Label-free detection of proteins using a terahertz chemical microscopy

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Abstract

A terahertz chemical microscopy (TCM) has been proposed and developed to visualize the distribution of chemical reactions such as antigen-antibody reaction on the sensing plate without any labels on the analyte. The TCM utilize the semiconductor-based device named a sensing plate. The sensing plate can convert the shift of the chemical potential in the magnitude on the surface of sensing plate into the amplitude of generated terahertz (THz) in the sensing plate. Thus, it enables to get images of the potential distribution on the surface of the sensing plate as the distribution of THz amplitude.

Here, we demonstrate the detection of the immune reactions between the mouse immunoglobulin G (IgG) and the anti-mouse immunoglobulin G (anti-IgG). IgG was immobilized on the sensing plate and reacted with anti-IgG in the solutions with various concentrations. We also fabricated the sample wells on the sensing plate and quantitative evaluation of immune reactions were demonstrated with the anti-IgG concentration of between 5 nM and 100 nM. As a result, the change in the THz amplitude during the reaction showed the proportional relation with the concentration of reacted anti-IgG.

1. Introduction

Various types of bio-sensing methods, which includes such as the detection method for antigen-antibody reactions, have been proposed and practically used. In the basic research of the life, these types of methods are used especially in order to elucidate how the biological substances act in vivo, and in order to develop antibody drugs which have low side-effects to human bodies because of their selectivity. Conventionally, an Enzyme-Linked ImmunoSorbent Assay (ELISA) [1] is used for immune reactions. In this method, the secondary antibodies are added to label the reacted antigens combined with the antibodies, and after that, the unbounded labels are washed away to distinguish from the labeled antigens. Therefore, due to this kind of complicated process of ELISA, it generally takes a few hours for detection, whereas it is possible to highly sensitive detection of sub-pM order. Meanwhile, Surface Plasmon Resonance (SPR) method [2, 3] enables to detect the immune reactions without any labeled secondary antibodies. However, the sensitivity of the SPR largely depends on the molecular weight and the antigens with the molecular weight less than 1000 Da are hard to detect by the SPR method.

In our group, a terahertz chemical microscope (TCM) has been proposed and developed to for label-free immune assay [4, 5]. The TCM utilize the semiconductor-based device named a sensing plate. The sensing plate can convert the shift of the chemical potential in the magnitude on the surface of sensing plate into the amplitude of generated terahertz (THz) in the sensing plate. Thus, it enables to get images of the potential distribution on the surface of the sensing plate as the distribution of THz amplitude. In this work, the sample wells were fabricated on the sensing plate of TCM and the quantitative measurement of immune reactions were demonstrated.

2. Experimental

Fig. 1 shows the schematic diagram of the sensing plate. The Si and SiO_2 thin films were respectively prepared on the sapphire substrate. The femtosecond laser pulses were introduced to the sensing plate from the substrate side of the plate, and the THz pluses were generated by the surface field effect of the depletion layer in the Si layer. If the antigen-antibody reaction occurs on the SiO_2, the electric field of depletion layer is changed, and it lead to the change in the peak amplitude of the THz pulses between before and after the reaction.

Fig. 2 shows the optical system of the TCM. The femtosecond laser pulses were split into the pump pulses and the probe pulses using a beam sampler. The average pump pulses power was about 200 mW with the repetition rate of 82 MHz. The center wavelength of the laser was 780 nm and the pulse width was 100 fs. The pump pulses were introduced to the sensing plate from the sapphire side of the plate with the incident angle of 45 degrees and the generated THz pulses were radiated to the free space from the same side of the plate. The THz pulses were focused on the THz detector by the off-axis paraboloidal mirrors. The bow-tie type photoconductive antenna made from a low-temperature grown GaAs was used as a THz detector. The probe pulses were focused on the THz detector after adjusting the arrival timing of the pulses to detect the peak amplitude of THz pulses. In order to get THz amplitude distribution image (THz image), the sensing plate was moved horizontally at optional interval and measured at each point. In this time, one third mm interval was chosen.

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Upon detection the antigen-antibody reaction, the plate was separated to the six sample wells using silicone glue as shown in Fig. 3, and the mouse immunoglobulin G (IgG, Thermo Fisher Scientific, 275 nM) ware immobilized on the five wells of the sensing plate by covalently bonded with the silanol group of the SiO_2 surface [6]. The surface of the one well was kept to be the SiO_2 surface without the antibody and used as the negative control. Then, the anti-mouse IgG (Cosmo Bio) solution was prepared with the concentration of 5, 25, 50, 100 nM. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 0.1M) buffer solution was used as solvent. Each prepared sample solution was dropped on the each well. In our experimental, we obtained the distribution of the amplitude change of the generated THz from sensing plate before and after the antigen-antibody reaction.

