Terahertz Time Domain Spectroscopy of Human Blood.

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Abstract

In the continuing development of terahertz technology to enable the determination of tissue pathologies in real-time during surgical procedures, it is important to distinguish the measured terahertz signal from biomaterials and fluids, such as blood, which may mask the signal from tissues of interest. In this paper, we present the frequency-dependent absorption coefficients, refractive indices and Debye relaxation times of whole blood, red blood cells, plasma and a thrombus.

Introduction

The spectroscopy of blood and its constituents is well known over many sections of the electromagnetic spectrum. The infrared spectroscopic analysis of blood serum plays a critical role in diagnosing and monitoring a wide variety of disorders [1]. In the visible and near infra-red range [2] the spectral differences in oxy- and deoxy-haemoglobin are readily utilised in pulse oximetry and imaging [3]. At longer wavelengths Wolf et al [4] measured the broadband dielectric spectroscopy of human blood over the frequency range 1 Hz to 40 GHz and Gabriel et al [5] developed a parametric model to describe the variation of dielectric properties of tissues including blood as a function of frequency. The experimental spectrum from 10 Hz to 100 GHz was modelled with four dispersion regions. To date little has been published on the spectroscopy of blood in the terahertz frequency range.

As terahertz frequency radiation (100 GHz – 10 THz) is highly absorbed by water and water is one of the main constituents of tissue, penetration depths in this frequency range are shallow, ranging from typically a few hundred microns in high water content tissues to centimetres in tissues with a high fat content [6], [7]. One would expect the terahertz spectrum of blood to be dominated by water and thus not have any specific spectral feature useful for diagnostic purposes. However, the potential use of THz technology in the clinical environment for in vivo tissue diagnosis makes the knowledge of blood absorption spectrum and refractive index in the THz frequency range important. The number of reported biomedical studies using THz includes teeth [8], [9], healthy skin and basal cell carcinoma in both vitro [10] and in vivo [11], excised breast cancer [12], colon cancer [13], liver [14] and burns [15]. Some of these potential applications of THz technology to medicine are to determine tissue pathologies in real time [16]. This has great implications for, for example, determining disease-free margins during surgery thus reducing the reliance on subjective surgical procedures. One major potential problem for use in the clinical setting is the presence of biomaterials and fluids, e.g. blood, which could contaminate a THz signal. It is, therefore, important to know the THz properties of blood.

In this paper, we present the spectroscopy of whole blood, two of its constituents (red blood cells and plasma) and a blood clot (thrombus) in the terahertz region. We also investigate the possible origins of the spectral differences of the samples by fitting to the Debye relaxation times and by determining the spectra of pure haemoglobin molecules.

Blood is composed of red blood cells, white blood cells and platelets suspended within plasma. Blood is, by volume, typically 45% red blood cells, 55% plasma and a very small amount of white blood cells. Plasma is composed predominantly of water (92%) and dissolved proteins and salts (8%). Red blood cells contain haemoglobin, each molecule of which consists of four protein subunits which contain iron and are responsible for blood's characteristic red colour. White blood cells defend the body against both infection. Platelets are involved in the formation of blood clots (thrombus), which consist of a solid mass of coagulated platelets and proteins (mainly fibrin) [17].

Methods

Experimental method

A stand-alone portable THz system, the TPI Imaga1000 (TeraView Ltd, Cambridge, UK), was used in this work. A full description of the system operation is given elsewhere [18], however is briefly described here for clarity. The system consists of a 250 mW Vitesse 800 (Coherent Inc, Santa Clara, CA, USA) mode-locked Ti:Sapphire oscillator, and uses photoconductive devices for both THz generation and detection. The 100 fs laser pulses with 800 nm central wavelength at a repetition rate of 80 MHz are split by a beam splitter into a pump and a probe beam. The probe beam is passed through a mechanical translation stage which is moved at fixed intervals over a finite distance. This acts as a delay line which changes the relative path length of the probe beam to the pump beam to facilitate the mapping of a whole THz pulse. Using multiple path lengths is equivalent to measuring the THz electric field at multiple instants in time. The delay line operates at a typical scan rate of 15 Hz, but as a pulse can be measured over both the up and the down sweep a pulse acquisition rate of 30 Hz is achieved. This rate was chosen as an optimum between acquisition time and signal-to-noise (SNR). By slowing the scan rate or by signal averaging the SNR can be improved at the cost of a corresponding increase in data collection time and vice versa. The time resolution was approximately 200 fs which is limited by the width of the laser pulse.

