Experimental Terahertz Z-Scan Imaging of Three-Dimensional Paraffin Embedded Breast Cancer Tissue

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***Abstract* — This paper presents experimental terahertz imaging of three-dimensional breast cancer tissue embedded in a paraffin block. The imaging was performed using a Z-scan time domain pulsed terahertz system with frequency range of 0.1 to 4 THz. The pathology images of 4-5 μm thick sections were used for visual correlation of cancer and fibroglandular tissue regions to the THz images. The THz results show good correlation with pathology images.**

Index Terms — Terahertz, breast cancer, biomedical imaging.

I. Introduction

The terahertz (THz) frequency range from 0.1 to 10 THz has recently become an area of great research interest in electromagnetics. Technology in the THz range has shown potential in non-destructive evaluation of electronics, material characterization, and security applications [1],[2]. In particular, THz signals have begun to receive attention for the purpose of biomedical imaging applications. There are several qualities of the THz frequency range that make it attractive for biomedical research. THz radiation is not as susceptible to strong scattering in tissue compared to optical techniques due to possessing longer wavelengths. At the same time, THz imaging possesses inherently higher resolution than microwave frequency techniques. There are strong absorption properties of water in the THz frequency range, which has been shown to provide additional imaging contrast in biomedical applications. Finally, due to the relatively low power and non-ionizing nature of THz sources the resulting applications are biologically safe and non-destructive for the use of in-vivo research or ex-vivo tissue analysis. [2],[3].

THz has shown promise in the detection of cancer tissue of the skin, liver, lungs, and breast [2]–[5]. The application for breast cancer is of particular interest. A study with freshly excised breast cancer tissue has shown the cancer to have distinct characteristics from surrounding tissue in frequencies up to 2 THz [3],[6]. Imaging and characterization has also been performed by our group on flat sections of embedded tissue [7],[8].

This work proposes to extend the use of THz imaging to three-dimensional (3D) tissue through a Z-scan procedure to show the capability of THz technology for the assessment of excised breast cancer tumors and the surrounding margin tissue. The technique will first be conducted on formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue in order to establish the methodology for the 3D imaging of the breast cancer tissue prior to applying the technology to fresh tissue in the future.

II. Methodology and Setup

THz imaging of 3D breast cancer tissue was obtained using a commercial pulsed THz imaging and spectroscopy system at the University of Arkansas. This system produces a time domain THz pulse with a width of <500 fs by exciting a biased GaAs antenna with a Ti:Sapphire laser. The Fourier transform of the time domain signal provides a frequency spectrum from 100 GHz to 4 THz. The imaging performed in this work made use of a reflection imaging setup, in which the incident and reflected beam are both located below the sample holder.

The biological tissue used in this work was a FFPE breast cancer tumor block obtained from the National Disease Research Interchange (NDRI). The 3D block can be seen in Fig. 1. The tissue block was obtained from a 54-year old black female via radical mastectomy. Subsequent pathology denoted the tumor as well-circumscribed Stage III infiltrating ductal carcinoma (IDC) with adjacent fibroglandular tissue. The tissue block of Fig. 1 was Z-scanned using the THz system to obtain the reflected time domain signal at each discretized point. These time domain signals reflected from the tissue block were collectively referred to as the Z-scan, since they were used to obtain electronic x-y images on the z-axis of the block without physically sectioning it. Following the Z-scan, the sample was processed using standard histopathology throughout the depth of the block. The sections obtained by

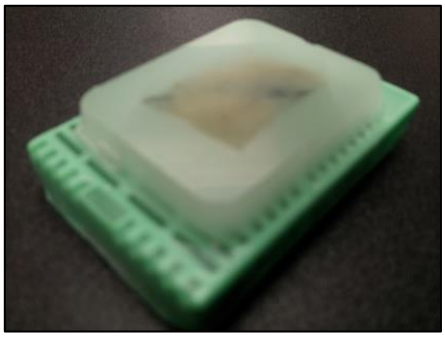

Fig. 1. Photograph of 3D block of breast cancer tissue embedded in paraffin used in this work.

