

Terahertz spectroscopy of intrinsic biomarkers for non-melanoma skin cancer.

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ABSTRACT

Continuous wave terahertz imaging has the potential to offer a safe, non-invasive medical imaging modality for detecting different types of human cancers. The aim of this study was to identify intrinsic biomarkers for non-melanoma skin cancer and their absorption frequencies. Knowledge of these frequencies is a prerequisite for the optimal development of a continuous wave terahertz imaging system for detecting different types of skin cancers. The absorption characteristics of skin constituents were studied between 20 and 100 cm^{-1} (0.6 THz – 3 THz). Terahertz radiation is highly absorbed by water. Thus, the high water content of human tissue necessitates a reflection based imaging modality. To demonstrate a reflection based, high resolution, terahertz imaging system, a prototype imaging system was constructed at 1.56 THz. The system resolution was determined to be 0.5 mm and the system signal to noise ratio was found to be 70 dB. Data from the terahertz spectroscopy experiments and reflection based terahertz images at 1.56 THz are presented.

Keywords: Terahertz spectroscopy, continuous wave terahertz imaging, skin cancer imaging

1. INTRODUCTION

1.1 Non-Melanoma Skin Cancer

Non-melanoma skin cancer is the most common form of cancer, with approximately 1 million new cases diagnosed each year. It is also nearly 100% curable if diagnosed in time and treated properly. According to the National Institute of Health (NIH), people with fairer skin have a higher risk of getting skin cancer, and approximately 40-50% of Americans who live to the age of 65 will have skin cancer at least once. Currently, early detection of skin cancer is based on a visual medical assessment, and diagnosis requires a biopsy. There is no standard *in vivo* skin cancer diagnosis technique. One of the most common treatment techniques for skin cancer is Mohs-Micrographic surgery. It involves removing the cancer layer by layer, while simultaneously processing the histology to map the residual tumor.

1.2 Terahertz Imaging

The terahertz region of the electromagnetic spectrum is generally considered to extend from 0.1 and 10 THz and lies between the microwave and infrared regions. The primary advantage of terahertz imaging is that terahertz radiation is inherently safe. Unlike x-rays, terahertz rays are non-ionizing and have no known harmful effects on living tissue.^{1,2} Also, the fact that terahertz rays have a shorter wavelength than microwaves implies an inherently higher spatial resolution for imaging applications.

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Biological interest in terahertz radiation arises from the high water sensitivity and the fact that many bio-molecules have characteristic resonance absorptions in the terahertz region.^{3,4,5,6} Studies have shown the potential for investigating molecular conformational states, *in situ*, using terahertz spectroscopy.^{7,8}

There are two primary approaches to medical terahertz imaging, Terahertz Pulse Imaging (TPI) and the continuous-wave (CW) terahertz imaging. The distinction is based on the nature of the source. Terahertz pulse imaging uses a sequence of wide-bandwidth, low-power pulses. Continuous wave systems use very narrow bandwidth, essentially single frequency, high power (several milliwatts), radiation sources. Most medical research in terahertz imaging thus far has been focused on terahertz pulsed imaging mostly due to the lack of commercially available continuous wave terahertz sources. TPI has already been used to identify Basal Cell Carcinoma (BCC) both *ex vivo* and *in vivo*.⁹The source mechanism for the contrast in TPI images of BCC is not yet clearly understood.¹⁰

1.3 Project Overview

The goal of this contribution is to develop a CW terahertz system capable of imaging skin cancers and revealing the source of contrast in the terahertz spectral range. There are numerous advantages to using a CW based terahertz system. For example, if the optimal interrogation frequency for detecting a specific biomarker or for maximizing image contrast is known, a CW system can be constructed to operate at that frequency. The high average power of CW beams leads to high signal to noise and fast detection techniques that can minimize image acquisition time. The relative simplicity of the data collected leads to a lower projected cost for a CW system as compared to a pulsed system.²

The high absorption of terahertz radiation by liquid water necessitates the use of reflection based imaging for *in vivo* applications, or restricts the technology to *ex vivo* thin sample transmission studies. The first step to building a CW terahertz imaging system is determining the optimal contrast terahertz frequency. In order to determine this frequency the transmittance characteristics of several human skin constituents in the terahertz region were characterized. The idea is to eventually correlate these measurements with absorption measurements of cancerous skin in order to determine the intrinsic biomarker and the optimal contrast frequency. The next step is the actual construction of a terahertz scanning device optimized for use at the terahertz contrast frequency. This involves determining and employing the appropriate source and receiver technology, designing and constructing the optical system and integrating a scanning device into the measurement system.

