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Abstract. A terahertz (THz) spectroscopic study is carried out to analyze DNA mutations in a label-free manner. Three newly designed liquid sample cells are considered and the best is selected as the sample carrier for THz transmission spectroscopic analyses. Discrimination based on spectral signatures of single-base mutations on single-stranded 20 nt oligonucleotides has been shown possible experimentally. The results clearly attest the ability of this promising approach for label-free analyses of single-base mutations of DNA molecules. This study has demonstrated that the THz spectroscopic technology can be considered as a potential diagnostic tool for investigating molecular reactions, such as DNA mutations. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.20.9.095009]

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1 Introduction

In recent years, genetic diagnostics has witnessed a dramatic, in some cases even a disruptive, increase in capability with the development of DNA biosensors, gene chips, and lab-on-a-chip DNA analyzers.¹ A majority of the approaches for identifying polynucleotide sequences are based on detecting the binding (i.e., hybridization) of unknown “target” DNA molecules to single-stranded (ss) oligo- or polynucleotide “probe” DNA molecules having a known base sequence, as hybridization occurs preferentially between molecules with complementary base sequences. Currently, hybridization detection is largely based on fluorescent labeling and identification of target DNA molecules. Although fluorescent labeling has evolved as an extremely efficient and high throughput method, and is implemented in many innovative devices such as gene chips,² it does have many drawbacks. Labeling is an unwanted step in sample preparation: it complicates the analytical procedure by possibly modifying DNA strand conformation, whereby it adversely affects the precision of gene detection.³ Furthermore, the yield of a labeling process is variable, with the labels being degradable and the fluorescence efficiency subject to site dependency.^{4,5}

Detecting base mutations of unknown DNA molecules is critical for many biomedical applications, as it is important for various analyses, especially those routinely employed in cancer research.⁶ Interest in label-free genetic diagnostic techniques have increased in recent years as a result. Terahertz (THz) spectroscopy employs radiations that lie between the microwave and infrared regions of the electromagnetic spectrum with an approximate frequency range between 0.1 and 10 THz.

THz radiation is known to be mild to biological systems.⁷ Many of the rotational and vibrational excitations of molecules, radicals and ions, as well as localized vibrations and conformational changes of DNA molecules involve optical transitions within the THz range, readily providing information that is generally absent in optical, x-ray, and nuclear magnetic resonance spectroscopic techniques. This is quite fortuitous, as the conformational information closely related to biological functions of biomolecules in tissues and cells is difficult to access with other techniques.⁸

While mostly limited to frequencies <2 THz, investigations to date have confirmed the previously postulated broad THz absorption of a number of proteins,^{9–12} nucleic acids (DNA and RNA),^{9,13,14} and whole tissues.^{15–18} Due to the strong absorption of water in the entire THz frequency range, many studies of biomolecules using THz waves have so far been limited to dehydrated powder samples,¹⁹ for example, in the form of pressed pellets of polyethylene-lyophilized biomolecular mixtures, including amino acids,²⁰ nucleic acids,⁹ and proteins,¹⁰ or dry DNA spots on a thin film microstripline resonator.²¹

In this report, we describe our recent successful attempt at label-free detection of single-base mutations on DNA molecules in aqueous solutions using THz time-domain spectroscopy (TDS). In our samples, the oligonucleotides are linear, ss, 20-mer polynucleotide, and are diluted in a buffer solution to have a concentration of 100 $\mu\text{mol/mL}$. To our knowledge, THz spectral detection of the oligonucleotides with single-base mutations in aqueous solution has not been reported to date. In so far as such single-base mutation is potentially significant in both diagnostics and therapeutics, for example, the missense mutations at codons 12 and 13 of the human homolog of the Kirsten rat sarcoma-2 virus oncogene have been confirmed as

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a predictor of nonresponse to epidermal growth factor receptor-targeted therapy with monoclonal antibodies cetuximab and panitumumab in patients with metastatic colorectal carcinoma,^{22–24} a spectroscopic means of clear identification of such anomalies is obviously useful. The remainder of this report is organized as follows. We first briefly describe the selected liquid sample cells, followed by a description of the use of a pulsed THz spectrometer to investigate the oligonucleotides in aqueous solution from 0.2 to 2.6 THz at room temperature. Summary and conclusions are presented in Sec. 4.

