was utilized to compress the subsamples made with the same materials, whose tip diameter is smaller than one tenth of the sample to reduce boundary effects [36]. A maximum compression was set to 1.5 mm, comprising a maximum strain of 2.5% (sample height is around 60 mm). Speed of compression was 2 mm/min such that time required to reach the maximum compression is round 45 s. The indenter was then held at the maximum compression position for another 250 s for stress relaxation. The overall time-force-position dataset was recorded and saved by the computer connected to the benchtop tester, after which the data was fitted to RH-KVFD model in MATLAB to calculate viscoelastic parameters.

3.3 Ex-vivo Biological Sample Preparation

Fresh chicken breast, bought from local butcher, was used as ex-vivo biological sample to test system performance for 2D and 3D relative RTC mapping. High intensity focus ultrasound (HIFU) (Transducer G701#, Chongqing Haifu Medical Technology Co., Ltd, China) was administrated to chicken breast for 7 s with a driving frequency of 10 MHz. The generated massive acoustic energy was converted into heat which tends to alter original tissue structure and component, resulting in viscoelasticity change [37]. Chicken breast after HIFU treatment was sealed into 0.5+5 % agar gelatin phantom which could serve as compact layer to ensure a good contact between shaker head and sample.

3.4 Vibration OCE Setup

The experimental setup of vibrational OCE is shown in Figure 2, where a PhS-OCT system was employed to detect sample deformation, a mechanical shaker was used to couple periodic

excitation into sample. Super luminescent diode was utilized as laser source (Thorlabs LS2000B) with a central wavelength of 1,340 nm and a bandwidth of 110 nm, leads to a theoretical axial resolution of 7 μm in air and 7.8 μm in practice. Focal length of objective lens was 50 mm, comprises a lateral resolution of 16 μm in air. The system was configured in conventional dual arms mode, where interference pattern was determined by optical path difference between reference arm and sample arm. The interference signal was collected by InGaAs linescan camera (SU1024LDH2, SUI, Goodrich Corp, NJ, USA) with 1,024 pixels and a maximum sampling frequency of 91 kHz. The collected signal was transmitted and saved in the connected computer. The maximum imaging depth of OCT was ~ 3 mm with 20 dB roll off. Phase noise level was tested to be smaller than 2 mrad with SNR of 51 dB and an exposure time of 6.96 μs , indicating a minimum detectable deformation in nanometre scale. Mechanical shaker was driven by signal generator with sinusoidal waves. The vibration amplitude of shaker head was set around 1,000 nm, as a relatively small vibration ensures independence of elasticity from strain [38]. Frequency selected for RTC mapping was below 200 Hz, which was lower than the resonant frequency of gelatin phantoms and chicken breast. In this frequency range, as stated in Equation 2 and Equation 3, ratio between phase slopes reveals contrast between relaxation time constants.

Figure 2 Experimental setup for vibrational OCE. The mechanical shaker is driven directly by signal generator, where sinusoidal signal is applied. Detail of setup design for vibration excitation is shown in right. The mechanical shaker is composed of shaker head, shaker rod and the main body. Sample is placed on the surface of shaker head, vibration perpendicular to top surface of shaker head is administrated to sample. Laser travels coaxially with vibration direction so that the detected deformation is parallel to vibration excitation.

3.4.1 Scanning Protocol

OCT was configured in M-B mode for scanning. For 2D scans, M-scan, corresponding to one lateral position in the B-mode image shown in Figure 3, consists of 512 consecutive A-scans at a sampling frequency of 20,730 Hz. 512 M-scans were incorporated in the end with a scanning range of 0.4 mm, thus, the overall data contains a size of 1,024 (pixel) * 512 (time sequence) * 512 (M-scans).

For 3D scanning, 128 2D-scans were incorporated with a scanning range of 0.4 mm × 0.7 mm. M-scans were composed of 256 consecutive A-scans and each 2D image consists of 128 M-

scans. The data size for single 3D scan is $1,024 \text{ (pixel)} * 256 \text{ (time sequence)} * 128 \text{ (M-scans)}$
 $* 128 \text{ (2D images)}$.

For both 2D and 3D scans, vibration is applied before M-B scan begins so that vibration internal sample could reach a steady state. Synchronization between vibration and data acquisition is not required.

