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Terahertz absorption and reflection imaging of carcinoma-affected colon tissues embedded in paraffin

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ABSTRACT

In the present study, dehydrated human colon tissues embedded in paraffin were studied at THz frequency. A compact THz imaging system with high numerical aperture optics was developed for the analysis of adenocarcinoma-affected colon sections, in transmission and reflection geometry. A comprehensive analysis of the THz images revealed a contrast up to 23% between the neoplastic and control tissues. Absorption and reflection THz images demonstrated the possibility to distinguish adenocarcinoma-affected areas even without water in the tissue, as the main contrast mechanism in THz measurements has been observed to be water absorption in *in vivo* or freshly excised tissues. The present results corroborate with previous histologic findings in the same tissues, and confirm that the contrast prevails even in dehydrated tissues.

1. Introduction

Early clinical diagnosis of cancer is crucial for in time patient treatment. The World Health Organization (WHO) estimates that there will be a substantive increase to 19.3 million new cancer cases per year by 2025 (GLOBOCAN 2012). The most commonly diagnosed cancers worldwide are those of the lung (1.8 million, 13.0% of the total), breast (1.7 million, 11.9%), and colorectal (1.4 million, 9.7%), with more than 85% of all cancers-associated deaths being due to the difficulty in detecting the disease in its early phases [1].

Historically, the discovery of a new electromagnetic wave spectrum has always originated the development of new tools for medical diagnostic and imaging. Recent technological achievements in the THz region have triggered new applications in biology and biomedicine and in particular those based on the specific spectroscopic fingerprints of biological matter [2–4]. Due to the absence of compact THz imaging systems operating at room environment, the THz waves have not been widely explored before. Strong THz signal attenuation by atmospheric water vapor and small energy of the THz quanta in comparison to room temperature photons. There are still many challenging issues to overcome, both at the fundamental and technological levels related to the understanding of THz interaction with bio-subjects, including safety guidelines. Eventually, a deeper understanding of THz – bio interaction and the appearance of novel THz systems will enable the development of powerful THz biomedical imaging systems to further biomedical diagnosis and therapy.

There is a growing interest in exploring THz radiation spectrum for cancer diagnosis. Its usage in medical imaging has certain

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advantages compared to X-rays, such as the considerably lower energy than the ionization energy of biological molecules and, the fact that the rotational and vibrational energies of water molecules lay within the THz spectral region, contributing to a high sensitivity to the motion of water molecules. The THz radiation is non-ionizing and power levels found in pulsed imaging systems (<1 μW) are orders of magnitude lower than the maximum permissible according to ionizing radiation safety guidelines [5]. Because of these unique characteristics the THz radiation has allowed the diagnosis of human skin and breast cancers by monitoring cell density and water content changes [6]. In addition, the application of THz waves in spectroscopic studies of cancer has also been reported recently [7]. The cancer environment generally causes increased blood supply to affected tissues and a local increase in tissue water content [8]. These facts act as a natural contrast mechanism for THz imaging of cancer [9,10]. Moreover, the structural changes occurring in affected tissue have also been shown to contribute to THz image contrast [11–14].

Current cancer diagnostic techniques rely essentially on X-ray imaging, magnetic resonance imaging (MRI) and in histological analyses of biopsies. Histopathologists use microscopic imaging methods of biopsed tissue parts to provide structural and functional information, whereas X-ray imaging and MRI provide images of live tissues at the macroscopic level, but at lower resolution and specificity [15]. Alternative techniques of highly-resolved imaging for *in vivo* diagnostic screening, capable of providing early detection of disease, are highly desirable. THz waves have the potential to bring new instruments that could contribute to the improvement of medical imaging and become particularly viable for comprehensive medical imaging of tumors. THz imaging provides additional useful information on bio-samples allowing the user to distinguish regions with different optical characteristics; for example, non-neoplastic and neoplastic tissues. Published results on cancer tissue imaging using THz pulsed radiation suggest that THz imaging can be used for macroscopic visualization of tumor margins in fresh tissues [6,16–18], which was later confirmed by other studies on various cancer types and organs [19–21], and contrast observation between healthy breast tissue and breast cancer [21]. These results suggested that the technique could be used to assist surgeons performing breast-conserving surgery when excising tumor margins.