This paper presents the results from two separate preliminary experiments for this project. The aim of the first experiment was to determine the terahertz absorption characteristics of human skin constituents and to determine if these characteristics were still observable in the presence of a highly absorbing media like liquid water. The second experiment involved the construction of a reflection based, high resolution, continuous wave terahertz imaging system at 1.56 THz. The aim of the second experiment was to determine imaging parameters such as system optical resolution and system signal to noise ratio. The experiment also verified motion control and data acquisition techniques that will be required to construct a terahertz imaging system capable of scanning non-melanoma skin cancer samples at the optimal contrast frequency.

2. EXPERIMENTAL SETUP

2.1 Terahertz spectroscopy of human skin constituents

The terahertz transmission characteristics of various components of human skin were measured. The substances examined were water, tyrosine, tryptophan, urocanic acid, melanin and collagen. All chemicals measured were purchased from Sigma Aldrich.

The terahertz spectroscopy was performed using a Bruker IFS 66v Fourier Transform Far Infrared (FTIR) spectrometer. The system has a signal to noise ratio of approximately 20 dB and uses an external, liquid helium cooled, silicon bolometer. Figure 1 shows the spectral output of the spectrometer in the region of interest without any sample in the beam path.

Initially all the skin constituent chemicals were measured as pressed powders in a fixed ratio (1:1) with photometric grade polyethylene powder. The mixed powder was pressed in between two polyethylene windows held together by a

stainless steel casing. The observed spectra were calibrated against a separate transmittance spectra acquired on the same amount of non-doped polyethylene powder housed in the same sample holder.

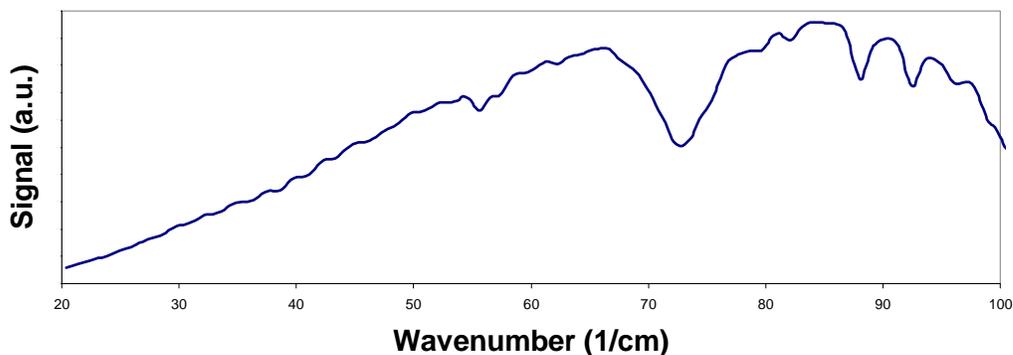


Figure 1. Spectral output of the spectrometer between 20 and 100 cm^{-1} .

Chemicals which exhibited absorption spectra in the terahertz region were later measured as suspensions in liquid water to ensure that the spectroscopic features were still observable in the presence of a highly absorbing medium like water.

To perform the liquid spectroscopy, a custom sample holder was designed. The primary considerations for the holder were that it should be vacuum tight, allowing one to place the sample in the standard evacuated chamber of the Bruker IFS 66v FTIR for measurement. The configuration should be reproducible so as to minimize the error from disassembly and reassembly required to take background and sample scans. Other factors were that the size of the clear aperture in the sample should be large as compared to the beam and the window material should be transparent in the terahertz region of interest.

The liquid sample holder designed for this experiment is shown in Figure 2. The top plate is removable and is held to the bottom plate using six screws. The spacing between the windows is maintained using Mylar spacers. When the top plate is screwed onto the bottom plate it pushes down on the windows and excess liquid enters the liquid vents which are capped off before placing the system under vacuum. The clear aperture of this holder is approximately 1 inch.

High resistivity silicon and high density polyethylene (HDPE) both have low loss in the terahertz region which makes them good candidates for sample holder windows. However, using plane parallel slabs of these materials produces a well known etalon effect, as seen in Figure 3a and 3b. This effect is easily avoided by using a wedged window. Figure 3 also shows the effect of a wedge angle on the transmittance of both polyethylene and silicon. Thus, the windows were manufactured with a 1.5 degree wedge angle on one face. The sample holder was designed to fix the orientation of the wedged windows with respect to each other.

