Terahertz sensing method for protein detection using a thin metallic mesh

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A label-free biological sensor, which is based on the resonant transmission phenomenon of a thin metallic mesh, is proposed in the terahertz wave region. By using this sensor, we demonstrate the highly sensitive detection of small amounts of protein horseradish peroxidase. For quantitative investigation of the sensitivity of our sensor, horseradish peroxidase was printed on the metallic mesh surface by using a commercial available printer. A distinct shift of the transmission dip frequency is observed for 500 pg/mm^2 (11 fmol) of horseradish peroxidase printed on the metallic mesh, indicating the significantly high sensitivity of our sensor. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2825411]

In the past two decades, terahertz waves, located in the frequency region between the microwaves and the infrared, have found an increasing number of applications in various fields of research.[1–3] Especially, terahertz technology has a broad applicability in the biomedical context because the collective vibration modes of many protein and DNA molecules are predicted to occur in the terahertz range.[4,5] The refractive index and absorption coefficients of single-stranded DNA and double-stranded DNA are different from each other in the terahertz region, indicating distinguishable characteristics.[6] Reports have also been published on the avidin-biotin complex[7] and the imaging of artificial RNA.[8] These studies have revealed the potential applications of label-free sensings for many biomedical molecules by using the terahertz waves. In biomedicine, label substrates, which might involve fluorescence, an enzyme reaction, or a radio-isotope are used for the detection of proteins. However, these procedures are complex and take a lot of time. The label-free detection with terahertz waves is a convenient technique in biomedicine, which enables us to realize easier and faster medical and food inspections than other techniques.

In this paper, we propose a label-free sensing method using a thin metallic mesh in the terahertz region. The transmission characteristics of thin metallic meshes, investigated since the 1960s,[9,10] are those of a band-pass filter in the far-infrared region. This is due to the resonant transmission caused by an excitation of surface plasmon polaritons[11] (SPPs). The transmission properties of a thin metallic mesh are determined mainly by its geometric parameters, but, when a material is placed near the mesh openings, are also affected by the refractive index of that material, in the sense that a shift of the resonant transmission frequency occurs.[12,13] Our sensing method is based on the change of the transmittance of terahertz radiation through a thin metallic mesh accompanied by the resonant frequency shift when a sample substance is applied on the mesh openings. The transmittance of the thin metallic mesh does not change due to the absorption, but dominantly due to the variation of the refractive index of the sample substances near the openings.[14] Using this thin metallic mesh sensor, we detected the protein horseradish peroxidase, which is an oxidation/reduction enzyme existing in many organisms and is typically used as an indicator in biochemical reactions. The horseradish peroxidase is one of the most widespread label substrates in biomedicine.

In our experiment, the thin metallic mesh was made from electroformed nickel, allowing the fabrication of a smooth surface and precise periodicity of the grating. The two dimensional square metallic mesh was 6 μm thick with a grating period of 76.3 μm and a metallic linewidth of 18.3 μm, in both dimensions. This metallic mesh behaves as a high-pass filter and the peak transmission is approximately 95% at 3.37 THz. We measured the transmission spectra of a thin metallic mesh using a Fourier transform infrared spectrometer (FARIS-1S; JASCO, Japan), in which the terahertz beam was focused into an area of about 7 mm in diameter on the thin metallic mesh (Fig. 1). We used the protein horseradish peroxidase (Nacalai Tesque) as a sample, by dissolving it in sterilized pure water at the concentrations of 1.0, 0.5, 0.25, and 0.125 mg/ml. These enzyme solutions were spread on the thin metallic mesh. For a quantitative investigation of the sensitivity of the metallic mesh sensor, the accurate spreading of sample substances on the sensor is criti-

