# Temperature Dependent Hyperspectral Terahertz Imaging of Human Bone for Disease Diagnosis

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## ABSTRACT

Water is a fundamental component of many biological systems. The ability to detect water therefore provides great insight into system functionality, particularly in the development of disease. In this work, the high interaction of terahertz radiation with water, paired with the dependence of the dynamics of water molecules with varying temperature, is utilised to monitor changes in the composition of bone tissue. Heterotopic ossification (HO) bone samples and deionised free water are measured using terahertz time-domain spectroscopy for varying environmental temperatures, for prospective use in disease diagnosis.

**Keywords:** Hyperspectral terahertz imaging, human bone, hydration mapping.

## 1. INTRODUCTION

After collagen and calcium phosphate, water is the third most common compound in bone structures, making up to 31% [1]. Studies of water within bone structures are limited compared to other constituents, despite its fundamental role in the structural makeup [2]. The ability to monitor hydration becomes important when diseases in bones, such as metastatic defects and osteoporosis, can modify the water content of the bone tissue. Current methods to determine free and bound water involve calorimetry and require preparation processes which cause significant limitations [3].

The molecular structure of a material, which depends on the formation and breakage of hydrogen bonds and translational and rotational motions of dipoles, is highly dependent on the thermal energy of its environment. This, in turn, influences interactions with electromagnetic fields (e.g. terahertz radiation). Hence, variation in dielectric properties with changing temperature is a fingerprint for material identification and can be exploited for biomedical application. Aided by high terahertz field-water interactions, modification in tissue hydration due to disease can therefore be monitored through temperature dependent terahertz spectroscopy. This work aims to take advantage of the sensitive dependence of molecular dynamics of water to environmental temperature, to monitor changes in the composition of tissue, with particular focus on bone, for prospective disease identification.

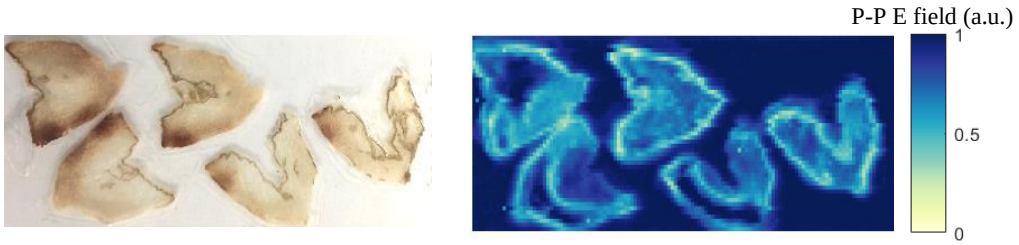


Figure 1 Photograph of the HO bone samples under investigation and image of the peak-to-peak value of the reflected electric field. The red circle indicates the pixel used in this work.

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## 2. METHOD

The hydration of ~100 μm thick HO bones (see Figure 1) was studied through temperature dependent spectroscopic measurements using a Menlo TERA K15 time-domain terahertz spectrometer in reflection configuration, illustrated in Figure 2. Details of the system and beam properties can be found in [4]. An image of the peak-to-peak value of the electric field is presented in Figure 1. The samples were heated from ~18°C to 26°C, monitored using an IR thermometer, through freezing the sample and bringing to room temperature. Tissue transfer and handling was conducted under approval of the National Research Ethics Service (15/NW/0079) and in accordance with the Human Tissue Act 2004. It should be noted that this study assumes that the dependence of the terahertz complex permittivity of the two main constituent materials of bone, i.e. hydroxyapatite (HA) and collagen [2], with respect to temperature is negligible compared to water.

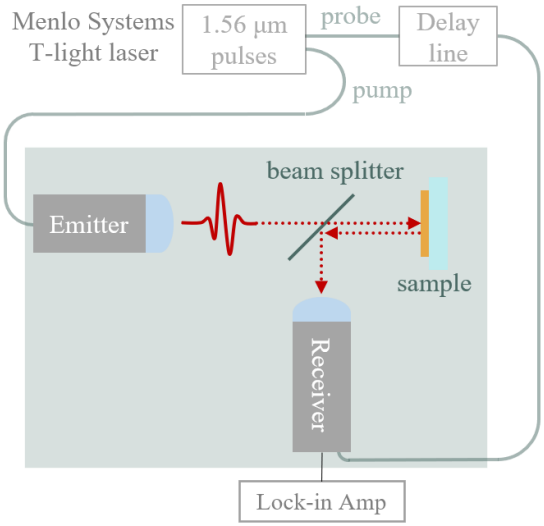


Figure 2 Experimental setup of the TDS system in reflection configuration. The terahertz pulse, generated by a photoconductive antenna (PCA) emitter, is reflected by the sample (HO bone on a glass substrate) and detected by a PCA receiver.

