Terahertz Imaging of Excised Breast Tumor Tissue on Paraffin Sections

Tyler C. Bowman, Student Member, IEEE, Magda El-Shenawee, Senior Member, IEEE, and Lucas K. Campbell

Abstract—This paper presents imaging and analysis of heterogeneous breast cancer tissue using pulsed terahertz (THz) imaging technology. The goal of this research is to validate and standardize a methodology for THz imaging capable of differentiating between heterogeneous regions of breast tumors. The specimens utilized here were obtained from breast tumors diagnosed as triple negative infiltrating ductal carcinoma (IDC). These tissues were fixed in formalin, embedded in paraffin, and cut into sections of three thicknesses: 10, 20, and 30 µm. All tissues were prepared on standard glass slides used in regular histopathology of hematoxylin and eosin (H&E) stained sections. The THz pulsed system is used to scan the two dimensional tissue sections with step size of 400, 200, and 50 µm. The experimentally measured THz fields reflected from single pixels identified in each region of the tumor are validated with the Fresnel reflection coefficient formulation. A variety of signal normalization and processing methods are investigated. The images are also validated with the standard histopathology images. The obtained results of three different tumors demonstrate strong capability of THz reflection imaging mode to distinguish between the heterogeneous regions in the tumor.

Index Terms—Breast cancer, histopathology images, reflection coefficient, terahertz (THz) imaging, tissue characterization.

I. INTRODUCTION

Breast cancer is a leading medical concern among women in the world today [1]. For breast cancer where the tumor is sufficiently small, the preferred method of treatment is for the patient to undergo breast conserving surgery (BCS), also called lumpectomy. This procedure seeks to remove the breast cancer via excision. Additionally, a minimal layer of surrounding normal tissue known as the margin is removed to ensure that no more tumor tissue is left in the breast while maintaining the best cosmetic outcome for the patient. In order to meet both of these goals, it is critical to have a method for assessing the margin tissue that is reliable. In other words, there is a significant need to develop a reliable imaging technique capable of differentiating between cancer, healthy fibrous and glandular (fibroglandular) tissue that directly surrounds the tumor, and healthy fatty tissue of the breast.

The standard process for margin assessment is to prepare slices of the excised tumor tissue for evaluation by the pathologist, who will classify the margin tissue into one of three categories. A positive margin indicates that cancer exceeds the edge of the excised tumor, a negative margin denotes no cancer within 2 mm of the edge, and a close margin is assigned when there is cancer within 2 mm of the edge without exceeding the excision [2]. When a positive margin is detected, the patient is required to undergo a second surgery in order to remove all remaining cancerous tissue. The need for a second surgery creates strain on the time and resources of the medical provider and has negative financial, cosmetic, and emotional impacts on the patient [3].

With conventional lumpectomy procedures, 20%–40% of excisions are found to have a positive margin [2]. Furthermore, when a new cancer growth also known as local recurrence does arise, 75%–90% of cases involve cancerous tissue in the site of the primary surgery [4]. There are several concerns with the use of pathology alone in assessing the tissue for positive margins, as it is difficult to perform a frozen section on all of the margins at the time of surgery. Additionally, the final pathology report on the margin status takes several days to acquire. This delay in particular calls for the development of techniques that will more reliably detect the extent of the tumorous tissue prior to or at the time of surgery.

To date, many techniques have been developed to address the problem of positive margins in BCS. The most common technique for determining the shape and location of a tumor prior to the surgery is mammography, an imaging technique using X-rays. However, there are some limitations when attempting to map the full extent of a tumor with mammography alone, especially in younger women [2]. Magnetic resonance imaging (MRI) is another common method for detecting the extent of a tumor prior to surgery using magnetic coils and a contrast agent. However, the use of MRI in tumor detection does not demonstrate an improvement in the overall occurrence of positive margins and recurrence of cancer following surgery [5]. A number of intraoperative techniques are already in use for attempting to minimize the occurrence of positive margins such as wire-guided marking of the tumor edge, ultrasound and radio frequency detection, and cryoprobe methods to freeze the tumor bulk for better localization of the tumor being excised. However, tumor localization alone has

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T. C. Bowman and M. El-Shenawee are with the Electrical Engineering Department, University of Arkansas, Fayetteville, AR 72701 USA (e-mail: tcbowman@uark.edu; magda@uark.edu).

L. K. Campbell is with the Northwest Arkansas Pathology Associates, P.A., Fayetteville, AR 72703 USA (e-mail: lkc@nwapathology.com).