Silicon has a lower absorption coefficient than HDPE in this range ($k = 0.00006$ for silicon, $k = 0.00077$ for HDPE).^{11,12} However, the comparatively higher refractive index ($n = 3.42$ for silicon and $n = 1.53$ for HDPE) leads to higher reflection and lowers the transmission through the wedge. Thus, high density polyethylene was the preferred choice for window material. The windows were made thick enough (2 mm maximum thickness) so as to not flex under vacuum.

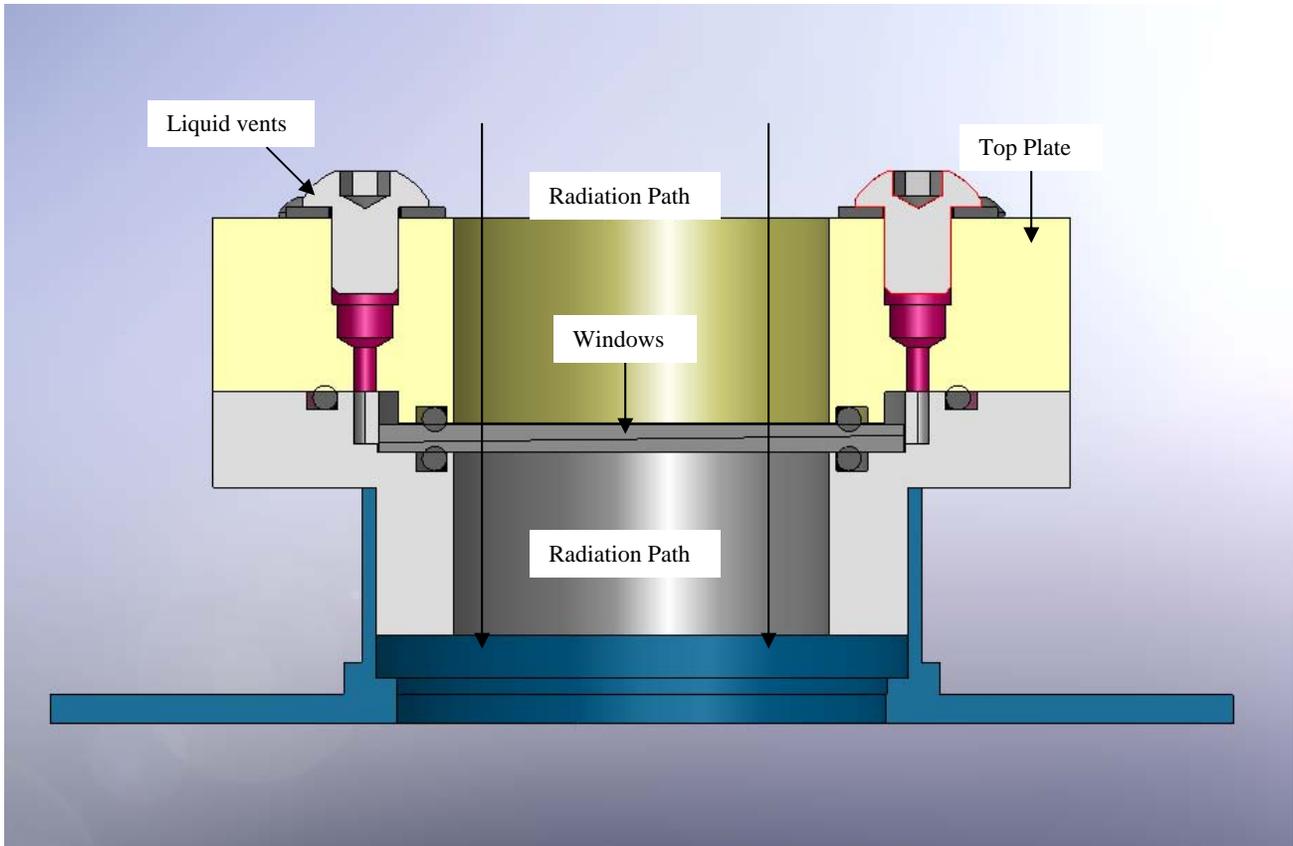


Figure 2. Cross-sectional view of liquid sample holder.

