Terahertz near-field microscopy of ductal carcinoma \textit{in situ} (DCIS) of the breast

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Abstract

Imaging with terahertz (THz) waves has been expected as a non-invasive/non-staining visualization tool for breast cancer margins during surgeries. Breast cancer is a generic name for a heterogeneous lesion comprising invasive adenocarcinoma, \textit{in situ} adenocarcinoma, most frequently in the form of ductal carcinoma \textit{in situ} (DCIS) and benign tissues. Until now, THz imaging has focused on invasive adenocarcinoma; however, THz analysis of DCIS has hardly been performed. One of the reasons is that the size of an individual DCIS lesion, ranging from 50 to 500 $\mu$m, is typically much smaller than that of an invasive carcinoma. This makes it difficult to identify these lesions by THz imaging, which has only a diffraction-limited spatial resolution of several millimeters. To overcome this drawback, we have developed a scanning point terahertz source (SPoTS) microscope with a resolution of 20 $\mu$m, in which a near-infrared-pump-laser-induced two-dimensionally-scannable point THz source ($\phi_{\text{THz}} \approx \phi_{\text{Pump}}$) generated in a GaAs crystal contacts a sample. In this study, utilizing this state-of-the-art microscope, we mainly performed THz near-field transmission imaging of a paraffin-embedded human breast cancer sample containing invasive carcinoma and DCIS, as a preliminary study. Consequently, for the first time, we succeeded in clearly visualizing a DCIS lesion of $\sim 500$ $\mu$m in the THz images. It was also found that the THz attenuation by DCIS was higher than that by invasive ductal carcinoma. Furthermore, also in a reflection-mode measurement, we successfully obtained a similar outcome to the above transmission-mode one. These results can be caused by the interaction between the THz waves and the cellular density, indicating that SPoTS microscopy may be suitable for DCIS diagnosis.

1. Introduction

Breast cancer is one of the most common cancers in women. More than two million new cases of breast cancer were estimated worldwide in 2018, representing 11.6\% of all the new cancer cases \cite{1}. The main procedure for early stage breast cancer treatment is a breast conserving surgery (BCS), in which only the malignant lesions are aimed to be removed from the breast while preserving the benign tissues \cite{2}. Breast cancer is designated as invasive adenocarcinoma which can be accompanied by \textit{in situ} adenocarcinoma, most frequently in the form of ductal carcinoma \textit{in situ} (DCIS). In a BCS, it is essential to identify the margins of the invasive carcinoma and DCIS. Current American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations consider surgical resection as complete when the invasive carcinoma is not in contact with the inked surgical margins. If DCIS is associated with invasive carcinoma, resection is also complete when the DCIS is not in contact with the inked margins; however, if DCIS is alone, resection is considered complete when the distance separating it from the inked surgical margins is $\geq 2$ mm \cite{3, 4}. Final assessment of the surgical margins can only be performed based on a postoperative histological
examination of an formalin-fixed paraffin-embedded (FFPE) breast tissue. However, this process takes several days, and if a positive margin is found, a second surgery is needed to remove the residual tumor missed during the first surgery. Such a reoperation affects patients physically, emotionally, and financially. Hence, there is an evident need for intraoperative diagnostic tools to accurately assess resection margins in a BCS [5].

Surgeons can currently use various intraoperative diagnostic techniques for a BCS. Intraoperative pathological examinations, such as frozen section analysis and touch imprint cytology, are the most reliable methods to determine tumor margins and can assist a surgeon in deciding whether the tumor resection is complete [2]. However, owing to their complexity and time-consuming nature, only a small number of hospitals use these types of histological methods [2]. In addition, they require experienced-pathologists and are not devoid of false negative results [2]. As non-pathologic intraoperative approaches, radiography and ultrasonography are also currently available [5].

However, a common problem of all the non-pathologic diagnostic techniques currently available is the difficulty in accurately detecting DCIS [5]. DCIS is the proliferation of neoplastic epithelial cells in mammary ducts and lobules. It is a precursor lesion to invasive carcinoma. DCIS can produce micro-calcifications and accounts for 25% of mammographically-detected breast cancers [6]. The natural history of DCIS can lead to invasive carcinoma; therefore, its diagnosis is essential in early stage breast cancer treatment. However, DCIS lesions are small, non-palpable lesions, compared to invasive carcinoma. These lesions are typically scattered in a breast tissue; however, they may sometimes agglomerate to form inconspicuous masses. The size of an individual DCIS lesion ranges from 50 to 500 µm, which makes it difficult to identify surgical margins intraoperatively. Scientists have recently proposed and developed new intraoperative DCIS diagnosis techniques, mainly adopting chemistry-based fluorescent probes [7, 8] or photonics approaches, such as radiofrequency spectroscopy [9], optical coherence tomography [10], diffuse reflectance spectroscopy [11], Raman spectroscopy with autofluorescence microscopy [12], and ultraviolet-photoacoustic microscopy [13].

