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Terahertz spectroscopy and imaging for gastric cancer diagnosis

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Terahertz waves are sensitive to differences in biological tissue hydration, we present promising results regarding the feasibility of applying this with terahertz time-domain spectroscopy and imaging for early detection of cancer through the characterisation of human gastrointestinal tissue with cancer-affected regions. To do that, healthy (normal) and carcinoma-affected gastric tissue samples at different stages were measured using transmission terahertz time-domain spectroscopy in the frequency range of 0.15–2.00 THz. Absorption coefficients and refractive index spectra of both normal and carcinoma-affected tissue were extracted and analysed. The results confirm that the techniques may be powerful tools to perform qualitative, early diagnosis of human cancer.

Keywords: gastrointestinal tract, gastric carcinoma, tumour staging, terahertz waves, terahertz spectroscopy and imaging

Introduction

According to global cancer data, the cancer burden rose to 18.1 million new cases and 9.6 million cancer deaths in 2018;[1] the commonest type of cancer is gastrointestinal (GI). Its early diagnosis is crucial for in-time treatment of affected patients. Several techniques, such as X-ray imaging, magnetic resonance imaging, tissue biopsy etc., are presently being used and are under continuous investigation for this purpose. However, there are still many challenging issues to overcome, as it is difficult to diagnose the disease's presence in its very early stages and/or to distinguish between benign and dysplastic tumours. Terahertz (THz) spectroscopy and imaging seem to be powerful options to add to this collection of diagnostic tools contributing to a solution for the important health and social problems associated with cancer.

Terahertz time-domain spectroscopy (TTDS) and imaging are attracting worldwide interest for their application in biomedicine, such as for the characterisation

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of biomolecules,[2] DNA/RNA, amino acids/peptides, proteins,[3] cells and tissues, including studies of different types of biomaterials.[4,5] The techniques are also important in a wide spectrum of applications, including homeland security,[6–9] law enforcement,[10] restoration of cultural heritage,[11,12] and for quality control of pharmaceuticals and identification of their counterfeiting.[13,14]

The promising results on the use of the techniques, particularly in the biomedical area,[15–17] have fostered the large-scale research activity of many groups worldwide. However, besides the previously mentioned issues, there are others that are challenging at the fundamental technological level: in understanding the interaction of THz waves with biological media, including appropriate safety guidelines. The fact that materials such as paper, vinyl, plastics, textiles, ceramics, semiconductors, lipids and powders are transparent to THz waves makes the THz region a good candidate for the above applications. Furthermore, THz waves do not present risks due to irradiation.

In the specific case of cancer diagnosis, there is a growing interest in exploring the THz spectrum. Its usage in medical imaging has certain advantages compared to X-rays, such as its considerably lower energy than the ionisation energy of biological molecules, coupled with the fact that the rotational and vibrational modes of water molecules lie within the THz spectral region, contributing to a high sensitivity. The feasibility of using THz waves to detect human tumour tissues by monitoring cell density and changes in water content has been reported recently.[18–20] The tumour environment usually generates increased blood supply to affected tissues and a consequent local increase in tissue water content. With the growth of tumours, the central cells no longer receive nutrients from the healthy blood vessels and, therefore, new blood vessels are formed. This could contribute to the contrast observed in THz cancer diagnosis. Moreover, conformational modes of water and biomolecules for this frequency region, as well as structural changes occurring in diseased tissue, have also been observed or suggested by several research groups to be contrast-contributing factors.[21–23]

Additionally, the high sensitivity of THz waves makes this technique suitable not only for the discrimination of abnormal from normal tissue, but also of benign from malignant tumours. This is achieved through differences in the interstitial water content in the tissue, due to the fact that the water-bound state is different in the two types of cancer.[15,24,25]

Malignant tumours are made up of cells that grow out of control, which can invade nearby tissues and spread to other parts of the body, moving away from the original (primary) tumour site and spreading to other organs. There, they can continue to grow and form another tumour on new sites, which is known as metastasis or secondary cancer.[26] Taking into account the unique features of THz technology, i.e. strong sensitivity to the presence of free or bound water, it can be seen how differentiation of the two types of tumour is possible.[27]

In previous works, we have investigated the potential of THz imaging and spectroscopy for the detection of dehydrated, paraffin-embedded, colorectal and gastric tissues. Essentially, these studies have shown that contrast between normal and tumour tissues could be detected, further indicating that other tissue elements contribute to the contrast observed rather than just water.[28–30]

The present work is aimed at demonstrating the capability of a compact THz spectrometer (a fibre-coupled THz spectrometer) to establish contrast between normal and gastric-carcinoma-affected freshly excised tissue. This research work is one of the few studies carried out on carcinoma-affected human stomach tissue by THz spectroscopy. Hou *et al.*[31] have studied dehydrated gastric tissue using THz spectroscopy and found differences in absorption spectra between normal and tumoural tissue, confirming that the intrinsic increase of hydration in cancer-affected tissues could be the main THz contrast-contributing factor.

