Hyperspectral terahertz imaging and optical clearance for cancer classification in breast tumor surgical specimen

Nagma Vohra^o,^a Haoyan Liu^o,^b Alexander H. Nelson^o,^b Keith Bailey,^c and Magda El-Shenawee^{o^a,*}

^aUniversity of Arkansas, Department of Electrical Engineering, Fayetteville, Arkansas, United States ^bUniversity of Arkansas, Department of Computer Science and Engineering, Fayetteville, Arkansas, United States ^cCharles River Laboratory, Mattawan, Michigan, United States

Abstract

Purpose: We investigate the enhancement in terahertz (THz) images of freshly excised breast tumors upon treatment with an optical clearance agent. The hyperspectral imaging and spectral classifications are used to quantitatively demonstrate the image enhancement. Glycerol solution with 60% concentration is applied to excised breast tumor specimens for various time durations to investigate the effectiveness on image enhancement.

Approach: THz reflection spectroscopy is utilized to obtain the absorption coefficient and the index of refraction of untreated and glycerol-treated tissues at each frequency up to 3 THz. Two classifiers, spectral angular mapping (SAM) based on several kernels and Euclidean minimum distance (EMD) are implemented to evaluate the effectiveness of the treatment. The testing raw data is obtained from five breast cancer specimens: two untreated specimens and three specimens treated with glycerol solution for 20, 40, or 60 min. All tumors used in the testing data have healthy tissues adjacent to cancerous ones consistent with the challenge faced in lumpectomy surgeries.

Results: The glycerol-treated tissues showed a decrease in the absorption coefficients compared with untreated tissues, especially as the period of treatment increased. Although the sensitivity metric of the classifier presented higher values in the untreated tissues compared with the treated ones, the specificity and accuracy metrics demonstrated higher values for the treated tissues compared with the untreated ones.

Conclusions: The biocompatible glycerol solution is a potential optical clearance agent in THz imaging while keeping the histopathology imaging intact. The SAM technique provided a good classification of cancerous tissues despite the small amount of cancer in the training data (only 7%). The SAM exponential kernel and EMD presented classification accuracy of ~80% to 85% compared with linear and polynomial kernels that provided accuracy ranging from 70% to 80%. Overall, glycerol treatment provides a potential improvement in cancer classification in freshly excised breast tumors.

© 2022 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JMI.9.1.014002]

Keywords: terahertz imaging and spectroscopy; breast cancer; optical clearance; glycerol; spectral angular mapping; hyperspectral imaging.

Paper 21187R received Jul. 15, 2021; accepted for publication Dec. 21, 2021; published online Jan. 12, 2022.

^{*}Address all correspondence to Magda El-Shenawee, magda@uark.edu

^{2329-4302/2022/\$28.00 © 2022} SPIE

1 Introduction

Imaging biological tissues in the electromagnetic spectrum is a continued challenge at any frequency. Complicating factors include the morphological nature of biological tissues, the increased absorption coefficient with moisture content, and the variations of the absorption coefficients and refractive indices with different tissue types.^{1,2}

Prior research has shown preliminary evidence that reducing moisture content in freshly excised specimens could enhance the contrast between tissue types, particularly between cancer and healthy collagen.³ Studies in optical and terahertz (THz) imaging used optical clearance agents, such as glycerol and urea, for skin and breast cancer.^{4–12} Glycerol is a biocompatible agent mixable with water.⁴ When glycerol is applied to fresh tissues, it binds to the structural protein and affects the free-to-bound water ratio around the region of application. This interaction changes the hydration of the tissue and hence increases the signal penetration in glycerol-treated tissue.⁵

The study in Ref. 6 revealed the assembly process of high-order collagen structures and proposed a molecular mechanism for increasing tissue transparency after glycerol application in laser diagnostics and therapeutics. Clearance agents, such as glycerol and urea, retain high-steady-state flux of the drug across skin membranes at dehydrating conditions.⁷ The change of optical parameters of rat *ex vivo* skin under different concentrations of glycerol solutions was reported.⁸ Both cancerous and non-cancerous *in vitro* human breast tissue show image improvement after tissue treatment using glycerol in conjunction with ultrasound in optical coherent tomography (OCT).⁹ Further, imaging biological tissues using 30% to 50% liquid paraffin glycerol solutions as optical clearance provided the best-known enhancement effect in OCT imaging.¹⁰ Similar work has shown THz imaging of tissue treated with glycerol solution.^{11,12} In Ref. 11, the results demonstrate that glycerol enhances THz wave penetration depth and therefore the potential to enhance image contrast of abnormal lesions below the skin. Several clearance agents (e.g., glycerol, propylene glycol, ethylene glycol, and polyethylene glycol) were investigated in Ref. 12 to comparatively uncover the strength and weaknesses of their use in the immersion optical clearing of tissues at THz frequencies.

In our previous work, regarding THz imaging of excised breast cancer tumors, we observed that the differentiation between tumor tissue regions became stronger in the formalin-fixedparaffin-embedded (FFPE) blocks, i.e., in dehydrated tissue.^{13,14} We conducted preliminary investigation on the effect of glycerol on THz imaging of freshly excised human breast tumors as reported in Ref. 15. In this work, we conduct a more in-depth investigation on the use of glycerol to enhance the image contrast of freshly excised breast tumors in THz reflection imaging. Our objectives are: (1) to investigate image enhancement when specimens are treated with glycerol solution, (2) to investigate optical properties (i.e., absorption coefficients and refractive indices) of glycerol-treated tissues compared with those of untreated ones, and (3) to demonstrate that the effect of glycerol treating tissue is insignificant on the histopathology process, which is the current gold standard in the detection of cancer in excised tissues.

Nevertheless, the task of classifying regions of breast tissues from THz spectroscopy and imaging is an open and challenging problem. Margins between cancer and healthy collagenous tissues in freshly excised tumors have remained difficult to classify with high accuracy. Prior work has considered multiple approaches to this task, each with advantages and limitations. We previously addressed methods of performing classification of cancer using two broad approaches: (1) an unsupervised approach based on expectation maximization (EM) or Monte Carlo Markov chain (MCMC) classifiers of Gaussian mixture model (GMM)¹⁶⁻¹⁹ and (2) a supervised approach based on a multinomial Bayesian probit regression learning²⁰ and on convolutional neural networks (CNN) and associated artificial neural networks (ANNs).²¹ For any of these methods, the underlying THz signal may be preprocessed. Prior work has used the Fourier transform¹⁷ and the wavelet synchrosqueezed transform²¹ to reduce the dimensionality or to accentuate certain frequency components and thereby enable better model fit. In this work, we address the classification of cancer in untreated and glycerol-treated specimens using a supervised approach based on hyperspectral imaging techniques.^{22–33} Specifically, we emphasize the use of the spectral angle mapping (SAM) and the Euclidean minimum distance (EMD) for image classification.22-29

All the above methods are considered "data driven" classification methods as opposed to "model driven" classifications. This is important for THz classification of cancer in tissue because to date we do not have a good physical model for how the reflected signal should be received for different types of tissue. One of the major advantages of the GMM for classification is that learning can occur in an unsupervised manner.³⁴ That is, the algorithm determines from the data those parameters that most optimally separate the data into number of classes (e.g., two classes as cancer and non-cancerous tissues, or three classes as fat, cancer, and muscle, or collagen, fat, and cancer).^{16–21} Unsupervised clustering algorithms do not have a concept of class but try to separate data into a certain number of clusters based on a selected set of features. For example, a potential problem will arise if the clustering algorithm does not have the actual number of classes in raw data. In contrast, both SAM and ANNs require expert labeling of some amount of data.^{35,36}

SAM requires one model example (the "reference spectrum") for each class.³⁵ Angles are calculated between the candidate and each reference. Spectral angles vary between 0 deg and 90 deg, where angles near 0 deg are a close match to the reference spectrum. The chosen class is the class of the closest reference spectrum to the candidate spectrum. Obtaining a single example of each class is not a significant challenge provided the knowledge generalizes to other tissue samples. ANNs can require a large amount of training data as some models may have millions of parameters that need to be tuned to achieve high classification accuracy.³⁷ Transfer learning can significantly reduce this amount but still requires several hundred labeled examples.^{21,38}

The statistical classification methods involve rigorous formulations, and the neural network methods are heavily data driven. However, both require relatively large number of ground truth data and therefore are less feasible when it comes to availability of human breast cancer data. Therefore, in this work, the classification is conducted based on SAM technique that does not require a large amount of training data.

