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Measuring water contents in animal organ tissues using terahertz spectroscopic imaging

KYUMIN LEE,[1] KIYONG JEOUNG,[1,2] SANG HOON KIM,[3] YOUNG-BIN JI,[2,4] HYEYOUNG SON,[5,6] YUNA CHOI,[6] YOUNG-MIN HUH,[2,6] JIN-SUCK SUH,[2,6,7] AND SEUNG JAE OH[2,8]

[1]Graduate Program for Nanomedical Science, Yonsei University, Seoul 03722, South Korea
[2]YUHS-KRIBB Medical Convergence Research Institute, Yonsei University, Seoul 03722, South Korea
[3]Applied Electromagnetic Wave Research Center, Korea Electrotechnology Research Institute, Ansan 15588, South Korea
[4]Gimhae Biomedical Center, Gimhae Industry Promotion and Biomedical Foundation, Gimhae 50969, South Korea
[5]Severance Biomedical Science Institute, College of Medicine, Yonsei University, Seoul 03722, South Korea
[6]Department of Radiology, College of Medicine, Yonsei University, Seoul 03722, South Korea
[7]jss@yuhs.ac
[8]issac@yuhs.ac

Abstract: We investigated the water contents in several organ tissues such as the liver, spleen, kidney, and brain tissue of rats using the terahertz spectroscopic imaging technique. The water contents of the tissues were determined by using a simple equation containing the absorption coefficients of fresh and lyophilized tissues and water. We compared the measured water contents with the difference in mass of tissues before and after lyophilization. All results showed a good match except for the kidney, which has several Bowman's capsules.

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1. Introduction

Terahertz (THz) spectroscopic imaging has emerged as a novel biomedical optical technique due to the favorable properties of THz waves. Several biochemical molecular modes, such as weak hydrogen bonding and lattice vibration, lie across the 0.1–10 THz region, and the energy of THz waves of several meV is so low that biological samples are rarely damaged when exposed to a THz beam, compared to ultraviolet and X-ray beams [1–7]. In particular, THz waves have a high refractive index and absorbance in water, and can, therefore, be used to obtain accurate water concentrations in materials [8]. Thus, THz imaging systems can be used to indicate chemicals in fresh tissues, such as water or ion concentration, as well as physical properties [9–19]. To date, a number of studies have been conducted to investigate the chemical information in normal and abnormal tissues using THz spectroscopy and imaging. Several of these works measured the optical constants of various organs and tumors. Stanly et al. reported that in the case of liver cirrhosis, the differences between abnormal and normal tissues were caused by the changes in tissue structure as well as water concentration [14]. Although water concentration was used to characterize tissues in THz biomedical studies, a direct quantitative investigation of water concentration in organ tissues has rarely been carried out [20]. In this study, we determined the water concentration in organ tissues of rats such as liver, spleen, kidney, and brain tissues using a THz time-domain spectroscopic imaging system. We formulated a hypothesis that fresh tissue consists of two kinds of material: water and substances except water, such as protein and fat and so on [6]. We determined the water contents of tissues using a simple equation containing absorption coefficient before and after eliminating water, and compared the water concentration measured with the difference in weight of tissues with and without water.

2. Materials and method

2.1 Sample preparation

Fresh tissues of liver, spleen, kidney, and brain surgically extracted from five Sprague Dawley rats were used to measure the water fraction of tissues. During the experiment, the sample stage was sealed by wrap to avoid the evaporation of moisture from the tissue. All samples were imaged within 12 h to minimize tissue necrosis that occurs with aging. Following the THz imaging experiment, we lyophilized fresh tissues to eliminate the water inside the tissues. Lyophilization, which is a freeze-drying technique, was used to dehydrate the materials. The technique sublimate directly the frozen water in the materials, decreasing the surrounding pressure. In our work, samples were frozen at approximately −70 °C for 3 days and then kept in the vacuum chamber for 3 days to sublimate the moisture inside the tissues. Dried tissues were formed to pellets to conduct THz spectroscopy using an oil hydraulic equipment with a pressure of 140 kg/cm^2 for 5 mm. The pellets were 8 mm in diameter and approximately 650 μm in thickness.

