Dual Frequency Continuous Wave Terahertz Transmission Imaging of Nonmelanoma Skin Cancers

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ABSTRACT

Continuous wave terahertz imaging has the potential for diagnosing and delineating skin cancers. While contrast has been observed between cancerous and normal tissue at terahertz frequencies, the source mechanism behind this contrast is not clearly understood.\textsuperscript{1} Transmission measurements of 240\,µm thick sections of nonmelanoma skin cancer were taken at two frequencies of 1.39 THz and 1.63 THz that lie within and outside the tryptophan absorption band, respectively. Two CO\textsubscript{2} pumped Far-Infrared molecular gas lasers were used for illuminating the tissue while the transmitted signals were detected using a liquid Helium cooled Silicon bolometer. At both THz frequencies 2-dimensional THz transmission images of nonmelanoma skin cancers were acquired with better than 0.5mm spatial resolution. The resulting images were compared to the sample histology and showed a correlation between cancerous tissue and decreased transmission. The results of the imaging experiments will be presented and discussed.

Keywords: continuous-wave terahertz imaging, skin-cancer imaging

1. INTRODUCTION

1.1 Nonmelanoma Skin Cancer

Nonmelanoma 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 appropriately. Currently, early detection of skin cancer is based on a visual medical assessment with a subsequent required biopsy for full diagnosis. One of the most common and effective treatment techniques for skin cancer is Mohs-Micrographic surgery. It involves removing the cancer layer by layer, while simultaneously processing histology to map the residual tumor. An in vivo skin cancer imaging technique could therefore aid in both the diagnosis and treatment of skin cancer.

1.2 Terahertz Imaging

The terahertz region of the electromagnetic spectrum is generally considered to extend from 0.1 to 10 THz and lies between the microwave and infrared regions. The primary advantage of medical terahertz imaging is that terahertz radiation is believed to be safe. Unlike x-rays, terahertz rays are non-ionizing and have no known harmful effects on living tissue.\textsuperscript{2,3} While the long wavelength (low energy) nature of the THz waves makes them non-ionizing, the fact that terahertz rays have a shorter wavelength than microwaves implies an inherently higher spatial resolution for imaging applications.

There are two primary approaches to medical terahertz imaging, Terahertz Pulse Imaging (TPI) and continuous-wave (CW) terahertz imaging. The distinction is based on the nature of the source. Terahertz pulse imaging uses a sequence of wide-bandwidth pulses. Continuous wave systems use very narrow bandwidth radiation sources. While the power
contained in a terahertz pulse may be high, the power at individual frequencies is comparatively low when compared to a CW source operating at that frequency. 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. The source mechanism for the contrast in TPI images of BCC is not yet clearly understood.

1.3 Project Overview

Water is considered to be a potential source of intrinsic contrast between cancerous and non-cancerous skin in terahertz images of skin cancer. While water has a large attenuation coefficient for the frequencies in this region, liquid water has no observable spectral characteristics in this frequency region. As one can see in Figure 1, the transmittance of terahertz rays through liquid water decreases with increasing frequency.

![Figure 1: Terahertz transmittance through liquid water (~0.07mm thick) encased in a liquid sample holder. Note the absence of any transmission features.](image)

Thus if water content alone were to serve as the intrinsic biomarker for skin cancer, higher frequencies are more sensitive to water content. Another advantage of higher frequencies is the increased spatial resolution as the resolution is ultimately limited by the wavelength. The primary disadvantage of high frequencies is that the decreased transmission necessitates an increase in the detection efficiency. While water is a potential source of the contrast, the contrast mechanism is not clearly understood. Studies show that there is a difference in the state of water between cancerous and noncancerous tissue. However, the ability of terahertz radiation to distinguish between the state of water in biological tissue is still unclear.

Tryptophan may also be the source of contrast in cancerous tissue. There is also evidence in the literature that tumors have higher tryptophan content than normal tissue. In contrast to water, Tryptophan does have spectral structure in the terahertz region. As one can see in figure 2, tryptophan has transmission dips at 1.4 and 1.8 THz. These absorption bands have been previously observed and assigned in literature.