Figure 3 MB scanning protocol for PhS-OCT, scanning begins from time sequence to acquire 512 consecutive A-lines for one axial position (M-scan), then follows by the next position with the same procedure. There will be 512 M-scans for one 2D OCE test.

3.4.2 Data Processing for RTC Mapping

Phase term of fast Fourier transform (FFT) of the interference signal represents deformation. Sinusoidal excitation at a single frequency is administrated by mechanical shaker, axial

displacement of each particle, allocated along the direction of excitation, is also in sinusoidal form which is expressed as oscillation along the frequency domain:

$$\phi(z, t) = \frac{4\pi}{\lambda} \sum_{i=0}^{j-1} A_i \sin(2\pi f_i t + \psi_i) + \varphi_i \quad (4)$$

Detail of derivation of this equation has been illustrated by Wang *et al.* in [39]. f_s is the sampling frequency, f_i is the resolved frequency that evenly distributed in the range of 0 Hz to $f_s/2$, j is frame number that each M-scan has, λ is the central wavelength of laser source, φ is the static phase term for each M-scan but is usually randomly distributed along $-\pi$ to π for individual M-scans, ψ is the vibration phase term, z is the depth in axial direction, t is time, A is amplitude of vibration for each frequency. Components of all frequencies are included in Equation 4, other than single frequency generated from shaker, to encompass noises and other cogenerated vibration distributed along frequency domain.

To eliminate the static phase term φ_i , time difference is applied to Equation 4:

$$\phi'(z, t) = \frac{8\pi^2 f_i}{\lambda} \sum_{i=0}^{j-1} A_i \cos(2\pi f_i t + \psi_i) \quad (5)$$

$\phi'(z, t)$ records velocity of particle movement, frequency domain spectrum is generated by applying another FFT to time-velocity sequence data, the corresponding value at excitation frequency is represented as:

$$\Phi(z, f_e) = FFT(\phi'(z, t)|_{f=f_e}) = |\Phi(z, f_e)| e^{i\psi_e} \quad (6)$$

ψ_e is the phase term for vibration, f_e is the excitation frequency. Phase slope is calculated by derivative of ψ_e along the axial direction.

$$p = \frac{d\psi_e}{dz} \quad (7)$$

p is the phase slope in axial direction that is parallel to laser beam and direction of vibration.

This value is served as the parameter to map 2D relative RTC.

Algorithm for strain mapping can be found in our previous publication [39], where moving slope of deformation amplitude along axial direction is calculated strain.

4 EXPERIMENTS AND RESULTS

4.1 Phase Slope Variation in Isotropic Material

We used isotropic tissue mimicking sample to validate phase slope to frequency relationship shown in Equation 1. It had been tested that the resonance frequency for the sample was around 160 Hz. Resonance frequency is measured by observing turning frequency where phase slope changes its sign from positive to negative, which is usually accompanied by maximized vibration amplitude when compared with its adjacent frequencies. From expectation of Equation 1 that phase slope tends to reach infinity and change its sign from positive to negative as excitation frequency passes through resonance, frequencies selected for vibration excitation varies from 90 Hz to 180 Hz with an equal interval of 10 Hz. System is configured for 2D MB scan, slope values for each M-scan were analysed with normalized distribution. A confidence interval (CI) of 95% was selected as the boundaries of error bar and the average value was utilized for mathematical fitting.

Results are shown in Figure 4, where the error bars in blue implies phase slope processed from experimental data with 95% CI; the red solid line is mathematical fitting result, fitting

model is composed of forced vibration model, shown in Equation 1, added with a constant value that used to compensate error between theory and experiment. Fitting result highlighted in dashed rectangular box is zoomed to the right for detail inspection. Fitting range is selected from 90 Hz to 150 Hz as phase slope near resonance frequencies are expected to reach infinity which is impossible in practice. R-square of fitting is 99.9% implying a good approximation of the mathematical model to real experiment.

Figure 4 Phase slope to frequency curve of 15% gelatin phantom. Blue solid error bars are phase slope data processed from experimental data, indicates 95% confidence interval (CI). Solid line in red is the mathematical fitting from 90 Hz to 140 Hz, the mathematical vibration model is introduced in Section2. Blue dashed line vertical to frequency axis shows the fitted resonance frequency of the tested sample, 158 Hz. The R-squared value is 99.9%, implies the experimental results is well correlated with mathematical model.

