Light-induced difference terahertz spectroscopy and its biomedical applications

Y. C. Shen, P. Upadhya, A. G. Davies, and E. H. Linfield

Abstract–**Laser-induced difference THz spectroscopy has been used to investigate three samples with different lifetimes. The spectroscopy system is based on a 10 nJ titanium sapphire laser with a pulse duration of 12 fs and a centre wavelength of 790nm. For semi-insulting (SI) GaAs and high-resistivity (HR) silicon samples, absorption in the THz range is mainly a result of mobile electrons. A lifetime of about 50 ps has been determined for the SI-GaAs sample, whereas the lifetime of the HR-Si samples was found to be much larger than the time interval between two successive laser pulses (12 ns). As a result, the differential THz signal is about twenty times larger than that for SI-GaAs. We also observed that the THz pulse arrives at the detector 100 fs earlier when it transmitted through an optically excited HR-Si wafer. For copper pathancyonine (CuPc) pellet samples, the excited state remains for at least 1ms. The absorption peak at 1.08 THz changes significantly under 790 nm laser excitation, suggesting that we have observed the first evidence of light-induced vibrational mode changes, in the THz range.**

Index Terms–difference spectroscopy, time-domain, terahertz spectroscopy

I. INTRODUCTION

Over the past few years, there has been much interest in developing the technique of difference spectroscopy for the investigation of photo-biological systems. Examples of such systems include bacteriorhodopsin and rhodopsin, as well as the study of photosynthetic reaction centers in bacteria and plants [1-6]. The principle behind difference spectroscopy consists of recording infrared spectra (of, for example, a protein) in two different states, before and after applying an external perturbation such as light. The difference is then calculated, and only vibrational modes that change in intensity or frequency are identified in the difference spectrum. Signals that do not originate from groups affected by the perturbation are subtracted out. This technique is particularly effective in probing minute structural differences between two states.

Light-induced difference spectroscopy (based on Fourier transform infrared (FTIR) technology) has been developed and applied successfully to investigate the structural changes associated with individual bonds of chromophores and proteins[7]. The detection sensitivity (defined as $\Delta T/T$, where T is the transmission coefficient) achieved in the mid-infrared frequency range is $10^{-5} - 10^{-6}$, at fixed delay and frequency, although greater sensitivity is required to apply difference spectroscopy to other important photo-biological systems. In the far-infrared, however, the poor performance of FTIR spectrometers, owing to the lack of suitable sources and detectors, makes it impractical to develop a FTIR difference spectroscopy system.

Recent advances in terahertz (THz) time-domain spectroscopy [9,10] have, though, stimulated interest in developing light-induced THz difference spectroscopy. Key benefits include the acquisition of time-resolved data and coherent detection. These give the amplitude and phase of the THz field, rather than simply the laser intensity. The dynamic range of coherent THz detection has been reported to be $10^5 - 10^6$, corresponding to an intensity range of $10^{10} - 10^{12}$. Such high dynamic range together with the intrinsic advantage of time resolved coherent detection make THz time-domain system attractive for differential spectroscopy.

There have been reports of differential THz time-domain spectroscopy for the characterization of thin dielectric films [10,11]. In these approaches, a mechanical shaker was used to exchange rapidly the sample of interest and a reference sample at a frequency of 20 – 100 Hz. The small difference between the THz pulses transmitted through sample and reference was monitored with a lock-in amplifier. Extremely high sensitivity, of the order $\Delta T/T \approx 10^{-9}$, was reported. In this paper, instead of using a mechanical shaker to change the sample position, we use a laser pulse to excite and change the state of the sample.

II. EXPERIMENT

The experimental arrangement for light-induced differential THz time-domain spectroscopy is similar to that for visible-pump-THz-probe experiments [12,13]. A Ti:sapphire laser (Femtolasers Produckions) provides visible/near-infrared pulses of 12 fs duration at a centre wavelength of 790nm. The output is split into three parts: a 250 mW beam is used to excite the sample with a focus diameter of 300μm at a variable time delay with respect to the THz pulse; a 250 mW beam is focused onto the surface of a biased SI-GaAs emitter for THz generation; and the remaining 25mW serves as the probe beam for electro-optic detection using a 1-mm-thick ZnTe crystal.