The goal of this study was to investigate the terahertz transmission of human skin tissue. Transmission imaging was demonstrated at two different frequencies, 1.4THz which lies within the tryptophan absorption region and 1.6THz which lies outside it. Both frequencies are sensitive to water content. If, however, the increased tryptophan level in cancerous tissue is the source of contrast then the 1.4THz image should show greater contrast than the 1.6THz image. The reason
that the off resonance line was chosen at 1.6THz is because then the two frequencies are close enough to each other and that enables one to easily match their optical paths.

**Figure 2:** Terahertz transmittance of tryptophan (measured in a 1:1 ratio with polyethylene powder, ~ 2.5 mm thick). Note the presence of transmittance dips at 1.4 and 1.8 THz.  

2. EXPERIMENTAL SETUP

2.1 Source and Receiver Technology

The sources used for this experiment were two CO\textsubscript{2} pumped far-infrared gas lasers. The CO\textsubscript{2} lasers and the pumped FIR lasers that are used have been described previously in literature.\textsuperscript{11} The first laser line selected for this experiment was a 1.39 THz (214.6\textmu m) line in CH\textsubscript{2}F\textsubscript{2}, pumped by the 9R34 transition of the CO\textsubscript{2} laser. Near the laser face, the measured terahertz output power of this laser line was 370 mWatts. The second laser line selected for this experiment was a 1.63 THz (184.3\textmu m) line in CH\textsubscript{2}F\textsubscript{2}, pumped by the 9R32 transition of the CO\textsubscript{2} laser. The measured terahertz output power of this line, near the laser face, was 31 mWatts. A liquid helium cooled silicon bolometer was used to detect the terahertz signal.

2.2 Optical path design

Since the beam emerging from the FIR lasers is a few mm in diameter and expands fairly rapidly as it propagates, an optical system was designed to focus the radiation into a sample imaging plane in order to improve the imaging resolution.\textsuperscript{12} The considerations for the system design were that the focuses of both beams should essentially lie in the same plane; practically this means that their Rayleigh ranges should overlap in the imaging plane. Dielectric waveguides were placed at the output of the FIR lasers to obtain a Gaussian output mode.\textsuperscript{13} We define the beam waist of a Gaussian beam to be the radius of the beam at the point at which the intensity drops to 1/e\textsuperscript{2} of its peak value. The measured waists of the terahertz beams coming out of the dielectric waveguides were 2.36 mm for the 1.4 THz beam and 2.55 mm for the 1.6 THz beam. These were then allowed to expand in free space before being collimated by a 30” focal length TPX lens. The resulting beam waists of the 1.4 THz beam and the 1.6 THz beam were 22.14 mm and 17.71 mm, respectively. The collimated beam was propagated 50” and then made incident on a 3.5” focal length, 3” diameter, off-axis parabolic mirror that focused the beam down onto the sample plane. The theoretically predicted beam waist of the 1.4 THz beam was 0.275 mm while the theoretically predicted beam waist of the 1.6 THz beam was 0.297 mm. The actual measured beam waists are slightly larger than the theoretically predicted ones.
Figure 3 shows a schematic of the optical layout. The removable mirror was on a kinematic mount. This allowed us to swap easily between the two frequencies. A two axis scan stage was used to move the sample in the imaging plane. The motion control and data acquisition software was written using LabView.

2.3 Sample Preparation

The samples were prepared from skin tissue specimens obtained from Mohs surgeries at Massachusetts General Hospital. The appropriate IRB approvals were obtained prior to imaging human tissue. The skin sections were sliced to a thickness of 240 µm using a cryotome. They were then mounted in between two 1mm thick slides of z-cut quartz. Using a 240 µm thick imaging spacer in between the slides, the samples were kept in pH balanced saline. This ensured that the samples did not dehydrate after being mounted in the slides. Sample images were collected within 24 hours after the sample was mounted. During the sample mounting procedure, the adjacent 5 µm slice was cut and stained for histology.

3. RESULTS

In order to measure the size of the terahertz beam and determine the location of the focal plane the beam was allowed to expand beyond the focal plane. By measuring the expansion of the beam, using a bolometer mounted on a computer controlled XY scan stage, and back-calculating using a Levenberg-Marquardt fitting routine, the location and the size of the focus was determined. The beam waist of the 1.4 THz beam was found to be 0.39 mm and the beam waist of the 1.6 THz beam was found to be 0.49 mm. The focal planes were within the respective Rayleigh ranges. Measuring the beam profile to determine the beam waist is done to minimize errors from the fact that the aperture on the bolometer is comparable in size to the focused beam.