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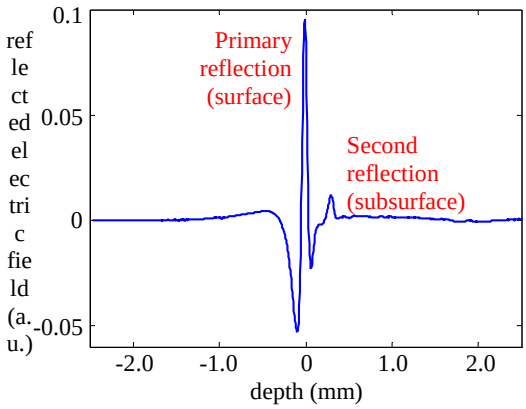

Fig. 2. Time domain signal measured from single point of tissue embedded in paraffin block.

the histopathology consisted of alternating sections stained by hematoxylin & eosin (H&E) with sections that were left as FFPE tissue. Each H&E slide was prepared with a standard pathology thickness of 4-5 μm, while the FFPE sections were prepared with thicknesses of 20, 30, and 40 μm. Each section was placed on a glass slide. Then the H&E slides were used for pathology images, and the FFPE slides were subject to further THz imaging as flat sections.

For 3D blocks with multiple interior heterogeneities, the reflected signal from each interface-like region is measured as a reflected pulse in the time domain. This reflection can be seen for a single point of the embedded tissue in Fig. 2, where the primary large peak was the reflection from the air/block interface and the secondary peak was due to reflection at the interface between the paraffin and cancer tissue region. For the sake of convenience the depth into the block was considered the –z direction with z = 0 at the air/block interface.

The arrival time difference between these two peaks could then be correlated to the depth at which the signal encountered a cancer region. By the same merit, taking the signal at a certain point of the z-axis can provide the magnitude of any returned signal from that depth in the scan. Since the z-axis reflection data exists at every x-y point in the scan, it is possible to construct an image of all of the reflections at a specific depth of the block. In this way the tissue block could be electronically sectioned at specific depths without the need for physical sectioning. These images were then compared to the histopathology images as well as the THz imaging scans obtained from the sectioned flat tissue at each depth.

III. Experimental Results

Fig. 3 shows the results for the comparison of histopathology sections (on the left) taken from specific depths in the tissue, the flat FFPE sections (in the middle) obtained at depths adjacent to the pathology sections, and the images obtained from the in-depth Z-scan (on the right) of the block prior to sectioning. The 4-5 μm H&E pathology slides in Fig. 3a were obtained from positions at z = -25 μm, -470 μm, and -770 μm in the paraffin block. The regions of IDC and fibroglandular tissue as defined by pathology are labeled for reference. Likewise the FFPE flat tissue sections shown in Fig. 3b were obtained from positions z = -20 μm, -455 μm, and -755 μm. All sections shown in Fig. 3b were 20 μm thick. Each flat section was individually imaged using the THz system. Finally the THz Z-scan images in Fig. 3c were obtained by taking the reflected electric field value in the time domain of the scan that corresponded to the same depth in the block as the FFPE tissue sections.

Comparison of the three sets of images found that the THz imaging of the flat FFPE sections showed a strong correlation to the pathology images obtained from the sample. In all cases, the regions of IDC (cancer) in the pathology corresponded to regions of high reflection in the THz imaging compared to the fibroglandular tissue. The results of the tissue reflections are consistent for the sections taken at all three depths. It should be noted that there were some areas of particularly high reflection in the flat section image taken from z = -755 μm. These corresponded to breaks in the tissue where the high reflection of the glass slide can be seen. Additionally, the image of the flat section from z = -455 μm showed a much stronger reflection near the edge between the IDC and fibroglandular tissue. This difference in reflection was attributed to the presence of necrosis and fibrosis in the center of the IDC region that were distinct from the active cancer cells near the outside of the IDC region. This same structure could be seen in the adjacent pathology image as well.

For the images obtained from the Z-scan of the paraffin block in Fig. 3c, the image obtained from a scan depth of z = -20 μm was very close to the surface reflection of the scan. Thus the two tissue regions seen at the surface of the block showed a clear reflection compared to the surrounding paraffin. The image obtained from a scan depth of z = -455 μm showed no significant reflections. This indicated that there was no change in tissue type at that depth, as seen by the comparison of the pathology images and THz images of flat sections in Fig. 4a and Fig. 4b. In other words, the Z-scan image indicates to in-depth changes in tissue type. Therefore if the Z-scan image was taken within the tumor tissue, the image would not show distinction between cancer and fibro tissues. However, the image obtained from a scan depth of z = -755 μm showed an area of notable reflection. Comparison to the adjacent pathology image revealed that the cross-section of the cancer was receding at this depth. Thus the reflection observed at this depth was correlated to the end of the tumor in the block. Therefore the Z-scan was able to provide imaging of the entire depth of the three-dimensional tumor.

