Continuous-Wave Terahertz Reflection Imaging of Ex-Vivo Nonmelanoma Skin Cancers


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ABSTRACT

Nonmelanoma skin cancers are the most common form of cancer. Continuous wave terahertz imaging has the potential to differentiate between nonmelanoma skin cancers and normal skin. Terahertz imaging is non-ionizing and offers a high sensitivity to water content. Contrast between cancerous and normal tissue in transmission mode has already been demonstrated using a continuous wave terahertz system. The aim of this experiment was to implement a system that is capable of reflection modality imaging of nonmelanoma skin cancers. Fresh excisions of skin cancer specimens were obtained from Mohs surgeries for this study. A CO2 optically pumped far-infrared molecular gas laser was used for illuminating the tissue at 584 GHz. The reflected signal was detected using a liquid Helium cooled Silicon bolometer. The terahertz images were compared with sample histology. The terahertz reflection images exhibit some artifacts that can hamper the specificity. The beam waist at the sample plane was measured to be 0.57 mm, and the system’s signal-to-noise ratio was measured to be 65 dB.

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

INTRODUCTION

Motivation: Skin Cancer

Nonmelanoma skin cancer is the most common form of cancer, with approximately 1 million new cases diagnosed each year. About 95% of all skin cancers are nonmelanoma skin cancers and fairer individuals have a higher risk of developing skin cancer. However, despite their common occurrence, they account for less than 0.1% of patient deaths caused by cancer1. The most effective form of treatment is Mohs Micrographic Surgery (MMS). MMS involves processing sample histology on frozen sections of the removed tissue during the surgical procedure. This allows the surgeon to map out cancer margins and completely excise the tumor while retaining normal skin tissue. MMS has very high resolution, sensitivity and specificity, however, it is also time consuming, labor intensive and cost ineffective. Thus an imaging modality that is capable of in vivo determination of skin cancer margins is of significant interest.

Terahertz Imaging

The terahertz region of electromagnetic spectrum extends from 30 µm to 3000 µm (10 to 0.1 THz) and lies between the microwave and infrared regions. Terahertz radiation is non-ionizing and medical applications of this frequency region are being explored2-11. Many biomolecules exhibit absorption in the terahertz region of the spectrum.
Biological effects of terahertz radiation are also being explored\cite{12,13}. Terahertz radiation is also highly absorbed by water, making it very sensitive to changes in water content. Studies show that there is a difference in the bound and free water content between normal and cancerous tissue\cite{14,15}. Liquid water has a large attenuation coefficient and its absorption increases with increasing frequency in this region of the spectrum.

There are two approaches to imaging nonmelanoma skin cancers in the terahertz region. The distinction is based on the nature of the source. Terahertz Pulsed Imaging (TPI) uses a pulsed laser source to image tissue. The laser pulse in TPI is short duration and wide bandwidth, thus access to frequency specific data within the terahertz regime is possible. Due to the lack of commercially available continuous wave terahertz sources, most medical research in terahertz imaging thus far has been focused on terahertz pulsed imaging (TPI). Even though TPI has already been used to identify basal cell carcinoma (BCC) both \textit{ex vivo} and \textit{in vivo}\cite{16}, the source mechanism for the contrast in TPI images of BCC requires further investigation\cite{17}.

The other approach is Continuous-wave terahertz imaging (CW-THz). CW-THz uses longer lasting, essentially single frequency sources to image tissue at specific frequencies of interest. While CW-THz at single frequencies does not offer spectroscopic information, its advantages include high signal-to-noise ratios, faster acquisition rates, simpler data processing and lower projected costs. CW-THz transmission imaging has been used to tumors in liver samples and also to investigate canine basal cell carcinoma (BCC)\cite{18,19}. CW-THz has also been used to show differences between cancerous and normal skin on fresh tissue sections in transmission modality\cite{20}, however, any \textit{in vivo} application of CW-THz requires that the system operate in reflection modality and this study is a step in that direction. This study is \textit{ex-vivo}, reflection mode and was designed to demonstrate reflection imaging of nonmelanoma skin cancers at 584 GHz using a continuous-wave (CW) terahertz imaging system. This paper outlines the system design and presents some preliminary results. The tissue response at this frequency was investigated and initial results indicate that contrast between cancerous and normal skin is observable. Fresh excisions of human skin cancer were used, and the terahertz images obtained were compared to Hematoxylin & Eosin (H&E) histology images of the samples. The goal of this study was to investigate the feasibility of continuous wave terahertz imaging for delineating skin cancers by looking at tissue reflectivity at 584 GHz.

