Case Report
Terahertz spectroscopy for cancer diagnosis

Han Li[1], Kun Zhang[3], Qian Zhou[1], Weiwei Mao[1], Ping Dong[1], Fubo Tian[2]

[1]Department of Nephrology, The First Rehabilitation Hospital of Shanghai, Shanghai 200090, China; [2]Department of Anesthesiology, Shanghai Obstetrics and Gynecology Hospital, Fudan University, Shanghai 200090, China; [3]Department of Nephrology, Tongji Hospital of Tongji University, Shanghai 200065, China

Abstract: This paper presents the application of terahertz (THz) technology for clinical cancer diagnose. Because of its advantages on the interaction with molecular network (such as hydrogen bonds), biologic tissues (especially the cancerous tissues) have significant characteristics in THz region. In this paper, after carefully choosing and comparing the samples, three kinds of cancers (i.e., colon carcinoma, gastric cancer, and renal cancer) can be efficiently detected by using THz method, which indicates that THz spectroscopy technique is of great importance with a broad prospect of applications in biological tissue investigation and clinical medicine (such as tumor disease diagnosis).

Keywords: Terahertz technology, clinical cancer diagnose, molecular network, colon carcinoma, gastric cancer, renal cancer

Introduction

Terahertz (THz) wave is generally defined as the electromagnetic wave in the frequency region from 0.3 to 3 THz (1 THz = 10^{12} Hz), with corresponding wavelength from 1 mm to 0.1 mm (or 100 μm). THz radiation exhibits the following properties [1]: Penetration-THz wave can pass through common clothing and packaging materials with relatively little attenuation. High-resolution imaging-the short wavelength of THz wave (in comparison to microwave) can be used to provide images with sub-millimeter resolution. Fingerprint spectroscopy-samples exhibit characteristic spectral features in the 0.3-3.0 THz region. This enables different chemical substances to be detected and distinguished in THz band. Non ionizing-THz radiation is non-ionizing and can be used at very low power levels (in the microwatt range) due to the availability of coherent detection schemes with high sensitivity [2]. This property of THz radiation makes it suitable for biological and medical applications [3] such as medical imaging for detecting infected tissues. Low scattering-the longer wavelength of THz signals (compared to visible light) allows it much lower scattering.

For most of the 20th century, electromagnetic spectrum in THz band remained almost inac-cessible due to the lack of efficient generation and detection methods. Only applications in astronomy and chemistry had access to this part of spectrum for the spectroscopic studies of a variety of light-weight organic molecules. Nowadays, THz radiation is widely used in bio-sensing applications because of numerous characteristics vibrational modes of macro-molecules, such as proteins and deoxyribose nucleic acid (DNA). The protein dynamical transition was observed for the first time in THz dielectric response by Markelz A G, et al. [4]. The complex dielectric permittivity $\varepsilon = \varepsilon' + i\varepsilon''$ is determined in 0.2-2.0 THz and 80-294 K ranges. Fischer et al. [5] demonstrated the use of THz time-domain spectroscopy (THz-TDS) for recording the far-infrared (0.5-4.0 THz) dielectric function of four nucleobases, which form the building blocks of DNA.