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not been found to fully address the problem of positive tumor margins [2], [6]. In addition to localization, other techniques attempt to provide assessment of the tumor margin during the surgery. Intraoperative specimen radiography evaluates the excised specimen using portable X-ray mammography, but the technique lacks the specificity to be reliable as a standalone method and requires a radiologist during the surgery [2]. For pathology assessment in the operating room, frozen section analysis (FSA) or touch prep cytology is used to perform rapid frozen pathology or pathology of the margin’s surface cells, respectively [2], [7]. While FSA is relatively cheap overall, it increases the length of the operation, requires the services of a pathologist close to the operating room, and can require large amounts of tissue which could interfere with standard pathology diagnosis and tumor staging. Additionally, frozen artifacts in the tissue can make interpretation difficult [2]. Other methods such as shaving, radiofrequency ablation, and local radiation therapy were proven effective in treating any detected cancerous tissue remaining in the lumpectomy cavity [8], [9].

Based on the above literature, there is a need to investigate new technologies capable of characterizing different regions of excised tumor tissue. The goal of this work is to apply terahertz (THz) imaging to this application. A comparable technology to THz is optical fluorescence imaging or chemical indicator sensing that have shown a potential for margin assessment [6], [10], [11]. However, experimental work in the literature demonstrated a significant enhancement in image contrast when using THz waves compared with near-infrared (NIR) [12]. This phenomenon is due to the longer wavelengths of THz waves compared with NIR and optical radiation. Scattering arises from spatial variations in the refractive index of the tissue, extracellular constituents, and mammalian cells. Photons are scattered most strongly by structures whose size matches the incident wavelength. Thus, scattering in biological materials is strong at visible and NIR wavelengths, and weak at longer wavelengths [13]. As a result, in biological tissue, Rayleigh scattering (i.e., weak scattering) is the major contributor to a THz pulse, whereas Mie scattering (i.e., strong scattering) becomes more important for an optical pulse. Thus the longer THz wavelength makes the scattering of waves in tissue much less than that of optical beams. In other words, biological scattering is particularly weak at THz wavelengths because THz waves are several orders of magnitude larger than most biological structures [13]. Due to this feature, THz tissue interactions are assumed to be an absorption-dominated case, which implies information at greater depth can be obtained with a THz beam [12], [13].

Another advantage is that THz radiation is nonionizing and carries relatively little power. Therefore, it poses little chance of damaging or changing the properties of tissue prior to the pathology diagnosis [14]–[17]. These studies showed promising results of THz time-domain images of freshly excised tumors. Also, it has been suggested in [14] that THz radiation is susceptible to several features of human tissues. In particular, the THz frequency range is known to respond to the density of tissue, the water content of the tissue, the protein structure as it differs between tissues, and the spin of certain diatomic compounds in the body. However, to the best of the authors’ knowledge, there has not been a thorough investigation into what specific components of cancer tissue compared to normal provide the differentiation in the THz range. While water content and density are key indicators of cancerous tissue, the work here will show differentiating contrast in THz imaging of fixed tissue where no water content was involved.

A recent study of single continuous frequency THz images was reported in [18]. Previous simulation and preliminary experimental work were presented in conferences by our group in [19] and [20]. Other research on using THz radiation for different types of cancer and heart disease has also been reported, e.g., liver, lung, skin, and heart tissue [21]–[23]. In all cases, THz imaging has been able to differentiate between cancerous and fibroglandular regions in the obtained images without the need to use contrast agents. This is a key characteristic in future efforts toward a fast and effective imaging technique for cancer margin assessment.

This work focuses on establishing and validating a THz reflection imaging methodology for differentiating between different regions in paraffin sections of fixed (dehydrated) cancerous breast tumors. As known, breast tumors can have significantly different morphology due to several factors, such as women’s age, race, genetic profile, heterogeneity, cell density, etc. This work is also addressing the challenging issue of imaging triple negative tumors, known for their high heterogeneity. In addition, tumors from women of different age are considered here. The pulsed THz system used here provides a frequency range from 0.1 to 4 THz. Visual correlation between the obtained THz and histopathology images demonstrated good agreement, in addition to validating experimentally measured THz fields with the theoretical reflection coefficient of multilayered sample. Although this research may overlap with some of the published work [14]–[17], it expanded the investigations to different venues that, to the best of our knowledge, were not investigated before. The novelty of this work is in demonstrating the contrast between cancer and normal tissue without the presence of water. The goal is to denote the distinction between the heterogeneous regions of triple negative breast tumors, investigate the effect of tissue section thickness on THz reflection imaging, and analyze experimental THz reflection data versus closed form expressions. The observation of the contrast in tumor tissue in the absence of water is of great significance to THz imaging technique.