The spectroscopy system consists of a sealed sample chamber that can be purged with nitrogen (or dry air) to remove water vapour. Following the delay line, the transmission pulses are focused, using a pair of gold coated off-axis parabolic mirrors onto a pair of z-cut quartz windows, between which a sample is held. The transmitted beam is focused by another pair of mirrors onto a photoconductive detection device.

The liquid sample transmission cell consists of two quartz windows separated by a Teflon spacer which can be between 100 μm to 1 mm thick. It is designed to be used vertically in transmission mode, so to hold liquid samples, rubber rings are placed between the quartz windows and a retaining plate which is tightened to create a water-tight seal. The spacer thickness used in this work was 200 μm. A reference measurement was made in each instance using two pieces of quartz plate held together. All measurements were made at room temperature.

Blood samples were taken from a healthy person by a qualified clinician; the samples were centrifuged to split them into separate volumes in order to make three measurements on each of the four constituents: whole blood, plasma, blood cells, and blood clots (or thrombus). Six samples of whole blood were taken and immediately stored in a blood collection tube with an anticoagulant. Whole blood from three of these tubes was transferred to the liquid sample cell for measurement. After centrifuging the three other collection tubes the plasma was decanted and three separate measurements of plasma were performed. The residual in each of the centrifuged collection tubes containing only cells were measured. Lastly, three samples were put into collection tubes with a coagulant to form a thrombus. Being more of a solid tissue-like substance, the cells and the thrombus were measured using a tissue sample holder, described in reference [19].

For the determination of the absorption coefficients of the haemoglobin molecules, measurements were made of haemoglobin powder, rehydrated to solutions of different concentrations. The haemoglobin powder (Sigma Aldrich) and was made into solutions using distilled water at concentrations of 2.5%, 5%, 6%, 7.5%, 8%, 9%, 10%, 12.5% and 15% haemoglobin by weight. It was assumed that the contribution from the haemoglobin and water in the solutions were linearly additive, that is, the contributions to the measured absorption coefficient (μ_a) follow equation 1. Previous studies of aqueous protein solutions have found this trend to be true [20]. The μ_a of the haemoglobin molecules was calculated by subtracting the μ_a of water, normalised for % concentration, from the measured μ_a of the haemoglobin solutions. The absorption coefficients calculated for each individual solution concentration were averaged to achieve a single μ_a value for haemoglobin.

$$\mu_{a(molecule)} = \mu_{a(solution)} - \mu_{a(H_2O\ bulk)} \quad (1)$$

Analysis method

Debye relaxation

To further investigate if the relaxation mechanisms present in the whole blood and its components from which these differences in optical properties arise can be determined, the Debye parameters of the samples were considered. Debye theory describes the reorientation of molecules which could involve translational and rotational diffusion, hydrogen bond arrangement and structural rearrangement. The Debye relaxation time, τ, describes the time necessary for 1/e of the dipoles to relax to equilibrium after an impulse. For a pure material, multiple Debye type relaxation processes are possible where the complex dielectric coefficient, $\hat{\varepsilon}(\omega)$, is described by equation 2 [21]. ε_∞ is the real part of the dielectric constant at the high frequency limit, $\Delta\varepsilon = \varepsilon_j - \varepsilon_{j+1}$, ε_j are intermediate values, occurring at different times, of the dielectric constant, τ_j is the relaxation time relating to the j^{th} relaxation process and ω is the angular frequency. The $\Delta\varepsilon$ term can be considered to be an 'amplitude', indicating the 'quantity' of that particular relaxation in the material under investigation.