The accuracy of classification models is often difficult to precisely define.³⁹ For cancer diagnosis, a false negative may be considered worse than a false positive, and therefore, a simple accuracy metric may not properly reflect the utility of a classification model. Several techniques have become standard practice to reduce this limitation, including F1 accuracy and ROC curves. A comparison between the ROC for the EM, MCMC, and CNN showed better performance for the CNN classifier than the other methods.²¹ This makes a sort of intrinsic sense because the CNN is a supervised model and has been provided more information to make the classification. It follows that a spectral model will likely obtain accuracy metrics somewhere between MCMC and ANN models, which is the conclusion found in Ref. 23 for a different application of SAM.

Another concern in classification is how well the underlying model can define uncertainty for a given region.⁴⁰ That is, if a section is defined to be a particular class, how certain is that classification? This is of particular importance in medical diagnosis so that surgeons can make informed decisions.⁴¹ Each of the three models has an intrinsic parameter that is used for classification that can be adapted to this task. MCMC and EM from GMMs obtain certain probability of each class and use a preselected threshold to obtain the ROC curve. EMD uses a minimum distance from the chosen cluster that indicates to a higher likelihood of belonging to that class than a larger distance. Similarly, SAM uses the spectral angle for classification. An angle near 0 deg is a high match where large angles are unlikely matches. CNNs have a probability metric for each potential class from the final layer of the model that can act as an uncertainty metric. A comparison for how each of these uncertainty metrics perform with the dataset could be conducted to determine if one model has a more robust metric for describing uncertainty.

Hyperspectral imaging techniques have been implemented in a variety of applications, including remote sensing, biomedical imaging, and others.^{29–33} The work in Ref. 29 reviewed hyperspectral imaging for cancer applications, where each material provides different response to light reflection, absorption, and scattering across the electromagnetic spectrum. These properties are used to differentiate and identify different substances present in a region using their spectral signature. In Ref. 30, the study highlighted the feasibility of using quantitative hyperspectral imaging as a diagnostic tool to delineate cancer boundaries in surgical specimens. In Ref. 31, the study demonstrated the possibility to measure spectral reflectance in gastric tumors and to differentiate between tumorous and normal mucosa. In Ref. 32, SAM was utilized as chemometrics to discriminate gastric cancer from normal. In Ref. 33, the study demonstrated

a simultaneous discrimination of tumor distributions and provided three-dimensional morphological information in colon surfaces. To the best of our knowledge, these works were in the optical frequency band.

In this work, we focused on THz reflection imaging and utilize hyperspectral imaging for the classification of three regions of interest: (1) cancer, (2) healthy collagen, and (3) fat, in freshly excised breast tumors. Our approach includes the implementation of linear, polynomial, and exponential kernels of SAM.²⁵ Furthermore, for the sake of comparison between different classification methods, we implemented EMD²² in addition to the expanded spectral angular mapping (ESAM) that measures the similarity between two images.²⁴ We utilize THz spectroscopy to obtain the absorption coefficients and indices of refraction of untreated and glycerol-treated tissues at each frequency up to 3 THz. The purpose is to investigate the treatment effect on tissue properties. We implemented the morphed pathology technique developed in Refs. 16 and 17 to achieve a means of point-wise comparison between the fresh and ground-truth images for quantitative evaluation of the classifiers. The ground-truth images were obtained from the hematoxy-lin and eosin (H&E) microscopic pathology slides taken from the FFPE blocks of fresh tissue.

This work is organized as follows: Section 2 describes the methodology and tissue preparation. Section 3 discusses the THz experimental and classification results along with sensitivity, specificity, and accuracy metrics for each algorithm. Section 4 provides our concluding remarks.

2 Methodology

2.1 Glycerol Treatment Experiments and Tissue Preparation

Five freshly excised human breast cancer specimens are used in this work. The specimens were obtained from three tumors purchased from the National Disease Research Interchange (NDRI) biobank and one tumor purchased from the Cooperative Human Tissue Network (CHTN) biobank. Tumors were received within 24 h after surgical excision and were immersed in Dulbecco's Modified Eagle Medium (DMEM) and antibiotic solution during shipping. These tissues were specifically selected because the cancer was adjacent to healthy collagen and fat in the same specimen allowing for investigating their differentiation on the margins.^{13–20}

Two specimens were not treated with glycerol (ND11066 and ND17668) and three specimens were treated with a 60% concentrated glycerol solution (CHTN-20-064, ND18228-part2, and ND18228-part4). The last two samples were obtained from the same bulk tumor ND18228. The size of the tumor was $\sim 2 \times 1 \times 1$ cm³ and was dissected into four parts. Two sections were treated with the glycerol (parts 2 and 4) while the other two sections (parts 1 and 3) were not treated. We could not use the untreated parts (1 and 3) because the THz image of part 1 was deteriorated due to the presence of too many air bubbles during scanning on the imager. In addition, part 3 showed no cancerous regions, based on the pathology assessment. The photos of the five samples are shown in Fig. 1. All specimens have normal tissue adjacent to cancerous tissue to enable the investigation of margins between cancer and healthy collagen or fat.

Our glycerol treatment protocol involved the preparation of concentrated glycerol solution, followed by application to tissue for a specific time duration. The 60% concentrated glycerol solution was prepared by diluting the 100% glycerol solution with phosphate-buffered saline







Fig. 2 Sample preparation for THz imaging of glycerol-treated and untreated tumors. (a) The freshly excised tumor taken out of DMEM and antibiotics solution, (b) sample immersed in 60% glycerol solution for 5 min, (c) sample placed in a clean petri dish for 20, 40, or 60 min without paper drying, (d) treated samples placed on grade 1 filter paper for 1 min, and (e) sample sandwich placed on THz scanner for imaging.

solution as a solvent.⁹ The specimen was taken out of the DMEM and immersed in the 60% glycerol solution for 5 min as shown in Fig. 2(b). The CHTN 20-064 sample was left in the dish for 20 min, the ND18228-part2 for 40 min, and the ND18228-part4 for 60 min as shown in Fig. 2(c). Each specimen was placed on a grade-1 filter paper for ~1 min to remove the glycerol solution as shown in Fig. 2(d). The specimen was carefully and gently pressed between two polystyrene windows and placed on the THz scanner (pulse TPS Spectra 3000 THz imager) as shown in Fig. 2(e). The idea is to make the imaging surface of the tissue as flat as possible consistent with the Fresnel reflection and transmission coefficients to be used later in data processing. The imaging was performed by setting the stepper motors to 200- μ m step size. The two untreated tumors were directly imaged as shown in Fig. 2(e).^{42,43}

Once the imaging process was completed, the tissues were immersed in formalin solution in a centrifuge tube and shipped to the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for the histopathology process.¹⁸ Then the formalin-fixed tumors were dehydrated and embedded in paraffin blocks. Furthermore, a 3- to 4- μ m-thick flat section of tissue was sliced from the block tissue and stained with the standard H&E ink for the pathology imaging. The H&E-stained tissue slides along with the FFPE tissue blocks were shipped to the University of Arkansas for further microscopic and THz imaging, respectively.⁴²

2.2 Hyperspectral Imaging and Quantitative Classification

The objective in this task is to classify three tissue types of interest in the tumor (i.e., cancer, healthy collagen, and fat). We implemented hyperspectral imaging classification using SAM and EMD as will be discussed in this section.