2 Materials and Methods

2.1 Sample Preparation

2.1.1 Design and synthesis of oligonucleotides

Each oligonucleotide had the same length of 20 nt, in which there is only one single different base (see Table 1). The oligonucleotides are synthesized and purified by high-performance liquid chromatography at Sangon Biotech Co., Ltd. (Shanghai, China). Each oligonucleotide is dissolved in a LoTE buffer, pH 8.0 (3 mM Tris-HCl, pH 8.0; 0.2 mM ethylene diamine tetraacetic acid, pH 8.0) at 100 μ M. Although the oligonucleotides we studied are actually not from DNA isolated from cancer patients or even from the simplest form of life (e.g., virus or bacteriophage), they targeted the single-base mutations at the first base of codon 13 and have significance as model biomolecules for THz detection.

Table 1 Sequences of oligonucleotides investigated in this study.

Oligo ID	Sequences (5' > 3') ^a
HQ-411	TGGAGCTGGTGGCGTAGGCA
HQ-418	TGGAGCTGGTCGCGTAGGCA
HQ-419	TGGAGCTGGT <i>T</i> GCGTAGGCA
HQ-420	TGGAGCTGGT <i>A</i> GCGTAGGCA

^aThe italic letters indicate the different bases.

2.1.2 Design of the liquid sample cell

Aqueous solution of these samples (about 7 μ L) is injected into the liquid sample cells. The cell has a diameter of 9 mm, thickness of 0.12 mm, and two slide windows sandwiching a ring-shaped Secure-Seal spacer. The cells are prepared for a LoTE buffer and the four oligonucleotides with single-base mutations (HQ-411, HQ-418, HQ-419, and HQ-420). The same liquid sample cell with fixed thickness and diameter is used for each sample and is disposed after use.

2.2 Terahertz Spectroscopy Measurement

THz transmission spectra are obtained using a standard setup of THz-TDS, as shown in Fig. 1. At a repetition rate of 82 MHz, the diode-pumped mode-locked Ti-sapphire laser (MaiTai, Spectra Physics) provided the femtosecond pulses with duration of <100 fs and a central wavelength of 800 nm. An InAs emitter is used as the excitation source of THz radiation. The THz field is detected based on electro-optic sampling with a ZnTe crystal. The system resolution is (at the output of the balanced detector) 50 pA, and the maximum signal response range is 160 nA.

The experimental measurements are conducted at an ambient temperature of 20.4°C, a relative humidity of 3.9%, with the purge of nitrogen gas. THz time-domain waveforms of our samples and the reference signal without samples are measured under the same experimental conditions. The frequency-domain spectra of measured signals are obtained by fast Fourier transform. The effective frequency region is 0.2 to 2.6 THz with this THz-TDS setup. Spectral frequency resolution of the spectrometer is 30 GHz. Triplicates are run per sample.

3 Results and Discussion

3.1 Cell Window Materials of Liquid Sample

Three different materials, glass (K and Na silicates), plastic (polyvinyl chloride), and silica (SiO₂), are used for windows of the sample cells for THz transmission spectral measurements. Figure 2 shows the THz transmission of liquid sample cells with windows made from these materials. Plastics seem best suited as a window material for cells containing aqueous solutions. Plastics are, due to its generally low absorbance in the