The characteristics of tumor tissues are more complex than macro molecules. Because of the differential histological and cytological characteristics, pathologists can distinguish normal human tissues and tumor tissues by microscope. Generally, tumor cells have abundant cytoplasm that is vacuolated, fluffy or granular, and usually with indistinct cell borders. Also tumor nuclei have variable atypia, irregular con-
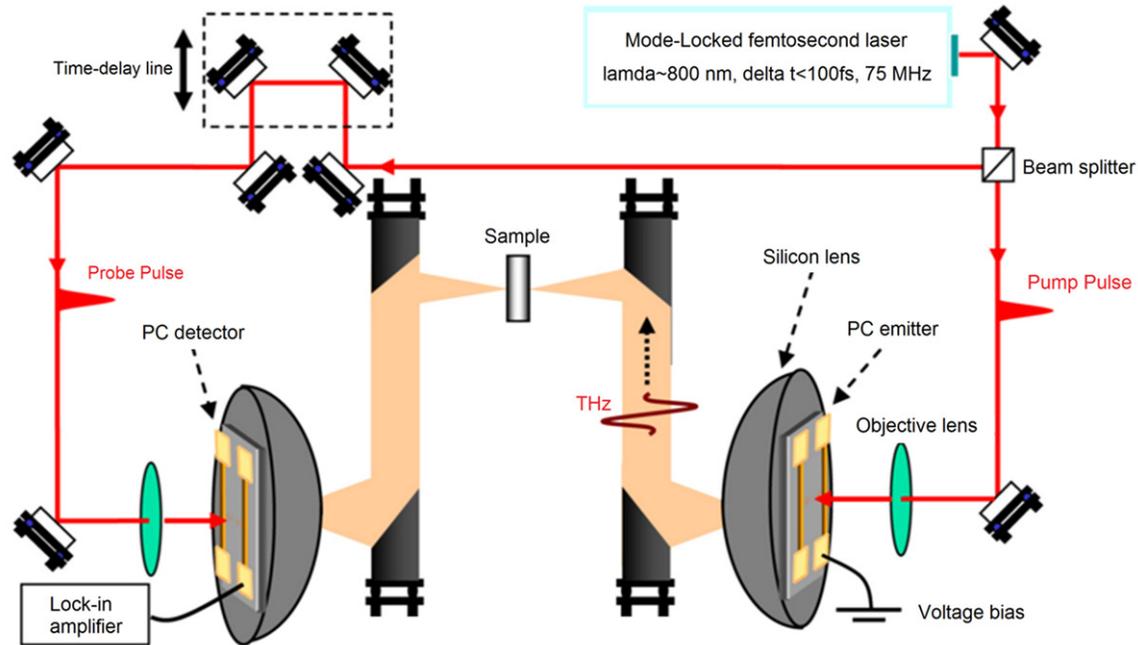
Figure 1. THz-TDS transmission mode set-up.

tours, haphazard orientation with abnormal chromatin, and variably prominent nucleoli. Moreover, tumor tissues have some specific cytological structures, such as heterogeneous cell population, small cytoplasmic vacuoles and hemosiderin deposits in kidney tumor [6]. However, normal cells have none of these aforementioned features.

Based on the cytological characteristics, researchers have made efforts to help doctors to distinguish normal tissues from tumor tissues via THz technologies. Fortunately, the feasibility of using THz pulsed spectroscopy for detecting cancer tissues has been demonstrated by many groups. For now, research on various kinds of cancers including skin [1], breast [7], oral melanoma [8], liver [9], stomach [10], colon [11-13] and animal tissues [14] has been carried out. Fitzgerald *et al.* [7] have imaged 22 excised women (mean age: 59 years old; range: 39-80 years old) breast tissue specimens with carcinoma by using THz pulsed imaging system, and investigated the map of margins of these exposed breast tumors. Sim *et al.* [8] have imaged a single case of oral malignant melanoma by using THz reflection imaging system at room temperature (20°C) and below freezing point (-20°C), respectively. Nishizawa *et al.* [9] have

demonstrated that THz imaging has a possibility to distinguish regions of metastatic cancer from normal tissue in liver, by comparing a THz wave image at 0.835 THz with that at 1.465 THz. Caroline B. Reid *et al.* [11] have presented the results from a feasibility study which measured properties in the THz frequency range of excised cancerous, dysplastic, and healthy colonic tissues from 30 patients. The absorption and refractive index spectra have been compared to identify trends which may enable different tissue types to be distinguished. In addition, the researchers have presented statistical models considering up to 17 parameters, which are calculated from the reflected time-domain and frequency-domain signals of all the measured tissues. Wahaia *et al.* [12] have applied THz-TDS to distinguish between normal and cancerous regions in dried and paraffin-embedded colonic tumor samples, respectively.