The laser energy used to excite the samples here is only a few nJ, rather than the few μJ used in most pump-probe experiments [14,15]. Low energy pulses are less likely to damage the samples under investigation, which is of a particular concern for some biomedical samples.

The light-induced THz time-domain difference spectrometer can be operated in two ways. The first, and simplest, approach is to use the THz spectrum of the sample in its ground state (without laser excitation) as the reference, and compare this with the spectrum of the sample under laser excitation. The

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latter can be achieved by electrically chopping the THz beam whilst maintaining constant pump laser excitation. The difference THz spectrum is then calculated, and only vibrational modes that change in intensity or frequency are detected in the difference spectrum. Signals not originating from groups affected by the laser excitation are subtracted out.

In the second approach, if the photogenerated process under investigation is fast and highly reproducible, the difference THz time-domain spectrum is measured directly, with a much higher sensitivity. In this case, the pump beam exciting the sample is chopped by a mechanical chopper whilst the THz beam is kept constant. The idea is to monitor the small THz transmission difference between the two sample states by alternately measuring the THz transmission through the excited and unexcited sample, and monitoring the difference signal with a lock-in amplifier. Owing to the intrinsic advantage of the coherent THz generation and detection, detection levels of the order $\Delta T/T \approx 10^{-8}$ can be demonstrated, which is already 2 – 3 orders of magnitude better than the performance of state-of-the-art FTIR spectroscopy system.

Copper pathancyonine (CuPc) powder was obtained from Sigma-Aldrich (product #252980) and used without further purification. Pellet samples were prepared by pressing CuPc powder into pellets with a manual pellet maker. All measurements were performed at room temperature.

III. RESULTS AND DISCUSSION

A. *Semiconductor samples*

In order to evaluate the performance of the light-induced THz time-domain spectrometer, SI-GaAs and HR-silicon wafers were studied using the second approach discussed above. Fig. 1 (a) shows the measured THz signal without the pump laser pulse and the differential THz signal 5 ps after visible laser excitation for SI-GaAs. The amplitude of the differential THz signal is less than 2 % of the original THz signal because only a 2 nJ pulse was used for excitation, corresponding to an energy density of about 3 $\mu J/cm^2$. The peak amplitude of the differential THz pulse was monitored as a function of time after the visible laser excitation and is plotted in Fig. 1 (b). A 50 ps lifetime was calculated by fitting the experimental results.

The differential THz signal arrives at the detector about 100 fs later than the original THz signal, as shown in Fig.1 (a). This can be explained as follows. The generated THz pulse is collected and focused onto the sample surface by two parabolic mirrors. Owing to diffraction during the THz wave propagation, the lower frequency components of the THz pulse will focus to a larger spot size at the sample surface than the higher frequency components. The visible pump laser has a spot diameter of 300μm and only this pumped area of the sample will produce the differential THz signal. The spatial confinement of the differential THz pulse in the pump area thus acts as a spectral filter, shifting the frequency distribution of the transmitted THz pulse towards higher frequency. This reshapes the THz waveform and, owing to the normal