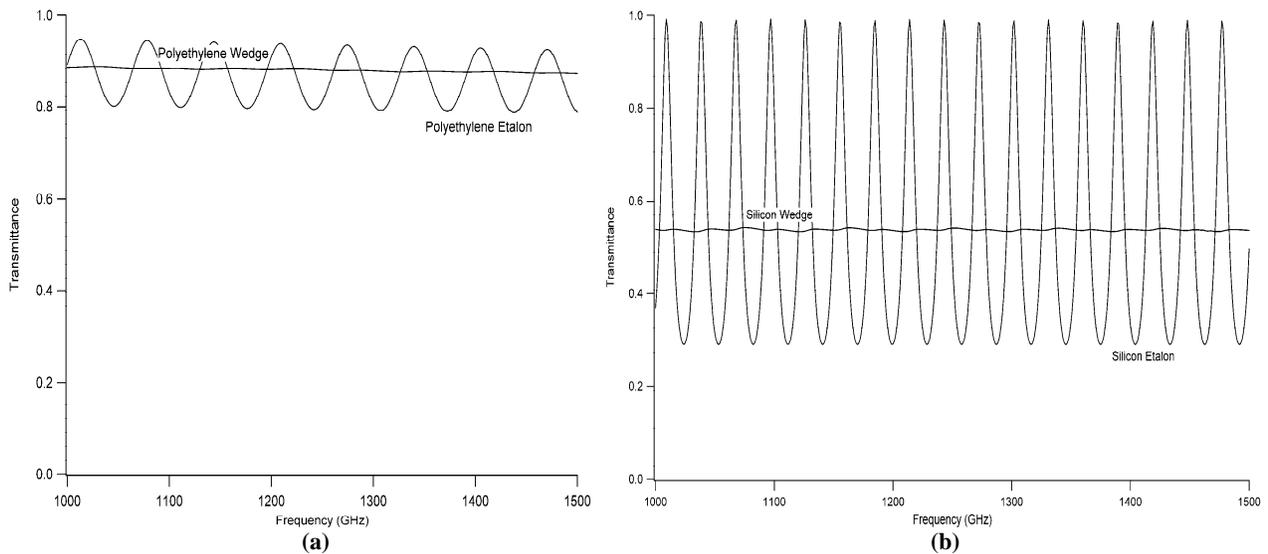


Figure 3. (a) Transmittance of a 1.5 mm HDPE etalon and the same etalon with a 1.5 degree wedge angle. (b) Transmittance of a 1.5 mm silicon etalon and the same etalon with a 1.5 degree wedge angle.

2.2 Reflection modality terahertz imaging at 1.56 THz

The aim of this experiment was to demonstrate reflection based, continuous wave terahertz imaging at 1.56 THz and ascertain imaging parameters such as the achieved system signal-to-noise ratio (SNR) and system optical resolution. The experiment was also used to determine possible imaging artifacts and determine optimal source and receiver technology for this application.

The laser source used for this experiment has been described previously.¹³ The source consisted of a CO₂ laser used as the optical pump for a far-infrared gas laser. The CO₂ laser was set to produce a 10 μm (10R10 CO₂ laser line) wavelength line that in turn was used to pump 1.5645 THz laser line in methanol (CH₃OH). The output power of the pump CO₂ line was 120 Watts and the resulting power of the far-infrared laser was measured to be 54 milliwatts. The layout for the experiment is shown in Figure 4.

The laser beam was focused onto the sample plane using an off-axis parabolic mirror. The detector was a liquid helium cooled silicon bolometer. The sample itself was mounted on a programmable XY scan stage, and raster scanned across the beam focus. The reflected beam retraces its incident path and is split using a 60% transmission Mylar beam splitter into the detector. The distance of the detector and the position of the beam splitter in the optical layout are such that the source and receiver arm are matched and the beam focuses into the detector. The optical system was designed to produce a Gaussian beam waist of approximately 0.2 mm at the sample.