2.2 Experimental setup

A reflection-mode THz imaging system was used to obtain THz signals reflected from fresh tissues, and a transmission-mode THz spectroscopy system was used to analyze the pellet samples. Both THz systems used a mode-locked Ti:sapphire laser producing 80-fs pulses at an 800-nm central wavelength to generate and detect THz signals. The laser beams were divided into THz signal pump and probe beams by a beam splitter. Laser beams were focused at photoconductive antennas (PCAs) fabricated on low-temperature-grown GaAs (LT-GaAs) antenna and semi-insulated GaAs (SI-GaAs) in the THz imaging system. The generated THz beams were guided by parabolic mirrors and focused on a z-cut quartz window at 31° [11,12]. The focused THz beam diameter on the sample stage was 0.8 mm. The THz pulses were acquired by a fast optical delay line with an amplitude of 37 ps and a frequency of 20 Hz in real time. In the transmission-mode THz spectroscopy system, a p-InAs wafer was used to generate the THz pulse and a lock-in-amplifier was used to measure the current in the LT-PCAs. Dry air was purged into the THz setup chamber to eliminate the effect of water vapor, and the humidity of the chamber remained under 3%.

2.3 Analysis method

The refractive indices and absorption coefficients were extracted from amplitude and phase signals of THz spectrums obtained by fast Fourier transform (FFT). The detailed processes for the transmission system and reflection system are indicated in Ref. 6 and Ref. 11, respectively [6,11]. We used the absorption coefficients to determine the water contents in this work because the difference values of absorption coefficients of each organ tissues were higher than the refractive index. To determine the water concentration in fresh tissues, we considered that fresh tissues comprised of water and biochemical substances without water such as protein, fat, and so on [6]. The water fraction of tissues was determined by two methods: (1) using the absorption coefficients in THz frequency and (2) using the difference in weight of tissues with and without water. First, the water fraction was determined by using the absorption coefficients of the tissues in THz frequency. To calculate the water fraction of tissues, it was considered that tissues consist of water and the other components except water, and that the total absorption by the tissue is the sum of absorption by water and the other components of tissue. The THz absorption coefficient of fresh tissue (α_{fresh}) can be described by the THz absorption by lyophilized tissue ($\alpha_{lyophilized}$) and water (α_{water}) and the water fraction (v_{water}) is given by the following formula:

$$\alpha_{fresh} = v_{water}\alpha_{water} + (1 - v_{water})\alpha_{lyophilized}. \quad (1)$$

It was also demonstrated that the water fraction of a tissue can be calculated by measuring the THz absorption coefficient of the tissue before and after it was lyophilized:

$$v_{water} = \frac{\alpha_{fresh} - \alpha_{lyophilized}}{\alpha_{water} - \alpha_{lyophilized}} \times 100. \quad (2)$$

To verify the water fraction value determined using THz waves, the difference in weight of the tissue sample was measured before and after lyophilization. The water fraction v_{water} can be described as

$$v_{water} = \frac{m_{fresh} - m_{lyophilized}}{m_{fresh}} \times 100. \quad (3)$$

where the weight before and after lyophilization are m_{fresh} and $m_{lyophilized}$, respectively.

3. Result and discussion

Figure 1 shows the visible and THz images of liver, spleen, kidney, and brain tissues, which were placed on the z-quartz window. The THz images of each organ are shown at the bottom in Fig. 1. We used the peak-to-peak values to obtain THz images, and all peak-to-peak values reflected from the sample were normalized by the peak-to-peak value reflected from the quartz window only. The brightness of the THz image corresponds to the reflection ratio, and it implies that the brighter the THz image, the more THz waves that were reflected. The reflectance of the kidney was the highest and the reflection ratio of other organs was similar.