Although plenty of works have been performed, proving that THz technology is a useful method for the identification of normal and tumor tissues, some cancers are still difficult to be efficiently diagnosed (e.g. renal cancer) due to the absorption of THz signal by free form water. This article has presented the use of ultrafast
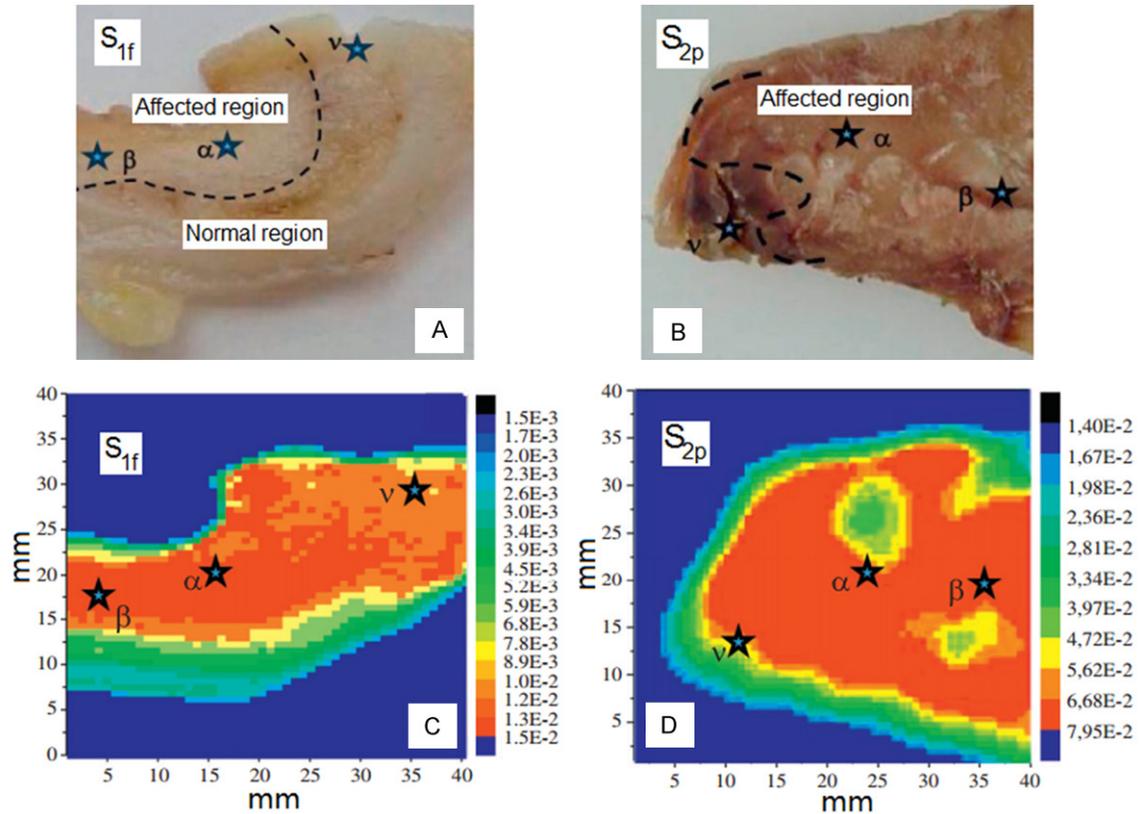
Figure 2. Samples that have been imaged by CWTI at the wavelength λ = 393 μm: (A and B); CWTI absorption coefficient images of the samples: (C and D). The reddish areas, corresponding to stronger absorption, correlate well with the cancer affect regions previously determined by histological observation. The green-yellow spots in red area of (D) have smaller thickness caused by paraffin embedding process [12].