4.2 RTC of Bilayer Phantoms

4.2.1 Viscoelasticity Characterization with Ramp Hold Relaxation Test

Raw data for indentation test and the RH-KVFD fitting curve have been plotted in Figure 5. Force-displacement relationship respect to time defined in RH-KVFD model is expressed as [36]:

$$P_r(t) = 4\sqrt{R}k^{3/2}Et^{3/2} \left[\frac{2}{3} + \frac{(t/\tau)^{-\alpha}}{\Gamma(1-\alpha)} B\left(\frac{3}{2}, 1-\alpha\right) \right], \quad 0 < t \leq T_r \quad (8)$$

$$P_r(t) = 4\sqrt{R}k^{3/2}ET_r^{3/2} \left[\frac{2}{3} + \frac{(t/\tau)^{-\alpha} (t/T_r)^{3/2}}{\Gamma(1-\alpha)} B\left(\frac{T_r}{t}; \frac{3}{2}, 1-\alpha\right) \right], \quad t \geq T_r \quad (9)$$

Where $B(x, y) = \int_0^1 t^{x-1} (1-t)^{y-1} dt$ $\text{Re}(x) > 0, \text{Re}(y) > 0$; $B(a; x, y) = \int_0^a t^{x-1} (1-t)^{y-1} dt$ for $a \in [0, 1]$. R is radius of tipped indenter, k is compression velocity of indenter, t is time, τ is relaxation time constant that $\tau = (c/E)^{-\alpha}$, c is viscosity E is elasticity and α is derivative order, Γ is gamma function which equals to $\Gamma(z) = \int_0^\infty e^{-t} t^{z-1} dt$.

Fitting parameters are presented in Table 2 with R square values above 99%, indicates the RH-KVFD model fits the experimental data well. Absolute time constant is defined in this study as $\tau_{abs} = \tau^\alpha = c/E$, which is related to the axial phase slope illustrated in Equation 1.

Figure 5 Indentation test data of 10% 15% 20 % gelatin phantoms and the KVFD model fitting results. It takes 45 s for the indenter to move to the position of maximum displacement.

Table 2 Fitting Results of KVFD Model with 95% Confidence Bounds

Solute Concentration agar/gelatin %	Elastic Modulus E_0 (Pa)	Derivative Order α	Relaxation Time Constant τ (s)	Fitting R^2 %	Absolute Time Constant * τ_{abs}
0/10	4136 ± 143	0.21 ± 0.03	0.01 ± 0.01	99.2	0.38
0/15	8735 ± 182	0.23 ± 0.02	0.04 ± 0.01	99.7	0.48
0/20	8571 ± 145	0.21 ± 0.01	0.05 ± 0.01	99.8	0.54
0.5/5	5405 ± 91	0.41 ± 0.03	0.50 ± 0.14	99.1	0.75

* Absolute time constant is equal to τ^α , which is equal to the ratio between viscosity and elastic modulus.

4.2.2 Phase Slope Variation in Bilayer Material

Detail of bilayer gelatin phantoms is shown in Table 1. Parallel to scanning configuration for single layer phantoms, 2D MB scan was implemented for each sample at the excitation frequency that was lower than resonance frequency to ensure the accuracy of approximation

for Equation 2. To acquire phase slope for each layer, linear fitting for vibration phase in axial direction was administrated within the depth enclosed in individual layers. Ratio of the slope values represents experimental RTC contrast between the two layers.

Results of phase slope ratio respect to expectation are shown in Figure 6, phase slope ratio of bilayer phantom (bottom layer/top layer) is illustrated in black dots and blue error bars. Expected phase slope ratio is defined as ratio between τ_{abs} for varying gelatin concentration from indentation test. Red dashed line highlights data points where experimental phase slope ratio equals to expected value. Average contrasts comparison between strain mapping and RTC mapping in different phantom groups are quantified by axial deformation slope ratio and phase slope ratio respectively. The results were illustrated in Table 3 (column 5 and 6).