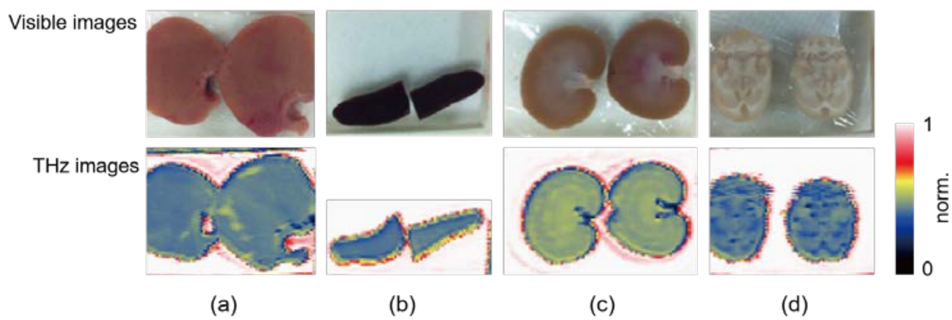

Fig. 1. Visible images (upper side) and THz images (bottom side) of surgically extracted (a) liver, (b) spleen, (c) kidney, and (d) brain.

We obtained and averaged the absorption coefficient of the fresh tissue at 10 points, which were chosen randomly within one sample. Figure 2 showed the absorption coefficients for tissues from 5 rats. Reflection spectroscopy results showed that THz absorption by kidney was the highest, and absorption by other organs slightly decreased in the order of spleen, brain, and liver, as seen in Fig. 1. The absorption coefficients of liver, spleen, kidney, and brain at 1 THz were 176.0 cm^{-1}, 187.3 cm^{-1}, 205.8 cm^{-1}, and 181.7 cm^{-1}, respectively. The absorption coefficient of water at 1 THz was 224.5 cm^{-1}, which is higher than those of the other tissues. This result was similar to that reported in Ref. 13.

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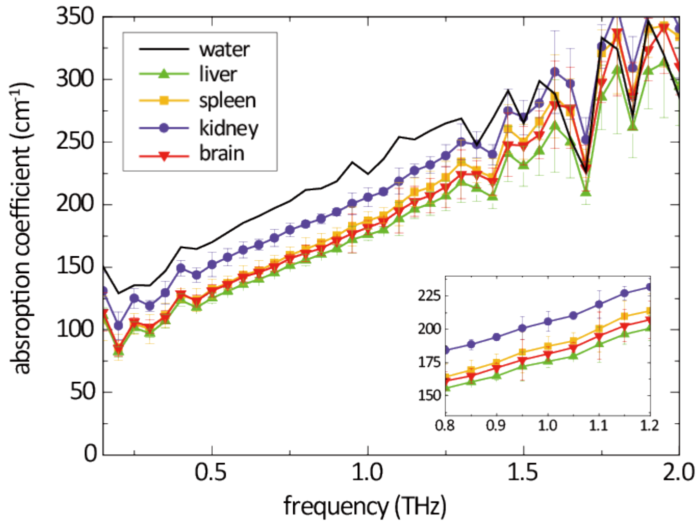

Fig. 2. THz absorption coefficients of fresh liver (green triangle), spleen (orange square), kidney (violet circle), brain (red inverted triangle), and water (solid line). The inset figure shows a magnified graph of the absorption coefficients of organs from 0.8 to 1.2 THz.

Figure 3 shows the absorption coefficients of lyophilized tissue pellets of 5 rats obtained using transmission-mode THz spectroscopy. The absorption coefficients of all tissues were reduced by more than a half because of the elimination of water after lyophilization. Unlike the result in Fig. 2, the absorption coefficients of lyophilized tissue pellets were the highest in spleen compared to other tissues, and the absorption coefficients of pellet decreased in the order of kidney, liver, and brain. The absorption coefficients of lyophilized spleen, kidney, liver, and brain at 1 THz were 45.5 cm^{-1}, 40.3 cm^{-1}, 38.3 cm^{-1}, and 35.0 cm^{-1}, respectively. This result is due to the difference of components of tissues such as lipid, protein, and so on [19].