## 3. RESULTS

Figure 3 presents the temporal response of the HO bone at the position indicated by the red circle in figure 1 for increasing temperature. One can observe an increase in reflected peak-to-peak electric field amplitude with increasing temperature. This is consistent with the behaviour of water at terahertz frequencies [5], whereby the real and imaginary parts of the permittivity increase as a function of temperature, attributed to formation and breakage of hydrogen bonds, translational and rotational motions of dipoles and structural rearrangement [6]. The higher the thermal energy, the higher the resistance of the polar molecules to align with the polarisation of the transmitting electric field. This results in higher resistance to transmission, and hence higher dielectric properties. The observable loss of high frequency components for lower temperature is arguably due to the reflection reduction (i.e. the refractive index contrast between air and bone decreases with temperature, resulting in a smaller Fresnel reflection coefficient) that causes high frequencies to fall below the noise level of the system.

To extract the amplitude and phase of the reflected electric field, a Fourier transformation was applied to the temporal waveforms in figure 3. Figure 3 presents the frequency dependent spectra, where the amplitude and phase are normalised to the amplitude and phase of the lowest temperature measurement, respectively. One can observe an increase in both amplitude and phase with increasing temperature, consistent with the increase in dielectric properties observed from the temporal results. The notable variation in increase in both amplitude and phase are thought to be attributed to Fabry-Perot reflections frequency oscillations in the frequency domain [7]. The removal of such oscillations is essential for accurate extraction of a material's dielectric response [8].

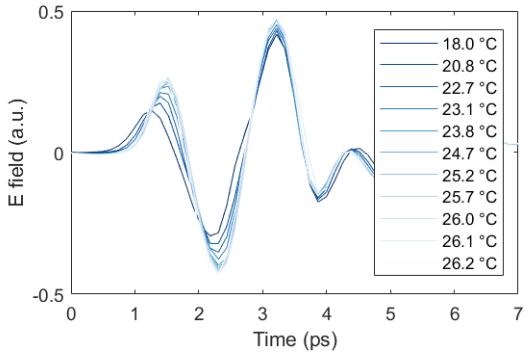


Figure 3 Temporal waveform of the electric field reflected from the HO bone sample, for varying temperature, indicated by the legend.

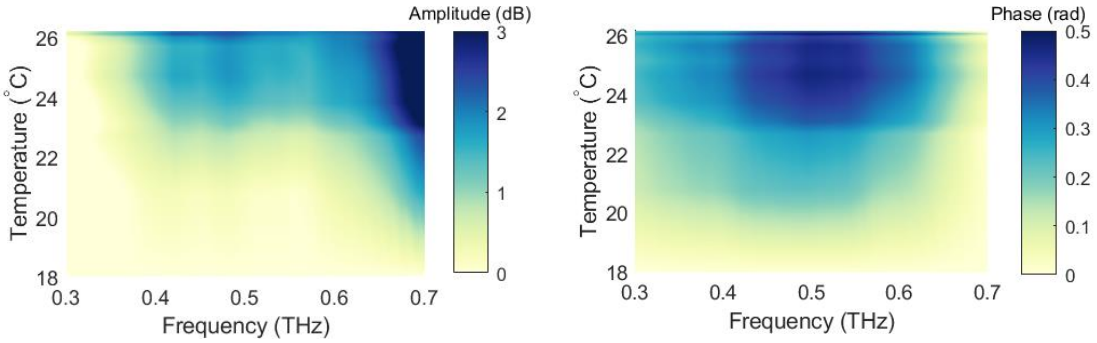


Figure 4 Amplitude and phase of the electric field reflected by the HO bone sample as a function of temperature. The amplitude and phase is normalised to the amplitude and phase obtained for the lowest temperature to illustrate the change in each.

Fabry-Perot oscillations occur when the investigated sample is comprised of a cavity, in which the field experiences internal reflection. For optically-thin samples, the detection of the initial pulse and subsequent reflected pulses overlap in time, making them unresolvable. This work utilises an algorithmic approach which analytically models the reflected electric field, as described in [7], to remove these Fabry-Perot effects.

