Continuous Wave Terahertz Reflection Imaging of Human Colorectal Tissue

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ABSTRACT

Continuous wave terahertz (THz) imaging has the potential to offer a safe, non-ionizing, and nondestructive medical imaging modality for delineating colorectal cancer. Fresh excisions of normal colon tissue were obtained from surgeries performed at the University of Massachusetts Medical School, Worcester. Reflection measurements of thick sections of colorectal tissues, mounted in an aluminum sample holder, were obtained for both fresh and formalin fixed tissues. The two-dimensional reflection images were acquired by using an optically pumped far-infrared molecular gas laser operating at 584 GHz with liquid Helium cooled silicon bolometer detector. Using polarizers in the experiment both co-polarized and cross-polarized remittance form the samples was collected. Analysis of the images showed the importance of understanding the effects of formalin fixation while determining reflectance level of tissue response. The resulting co- and cross-polarized images of both normal and formalin fixed tissues showed uniform terahertz response over the entire sample area. Initial measurements indicated a co-polarized reflectance of 16\%, and a cross-polarized reflectance of 0.55\% from fresh excisions of normal colonic tissues.

Keywords: Continuous-wave terahertz imaging, colorectal cancer, Reflection imaging, formalin fixation, polarization

1. INTRODUCTION

The terahertz (THz) frequency regime of the electromagnetic spectrum extends from 0.1 to 10 THz and lies between the microwave and infrared regions of the spectrum. The high sensitivity of THz radiation to water concentration, resulting from low energy interactions with the low frequency molecular motions, has expanded its applications into the areas of imaging and spectroscopy [1, 2]. Due to its non-ionizing property (unlike X-rays), THz technology has become increasingly important for biological applications [3]. Some frequencies in the THz can penetrate several millimeters of tissue to enable detection of the differences in water content and tissue density. According to recent investigations, it was shown that cancer cells absorb more water than normal cells [4-6]. As THz radiation is highly sensitive to water concentration, active THz imaging is an alternative and convenient way to ascertain the location of affected cells in the tissue.

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in world [7] with more than 1.2 million people diagnosed each year. It is the fourth most common cancer in men after skin, prostate, and lung cancer and also the fourth most common cancer in women after skin, breast, and lung cancers [8]. Most colon studies showed the growth of CRC from polyps and adenomas. The staging of CRC and subsequent treatment of CRC is dependent upon current imaging technologies, such as CT scan, PET, MRI, and colonoscopy. The current standard of care for diagnosis and treatment of early CRC and pre-cancerous lesions is use of conventional colonoscopy which relies on visual inspection by the physician. During the colonoscopy, the decision to remove the mucosal growths (abnormal tissue) is based on the physician’s experience. As terahertz imaging offers intrinsic contrast between cancerous and normal tissue, a THz
endoscope can be used as a potential tool in the examination and detection of cancerous or pre-cancerous regions of a biological (colorectal) tissue. In addition, the recent development of low-loss hollow flexible THz waveguides [9] provides a clear path for in vivo transmission of the THz radiation complementing the endoscopic design.

The potential of THz endoscopic imaging for colorectal studies was encouraged by the positive results obtained from the terahertz imaging of skin [10, 11], breast [12], liver [13], and other biological materials. As water is a polar molecule, it is highly absorbing in the THz region and thus a likely source of imaging contrast. Nevertheless, THz imaging studies of dehydrated samples (Paraffin embedded) showed the evidence of contrast between normal and cancerous colon tissues [14]. These studies suggest the possibility of other factors for contrast such as increase in lymphatic systems, vasculature, and other molecular/structural changes of diseased, in comparison to normal tissue [15].

2. BACKGROUND

Based on the nature of the source, THz imaging systems can be classified into pulsed and continuous-wave (CW). THz pulsed imaging (TPI) systems use a sequence of wide-bandwidth, low-power, electromagnetic pulses generated from either an ultra-fast Ti:sapphire or similar pulsed lasers. On the other hand, CW systems use very narrow bandwidth essentially single frequency, high power (several milliwatts) radiation sources. The ultra-fast pulsed lasers are relatively complex and expensive. The optical power of TPI systems is limited, so large arrays of sources and detectors cannot be easily constructed; and the need to sample the pulse in the time domain using some form of mechanical delay line means systems are relatively slow. However inexpensive compact CW laser systems can provide high resolution, spectral selectivity, superior SNR values, narrow line width, and versatility. Most medical research in terahertz imaging thus far has been focused on terahertz pulsed imaging due to their higher depth of fields, as well as the lack of commercially available CW terahertz sources.