THz pulsed technology aimed at investigating some cancer tissues. In order to remove the influence of the sample thickness, the diagnosis of colon carcinoma and gastric cancer were carried out by using mucus instead of tissues themselves. Furthermore, renal cancer has also been tested by using dehydrated tissues instead of fresh tissues, which can cancel the influence of the free form water. Finally, this work has analyzed the advantages and shortages, and makes the outlook of disease diagnose by means of THz technologies.

THz pulsed spectroscopy and performance

Since established in the early 1990s, THz-TDS uses short pulses of broadband THz radiation, and the transmitted THz electric field is measured coherently. Thus, THz-TDS could provide THz broadband spectroscopic information both on amplitude and phase with high sensitivity.

The experimental setup used in this work is a conventional THz-TDS system, as shown in Fi-

gure 1, which is similar to the previous work reported by Du *et al.* [16]. A mode-locked Ti: sapphire laser was applied with the central wavelength at 800 nm, FWHM of spectral bandwidth at ~20 meV, pulse duration around 80 fs, repetition frequency at 76 MHz and output power around 1.3 W. The laser pulse was split into pump beam and probe beam by a beam splitter. Pump beam, which was modulated by optical chopper, was focused on Gallium Arsenide (GaAs) crystal. The photo-excited electrons were accelerated by the applied electrical field, generating THz wave emission from the GaAs crystal [17, 18]. The THz pulse was focused on sample by off-axis parabolic mirrors. After passing the sample, the THz wave with the information of sample was focused on Zinc Telluride electro-optic crystal, together with probe beam. The free space EO sampling technique was used to record temporal waveforms of THz electric fields transmitted from the samples [19, 20]. The EO sensor used in this experiment was a 700-μm-thick <110>-ori-
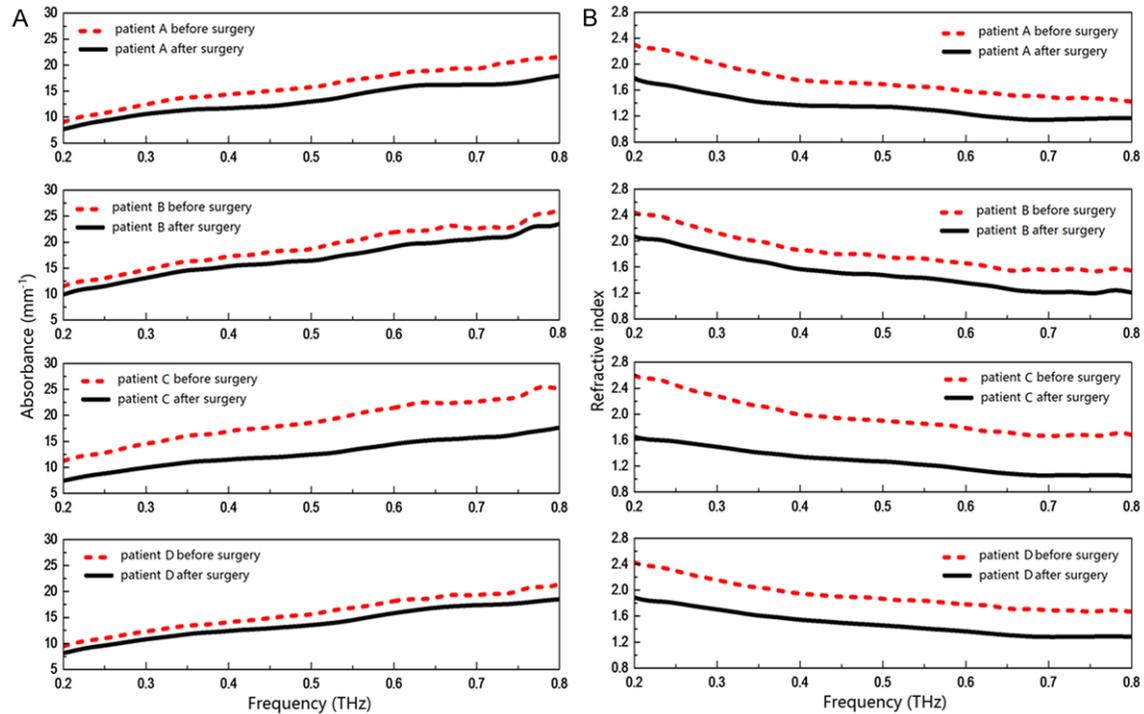
Figure 3. A. THz absorbance of surface mucus. B. THz refractive index of surface mucus.