It is important to emphasize that this work used formalin fixed, paraffin embedded breast cancer tissue, which can be purchased from biobanks, to allow for examining and developing the imaging technique without using valuable freshly excised tissue, which is much harder to obtain. It is well known that the water content of the tissue does have a significant contribution to the electrical properties of the tissue in the THz range. In particular, the properties of fresh tissue up to 2 THz reported in [16] indicate somewhat higher refractive index and significantly higher absorption coefficients compared with those of fixed tissue [24], [25]. Thus, any contrast seen in imaging fixed tissue to be shown in this work would be further strengthened when imaging freshly excised tumors in the THz frequency band.

In the future phase of this research, we will apply for Institutional Research Board protocol through collaboration.
with the local hospital to obtain freshly excised breast cancer tumor tissue.

This paper is organized as follows: the system setup and methodology will be discussed in Section II, theoretical model and validation with experimental data will be presented in Section III, breast tissue imaging results and correlation with histopathology images will be presented in Section IV, and conclusion, discussion, and future work will be discussed in Section V.

II. SYSTEM SETUP AND METHODOLOGY

A. Pulsed THz System

TPS Spectra 3000 model is used in this work. A simplified diagram of the system in the reflection mode is shown in Fig. 1. This system produces a signal using a Ti:sapphire femtosecond laser (800 nm) to excite a biased GaAs antenna, which subsequently emits a time-domain THz pulse. Upon taking the Fourier transform of this pulse, a frequency-domain signal with a spectral range from 100 GHz to 4 THz is obtained as shown in Fig. 2. For the results presented in this work, the reflection imaging module (RIM) is utilized to scan \( \sim 2\text{ cm} \times 2\text{ cm} \) two-dimensional (2-D) tissue sections. Micro-motors on the module permit a variable step size with minimum value of 50 \( \mu \text{m} \) when raster scanning the sample.

B. Imaging Calibration

Prior to obtaining the image, a reference and baseline are selected. For the reflection mode, the reference could be either a gold mirror providing perfect reflection or a point on the same glass slide next to the tissue. The baseline is selected as air to provide the measurement of the system noise inside the compartment where the tissue samples are illuminated with the THz pulse. A flowchart of the standard calibration and signal processing for the time-domain signal is shown in Fig. 3.

The focus point of the signal is set to provide a peak reflection at the surface of the sample in order to obtain the best resolution for the image. For thin tissue sections, no depth information can be obtained, but this functionality can be expanded upon using thicker tissue sections. A main condition in the available system is that the surface of the tissue needs to be perfectly flat. The RIM platform can be leveled to assure a horizontal flat surface such that the signal reaches all points of the surface at the same time, ensuring that the entire image is in focus. However, tissue surfaces cannot be perfectly flat, so some errors are inherently introduced in the images due to surface roughness.

In order to obtain a THz image, the sample is raster scanned to measure the reflected waveform at each step in a defined 2-D space \((x - y)\). From the collected reflection measurements, the THz image is constructed from the values of the reflected time-domain pulse at each pixel of the sample. This pulse can also be normalized to the maximum value of the reference pulse (e.g., the mirror or the glass slide). Since the signal may be subject to effects from system noise and background reflections, a deconvolution process is utilized here as outlined in Fig. 3. This process can be employed with either the gold mirror reference or the glass slide reference in order to minimize both of these noise effects. Since this operation was performed in the frequency domain, a low-pass (LP) filter was implemented prior to obtaining the new sample signal in order to avoid low signal errors at higher frequencies of the reference. In comparing images obtained in this work via deconvolution using the mirror reference or the glass slide reference or just simple normalization, little overall difference was observed. However, using the deconvolution process provided slightly higher contrast between the different tissues. More advanced deconvolution techniques and regulated methods such as Tikhonov regularization and Wiener filtering could also be considered for further medical imaging enhancement [26], [27].
C. Tissue Preparation

The tissue samples used here were obtained from two different biobanks: the Cooperative Human Tissue Network (CHTN) division at the University of Alabama and the National Disease Research Interchange (NDRI). Uniformly flat tissue sections of 10, 20, and 30 µm thickness were fixed in formalin and embedded in paraffin. Also, standard hematoxylin and eosin (H&E) stained pathology slides of 4–5 µm were cut from the same blocks in order to provide the histopathology images for the purpose of validation. Three breast tumor blocks were obtained from patients at ages of 40, 46, and 54 years. The tumors of the first two patients were diagnosed as triple negative invasive ductal carcinoma, which indicates highly heterogeneous cancer adjacent to normal tissue. These two samples were obtained from Caucasian females who underwent mastectomy. The third block was obtained from a black woman also using radical mastectomy.