2.2.1 Training dataset

Three sets of data are generated in this work. The first set is the reference data (training data) of cancerous, healthy collagenous, and fatty tissues, as shown in Fig. 3. Cancerous tissues were obtained from breast cancer patients who went through mastectomy or lumpectomy surgeries. Healthy collagenous and fatty tissues were obtained from breast reduction surgeries. Training data were not used in any of the testing data to avoid overfitting or selection bias. The training data represent the magnitude of the THz reflection at each pixel in the specimen and at each frequency in the THz band of the system (0.1 to 4 THz). Results were averaged based on the total number of pixels in each type.

The results of Figs. 3(a) and 3(b) show the average THz reflection magnitude of fresh and FFPE breast tissues, respectively, plotted corresponding to the frequency along the *x* axis. The plots in Fig. 3 demonstrate that fresh tissue has a higher magnitude than those embedded in FFPE. This observation may be explained by the dehydration of the FFPE tissue during the histopathology process. Another observation is that the magnitude of cancer tissue in FFPE blocks fluctuates, as shown in Fig. 3(b). Although the THz signals reflected from the fat and collagen also fluctuate, their fluctuations are insignificant compared of that of cancer as shown in



Fig. 3 Reference data (training data) based on the average of normalized THz reflection magnitude of excised human breast tumors: (a) fresh tissue and (b) FFPE tissue block. The normalization of THz signals for fresh tissue was conducted with respect to the reflection from a single point on the polystyrene slide with no tissue. Normalization of FFPE tissue was conducted with respect to the reflection from a golden mirror at a single point.

Fig. 3(b). This fluctuation is likely due to the presence of paraffin in the tissue region because of the histopathology procedure and results in a smaller magnitude of reflection.

The second set of data is the unknown raw THz data (testing data), and the third set of data is the ground truth obtained from the pathology images.^{16,17}

2.2.2 Testing and ground truth datasets

The THz reflection images of the five specimens obtained from four tumors are shown in Fig. 4 along with their corresponding pathology images. Figures 4(a) and 4(b) show the THz reflection images of the untreated tumor 1 and tumor 2. Their corresponding pathology images are shown in Figs. 4(f) and 4(g), respectively. Figures 4(c)-4(e) show the THz reflection images of the glycerol-treated tumor 3 and tumor 4 (parts 2 and 4). Their corresponding pathology images are shown in Figs. 4(h)-4(j), respectively. These three specimens were treated with 60% concentration glycerol for 20, 40, or 60 min.



Fig. 4 Testing data (THz images) and ground truth data (pathology images). Freshly excised human breast specimens with normal tissue adjacent to cancerous tissue. (a), (b), (f), and (g) Untreated specimens and (c)–(e), (h)–(i) specimens treated with 60% glycerol concentration for 20, 40, or 60 min. The color bar represents the power spectra of the reflected electric field magnitude in arbitrary units (a.u.).

Tumor 1 (ND11066) was procured from a 49-year-old female patient via a right breast mastectomy. As can be seen in the pathology image in Fig. 4(f), there are three regions assessed as cancer (purple color), collagen (pink color), and fat (white color). Upon comparing the THz image in Fig. 4(a) with the pathology image in Fig. 4(f), it is observed that cancer in the THz image presents the area with highest reflection (red color) and fat with the lowest reflection (blue color). However, collagen can be visualized in two different colors—yellow greenish and light red color. Consequently, it is challenging to visually differentiate cancer from the collagen on the margins.

Tumor 2 (ND17668) in Fig. 4(b) was obtained from a 71-year-old female patient via partial mastectomy. The pathology image in Fig. 4(g) represents three regions—cancer, fibro-fatty, and fat. This tumor has different physiology from tumor 1 in terms of the presence of fibro-fatty tissue, defined here as a composition of tissues containing collagen mixed with fatty tissues. Furthermore, the region assessed as cancer includes dense collagen in its background. This collagen is not pre-existing as the fibro-fatty tissue but is induced during the growth of cancer. Upon comparing the THz image in Fig. 4(b) with the pathology image in Fig. 4(g), it is observed that, like tumor 1, cancer represents the highest reflection (red color), followed by collagen (cyan/ yellow-greenish color), and fat representing the lowest reflection (blue color). Furthermore, the shape of the pathology image is slightly different from that of fresh tissue in the THz image. This shape mismatch occurred during the histopathology process.^{16,17}

The third tumor (CHTN-20-064) was obtained from a 58-year-old female patient via breast mastectomy surgery. From the pathology image in Fig. 4(h), it is observed that this tumor also includes cancer, mature collagen with healthy ducts and glands, and fat regions. Furthermore, this tumor was treated with 60% glycerol for 20 min before imaging as described in Sec. 2. Based on the color bar scale, the THz image of the tumor in Fig. 4(c) represents lower reflection than that of the first two tumors in Figs. 4(a) and 4(b). This low reflection could be explained by the effect of glycerol on the treated tissue where the penetration depth of the signal in the tissue has increased leading to smaller reflection values, in agreement with the results reported in Ref. 11. Like the first two tumors, cancer represents the highest reflection (light red color) compared with collagen (yellow color) and fat (blue color).

The fourth and fifth specimens in Figs. 4(d) and 4(e) represent parts 2 and 4 of the bulk tumor ND18228, obtained from a 70-year-old female via mastectomy surgery. As mentioned earlier, ND18228-part 2 specimen was treated with 60% glycerol for 40 min and ND18228-part 4 tumor was treated for 60 min. The pathology image of part 2 sample is presented in Fig. 4(i) which shows that the percentage of healthy tissues (collagen and fat) is larger than that of cancer. When the THz image is compared with the pathology image, a large area is observed in the center of the specimen that has high reflection, which is typical of cancerous regions (light red). However, during pathology, this region was assessed as collagen. The other collagenous (cyan color) and fatty (blue color) regions are otherwise quite differentiated from cancer. Like tumor 3, the highest reflection in the THz image in Fig. 4(d) is lower than those in tumors 1 and 2 in Figs. 4(a) and 4(b), which is due to the glycerol treatment. Nevertheless, the identification of the region in the center of the specimen poses a challenge in THz imaging. We can think of three possible reasons for this discrepancy: (1) the region was originally cancer in the fresh tissue but it was shaved off during the histopathology process; (2) the region represents dense collagenous tissue that was not differentiated correctly from cancer in THz imaging, since both tissues have close electrical properties at higher frequencies as shown in Fig. 3;⁴³ and (3) the tissue orientation was inadvertently altered during paraffin embedding process for histopathology. For the third possibility, what we see in the pathology image could be the backside of the specimen which does not have cancer in the center region.

