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Label free immune assay using terahertz chemical microscope

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ABSTRACT

A terahertz chemical microscope (TCM) has been proposed and developed to visualize the distribution of the antibody–antigen bindings on the silicon based sensing plate without any labels and/or markers on the antibody. The sensing plate can emit the terahertz pulses by femtosecond laser pulses and the amplitude of the terahertz pulses can be related to the chemical or electric potential of the sensing plate surface at where the laser pulses are irradiated. Thus the potential distribution can be visualized as the map of the terahertz pulses amplitude.

As the first demonstration of the label free immune assay, the mouse immunoglobulin G (IgG) was immobilized on the half part of the sensing plate and the mouse anti-IgG was combined with the IgG. The terahertz pulses were enhanced at where anti-IgG was combined with IgG on the sensing plate and the distributions of the IgG and anti-IgG bindings on the sensing plate were clearly seen as the TCM images. The amplitude of terahertz pulses as a function of the concentration of anti-IgG was also investigated.

1. Introduction

High sensitivity and throughput analysis of the interactions of proteins, label-free detection of antibody–antigen bindings are demanded. As the label-free sensing, the systems, which utilize a surface plasmon resonance (SPR), were developed and succeed in the field of biology [1–3]. An SPR system consists of an optical prism with a metal film, with the immobilized antibody membrane on the film. An optical beam at a specific incidence angle excites surface plasmon on the surface of the gold thin film by an evanescent wave. When the antigen combines with the immobilized antibody, the condition of the incident angle shifts because of a change in the effective refractive index of the membrane. This technique is theoretically a highly sensitive method for detecting proteins; however, the sensitivity was reduced as decreasing molecular mass of the sample proteins. Generally, the measurement was limited to the sample molecules larger than thousands Daltons (Da).

Recently, thanks to the progress in both of the semiconductor and the laser technologies, the terahertz (THz) pulses have been easily handled and thus, a lot of sensing applications has been proposed [4]. Among a various THz system, a laser THz emission microscopy (LTEM) was recognized as a useful tool for evaluating the local field in semiconductor devices such as large scale integration circuits [5–8]. This type of THz technique can realize higher spatial resolution than standard THz imaging systems because the spatial resolution is not determined by the wavelength of the THz pulse but by that of the femtosecond laser, which is typically around 790 nm. In our group, a terahertz chemical microscope (TCM) has been developed in order to measure the distribution of the chemical or electric potential shift on the Si-based sensing plate. The sensing plate consists of the SiO_2/Si thin films on the sapphire substrate. The thickness of the SiO_2 and Si films are typically 250 nm and 170 nm, respectively. The size of the plate is 15 mm × 15 mm. Because of the defect at the Si film, the depletion layer is formed and the local field exists in the film. When the femtosecond laser illuminate the Si layer from the substrate side of the plate, the carriers are excited and accelerated by the local field, which can be considered as the ultrafast modulation of the current density J. According to Maxwell's law, the amplitude of the electromagnetic wave E in the far field is proportional to the time derivative of the current density J and expanded to following equation.

$$E = \frac{\partial J}{\partial t} = \frac{\partial n}{\partial t} ev + ne\frac{\partial v}{\partial t} \quad (1)$$

where n is the carrier density, e is the elementary charge, and v is the velocity of the carriers. The second term of the last equation indicates that the E is proportional to the local field. Thus, the THz pulses are generated by the local field of the depletion layer in the Si layer and radiated into the free space. If the chemical or electric potential on the surface of SiO_2 shifts by the chemical reactions and/or the adsorption of the proteins, the local field also changes. Up to now, detection of various chemical reactions, which includes neutralization process, catalytic reactions, and protein bindings, has been demonstrated [9–13].

In this paper, as the first demonstration of the label free immune assay, the mouse immunoglobulin G (IgG) was immobilized on the

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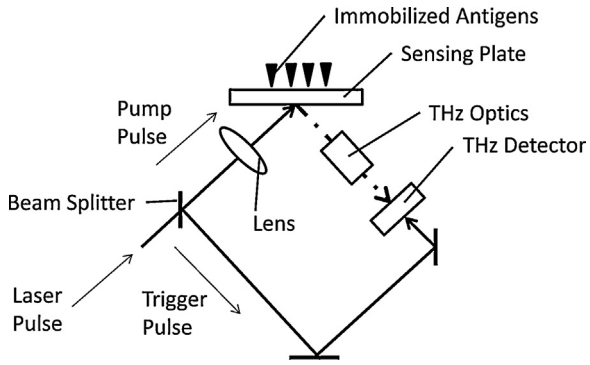

Fig. 1. The optical set-up of the terahertz chemical microscope.

half part of the sensing plate and the distributions of the IgG and anti-IgG bindings on the sensing plate were observed as the TCM images.