\section*{MATERIALS AND METHODS}

\subsection*{Frequency Selection}

Sample reflectance depends upon the refractive index of the tissue at that frequency. There is evidence in literature that the difference between the real part of the refractive index for cancerous and normal skin is maximum in between 500-600 GHz\cite{11}. The resolution of terahertz images is wavelength limited. Thus, while higher frequencies can offer better resolution they may not offer measurable contrast. Therefore the frequency that was selected was 584 GHz.

\subsection*{System Design and Construction}

The source used for this experiment was a CO\textsubscript{2} optically pumped far-infrared (FIR) gas laser. The CO\textsubscript{2} and FIR lasers are custom designed and built at the Submillimeter-wave Technology Laboratory. The output power of these CO\textsubscript{2} lasers is in the range of 100-150 W. Tuning the output frequency of the CO\textsubscript{2} laser allows one to pump different transitions of the gas in FIR cell. Selecting the gas in the FIR cell and the tuning of the CO\textsubscript{2} laser to the appropriate pump frequency provides one with the ability to lase different frequencies in the terahertz region. These CO\textsubscript{2} lasers and the pumped FIR lasers have been described previously in literature\cite{21}. The laser line used was the 584 GHz (513 \textmu m) transition in Formic acid (HCOOH), pumped by the 9R28 transition of the CO\textsubscript{2} laser.
A liquid helium cooled silicon bolometer manufactured by IRLabs was used as a detector. The Noise Equivalent Power (NEP) of the detector was $1.13 \times 10^{-13}$ W/Hz$^{1/2}$ and the system responsivity was $2.75 \times 10^{7}$ V/W. The bolometer had a response time of 5 ms and the gain was 200. A crystalline quartz with Garnet powder window on the bolometer cut-off wavelengths below 100 µm.

Since the beam emerging from the FIR laser is a few millimeters in diameter and expands rapidly as it propagates, an optical system was designed to focus the beam onto the sample plane. A dielectric (glass) waveguide was placed at the output of the FIR lasers to obtain a Gaussian beam profile$^{22}$. The measured output power after the dielectric tube was 10.2 mW. The waist is the radius of the Gaussian beam profile at the point at which the intensity drops to $1/e^2$ of its peak value. Figure 1 shows a photograph of the experimental layout. The laser beam was collimated using a 30” focal length TPX lens placed 30” away from the initial beam waist. The collimated beam was then focused onto the sample plane using a short focal length (3.5”) off axis parabolic mirror. The beam waist was measured to be 0.57 mm at the sample plane. The sample was placed normal to the incident beam. An automated two axis scan stage raster scanned the sample in the imaging plane. The scanning resolution of the horizontal axis was set to 0.1 mm and the resolution of the vertical axis was 0.1 mm. Signal reflected back from the sample was split using a 50-50 Mylar beam-splitter and collected into a liquid Helium cooled Silicon bolometer.

Figure 1: Photograph of experimental layout. The blue line indicates the beam path and the red line indicates the reflection arm of the system.

The data collected by the bolometer was sent to a lock-in amplifier that had a time constant of 30 ms. An optical chopper was used to chop the terahertz radiation and the chop frequency served as the reference signal for the lock-in amplifier. Data acquisition times for the images collected were determined by the speeds of the translation axes.
used for this experiment. The dwell time per point in the image was around 150 ms. Motion control and data acquisition software was programmed using National Instruments LabView®. The data-acquisition was synchronized with the sample position in the imaging plane. The system signal-to-noise ratio (SNR) using a lock-in amplifier was found to be 65 dB.