TPI has already been used to identify colorectal cancer in ex vivo studies. A study based on THz pulsed imaging of human colonic tissues showed a contrast between dysplastic and healthy tissue, based on the increased absorption and refractive index of the diseased tissue. The tissue staining studies identified an accession in lymphatic systems and vasculature of the diseased tissue compared to normal. These studies indicate the importance in physical differences between the tissue pathologies in determining the underlying contrast mechanisms in THz imaging [15]. Another study that distinguishes normal and cancerous regions in the sample when fixed in formalin or embedded in paraffin, confirms the possibility of additional contrast-contributing factors other than water [14]. Transmission time-domain spectroscopy shows contrast between normal and cancerous regions of colon tissue in the frequency range 0.5 – 1.5 THz with the greatest difference at 0.6 – 0.8 THz, and rectum tissue in the frequency range 0.5 – 3.0 THz with the maximal difference in refractive index at 0.6 THz and absorption coefficient in 0.5 - 2.5 THz range [16]. However, the source mechanism for the contrast in TPI images of colorectal cancer is not yet clearly understood. Also, their do not seem to be any resonances in this region, which is expected as the THz response of water is the dominant mechanism. Thus, a continuous-wave imaging system operating in between 0.6 – 0.8 THz should be able to detect contrast between normal and cancerous tissue. This will facilitate the delineation of cancerous and normal regions of a colorectal tissue with the clear understanding of contrast mechanisms.

3. EXPERIMENTAL SETUP

The terahertz reflection imaging system at 584 GHz consists of a CO₂ optically pumped far-infrared (FIR) gas laser. The output power of these CO₂ lasers is in the range of 100 – 150 Watts. Pumping different transitions of the gas in the FIR cell can be achieved by tuning the output frequency of the CO₂ laser. The combination of the right gas in the FIR cell and correct frequency of the CO₂ laser provides the ability to lase different frequencies in the terahertz region. The required 584 GHz (513 µm) vertically polarized transition in Formic acid (HCOOH) was obtained by pumping the 9.23R28 transition of CO₂ laser. A dielectric waveguide was placed at the output of the FIR laser to attenuate the higher order modes and obtain a Gaussian output mode [17]. The measured output power before and after the dielectric tube were 33 mW, and 10.23 mW respectively. Since the 2.15 mm diameter beam emerging from the FIR laser expands
rapidly as it propagates in air, an optical system was designed to focus the radiation onto the sample, achieving a resolution of 0.68 mm. The detector used was a liquid helium cooled silicon bolometer detector manufactured by IRLabs which had a noise equivalent power (NEP) of $1.13 \times 10^{-13}$ W/Hz$^{1/2}$, responsivity of $2.75 \times 10^5$ V/W, response time of 5 ms, and gain of 200. The crystalline quartz (Garnet powered) window on the bolometer rejected wavelengths below 100 µm.

The optical layout is depicted in Figure 1, a beam waist of 2.36 mm was measured exiting from the dielectric waveguide. This beam expanded in free space before being collimated by a 24” focal length TPX lens and passed through a wire grid polarizer. Then, the beam passed through 50-50 Mylar beam splitter, and finally focused onto the imaging plane using a short focal length off-axis parabolic (OAP 1) mirror (Laser beam path was shown with pink arrows). The full width at half max (FWHM) was measured to be 0.68 mm at the sample plane. The component of the incident radiation that was reflected by the beam splitter was dumped into a THz absorber (Anechoic).

Figure 1. Schematic of experimental setup for reflection imaging (inset: reflection measurement of an aperture with 0.2 mm, 0.5 mm holes separated by 12 mm distance)

The signal remitted from the sample was redirected and focused into the detector, using OAP 2 after passing through OAP 1, the beam splitter, and an analyzing wire grid polarizer (beam path was shown with gold arrows). In order to detect either co- or cross-polarized light, the signal remitted from the sample will be passed through an analyzing wire grid polarizer sitting in the reflection arm (shown as red dotted line in Figure 1). An automated two-axis stage was used to raster scan the tissue holder (as discussed in Section 4) in the imaging plane with a resolution of 0.1 mm and dwell time of 150 ms per point. The laser beam was optically modulated using a chopper. The modulating frequency (83 Hz) served as the reference for a lock-in amplifier. The system’s signal to noise ratio using a lock-in amplifier was 65 dB. The inset of Figure 1 shows the terahertz reflectance image of a metal plate, containing 0.2 mm and 0.5 mm diameter holes (separated by 10 mm), scanned using the described system.