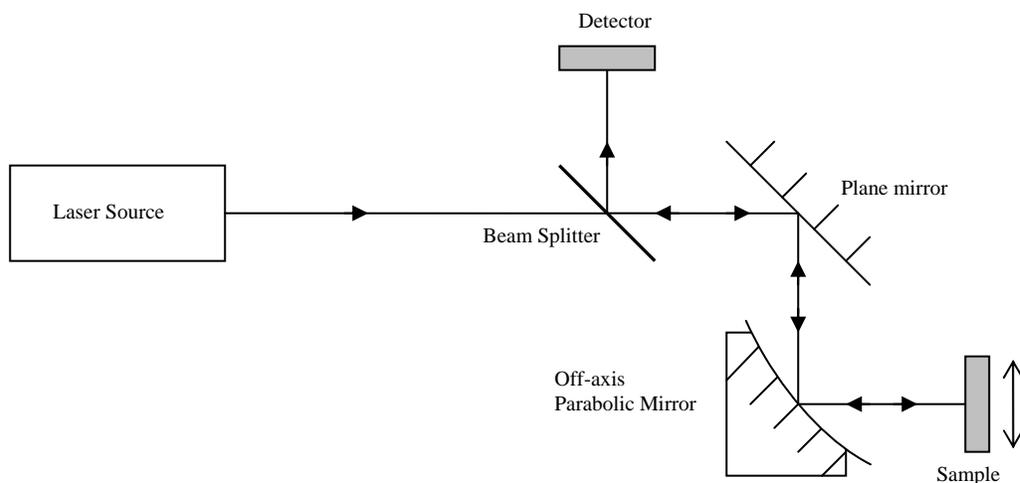


Figure 4. Schematic of the experimental setup for reflection based imaging at 1.56 THz

Data acquisition and processing software along with motion control software for the two axis XY scan stage was written using National Instruments LabVIEW software. The amplitude information from the silicon bolometer was sent to a Lock-In amplifier and the data from the amplifier was collected by the program using a data acquisition card from National Instruments. The software synchronized the motion control and data acquisition, and generated an amplitude intensity map of the sample area.

3. RESULTS AND DISCUSSION

3.1 Results of spectroscopy studies

The chemicals measured were tyrosine, tryptophan, melanin, collagen and urocanic acid. As water is also present in human skin, the terahertz transmission characteristics of liquid water were also measured and are shown in Figure 5. As one can see there are no sharp spectral features for liquid water in this frequency region, however, the absorption increases with increasing frequency. This implies that higher frequencies are more sensitive to differences in water content. However, in order to detect the difference one requires more sensitive detectors or more powerful terahertz sources. Thus, if water content was to serve as the biomarker for skin cancer, then the highest frequency that one could achieve the requisite signal to noise ratio at is the optimal contrast frequency.