To reveal the characteristic dielectric behaviour of water as a function of temperature, the TDS measurements were repeated in transmission configuration for deionised water within a temperature-controlled 500 μm thick cell. The complex permittivity was subsequently calculated for both the HO bone sample and free water, using the Fabry-Perot algorithm and Teralyzer (a material parameter extraction software by Menlo Systems), respectively. Figures 5 and 6 present the change in the frequency dependent real and imaginary parts of the permittivity as a function of temperature to reveal the water content, under the reasonable assumption that no other compound has a significant temperature-dependence. Each spectrum is normalised by the spectrum of lowest temperature.

The figures illustrate an increase in both real and imaginary permittivity with temperature, both of which are more pronounced for lower frequencies. The consistency between the bone and water samples indicates the presence of water in the bone structure. The increase in permittivity with increasing temperature and decreasing frequency can be attributed to the relaxation mechanisms of molecules that determine the permittivity of water, specifically the reorientational dynamics of dipole moments. This is characterised by the reorientation time of hydrogen-bond structures. Water exhibits two relaxation mechanisms modelled by Debye theory: slow and fast relaxation, occurring at ~10 ps and sub-picosecond (THz) timescales, respectively. The slow relaxation time is highly dependent on temperature and increasingly pronounced for lower frequencies [9]. This is further illustrated by figures 7 and 8, which present the real and imaginary

permittivity as a function of temperature, for fixed frequencies. The permittivity characterises a material's ability to permit the electric field and the loss of the field within the material.

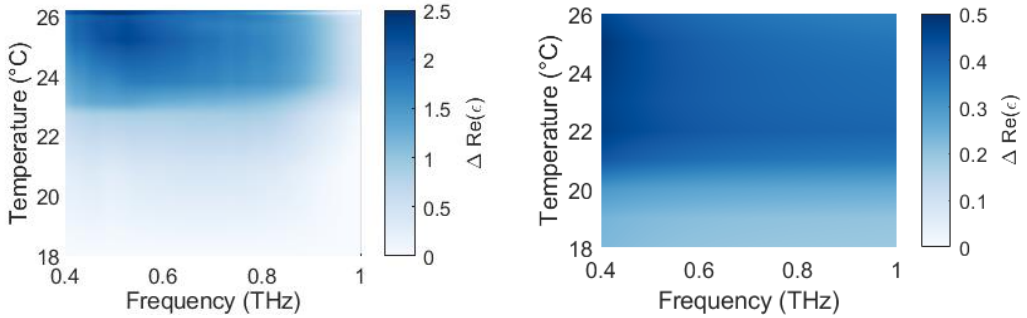


Figure 5 Change in the real part of the permittivity of the HO bone (left) and water (right) as a function of frequency and temperature. Each spectrum is normalised by the spectrum of lowest temperature.

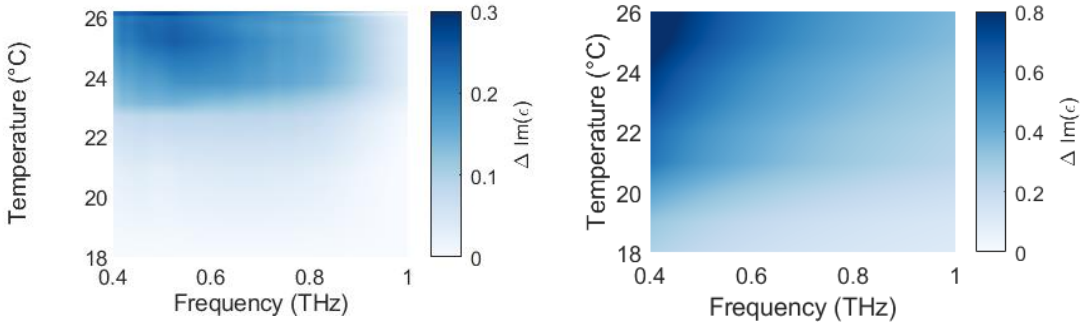


Figure 6 Change in the imaginary part of the permittivity of the HO bone (left) and water (right) as a function of frequency and temperature. Each spectrum is normalised by the spectrum of lowest temperature.

The difference between the change in permittivity for bone and water is thought to be attributed to the different dielectric properties of bound and free water [10]. The HO bone samples are expected to be comprised of bound water, typically found in tissue molecular components, while the water measurements carried out in this study are of free water only. Unlike free water, bound water is not moveable, and hence the dynamics of each differs. Further study of the temperature dependence of different water states must be carried out to characterise this.