ented ZnTe crystal. The high cutoff frequency (~3 THz) was determined by the thickness of the EO crystal and the pulse duration of the femtosecond laser [21].

The THz spectroscopy system was enclosed in dry-nitrogen-purged boxes to diminish the absorption of far infra-red (FIR) wave due to vibration of the water vapor molecule. The humidity of covered box was kept less than 5% and temperature was remained at about 295 K.

The time-domain signal is continuously obtained with the stepmotor's move, and then the spectrum was calculated via Fourier transformation. The resulting signal-to-noise ratio is 8 orders of magnitude at the peak frequency.

Measurements on cancer

THz pulsed spectroscopy has been demonstrated to play a significant role of pre-diagnose. To be noticed, the thicknesses of the samples should be controlled more or less the same, which are usually 0.2~0.3 mm. If the sample is too thin, the THz wave cannot interact with the sample sufficiently. On the other hand, if the thickness of the sample is too large,

the THz wave is absorbed by the sample, and eventually the signal-to-noise ratio is too low for the purpose of diagnosis.

Colon carcinoma

Colon carcinoma is the second most common cause of death among cancers [22]. Microscopic analysis of human colon-cancer specimens with immunohistochemistry method by using haematoxylin-eosin staining can exactly identify colorectal cancer tissues and normal (or inflammatory) tissues, but the histological and cytological methods need a lot of time to prepare the specimens.

The novel THz pulsed spectroscopy technology has been demonstrated to help the diagnosis. Wahaia *et al*. [12] distinguished the benign colonic tumor tissues and the malignant colonic tumor tissues by using continuous wave THz imaging system to acquire 2-D transmission THz image. They dealt the tissues with paraffin embedding process as well as the formalin-fixed process. The images of paraffin embedded results are shown in **Figure 2**.