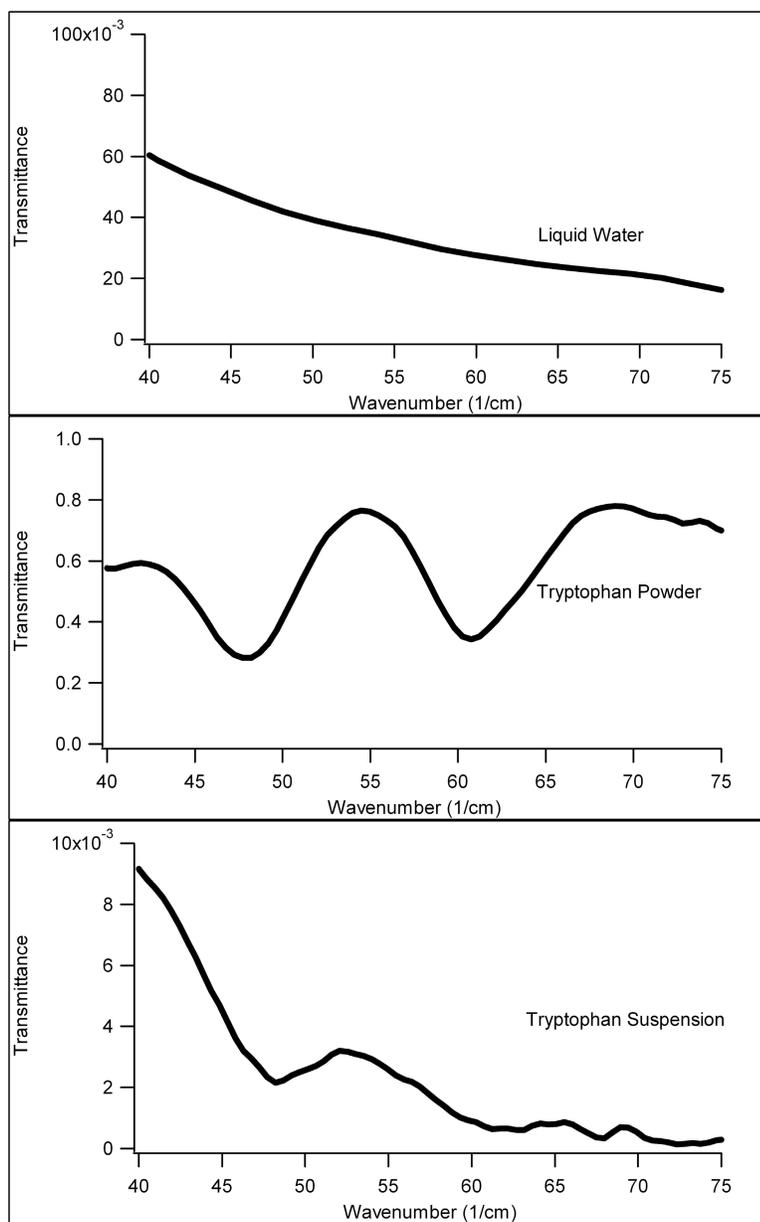


Figure 5. Terahertz transmittance spectrum of water, tryptophan powder and tryptophan suspension (0.74 gm/ml).

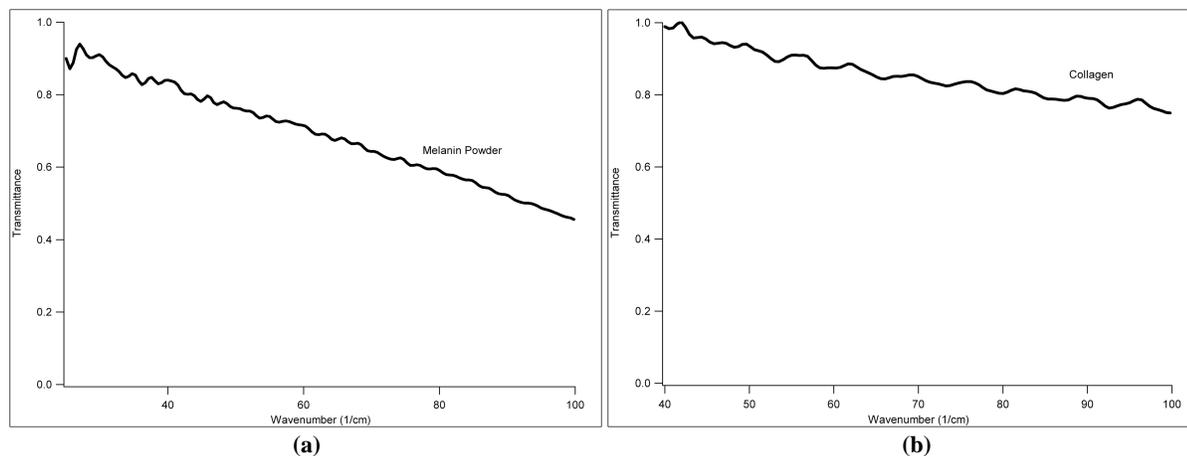


Figure 6. (a) Terahertz transmittance spectra of melanin powder (b) Terahertz transmittance spectra of Collagen

Of the other chemicals that were measured only tyrosine, tryptophan and urocanic acid exhibited any terahertz resonant absorption structure. The transmittance spectrum of tryptophan is of special interest for this project and is shown in Figure 5. In Figure 5 one can observe dips in the terahertz transmission of tryptophan which correspond to known resonance absorption modes at 47.7 and 60.8 cm^{-1} (1.42 and 1.84 THz respectively). These have been previously observed and assigned in literature. The mode at 1.42 THz is due to C_{11} - C_{12} torsional motion in the tryptophan molecule, while the 1.84 THz absorption peak is due to C_1 - C_9 ring torsion.¹⁴ The terahertz transmission spectra of melanin and collagen are shown in Figure 6. As one can see they exhibit no resonance absorption phenomena in the terahertz region and hence are unlikely to affect the results of the imaging experiment.

Tyrosine and Urocanic acid also exhibited terahertz absorption structure and were measured in liquid water suspension as well. Urocanic acid exhibited absorption modes at 37.123 cm^{-1} (1.11 THz) and 51.1044 cm^{-1} (1.53 THz). These modes have not been previously observed and are as yet unassigned. Tyrosine has been studied in the terahertz region by other authors previously. Table 1 lists the observed absorption peaks of tyrosine and the corresponding mode assignments where found in published literature.

Table 1. Observed absorption peaks and mode assignments for Tyrosine.