4. SAMPLE PREPARATION

Fresh thick excess colorectal specimens were imaged within 2 hours after standard surgical procedures performed at University of Massachusetts Memorial Hospital under an Institutional Review Board approved protocol. For this study, the two sets of normal colon samples were imaged fresh, and fixed with formalin. The thickness of the colon specimens obtained was approximately 1 cm, with the lateral dimensions between 8 and 15 mm. The colon specimens were transported to the University of Massachusetts Lowell in saline, to prevent dehydration, within 45 min. Then the en-face specimen sections were imaged within 2 hours.

For terahertz imaging, the colon tissue specimens were mounted in an aluminum two-piece sample holder (with 3” x 1” front opening), as shown in Figure 2. The tissue specimens were placed behind the 1 mm thick z-cut quartz slide of the sample holder. The colorectal tissue specimens were covered with wet gauze, soaked in pH balanced (pH 7.4)
saline, to prevent tissue dehydration during the experimental study. During the mounting process, both the front and back pieces of sample holder were gently pressed onto the tissue, to avoid air gaps between colon tissue and quartz slide. Finally to ensure the security of tissue specimen position, the whole assembly (quartz slide, tissue specimen, and wet gauze) was then placed in the sample holder and gently tightened with four screws.

The most commonly used fixative to preserve the biological samples for routine histopathological diagnosis is formalin. The resulting formalin fixed samples after histology process are often useful for retrospective studies. In order to study the affect of formalin fixation in THz imaging of colorectal samples, the tissue specimens were fixed with 3% neutral-buffered formalin solution. Before fixing the tissue with formalin, the THz response from all the fresh colon sections were obtained using co- and cross-polarized reflection imaging. The volume of formalin applied to each sample was at least 10 times the initial sample volume. The sample was then left to soak in the fixative in a sealed container and refrigerated at a constant temperature (4°C). During imaging, gentle pressure was applied to the sample to ensure it made good contact with quartz as it becomes more difficult due to the increment in the rigidity of a fixed sample. In order to monitor the changes caused by the formalin, each sample was measured after being fixed for 24 h, 48 h (2 days), and 120 h (5 days).

![Figure 2. Schematic of sample mounting for reflection measurements a) 2-piece aluminum holder, b) tissue placed behind the 1 mm thick z-cut quartz slide, c) colon tissue covered with a gauze soaked in pH 7.4 balanced saline solution, d) closing the assembly with holder’s second piece, e) tissue specimen mounted in the sample holder.](image)

5. POLARIZATION IMAGING

With a time domain terahertz spectroscopic system one can reject the specular reflections from air-glass interface, and obtain the terahertz response of the glass-sample interface alone, by using time gating techniques. However, for continuous-wave systems the polarized light imaging technique, suggested by C. S. Joseph and coworkers [11], has been used to avoid the unwanted reflections. Usually, the co-polarized terahertz response of the sample includes Fresnel reflections from the air-quartz and quartz-sample interfaces. In contrast, the cross-polarized terahertz response effectively rejects the specular reflections, as the Fresnel component is co-polarized with the incident radiation. Thus cross-polarized terahertz imaging effectively samples the tissue volume. In imaging human nonmelanoma skin cancer, the cross-polarized THz response from the sample identified the correct location of cancerous region based on image contrast between healthy and diseased tissue [11]. This shows the ability of cross-polarized THz imaging in delineating the normal and cancerous region of a biological tissue.

6. RESULTS & ANALYSIS

The co- and cross-polarized reflection THz images were obtained by collecting the signal remitted from the tissue specimen sitting on the raster scanned XY-stage and by placing the appropriate analyzing polarizer grid in the reflection arm before the Si detector. The THz images obtained from reflection imaging were then processed using a LabView™ program that synchronized the sample position in the imaging plane with the return signal obtained from the lock-in amplifier. The co- and cross-polarized images were then calibrated against the full-scale return from a flat gold
front-surface mirror to determine the reflectance. The image was plotted in logarithmic space and the off sample areas were removed in post processing with the reflected THz signal quantified pixel by pixel using the reflectance formula:

$$R = \frac{I_{me}}{I_{Full}} \times 100\%$$

where $R$ is the terahertz reflectance value in percent (%), $I_{me}$ is the measured reflectance intensity from the sample, and $I_{Full}$ is the full-scale return or the measured intensity of the incident beam reflected from a gold front-surface flat mirror.