Terahertz (THz) imaging, which utilizes electromagnetic radiation ranging from 0.1 to 30 THz, has attracted significant attention in the field of cancer detection over the past two decades, owing to its excellent characteristics. THz radiation is non-ionizing owing to its low-photon energy (1 THz ≈ 4 meV) [14], sensitive to the changes in the hydration level/cellular structure (morphology) associated with tumor emersion [15, 16], and less scattered in biological tissues compared to infrared and visible light [17]. Moreover, in the THz frequency band, various bio-related samples present fingerprint spectra derived from low-energy intra/intermolecular interactions, such as hydrogen bonds and van der Waals forces [18]. Until now, numerous researchers have conducted THz imaging of various skin [19], oral [20], gastrointestinal [21], breast [22], and brain [23] cancers. These studies indicate that THz imaging technology can directly visualize carcinous lesions without labelling process which is required in optical imaging, such as fluorescent probes, and has the potential to distinguish malignant tumors from normal tissues more simply, quickly, and accurately than conventional modalities, such as x-ray computed tomography, magnetic resonance imaging, and ultrasound imaging [18]. Particularly for breast cancer, Fitzgerald et al reported in 2006 that THz imaging can discriminate freshly-excised breast cancer tissues, including large DCIS lesions (~several millimeters), from benign ones with high contrast [22]. This study has accelerated the research on THz application to intraoperative diagnostic devices for breast tumors, e.g. accurate understanding of THz signals from breast tissues with a signal/image processing technology [24, 25] and development of practical THz detecting devices in a hospital environment [26, 27]. These studies mainly focused on invasive-type breast cancers, with few being on DCIS lesions [22, 28, 29]. One of the reasons is considered to be the spatial resolution of THz imaging. Because its resolution is diffraction-limited to several millimeters, it is difficult to measure DCIS lesions in the size range of 50–500 µm. In addition to this THz drawback, practical clinical testing in a BCS requires clear margins at the cellular level to be identified extemporaneously during surgery [2, 5]. Consequently, to precisely evaluate the potential of THz technology for DCIS diagnosis, it is undeniably required that a THz imaging system with a micrometer-scale spatial resolution which can be constructed as a miniaturized intraoperative diagnostic device.

To overcome this problem, we have developed a scanning point terahertz source (SPoTS) microscope with a fiber-coupled femtosecond laser source which enables THz near-field imaging beyond the diffraction limit without any sub-wavelength probes [30, 31]. This microscope operates on the basis of a near-field interaction between a sample and a point THz source, which is scanned by a galvanometer. The sample is placed in the vicinity of a GaAs THz-emitter crystal, and the point THz source is created at the irradiation spot of the femtosecond pulse laser beam in the crystal via optical rectification. Because the localized THz wave pulses emitted from the source interact with the sample, THz measurement with a resolution close to the excitation laser wavelength can be realized. Concretely, transmission/reflection THz imaging at a spatial resolution of 20 µm ≈ λ_{THz}/34 (λ_{THz} = 680 µm) and a maximum imaging speed of 4 s/image (128 × 128 pixels) have been achieved [30, 31]. This resolution is top-level among the THz imaging instruments applied
to biological tissues [32]. Utilizing this microscope, we have already succeeded in visualizing a human hair strand of \(\sim 80 \mu m\) [30, 33] and paraffin-embedded human breast tissues of several tens of micrometers [31], and in detecting the concentration of various aqueous solutions with a sensitivity of 1.4 fmol and a solution volume of 128 pl. [34–36]. These are reasonably difficult to realize with conventional THz far-field systems. Thus, SPoTS microscope provides more simple, high-speed, and low-cost way to achieve micrometer spatial resolution and high sensitivity in THz bio-imaging. In the future, as developed with far-field THz system in [27], a miniaturized flexible handheld probe for onsite cancer diagnosis can be constructed by incorporating reflection-type SPoTS microscope [31] with fiber optics.

