Characterization of the elasticity of small objects buried in media based on the measurements of photoacoustic temporal waveforms

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Since elasticity of biological tissues is related to their pathological states, development of new methods allowing the non-invasive measurements of the elasticity has been desired in medical field. We present the characterization of the elasticity of objects buried in media from the temporal waveforms of photoacoustic signals. As the increment in Young’s moduli of the objects, the frequency corresponding to the gravitational center of the power spectra obtained by the Fourier-transformation of the waveforms is increased. In our experiment configuration, the elasticity of buried objects is able to be identified up to about 1 MPa of Young’s modulus from the frequency. These results suggest the measurements on the temporal waveforms of photoacoustic signals and the resultant power spectra would provide a useful method for the evaluation on the elasticity of deeply-situated microscopic pathological lesions, such as stage 0 or 1 mammary gland cancer, which is difficult by conventional ultrasound elastography.

Keywords: elasticity, photoacoustic, temporal waveform, power spectrum
**Introduction**

Since elasticity of biological tissues is closely related to their pathological states as observed in solid tumors and inflammation-involved tissue fibroses, the measurements of the elasticity are crucial for medical diagnosis. As a conventional method for the measurements of the elasticity, palpation has been used. However, the diagnosis with palpation depends on the subjective decision of medical doctors. In addition, by palpation, the diagnosis for the elasticity of deeply-situated or microscopic pathological lesions is extremely difficult.

As a method for the noninvasive diagnosis for the elasticity of deeply-placed biological tissues, elastography, which is able to create three-dimensional pictures of the elasticity with ultrasound or magnetic resonance, has been developed.\(^1\,^2\) Especially, ultrasound elastography allows for the three-dimensional imaging of the elasticity in real time. Thus, it has been widely used for the clinical diagnosis of disorders such as mammary gland cancer, arteriosclerosis, and liver fibrosis.\(^1\,^3\,^4\) In ultrasound elastography, ultrasound is irradiated to biological tissues in order to measure the distribution of their elasticity from the strain distribution under pressure or from the distribution of the propagating velocity of shear wave. Since ultrasound with submillimeter of wavelength is utilized, the scattering of the ultrasound to
biological tissues is extremely small, leading to the achievement of the measurements in target
regions at a depth of 5–10 centimeters from skin surfaces.\textsuperscript{5–7}

However, in ultrasound elastography, its spatial resolution is in the range from a few
millimeters to 1 centimeter due to the wavelength of utilized ultrasound.\textsuperscript{5–7} The low spatial
resolution makes it difficult to obtain the distribution of elasticity for microscopic pathological
lesions, such as \textit{c.a.} capillaries and lymph vessels with the diameters less than 0.5 millimeter,
which are related to vascular diseases such as diabetes and vitamin deficiency, and 1 millimeter
size of stage 0 or 1 mammary gland cancer.\textsuperscript{8,9} For the detection of such microscopic lesions,
development of a novel method that allows for the measurements on the elasticity of deep
tissues with sub-millimeter spatial resolution has been desired.

Photoacoustic spectroscopic method is an advantageous one for the non-invasive
measurements on tissues-selective positional distribution with sub-millimeter spatial resolution
at a penetration depth of a few centimeters, based on the detection of ultrasound generated by
optical absorption of targets.\textsuperscript{10,11} For these reasons, the method has been tested \textit{in vivo} toward
its application to clinical diagnosis.\textsuperscript{12–14} In addition to the positional distribution, the elasticity
of objects has been studied by photoacoustic method.\textsuperscript{15,16} Wadamori showed the relationship
between the Young’s moduli of objects and the resonance frequency of generated photoacoustic signals.\textsuperscript{15} Hai and co-workers imaged the strains of objects buried into media under an external pressure based on the measurement of their displacement from the changes in the intensity of photoacoustic signals.\textsuperscript{16} However, in the former studies, because the amplitude of photoacoustic signals inversely depends on the modulation frequency of a laser irradiated into objects,\textsuperscript{15} the identification of the resonance frequency for objects with higher elastic moduli is difficult. In the latter, as reported in ultrasound elastography, the application of a normal uniform external pressure \textit{in vivo} is difficult, which makes the application of the reported method to clinical practice difficult.\textsuperscript{17}