Similarly, the pathology image of ND18228-part 4 shown in Fig. 4(j) displays cancer as an oval shaped region in the lower middle part of the specimen. However, in the THz image, the cancer region (light red color) is visualized more extended to the right side along with the lower middle region. The collagen region is represented in yellow and cyan colors and fat in blue. Figure 5 shows the pie chart summary of the percentage of pixels in the three regions of fresh tissue (cancer, collagen, and fat). The training dataset of the fresh tissue is shown in Fig. 5(a) with 4588 cancer pixels, 38,409 collagen pixels, and 26,272 fat pixels. The testing dataset of the untreated tissues is shown in Fig. 5(b) with 2706, 571, and 993 pixels in cancer, healthy collagen,



Fig. 5 Summary of the % number of pixels in the three regions of interest in fresh tissues: (a) training data, (b) testing data of untreated tissue, and (c) testing data of treated tissue.

and fat regions for tumor 1, and 2315, 732, and 1834 pixels in cancer, healthy collagen, and fat regions in tumor 2. The testing dataset of treated tissues is shown in Fig. 5(c) with 1615, 597, and 586 pixels in cancer, healthy collagen, and fat regions for tumor 3; 718, 799, and 3703 pixels, and 829, 1837, and 3250 pixels in cancer, healthy collagen, and fat regions for tumor 4 parts 2 and 4, respectively. As observed in Fig. 5, the cancerous pixels in the training dataset represent only 7% of the specimen, which motivates the use of SAM as a classifier in this work. Although the number of cancerous pixels is much smaller than healthy collagenous and fatty tissues, this difference is immaterial in classification using SAM and is one of the motivating factors for our evaluation metrics. Given a large enough number of independent and identically distributed samples from each of the types of tissue, the calculation of the average reference spectrum is robust to outliers in the dataset.^{44,45} This is an advantage over other data driven classification methods that may be biased if classes are not properly balanced in proportion to the expected ratio of tissues.⁴⁶

2.2.3 Hyperspectral SAM classification technique

The THz imaging system provides a time domain pulse reflected from each pixel in the specimen. The frequency domain reflection is obtained through the Fourier transform of each pulse. The diagram in Fig. 6 clarifies the scheme of the hyperspectral imaging where several frequencies in the THz band are used (*K* frequency bands). The raw data of THz images at each frequency is converted from a matrix of order $M \times N$ to *K* vectors each of length *MN*. The training dataset matrix is of order $K \times W$, where *K* represents the number of frequency bands used in the classifier and *W* represented the number of regions of interest to be classified. Here we used W = 3 for cancer, healthy collagen, and fatty tissues. The details of SAM algorithm are given in Refs. 22–29. There are several kernel-based methods that can be used in SAM, such as linear, polynomial, and exponential. The kernel-SAM (KSAM) is given by²⁵



Fig. 6 THz hyperspectral imaging: (a) raw data of THz images of order $M \times N$ at K frequencies (f_1, f_2, \ldots, f_K) ; (b) THz images reorganized in K columns each column of order $NM \times 1$ producing a matrix of order $NM \times K$; and (c) training THz data of freshly excised specimen of cancer, fat, and collagen.

$$\theta = \arccos\left(\frac{K(x,z)}{\sqrt{K(x,x)K(z,z)}}\right), \quad 0 \le \theta \le \pi/2.$$
(1a)

The parameter θ presents the angle between the raw data vector (testing data) and the reference data vector (training data). The obtained value of the angle determines how close the unknown pixel in the image to the region type in the reference data. We did not use an angle threshold in the implementation of the SAM classifier. Instead, we used the minimum angle of the three classes to determine if the pixel in the THz image is to be classified as cancer, healthy collagen, or fat. In other words, the predicted class is determined to be the class of the reference spectrum that produces the minimum angle between the candidate and reference spectrum.²² In Eq. (1a), the *x* and *z* are data matrices, one represents the raw data (testing data) and the other represents the reference data (training data). Let *x* represent the THz images of order $NM \times K$, and *z* represent the training data of the three regions of interest and of order $K \times 3$. For the linear kernel, $K(x, z) = x^T z$, for the polynomial kernel, $K(x, z) = (x^T z + b)^d$, where *b* and *d* are constants (d > 0), and for the exponential kernel $K(x, z) = \exp(-(1/2\sigma^2) ||x - z||^2)$, where σ is a constant. The ESAM is given by²⁴

$$\theta = \arccos\left(\frac{2x^{T}z}{\|x\|^{2} + \|z\|^{2}}\right), \quad 0 \le \theta \le \pi/2.$$
(1b)

Furthermore, we investigated the EMD given by²²

$$\|x - z\| = \sqrt{\sum_{i=1}^{K} ((x_i - z_i)^2)}.$$
 (1c)

For quantifying the classifier, we calculated the standard sensitivity, specificity, and accuracy metrics. The pixels in the classified regions of interest are compared with those in the ground truth data of the pathology images. These metrics are calculated as

sensitivity
$$=\frac{\text{TP}}{P} = \text{TP}/(\text{TP} + \text{FN}),$$
 (2a)

specificity
$$= \frac{\text{TN}}{N} = \text{TN}/(\text{TN} + \text{FP}),$$
 (2b)

accuracy =
$$\frac{\text{TP} + \text{TN}}{P + N} = (\text{TP} + \text{TN})/(\text{TP} + \text{TN} + \text{FN} + \text{FP}),$$
 (2c)

where P (or N) represents the number of pixels in the ground truth pathology images that is positive (or negative). The symbols TP, FN, TN, and FP represent the number of pixels in the classified image that is true positive, false negative, true negative, and false positive, respectively. In this work, we utilize morphed pathology images to compensate for the changes that occurred to the fresh tissue during the histopathology process as reported in our previous work.^{16,17}

3 Experimental Imaging and Classification Results

Overall, it can be observed in Figs. 4(c)-4(e) that with the 60% glycerol treatment, the reflection from different regions in the three tumors were decreased by ~30% compared with the reflection of the untreated samples in Figs. 4(a) and 4(b). Specifically, the CHTN-20-064 specimen presents higher reflection values than the ND18228-part 2 followed by the ND18228-part 4 specimens. First, we investigate the effect of the glycerol treatment on the specimen before we implement and quantify the classification accuracy of THz images. We utilized the tomography technique to obtain the absorption coefficient and refractive index at each pixel in the image and at each frequency in the frequency band of the system.⁴³

3.1 Glycerol Treatment Results

The absorption coefficients and the refractive indices of cancerous and non-cancerous pixels in the untreated and treated specimens are shown in Fig. 7. According to our protocol, the tissue was treated with glycerol for 20, 40, or 60 min. The absorption coefficients of all cancerous pixels are calculated and averaged at each frequency. Similarly, the average of the absorption coefficients of all non-cancerous pixels is obtained at each frequency. The same is repeated for the refractive indices at cancerous and normal pixels. The absorption coefficients of cancerous pixels are shown in Fig. 7(a) and those of non-cancerous pixels of healthy collagen and fatty regions (normal) are shown in Fig. 7(b). The refractive indices of cancerous pixels are shown in Fig. 7(c) and those of normal pixels are shown in Fig. 7(d). For comparison, we added the results of the absorption coefficients and refractive indices of water and glycerol of 60% concentration (dashed lines). Figure 7(a) demonstrates the trend of glycerol effect on the absorption coefficients of tissues treated for 0 min (untreated) to 60 min treatment. As anticipated, the absorption coefficients are negatively correlated with the time duration of glycerol treatment. Furthermore, the absorption coefficient of water represents the upper limit while that of glycerol represents the lower limit of the optical properties. The results of normal tissue in Fig. 7(b) show the same negative correlation. However, the glycerol solution shows comparable absorption coefficients to normal tissues treated at 40 or 60 min, which was not the case in the cancerous tissues in Fig. 7(a). This could be explained by the morphological difference between normal and cancerous tissues where the former allows the glycerol to penetrate the tissue more. Furthermore, upon comparing the results of Figs. 7(a) and 7(b), the absorption coefficients of cancerous tissues are



Fig. 7 Absorption coefficient and refractive index of the specimen shown in Fig. 4 (testing data): (a) absorption coefficient of cancerous region, (b) absorption coefficient of non-cancerous regions (healthy collagen and fatty tissues), (c) refractive index of cancerous region, and (d) refractive index of non-cancerous regions (healthy collagen and fatty tissues). Results are obtained through reflection spectroscopy at each pixel.

higher than those of normal tissues with or without treatment, which is consistent with our reported data in Ref. 43.

