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Terahertz spectroscopy for the study of paraffin-embedded gastric cancer samples

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HIGHLIGHTS

- Gastric cancer detection.
- Paraffin-embedded samples.
- Cancer affected tissue regions, low transmittance.
- High absorption coefficient and refractive index for affected tissue.
- Dehydration of bio-tissues lead *t* low contrast.

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ABSTRACT

Terahertz (THz) spectroscopy constitute promising technique for biomedical applications as a complementary and powerful tool for diseases screening specially for early cancer diagnostic. The THz radiation is not harmful to biological tissues. As increased blood supply in cancer-affected tissues and consequent local increase in tissue water content makes THz technology a potentially attractive. In the present work, samples of healthy and adenocarcinoma-affected gastric tissue were analyzed using transmission time-domain THz spectroscopy (THz-TDS). The work shows the capability of the technique to distinguish between normal and cancerous regions in dried and paraffin-embedded samples. Plots of absorption coefficient α and refractive index n of normal and cancer affected tissues, are presented and the conditions for discrimination between normal and affected tissues are discussed.

Introduction

Among the most common worldwide causes of cancer death is gastric cancer (with about 738,000 deaths per annum, 9.7% of all cancer cases) [1]. Early clinical diagnosis of gastric cancer is crucial for in time patients' treatment. Usually microscopic pathology is the most commonly used technique for tissue analysis. Embedding tumor bio-tissues in paraffin for histopathologic analysis is a widely used fixation method owing to its better storage and long-term tissue morphology preservation in clinical settings [2]. There are many other optical techniques being investigated for this purpose, and, the introduction of THz spectroscopy appears to be a powerful instrument to contribute for the solution of the important health and social problem associated with cancer. However, there are still many challenging issues to overcome such as better understanding of THz bio-interaction for THz spectroscopy and safety guidelines, which could enable the development of reliably powerful THz biomedical spectroscopy systems for further improvement of early cancer diagnosis. And are, thus, expected to bring a more comprehensive screening and diagnosis of human

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disease, particularly in the case of gastric and other cancers. This technique are based on the specific spectroscopic fingerprints of biological medium in the THz spectral region [3].

The terahertz region lies between the microwave and infrared regions of the electromagnetic spectrum. It has very low photon energy – 1 THz is 4.1 MeV – and thus it does not pose any ionization hazard for biological tissues [4], and they have small dispersion and large absorption in the terahertz range [5]. THz radiation has a shorter wavelength than that of microwave frequencies enabling higher spatial resolution capabilities. The radiation is very sensitive to water content and strongly attenuated by water, non-ionizing, and power levels found in pulsed systems (<1 μW) are orders of magnitude lower than the maximum permissible beam power as determined by ionizing radiation safety guidelines [6]. Furthermore, owing to the coherent time gated detection technique there is an efficient elimination of background noise which commonly leads to a very high signal to noise ratio (>10,000:1 at 1 THz). Because of these characteristic properties, there has been growing interest in THz imaging and spectroscopy for biomedical application in recent years. In addition increasing studies are being reported using THz technology in spectroscopic studies of cancer [7,8]. The presence of cancer often causes increased blood supply in affected tissues and a local increase in tissue water content [9], fact acting as a natural contrast mechanism for THz imaging of cancer. Moreover, the structural changes that occur in affected tissues have also been shown to contribute to THz-TDS's spectra contrast [10,11].

Histo-pathologists use microscopic imaging methods of biopsied tissue parts to provide structural and functional information, X-ray imaging, magnetic resonance imaging MRI to provide images of living tissues at the macroscopic level, but at much lower resolution and specificity [12,13]. Alternative techniques of highly-resolved imaging for *in-vivo* diagnostic screening capable of providing early detection of disease are highly still desirable. THz imaging may constitute a powerful tool to complement these and other currently used techniques in years to come.