We define these samples here as: Sample 1, 10 µm thickness obtained from 40 years old patient; Sample 2, 10 µm thickness obtained from 46 years old patient, and Sample 3, 20 µm and 30 µm thicknesses obtained from 54 years old patient. Samples 1 and 2 were provided by the CHTN while Sample 3 was provided by the NDRI. The histopathology images presented for all samples were provided by the Northwest Arkansas (NWA) Pathology Associates Lab.

III. THEORETICAL MODEL AND VALIDATION

A. Reflection Formulation in TE/TM Polarization

As shown in Fig. 1, the RIM setup is based on oblique incidence angle $\theta_1$, which is 30° in this system. An important step in making use of the reflection imaging is to validate the measured reflected THz signal at a single point with the well-known reflection coefficient of multilayered lossy media. The configuration shown in Fig. 4 shows the TM polarization (i.e., parallel, or $p$-polarization) case. While the TE case is also considered here but the configuration is not shown.

The reflection coefficient is obtained at single point on the tissue interface with air and also at a single point on the glass interface with air. Both points are marked by $x$ in Fig. 4. The reflected field expressions are obtained following the procedure in [28] and [29].

Dividing the reflection coefficient from the tissue to that from the reference point obtains the following expression:

$$
\frac{E_{\text{sample}}}{E_{\text{ref}}} = \frac{\rho_{T,12} + \rho_{T,23} e^{-j2k_3 \cos \theta_2 d_2}}{1 + \rho_{T,23} e^{-j2k_3 \cos \theta_2 d_2}} e^{-j2k_1 \cos \theta_1 d_1} \times \frac{1 + \rho_{T,13} e^{-j2k_3 \cos \theta_3 d_2}}{\rho_{T,13} + \rho_{T,31} e^{-j2k_3 \cos \theta_3 d_2}} e^{j2k_1 \cos \theta_1 d_1},
$$

where $E_{\text{sample}}$ and $E_{\text{ref}}$ are the magnitudes of the electric fields reflected from the tissue interface and the reference point, respectively. The thickness of the tissue and glass is denoted by $d_1$ and $d_2$, respectively, as shown in Fig. 4. The incident angles $\theta_1$ and the transmitting angles in regions 2 and 3 ($\theta_2$ and $\theta_3$) are related through Snell’s law (given in Appendix A). The complex wavenumber $k_1$, $k_2$, and $k_3$ are given by $(\omega \tilde{n})/c$ where $\omega$ is the angular frequency and $c$ is the speed of light. The symbols $\tilde{n}_1$, $\tilde{n}_2$, and $\tilde{n}_3$ are the complex index of refraction of regions 1, 2, and 3, with $\tilde{n}_1 = 1$ for air.

The expressions $\rho_{T,j}$ in (1) are given in Appendix A for the TE and TM cases, where $i$ and $j$ are the indices of the regions 1, 2, 3, or 4. Experimental THz measured electric fields will be compared with fields calculated using (1) and will be presented in Section IV.

IV. THz RIM IMAGING RESULTS

A. Imaging of 10 µm Thickness Tissue Samples

In this section, the THz images of Samples 1 and 2 are presented. Each of the two samples was imaged at a step size of 400 and 200 µm to obtain the initial and final images, respectively. Scanning at a smaller step size of 50 µm is utilized to zoom in on interesting regions on the tissue section of Sample 1. These regions were based on the histopathology images, as will be discussed later in this section.

In this work, scanning $2 \times 2$ cm sections using 400 µm step size requires an average of 4 min, while the raster scanning time increases in proportion to decreased step size depending on the scanned area. In addition to obtaining the THz images in the time domain, frequency-domain images can be produced at any selected frequency in the band from 0.1 to 4 THz to possibly acquire better contrast at a single frequency. All experimental THz data here are collected using the system and then exported to MATLAB for postprocessing as described in Section II.

The THz images obtained using the reflection mode are shown in Figs. 5–7. In addition, the associated low-power pathology images are shown in the same figures. In these figures, the color bar of the time-domain images shows the electric field amplitude of the deconvolved pulse at each point on the sample. Additionally, for each sample THz frequency-domain...
images are presented as well. The glass was used as a reference in all images. In these figures, all THz images are obtained using a step size of 200 µm.