2. Experimental

Fig. 1 shows the optical set-up of the TCM. The femtosecond laser pulses were split to the pump pulses from the trigger pulses using a beam splitter. The center wavelength of the laser was 790 nm. The average laser power was about130 mW with the repetition rate of 82 MHz and the pulse width was around 100 fs. The pump pulses were introduced to the sensing plate from the substrate side of the plate with the incident angle of 45° and the generated THz pulses was radiated to the free space from the same side of the plate. The THz pulses were focused on the THz detector by THz optics such as Si lens and off-axis paraboloidal mirrors. As a THz detector, the bow-tie type photoconductive antenna made from a low-temperature grown GaAs were used. The trigger pulses were focused on the THz detector after adjusting the arrival timing of the pulses in order to detect the peak amplitude of THz pulses.

In order to detect the antibody-antigen bindings, the mouse IgG (Pierce Biotechnology, 275 nM) immobilized on the half part of the plate by covalently bonded with the silanol group of the surface [14]. After preparation, the biotinylated goat anti-mouse IgG (Wako, whole molecule) with 200 nM in the 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) solution was dropped to the immobilized area and thus the IgG and the anti-IgG complex was formed on the sensing plate. The formation of the IgG and the anti-IgG was confirmed by AFM images of the surface of the sensing plate with and without the bindings as shown in Fig. 2.

3. Results and discussion

Fig. 3(a) shows the signal shift of TCM images before and after forming the IgG and anti-IgG complex. The average value at the area without the complex was set to be zero. One can see that the signal shift at where the antibody existed was clearly different from that at where no antibody existed. This change in the THz amplitude could result in the change in the chemical potential by forming the antibody–antigen bindings. After observing the TCM images, secondary antibodies were combined to the anti-IgG and the substances were injected on the sensing plate. Fig. 3(b) indicates the photograph of the sensing plate after labeling. The color of the substance were changed at where the IgG was immobilized and the result was consistent with the data obtained using TCM. In order to evaluate the anti-IgG concentration dependence of the THz amplitude, the IgG was immobilized on the whole area of the sensing plate and the plate was separated to the four region using

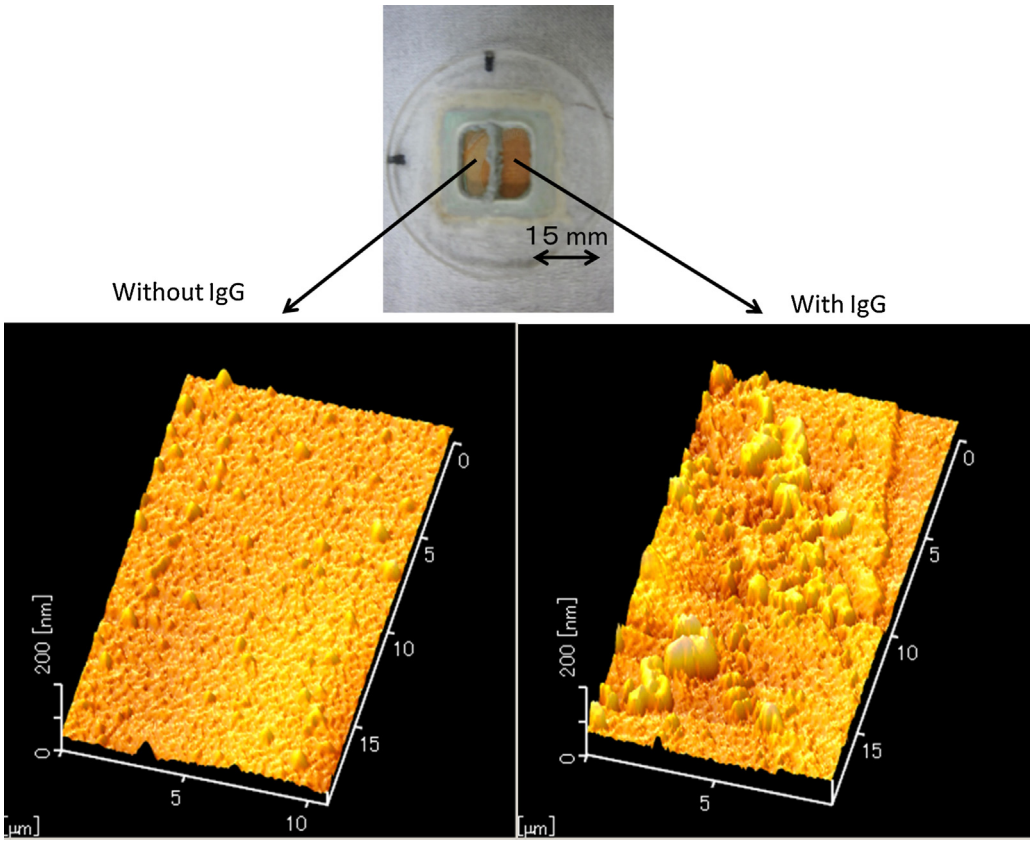

Fig. 2. AFM images of the surface of the sensing plate with and without the bindings.