The purpose of the present work should be to demonstrating the capability of a compact THz imaging system to find contrast between dysplastic and non-dysplastic tissue even in totally dehydrated bio-tissue, confirming the existence of other contrast-contributing factors although at very low percentage. The novelty of the present research work resides in (i) being among the few studies carried out concerning the application of THz imaging for colon cancer, (ii) showing that additional contrast-contributing factors (in bio-tissue dehydration condition) other than water (in freshly excised or *I -vivo*) influence the diagnostic potential of this technique. In more general terms, the present study intends to contribute and widen opportunities for THz science and technology in medicine by exploring its spatial resolution and data acquisition rate and by providing a better understanding of THz waves' propagation through complex media. Overall, it is intended to develop cost effective and reliable diagnostic THz systems with endoscopic ability to provide access to internal epithelial surfaces for early cancer detection.

2. Materials and methods

2.1. Experimental set-up

The THz imaging (TI) system was developed for fast THz image acquisition at room environment. The setup was configured in such a way that the images in reflection and transmission mode were recorded simultaneously as it is shown in Fig. 1. The TI system was based on commercially available amplifier/multiplier chains (Virginia Diodes, Inc.) and THz antenna-coupled micro-bolometer detectors [22] associated with the 3-axis translation stage used for accurate positioning of the subject under test. The source was set to operate in meander-type 1.5 kHz amplitude modulation regimen, emitting power of about 1.2 mW at 590 GHz frequency. The particular frequency range was selected due to higher contrast between control and neoplastic tissues measured with the coherent THz spectrometer (see Fig. 2). Imaging at lower frequency (200–300 GHz) was also tested but contrast between different tissues was found insufficient and the spatial resolution obtained was low (1.0 mm), compared to the one obtained at 600 GHz (0.5 mm). Emitted THz radiation was collimated with high density polyethylene (HDPE) lens (L1) and directed with the mirror M3 to the 2″ diameter 5 cm focal length off-axis parabolic (OPA) mirror M4 used to focus the THz beam onto the sample (Fig. 1). Radiation transmitted through the sample was collected by the identical OPA mirror M6 and directed to the THz detector D2 by using the mirror M5 (Fig. 1). The high resistivity silicon beam splitter (BS) was inserted in an optical path to redirect reflection from the sample THz radiation to the THz detector D1 (Fig. 1). The antenna-coupled titanium micro-bolometers were used for sensitive reflected and transmitted THz signal detection in real time at room temperature [22]. The signals were registered by the lock-in method employing the position synchronized measurement technique [23].

The transmission and reflection spectra were obtained with the THz-TDS system [24]. Transmission of THz pulses with and without the sample provided the absorption spectrum of the tissue. THz-TDS provided the signal to noise ratio up to 750 related to the absorption coefficient and the thickness of the tissues. Reflection spectra were obtained by measuring reflected THz pulses form the surface of the tissue and a gold mirror used as the reference.

2.2. Sample preparation

A set of 14 anonymous paraffin-embedded control and neoplastic carcinoma-affected human colon tissue blocks were obtained from the archives of the Department of Pathologic Anatomy of São João Hospital (Porto, Portugal). The work with the biological samples was authorized (CES 211-13) by the Ethics Committee of Centro Hospitalar S. João-EPE (Porto, Portugal).

Histological samples with constant thickness of 2 mm were

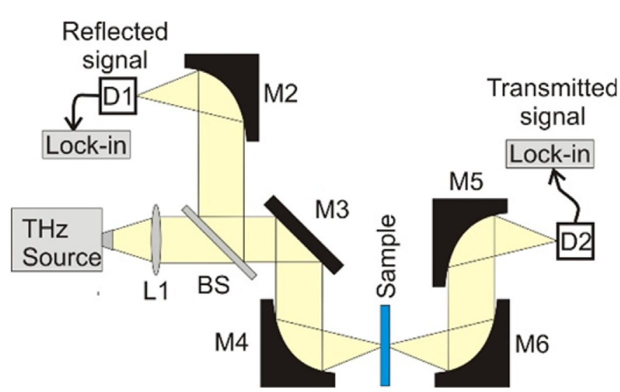

Fig. 1. Setup of the STIS used for THz imaging in transmission and reflection mode. Here M3 is flat gold mirror, M4, M6 – 5 cm focal length off-axis parabolic (OPA) mirrors, M2, M5 – 10 cm focal length OPA mirrors. All mirrors used were 2″ in diameter.