The results of Fig. 5 show the images of Sample 1, taken from the 40-year-old Caucasian woman via mastectomy. The postsurgery pathology report of this sample described the tumor as infiltrating ductal carcinoma (IDC). Furthermore, the cancer was defined to be poorly differentiated, indicating a low amount of distinction between cancerous and noncancerous tissue in the sample. A macroscopic low-power histopathology image obtained here is shown in Fig. 5(a) denoting the general regions of the tissue types observed in this sample using the stained slide. The image shows regions of IDC, fibroglandular (fibro), and fatty tissue.

It should be noted that the pathology examination used to define the regions in all tumor tissues utilized much more high-power pathology in order to examine the tissue on a cellular level and define the different regions of the tissue. Due to space limitation, the high-power pathology images will not be shown for all samples presented here. However, some critical border regions ①, ②, ③ and ④ [see to-scale rectangular markers in Fig. 5(a)] are demonstrated with high-power images. It should be emphasized that the highly heterogeneous nature of the tissue in this sample and its small thickness of ~10 µm make the regions more challenging to distinguish in Fig. 5(b). However, the THz images obtained using the smallest scanning step size of the system (50 µm) for the border regions ①–④ are indeed highly correlated to the associated high-power pathology images shown in Fig. 5.

In Fig. 5(a), the fibroglandular tissue exhibits different levels of density, with the low-density tissue is defined by a mixture of fibro and fatty tissue. In particular, the darkest colors

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Fig. 5. THz image of Sample 1, 40-year-old Caucasian woman diagnosed with poorly differentiated infiltrating ductal carcinoma (IDC). (a) Low-power pathology image used for correlation. Selected locations of high-power pathology of borders between regions are denoted by ① between fatty and IDC and by ②, ③, and ④ between IDC and fibro. (b) THz time-domain image. (c) THz frequency-domain image at 1.5 THz. (d) THz frequency-domain image at 2.0 THz. THz images in (b)–(d) are scanned at 200 µm. High-power pathology images at 100× magnification are shown for ①, ②, ③, and ④ borders compared to the frequency-domain images at 1.5 THz using 50 µm scanning step size.

Fig. 6. THz high-resolution images using 50 µm step size for: (a) Region I and (b) Region II, labeled in Fig. 5(a).
represent the IDC (cancer) regions, the lighter colors represent the healthy fibro glandular regions, and the almost white colors represent fatty tissue. Fig. 5(b) provides the time-domain THz image, while Fig. 5(c) and (d) demonstrates the frequency-domain images at 1.5 and 2 THz. The results also show that IDC regions reflected a higher magnitude of the electric field compared with the fatty or fibro regions. The IDC tissue appeared distinct as the high reflection indicated by dark red compared with the lower reflections fatty or fibro regions.

The results also agree with the original pathology report describing the tumor as poorly differentiated, as the contrast between adjacent regions of tissue is fairly low and there is a significant amount of mixed IDC and fibro tissue as well as low-density fibro tissue present in the sample.

In addition, two interesting regions in Fig. 5 are selected to be more closely investigated. The first region is located at the margin of the tissue section where the IDC tissue is denoted on the upper left side of the figure [labeled as I in Fig. 5(a)]. The second region is selected where the histopathology image in Fig. 5(a) shows a different tissue region to the three other regions defined in the pathology [labeled as II in Fig. 5(a)]. This region is diagnosed by the pathologist as lymphoid aggregate surrounding carcinoma tissue in the core (the dark purple oval in Fig. 5(a)). Therefore, high-resolution THz images are produced focused on these regions I and II, as shown in Fig. 6(a) and (b). In this case, the THz images are obtained upon scanning the regions using the smallest step size of 50 µm. The resulting images are presented in the frequency domain at 1.5 THz in Fig. 6(a) and (b). These tissues appeared also well differentiated with the IDC tissue providing a higher reflection than the fibro and fatty tissues. In Fig. 6(b), the lymphoid aggregate surrounding the carcinoma tissue is also clear in the THz image.

The observed smearing effect in the THz images in Fig. 6(a) and (b) is likely attributable to the interference in the reflected signal from the tissue interface and the glass slide below the tissue due to the small thickness of the tissue (10 µm). This problem is predicted to improve when thicker sections of tissue are used as will be shown later in this work. The high-resolution results of Figs. 5–6 demonstrate the potential capability of THz imaging for differentiation between tissue regions at smaller scale without the need for a contrast agent. It should also be noted that high-power pathology does offer much higher resolution than THz imaging.