In this study, utilizing the SPoTS microscope, we mainly performed preliminary experiments of THz near-field transmission imaging and THz time-domain spectroscopy (THz-TDS) of a human breast cancer sample including DCIS at a micrometer-scale spatial resolution. We used an FFPE histological tissue section fabricated on a GaAs wafer as a sample. Consequently, we successfully obtained, for the first time, clear THz images of a DCIS lesion with a size of \(\sim 500 \mu m\), in good agreement with the corresponding conventional histological image. Moreover, our THz near-field time-domain spectroscopy data showed that the THz attenuation was significantly different for DCIS and invasive ductal carcinoma (IDC). On the other hand, a practical intraoperative cancer assessment using THz waves has to be done with a reflection setup because THz radiation is strongly attenuated by water-based objects such as freshly-excised tissues. Therefore, to more clearly demonstrate the potential of the SPoTS microscope for such clinical use, we also carried out reflection-mode THz near-field imaging and THz-TDS of the FFPE cancer sample, and succeeded in obtaining a similar outcome to the above transmission-mode one. These results indicate that SPoTS microscope enables the visualization of small lesions, such as DCIS, and may open the route to THz intraoperative diagnosis to accurately assess DCIS margins.

2. Experimental method

First, we explain the setup of the transmission-type SPoTS near-field microscope used in this study. Figure 1(a) shows the overall schematic of the microscopy system. As the optical source for THz generation and detection, we used a femtosecond fiber laser (TOPTICA FemtoFiber pro IR: center wavelength 1.56 \(\mu m\), maximum power of 350 mW, pulse width of 100 fs, and repetition rate of 80 MHz). The laser beam is divided into pump and probe beams by a beam splitter. The pump beam is modulated via an optical modulator and irradiates a two-dimensional (2D) THz emitter, a (110)-oriented GaAs crystal of 500 \(\mu m\) thickness [37]. As shown in figure 1(b), measured sample is placed directly on this GaAs crystal. Pump pulse beam is focused near the upper surface of the crystal using a focal lens, and intense THz emission is caused at the laser-focusing spot of approximately \(10 \mu m\) by optical rectification. Accordingly, a point THz source is created exactly underneath the sample. Subsequently, the THz pulse beam emitted from this point THz source is transmitted through the sample and focused onto a bowtie-shaped low-temperature-grown (LT-) GaAs photoconductive antenna detector using a pair of off-axis parabolic mirrors. This detector is driven by probe pulse whose wavelength is converted to 780 nm by a periodically-poled-LiNbO\(_3\) crystal. THz imaging can be performed by high speed 2D scanning of the point THz source \(\text{at} i.e. \text{pump laser pulse}\) over the GaAs crystal in the \(X\)-\(Y\) direction with a galvanometer. We can also obtain THz transmission time-domain waveforms of the sample by monitoring the amplitude of the THz pulses while moving a delay stage. For precise alignment and positioning of the THz point source, a pump-laser-reflection image of the sample is also obtained by scanning the laser pulse over the sample and detecting the laser reflection signals with a photodiode. The laser image can be directly compared with the THz image. The current achieved performance of the SPoTS microscope is as follows: 20 \(\mu m\) spatial resolution, \(275 \mu m\) visual field, imaging speed of 4 s per image for \(128 \times 128\) pixels, and detectable frequency band up to approximately 3 THz in the THz-TDS (see the characteristic of typical THz emission waveform from the GaAs crystal described in section 1 in supplementary material available online at https://stacks.iop.org/JPHONTON/2/044008/mmedia). It is noted that, in the present study, THz imaging was performed at image acquisition speeds of 90 s and 10 min per image for \(128 \times 128\) pixels and \(512 \times 512\) pixels, respectively, to maintain a good signal to noise ratio. Detailed information of THz image acquisition process with this SPoTS microscope is described in section 2 of supplementary material. System details can be found in our previous reports [30, 31].