For the refractive indices in Figs. 7(c) and 7(d), the results did not show significant difference between the untreated and treated tissues. However, upon comparing Fig. 7(c) with Fig. 7(d), the refractive indices of cancerous tissues are closer to those of water and are higher than those of normal tissues. The refractive index indicates the velocity of the wave in tissue in this case. In other words, the results of Figs. 7(c) and 7(d) can be interpreted that the wave travels slower in cancerous tissue compared to healthy tissues, regardless of the treatment. Based on the results of Fig. 7, we can conclude that the glycerol treatment indeed reduces the absorption coefficients of freshly excised tissue with increased treatment duration. To confirm the trend effect observed in Fig. 7 for human tissues, we conducted two experiments on other biological tissues (salmon and bovine) where the treatment time was increased from 0 to 150 min (results are not shown for space limitation). We recognize that longer durations of glycerol treatment can make the tissue dry and hence lose its properties as fresh tissue.

It is important to demonstrate that the histopathology process is not affected by glycerol treatment of the tissue. Therefore, in the histopathology lab at Oklahoma State University, we experimented with fresh mammary gland from a cow (adult Holstein cow). A dairy cow was selected because of the well-developed mammary glands and ducts, as well as the prominent collagenous stroma. The tissue was recovered from the cow immediately after euthanasia for an unrelated (severe) leg injury. We treated tissue with 60% glycerol dissolved in phosphate-buffered saline for 0 min (negative control), 10, 30, 60, or 120 min. Following the exposure periods, the slice of fresh mammary gland was immersed in 10% neutral-buffered formalin and gently agitated. Tissues were fixed in formalin overnight and were placed in a cassette for routine tissue processing. No differences were observed in untreated (formalin fixation only) and glycerol-treated (varying exposure times) sections of lactating mammary gland. In addition, no differences were detected between the various treatment groups.

We recognize that long treatment time of fresh tissue is not realistic in a clinical setting, and therefore, further investigation of glycerol concentration, treatment protocols, and other types of clearance agents will be needed and are left to future work.

3.2 Quantitative Classification Results

The five classifications schemes investigated in this work are SAM linear, polynomial, and exponential kernels, EMD, and ESAM (discussed in Sec. 2). In all results, each frequency band included 10 frequencies and we investigated 95 frequency bands. The frequency bands are cumulative which means that we started with the first band ranging from 0.23 to 0.277 THz, then we added 10 more frequencies such that the second band ranges from 0.23 to 0.324 THz, the third band from 0.23 to 0.37 THz, etc. Due to space limitation, we only present the classification images for the frequency band #22 which starts from 0.23 to 1.25 THz. The signal around 0.1 to 0.2 THz is unreliable and the signals at frequencies higher than 3 THz are noisy due to the high absorption of the signal in freshly excised tissues.⁴³ Therefore, the results for those bands are not included in the classifier quantification plots. For consistency, we presented the quantification results of all classification schemes from frequency band #15 (0.23 to 0.69 THz) to frequency band #55 (0.23 to 2.77 THz). We experimented with some values of polynomial parameters dand b and selected d = 0.35 and b = 0.2 as the best performance in this application.²⁸ Additionally, we selected $\sigma = 100$ in the exponential kernel as it provides best classification compared with smaller values of σ .²⁵ The ground truth morphed pathology masks of cancer, fat, and healthy collagen obtained from the pathology images in Figs. 4(f)-4(j) are not shown here for space limitation, but details of the algorithm are available in Refs. 16 and 17.

The results in Fig. 8 correspond to the classifications of the five specimens shown in Figs. 1 and 4. The spectral mapping classification results based on the linear, polynomial, and exponential kernels of SAM, EMD, and ESAM schemes are shown in Figs. 8(a)-8(y). Each classification image contains three colors representing the three regions of interest: cancer (yellow), fat (turquoise), and healthy collagen (dark blue). Figures 8(a)-8(e) correspond to the classifications of the untreated tumor 1. Figures 8(f)-8(j) correspond to the classifications of the untreated tumor 2. On the other hand, Figs. 8(k)-8(o) correspond to the classifications of the



Fig. 8 (a)–(y) Spectral mapping classifiers based on linear, polynomial, exponential kernels, and EMD and ESAM methods for band # 22 which starts from 0.23 to 1.25 THz. The yellow color indicates to cancer, the turquoise color indicates to fat, and dark blue color indicates to healthy collagen.

glycerol-treated tumor 3 for 20 min, Figs. 8(p)-8(t) correspond to the classifications of the treated tumor 4 part 2 for 40 min, and Figs. 8(u)-8(y) correspond to the treated tumor 4 part 4 for 60 min.

To interpret the classification results in Fig. 8, we look at the THz images in Figs. 4(a)-4(e) that represent the testing data (raw data). The classifications images of Fig. 8 are obtained by performing the dot product in the SAM algorithm or the minimum distance in the EMD algorithm with respect to the training data of cancer, healthy collagen, and fat. First, we observed that the classification images for tumors 1 to 3 based on the exponential kernel, the EMD, and the ESAM provide comparable accuracy and are strongly correlated to the THz images in Figs. 4(a)-4(c). However, they are noticeably different from those obtained using the linear and the polynomial kernels. Furthermore, for glycerol-treated specimens of tumor 4 parts 2 and 4, we noticed that the THz images in Figs. 4(d) and 4(e) do not visually seem well correlated with the pathology images in Figs. 4(i) and 4(j), respectively. We can interpret this observation by the deformation occurred to the freshly excised specimen ND18228 during the histopathology process

such that the mismatch between the THz and morphed pathology images became more noticeable compared to the other three tumors. Additionally, the THz image was taken at the surface of the fresh bulk tissue, whereas the pathology image was taken at a different surface in the FFPE block tissue after completing the histopathology process. Indeed, there is a difference in the imaged surface between the two methods which could introduce additional mismatch between THz and pathology images.

Due to the significance of cancer as the main region of interest and due to space limitation, we present the classification metric results of sensitivity, specificity, and accuracy of cancer only in Figs. 9(a)–9(o). The results show that for tumor 1, the sensitivity metric (TP/P) of cancer in Fig. 9(a) demonstrates ~84% across all frequency bands, except those of the linear and polynomial which show deterioration starting at frequency band # 45. In Fig. 9(b), the specificity metric (TN/N) of cancer demonstrate ~84% in the exponential (EXP), the EMD, and the ESAM, consistently across the frequency bands. However, the results of the linear and polynomial kernels show increasing specificity across the frequency bands ranging from ~70% to 85%. The accuracy metric of cancer in Fig. 9(c) demonstrates a slightly better performance of the EXP, the EMD, and the ESAM (~84%) compared with the linear and polynomial kernels (~80%). It is important to mention that while the classification image visually show strong correlation with the THz image, the comparison in these metrics was conducted with respect to the pathology images through the morphed pathology masks.^{16,17}

Furthermore, there is an unavoidable human error in producing the morphed pathology masks due to the deformation of fresh tissue during the histopathology process. Indeed, the pathology images obtained from slicing the dehydrated tissue blocks are the only available ground truth data to compare with. This is one of the continuing challenges when assessing THz images of freshly excised tissues as will be discussed in Sec. 4.

In Fig. 9(d), the results for tumor 2 show the sensitivity of cancer demonstrating ~84% across the frequency bands for all methods. However, the specificity in Fig. 9(e) shows lower values in the linear and polynomial methods, ranging from ~60% to 70%. The accuracy results in Fig. 9(f) also show better performance in the EXP, the EMD, and the ESAM methods with almost constant values at ~82% across the bands. On the other hand, the accuracy is ranging from ~70% to 75% in the linear and polynomial kernels.