The co- and cross-polarized terahertz reflection images of fresh (Figure 3a,b), and formalin fixed (Figure 3c,d) colorectal samples are shown in Figure 3 with corresponding digital photographs. Figure 3a shows the THz images of fresh colorectal sample I. Both the co- and cross-polarized terahertz images did not show any contrast over the entire sample surface. The co-polarized reflectance image, plotted in logarithmic dB scale, shows a reflection of -7.5 – -8.5 dB and the cross-polarized image shows a scale of -21.5 – -22.5 dB. The corresponding reflectance values obtained were 16.50 ± 0.25% and 0.68 ± 0.034%.

![Figure 3. Digital photograph, co-, and cross-polarized terahertz reflection images of human](image)

The co- and cross-polarized images obtained from fresh colorectal sample II also lie in the -8 dB and -22 dB signal ranges respectively, with the corresponding 15.76 ± 0.27%, and 0.54 ± 0.028% reflectance values, as shown in Figure 3b. Again, both polarization images of sample II did not show any contrast over the sample except the yellow dotted line visible on the top left corner in each image. As expected the effect has been observed in the THz images of both fresh and formalin fixed sample II, due to the appearance of side and back surfaces of colorectal tissue in the front view, as shown in the digital photographs of Figure 3b and 3d. This was caused by the non-uniform thickness of colorectal tissue, and the gentle pressure applied on the tissue against quartz slide during the mounting process to avoid air gaps.

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In order to understand the effects of formalin fixation on the THz properties of biological samples, the colon tissues were fixed with 3% neutral buffered formalin solution and stored in refrigerator at 4º C. Once the THz images of fresh tissues were obtained they were fixed with formalin, and imaged after 24 hr, 48 hr, and 120 hrs. Figure 3c & 3d shows the digital photograph, co-, and cross-polarized terahertz reflection images of 2-days fixed colorectal sample I, and 5-days fixed colorectal sample II. The co- and cross-polarized terahertz images of 48 hr formalin fixed sample I showed a constant terahertz response throughout the entire surface, identical to the fresh sample I. Also the 5-day formalin fixed tissue II did not show any contrast over the sample, except the yellow dotted line resulted from the non-uniformity of the tissue thickness, similar to the fresh colon tissue II. The reflectance values of 15.32 ± 0.299%, 0.54 ± 0.018% were obtained from formalin fixed colorectal sample I and 15.27 ± 0.24%, 0.52 ± 0.016% from colorectal sample II. The abatement in the reflectance values of formalin fixed samples, as compared with the fresh tissues, resulted from the change in refractive index of fixed tissue due to the replacement of water content by the formalin solution [18].

The reflectance values obtained from the formalin fixed samples after 24 hr were noticeably smaller when compared with fresh tissues, however there is a significant reduction in the reflectance after 48 hr as shown in Figure 3c. The reflected THz response values were reduced with formalin fixation. This is expected as the water content in the tissue has refractive index of 2.4 at 0.6 THz will be replaced by formalin solution which has lower refractive index of 2 at 600 GHz. Despite this, the reflectance values of polarized THz images collected from the formalin fixed colonic samples were consistent after 48 hr up to 120 hr. The refractive index of human colonic tissue is 1.5 at 584 GHz, when measured by a pulsed terahertz system [16]. Therefore, the lower reflectance values obtained for fixed tissues were potentially resulted from the reduced internal refractive index of the colon tissue. Also, it was noticed that the terahertz response obtained from a formalin fixed colorectal tissue sample changes during the first 48 hours. Once the water content in the tissue was entirely replaced by formalin the THz response attained was consistent.

7. CONCLUSION

Continuous-wave terahertz imaging has been used to study the terahertz response of human colonic tissues. Both the co- and cross-polarized THz images were obtained using a reflection based 584 GHz system. The THz imagery of fresh, 48 hr (2-day) formalin fixed, and 120 hr (5-day) fixed normal colon samples preserved at 4º C were studied. Neither of the co and cross polarized THz images of fresh colon samples show any contrast over the sample, as expected. Reflectance values of ~16% and ~0.55% were obtained from the co- and cross-polarized reflection images of normal human colonic samples. Finally, the effects of formalin fixation on the terahertz properties of colorectal specimens were investigated.

REFERENCES