Here, we present the acquisition of the information on the elasticity of objects buried into media from the temporal waveforms of photoacoustic signals. In photoacoustic spectroscopic method, a pulsed laser beam is irradiated to an object, then ultrasound (photoacoustic signals) generated by instantaneous thermal expansion, followed by thermal relaxation, is detected. To obtain the information on the elasticity of an object, we focused on the temporal waveform of generated ultrasound, especially its frequency components. Generated ultrasound is a compressional wave generated by the expansion and contraction of local volume in an object.
Thus, it is expected that the elasticity of an object acting as a restoring force to the volume changes influences the frequency components of generated ultrasound. For this verification, the following two samples were prepared. One was that the optical absorbers (sound sources) with different Young’s moduli in the range from sub-MPa to a few MPa were buried at the depth of 2 cm from surfaces of media (sample 1, Fig. 1a). The other was that bodies with different Young’s moduli in the range from about 100 kPa to 1 MPa were buried at the depth of 0.5 cm from surfaces of media (sample 2, Fig. 1b). In sample 2, each body was in contact with an optical absorber with the equal optical absorption properties (Fig. 1b, hemoglobin (Hb) / phosphate buffer saline (PBS) solution).

Experimental

Preparation of sample 1 and 2

Sample 1: As optical absorbers, six black-colored rubbers (L10 × W10 × H3, mm) with different Young’s moduli were prepared (S. I. Fig. 1). A 2 wt% agarose gel was used as a medium. To prepare the gel, a purified agarose powder (Kokusan Chemical) was dissolved in ultrapure water at 100 °C for 15 minutes. The agarose aqueous solution was cooled to 65 °C,
then well stirred for 1 hour for the acquirement of its high homogeneousness. It was gently introduced into an acrylic container. Before the complete gelation of the solution, a rubber was set inside the gel. Water was further added into the container. It completely turned to gel after standing still overnight at room temperature. Each rubber was placed at the depth of 2 cm from the gel surface. The surface of the rubber was polished for the decrease in its surface roughness and was well cleaned for the removal of attached small objects such as dust.

Sample 2: Agarose gels (L10 × W10 × H10, mm) with different concentrations (2, 4, 5, 6, and 8 wt%) were prepared in the same manner described above. Each gel was placed into the center of a 2 wt% agarose gel as a medium (L55 × W55 × H20, mm). A tube was formed by inserting a needle into the gel. Hb (from bovine blood, SIGMA-ALDRICH, H3760) / PBS solution (pH = 7.4, 15 g/dL) was injected into the tube. The diameter of the tube was estimated to be c. a. 0.5 mm.

Young’s moduli measurements of the rubbers and agarose gels

To estimate the Young’s moduli of the rubbers and agarose gels, the yielding stress of each sample was measured by rheometer (Thermo Fisher Scientific Inc., HAAKE MARS III). Each Young’s modulus was estimated with the following equation,
where $E$ [Pa] is Young’s modulus, $\sigma$ [Pa] stress, $\varepsilon$ [–] strain. The equation was fitted to the obtained stress-strain curves. All measurements were carried out at 25 °C.