Figure 7 shows 2D result of the sample assigned with 10% gelatin at top and 0.5% + 5% agar & gelatin compound with an excitation frequency of 98 Hz. Figures from top to bottom are structure image, strain mapping and relative RTC mapping (phase slope mapping) respectively. Both strain mapping and relative RTC mapping reveals difference between the two layers but the contrast of relative RTC mapping is higher than strain mapping, which is illustrated and compared in both Table 3. An average contrast enhancement is 15%.

Table 3 Data of Phase Slope Ratio & Strain Contrast

Group Number #	Top Layer τ_{abs}	Bottom Layer τ_{abs}	Expectation Slope Ratio (RH-KVFD)	Phase Slope Ratio Experiment	Deformation Slope Ratio Experiment
1	0.54	0.48	0.88	0.86 ± 0.04	0.98 ± 0.04
2	0.48	0.54	1.14	1.19 ± 0.01	0.88 ± 0.01
3	0.38	0.48	1.26	1.28 ± 0.02	0.95 ± 0.04
4	0.38	0.54	1.43	1.39 ± 0.03	0.79 ± 0.03
5	0.48	0.75	1.58	1.48 ± 0.04	1.45 ± 0.02
6	0.38	0.75	1.98	2.00 ± 0.11	1.70 ± 0.01

Figure 6 Phase slope ratio from experiment and expectation. Experimental phase slope ratio between bottom and top layer is illustrated in blue error bar with black marker. Error bars indicates the bounds of 95% CI. Expectation phase slope ratio is calculated by ratio between absolute τ shown in Table 2. Red dashed line emphasizes results that expectation value equals to experiment. Data detail is shown in Table 3.

Figure 7 2D mapping for bilayer phantom sample, with 10% gelatin overlaid on 0.5% + 5% agar & gelatin compound base. A) is the structure image in dB scale, boundary between two layers is clearly viewed with higher brightness. B) is the strain image. C) is the 2D contrast RTC mapping.

4.3 2D RTC Mapping in Biological Tissue

Biological sample was prepared with chicken breast which has been covered in section 3.2. PhS-OCT was performed in 3D M-B scan mode, scanning protocol is illustrated in section 3.4. Frequency selected for relative RTC mapping was 98 Hz and for strain mapping, to reveal elasticity, was 450 Hz. A much higher frequency excitation for elasticity mapping was not used as stress concentration are probable to happen, which leads to false results and strain will be incapable to reveal elasticity. Please refer to our previous publication for more detail [28].

Results of biological sample are shown in reciprocal of strain ($1/\text{strain}$) for strain mapping and in reciprocal of RTC ($1/\text{RTC}$) for viscoelasticity respectively. Chicken breast been treated with HIFU has a higher stiffness [40], region been treated with HIFU will be highlighted with brighter luminance in $1/\text{strain}$ mapping. Parallel to strain mapping represented by $1/\text{strain}$, RTC mapping is also rendered by its reciprocal value which is proportional to E/c so that treated chicken breast will be featured out in 2D and 3D mapping.

Results in 3D are shown in Figure 8 which is constructed by 128 gray scale 2D images, where luminance is selected as parameter to render 3D model. Maximum intensity projection [41] is administrated to enhance visualization that inclusion is heightened and reference material is invisible. Structure image is rendered in red glow which serves as background. In Figure 8A, relative RTC mapping is represented in green with its reciprocal to highlight area been treated with HIFU. Strain mapping is shown in magenta in Figure 8B with the reciprocal of strain. Scale bar is attached to the frame in the bottom right, it represents a length of 1 mm. Please refer to appendix for the short video to show the rotation of the 3D model.

Figure 9 shows one of ortho slices that used to construct 3D visualization, which is parallel to ZY plane in Figure 8. Figure 9A is the structure image in dB scale; Figure 9B is strain mapping that rendered by reciprocal of strain, the color bar is attached to its right. Figure 9C is the relative $1/\text{RTC}$ image, luminance represents reciprocal of phase slope with the unit of $\mu\text{m}/\text{rad}$. Dashed line in red roughly delineates boundaries between phantom and treated/untreated chicken breast.

Table 4 shows the contrast estimation between treated chicken breast and phantom covered to its above. Volume is calculated by counting data points whose values are two times larger

than mean value of phantom in 2D ortho slices. Coefficients of variance (COV) is employed for both treated chicken breast and covered phantom to estimate uniformity.