In Fig. 9(g), the results for tumor 3 show the sensitivity of cancer range $\sim 75\%$ to 80%, with the linear and polynomial methods showing slightly better values than the EXP, the EMD, and the ESAM methods. Like the results of Fig. 9(e) of tumor 2, the specificity results in Fig. 9(h) show smaller values in the linear and polynomial methods ranging from $\sim 68\%$ to 85%, while the EXP, EMD, and ESAM methods show specificity results $\sim 85\%$ consistently across the frequency bands. The accuracy results in Fig. 9(i) also show better performance of the EXP, EMD, and ESAM methods with almost constant values at $\sim 80\%$, while it is around $\sim 75\%$ for the linear and polynomial kernels.

The results in Fig. 9(j) for tumor 4-part 2 sensitivity of the linear and polynomial show better values (~65%) than those of the EXP, EMD, and ESAM (~30% to 35%). However, the specificity of the latter show ~95% in Fig. 9(k) and the accuracy ~85% as shown in Fig. 9(l). The linear and polynomial specificity and accuracy results range from ~60% to 80% as shown in Fig. 9(l). Similarly, for tumor 4-part 4 sensitivity of the linear and polynomial show better values (~80%) in Fig. 9(m), than those of the EXP, EMD, and ESAM (~60% to 65%) in the same figure. Interestingly, the specificity and accuracy are much better in the EXP, EMD, and ESAM, ~90% in Fig. 9(n) and ~84% in Fig. 9(o).

Finally, we conclude this section with a quantitative comparison between the untreated and glycerol-treated specimens. The results are shown in Fig. 10 for the cancer classification. Figures 10(a)-10(c) demonstrate the sensitivity, specificity, and accuracy metrics, respectively. Due to space limitation, the results of Fig. 10 are based on SAM exponential kernel only. In Fig. 10(a), we can see that the glycerol-treated specimen of ND18228 part 2 (green color) shows the lowest sensitivity among all specimens investigated in this work. As defined earlier, the sensitivity represents the ratio between the number of the positive cancer pixels in THz image and true positive cancer pixels in the ground truth pathology image. On the other hand, we demonstrated in Figs. 9(j)-9(1) that ND18228 part-2 specimen shows the lowest sensitivity compared with all other specimens investigated in this work. This observation was explained earlier



Vohra et al.: Hyperspectral terahertz imaging and optical clearance for cancer classification...

Fig. 9 (a)–(o) Classifier metrics of all specimens: sensitivity, specificity, and accuracy of cancer classification.

by the mismatch between the THz and pathology images shown in Figs. 4(d) and 4(i), respectively.

Similarly, the treated specimen of tumor 3 (black color) shows sensitivity of \sim 73% which was also unexpected. However, the sensitivity of the treated specimen ND18228 part-4 shows higher sensitivity along with those of the untreated tumors 1 and 2. Figure 10(a) indicates that the glycerol treatment is not as effective in classifying the cancerous tissues as anticipated. However, there are several factors affecting the sensitivity of the classifier, e.g., the amount of cancerous



Fig. 10 Quantitative comparison between untreated and glycerol-treated specimens. Cancer classification: (a) sensitivity, (b) specificity, and (c) accuracy. All results are based on the exponential kernel of SAM.

tissue versus collagenous and fatty tissues, especially dense collagen. For example, the cancerous tissues in the untreated tumors 1 and 2 are much larger than those of the collagen and fatty tissues. For this reason, the sensitivity of the classifier could be higher in tumors 1 and 2 compared with those of tumor 3 and tumor 4 part-2 and part-4, regardless of the glycerol treatment. Also the level of mismatch between the THz image and the pathology image plays a significant role in classification.

Figure 10(b) demonstrates that the specificity of the three treated specimens (tumors 3, 4 parts 2 and 4) is higher than that of the untreated samples. The specificity was defined earlier by the ratio between the number of the negative (non-cancerous) pixels in THz image and the true negative non-cancerous pixels in the ground truth pathology image. The results of Fig. 10(b) indicate that the glycerol treatment was more effective in classifying the non-cancerous tissues than classifying the cancerous ones shown in Fig. 10(a).

Figure 10(c) demonstrates that the classifier accuracy of treated tumor 4 part 2 and part 4 is higher than that of the untreated tumors 1 and 2. However, the accuracy of the treated tumor 3 shows the lowest accuracy, although still ~80%. The accuracy was defined earlier as kind of the average between the sensitivity and specificity; therefore, low sensitivity directly affects the accuracy of the classifier. Based on Fig. 10, the overall results indicate that glycerol treatment of tissue provide better classification compared with no treatment. However, more tissue specimens are needed for investigating the classifier sensitivity issues of Fig. 10(a).

4 Conclusions

The differentiation between cancer and collagen regions continues to be a challenge in THz imaging of freshly excised breast tumors. This work was focused on two goals: the first one was using the clearing agent glycerol to demonstrate its potential in enhancing the contrast between cancer and healthy collagen tissues. The second goal was implementing the hyperspectral SAM algorithm to classify cancer, collagen, and fat in tumor tissue. In this algorithm, there are several kernels cited in the literature²⁵ that deserved to be investigated to provide the best classification.

In this work, we used glycerol solution of 60% concentration as an optical clearance agent to treat excised tissues before scanning on the THz imager. Other imaging techniques have used glycerol and other types of optical clearance agents to improve the image contrast in frequencies across the electromagnetic spectrum. All tumors used in the testing data have healthy tissues adjacent to cancerous ones, consistent with the challenge faced in lumpectomy surgeries. On the other hand, to produce accurate training dataset, the THz cancer data were obtained entirely from cancerous tissues through lumpectomy or mastectomy surgeries, whereas the THz healthy data were obtained entirely from healthy tissues through breast reduction surgeries. The ground-truth data were obtained from the pathology images using the morphed pathology technique.^{16,17}

We investigated the effect of glycerol treatment on excised human breast tissues for application time of 20, 40, or 60 min. The three specimens that were treated with glycerol of 60% concentration were obtained from tumor 3 (20 min) and tumor 4 part-2 (40 min) and tumor 4 part-4 (60 min). On the other hand, we had tumors 1 and 2 that were not treated. The results of the absorption coefficients and the refractive indices of the treated human specimens were compared with those of the untreated ones. The obtained results showed that for any tissue type, the absorption coefficients of the treated specimens decreased with the application of glycerol and with increasing the time duration of the treatment. This observation is consistent with the work reported in the literature,¹¹ where the application of the clearance agent increased the penetration depth of the THz signal in the tissue. In other words, the application of glycerol increased the signal transmission and reduced the reflection from the tissue. Furthermore, the results showed that the absorption coefficients in cancer regions remains higher than that in healthy regions regardless of the glycerol treatment.⁴³

Hyperspectral imaging technique was used in image classification based on cumulative frequency bands. All bands start from the same frequency and each band had 10 more added frequencies than the previous band. The THz images of the five specimens considered in this work were classified using five classifications schemes: (1) SAM linear kernel, (2) SAM polynomial kernel, (3) SAM exponential kernel, (4) EMD, and (5) ESAM. The results were quantitatively compared using the standard metrics of sensitivity, specificity, and accuracy. The linear and polynomial classifiers did not show as good classification as the EXP, EMD, and ESAM in all cases investigated here.