Photoacoustic measurements of sample 1 and 2

Figure 1 shows the schematic diagram for photoacoustic measurements of each sample. A collimated Nd-YAG pulsed laser beam (Photonic Solutions Ltd., Minilite, wavelength: 532 nm, repetition: 10 Hz, pulse width: 7 ns, power: 3 mW, beam diameter: 3 mm) was incident to each sample at an angle of 66° (sample 1) or 45° (sample 2) to the gel surface. To detect the generated ultrasound, a hydrophone (TORAY, H9C, diameter of sound receiving point: 9 mm, received frequency: 0.5–10 MHz) was set 4 cm above the center of the rubbers with dark-field configuration (Fig. 1a). For sample 2, the hydrophone was located 1 cm above the center of the buried gels (Fig. 1b). Ultrasound detected by the hydrophone was amplified by pulser / receiver (OLYMPUS, 5073PR), then the temporal waveforms of the ultrasound were recorded by oscilloscope (Tektronix, MDO4104-6). The obtained temporal waveforms were Fourier-transformed with Igor Pro 8 to obtain corresponding power spectra. For sample 1, the gel was covered with water for acoustic coupling between the gel and the hydrophone, while for
sample 2, ultrasound gel (JEX Co., Ltd., PROJELLY) was used for the acoustic coupling. All measurements were carried out at 25 °C.

Acoustic characterization of the agarose gels

The acoustic attenuation coefficient of each gel was measured. Each gel was placed between an ultrasound transducer (OLYMPUS, V318-S (center frequency: 0.5 MHz) or V306-SU (2.25 MHz)) used as a transmitter and a hydrophone (TORAY, H9C) as a receiver. Ultrasound gel (JEX Co., Ltd., PROJELLY) was used for the acoustic coupling between the sample and the transducer or hydrophone. The hydrophone was aligned along the axis of the transducer to maximize the intensity of the detected signal. The transducer and hydrophone were placed at the center of gel surfaces. The transducer was operated by pulser / receiver (OLYMPUS, 5073PR) and the transmitted ultrasound was detected by the hydrophone. The ultrasound was amplified by the same pulser / receiver, then its temporal waveform was acquired using oscilloscope (Tektronix, MDO4104-6).

Reflectance of the rubbers

The reflectance of the rubbers was measured with an integrating sphere-installed spectrometer (SHIMADZU CORP., SolidSpec-3700 DUV). Each rubber was cut into the size
of (L30 × W15 × H3, mm) for the measurements. The range of wavelength and its resolution for the measurements were set 400–700 nm and 0.5 nm, respectively. The reflectance was analyzed with the software installed into the spectrometer (SHIMADZU CORP., UVprobe).

Results and Discussion

Firstly, we investigated using sample 1 whether the information on the elasticity of buried objects was extracted from the temporal waveforms of photoacoustic signals. In sample 1, as sound sources, six black-colored rubbers with different Young’s moduli were prepared. From the rheometer measurements, the Young’s modulus of each rubber was estimated to be 0.38 ± 0.15, 0.71 ± 0.17, 0.99 ± 0.13, 1.70 ± 0.21, 1.86 ± 0.17, and 2.03 MPa ± 0.24 (mean ± s. d., n = 8). Figure 2 shows the temporal waveforms of generated ultrasound in each sample. After the illumination of the pulsed laser beam to each sample, the intense signals were observed in the range from 25 µs to 28 µs. Then, the signal intensity returned to the base-line level by 30 µs after the illumination. Because the distance between the rubbers and the hydrophone was 4 cm and the velocity of ultrasound inside agarose gel and water is about 1500 m/s,¹⁹,²⁰ the measurement time for the straight ultrasound wave from the sound source is calculated as about
26.6 µs after the pulse illumination, which was in agreement with the measurement time of the signals. Thus, the signal observed in each rubber was derived from the ultrasound generated by photoacoustic effect.

In general, since a body with higher elasticity has a larger restoring force, it deforms faster. Then, the velocity of generated acoustic wave is faster, leading to the propagation of the wave with higher frequencies. From this principle, it is expected that the photoacoustic signals generated from the rubbers with higher Young’s moduli contain higher frequency components. Figure 3 shows the power spectra obtained by the Fourier transformation of the temporal waveforms in Figure 2. The temporal waveforms were Fourier-transformed in the time range from the onset for the appearance of the first positive peak signal to the return of intensity to base line. The power spectrum for each rubber was obtained in the frequency range of 0–20 MHz. As the increase in the Young’s modulus, it was observed that the power spectrum spread to higher frequencies. As a parameter to present the spread of the spectra toward higher frequencies, the frequency corresponding to the gravitational center of each spectra was evaluated (dashed lines in Fig. 3). Figure 4 shows the dependence of the frequency to the Young’s modulus. The frequency increased up to about 7.5 MHz as the increment in the
Young’s modulus up to about 1.9 MPa. On the other hand, in larger Young’s modulus than 1.9 MPa, the frequencies hardly changed. In our experiment configuration, it was shown that the elasticity of buried objects was able to be identified up to about 1.9 MPa of Young’s modulus from the frequency corresponding to the gravitational center of photoacoustic power spectra.