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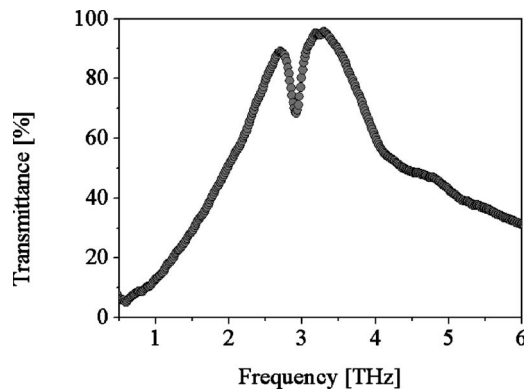
FIG. 1. Measured transmission spectrum of a thin metallic mesh with a grating period of 76.3 μm and a metallic linewidth of 18.3 μm.

cally important. We printed horseradish peroxidase on the metallic meshes using a commercial inkjet printer (Pixus 860i; Canon, Tokyo, Japan), the same that was previously used to fix DNA on a chip.[15] The amount of the sample was controlled using an image processing software. By using the inkjet printer, a considerably small amount of sample can be spread on various materials more readily than with other printing systems.

Figure 1 shows the transmission spectrum of the thin metallic mesh. The broad transmission peak is observed at 3.37 THz and the peak transmittance exceeds the fraction of a mesh opening. This resonant transmission phenomenon is derived from the resonant excitation of SPPs. The resonant frequency of the SPP is expressed as

$$f_{\mathrm{SPP}} = |\mathbf{k}_{\mathrm{in}} + \mathbf{G}| \frac{c}{2\pi} \left(\frac{\varepsilon_m + \varepsilon_d}{\varepsilon_m \varepsilon_d} \right)^{1/2}, \tag{1}$$

where $\mathbf{k}_{\mathrm{in}}$ is the in-plane wave-vector of the incident terahertz wave, $\mathbf{G}$ is the reciprocal lattice vector of the periodic structure, and ε_m and ε_d are the dielectric constants of the metal and the interface medium. The discrepancy between the observed peak frequency (3.37 THz) and the resonant frequency of SPPs (3.93 THz) expected from the Eq. (1) is attributed to the Fano-like interference effect between the terahertz waves transmitted directly through metal openings and reemitted from the SPPs excited on the metal surface.[16,17] In Fano's original study, he investigated the situation of the interference between two contributions, a non-resonant continuum state and a resonant discrete one. For the problem of transmission of terahertz wave through metal mesh, the former corresponds to the directly transmitted terahertz wave and later corresponds to the reemitted terahertz wave from SPPs. This Fano-like interference leads to the asymmetric spectral shape around the resonant frequency and, consequently, the redshift of the transmission peak with respect to the SPP resonant frequency.

When we used collimated incident terahertz waves, this transmission dip is not observed at normal incidence while it is observed at oblique incidences (not shown). In addition, the transmission dip frequency shows the incident angle dependence. From these results, the observed transmission dip arises as a result of the transmission peak splitting for oblique incidence. For the square lattice structured surface, the first order SPP mode splits into two modes analogous to photonic band modes in photonic crystals.[18,19] In this case, the spectral dip is possibly observed between transmission
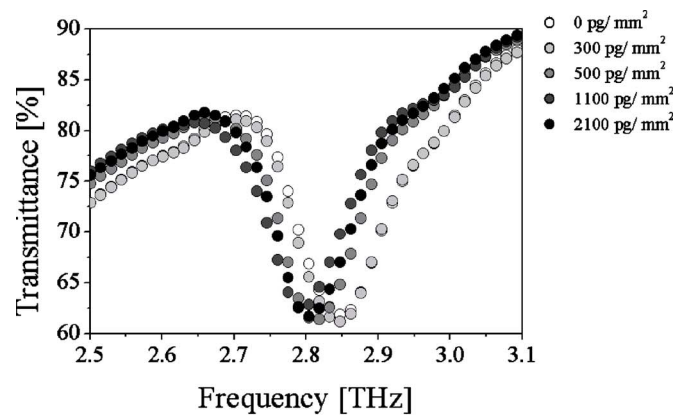
(a)
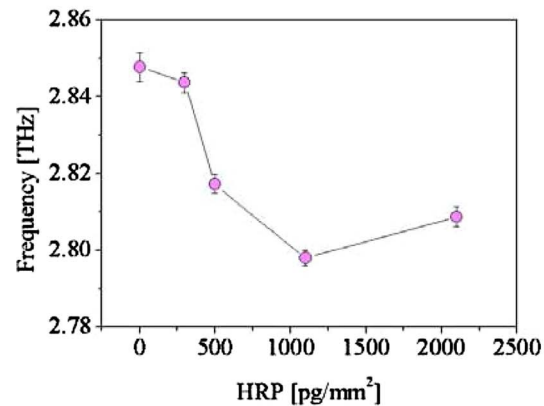
(b)