The THz images of fresh tumors 1 to 3 showed good correlation with the pathology images and that reflected on their good classifications results. On the other hand, the THz images of tumor 4 parts 2 and part 4 did not show good correlation with their pathology images and hence the sensitivity metric in their classifications was not as good as those of tumors 1 to 3. The classification of tumor 4 parts 2 and 4 showed cancerous regions that were not seen in the pathology images. We can think of three possibilities for the discrepancy between THz and pathology images as: (1) during the histopathology process, the specimen was shaved off producing a different imaging surface for pathology than what was used in the THz imaging; (2) THz could not accurately differentiate between cancer and dense collagen due to their close properties especially at high frequency as shown in Fig. 3(a); and (3) the tissue orientation was inadvertently altered during the paraffin embedding process for histopathology.

To ensure that these possibilities occur only in imaging freshly excised specimens, we investigated the THz imaging of the FFPE blocks of tumor 4 parts 2 and 4 where this discrepancy was mainly observed in their fresh tissue images. In this case, the number of pixels in the FFPE training data of cancer, collagen, and fat are 4941, 15,522, and 45,403, respectively. The results are shown in Fig. 11, demonstrating excellent correlation between FFPE THz and pathology images. The classification results using the exponential SAM kernel also showed excellent correlation with both the FFPE THz and the pathology images. Furthermore, the classification metrics were compared in Table 1, showing much higher values for the sensitivity, specificity, and accuracy of the FFPE compared with those of fresh specimens. While the classification images show some spots of cancer in Figs. 11(c) and 11(f), these wrongly classified spots are much smaller than the correctly classified regions of cancer, collagen, and fat.

It is important to be aware that during the histopathology process, the freshly excised tumor is dehydrated by removing all the lipids and fluids before embedding in the paraffin block. This dehydration process creates lumens or cracks in the cancer region. These lumens are then filled with paraffin, which has lower refractive index and absorption coefficient values compared to cancer. Therefore, it causes the fluctuations in the average normalized reflection magnitude as shown in Fig. 3(b). The cancer reflection magnitude in Fig. 3(a) is \sim 45 times larger than that in Fig. 3(b), however, the classification results of the FFPE tissue are correct as shown in Fig. 11.

Discussing possibility (1), indeed at the OADDL histopathology lab, the FFPE blocks were faced in on the microtome until all margins/edges of tissue were visible on a microscope slide. The depth of tissue sectioning would depend on the uniformity of the excised fresh surface. Additionally, fatty tissues are more susceptible to slight tissues distortion during formalin fixation when compared to rigid (i.e., collagenous or cancerous tissues), therefore some degrees of tissue distortion may have occurred in freshly excided tissues with higher fat content. Finally, slight tissue distortion may have occurred during attachment and removal of tissue specimen to the cardboard stock used to maintain proper orientation of the tissue during shipment in 10% formalin solution.

Based on the obtained results in this work, the application of glycerol on freshly excised tissues for specific time periods make the tissue temporarily dehydrated and hence less reflective



Fig. 11 FFPE block tissue of tumor 4 (ND18228: (a)–(c) part 2 and (d)–(f) part 4. Pathology image, THz reflection image, and SAM exponential classifier. THz images are based on the peak time domain signal at each pixel. The classification images (c) and (f) are in the frequency band # 22 (0.23 to 1.25 THz).

Tumor 4 (ND18228)	Fresh			FFPE		
	Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Part 2	27 to 22	95 to 96	86 to 86	91 to 94	99 to 95	98 to 95
Part 4	60 to 56	88 to 89	84 to 85	81 to 84	92 to 88	91 to 89

Table 1 Comparison between fresh and FFPE tissue classification across frequency bands 15to 55.

compared with non-treated tissue. This can be seen in Fig. 4, where the two images of nontreated tissues show that the cancer has higher reflection intensity, represented by the darker red color. On the other hand, the three images of glycerol-treated tissues show that the cancer has lower reflection intensity, represented by the lighter red color. These results are consistent with the results of Fig. 7. Based on the results of Figs. 4 and 7, it can be concluded that indeed the penetration depth of THz signal has been enhanced in treated fresh tissue.

Overall, the improvement in THz classifications of fresh tissues treated with glycerol solution is promising. The adopted treatment protocol, the type of the optical clearance agent, and the time duration of the treatment are all vital factors that can use further investigation in the future.

Upon conducting qualitative comparison between THz images and x-ray images of human tissue and with computer tomography images of mice tissue, the results were in favor of THz images (not included due to space limitation). Providing a quantitative comparison among different imaging modalities of excised tissues is important, but it is outside the scope of this work.

Disclosures

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was funded by the National Institutes of Health (Award No. R15CA208798). We would like to acknowledge the collaboration with OADDL at the Oklahoma State University for conducting the histopathology procedure tissues. The authors also would like to acknowledge the NDRI biobank for providing the freshly excised breast tumors used in this work. In addition, the authors would like to acknowledge Dr. J. Wu's valuable discussion on the classifier quantification.

References

- 1. V. V. Tuchin, "Tissue optics and photonics: biological tissue structures," *J. Biomed. Photonics Eng.* **1**(1), 3–21 (2015).
- V. V. Tuchin, "Tissue optics and photonics: light tissue interaction," J. Biomed. Photonics Eng. 1(2), 98–134 (2015).
- 3. L. M. C. Oliveira and V. V. Tuchin, *The Optical Clearing Method: A New Tool for Clinical Practice and Biomedical Engineering*, Springer, Switzerland (2019).
- 4. R. Christoph et al., Glycerol, Ullmann's Encyclopedia of Industrial Chemistry (2006).
- 5. O. A. Smolyanskaya et al., "Glycerol dehydration of native and diabetic animal tissues studied by THz-TDS and NMR methods," *Biomed. Opt. Express* 9, 1198–1215 (2018).
- A. T. Yeh et al., "Reversible dissociation of collagen in tissues," J. Invest. Dermatol. 121(6), 1332–1335 (2003).
- 7. S. Björklund et al., "Glycerol and urea can be used to increase skin permeability in reduced hydration conditions," *Eur. J. Pharmaceut. Sci.* **50**(5), 638–645 (2013).
- 8. V. D. Genin et al., "Ex vivo investigation of glycerol diffusion in skin tissue," *J. Biomed. Photonics Eng.* **2**(1), 010303 (2016).
- H. Q. Zhong et al., "Enhancement of permeability of glycerol with ultrasound in human normal and cancer breast tissues in vitro using optical coherence tomography," *Laser Phys. Lett.* 7(5), 388 (2010).
- J. Wang et al., "Evaluation of optical clearing with the combined liquid paraffin and glycerol mixture," *Biomed. Opt. Express* 2(8), 2329–38 (2011).
- S. J. Oh et al., "Measurement depth enhancement in terahertz imaging of biological tissues," *Opt. Express* 21(18), 21299–21305 (2013).
- G. R. Musina et al., "A comparison of terahertz optical constants and diffusion coefficients of tissue immersion optical clearing agents," *Proc. SPIE* 11065, 110651Z (2019).
- T. C. Bowman, M. El-Shenawee, and L. K. Campbell. "Terahertz imaging of excised breast tumor tissue on paraffin sections," *IEEE Trans. Antennas Propag.* 63(5), 2088–2097 (2015).
- M. El-Shenawee et al., "Cancer detection in excised breast tumors using terahertz imaging and spectroscopy," *Biomed. Spectrosc. Imaging* 8(1-2), 1–9 (2019).
- N. Vohra, K. Bailey, and M. El-Shenawee, "Dehydration approach for enhancing terahertz detection of cancer in freshly excised breast tumors," in *Proc. IEEE-APS/URSI 2020*, Montreal, Quebec (2020).
- T. Chavez et al., "Assessment of terahertz imaging for excised breast cancer tumors with image morphing," J. Infrared Millimeter Terahertz Waves 39(12), 1283–1302 (2018).
- T. Chavez et al., "Breast cancer detection with low-dimensional ordered orthogonal projection in terahertz imaging," *IEEE Trans. Terahertz Sci. Technol.* 10(2), 176–189 (2020).
- 18. N. Vohra et al., "Mammary tumors in Sprague Dawley rats induced by N-ethyl-Nnitrosourea for evaluating terahertz imaging of breast cancer," *J. Med. Imaging* **8**(2), 023504 (2021).
- 19. T. Bowman et al., "Pulsed terahertz imaging of breast cancer in freshly excised murine tumors," *J. Biomed. Opt.* 23(2), 026004 (2018).
- T. Chavez et al., "Supervised Bayesian learning for breast cancer detection in terahertz imaging," *Biomed. Signal Process. Control* 70, 102949 (2021).

