Redox Reactions of Enzymes Measured by Terahertz Chemical Microscope

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***Abstract*—A terahertz chemical microscope (TCM) was proposed and developed to measure redox reaction of enzymes. TCM utilize the sensing plate which can measure the surface potential changes at the laser illuminated area by detecting the terahertz waves generated from the plate. An immobilized urease was applied to the plate and the redox reaction with the urea in the buffer solution was demonstrated.**

I. Introduction And Background

Demands on the easy and real-time screening of the diseases are rapidly increasing with increasing complexity of medical procedure. Chemical sensors and transducers with immobilized enzymes are one of useful tool for real-time monitoring of bio-materials.

Recently, a terahertz (THz) chemical microscope (TCM) has been proposed and developed to observe the surface potential distribution of the insulator/semiconductor surface [1] by utilizing the laser-terahertz techniques [2-4]. A sensing plate, which consists of insulator/semiconductor thin films on a sapphire substrate, was fabricated. THz is generated and radiated to free space by a femtosecond laser pulse illumination of the semiconductor layer, because of the photo-carrier acceleration by the local field of depletion layer. When the surface potential is changed, the local field of the depletion layer also changes and simultaneously the amplitude of the radiated THz changes. Thus the surface potential of the sensing plate can be measured by detecting the amplitude of the THz.

In this work, immobilized enzyme was fabricated on the sensing plate and the surface potential change due to the redox reaction of enzyme was detected. Because the spatial resolution of TCM is theoretically determined by the wavelength of the laser, the combinatorial sensing of reaction with highly integrated enzymes may be possible.

II. Experiments

Figure 1 shows the schematic diagram of the sensing plate. Si thin film was deposited on the double-side-polished sapphire substrate and SiO_2 thin films was prepared on the Si thin film. The film thicknesses of Si and SiO_2 were 150 nm and 275 nm, respectively. We also fabricated the immobilized enzyme on the SiO_2. The urease was used as the enzyme. The urease was immobilized by photocrosslinking technique using AWP. The photograph of the sensing plate with the immobilized urease is shown in Fig. 2. The framed area by the dashed line is where the urease was immobilized. Figure 3 indicates the optical set up of the system. Ti:Sapphire laser which produces the ultrafast optical pulse of 100-fs (FWHM) was used as the source. The center wavelength was around 790 nm. The pulses were split to the excitation pulse from probe pulse, and introduce on to the sensing plate by an object lens. The probe pulses triggers the photo conductive switch (PC) after through the optical delay. The optical delay was fixed at where the peak amplitude of THz

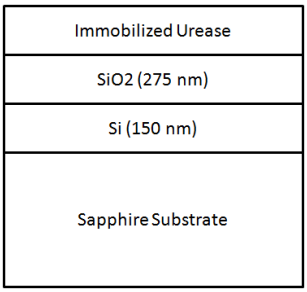

Figure 1: Schematic diagram of the sensing plate.

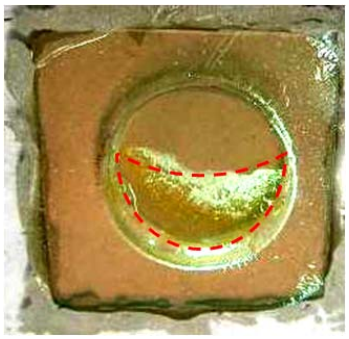

Figure 2: Photograph of the sensing plate with the immobilized urease.

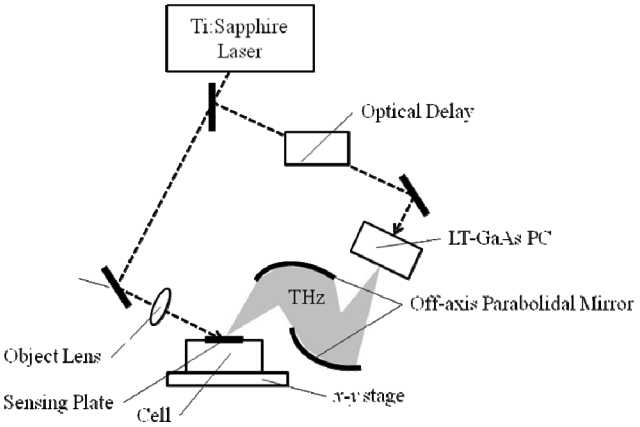

Figure 3:Optical set up.

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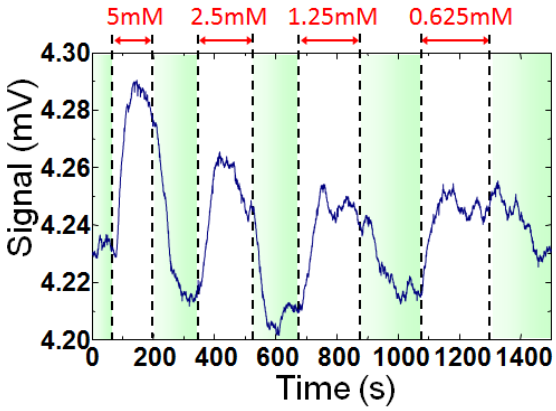

Figure 3: Response of TCM by the urea solution.

was obserbed.

III. RESULTS

The urea solution with phosphate buffer solution (pH 6/ 5 mM) was applied to the sensing plate and measure the amplitude of radiated THz. The amplitude of THz was plotted in Fig. 3. The urea solutions and buffer solution was repeatedly applied to the plate. One can clearly see that the amplitude changed when the urea concentration changes. This result indicates that the redox reaction of urease and urea can be measured by TCM. Visualization of the urea concentration using raster scanning technique was also demonstrated.

IV. SUMMARY

THz chemical microscope was developed and rmeasurments of edox reaction of enzyme was demonstrated. The result indicates that the system can be one of useful tool for a bio detection tool.

This work was supported by Industrial Technology Research Grant Program in 2006 from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

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