Materials and methods

Tissue sample preparation

A set of 15 anonymous samples of human gastric tissues, comprising normal and carcinoma-affected areas, was obtained from the Department of Pathologic Anatomy of Centro Hospitalar São João (Porto, Portugal). The tissues were donated for lab use and prepared under the approval of the Ethics Commission of Centro Hospitalar S. João – EPE, (Ref. CES 211-13).

Histological samples with constant thickness of 0.50 mm were taken from partial distal and total gastrectomy specimens. They had been previously analysed, classified and staged as gastric adenocarcinomas pT3 and pT4. Those staged as pT3 corresponded to a tumour penetrating sub-serosal connective tissue without invasion of visceral peritoneum or adjacent structures.[32,33] Those staged as pT4 corresponded to a tumour which had invaded the serosa (visceral peritoneum).[32] The tissue samples were prepared according to standard protocols approved at the Centro Hospitalar S. João, Porto. Briefly the procedures for collection and preparation of tissue samples consisted of three steps: 1) tissue collection, preparation and fixation; 2) selection of samples from the tissue; and 3) processing of the sample.

The normal and carcinoma-affected areas in each sample had been previously identified by histological examination (Figure 1).

Before starting any measurements, samples were sandwiched between two 1 mm thick and 20 × 20 mm sized high-density polyethylene (HDPE) plates, separated with spacers of 0.50 mm. The tissue was slightly pressed between the two plates in order to avoid air gaps between the sample surface and the HDPE surface.

THz spectroscopic analyses

A fibre-coupled THz spectrometer (model T-Fiber, Teravil Co., Vilnius, Lithuania) was used for the measurements. The spectrometer has an integrated femtosecond fibre laser with two fibre output ports. The femtosecond laser with fast delay line and signal registration electronics is integrated in a single compact housing with a footprint of 40 × 40 cm. The fast delay line allows real time data acquisition with a speed of 10 spectra s^{-1}, 110 ps time window and a spectral resolution <10 GHz. The transceiver (emitter and detector) is fibre-coupled to the femtosecond laser. The laser is a LightWire FF50 (EkSPLA), with 1064 nm central wavelength, and a pulse duration of <160 fs, 60 mW output power, 40 MHz pulse repetition rate and 0.15–3.00 THz frequency range. The TTDS and imaging measurements were performed in randomly chosen zones of the same samples. All the samples were measured at the room temperature under the same conditions and mechanical positioning.

Tissues parameter extraction

Several authors have presented material parameter extraction algorithms to determine the complex refractive indices of samples with TTDS.[34,35] For that purpose, a THz pulse propagating through a sample is compared to another THz pulse propagating without the sample in its path. This was achieved by tracing the temporal shape of the electric field with sample, $E_s(t)$, and without sample, $E_{ref}(t)$, where t is the optical delay time. These two pulses are transformed into the frequency domain using a fast Fourier transform (FFT) to obtain the complex transmission spectra for the signal, $E_s(\omega)$, and for the reference (HDPE only), $E_{ref}(\omega)$. Considering normal incidence, the ratio of these fields is related to the absorption coefficient $\alpha(\omega)$ and refractive index $n(\omega)$ of the sample as follows,[36,37]

$$\frac{E_s(\omega)}{E_{ref}(\omega)} = \frac{1}{4n(1+n)^2}\exp\left(\frac{\kappa\omega d}{c(n-1)}\right) = A(\omega)\exp\left[-\kappa\phi(\omega)\right] \quad (1)$$

where ω is the frequency, c is the speed of light in a vacuum, d is the thickness of the sample, $A(\omega)$ is the amplitude ratio between the spectrum of the sample signal and that of the reference, and $\Delta\varphi(\omega)$ is the relative phase difference. From Equation (1), the optical constants of the tissue were calculated, and, therefore,