Since the water-bound states of the benign and malignant samples are quite different, and the
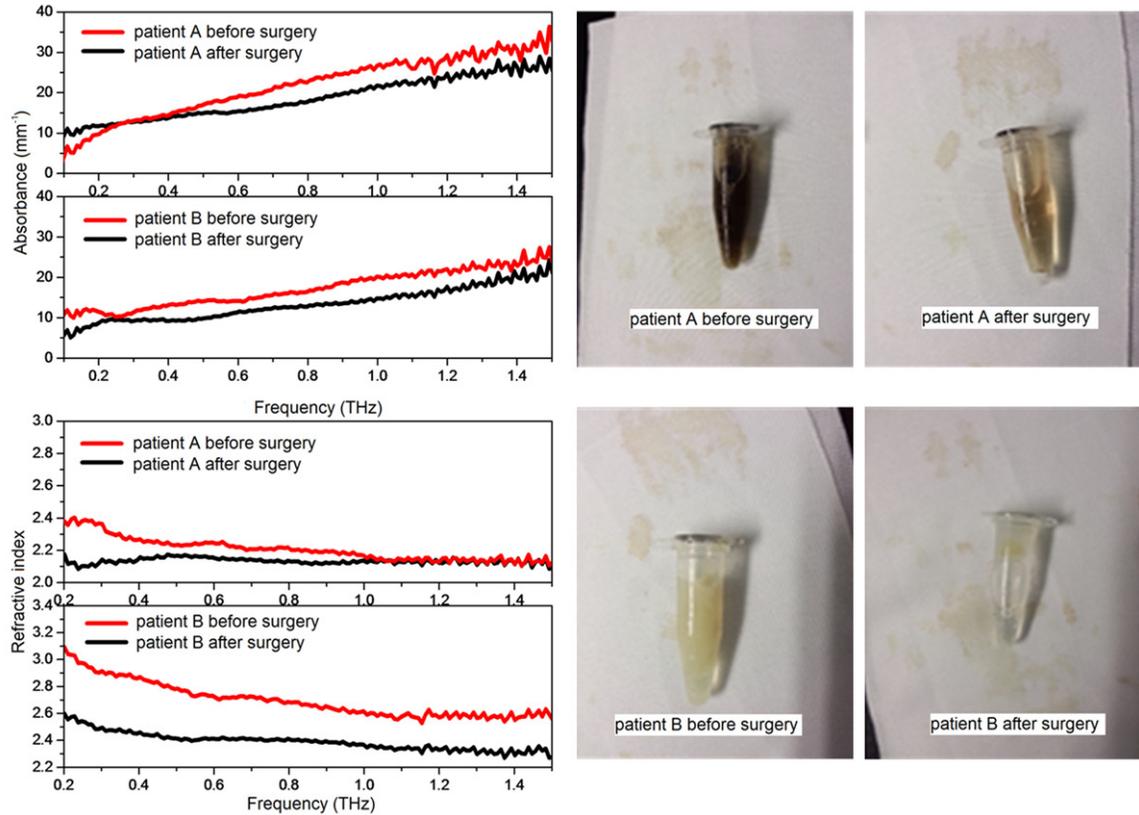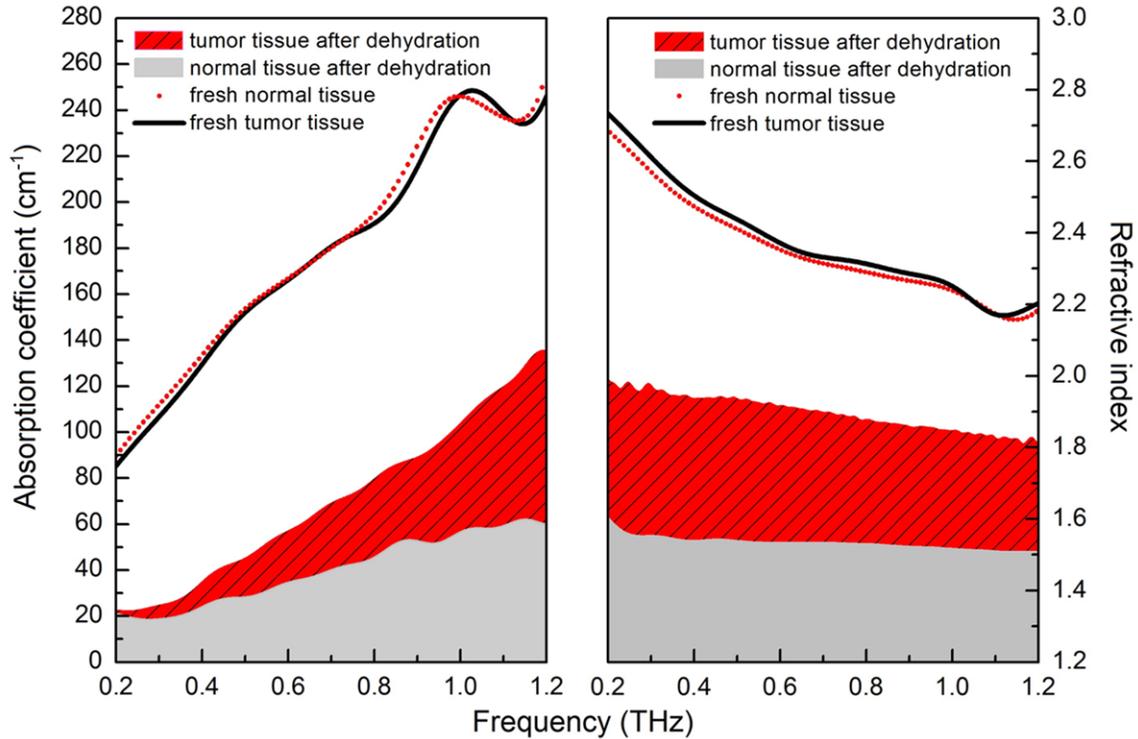
Figure 4. THz absorbance and refractive index of gastric juice after centrifuging and extracting.