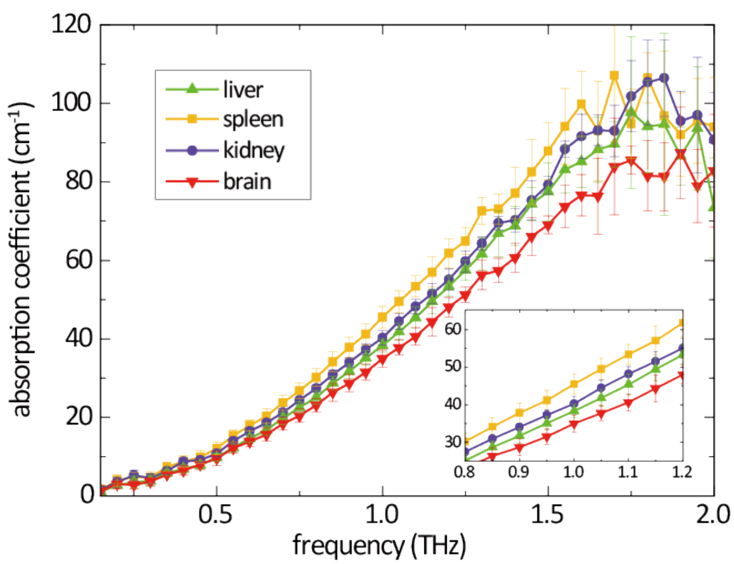

Fig. 3. THz absorption coefficients of lyophilized liver (green triangle), spleen (yellow square), kidney (violet circle), and brain (red inverted triangle). The inset figure shows a magnified graph of the absorption coefficients of organs from 0.8 to 1.2 THz.

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We calculated the water fraction using these absorption coefficients of fresh tissues and lyophilization organ pellets in Eq. (3). Figure 4 shows the THz spectrum of the transmission- and reflection-mode THz system. The amplitude of the lyophilized liver is the highest at 0.3 THz, while that of the fresh liver is the highest at 0.6 THz. Therefore, we used the absorption coefficient at 0.5 THz to calculate the water fraction of each tissue, because the amplitude of the THz signal in both transmission- and reflection-modes were high at 0.5 THz, as shown in Fig. 4. The obtained results were that the water fraction of the kidney was 87.6%, which is the highest among the tissues, and the water fractions of spleen, brain, and liver were 74.8%, 75.8% and 72.0% as shown in Fig. 5, respectively. The results were similar to the THz imaging results shown in Fig. 2.

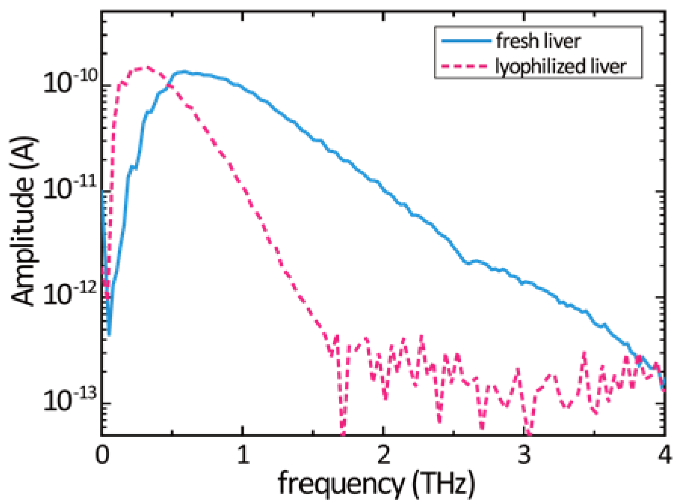

Fig. 4. THz spectrum of the transmission (lyophilized liver) and reflection (fresh liver) THz system.

To verify the water fraction results, we measured the weight of water in the tissues. The masses of organs of five rats before and after lyophilization were measured to determine the weight of water in each tissue. The masses before and after lyophilization were substituted into Eq. (1) to determine the water fraction of the tissue. The water fractions of five rats were averaged. The organ that contained the most water was kidney, and 81.2% of water was removed. Liver had 77.3% of water, spleen had 75.1%, and brain had 77.3% as shown in Fig. 5. We found that the water fraction results in each organ matched well in the error range, with the exception of kidney.