$$\hat{\varepsilon}(\omega) = \varepsilon_\infty + \sum_{j=1}^{n} \frac{\Delta\varepsilon}{1 + j\omega\tau_j} \quad (2)$$

The complex dielectric coefficient of a material is simply related to the complex refractive index, $\hat{n}(\omega)$, as described by equation 2, where $\varepsilon'(\omega)$ and $\varepsilon''(\omega)$ are the real and imaginary parts of the complex dielectric coefficient. The complex dielectric coefficient of a material can be determined simply from the measured values for absorption coefficient, μ_a, and refractive index, *n*, using equations 2 and 3, respectively. The complex dielectric coefficients were calculated from the measured absorption coefficient and refractive indices following equation 2 for all the samples, and double Debye theory was applied following equation 1, where the ε_S values were held constant.

$$\hat{\varepsilon}(\omega) = \hat{n}^2(\omega) = \varepsilon'(\omega) + i\varepsilon''(\omega) \quad 3$$

$$\varepsilon'(\omega) = n^2(\omega) - \mu_a^2(\omega) \quad 4$$

$$\varepsilon''(\omega) = 2n(\omega)\mu_a(\omega) \quad 5$$

Results and discussion

Transmission spectroscopy measurements were made of whole blood, blood plasma, blood cells and a thrombus, from which the absorption coefficients and refractive indices of each component was determined. The absorption coefficients of the blood components illustrated in figure 1 are shown in comparison to pure water. Even though the spectroscopy system was purged during all measurements, some water vapour lines were still present; therefore, we applied a numerical method for removal of these effects as described in [22]. Figure 1 shows that the absorption of pure water is greater than whole blood or any of its components. It is apparent that there is a decrease in overall absorption coefficient as the water content of the blood components is reduced; plasma (≅92% water), whole blood (≅50% water) and packed blood cells. The exception, however, is the blood clot which has the same water content as whole blood. Here, clearly, the effects of coagulation and the formation of a thrombus have affected the absorption coefficient. It is likely that the formation of a thrombus, a protein/fibrin covalently bonded structure, encapsulates much of the water present impeding its ability to absorb THz radiation in the measured range.

The refractive index, shown in figure 2, of whole blood and blood components, in comparison to pure water shows, overall, that the refractive index of water is greater than that of the whole blood and components. It would be expected that components with high water content would have refractive indices very similar to pure water and, while this is true of the whole blood sample, this does not appear to be the case with the plasma sample. The refractive index of the plasma at low frequency (0.1-0.5 THz) is consistent with water and whole blood, however, begins to diverge at higher frequencies. The refractive indices of the blood clot and blood cells appear to be consistently 0.05 below that of pure water.