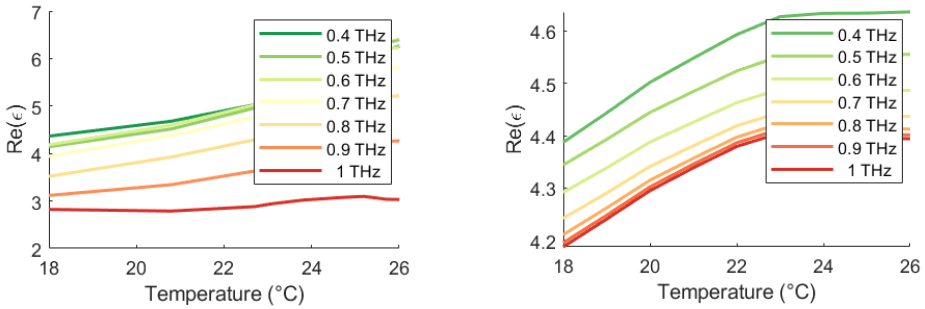


Figure 7 Real permittivity of the HO bone (left) and water (right) as a function of temperature, for fixed frequencies.

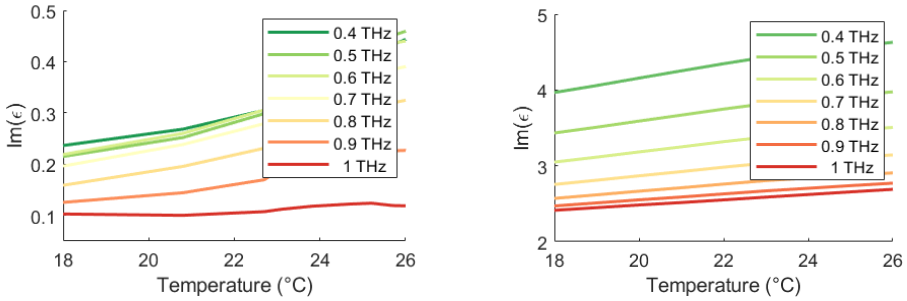


Figure 8 Imaginary permittivity of the HO bone (left) and water (right) as a function of temperature, for fixed frequencies.

## 4. CONCLUSION

Terahertz temperature dependent spectra have been taken to probe the hydration of HO bone samples. The observation of increasing permittivity with increasing temperature and decreasing frequency is identified as a signature of the presence of water. Future work aims to use this as a hydration mapping tool, for probing changes in tissue hydration, for prospective disease monitoring.

## 5. ACKNOWLEDGEMENTS

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## REFERENCES

[1] H. H. Mitchell, T. S. Hamilton, F. R. Steggerda and H. W. Bean, "The chemical composition of the adult human body and its bearing on the biochemistry of growth," Journal of Biological Chemistry, 158, 625–637, 1945.

[2] E. E. Wilson, A. Awonusi, M. D. Morris, *et al.*, "Three Structural Roles for Water in Bone Observed by Solid-State NMR," Biophysical Journal, 90, 3722–3731, 2006.

[3] M. Granke, M. D. Does and J. S. Nyman, "The Role of Water Compartments in the Material Properties of Cortical Bone, Calcified Tissue International," 97, 292–307, 2015.

[4] S. Freer, A. Gorodetsky and M. Navarro Cía, "Beam profiling of a commercial lens-assisted terahertz time domain spectrometer," IEEE Transactions on Terahertz Science and Technology, 11, 90–100, 2021.

[5] J. Zhou, X. Rao, X. Liu, *et al.*, "Temperature dependent optical and dielectric properties of liquid water studied by terahertz time-domain spectroscopy," AIP Advances, 9, 035346, 2019.

[6] B. L. Yu, Y. Yang, F. Zeng, *et al.*, "Reorientation of the H2O cage studied by terahertz time-domain spectroscopy," Applied Physics Letters, 86, 061912, 2005.

[7] S. Freer, C. Sui, S. M. Hanham, *et al.*, "Hybrid reflection retrieval method for terahertz dielectric imaging of human bone," Biomedical Optics Express 12, 4807–4820, 2021.

[8] Mira Naftaly, "Terahertz Metrology," Artech House, 2015.

[9] W.J. Ellison, "Permittivity of Pure Water, at Standard Atmospheric Pressure, over the Frequency Range 0–25THz and the Temperature Range 0–100°C," Journal of Physical Chemistry Reference Data, 36, 1–18, 2007.

[10] S. Carvalho, N. Gueiral, E. Nogueira, *et al.*, "Glucose diffusion in colorectal mucosa - A comparative study between normal and cancer tissues," Journal of Biomedical Optics, 22, 091506, 2017.