It has been shown that the power spectra of photoacoustic signals are sensitive to optical absorption coefficients of samples. To examine whether the optical absorption properties of the used rubbers influenced the power spectra, the effective optical absorption coefficients of rubbers buried into the gels ($\mu_{\text{eff}}$) were evaluated. For the estimation of $\mu_{\text{eff}}$, each rubber was placed at the depth of 1, 2, 3, 4, or 5 cm from the surface of a 2 wt% agarose gel. As for sample 1, photoacoustic measurements were performed to the rubber placed at the depth. The dependence of the detected photoacoustic signal intensity to the depth is shown in S. I. Fig. 2. The $\mu_{\text{eff}}$ values of the rubbers were in the range from 1.1 cm$^{-1}$ to 1.2 cm$^{-1}$ ($\mu_{\text{eff}} = 1.1$ cm$^{-1}$ for the rubbers with 0.38, 0.71, 1.70, and 2.03 MPa and $\mu_{\text{eff}} = 1.2$ cm$^{-1}$ for 0.99 and 1.86 MPa). The coefficients were similar in the rubbers. Here, they should be affected by optical attenuation in the gel. Thus, we also evaluated the rubbers’ own optical absorption properties. As shown in S. I. Fig. 1, we used the black-colored rubbers for sample 1. In general, black-colored rubbers
do not have transmission properties for visible light. Thus, the reflectance of the rubbers was measured by a spectrometer. The reflectance of the rubbers was estimated as ca. 3–4 % (S. I. Fig. 3). Because the transmission of the rubbers is almost zero, the degree of the optical absorption was calculated ca. 96–97 %. The remarkable differences in optical absorption properties between the rubbers were not observed. Thus, the optical absorption properties of the rubbers would not influence the differences in the power spectra between the rubbers.

Next, it was examined with sample 2 whether the information on the elasticity of objects in contact with an optical absorber is acquired by photoacoustic measurements. In the photoacoustic studies, blood vessels have been widely used as an optical absorber and the positional distribution of tissues such as mammary gland cancer have been three-dimensionally imaged by probing blood vessels buried into tissues. Then, the instantaneous thermal expansion and relaxation of blood vessels should be influenced by the elasticity of tissues surrounding blood vessels. Thus, it is expected that the frequency components of the generated ultrasound by photoacoustic effect reflect the elasticity of not only blood vessels as an optical absorber but also surrounding tissues in contact with the vessels.

In the preparation of sample 2, Young’s moduli of the buried gels were controlled toward the
medical application of the method presented here, especially the application to the early-case
detection of mammary gland cancer. In mammary gland tissues, it has been reported that the
Young’s moduli were in the range of 20 kPa–300 kPa for normal ones and 200 kPa–1.3 MPa for
cancerous ones. The agarose gels covering the Young’s moduli of the normal and
cancerous ones were prepared. The Young’s modulus of each gel prepared was evaluated with
the rheometer to be 128 ± 38, 323 ± 75, 610 ± 54, 878 ± 24, and 1128 kPa ± 113 (mean ± s. d., n
= 8). Figure 5 shows the temporal waveform of each sample obtained by the illumination of
the pulsed laser beams to the Hb buffer solutions inside the gel. After the illumination, the
intense signal was observed in the time range from 5 µs to 7 µs. Then, the signal intensity
returned to the base-line level by 10 µs after the illumination. Because the distance between
the tube containing Hb buffer solution and the hydrophone was 1 cm, the measurement time for
the straight ultrasound wave from the sound source is estimated as about 6.7 µs after the pulse
illumination based on the velocity of ultrasound inside agarose gel and water. Thus, it was
shown that the signal observed in each sample arose from the ultrasound generated by
photoacoustic effect.