The results for Sample 2 obtained from the 46-year-old Caucasian woman via mastectomy are shown in Fig. 7. The pathology report of this section performed after surgery diagnosed the tumor with IDC. The low-power pathology image of the H&E stained slide is shown in Fig. 7(a). This image further defines regions of IDC and fibro tissue that were identified by the pathologist. It is noted that the IDC region (cancer) almost occupies the whole left side of the tissue shown in Fig. 7(a) (dark color), while the healthy fibro glandular tissue occupies the right side (light color). The time-domain image is shown in Fig. 7(b) with a color bar scale of the deconvolved electric field amplitude ranging from 0.02 to 0.027. The THz frequency-domain images at 1.5 and 1.75 THz are shown in Fig. 7(c) and (d). As clearly shown in Fig. 7, the frequency-domain images add more contrast to distinguish between IDC and fibro regions. This distinction was not as clear in the time-domain image of Fig. 7(b). As mentioned earlier, the frequency-domain images can be obtained in postprocessing of collected data. The comparison between the histopathology image of Fig. 7(a) and the obtained THz images in Fig. 7(c) and (d) demonstrates a strong correlation. In these figures, the IDC cancerous region reflected the highest electric field in the tissue and is clearly distinct from fibro tissue region, consistent with the results of Fig. 5. The dark
red color at tissue boundaries with paraffin observed in the THz images occurred at places where tissue is separated from the glass slide, indicating that the observed strong field is due to a high reflection from the glass itself beyond the range of values reflected by tissue. In Fig. 7(c), the high reflection observed in the upper right corner of the frame represents reflection from just paraffin on glass where no tissue is present.

While the 10-µm thickness of the tissue samples has rendered the THz imaging rather challenging, the strong correlation with pathology images is noticed. On the other hand, and in agreement with the literature [21], preparing thick tissue sections requires more elaborate procedures from the histopathology lab to assure uniform flat thick tissue sections that are well adhered to glass slides.

B. Validation of THz Reflected Fields With Theoretical Model

The samples used earlier to produce the THz images included 11 slides each. All slides were scanned producing similar images, but the results presented in Figs. 5–7 were for slide #3 in both samples. The experimentally measured electric fields reflected from the tissue interface normalized to that reflected from the reference point, as shown in Fig. 4, are compared with the theoretical model of (1). There are some assumptions made here to implement this comparison. The formulation of (1) is based on plane wave excitation to infinitely large flat homogeneous surface. However, in the experimental measurements, the incident THz beam is a pulse and not a plane wave, and the single point on the tissue was identified inside a relatively small homogenous region. This assumption is more accurate at higher frequencies where the spatial spot size of the beam is smaller.

The fields in this model are calculated using the index of refraction $n$ of glass and for different regions of tumor tissues as IDC, fibro, fat, etc. These values were obtained from spectroscopy measurements reported in [24] and [25]. For consistency, this comparison is also made for slide #3 in Samples 1 and 2. The polarization of the experimentally collected THz signals using the system could not be confirmed due to the use of a complex mirror setup in the hardware as shown in Fig. 1. Although we can assume TM polarization here based on the normal incidence configuration of the spectroscopy module of the system [25], the comparison is conducted for both the TM and TE polarizations of the model in (1). Only the results for Sample 1 are shown in Fig. 8, but all comparison results similarly demonstrated good agreement.

The results of Fig. 8 show that the magnitude of $E_{\text{sample}} / E_{\text{ref}}$ for TE and TM modes, calculated using (1), are almost the same due to the small incidence angle of the system (30°). The observed oscillations in the TE and TM mode plots can be explained by the multiple reflections in the glass slide that attenuate rapidly as the frequency increases. The glass slide has 1 mm thickness while the tissue section has much smaller thickness of 10 µm making the reflection from the configuration to be dominated by the glass. Therefore, as observed in Fig. 8, the ratio $E_{\text{sample}} / E_{\text{ref}}$ is oscillating around a value of 1. By contrast, there are relatively small observed oscillations for the measurement plot due to the fact that the time-domain signals collected using the system are time gated, leading to truncation of some of the multiple reflections. Depending on the frequency of the signal and the length of tissue sections, the edges of the tissue could create surface wave excitations that produce unpredictable oscillation patterns in the received signal. While it is known that the multiple reflections in the tissue and glass cause some oscillations, it should be noted that there is a possibility of some of the oscillations seen in Fig. 8 being due to the effect of these surface waves.