- 21. H. Liu et al., "Deep learning classification of breast cancer tissue from terahertz imaging through wavelet synchro-squeezed transformation and transfer learning," *J. Infrared, Millimeter, and Terahertz Waves* (in press).
- N. Keshava, "Distance metrics and band selection in hyperspectral processing with applications to material identification and spectral libraries," *IEEE Trans. Geosci. Remote Sens.* 42(7), 1552–1565 (2004).
- G. P. Petropoulos et al., "Comparison of spectral angle mapper and artificial neural network classifiers combined with Landsat TM imagery analysis for obtaining burnt area mapping," *Sensors* 10(3), 1967–1985 (2010).
- S. Chen et al., "The tradeoff analysis for remote sensing image fusion using expanded spectral angle mapper," *Sensors* 8(1), 520–528 (2008).
- 25. G. Camps-Valls, "Kernel spectra angle mapper," *Electron. Lett.* 52(14), 1218–1220 (2016).
- M. A. Cho et al., "Improving discrimination of savanna tree species through a multipleendmember spectral angle mapper approach: canopy-level analysis," *IEEE Trans. Geosci. Remote Sens.* 48(11), 4133–4142 (2010).
- P. Beatriz Garcia-Allende et al., "Data processing method applying principal component analysis and spectral angle mapper for imaging spectroscopic sensors," *IEEE Sens. J.* 8(7), 1310–1316 (2008).
- 28. X. Liu and C. Yang, "A kernel spectral angle mapper algorithm for remote sensing image classification," in *IEEE 6th Int. Congr. Image and Signal Process. (CISP 2013)* (2013).
- 29. N. Liu et al., "Gastric cancer diagnosis using hyperspectral imaging with principal component analysis and spectral angle mapper," *J. Biomed. Opt.* **25**(6), 066005 (2020).
- 30. M. Halicek et al., "In-vivo and ex-vivo tissue analysis through hyperspectral imaging techniques: revealing the invisible features of cancer," *Cancers* **11**(6), 756 (2019).
- 31. G. Lu et al., "Detection of head and neck cancer in surgical specimens using quantitative hyperspectral imaging," *Clin Cancer Res.* **23**(18), 5426–5436 (2017).
- A. Goto et al., "Use of hyperspectral imaging technology to develop a diagnostic support system for gastric cancer," J. Biomed. Opt. 20(1), 016017 (2015).
- J. Kim et al., "Multimodal endoscopic system based on multispectral and photometric stereo imaging and analysis," *Biomed. Opt. Express* 10(5), 2289–2302 (2019).
- 34. H. B. Barlow, "Unsupervised learning," Neural Comput. 1(3), 295-311 (1989).
- R. H. Yuhas, A. F. H. Goetz, and J. W. Boardman. "Discrimination among semi-arid landscape endmembers using the spectral angle mapper (SAM) algorithm," in *Proc. Summaries 3rd Annu. JPL Airborne Geosci. Workshop*, Vol. 1, pp. 147–149 (1992).
- A. Krizhevsky, I. Sutskever, and G. E. Hinton. "Imagenet classification with deep convolutional neural networks." *Commun. ACM* 60, 84–90 (2012).
- 37. Y. L. Cun et al., "Handwritten digit recognition with a back-propagation network," in *Adv. Neural Inf. Process. Syst.* (1989).
- H. C. Shin et al., "Deep convolutional neural networks for computer-aided detection: CNN architectures, dataset characteristics and transfer learning," *IEEE Trans. Med. Imaging* 35(5), 1285–1298 (2016).
- M. Sokolova, N. Japkowicz, and S. Szpakowicz, "Beyond accuracy, F-score and ROC: a family of discriminant measures for performance evaluation," in *Aust. Joint Conf. Artif. Intell.*, pp. 1015–1021, Springer, Berlin, Heidelberg (2006).
- C. Johnson, "Top scientific visualization research problems," *IEEE Comput. Graphics Appl.* 24(4), 13–17 (2004).
- C. Lundström et al., "Uncertainty visualization in medical volume rendering using probabilistic animation," *IEEE Trans. Vis. Comput. Graphics* 13(6), 1648–1655 (2007).
- N. Vohra et al., "Terahertz imaging and characterization protocol for freshly excised breast cancer tumors," J. Vis. Exp. 158, e61007 (2020).
- T. Bowman et al., "Terahertz tomographic imaging of freshly excised human breast tissues," J. Med. Imaging 6(2), 023501 (2019).
- P. L. Hsu and H. Robbins, "Complete convergence and the law of large numbers," in *Proc. Natl. Acad. Sci. U. S. A.* 33(2), 25–31 (1947).
- 45. J. Durbin, *Distribution Theory for Tests Based on the Sample Distribution Function*, Society for Industrial and Applied Mathematics (1973).

Q. Yang and X. Wu, "10 challenging problems in data mining research," *Int. J. Inf. Technol. Decis. Making* 5(04), 597–604 (2006).

Nagma Vohra received her BTech degree in electronics and communication engineering from Guru Nanak Dev University, Amritsar, India, in 2014 and her MTech degree in communication engineering from Vellore Institute of Technology, Vellore, India, in 2017. She recently graduated with her PhD in electrical engineering from the University of Arkansas, Fayetteville, Arkansas, USA, in August 2021. Her dissertation focuses on the electromagnetic characterization of materials at microwave, millimeter-wave, and terahertz frequencies.

Haoyan Liu received his BE degree in automation from Harbin University of Science and Technology in 2012 and his MS degree in electrical engineering at the University of Arkansas in 2016. He is currently pursuing his PhD in computer engineering at the University of Arkansas. His research interests include deep learning, machine learning, embedded systems, wearable technologies, power conversion, and control systems. He is a member of Tau Beta Pi and IEEE-HKN.

Alexander H. Nelson received his BS degree in 2012 and his MS degree in 2013 in computer engineering from the University of Arkansas and his PhD in computer engineering from the University of Maryland, Baltimore County, in 2017. He is an assistant professor of computer science and computer engineering at the University of Arkansas, Fayetteville, Arkansas, USA. He is a member of IEEE-HKN and Tau Beta Pi.

Keith Bailey currently serves as a senior pathologist at Charles River Laboratories. He has more than 20 years of experience as a board-certified veterinary pathologist in the public and private sectors. He has served on faculty at the University of Illinois and Oklahoma State University and as a toxicologic pathologist in the biopharmaceutical industry for 7 years while employed by Pfizer and Amgen.

Magda El-Shenawee received her PhD in electrical engineering from the University of Nebraska, Lincoln, Nebraska, USA. She is currently a professor of electrical engineering at the University of Arkansas in Fayetteville. Her research interests include terahertz imaging and spectroscopy, breast cancer detection, material characterization in the microwave, millimeter-wave, and terahertz frequency bands, computational inverse scattering algorithms, MEMS antennas, nano-antennas for energy enhancement of photovoltaic solar cells, and biopotentials modeling of breast tumor cancerous cells.