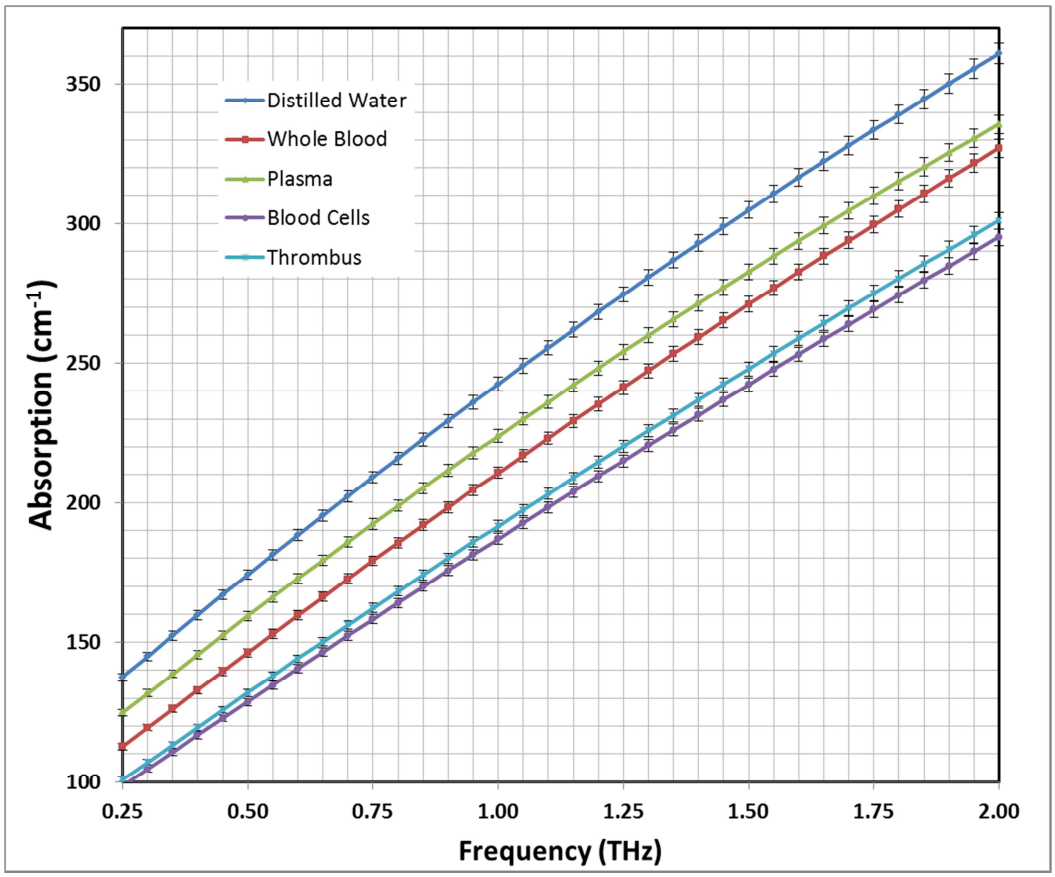

Figure 1. Absorption coefficient for whole blood, blood cells, plasma and a thrombus.