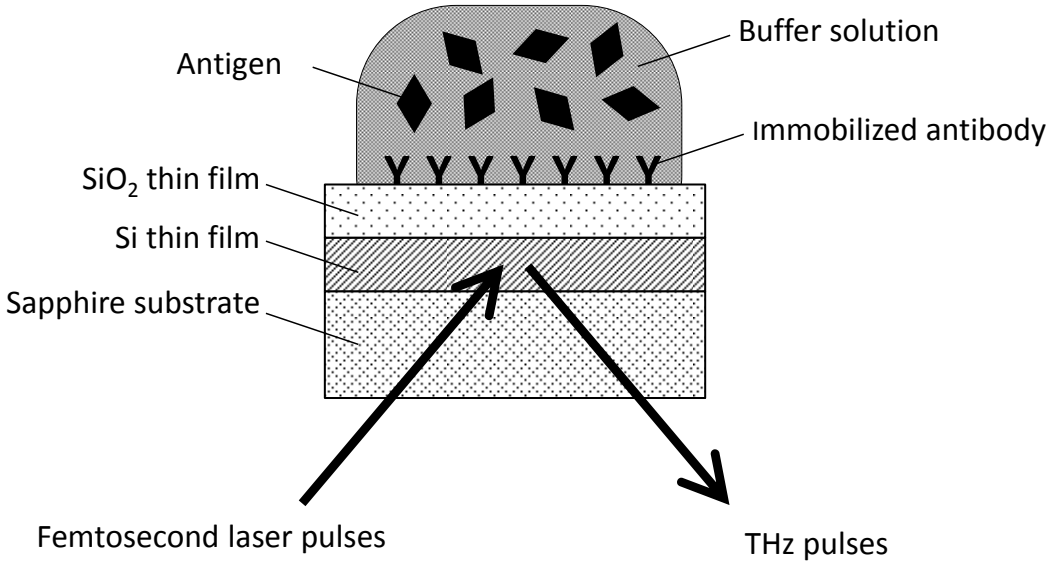

Fig. 1 Schematic diagram of the sensing plate

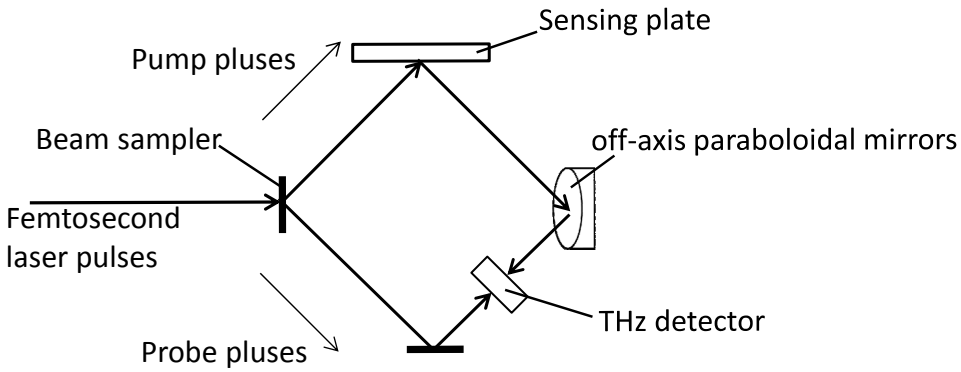

Fig. 2 Optical system of the TCM

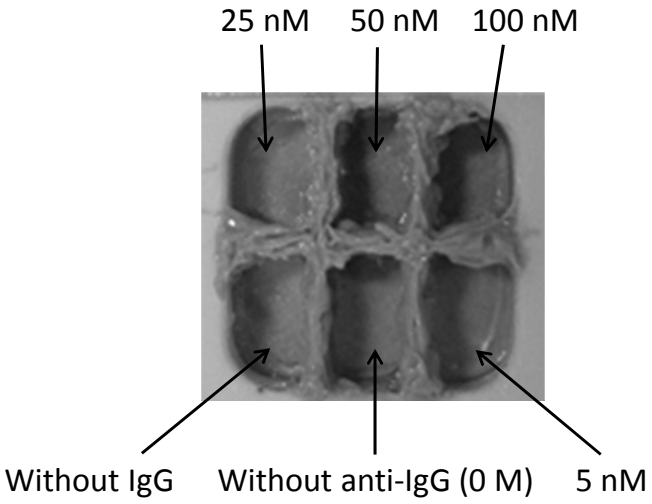

Fig. 3 Separated plate

3. Results and discussion

Fig. 4 shows the obtained THz image. One can see that the THz amplitude shift was enhanced at the wells where the immune reactions were occurred. In order to evaluate the relation between THz amplitude shift and the concentration, the averaged THz amplitude shift at the center of well were calculated and plotted in Fig. 5 This result shows that the change in the THz amplitude shift showed the proportional relation with the concentration of reacted anti-IgG and, therefore, TCM has the possibility to be one of the label-free immune assay tools.