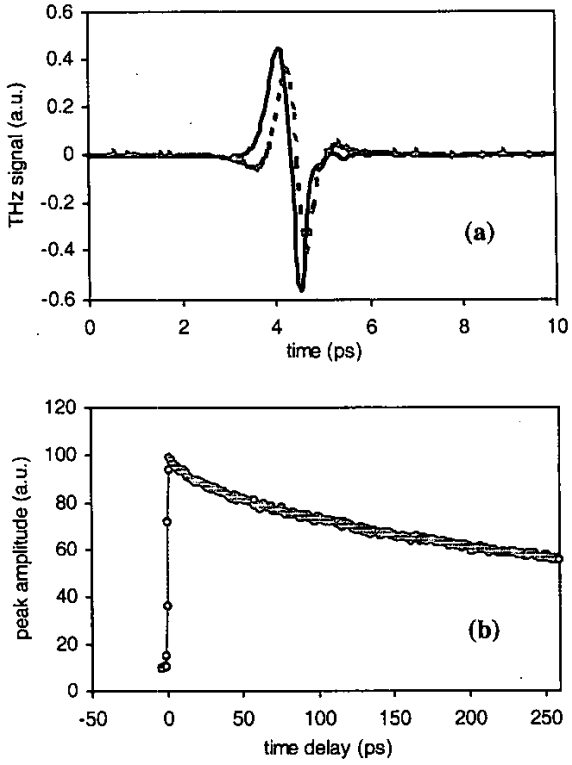

Fig. 1 (a) THz transient (solid line) and differential THz transient (dotted line) measured for SI-GaAs wafer. The differential signal was amplified by a factor of 50 for comparison. (b) The peak amplitude of the differential THz transient recorded as a function of time delay between the pump and probe pulses. For negative delays, the probe pulse is in advance of the pump pulse.

dispersion, slows the THz pulse down so that it arrives at a later time. The shape and peak position of the differential THz signal can be well simulated by applying a high-pass digital filter to the original THz signal, confirming that the effect of a spectral filter is cause to the later arrival of the differential THz pulse.

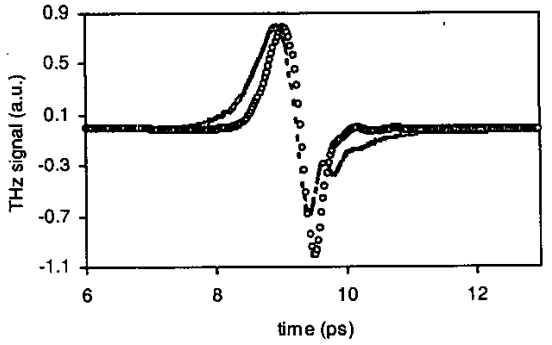

Fig. 2 THz transients transmitted through the unexcited (open circles) and excited (solid line, multiplied by 2.5) HR-Silicon wafers.

In contrast to our observations here, Schall *et al.* [12] observed the THz pulse to arrive earlier when transmitted through an optically excited SI-GaAs wafer. This is a result of the different experimental arrangement used. Schall et al measured the THz pulses transmitted through an unexcited and a continuously excited GaAs wafer. In this case, the frequency-dependent transmission and phase shift at the air–GaAs (excited) interface has a substantial contribution to the observed earlier arrival of the THz pulse. Indeed we also observe the earlier arrival of the THz pulse for excited HR-silicon wafers, as shown in Fig. 2. For a silicon wafer the lifetime of the photo-generated carriers is much longer than the time interval between two successive optical pulses (12 ns). A much larger differential THz signal was observed owing to the accumulation of photo-generated carriers in the silicon. Considering that HR-silicon is widely used in THz time-domain spectroscopy experiments, care must be taken to avoid the frequency-dependence of the transmission and phase shift caused by long-lived photo-generated carriers. Note that the effective spot size of the pump beam on the sample surface is now much larger owing to the diffusion of the photo-generated electrons.

In summary, for GaAs wafers, we directly measured the differential THz signal resulting from optical excitation. The differential signal is only a small fraction (1 – 2 %) of the original THz signal, therefore, the contribution from the frequency-dependent transmission and phase shift at the interface is much smaller for GaAs than HR-silicon. The main contribution to the differential THz signal is thus from the spectral filter owing to the spatial confinement of the THz pulse in the pump area.