FIG. 2. (Color online) (a) Measured transmission spectra of a thin metallic mesh with various concentrations of horseradish peroxidase. (b) The transmission dip frequency as a function of the horseradish peroxidase concentration.

peaks of these two modes. For the focused incident beam, like our experiment, the component of oblique incident terahertz wave involved and, thus, the transmission dip appears as the integral of the contributions from several oblique components. Since the resonant frequencies of the transmission peak and dip strongly depend on the refractive index in the vicinity of the metallic mesh openings, the extremely small amount of the sample substances can be detected by monitoring the position of the transmission peak and/or dip.

Figure 2(a) shows the measured transmission spectra of the thin metallic mesh with various amounts of peroxidase printed on it. We present the transmission spectra only in the frequency range around the transmission dip observed in Fig. 2. The transmission dip frequency shows a tendency to decrease with increasing the amount of sprayed peroxidase. The transmission dip frequency, which is estimated from the fitting of the measured spectrum with a Lorentz function, is plotted in Fig. 2(b) as a function of the amount of peroxidase. A clear frequency shift of the transmission dip is observed for 500–2100 pg/mm^2 horseradish peroxidase with respect to that of the bare metallic mesh (0 pg/mm^2). This result indicates that the sensing with thin metallic meshes is extremely sensitive for the detection of very small amounts of biomolecules, equal to the sensitivity of the conventional method using an antibody labeled horseradish peroxidase. Nagle *et al.* reported the detection of the femtomole order of

DNA by using a coplanar waveguide with a resonant structure[20] and at functionalized electrodes via hybridization in the terahertz region.[21] Our thin metallic mesh sensing can detect proteins in the order of femtomole, which should allow the application of our sensor to protein detection in biomedicine.

The peak frequency basically shifts to the lower frequency side with increasing the volume of the horseradish peroxidase in the range from 300 to 2100 pg/mm^2. The peak frequency for 2100 pg/mm^2, however, returns slightly to the higher frequency with respect to that for 1100 pg/mm^2. This result is due to the inhomogeneous spreading of the horseradish peroxides on the metallic mesh in our printing system. More precise spreading can help to avoid this ambiguity in the detection of the frequency shift.

The transmittance at the dip frequency changes only slightly with the volume of the horseradish peroxides. This indicates that the absorption of the terahertz wave by the sample substances plays a minor role for the variation of the transmission spectrum. Such a small variation of the transmittance makes it difficult to detect the sample substances by monitoring the transmission intensity at a single frequency.

In conclusion, we demonstrated the highly sensitive detection of protein molecules in amounts in the femtomole order by using a thin metallic mesh sensor. For spreading the extremely small amount of sample substances on the metallic mesh sensor, we used a commercially available printer with which horseradish peroxidase could be printed. For the metallic mesh sensor printed with 500 pg/mm^2 (11 fmol) horseradish peroxidase, a redshift of the transmission dip frequency is observed clearly, which is based on the variation of the refractive index in the vicinity of the metallic mesh openings. This result exceeds the detection limit of conventional terahertz spectroscopy for protein molecules. As a next challenge, we aim to achieve a label-free selective detection of proteins by using an antigen/antibody reaction.

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