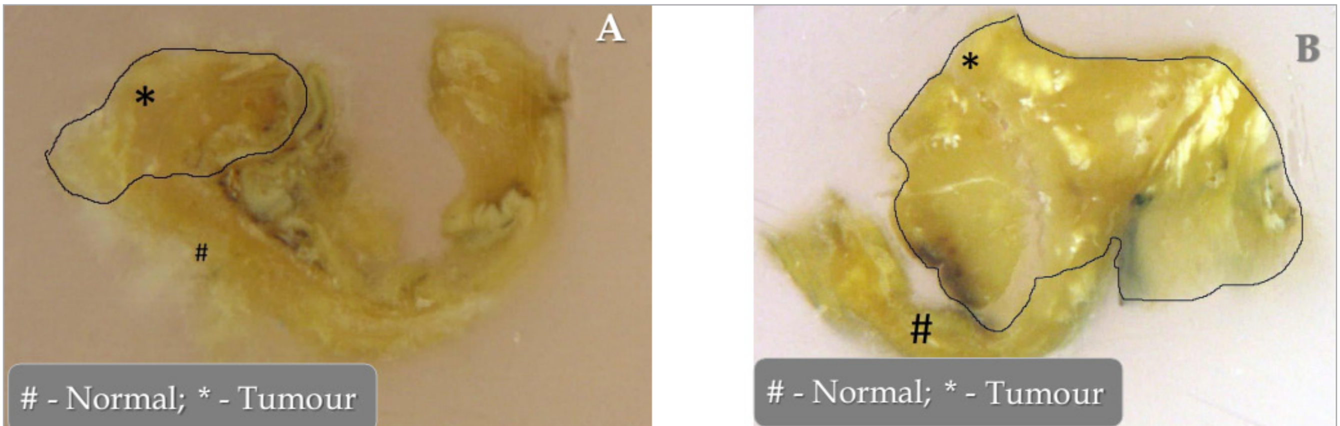

Figure 1. Macroscopic images of samples from different stages of gastric carcinoma. (A) pT3 stage and (B) a pT4 stage carcinoma.

the refractive index and the absorption coefficient were determined using the following equations,[34,35,38]

$$n_{samp}(\omega) = \left[c\Delta\phi(\omega)/\omega d\right] + n_{ref} \quad (2)$$

$$\alpha_{samp}(\omega) = 2d^{-1}\ln\left\{4n_{samp}(\omega)\Big/A(\omega)\left[n_{samp}(\omega) + n_{ref}\right]^2\right\} \quad (3)$$

where $\kappa(\omega)$ is the attenuation coefficient, which is the imaginary part of the complex refractive index, $\tilde{n}(\omega) = n_s(\omega) - i\kappa(\omega)$. From the recorded pulses, $A(\omega)$ and $\varphi(\omega)$ were directly obtained through FFT operation.

Results and discussion

A comparison was made in terms of the attenuation of absorption functions due to water and bio-structural alterations due to the tissue pathologic stage between normal, and pT3 and pT4 development stages. It allowed us not only to discriminate the three phases and their exact margins, but also to achieve early diagnosis by detecting initial differences between the malignant and benign phase.

In this study, the working frequency range was restricted to between 0.15 THz and 2.00 THz due to the relatively high noise level on both sides of the working range (before 0.15 THz and after 2.00 THz).

In Figure 2, a slight contrast between the normal (healthy) regions and those in tissue pathological stage pT3 as well as that in stage pT4 can be observed. In turn, one can also observe a slight contrast between the two pathological stages pT3 and pT4. For example, at a frequency of 2 THz, the absorption coefficient value for the normal tissues varies between 90 cm^{-1} and 110 cm^{-1} from the regions of samples with pathological stages pT3 and pT4, respectively.

In previous studies on dehydrated gastric tissue,[29] we observed very low contrast, a fact that was explained by the absence of water, which intensifies the contrast due to its strong absorption in the THz frequency region. As was found by other research groups,[18,39] refractive indices and absorption coefficients of tumour tissue are higher than those of normal tissue. This occurs because tumour tissue tends to have slightly higher water content compared with normal. HDPE has low absorption and no dispersion at working frequencies and, therefore, losses due to this medium had no significant impact on the results. Other factors on dehydrated samples, namely conditions within the tumour microenvironment, rapid and uncontrolled cell division leading to an increased cell density and/or to the presence of certain proteins, factors associated with the differences caused by cell iterations and abnormal proteins, increase in the vascularisation around tumours and the intrinsic release of growth factors, contributed to the observed contrast, although at lower percentages.