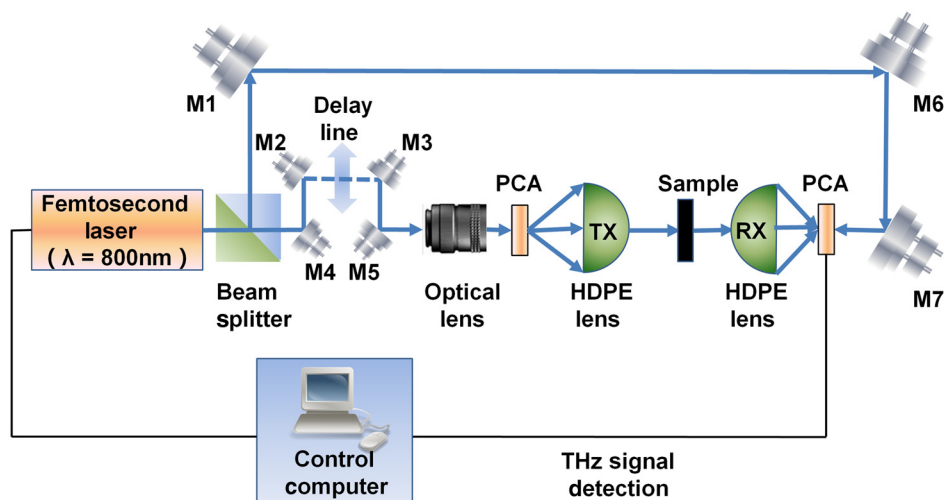


Fig. 1 Schematic of the terahertz (THz) time-domain spectroscopy experimental setup.

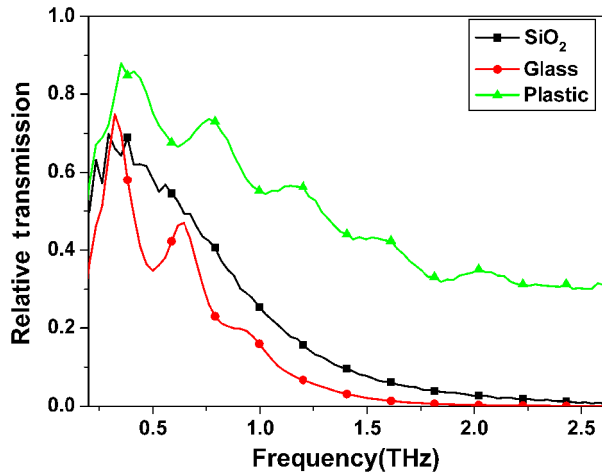


Fig. 2 THz transmission spectra of liquid sample cell windows made from different materials relative to the transmission of air.

THz region, often considered to be practically transparent in the THz-frequency domain, primarily attributable to its nonpolar nature and amorphous material structure.

3.2 Oligonucleotides in Solution

Figure 3 shows THz time-domain spectra of LoTE buffer solution and 20 nt oligonucleotides with only single-base mutations (HQ-411, HQ-418, HQ-419, and HQ-420). Figure 4(a) shows the transmission as a function of frequency obtained by fast Fourier analysis of the time-domain spectra shown in Fig. 3. The transmission spectra showed mean values of three independent experiments of LoTE buffer and four oligonucleotides. Figure 4(b) illustrates the transmission values at 586 GHz, 878 GHz, and the transmission ratio (878 GHz/586 GHz). Values represent mean \pm SD (bar) of three samples. The transmission at 878 and 586 GHz was significantly different between LoTE buffer solution and the oligonucleotides. There are obviously different transmission ratios for the four oligonucleotides with only single-base mutations.

Figure 5 shows the absorption coefficient of the LoTE buffer solution obtained by subtracting the contribution from the plastic window cell. It can be seen from this figure that the

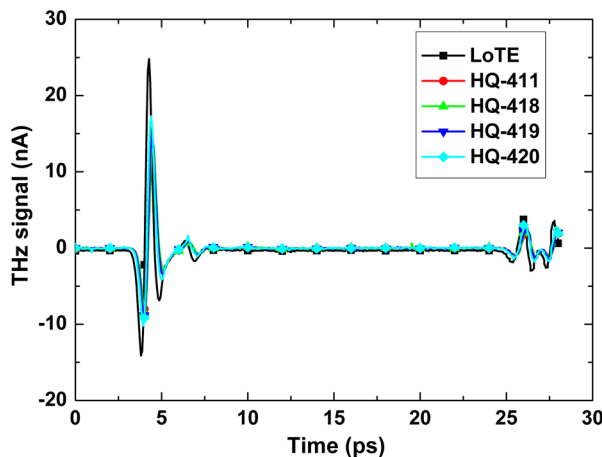


Fig. 3 THz time-domain spectra of LoTE buffer solution and oligonucleotide samples.