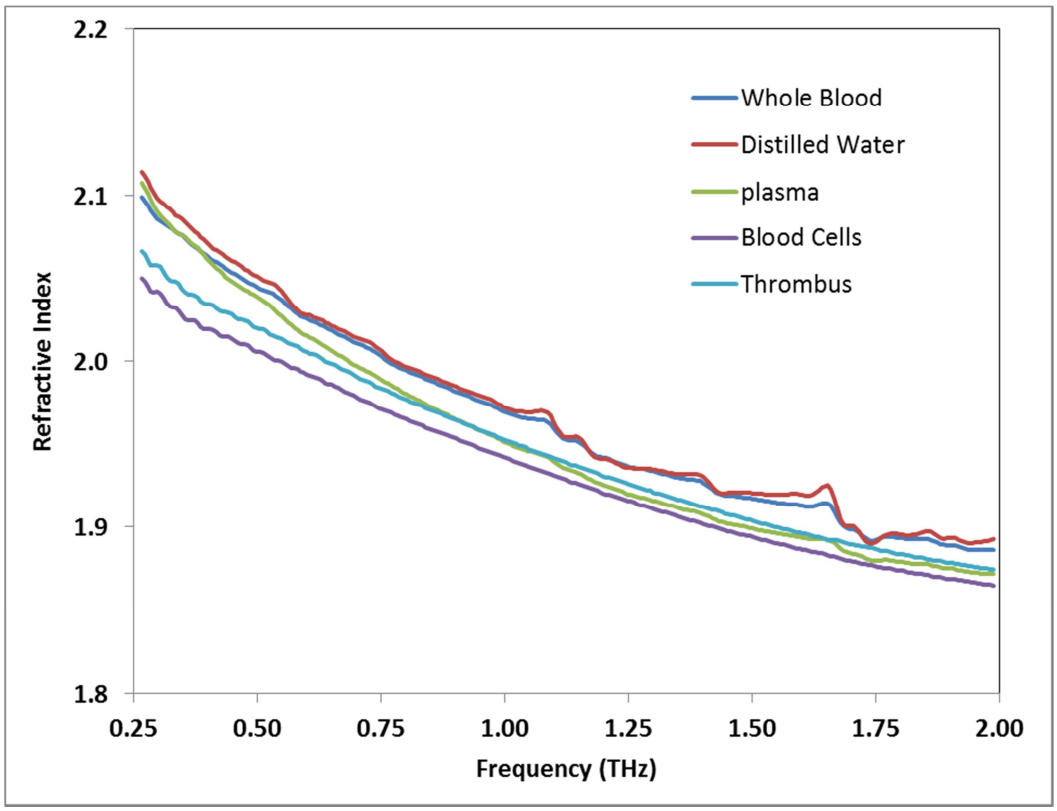

Figure 2. Refractive index for whole blood, blood cells, plasma and a thrombus.

The absorption coefficient of the haemoglobin molecules are illustrated in figure 3, and are shown in comparison to the absorption spectra for pure water and the red blood cells. The absorption coefficient for pure haemoglobin is lower than that of both water and the red blood cells. One might expect that the spectra for the haemoglobin and blood cells would be similar; however, differences in the spectral shape exist, particularly at the low frequency range. Previous studies of dry protein and sucrose samples in the THz region have described a monotonic frequency dependent increase below 2 THz for dry samples of DNA, BSA, cytochrome c, polypeptides, myoglobin, DNA and collagen [23–28]. The monotonic increase with frequency is proposed to be due to a dense collection of overlapping vibrational modes in macromolecules which result in a rapid rise in the density of vibrational modes with frequency followed by a plateau or saturation at higher frequencies. This has been recreated in molecular dynamics simulation studies of several small proteins [24], [29–32]. The spectra measured in this study show a strong monotonic frequency-dependent increase up to at least 2.5 THz without any apparent resonances which is consistent with the proposed theory of a dense collection of overlapping vibrational modes.