Terahertz imaging is able to provide a greater spatial resolution, up to tens of microns. Therefore, THz imaging can, in support of spectroscopy, be used to assess both the structure and the attenuation performance of bio-tissue samples by recording the image at selected frequencies where optical constants differ considerably. The imaging was performed with a VDI (Virginia Diodes, Inc.) electronic multiplier chain driven by the synthesiser and producing 580–620 GHz radiation. Radiation was electrically modulated at a frequency of 2 kHz.

The beam was collimated by a Teflon lens with 12 cm focal length distance and 5 cm diameter. The collimated beam was angled with a 5 cm mirror in order to extract just the central area of the emitted beam and directed to the investigated sample by a 5 cm focal length off-axis parabolic mirror. The transmitted and reflected radiation was also collected using parabolic mirrors and focused to the titanium microbolometer detector.[39] We estimated that a distance of 1 m between the THz source and the flat mirror was required for far field radiation conditions. The titanium microbolometer was chosen for THz radiation detection as it possesses a small effective-antenna cross-section, enabling a high-resolution scan of a local intensity.[40] The signals were registered with a lock-in amplifier and processed with a computer. Samples were scanned by moving them on two translational stages along the x–y axes.

Figure 3 displays the THz imaging results for one of the samples, obtained at 0.58 THz. The randomly selected tissue for imaging appeared to be opaque in the transmission experiments. However, the image in reflection shows a reasonable contrast between the zones of affected and normal tissue (scales in the change of the signal are shown on the left axis in all panels). Diseased zones can be clearly seen as areas demonstrating higher reflectance (red colour). The low contrast can be explained by the fact that measurements were performed directly on an irregular surface of the tissue, producing THz wave scattering. The selection of only one sample for imaging was intended to show the differences between affected and normal (healthy) regions in the same sample.