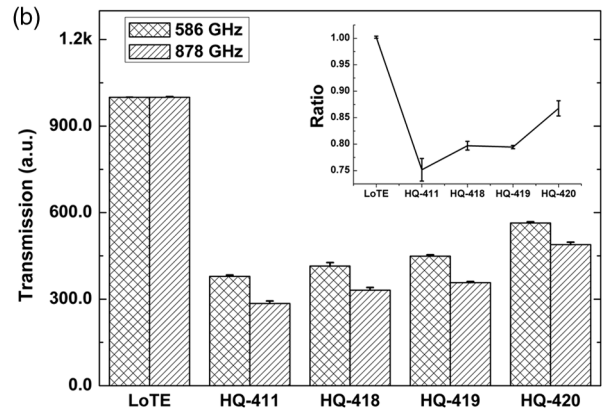
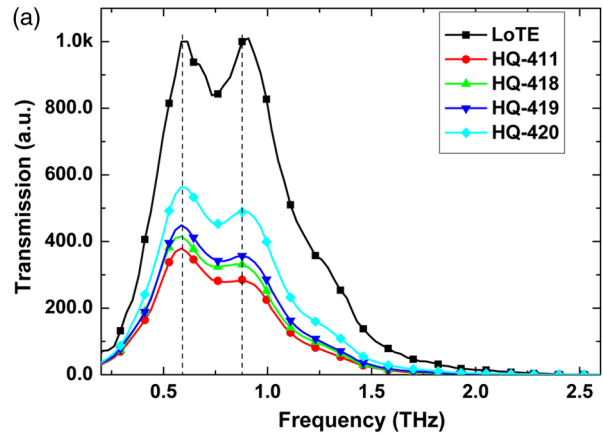


Fig. 4 (a) THz transmissions of LoTE buffer solution and oligonucleotide samples. The two vertical dashed lines mark the peak transmission positions at 586 and 878 GHz. (b) THz transmission at 586 GHz, 878 GHz, and the corresponding transmission ratio (878 GHz/586 GHz) (inset). Error bars of (b) are standard deviation from the mean values.

absorption spectrum of the LoTE buffer solution closely follows that of water in this frequency range.

Figure 6 shows the oligonucleotides transmission spectra obtained by subtracting the LoTE buffer solution. According to the Beer-Lambert's law, the absorption coefficient of the oligonucleotide sample after subtracting the contribution from

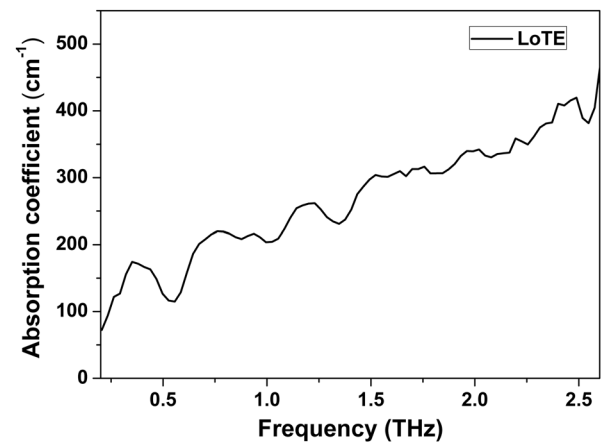


Fig. 5 Absorption coefficient of LoTE buffer solution after subtraction of the contribution from the sample cell windows. It closely follows the absorption spectrum of water in this frequency range.

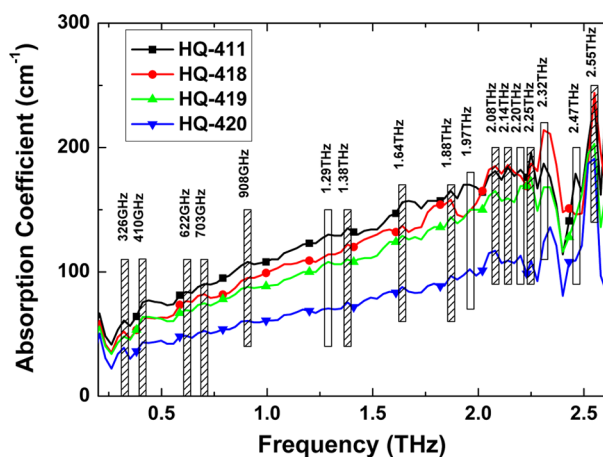


Fig. 6 THz absorption spectra of oligonucleotide samples. Blank and patterned bars with a width of 30 GHz (spectral resolution) are used to indicate the same and different absorption peaks of the four oligonucleotide samples, respectively.