Absorption Peak (cm^{-1})	Frequency (THz)	Absorption line in literature (THz)	Mode Assignment	Reference
27.7 ± 0.1	0.83	-	Not assigned	
32.3 ± 0.1	0.97	0.97	Torsion of entire chain around C-ring bond	Grace et al. J. of Mole. Spec. Vol. 215(2) 204-219
63.64 ± 0.1	1.90	-	Not assigned	
68.9 ± 0.1	2.06	2.03	C-H oop bend, in-phase	Fukushima et al. Spectrochim. Acta. Vol. 15 236-241
88.2 ± 0.1	2.64	2.62	COOH torsion	Grace et al. J. of Mole. Spec. Vol. 215(2) 204-219

Tryptophan is of special interest is because there is evidence in literature that some malignant tumors have a higher tryptophan concentration than benign tumors.^{15,16} Tryptophan, tyrosine and urocanic acid were measured as suspensions in liquid water as well.

The measurements of liquid water suspensions were performed to ensure that the absorption frequencies of the different chemicals were still observable in the presence of a highly absorbing medium like water. Figure 5 shows the observed terahertz absorption of tryptophan as a suspension in liquid water alongside the spectra of tryptophan powder and liquid water as a reference. If tryptophan were to serve as the biomarker for skin cancer, the CW imaging system would be designed and constructed at a resonance absorption frequency in order to maximize sensitivity.

3.2 Reflection modality continuous wave terahertz imaging results

The output of the pump CO₂ gas laser was measured to be 120 Watts and the 1.56 THz FIR gas laser output power was measured to be approximately 54 milliwatts.

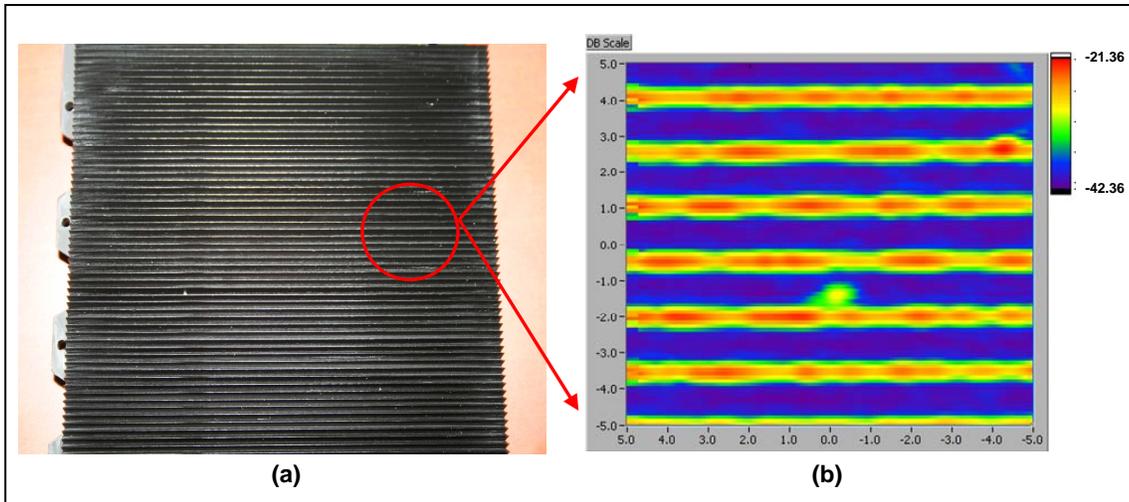


Figure 7. (a) Photograph of Terasorb-1500 (b) 1.56 THz reflection measurement in logarithmic amplitude. The terahertz image is of 10 mm x 10 mm patch of Terasorb-1500.