3. Sample information

In our previous study, we have already achieved imaging of a paraffin-embedded human breast tissue sample with a size of several tens of micrometers deposited on a plastic substrate. However, the discrimination of tumors from other normal tissues was challenging, because the sample was not appropriately in contact with
the GaAs surface owing to the surface roughness of the sample \([31]\). Therefore in this study, to effectively observe their interaction with THz waves, we prepared an FFPE human breast cancer tissue directly deposited on a GaAs THz-emitter crystal. Figure 2(a) shows a photograph of a 30 \(\mu m\)-thick FFPE breast tissue section deposited on a 500 \(\mu m\)-thick GaAs (110) crystal as well as its cross-sectional view (inset at the upper right). The formalin-fixation and paraffin-embedding technique enabled the preservation of biological tissues without alteration for a relatively long time \([38]\). This breast tissue sample was excised from a 44-year-old woman and includes DCIS, IDC, and other normal tissues such as normal ducts and lobules as well as adipose and collagen fibrosis tissues. The invasive carcinoma was diagnosed as a hormone receptor...
positive/ERBB2 negative type carcinoma, which is the most common case (70% of patients) among the three major breast cancer subtypes: hormone receptor positive/ERBB2 negative, ERBB2 positive, and triple-negative [39]. It is noted that lipids were purged from the adipose tissues, during the paraffin embedding process. Minor orientation and positioning differences could have occurred during this very precise ultrathin layer assembly. The distribution regions of these tissues were identified using a hematoxylin-and-eosin (H&E)-stained section of the sample obtained from the same tissue as the GaAs-substrate sample, as shown in figure 2(b). In the H&E staining, orange/pink-stained regions depict a cytoplasmic or fibrous environment. Purple-stained areas denote the presence of nucleic acids/nuclei, and white regions correspond to adipose tissues and/or tissue retraction artifacts. In this study, THz imaging was mainly performed on a region, depicted as a surrounded black box in figure 2(a). This region contains numerous DCIS lesions, as can be seen from the corresponding H&E image in figure 2(b).

4. Results and discussion

Figures 3(a)–(c) show the optical-, H&E-stained-, and THz-transmission- images of the breast tissue sample region highlighted in the black boxes in figure 2, respectively. As illustrated in the H&E image, this region includes several DCIS lesions, IDC, and fibroadipose tissues. The each DCIS and IDC structure is highlighted by purple and blue dotted lines and numbered, respectively, and fibroadipose tissues correspond to the orange/pink-stained areas that fill the gaps between the malignant structures. The THz image was acquired at the highest positive peaks of the THz transmission time-domain waveforms with a scanning step size of 2 µm, at a temperature of 19.4 °C and relative humidity of 36%. In this THz image, the DCIS and IDC structures are distinguished from the fibroadipose regions, and therefore good correlation is found between the three images at the almost all cancer structures. (On the other hand, in the shapes of the No.8 and 10 DCIS lesions, there are differences between the H&E image and the THz (and optical) images. This may be due to minor changes in tissue structure that occurred during the preparation of paraffin-embedded tissue slices.) Concretely, the DCIS regions are displayed with dark blue colors, the IDC regions with blue to yellow colors, and the fibroadipose regions with orange/red colors. The dark blue DCIS lesions are also delineated by a smooth orange/red fibrous rim. This indicates that the intensities of the transmitted THz waves are weaker in the DCIS and IDC compartments compared to those in the fibroadipose compartments.