Figure 8 Three-dimensional illustration of scanned biological tissue. Structure of the sample is shown in red which serves as background, maximum intensity projection is administered to enhance visualization. A) Relative 1/RTC mapping in green, overlapped with structure. B) Elasticity mapping in magenta, overlapped with structure. Scale bar in red represents a length of 1 mm.

Figure 9 Representative ortho slice that covers chicken breast that been treated with HIFU. A) Structure image in dB scale B) 1/RTC mapping ortho slice. Region been treated with HIFU has higher brightness than its surrounding area. C) Strain mapping ortho slice, the pixel value equals to 1/strain. The treated chicken breast is stiffer than the normal. The scale bar in red represents 0.5 mm. ① Covered phantom ② Chicken breast treated by HIFU ③ Untreated chicken breast

Table 4 Contrast Estimation

	1/Strain	Relative 1/RTC
Mean Contrast	4.12	2.63
Volume (mm ³)	0.70	0.67
COV Treated Chicken Breast	0.36	0.21
COV Covered Phantom	0.23	0.15

COV represents coefficients of variance

4 DISCUSSION

This paper proposes a novel method for relative RTC mapping by measuring phase slope of periodic vibration coupled to sample. Mathematical model was constructed to explain correspondence between phase slope of vibration and RTC. Relationship between phase slope respect to excitation frequency had been illustrated that frequencies much lower than resonance is recommended. Verification was achieved by experiment with isotropic gelatin phantom, where a close correspondence with mathematical model was discovered by customized fitting of the result to model speculation with a R square of 99%. Experiment of layered phantom was utilized to validate system's performance and accuracy of generated 2D RTC mapping. Expectation was acquired by RH-KVFD model fitting for indentation test result of the homogeneous phantoms which were assigned to the bilayer samples. Close correspondence was found between the two, demonstrated the capability of the system to reveal RTC contrast. Biological sample was prepared to prove system's ability targeting at real tissue sample, where generated 3D mapping clearly delineated area been treated with HIFU. Methodology proposed in this study extends functionality of vibrational OCE for RTC mapping, the results could be served as an additional signature for diagnose of pathological conditions besides conventional approaches. Mathematical models and algorithms for data processing could also be employed as a guidance for other image based elastography techniques, such as ultrasound elastography and magnetic resonance elastography.

RH-KVFD model was employed to fit indentation test results for homogenous phantoms that had the same constitution assigned to the layered phantoms. A good approximation had been illustrated with a R square above 99% of the model fitting, indicated the rational of fitted

parameters to estimate viscoelasticity. In addition to indentation test results, practical experiment had been carried out with RTC-OCE. Experimental RTC contrast (represented by slope ratio) and the expectation from KVFD model fitting are shown in Figure 6, data points distributed along the tilted dashed line implies accordance between two results and demonstrates the accuracy of OCE results. Vibration phase slope ratio collected by OCE matches quantitative RTC ratio generated from RH-KVFD model, demonstrates accuracy of RTC contrast revealed by the system. For RTC mapping in future, quantitative RTC of tested sample could be calculated with reference material with known viscoelasticity.

Three-dimensional relative RTC mapping and strain mapping for biological tissue are shown in Figure 8. Xu *et al.* [42] have reported a noticeable increase storage modulus and a decreasing loss factor for pig skin been heated by bath, the loss factor equals to τ_{abs} in this paper. Boundary assessed from relative inverse RTC mapping is well correlated with strain mapping, which is demonstrated by the close volume estimated from the two. The combination of the two indicates both elasticity and viscosity are enlarged after HIFU treatment. Coefficient of variance estimates uniformity for both strain mapping and relative inverse RTC mapping, shown in Table 4. Uniformity of RTC mapping for both treated chicken breast and reference phantom are higher than the counterparts of strain mapping, indicates RTC mapping is more likely to visualize inhomogeneity internal sample. Compared to strain mapping, the RTC mapping has a lower contrast.