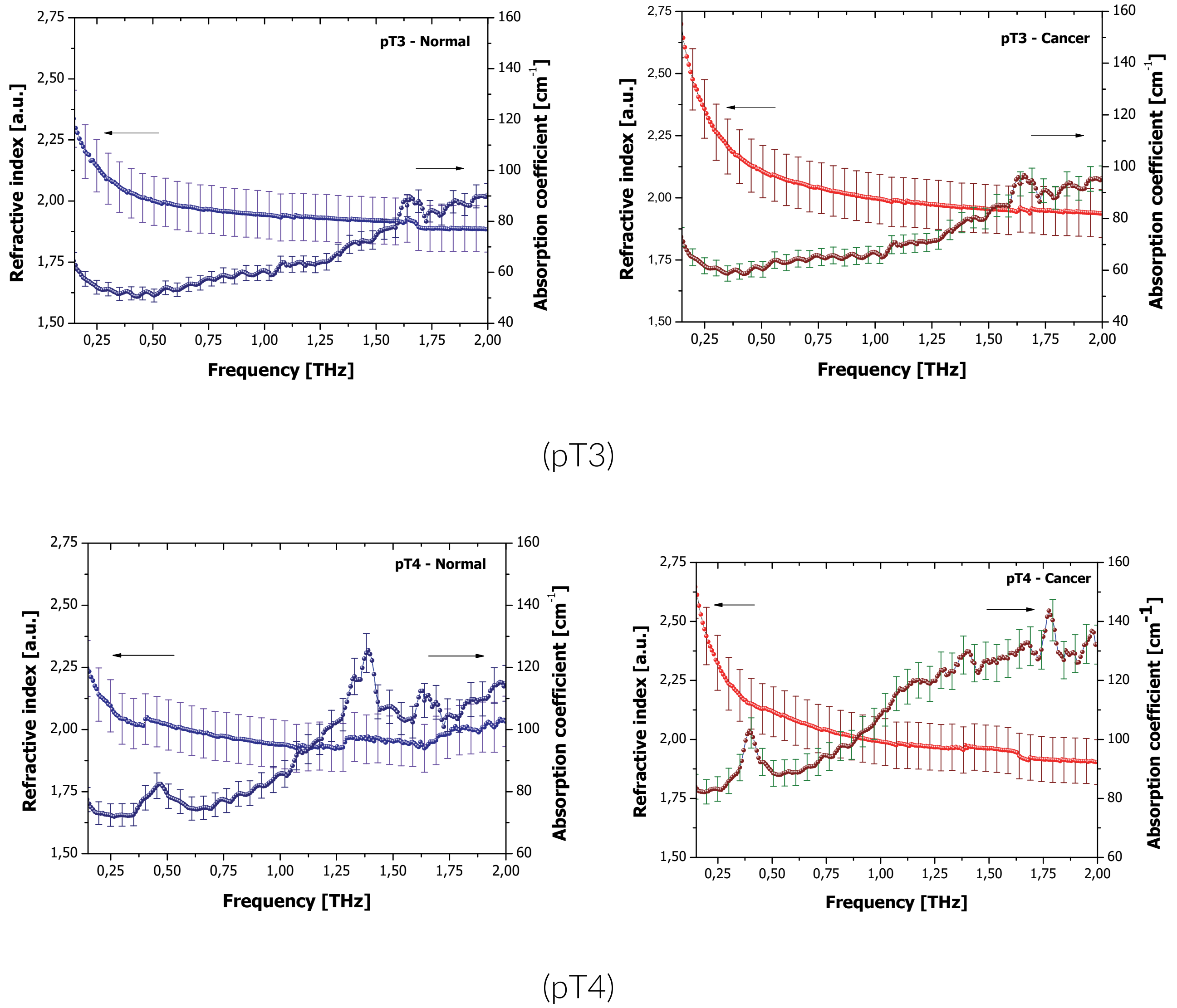

Figure 2. The absorption coefficients and refractive indices spectra in both, normal and tumour tissue for pT3 and pT4.

The contrast between affected and normal tissue regions is resolved in the reflection image, indicating different water content in the sample. This illustrates that THz imaging can successfully be employed for cancer diagnosis in very early phases.

Conclusions

In the present study, two groups of human normal and tumoural gastric tissue, taken from a section with the same bio-structural typology, were investigated. Using TTDS, we have shown that carcinoma regions of all samples intrinsically show higher absorption coefficients and refractive indices, allowing them to be distinguished from the normal regions of the samples. These findings, together with those observed using THz imaging, may take us a step closer to early cancer detection. Thereby, the techniques (TTDS and THz imaging), after exhaustive and accurate experimental work, could be good candidates as complementary tools to those already used in cancer diagnosis.

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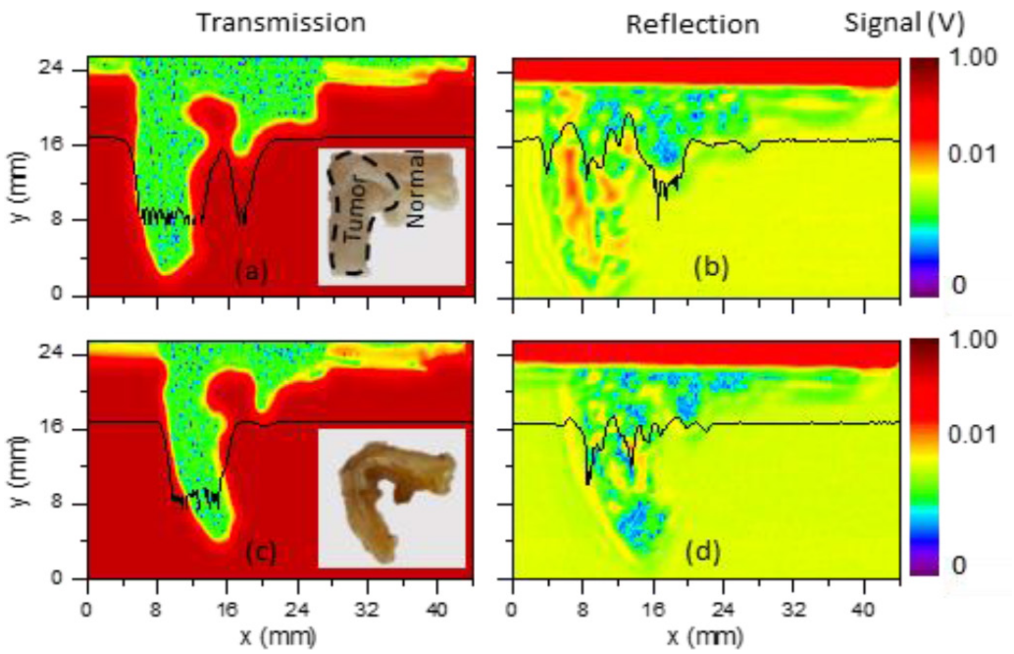

Figure 3. 0.58 THz image of the tissue from the group staged pT4. Image of the fresh tissue, taken straight from the container cell, in (a) transmission and (b) reflection configurations. Image of the tissue 24 h after the first measurement, in (c) transmission and (d) reflection configurations. Digital photos of the sample are shown in the insets in (a and c) . Image consists of 440 × 85 pixels with a pixel size of 0.1 × 0.3 mm.

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