To understand such differences in the THz intensities, we focused on the yellow boxes depicted in figure 3 and conducted THz imaging with a smaller step size of 500 nm. Here, we also performed THz-TDS of the DCIS, IDC, and fibroadipose tissue. Figures 4(a)–(c) show the optical-, H&E-stained-, and THz-transmission- images of the region, respectively. Each tissue compartment is represented similarly to the H&E slide, as presented in figure 3(b). In the THz image, a DCIS structure (No.2, ~ϕ500 µm) is clearly visualized, in good agreement with the corresponding H&E image. It should be noted that the strong THz transmission part in the center of this DCIS may be affected by an abnormal paraffin state, probably arising from the removal of some paraffin in the region during the sample fabrication. As can be seen from the
Figure 3. (a) Optical-, (b) hematoxylin-and-eosin (H&E)-, and (c) THz-transmission- images (with 2 µm scanning step size) of the breast tissue sample highlighted by black boxes in figure 2. The THz image was obtained at the highest positive peaks of the time-domain THz waveforms, and histogram equalization process was applied to the THz image. Additionally, the THz image with an area of $2750 \times 1375$ µm consists of fifty THz images in the size of $275 \times 275$ µm. In the optical- and THz- images, each structure of ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) lesions is labelled with white dotted lines and numbered. In the H&E image, the DCIS and IDC compartments are highlighted by purple and blue dotted lines and numbered, and the fibroadipose region corresponds to the orange/pink-stained areas that fill the gaps between the malignant structures. The yellow boxes correspond to the region shown in figures 4(a)–(c). Optical image in figure 4(a), the corresponding region appears black, which is different from other parts. Furthermore, another DCIS structure (No.3) and IDC (No.1) are also significantly discriminated with weaker THz intensities than the surrounding fibroadipose tissue. To compare the THz signal levels in the three regions of DCIS, IDC and fibroadipose tissue, we extracted line profile along the white dotted line in the THz image, as shown in figure 4(d). The signal intensities of the fibroadipose compartment are stronger than those of the DCIS and IDC compartments. Interestingly, the observed intensities are different for the DCIS and IDC. THz transmission in the DCIS compartment is lower than that in the IDC compartment. Figures 5(a)–(b) present the THz time-domain waveforms and their frequency spectra acquired in the three regions (upper column). The lower column in figure 5(b) presents the DCIS and IDC spectra normalized by the spectrum of the fibroadipose (Fibro) tissue. We collected THz waveforms at several different areas of the DCIS (5 points), IDC (9 points), and Fibro (8 points). The solid curves are their averaged plots, and the shaded areas indicate the standard deviations. The overall shapes of the waveforms are almost identical; the THz waveform in the GaAs-bare region shown in figure S1(a) in supplementary material also exhibits similar shape. In addition, when the positive peaks of the THz pulses are magnified, it is found that the appearance timing of the Fibro peak is 0.04 ps delayed compared to those of the DCIS and IDC peaks. Furthermore, the electric field intensities ($E$) at the highest peak points are remarkably reduced in the order of $E_{\text{DCIS}} < E_{\text{IDC}} < E_{\text{Fibro}}$. In the frequency spectrum data shown in figure 5(b), we can note a similar signal trend for the electric field intensity. Specifically, the three signals are broadly separated over the range of 0.5–2.5 THz, and the transmission of DCIS is weaker than that of IDC in the same range as exhibited by the normalized spectra in the lower column. The absorption peaks observed at approximately 1.1 and 1.7 THz are attributed to water vapor [40]. Similar tendencies were observed in the THz imaging and spectroscopy results measured at different sample region, which includes both the DCIS and IDC compartments highlighted by white boxes in figure 2, as presented in section 3 in supplementary material.
Figure 4. (a) Optical-, (b) hematoxylin-and-eosin (H&E)-, and (c) THz-transmission- images (with 500 nm step size) of the tissue region highlighted by yellow boxes in figure 3. The THz image was obtained at the highest positive peaks of the time-domain THz waveforms, and histogram equalization process was applied to the THz image. Additionally, the THz image with an area of 1100 × 825 µm consists of twelve THz images in the size of 275 × 275 µm. In the H&E image, the ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) compartments are surrounded by purple and blue dotted lines and numbered, and the fibroadipose region corresponds to the orange/pink-stained areas that fill the gaps between those lesions. (d) Line profile along the white dashed line inserted in the THz image (ten points adjacent average-smoothed).
Figure 5. (a) THz transmission time-domain waveforms observed with ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC), and fibroadipose (Fibro) tissue around the sample region in figure 4. (b) Corresponding frequency spectra of them (upper column) and the spectra of the DCIS and IDC normalized by the spectrum of the Fibro (lower column). To extract the above data, we obtained THz waveforms measured at several different areas of DCIS (5 points), IDC (9 points), and Fibro (8 points). The solid curves are their averaged plots and the shaded areas indicate the standard deviations.

Figure 6. Hematoxylin-and-eosin (H&E) images of (a) region I (ductal carcinoma in situ (DCIS)), (b) region II (fibroadipose), and (c) region III (invasive ductal carcinoma (IDC)) at the cellular level, as labeled in figure 4(b).

Numerous studies have discussed the origin of the THz image contrast between breast cancer and normal tissue, indicating that the dielectric properties of the tumor are different from those of benign tissue. Specifically, both the absorption coefficient and the refractive index of breast cancer are higher than those of normal tissue. This tendency is commonly observed in both freshly-excised and FFPE (i.e. hydrated and dehydrated) sample states [41, 42]. It is considered that several factors affect the intrinsic optical constants of malignant tissue: increased water and decreased lipid concentrations caused by tumor-related physiologic changes, increased cell density and structural changes in the tissue (such as increase in the vasculature owing to cancer cell growth), and presence of certain biomarker proteins [22]. In particular, water, which is a strong absorber of THz waves, is assumed to be a dominant factor of the image contrast in hydrated breast cancer tissues [41]. On the other hand, the paraffin-embedding process dehydrates the samples and purges the lipid components. This indicates that the water and lipid factors do not significantly affect the image contrast of the FFPE sample analyzed in this study. Considering the optical constant of paraffin, the absorption coefficient is almost 0 and the refractive index is approximately 1.5 up to 3 THz in the broadband spectrum. Considering these facts, we discuss the results observed in this study below.