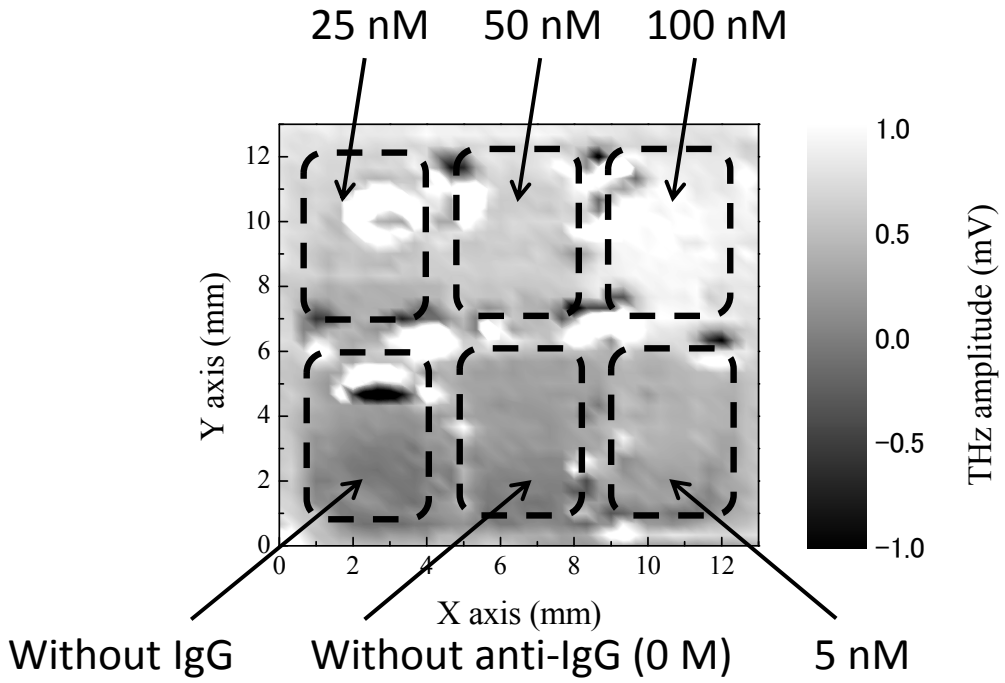

Fig. 4 Differential THz obtained image before and after antigen-antibody reaction

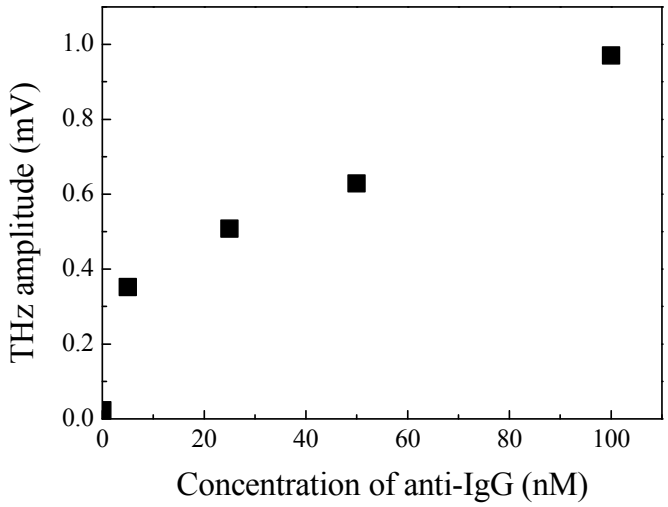

Fig. 5 The THz amplitude shift as function of the concentration of anti-IgG

4. Summary

In this demonstration, we got the differential THz image before and after the reaction, and the relation between the concentration and the THz amplitude shift was obtained. The results indicated that the change in the THz amplitude shift showed the proportional relation with the concentration of reacted anti-IgG. Thus, the label-free detection and visualization of antigen-antibody reaction using TCM were posible. It suggests that the TCM can be useful system for label-free immunoassay.

5. Acknowledgements

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6. References

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