Terahertz Near-field Imaging of Biomolecular Nanostructures

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***Abstract*—We present the biomolecular imaging by using a terahertz pulse apertureless near-field microscope (THz NFM) with a nanometer resolution. THz NFM provides THz spectral imaging as well as topography of the nanostructured sample surface. By scanning the probe over a single biomolecular nanoparticle, the THz images and the scattering spectra were obtained.**

RECENTLY terahertz (THz) science and technology has been attracting a lot of attention for various applications such as THz imaging and characterization of materials since explored THz time-domain spectroscopy (THz TDS). By using THz TDS one can measure simultaneously the amplitude and the phase of the electric field, and thus THz TDS provides powerful means to probe the electronic and optical dynamics of both semiconductor and biomolecular structures[1-4].

Interestingly, biomolecules are expected to exhibit low frequency vibrational and torsional motions in THz range, which correspond to functionally relevant collective modes with periods on a picosecond timescale[5]. It is supported by theoretical molecular dynamics and normal mode calculations that predict the existence of collective modes in THz range[6]. It follows that the investigation of spectral features in THz region may lead to insights for our understanding of the molecular dynamics involved in biological functions and specificity in conformations[7]. Recently, several reports demonstrated low frequency spectral features of biomolecules in THz region, including nucleic acids, sugars, vitamins, amino acids, and even proteins[8-13].

In principle, altering the structure of biomolecules induces a significant change in their collective modes in THz region[5-7]. Moreover, it is turned out that low frequency collective modes can explain the conformational change occurring in protein when ligands bind to enzyme[14]. For example, upon calculating by density functional theory, as described in Fig. 1, it is found that there are distinct features of normal modes for 11-*cis* and all-*trans* retinal chromophores, which is embedded in a pocket of rhodopsin protein. This suggests that conformational change of biomolecules can be probed successfully by THz TDS. Conformational change from 11-*cis* to all-*trans* of retinal chromophore has been already investigated in THz range[15]. More recently, the conformational change in cytocrhome c and chlorophyll proteins due to denaturing has been examined[16-17].

Imaging and sensing techniques at THz frequencies have been applied extensively in chemistry, biology, medical science, security, and semiconductor electronics[18-19]. However, the spatial resolutions of conventional THz TDS imaging systems are still limited by diffraction. One of the promising techniques overcoming the diffraction limit is the near-field microscopy (NFM). Using a sub-wavelength aperture or apertureless probes, the NFM techniques have been studied widely in visible[20], infrared[21], microwave[22], and most recently THz spectral regimes[23-28]. Since the spatial resolution is determined by the size of the probe tip, the scattering-type or apertureless NFM with a metallic or dielectric probes has been shown to significantly improve resolution than the aperture-type NFM.

TDS-based THz NFM can provide ultrabroad-band spectral imaging in nanometer scale as well as topography of the sample surface. We previously reported a THz pulse apertureless near-field microscope (THz NFM), which combines THz TDS with atomic force microscopy (AFM) technique and an 80 nm lateral resolution for a metallic step edge with 35 nm height was reported[23]. In this work, we present our preliminary results on nano-scale THz imaging with scattering spectra of biomolecular nanoparticles.

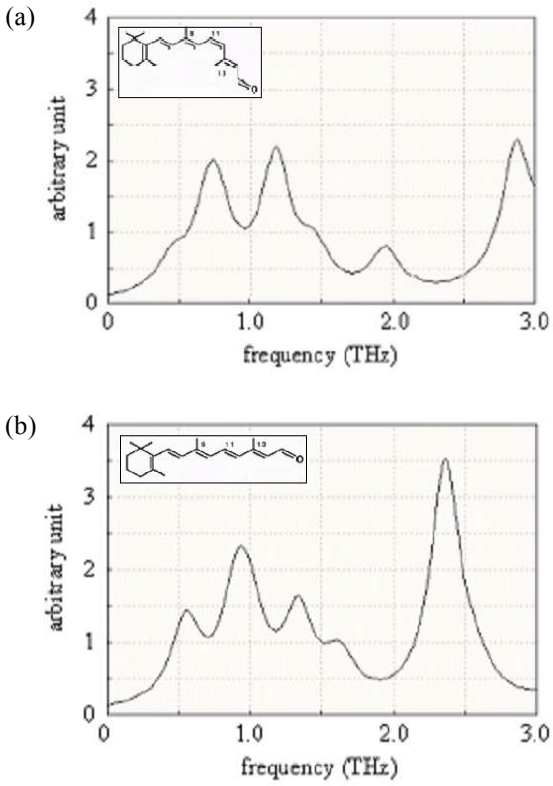

Fig. 1. Low frequency normal modes of (a) 11-*cis* and (b) all-*trans* retinal chromophores calculated by density functional theory.