First, regarding the extinction, from the THz transmission images in figures 3(c) and 4(c), it can be seen that the transmitted THz intensities in the breast cancer compartments (both DCIS and IDC) are weaker than those in the fibroadipose compartment. This tendency is consistent with the above-mentioned dielectric properties of breast tissues, and similar experimental data have been reported in previous studies on far-field THz imaging of FFPE breast cancer tissues [29, 43]. Furthermore, from the line profile in
Figure 7. (a) Optical-, (b) hematoxylin-and-eosin (H&E)-, and (c) THz-reflection- images (with 500 nm step size) of the tissue region highlighted by white boxes in figure 2. The THz image was obtained at the highest positive peaks of the time-domain THz waveforms reflected at the interface between the tissue sample and GaAs crystal, and histogram equalization process was applied to the THz image. Additionally, the THz image with an area of \(840 \times 840\ \mu\text{m}\) consists of nine THz images in the size of \(280 \times 280\ \mu\text{m}\). In the H&E image, the ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) compartments are surrounded by purple and blue dotted lines and numbered, and the fibroadipose region corresponds to the orange/pink-stained areas that fill the gaps between those lesions. (d) Line profile along the white dashed line inserted in the THz image (ten points adjacent average-smoothed).

figure 4(d) and the time- and frequency-domain spectroscopy data in figure 5, we found that THz attenuation increases in the order of DCIS > IDC > fibroadipose tissue. One of the critical causes of this phenomenon is assumed to be the difference in the epithelial cell density. Figure 6 shows the cellular-level H&E images of the regions I-III labeled in figure 4(b). Initially, in the DCIS compartment, almost all the cells
are neoplastic epithelial cells, as shown in figure 6(a). Second, there is a mixture of neoplastic epithelial cells and fibrous stroma in the IDC compartment, as presented in figure 6(c). Finally, there are no neoplastic epithelial cells, only collagenous tissues with some fibroblasts and adipocytes in the fibroadipose compartment, as presented in figure 6(b). This suggests that the epithelial cell nuclei, which are stained purple, are remarkably more numerous in the order of DCIS > IDC > fibroadipose tissue. The cell nucleus is denser than other cellular components, because it contains numerous nucleic acids in a small area. Therefore, it is considered that the high cell nucleus density enhances the absorption and/or scattering of the THz waves. Indeed, Oh et al reported that THz reflection intensities become high in the high-cell-density regions of an FFPE brain cancer tissue [44]. Hence, it is considered that the difference in the epithelial cell density (DCIS > IDC > fibroadipose tissue) contributes to the aforementioned trend of THz attenuation. Consequently, from the viewpoint of extinction, the observed THz transmission tendency ($E_{\text{Fibro}} > E_{\text{IDC}} > E_{\text{DCIS}}$) may be explained.

Next, regarding the refraction, from the time-domain waveforms in figure 5(a), it is considered that the DCIS and IDC refractive indices are almost the same, being smaller than that of the fibroadipose tissue. However, in the result obtained around the different THz imaging region in figure S3(d) in supplementary material, such a tendency was not observed. These indefinite results are considered to be due to the non-uniformity of the paraffin embedding over the sample or due to the extent of adhesion of the sample to substrate slightly different by place. Therefore, to precisely verify the optical constant, it will be necessary to obtain higher quality FFPE samples and conduct statistical analysis in the future.