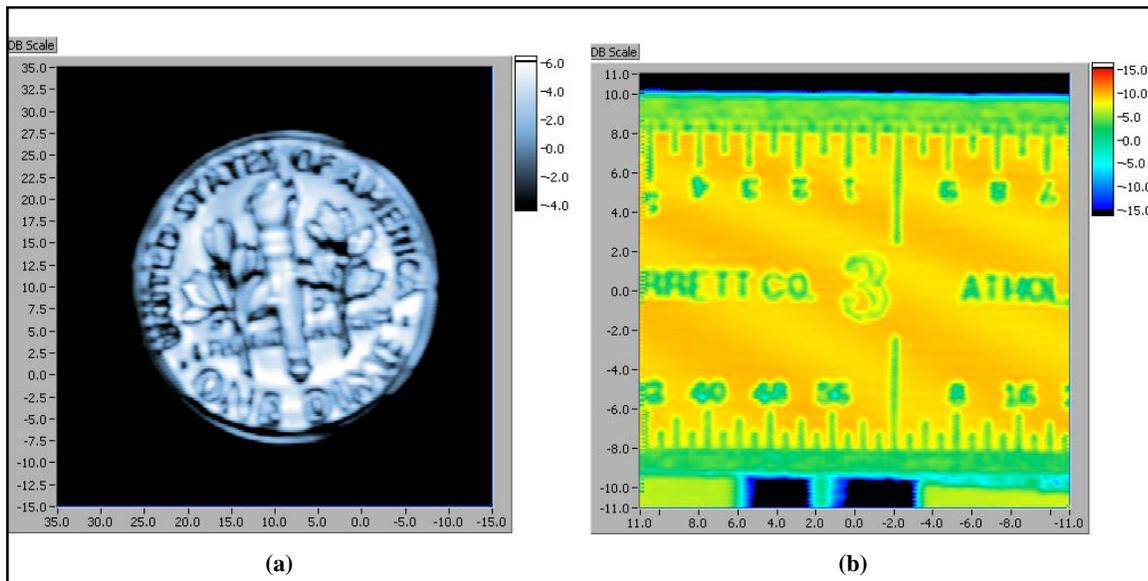


Figure 8. (a) THz Image of a Dime, shown in logarithmic amplitude (b) THz Image of a Ruler shown in logarithmic amplitude.

Figure 7(b) shows the 1.56 THz reflection image of a 10 mm x 10 mm cross-section of anechoic material which is designed to minimize the backscattered radiation at 1.5 THz. The valley in the material is 42 dB below full scale return. The system's signal to noise ratio was measured to be approximately 70 dB after using a lock in amplifier to measure the signal.

The Gaussian beam waist at the sample position was measured to be 0.51 mm, approximately 0.6 mm Full Width at Half Max (FWHM). This is the optical resolution of the system. The system field of view was determined by the XY scan stage used and could be varied easily to a maximum of 10 cm x 10 cm. Most measurements were done with a smaller field of view as determined by the sample size. The resolution of the horizontal axis was typically set to 0.01 mm and the resolution of the vertical axis was set to 0.1 mm. It is possible to increase the vertical axial resolution but that also increases the data acquisition time. Figure 8 shows terahertz images of a dime and part of a ruler. These serve to confirm the optical resolution of the system as one is able to discern fairly fine features.

This experiment demonstrated the practicality of a high resolution continuous wave terahertz imaging with a high signal-to-noise ratio. Once the frequency of optimal resolution has been determined the system optical path can be adjusted to yield the required imaging system. The system resolution is ultimately a function of wavelength and this experiment demonstrated that achieving a resolution of the order of a few wavelengths is feasible using off-the-shelf optics. Higher resolution should be possible using special techniques and custom optics. For the purpose of this application however, such techniques are unnecessary. Future iterations of the imaging system might involve constructing a heterodyne based receiver system at the desired optimal contrast frequency. A heterodyne system requires two laser sources and is capable of giving both amplitude and phase information. The detectors used would be whisker-contacted Schottky diodes that have the added advantages of increased sensitivity as compared to a bolometer and room temperature operation. The projected signal to noise ratio of a heterodyne system using whisker contacted Schottky diode detectors is 120 dB, which is 10^5 times more sensitive than the imaging system described here.

4. SUMMARY

The spectroscopy results confirmed that certain biomolecules have characteristic transmittance spectra in the terahertz region of the electromagnetic spectrum. As discussed previously, both water content and tryptophan concentration are potentially intrinsic biomarkers for skin cancer. Liquid water has high absorption but no resonance absorption structure in the terahertz region. Tryptophan, however, has resonance absorption modes at 47.7 and 60.8 cm^{-1} (1.42 and 1.84 THz respectively). A terahertz imaging system constructed at 1.84 THz would be sensitive to both water and tryptophan content. One would prefer the 1.84 THz absorption as the absorption of liquid water increases with frequency, thereby making the system more sensitive to water content than one operating at 1.42 THz. Prior to designing an imaging system however, we propose to measure the terahertz absorption spectrum of cancerous tissue using point spectroscopy and measuring across $5 \mu\text{m}$ thick histological sections. These measurements will then be correlated to measurements of human skin constituents to determine the optimal contrast frequency. The imaging experiment demonstrated that high resolution continuous wave terahertz imaging with high signal to noise ratios is a feasible application. The next iteration of the system will be at the optimal contrast frequency and utilize a heterodyne based detection scheme. This will yield higher signal to noise ratios and the availability of phase information which can be used for additional, more complex image processing techniques.

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