Sample Preparation

The samples were prepared from fresh thick excess cancer specimens obtained within 2 hours from Mohs surgeries at Massachusetts General Hospital under an Institutional Review Board approved protocol. En-face skin sections were mounted in a custom designed sample holder, which pressed the sample up against a z-cut quartz window. The tissue samples were imaged within 6 hours of being mounted. Before mounting several adjacent horizontal 5 µm slices were cut and stained for histology.

Histology Processing

Horizontal sections were processed in the following way. Tissue was frozen in an optimal cutting temperature compound and processed in the standard en-face sectioning technique\textsuperscript{23, 24}. Five micron-thick sections were transferred to glass slides and stained with hematoxylin and eosin. These frozen H&E sections were then compared to the terahertz images.

RESULTS

In Figure 2 example images of skin tissue with residual cancer are presented. Figure 2(a) shows the reflectance image of a thick skin sample at 584 GHz. The signal was calibrated against the full scale return from a front-surface flat gold mirror placed in the sample plane. Off sample areas were set to zero during post-processing and the data is shown in logarithmic space. Figure 2(b) shows the Hematoxylin & Eosin stained histology of an adjacent 5 µm thick section of the tissue. The black dotted line in the histology outlines cancerous (basal cell carcinoma) tissue.

Figure 2: (a) Terahertz reflectance image at 584 GHz and (b) H&E stained histopathology of adjacent 5 µm section. The black dotted line outlines the tumor area.
As one can clearly see, the cancerous region correlates well with an area of low reflectance in the terahertz image (Figure 2a). However, other areas of the sample, notably the top left area, which does not have cancer, also present the same reflection values thereby impacting image specificity.

**DISCUSSION**

As shown in Figure 2a, the cancerous area exhibits a lower reflectance than normal skin in this imaging set-up. This fact requires explanation as at 584 GHz, the refractive index of normal skin is approximately 2.2 and the absorption coefficient is 12 mm⁻¹ and the refractive index of cancerous skin is approximately 2.27 while its absorption coefficient is 15 mm⁻¹. Thus, one would expect higher reflectivity from the tumor as compared to normal tissue.

![Figure 3: Plot showing the expected reflectivity of a layered system as a function of window thickness.](image)

In the terahertz image the reason for the lower reflectivity is an interference effect from the z-cut quartz window. The signal collected by this system is incoherent and in frequency-domain. Thus it is not possible to time-gate out multiple reflections from the air-quartz and quartz-tissue interfaces as is done when using TPI systems. What the system measures is the cumulative reflectance of the window-tissue system. This depends on the refractive indices of the quartz-window and the tissue as well as the thickness of the window material. Figure 3 shows the expected reflectivity from such a layered system as function of the window thickness. This was estimated using a Fresnel equation based layered system with known refractive index values for the layers. As the samples measured were thick excisions of tissue ( > 5 mm), we can treat the second layer is essentially infinite as the tissue is very absorbing at this frequency. As one can see, for the 1 mm thick quartz slide used, the reflectivity from the tumor area is expected to be lower than the reflectivity of the normal tissue.
The other issue that is apparent in the terahertz image (Figure 2a), is that some noncancerous areas of the sample also present the same reflectance as cancerous areas. This is probably due to surface Fresnel effects at the window-tissue boundary, and needs further investigation as it impacts the specificity of the technique presented.

SUMMARY

This paper describes the construction of a continuous-wave terahertz imaging system that is capable of investigating the reflectance of fresh excisions of nonmelanoma skin cancers at 584 GHz. The system signal to noise ratio was found to be 65 dB. The resolution of the system is wavelength limited and the beam waist at the sample plane was measured to be 0.57 mm. Initial results indicate that cancerous regions of the sample show up as areas of lower reflectance in the terahertz image. This is due to interference with the quartz window. Moreover, the specificity of the technique needs to be improved as some normal areas exhibit the same reflectivity as cancer. Further efforts are currently underway to improve the image specificity. Further tests are also required to establish the sensitivity of the device.

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REFERENCES