The experimental plot is the result of averaging over five single points on the tissue in the IDC, fat, and fibro regions. The experimental data demonstrate good agreement with the calculated TE and TM models throughout the range 0.25–1.3 THz. At frequencies higher than 1.3 THz, the glass becomes significantly attenuating to the THz signal making the measured spectroscopy data unreliable as reported in [24] and [25]. Also, it is observed that the measured data in Fig. 8 were not in good agreement with the model at frequencies lower than 0.25 THz. This disagreement is due to larger size of the spatial THz spot on the tissue at lower frequencies, while the incident signal in the model is assumed to be a plane wave at all frequencies. Fig. 8 shows that the model and experimental results differ in the fibro and also in the fatty tissue compared with the IDC results, where they are in very good agreement. This can be explained in that the electrical properties of the IDC, fibro, and fatty tissues were obtained using spectroscopy measurements based on averaging several points in each region. Due to the high heterogeneity of the tumor of Fig. 5, the electrical properties used in the models could be higher than the true values due to random inclusion of points from cancerous regions. This fact made the model results appear at higher values compared with the experimental data for both fibro and fatty regions. In all cases, these errors are ~2.7%–5.4%. The measurements in Fig. 8 are raw data without using any type of filtering. In general, the measurements and the model are in good agreement in all samples except at frequencies below 0.25 THz.

C. Imaging of 20 and 30 µm Thickness Tissue Samples

In this example, the tumor presented in Fig. 9 is different from the previous examples. The tumor here has a well-circumscribed border where the IDC and fibroglandular tissue have very little proliferation into each other.

In testing multiple tissue thicknesses, the adhesion of tissue of thickness more than 30 µm was a challenge leading to non-flat tissue surface on the glass slides. Therefore, in this section, we present THz images of Sample 3 at two thicknesses 20 and 30 µm as shown in Fig. 9. The same scanning procedure used for Samples 1 and 2 is used here (i.e., 200 µm step size). Both the 20 and 30 µm sections were cut from the same tumor block; therefore, we present one histopathology image cut of thickness 3–4 µm and at a depth between these two sections. The pathology report identified two regions in this tumor as IDC and fibro as shown in Fig. 9(a).

In this figure, the scattered spots within the fibroglandular tissue indicate benign lobular and ductal tissues of the breast that are not related to the region of cancer. The time-domain THz images are shown in Fig. 9(b) for the 20 µm section and in Fig. 9(c) for the 30 µm section. In Fig. 9(b)–(g), the scattered
pinpoint spots in the THz 20 and 30 µm images are due to regions where the tissue was breaking (i.e., no tissue on glass) causing high reflection (red spots) or due to separation of tissue from the glass producing air pockets beneath the tissue causing low reflection (blue spots). Both cases are problems that arise from the lack of adhesion when working with thicker sections of tissue. In the THz images of the 20 µm section, lobular tissue (healthy tissue containing glands and ducts) can be seen as red colors indicating reflection similar to cancer, which is a false positive. However, the lobular tissue areas are not seen as cancer in the 30 µm images, which shows the improvement of the imaging with the thicker tissue section. This lobular tissue is seen as dark purple spots inside the fibroglandular tissue in Fig. 9(a).

Within the IDC region of Fig. 9(a), a less pigmented central region (slightly dark pink) can be seen on the right side of the image compared with a much darker pigmentation (purple) region near the border between the IDC and fibroglandular tissue. This pink region is most likely necrosis (dead tissue) or fibrosis (connective tissue formed by the tumor) that is typically found on the interior of reasonably large tumors. It is notable that the greatest distinction in the THz 30 µm images is seen in the active cancer cells at the margin between the IDC and fibroglandular regions. The reason that this distinction exists in the 30 µm slice but not the 20 µm slice is likely due to the effect that increased thickness has on the reflected signal.

Upon comparing the THz images presented in Fig. 9 against those presented in Figs. 5 and 7, we can qualitatively conclude that the increased thickness in tissue sections has led to better contrast in the THz reflection mode images. However, one has to be cautious with this conclusion as it is not confirmed that the tumor of Sample 3 was triple negative as those of Samples 1 and 2.

V. DISCUSSION AND CONCLUSION

The results of this work demonstrate that THz reflection imaging successfully distinguished between cancer and non-cancer breast tissue even when the tissue section was dehydrated heterogeneous and as thin as 10 µm. The presented THz images showed that cancerous regions are greatly discerned from normal (fibroglandular and fatty) tissue in all three tumors considered here. Further comparison between the high-power pathology and the THz images obtained using fine scanning steps of 50 µm demonstrated strong correlation even for dehydrated heterogeneous thin section tissue. It is believed that this technology has a potential for tumor margin assessment in the future.