Finally, to more clearly show the potential of the SPoTS microscope for practical intraoperative DCIS assessment, we also present preliminary results of THz near-field reflection microscopy and THz-TDS of the FFPE breast cancer sample measured with a reflection-type SPoTS microscope. The details of the reflective microscope can be accessed in our previous paper [31]. The reflection image acquisition process is same as the transmission one described in section 2 of supplementary material. Figures 7(a)–(c) show optical-, H&E-, and THz-reflection- images of the region highlighted by white boxes in figure 2 which includes both DCIS and IDC, respectively. The THz image was obtained at the highest positive peaks of the THz time-domain reflection pulses from the interface between the GaAs crystal and the sample, with a 500 nm scanning step size. In the THz image, it is found that both the DCIS (No.2) and IDC (No.1) compartments are clearly distinguished from the surrounding fibroadipose tissue of which width is approximately 100–200 µm. The higher THz reflection intensities in both the DCIS and IDC compartments than that in the fibroadipose tissue contribute to the image contrast, which is consistent with the above transmission results and previous report [43]. Focusing on the line profile (figure 7(d)) along the white dashed line inserted in the THz image, there are substantial signal differences between malignant and benign tissues. However, unlike the above transmission data, the reflection intensities are almost same in both the DCIS and IDC regions. On the other hand, in the THz-TDS results in figures 8(a)–(b), THz reflection signals at the DCIS are slightly higher than those at the IDC, in both time- and frequency- (over the range of 0.5–1.6 THz)
domain. (The absorption peaks observed at approximately 1.17 THz and 1.66 THz are also due to water vapor [40]). It is considered that these phenomena are also attributed to the difference in the cell density, as explained in the above discussion of the transmission data. Compared to the transmission SPoTS measurement, in the reflection SPoTS sensing, the signal-to-noise ratio is low, and the interaction length of the THz wave with the sample is small. These factors may cause such small THz signal differences between the two kinds of carcinoma. Consequently, also in reflection mode, we could successfully visualize both DCIS and IDC compartments. Although the measurements of freshly-excised (hydrated) samples must be done in the future, the results indicate that the SPoTS method may be a useful intraoperative diagnostic method for DCIS lesions, as preliminary study.

5. Conclusions

As a preliminary study, THz near-field imaging measurements of a human-FFPE-DCIS-sample in transmission mode were mainly performed using a SPoTS microscope with a 20 µm spatial resolution. Consequently, we succeeded in noticeably visualizing a DCIS structure of ∼500 µm in the THz images, for the first time. We also observed that THz attenuation becomes stronger in the order of DCIS > IDC > fibroadipose tissue, by performing THz-TDS. This phenomenon is considered to be primarily due to the difference in the epithelial cell densities of the tissues. Next, we conducted THz reflection imaging of the FFPE-DCIS-lesion on GaAs substrate, by using a reflection-mode SPoTS microscope. It demonstrated that the image contrast mechanism observed in the transmission result also contributes to the contrast in the THz reflection mode image. Furthermore, such contrast may be strengthened when imaging fresh (hydrated) samples, because the water content of the tissue is a dominant factor of the contrast between malignant and benign tissues in the THz image of a fresh tissue. Therefore, as a future study, it is necessary to rigorously and statistically evaluate the THz physical properties of breast cancer tissues by extracting the complex heterogeneous refractive indexes of the tissues and their components (cell, protein, DNA, etc) in both hydrated and dehydrated states. To improve the capability of the SPoTS microscope to identify DCIS intraoperatively, following tasks also need to be addressed as future work: enhancing the spatial resolution to sub-micrometer- (i.e. cellular-) level by adopting a thinner GaAs crystal and high-NA lens for focusing the pump light, improving the imaging speed to semi real time by replacing motor-type galvano lens with resonant-type one which enables higher speed laser scanning and adopting stronger THz emitter, expanding the visual field to a several centimeters level by using a 2D THz detector such as a THz camera, and developing a fiber-coupled flexible handheld diagnostic probe for clinical use by incorporating reflection-type SPoTS microscope [31] with fiber optics and employing small optical components such as a micro-electro-mechanical-systems-driven galvano scanner. Furthermore, with such efforts for a practical clinical use, we envision realizing THz near-field endoscope, as one of the ultimate goals of our research. These challenging attempts can be achieved in collaboration with pathologists, and may evolve SPoTS microscopy into THz cytology available during/after surgery, helping surgeons and pathologists to obtain accurate information of the lesions. Currently, newly emerging THz microscopic sensing systems have opened up unprecedented THz bio-science and medical sensing functions [26, 45–50]. We hope that in the research stream, SPoTS microscopy will be a powerful tool accelerating THz biomedical science and technology.

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The authors declare no competing financial interest.

Ethical statement

Human tissue analysis and measurements were performed in view of the fundamental ethical principles stipulated in the Declaration of Helsinki and its subsequent revisions. Samples were used in accordance with the ethical regulations of the Bergonié Institute Tissue Bank.

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11
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