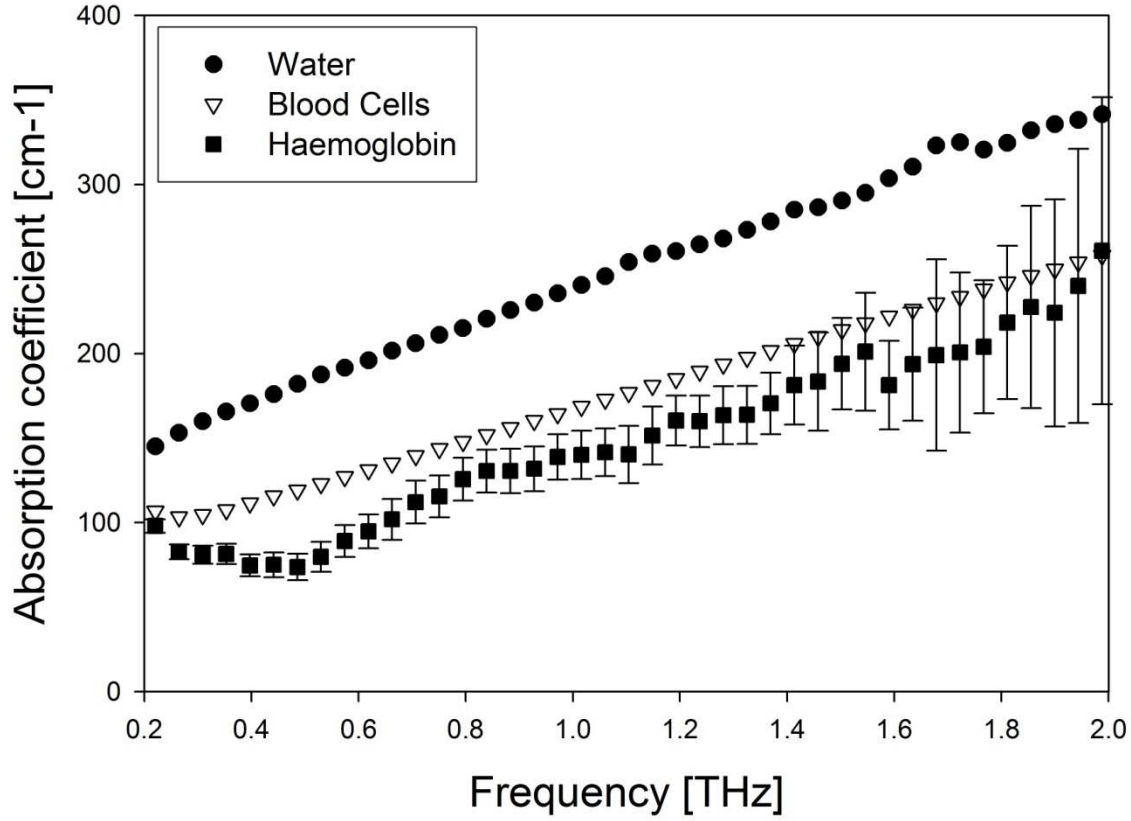

Figure 3. Absorption coefficient for haemoglobin molecules, blood cells and pure water.

The determined Debye relaxation coefficients are given in table 1. The τ_1 relaxation for the whole blood, at 14.4 ps, is higher than that of the pure water, suggesting that the red blood cells have a retardation effect on the free water relaxations. The $\tau 1$ relaxation time for thrombus is longer than for water or whole blood, suggesting that the coagulation of the blood results in a greater impedance of the free water present. Interestingly, the τ_1 relaxation for the blood cells is very large. While this is unlikely to correspond to a relaxation of the cell itself or of haemoglobin molecules

within the cells as these would be much slower processes, this relaxation may well be due to the slowed relaxation of the associated water surrounding the molecule, which is, approximately, 10^2-10^4 slower than the free water [33–35].

Table 1: Double Debye coefficients for whole blood and blood components

	E_∞	E_S	E_2	τ_1(ps)	τ_2(ps)
Whole Blood	2.1	130[a]	3.8	14.4	0.1
Blood Plasma	1.7	78.8	3.6	8.0	0.1
Blood Cells	3.4	2.5[b]	23.8	410.8	1.8
Thrombus	2.2	130[a]	3.7	16.1	0.1
Water	*3.3*	*78.8*	*4.5*	*8.4*	*0.1*

a (Schwan 1983 [36])
b (ICT 1933)

Conclusion

In this work, we have measured the absorption and refractive index of whole human blood, plasma and blood cells over the THz frequency range of 0.2 to 2 THz. We have found that there are measurable differences in the spectra of blood and its components, though the spectral shapes of the measured absorption coefficients and refractive indices and show little distinction. There appears to be some distinction in the Debye relaxation coefficients for the whole blood and its components. It has been suggesting that FDTD modelling of tissues, which incorporates the Debye coefficients, may be of use as an analysis tool in the biomedical field [18], [37] meaning it may be possible to distinguish blood from surrounding biological tissue. A significant difference could be seen in both the absorption and refractive index spectra of the whole blood and its thrombus, as well as the Debye coefficients. THz frequency is therefore sensitive to the bonding mechanisms within the whole blood. In conclusion, given the high absorption of whole blood and plasma, when THz technology is being used in vivo during surgical procedure the presences of blood needs to be minimised as not to interfere with the diagnostic ability and accuracy of tissue differentiation.

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