Figure 6 shows the power spectra corresponding to the temporal waveforms shown in
Figure 5. The waveforms were Fourier-transformed in the range from the onset for the appearance of the first positive peak signal to the return of intensity to base line. As the increase in the Young’s modulus, the spread of the power spectrum up to higher frequencies was observed. As with sample 1, the frequency corresponding to the gravitational center of each power spectrum was estimated. As shown in Figure 7, the increase in the Young’s moduli up to ca. 1MHz demonstrated an increasing trend in the frequency, suggesting that mammary gland cancers (Young moduli: 200 kPa–1.3 MPa) were able to discriminate from normal mammary gland tissues (Young moduli: 20 kPa–300 kPa) from the viewpoint of their elasticity.\textsuperscript{25,26}

In sample 2, the generated ultrasound transmitted the inside of the agarose gels, followed by the arrival to the hydrophone. Thus, the photoacoustic power spectra may receive the influence of acoustic attenuation dependent on agarose concentrations and the frequency of generated ultrasound.\textsuperscript{27} We evaluated the frequency-dependent acoustic attenuation coefficients of the used gels. The ultrasound with 0.5 MHz or 2.25 MHz of frequency was irradiated to 2 wt % or 8 wt % of agarose gels with thickness from 1 cm to 5 cm, then the temporal waveforms of the transmitted ultrasound were measured (S. I. Fig. 4a). As shown in S. I. Fig. 4a, by the increase in gel concentration, the intensity in the transmitted ultrasound
decreased. From the dependence of the intensity to the gel thickness, the acoustic attenuation coefficients ($\alpha$) of the gels were estimated. S. I. Figure 4b shows the dependence in the intensity for the 2 wt % or 8 wt % gels in a given frequency of ultrasound (2.25 MHz). From the analysis by exponential functions, the $\alpha$ values for the 2 wt% and 8 wt% gels were estimated to be 0.26 and 0.37 cm$^{-1}$, respectively. As reported previously, the $\alpha$ value increased as the increase in gel concentration.$^{27}$ Moreover, it was examined whether the $\alpha$ value depended on the frequency of irradiated ultrasound to the gel. S. I. Figure 4c shows the dependence in the intensity of the transmitted ultrasound under the irradiation of ultrasound with different frequencies (0.5 MHz or 2.25 MHz) to the 2 wt % gel. The $\alpha$ values for the 2.25 MHz and 0.5 MHz frequency were estimated to be 0.26 and 0.23 cm$^{-1}$, respectively. According to the previous report, in the irradiation of ultrasound with the frequency over 4 MHz to gels, $\alpha$ values increased as in the increase in frequency of irradiated ultrasound, while $\alpha$ values were similar in the cases below 4 MHz.$^{27}$ The similar $\alpha$ values for the 2.25 MHz and 0.5 MHz frequencies were valid, but it is likely that the values depend on the frequency in the irradiation of ultrasound with higher frequencies. It was expected that the frequency-dependent acoustic attenuation coefficients influenced the interpretation for photoacoustic power spectra in higher
frequency regions over 4 MHz. However, in such frequency regions, the elasticity of objects may be evaluated from the apparent peak shift in power spectra based on the difference in the coefficients between objects.