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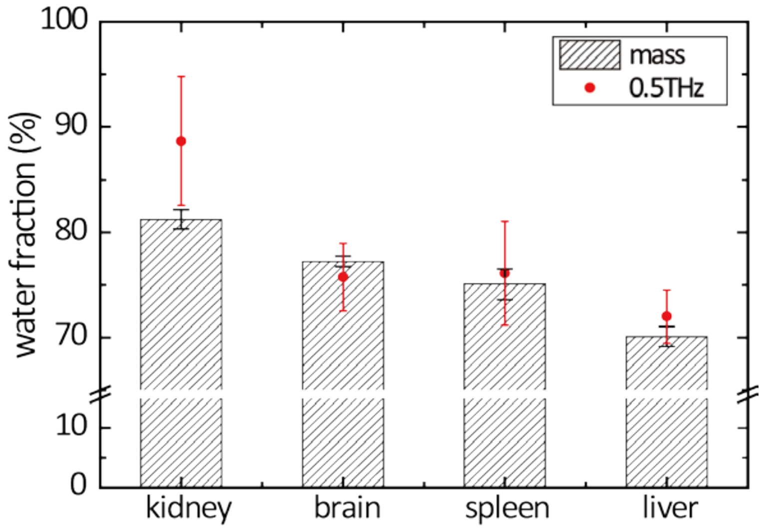

Fig. 5. Comparison the water fractions calculated using weights (bar) and THz absorption coefficients (red point).

Hematoxylin and eosin (H&E) staining images of each tissue were obtained to investigate the difference between water fraction values measured using THz absorbance and mass values, as shown in Fig. 6. Unlike brain, spleen, and liver, which have dense structure, several tens of micron size capsules were found in kidney. The capsules are Bowman's capsules and renal tubes containing urine and comprise a urea fraction of approximately 3.36–6.85% in water [21]. Therefore, we assumed that the mismatch between the THz absorbance and mass values in kidney are due to the structure and chemical composition. The several tens of micron size capsules with urine act as scatterers in the THz frequency and increased the THz absorbance of kidney [22,23].

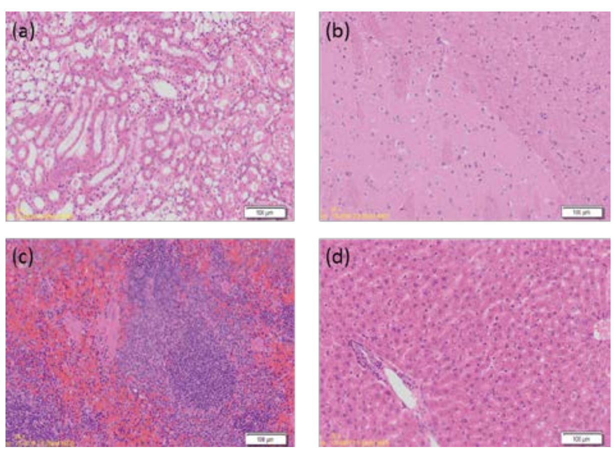

Fig. 6. H&E stained microscopy image of (a) kidney, (b) brain, (c) spleen, and (d) liver. The unit of scale bar was 100 μm.

4. Conclusion

This study demonstrated that the water fraction of tissues can be determined using a THz spectroscopy and imaging system. We quantified the water contents in fresh tissues using absorption coefficients of the tissues before and after lyophilization. The water contents were verified by the difference in mass before and after lyophilization. The water fraction of liver, spleen, and brain obtained by both methods matched well, with the exception of kidney. Using H&E stained microscopy, we observed that kidney, unlike other organs, has a special structure, constituting several capsules containing urine and these capsule structures act as THz scatterers. This result shows that the effective medium theory considering the geometrical conditions should be investigated to characterize inhomogeneous tissues such as kidney in future studies [20,24].

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Disclosures

The authors declare that there are no conflicts of interest related to this article.