Due to attenuation in both, the dielectric tube and the TPX lens, the power incident on the sample plane was found to be approximately 25 mWatts at 1.4 THz and 7 mWatts at 1.6 THz. The system signal to noise ratio (SNR) using a Lock-in amplifier was estimated to be 68 dB for the 1.4 THz beam and 67 dB for the 1.6 THz beam without a sample in the beam. Figure 4 shows the transmission image of a leaf at 1.4 THz alongside a photograph. The leaf was mounted in between two slides of z-cut quartz. The contrast in the leaf is generated primarily by transmittance differences due to water content in the veins and some slight thickness variations. The terahertz image of the leaf is also a good indicator of the system’s spatial resolution of 0.4 mm, as one is able to make out a significant amount of structure in the terahertz image and easily correlate it to the sample photograph.
Figures 5c and 5d show transmission images of a 240\(\mu\)m thick excision of human skin cancer tissue. Figure 5b shows the histology of the corresponding adjacent 5\(\mu\)m slice of the same sample. The cancerous region is demarcated by the dotted line in all images. The sample orientation with respect to histology was fixed using the blue stain at the corner of the tissue section.

The data amplitude is plotted in dB and is calibrated against full scale signal return. Full scale return is the amplitude measured by the lock-in amplifier with no sample in the beam path. The part of the terahertz image that is not on the sample was set to zero during post-processing of the image and only contrast on the sample image was studied. Thus one can see that the 1.6 THz frequency exhibits considerably less transmission through the sample. This is expected as the sample is predominantly water. The bright white spots in the images are air bubbles trapped in the slide. These results are further discussed in the discussion section.

4. DISCUSSION

As one can see in Figure 5, the cancerous tissue area demarcated in the histology image matches the area of lower transmission demarcated in the terahertz images of the tissues fairly well. The sample section that is imaged is 240\(\mu\)m thick, while histology is processed on an adjacent 5\(\mu\)m thick layer. Decreased transmission in the cancerous region indicates that the biomarker responds differently to terahertz radiation in that area. Also the difference in transmission between normal and cancerous regions of the tissue is similar at both frequencies (\(-4\) dB). Given the expected strong difference in terahertz transmission for Tryptophan at these frequencies this suggests that tryptophan may not be the source of the contrast.

One of the concerns in this experiment was the dynamic range of the imaging system. Figure 6 shows a 1.6 THz transmission image of the same sample described in Figure 5. The difference is that less attenuation was used in the system so as to improve transmission and the SNR of the image. As one can see, the detector saturates across the air bubbles in the sample area. While saturation effects have been removed from off sample areas of the image, some saturation effects can still be seen on the sample. These manifest themselves as streaking in the sample image in the direction of the raster scan. While this limits the dynamic range of the system, there is enough contrast to demarcate the lower transmittance layers from the rest of the sample even with a more attenuated beam as seen in Figure 5(d).
For future work we propose to image a larger number of samples in order to determine transmission threshold values that demarcate cancerous and non-cancerous regions. Using these threshold values and a large number of samples, one should be able to determine the sensitivity and specificity of the technique. Eventually we propose to move towards a reflection based imaging system. Also further sample studies, which would include reflection, should enable one to identify the likely contrast source.

**Figure 5:** (a) Photograph of skin sample (b) H&E Histology image of subsequent section (c) 1.4 THz transmission image of sample in saline (d) 1.6 THz transmission image of sample in saline
5. CONCLUSIONS

A terahertz transmission imaging system was built that is capable of imaging human skin tissue at two frequencies, 1.4 THz and 1.6 THz. The system’s resolution was found to be of the order of 0.5 mm at both frequencies and the system SNR was also found to be of the order of 70 dB at both frequencies. Preliminary measurements indicate that the imaging system is capable of measuring transmission differences between non-melanoma skin cancer and normal skin. Further tests are required to determine the system’s sensitivity and specificity.

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