In previous studies, the intensity of photoacoustic signals has been measured for the evaluation of the elasticity of objects. However, the external compression force was applied into objects or surrounding media. In addition, the identification of the elasticity of objects with high Young’s moduli was difficult from resonance frequency of photoacoustic signals. On the other hand, in the method presented here, the temporal waveforms of photoacoustic signals were measured, and then its frequency components were correlated with the elasticity of objects. This technique does not require any external stimulation such as compression force. Furthermore, as shown in Figures 4 and 7, the elasticity of objects up to about 1 MPa was probed in the present experimental configuration, while in the previous method, the elasticity of objects up to about 500 kPa was characterized. The presented method shows that the elasticity of objects buried into media were non-invasively identified in the range up to about 1 MPa from the temporal waveforms of photoacoustic signals and corresponding power spectra. On the other hand, in the present experimental configuration,
the extraction of the information on viscosity of objects is difficult. In general, living matters are viscoelastic. Thus, the measurements of not only elasticity but viscosity of objects have been desired. Although the peak width in power spectra has the information on viscosity, the evaluation of the width is difficult from the measured broad power spectra of photoacoustic signals. Recently, Guzman-Sepulveda and co-workers reported the measurements on storage and loss moduli of hydrogels from the power spectra of light intensity fluctuation in dynamic light scattering. The method may be useful for the extraction of viscous information from photoacoustic power spectra.

It has been reported that the temporal waveforms and corresponding power spectra of photoacoustic signals were affected by optical absorption properties of samples (sound sources) such as optical absorption coefficients. Although photoacoustic measurements of biological tissues and cells such as breast cancers and vascular cells have been widely desired, their optical absorption properties are unknown and different among samples. Thus, the acquirement of the information on the elasticity and viscosity of such samples is needed from their photoacoustic power spectra. We suppose that the present analytical method is limited to the specific cases where the optical absorption properties of samples were similar between the
samples. For applying the present method to the extraction of the information on elasticity and viscosity from unknown samples, it should be necessary to establish the technique that compensates the effect of optical absorption properties of samples in photoacoustic signals.

Summary

The elasticity of objects buried into media was characterized from the temporal waveforms of photoacoustic signals, especially with their frequency components. The bodies with different Young’s moduli were buried into media and their photoacoustic temporal waveforms were examined. The increase in the frequencies corresponding to the gravitational center of the power spectra for the temporal waveforms was observed as the increment in Young’s moduli of the objects. In our configuration for photoacoustic measurements, it was shown that the elasticity of buried objects is able to be identified up to about 1 MPa of Young’s modulus. In addition, it was suggested that the method presented here was also able to be applied into the discrimination between normal and cancerous tissues.
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Figure captions

Fig. 1 Schematic illustration of (a) sample 1 and (b) sample 2 and for their photoacoustic measurements (not to scale).

Fig. 2 Temporal waveforms of ultrasound generated in sample 1. Young’s moduli of each rubber: (a) 0.38, (b) 0.71, (c) 0.99, (d) 1.70, (e) 1.86, and (f) 2.03 MPa.

Fig. 3 Power spectra corresponding to the temporal waveforms in Fig. 2. Young’s moduli of each rubber: (a) 0.38, (b) 0.71, (c) 0.99, (d) 1.70, (e) 1.86, and (f) 2.03 MPa. Each spectrum was normalized by the intensity of peak frequency. The dashed lines show the frequency corresponding to the gravitational center of each spectrum.

Fig. 4 Dependence of the frequencies corresponding to the gravitational center of each spectrum in Fig. 3 to the Young’s modulus of the rubber. For the Young’s modulus and the frequency, mean ± s.d., (n = 8).

Fig. 5 Temporal waveforms of generated ultrasound in sample 2. Young’s moduli of the buried gel: (a) 128 kPa, (b) 323 kPa, (c) 610 kPa, (d) 878 kPa, and (e) 1128 kPa.

Fig. 6 Power spectra corresponding to the temporal waveforms in Fig. 5. Young’s moduli of the buried gel: (a) 128 kPa, (b) 323 kPa, (c) 610 kPa, (d) 878 kPa, and (e) 1128 kPa. Each spectrum was normalized by the intensity of peak frequency. The dashed lines show the frequency corresponding to the gravitational center of each spectrum.

Fig. 7 Dependence of the frequencies corresponding to the gravitational center of each spectrum in Fig. 6 to the Young’s modulus of the buried gel. For the Young’s modulus and the frequency, mean ± s.d., (n = 8).
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Figure 2

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