the buffer solution was calculated as $\Delta\alpha = \ln(I_{\text{buff}}/I_s)/d$, where I_{buff} and I_s are power transmissions of buffer solution and the oligonucleotide solution, respectively, and d is the thickness of the plastic window cell ($d = 0.12$ mm). The absorption peaks of the oligonucleotides are significantly different from the ones of the LoTE buffer solution (compared to Fig. 5). Using the frequency resolution of the THz-TDS spectrometer as a standard, similarities and differences of absorption peaks were identified between the four samples. All four oligonucleotide samples share the same 12 peaks (326 GHz, 410 GHz, 622 GHz, 703 GHz, 908 GHz, 1.38 THz, 1.64 THz, 1.88 THz, 2.08 THz, 2.14 THz, 2.25 THz, and 2.55 THz). Some of the characteristic features are close with those reported previously. For example, an absorption signature <720 GHz is due to a local hydrogen-bond, cross-linking between ssDNAs in solution whereby two ssDNA strands are bonded together or a ss self-bonds by looping. It is invariably observed in the THz spectra of ssDNA.²⁵ Five different peaks (1.29 THz, 1.97 THz, 2.20 THz, 2.32 THz, and 2.47 THz) can be identified between each of the four oligonucleotides, where the peaks were shifted in frequency compared with one another, allowing for comparison.

Although observed absorption peaks are clearly distinguishable for the four oligonucleotides, the origin of these peaks needs to be further investigated for conclusive inference. It is speculated that the different peaks are indicative of different bases between the oligonucleotide constituents. The stronger absorption peak at low frequency of the oligonucleotide samples is most likely originated from the water content of the sample under ambient conditions. However, further investigation is necessary to validate the foregoing hypothesis.

4 Conclusions

In this paper, identification of oligonucleotides with only single-base mutations has been demonstrated via THz-TDS. Based on our results and analysis, the following conclusions can be drawn: (1) plastics are suitable as a window material for aqueous solutions due to their generally low and featureless absorbance in the THz region. However, due to the strong absorption of water in the THz-frequency domain and the relatively large volume of the liquid sample cell in this experiment, the effect of

water absorption cannot be completely isolated in our experiment. It is perhaps advantageous to consider the design of a plastic sample cell based on a single or an array of micro- and/or nanolevel fluidic channel(s) as a carrier device, in order to enhance the information-bearing THz signal of aqueous solutions. Undertakings along this line of thought are currently being pursued in our laboratory and will be reported elsewhere. (2) It is found that different transmission ratios for the LoTE buffer solution and the four oligonucleotides with only single-base mutations (HQ-411, HQ-418, HQ-419, and HQ-420) can be detected. The transmission of the LoTE buffer solution is significantly higher than those of all the oligonucleotides. The THz absorption spectra of the oligonucleotides are much richer than that of the LoTE buffer solution, because the rotational, vibrational, and conformational changes of the oligonucleotides are known to involve optical transitions in the frequency regime considered here. (3) It is found that the four oligonucleotides exhibit distinguishably different absorption behaviors over a frequency range of 0.2 to 2.6 THz.

As can be seen from the above results, single-base mutation can cause a readily observable change in the relative absorption peaks. Therefore, single-base mutation has resulted in the alteration of the charge distribution and frequency response of the DNA polymers associated with, for example, the conformational change of the oligonucleotide. It is reasonable to conjecture that the conformational change of the oligonucleotide may be significantly present only in aqueous solutions, albeit that the attendant frequency response might be damped and frequency-shifted due to the attachment of water molecules. This unique advantage contributed to the positive outcome of the experiments, namely the oligonucleotides with only single-base mutation in an aqueous solution is distinguished using THz-TDS. The transmission and the transmission ratios of the oligonucleotides with the single different base being A and T are higher than the oligonucleotides with the single different base being G and C. It may be related with the structure of complementary base pairing (A-T, G-C) and its resulting charge redistribution. The capability demonstrated in our experiments, that is, the label-free, clear identification of single-base mutation in DNA molecules using THz spectroscopy can be used as a useful tool for diagnostics or for studying molecular interactions such as mutation.

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