RTC has also been illustrated in other publications, Wijesinghe *et al.* [32] and Matveev *et al.* [43] estimated viscoelasticity by measuring creep response of sample. Acquisition time to get a 2D scan for tissue is 120s [32], as to fully encompass creep response. Compared to this

method, we focused on phase of periodic vibration instead of creep response with an average acquisition time less than 2s for each B-scan with a lateral scanning range of 0.4mm.

Resonance frequency is vital for coupling periodic vibration into sample, where a frequency lower than resonance is recommended to ensure a linear relationship between frequency and phase slope. Prediction of resonance frequency is complicated, as resonance frequency is dictated not only by viscoelastic nature of sample, but also sample geometry [44, 45]. Thus, determination of proper frequency range on various samples are required to ensure credibility of the generated RTC mapping.

Future work will be focused on application of relative RTC mapping, especially its potential in clinical use. On the other hand, this methodology can also be extended to other imaging modalities such as ultrasound imaging, for its capability of larger field of view that might yield more information.

5 Conclusion

This study proposes a novel method to visualize 3D mechanical difference by RTC mapping, forced vibration model is constructed to predict and explain experimental results. Accuracy and validation of mathematical model has been accomplished by experiments with tissue mimicking phantoms as well as biological sample. Methodology proposed in this paper may serve as guidance for future research, methods can also be extended to other imaging based elastography techniques.

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APPENDIX

Forced vibration model is introduced to analyse periodic vibration of sample with isotropic viscoelasticity. Sample is divided into infinitesimal elements with equal mass, denoted as m , which are connected by elastic spring and viscous damper in parallel. Figure A1 shows the aligned elements that are located along the axial direction, which is parallel to the direction of mechanical excitation. Force and deformation in shear direction are out of consideration to simplify the mathematical model.

Figure A1 Model of forced vibration with viscous damping. Connection between elastic spring and viscous damper is parallel, k represent elastic modulus and c stands for damping viscosity. m is the mass, $x_1(t)$ $x_1(t)$ and $y(t)$ are periodic vibrations for each element.

Periodic vibration for each element in steady state is represented as:

$$x_1(t) = A_1 e^{i(\omega t + \theta_1)} \quad (\text{A.10})$$

$$x_2(t) = A_2 e^{i(\omega t + \theta_2)} \quad (\text{A.11})$$

Where t is time, A is the amplitude of vibration, ω is angular frequency of external excitation, and θ is the phase term. $y(t)$ is unknown but can be expressed by $x_1(t)$ and $x_2(t)$ by solving the differential equation of the mass motion:

$$m\ddot{x}_1 + c(2\dot{x}_1 - \dot{x}_2 - \dot{y}) + k(2x_1 - x_2 - y) = 0 \quad (\text{A.12})$$

$\ddot{x}$ is the second order time derivative of motion, acceleration; $\dot{x}$ represents velocity of motion. k is elastic modulus of sample and c is viscosity. Noted that this mathematical model assumes that every single mass element only affected by its adjacent elements. Substitute x_1 and x_2 in Equation A.3 by Equation A.1 and Equation A.2, and then the generated equation is imported into MATLAB for solving y , the result is shown as:

$$y(t) * k = ic\omega * (2A_1 e^{i(\omega t + \theta_1)} - A_2 e^{i(\omega t + \theta_2)}) + k * (2A_1 e^{i(\omega t + \theta_1)} - A_2 e^{i(\omega t + \theta_2)}) - A_1 m \omega^2 e^{i(\omega t + \theta_1)} \quad (\text{A.13})$$

Vibration phase shift between $y(t)$ and $x_1(t)$ is calculated by the angle value of product of $y(t)$ and the conjugate of $x_1(t)$:

$$\begin{aligned} d\theta &= \text{angle} \{ y(t) * \text{conj}[x_1(t)] \} \\ &= \text{angle} \left[(c\omega i + k) * (2A_1 - A_2 e^{i(\theta_1 - \theta_2)}) / k - A_1 m \omega^2 \right] \end{aligned} \quad (\text{A.14})$$

Substitute e^{ix} in Equation A.5 into $\cos x + i \sin x$ according to Euler's formula:

$$d\theta = \text{angle} \left\{ (c\omega i + k) * [2A_1 - A_2 \cos(\theta_1 - \theta_2) - i \sin(\theta_2 - \theta_1)] - A_1 m \omega^2 \right\} \quad (\text{A.15})$$