The results thus far have shown potential for the use of Z-scan of THz time domain imaging of embedded 3D breast cancer tissue. Further research will investigate several more tissue blocks in order to build the methodology for imaging 3D excised breast cancer tumors.

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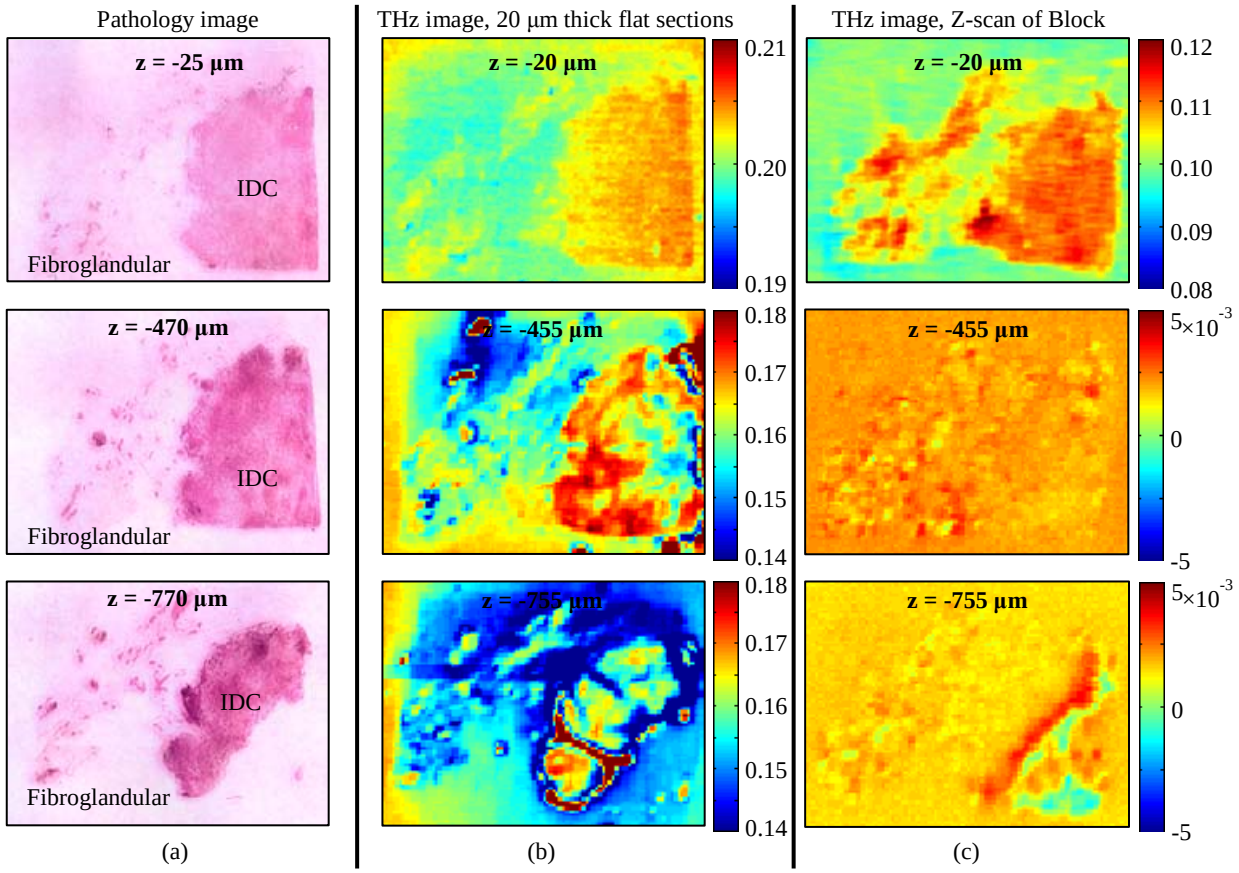

Fig. 3. Comparison of 3D THz imaging using (a) physical pathology sections obtained from z=-25 µm, -470 µm, and -770 µm in the block, (b) THz scans of physical flat FFPE sections obtained from z= -20 µm, -455 µm, and -755 µm in the sample, and (c) cross-sections of the THz Z-scan of the tissue block corresponding to z=-20 µm, -455 µm, and -755 µm in the block. All flat sections were 20 µm thick.

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