biological tissue responses in THz frequencies are strongly sensitive to the presence of free and bounded water [23], the malignant region as showed in **Figure 2** can be well distinguished. However, the results of **Figure 2** might not be very precise, because besides waterbound, there are other important variables which could have influence on the signals when using THz or other FIR devices, e.g. the thickness of sample and the sample processing method.

Solid state tissues are relatively hard to be cut accurately. Thus the thicknesses of samples are difficult to be controlled. On the other hand, the thickness of the sample directly relates to the THz absorption spectrum. In order to solve this problem, here we present a practical way to detect the surface intestinal mucus by using THz-TDS. The samples of intestinal mucus are from four patients acquired before (marked as red dashed lines in **Figure 3**) and after (marked as black solid lines in **Figure 3**) surgeries, which are supplied by Huashan Hosptical, Shanghai, China. Surface mucus (liquid state) is unlike

tumor tissue (solid state), and the thickness of the sample is more controllable by using sample holder with two polytetrafluoroethylene films. When mucus is fully filled in the holder, the thickness can be controlled as 0.3 mm and it can be kept the same for each sample. The absorbance and the refractive index of samples are shown in **Figure 3**.

The results show that absorption coefficients and refractive indices of cancerous samples are generally higher than that of normal samples. According to previous reports, water content in tumor tissue is usually greater than that in normal tissue. Therefore, the differences of water content is the main factor accounting for the experimental results [24]. To be precise, OH bonds in water interact with each other and form a network structure. When THz wave irradiates the sample, the hydrogen bonds of water molecules experience stimulated resonance and the dipole orientation rotation, and finally form a new dynamic relaxation hydrogen bond network structure [25].

Figure 5. Absorption coefficient and refractive index of fresh tumor tissue, fresh tumor adjacent normal tissue, tumor tissue after dehydration, and tumor adjacent normal tissue after dehydration, respectively.

Gastric cancer

Similar to colon cancer, the traditional identification method of gastric cancer is histology method, which is not only time consuming, but also difficult to estimate the fuzzy boundaries. Since the cancerous tissues are different from normal tissues in terms of water content and cell structure, and water exhibits a high absorption coefficient in THz region as shown in the text above. Therefore, here, the simply obtained gastric juice is used to replace the gastric tissue.

THz-TDS has also been used to detect the gastric juice from gastric cancer patients. The gastric juices were acquired before (marked as red line in **Figure 4**) and after (marked as black line in **Figure 4**) surgeries from the same patient. All samples were operated in a certain degree of centrifugation and the liquid supernatant was extracted. **Figure 4** shows the THz absorbance and refractive indices of the samples. After the experiment, we found that the color of liquid supernatant before surgery is darker than the samples after surgery. And the variation trend of THz absorbance and refractive index follows

the color change. This result shows that THz technology is a practical method to help diagnose gastric cancer.

Renal cell carcinoma

THz pulsed spectroscopy technique is also used for the investigation of human renal cancer by comparing the response of tumor tissue and tumor adjacent normal tissue. In this section, the experimental system is still THz-TDS as shown in **Figure 1**.