The angle operation of complex number is equivalent to apply $\tan^{-1}$ to the quotient between imaginary and real part of the input value. So $d\theta$ could be rewritten as:

$$d\theta = \tan^{-1} \left(\frac{\text{IMAG}(H)}{\text{REAL}(H)} \right) = \tan^{-1} \left(\frac{c\omega [2A_1 - A_2 \cos(\theta_2 - \theta_1)] - k \sin(\theta_2 - \theta_1)}{c\omega \sin(\theta_2 - \theta_1) + k [2A_1 - A_2 \cos(\theta_2 - \theta_1)] - A_1 m \omega^2} \right) \quad (\text{A.16})$$

Where $H = (c\omega i + k) * [2A_1 - A_2 \cos(\theta_2 - \theta_1) - i \sin(\theta_2 - \theta_1)] - A_1 m \omega^2$, and *IMAG* and *REAL* are operations to retrieve imaginary and real part of input value. As distances between each

element is infinite small, $\theta_1 - \theta_2 \approx 0$, $A_1 - A_2 \approx 0$ and $d\theta \approx 0$, thus equation can be simplified as:

$$d\theta \approx \tan^{-1} \left(\frac{c\omega A_1}{A_1(k - m\omega^2)} \right) = \tan^{-1} \left(\frac{c\omega}{k - m\omega^2} \right) \quad (\text{A.17})$$

Inverse $\tan^{-1}$ into $\tan$ to the right side from Equation A.8:

$$\tan(d\theta) = \frac{c\omega}{k - m\omega^2} \quad (\text{A.18})$$

As $d\theta \approx 0$, the formula can be approximated as:

$$d\theta \approx \frac{c\omega}{k - m\omega^2} \quad (\text{A.19})$$

Equation A.10 indicates that vibration phase shift between adjacent elements is determined primarily by viscoelasticity coefficients, mass or density of the tested sample as well as the excitation frequency of coupled vibration.

If we consider k as a representation of natural frequency and mass for free vibration without damping:

$$k = m\omega_n^2 \quad (\text{A.20})$$

ω_n is the angular natural frequency of free vibration without damping. Then replace k in Equation A.10:

$$d\theta = \frac{c\omega}{m\omega_n^2 - m\omega^2} = \frac{c}{m} * \frac{\omega}{\omega_n^2 - \omega^2} = \frac{cf}{m\pi(f_n^2 - f^2)} \quad (\text{A.21})$$

In the end of Equation A.12, the formula is transferred into expression of frequency. f is frequency in Hz, f_n is the resonance frequency of the sample.

If the driving frequency supplied by external shaker is small enough so that $\omega_n \gg \omega$, Equation A.12 can be simplified into:

$$d\theta \approx \frac{c}{m} * \frac{\omega}{\omega_n^2} = \frac{c\omega}{k} = \omega \tau_{abs} \quad (\text{When } \omega_n \gg \omega) \quad (\text{A.22})$$

τ_{abs} is the relaxation time constant that equals to c/k . It implies the phase shift between adjacent elements is proportional to the relaxation time constant at frequencies that are much lower than natural frequency of the sample. Parallel to phase shift between adjacent elements in micro-scale illustrated above, phase shift in macro scale, e.g. phase shift in OCT depth of view, is interpreted as phase slope along the direction of vibration.

Based on findings shown in Equation A.12 and Equation A.13, interpretation could be accomplished as follows. For sample with isotropic viscoelasticity, the phase slope along axial direction is approximately proportional to the driving frequency at low frequency range. For high frequency range close to natural frequency from left side, phase slope to frequency curve undergoes a rapid growth and reaches its maxima around natural frequency, then the slope value inverses its sign from positive to negative.

For a bilayer sample with axially varied viscoelasticity, the ratio of phase slope at low driving frequencies is expressed as:

$$\frac{d\theta_1}{d\theta_2} = \frac{\omega\tau_1}{\omega\tau_2} = \frac{\tau_{abs1}}{\tau_{abs2}} \quad (\text{When } \omega_{n1} \gg \omega \quad \omega_{n2} \gg \omega) \quad (\text{A.23})$$

Thus, vibrational OCE generates 2D relative RTC mapping by displaying moving gradient of phase.