Ongoing research is to expand the THz imaging methodology to include the transmission mode. One of the goals is to identify which imaging module would provide better images. Also, imaging excised fresh tissue is a step further in this research. It is important to emphasize that obtaining excised tissue from lumpectomy is challenging due to the fact that these surgeries are conducted when the tumor is small in order to conserve the breast. As a result, most of the excised tissues are needed for diagnosis and staging the cancer, meaning that obtaining adequate samples will require the direct cooperation of pathologists and surgeons.

APPENDIX

Snell’s law is given as [28], [29]

\[ n_1 \sin \theta_1 = n_2 \sin \theta_2 = n_3 \sin \theta_3. \]  \hspace{1cm} (A1)

The expressions \( \rho_{T,i,j} \) in (1) are given here for the TM and TE cases, respectively, with \( i \) and \( j \) the indices of the regions 1, 2, 3, or 4, [25]

\[
\rho_{TM,i,j} = \frac{n_i \cos \theta_j - n_j \cos \theta_i}{n_i \cos \theta_j + n_j \cos \theta_i} = \frac{n_i^2 \sqrt{n_j^2 - \sin^2 \theta_j} - n_j^2 \sqrt{n_i^2 - \sin^2 \theta_i}}{n_i^2 \sqrt{n_j^2 - \sin^2 \theta_j} + n_j^2 \sqrt{n_i^2 - \sin^2 \theta_i}}, \quad \text{and} \quad \rho_{TE,i,j} = \frac{n_i \cos \theta_j - n_j \cos \theta_i}{n_i \cos \theta_j + n_j \cos \theta_i} = \frac{\sqrt{n_i^2 - \sin^2 \theta_j} - \sqrt{n_j^2 - \sin^2 \theta_j}}{\sqrt{n_i^2 - \sin^2 \theta_j} + \sqrt{n_j^2 - \sin^2 \theta_j}}. \ \hspace{1cm} (A2)\]
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REFERENCES


Tyler C. Bowman (S’11) received the B.S. and M.S. degrees in electrical engineering from the University of Arkansas, Fayetteville, AR, USA, in 2012 and 2014, respectively. He is currently pursuing the Ph.D. degree at the University of Arkansas with a primary research focus on terahertz imaging and characterization of cancer tissue in the Terahertz Imaging and Spectroscopy Lab as part of the greater Computational Electromagnetics Lab.

Mr. Bowman is a member ofEta Kappa Nu Electrical Engineering Honor Society and Tau Beta Pi Engineering Honor Society. He served as a National Science Foundation GK-12 Fellow from 2012 to 2013. He was the recipient of NSF Graduate Research Fellowship in 2013.

Magda El-Shenawee (SM’02) received the Ph.D. degree in electrical engineering from the University of Nebraska, Lincoln, USA, in 1991. She joined as a Research Associate with the Center for Electro-Optics, University of Nebraska, focusing on the enhanced backscatter phenomenon from random rough ground surfaces. She furthered her research at the University of Illinois at Urbana-Champaign, Champaign, IL, USA, working on computational electromagnetics. Before joining the University of Arkansas, Fayetteville, USA, she worked in Boston, MA, with the Multidisciplinary University Research Initiative Team on antipersonnel landmine detection at Northeastern University. She is currently a Professor of Electrical Engineering with the University of Arkansas, where she joined as an Assistant Professor in 2001. Her research interests include terahertz imaging and spectroscopy, breast cancer detection, computational inverse scattering algorithms, MEMS antennas, nano-antennas for energy enhancement of photovoltaic solar cells, and biopotentials modeling of breast tumor cancerous cells.

Dr. El-Shenawee is a member of Eta Kappa Nu Electrical Engineering Honor Society.
Lucas K. Campbell received the M.D. degree from the University of Arkansas for Medical Sciences, Little Rock, USA, in 2002.

He then served as a Pathology Resident with the University of Arkansas for Medical Sciences until 2005, a Gastrointestinal & Hepatic Pathology Fellow with the University of Chicago from 2005 to 2006, a Pathology Resident with the St. Louis University from 2006 to 2007, and a Pathology Fellow with the Washington University/Barnes-Jewish Hospital, St. Louis, from 2007 to 2008. He is currently a Partner and President at the Northwest Arkansas Pathology Associates, P.A., Fayetteville and serves as a Staff Pathologist in several hospitals in the Northwest Arkansas region.

Dr. Campbell is a member of the American Society of Clinical Pathologists, the College of American Pathologists, the Hans Popper Hepatopathology Society, the Roger C. Haggitt Gastrointestinal Pathology Society, the United States and Canadian Academy of Pathology, the Washington County Medical Society, and the Arkansas Medical Society.