As shown in **Figure 5**, the absorption coefficient of fresh renal tumor tissue is almost the same as tumor adjacent normal tissue, because the water content in fresh renal tissue is rather high, and water molecule network formed by hydrogen bonding induces a strong absorbance in 0.2~1.2 THz region. The evidence is that the absorption of fresh tissue is approaching the pure water absorption in THz region. The similar phenomenon happens in refractive coefficients. Therefore, in order to avoid the influence of free form water in renal tissue, the tissue sample needs a dehydration progress to remove the free form water.
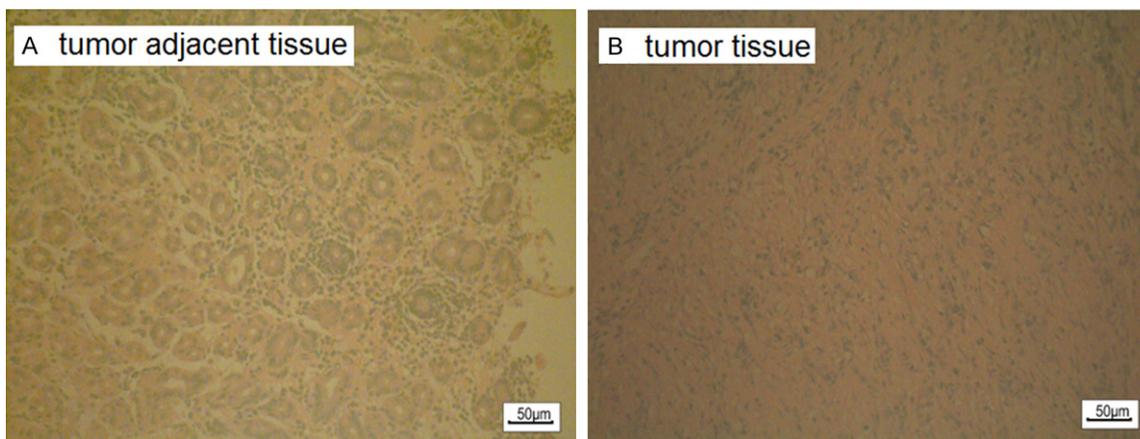
Figure 6. Pathology of stained tumor adjacent tissue (A) and tumor tissue (B) sections.

Comparing the binding water and the free form water within the cell, the binding water is more difficult to be removed during the dehydration process. So even after precise dehydration progress, we can just remove the free form water rather than the binding water. The shaded red and grey areas respectively represent for tumor tissue and normal tissue after dehydration. Obviously the THz absorption coefficient and refractive index are higher in tumor tissue than that of normal tissue.

Furthermore, because of the differential histological and cytological characteristics, pathologists can distinguish normal human tissues from tumor tissues by microscope, which is an accuracy standard in modern medicine system. **Figure 6** is the histology photomicrograph of the renal sample. Tumor cells arrange in groups, without or with only a small amount of adenoid structure. Furthermore, one can also find in tumor cells that nucleuses are large and nucleoli are prominent. Lymphoid cell infiltration exists. These differences in composition and structure affect the THz refractive index, absorption and scattering. As a conclusion, after removing the free form water, the tumor tissue and tumor adjacent tissue can be distinguished clearly by using THz technology due to the differences of their structure and composition.

Conclusion

Several cancers, namely colon carcinoma, gastric cancer and renal cancer, are diagnosed by using THz technology after carefully choosing and preparing the states of the samples in this paper. Tumor cells, such as colon carcinoma

and gastric cancer in form of mucus instead of tissue, can be directly distinguished because of the difference of the content of free form water, after carefully controlling the thickness of the sample. Some kinds of the tumor cells need an additional dehydration process to observe the difference of the composition and structure. Because of its advantages on the interaction with molecular network (such as hydrogen bonds), THz spectroscopy has been demonstrated to be an important technology and has a broad prospect of applications in biological tissue investigation and clinical medicine.

Disclosure of conflict of interest

None.

Address correspondence to: Ping Dong, Department of Nephrology, The First Rehabilitation Hospital of Shanghai, Shanghai 200090, China. E-mail: dongping809@163.com; Fubo Tian, Department of Anesthesiology, Shanghai Obstetrics and Gynecology Hospital, Fudan University, Shanghai 200090, China. E-mail: cedartian@126.com

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