Terahertz spectroscopy provides broadband information on biosamples making it possible to distinguish between tissue regions with different optical characteristics (e.g., healthy and neoplastic tissue) over the reliable working THz frequency range. THz-TDS has previously been used to obtain the THz optical characteristics of skin tissue [11,14]. THz-TDS has also been used to successfully characterize DNA and proteins, allowing intermolecular interactions to be probed [15,16]. Because of the advantages listed above, THz techniques have the potential to become a particularly viable tool for early cancers diagnostic. First published results specially, in cancer tissue imaging using THz pulsed radiation, suggest that THz imaging can be used for macroscopic visualization of tumor margins in fresh tissues [17–20], which was later confirmed by other studies on various cancer types and organs [21–23] and to identify contrast between healthy breast tissue and breast cancer [23]. It has been suggested that this technique could be used to assist surgeons performing breast-conserving surgery when excising tumor margins.

The present work is aimed at, firstly demonstrating the capability of THz-TDS for gastric cancer diagnosis and, secondly, exploring the technique's capability as a confirmatory (auxiliary) technique for early gastric cancer detection by distinguishing healthy tissue from the neoplastic one. The novelty of the present research work resides in (i) being the first study available concerning the application of THz-TDS for gastric cancer, (ii) showing that additional contrast-contributing factors other than water influence the diagnostic potential of this technique [24]. In more general terms, the present study intends to contribute to widen opportunities for THz science in medicine by the spatial resolution and data acquisition rate and by providing a better understanding of THz pulse propagation through complex media with the overall aim of developing cost effective and reliable diagnostic THz devices with endoscopic ability to provide access to internal epithelial surfaces for early cancer detection within the established safety guidelines.

Theoretical background: Samples' parameters extraction

Several authors presented material parameter extraction algorithms to determine the complex refractive indexes of samples with THz-TDS [25,26]. For that purpose, a THz pulse propagating through a sample is compared to another THz pulse propagating without the sample in its path. This is achieved by tracing the temporal shape of the electric field with sample $E_{sample}(t)$ and without sample $E_{ref}(t)$ where t is the optical delay time. These two pulses are transformed into the frequency domain using fast Fourier Transform to obtain the complex transmission spectra for the signal (sample embedded in paraffin) $E_{sample}(\omega)$, and for the reference (paraffin 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 follow [25–28],

$$\frac{E_{sample}(\omega)}{E_{ref}(\omega) = 4n(1+n)^{-2}e^{\frac{-k\omega d}{c(n-1)}}} = A(\omega)e^{-k\varphi(\omega)} \tag{1.1}$$

where ω, is the frequency, c, the speed of light in 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 $\varphi(\omega)$ is the relative phase difference.

From the expression (1.1), refractive index and the absorption coefficient can be calculated through the following expressions [25,26,21–24]

$$n_{sample}(\omega) = 1 + (2\pi\omega d)^{-1}c\varphi(\omega) \tag{1.2}$$

$$\alpha_{sample}(\omega) = -2d^{-1}\ln\{[4n_{Sample}(\omega)]^{-1}A(\omega)[1+n_{sample}(\omega)]^2\} \tag{1.3}$$

Materials and methods

Experimental set-up

In this work, a Teravil-Ekspla T-Spec THz TDS system (Vilnius, Lithuania) was used in the frequency range 0.1–3.5 THz with a spectral resolution of <10 GHz. The layout of the system is shown in Fig. 1.

A photoconductive antenna made from GaBiAs, illuminated by ultra-short laser pulses, is used for THz radiation and detection. The antenna is formed using AuGeNi metallization. The fiber-based pumping laser provide pulses of 1064 nm wavelength, 150 fs pulse duration and 40 mW output power at 30 MHz pulse repetition rate. For more efficient collimation and focusing of THz radiation, a hemispherical lens fabricated from high resistance silicon is attached to the backside of the THz antenna. The fiber-based pumping laser has two outputs where, one part of the laser beam is used for illumination of THz emitter, the second part of laser beam is fiber coupled and is used to illuminate the THz detector and the other part goes through slow delay line and guided to the fast delay line. After the delay line the beam is guided to the THz emitter, where is focused to the gap of the photoconductive antenna. The second part of the beam goes to the THz detector. The generated sub-picosecond pulses of THz radiation are focused to the sample by parabolic mirror M2. The transmitted radiation from the sample is collected by the parabolic mirror M3, and then, registered by a fiber coupled THz detector after which the THz signal goes to a digital signal processing unit. By scanning fast optical delay line in 10 Hz frequency, the waveform of electrical field of THz radiation is build and a numerical Fourier transform operation