B. Copper pathancyonine pellet

Pathancyonines are important dye molecules with excellent light harvesting capabilities, and their biomedical applications have been extensively investigated [16,17]. The molecular structure of copper pathancyonine (CuPc) is shown in Fig. 3. The optical absorption of CuPc peaks at 678 nm and overlaps with the spectrum of the pump laser pulse (centre wavelength 790 nm, bandwidth 100nm). Fig. 4 shows the THz transient

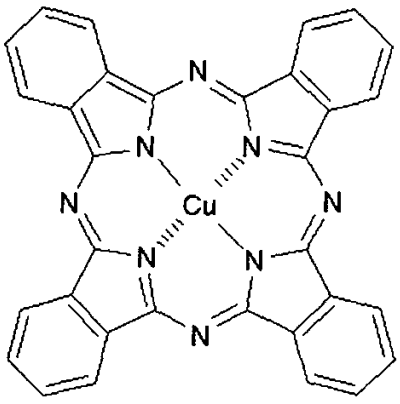

Fig. 3 Molecular structure of the copper pathancyonine dye molecule.

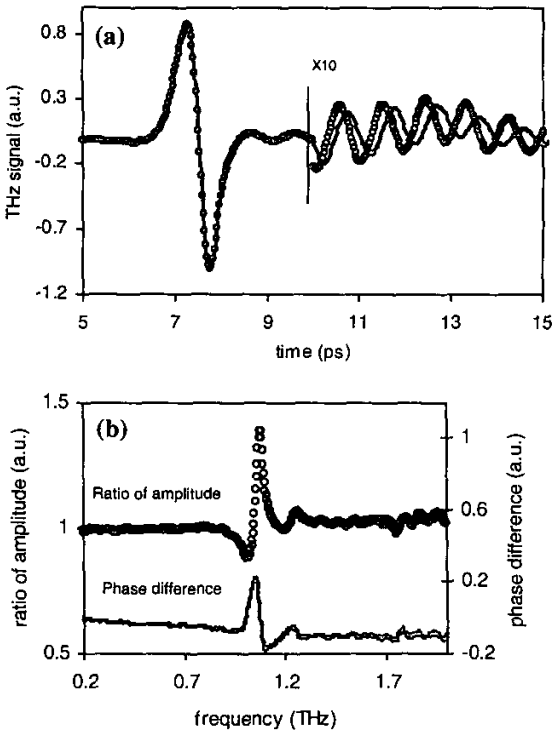

Fig. 4 (a) THz transients after transmission through CuPc pellet, measured with (line) and without (open circle) the pump laser beam. The signals after 10 ps were multiplied by a factor of 10. (b) Laser induced difference THz spectrum of a CuPc pellet calculated from the time domain signals of (a).

transmitted through a CuPc pellet measured in the presence and absence of visible laser excitation. The main THz pulses measured with and without laser excitation are almost identical, suggesting that most frequency components in the absorption spectrum do not change under laser excitation. However, the smaller amplitude ripples after the main pulse in the time domain are different, indicating that some absorption features are changed by the laser excitation. The Fourier transforms of the measured THz transients are calculated and the ratio of their amplitudes is plotted in Fig. 4, together with the relative phase difference. We observe features at 1.08 THz and 1.26 THz. To the best of our knowledge, this result represents the first evidence of a light-induced vibrational mode change in the THz frequency range.

We do not believe that the observed change is results from the mobile electrons, which is the main cause for the differential THz signal in semiconductors. Instead, the observed peak may be due to the change in the environment surrounding the vibrational modes. The energy associated with vibration modes in the THz frequency range is about 4 meV, corresponding to a temperature difference (kT) of 47 °C. Therefore a few degrees change in temperature is sufficient to cause substantial change in either the intensity or the frequency of the THz vibrational modes.

IV. CONCLUSIONS

Our results have significant implications for THz medical imaging. The resolution of a THz imaging system is ultimately limited by the wavelength of the THz wave and although near field optics can be used to obtain higher resolution images, this can not be applied to *in vivo* THz imaging beneath, for example, the surface of skin. As we demonstrated here, the effective spot size of a THz pulse can be spatially confined to the pump area of a sample, which is determined by the focused size of the visible pump laser beam. Therefore the resolution of a differential THz imaging system is ultimately limited by the spot size of the visible pump beam rather than the THz wavelength.

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