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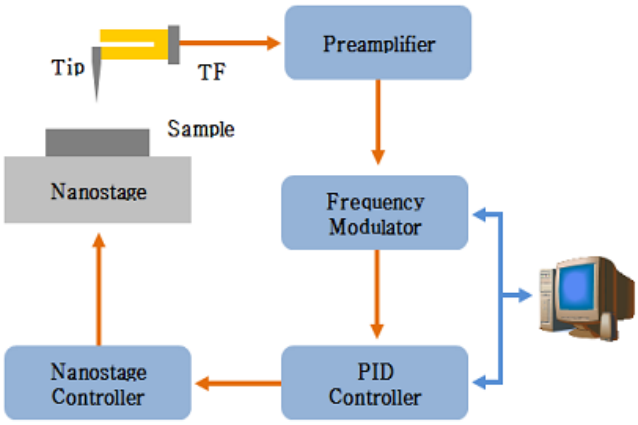

Fig. 2. The experimental setup of terahertz near-field microscope.

The sample was prepared by distributing lactose nanoparticles on the surface of a thick gold (Au) film evaporated on a semi-insulating gallium arsenide (SI-GaAs) substrate. The probe tip was fabricated by an electrochemical etching method. The radius of the fabricated probe tip was typically a few tens of nanometers. The probe tip was attached to a quartz crystal tuning fork so that the tip oscillates perpendicularly to the sample surface. As the tip approaches the sample surface, the oscillation amplitude, phase, and resonant frequency of the tuning fork change and provide the feedback control for the regulation of the tip-to-sample distance.

The experimental setup of THz NFM is similar to that of the conventional THz TDS except for the AFM components shown in Fig. 2. A frequency modulator excites the quartz tuning fork with a probe tip and provides a reference signal to a lock-in amplifier which measures the scattered THz signal. The resultant signal enters the input terminal of the detector of a frequency modulator, which detects the changed signals of tuning fork. The output signal of a frequency modulator is input of a proportional integral derivative (PID) controller. The tip-to-sample distance was tightly regulated by the PID controller which adjusts the piezo actuator in order to track the sample surface. The AFM image of the sample surface was obtained from the feedback signal for the piezo actuator, and simultaneously the THz field scattered by the nano-probe tip was recorded by a THz detector.

For THz pulse generation, p-type InAs with a doping concentration of ~ 10^{16} /cm^3 was used. When a femtosecond Ti:sapphire laser pulse with 800 nm center wavelength and 80 MHz repetition rate was incident on the InAs surface, the THz pulse was generated by Photo-Dember effect. The generated THz wave was collimated and focused onto the tip-to-sample gap by two off-axis parabolic mirrors. The THz near-field in the vicinity of the tip apex is scattered by a nano-probe tip. The near-field signal scattered by a tip and the THz background signal specularly reflected from a sample surface are also collimated and focused on a gap of a photoconductive dipole antenna which was fabricated by ultraviolet lithography and metal lift-off on the low-temperature-grown (LT) GaAs substrate. The scattered signal modulated by dithering of the probe tip is measured by a lock-in amplifier to eliminate the large background THz signal. With regulating the tip-to-sample distance, the reflected signal was measured at the chopping frequency of a mechanical optical blade. The scattered signals for gold surface and biomolecules were obtained under the same condition except for the replacement of the chopping frequency by dithering frequency of probe tip.

The scattered time-domain THz signals for gold surface and biomolecular nanoparticles were measured. The peak amplitude of the signal measured from the Au surface was larger than that from the biomolecular nanoparticles since the near-field signal scattered by tip is dependent on the magnitude of the dipole moment induced in the nanoparticles[21, 27, 29]. To measure the absorption spectrum of the observed lactose sample, we performed the conventional transmission-type THz TDS experiments[1]. For a 1 mm-thick lactose pellet, the three sharp absorption peaks were measured in the frequency range of 0.2 ~ 1.5 THz such as reported in Ref. 3 and 4. However in the spectrum measured by THz NFM for a single biomolecular nanoparticle, the bulk absorption peaks were not observed even though some spectrally distinct features were obtained. This is possibly due to the poor signal-to-noise ratio of the signal scattered from biomolecules and/or the effect of the water molecules absorbed in the biomolecular nanoparticles during the measurements.

Based on the time-domain scattered signal, we performed the AFM and THz near-field imaging for a single lactose nanoparticle. We set the time delay at a fixed position corresponding positive or negative peaks. The 2×2 μm^2 image was scanned with a step of 50 nm for the x-axis and 100 nm for the y-axis, and the sizes of the nanoparticles were ~ 10 to 1000 nm, measured from the AFM image. It is found that our THz NFM system has a great potential for spectrally resolved imaging of various nanostructured samples as well as biomolecular nanoparticles. To obtain the complete spectral images, the time-domain signal must be measured least for more than several picoseconds time delays. However it is rather difficult to measure such a long time signal without image distortion and drift because it takes a great deal of measurement time for even a single image point with a mechanical delay line.

In summary, we performed the THz near-field imaging for biomolecular nanoparticles on a gold surface by measuring time-domain scattered signals. THz scattering spectra for a biomolecular nanoparticle were obtained, which were different from those of a bulk biomolecular pellet. We successfully demonstrated that the THz NFM can measure the scattering spectra of an individual biomolecular nanoparticle as well as the imaging with a nanometer resolution.

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