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High-Sensitivity Membranes of Light Addressable Potentiometric Sensor for Penicillin Detection

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In this study, light addressable potentiometric sensors (LAPSs) that have devices with $Si_3N_4/SiO_2/Si$ and $Ta_2O_5/SiO_2/Si$ structures were fabricated. We investigated the response characteristics of penicillin and evaluated the possibile application to a bioimage sensor using the fabricated LAPSs. Penicillinase was immobilized on the devices to hydrolyze the penicillin using the self-assembled monolayer (SAM) method. Then, the response characteristics according to the concentration of the penicillin were measured and compared. The measuring system made up of parts such as a potentiostat, signal processing parts, and an xyz stage controlling part was simplified using LabVIEW. As a result of the enzyme reaction, the sensitivity of the sensor that has a Si_3N_4 surface was 60 mV/decade and 74 mV/decade which has a Ta_2O_5 surface in the penicillin concentration range of 0.1–10 mM. Also, we could obtain an image of with a resolution of 128 × 128 pixels within a 1 × 1 cm sensing area.

1. Introduction

Since the enzyme field effect transistor (EnFET)[(1)] and capacitive electrolyte-insulator-semiconductor (EIS) sensors were introduced, there has been a lot of progressive research on semiconductor—type biosensors that use the discernible ability of active molecules in

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an organism. Recently, the light addressable potentiometric sensor (LAPS)[2] system has represented a relatively new type of a sensor with an EIS structure. The characteristics of this sensor include a fast response, high accuracy, and an easy fabrication using a semiconductor process.

In particular, the LAPS system can detect potential changes at the interface between electrolytes and an insulator, and has several advantages including high electrochemical stability and the ability to measure various materials by local light illumination without any additional processes. The LAPS system has a wide range of analytic applications, ranging from the detection of microcells such as pathogenic microorganisms, to macromolecules such as biologic toxin elements, protein hormones, and *E. coli* colonies, and as bioimage sensors.[3–5]

Nonetheless, the sensing membrane of most LAPS devices uses a LPCVD-deposited Si_3N_4 film owing to the films with good long-term stability and excellent roughness. However, this Si_3N_4 film has the disadvantages of drift and slow response.[6] In the case of biomaterial measurement using the LAPS system, sensitivity and stability are affected by the method used to immobilized the biomaterials.

Previously reported polymer matrices such as PVC,[7] gelatin,[8] photo-curable polymers,[9] are used to immobilize biomaterials. They have disadvantages, for example, the adhesion is not adequate for the sensor surface and activity can be restrained during a catalytic reaction.

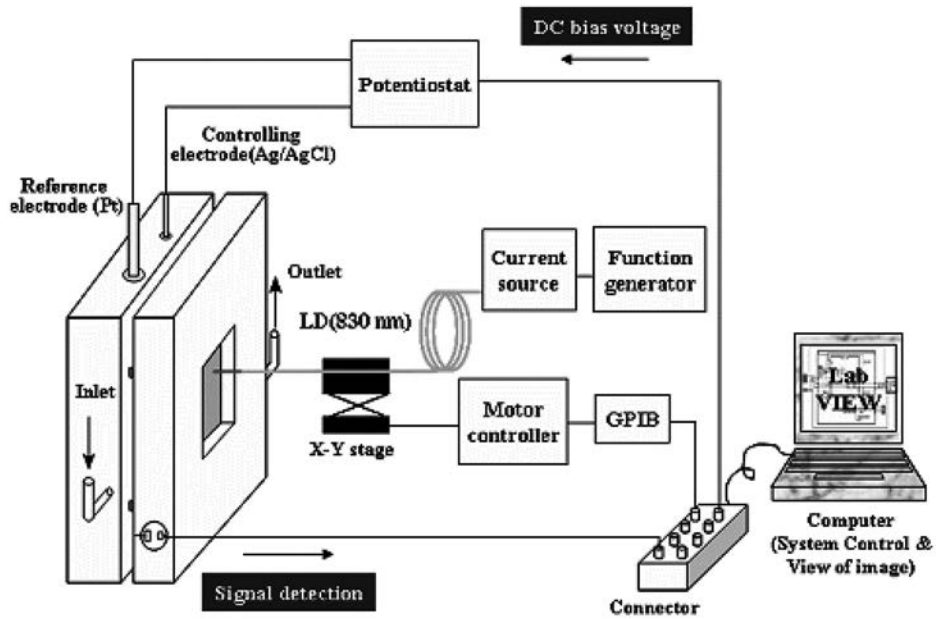
In this study, to solve such problems related to the sensitivity and the immobilization of biomaterials, we fabricated a LAPS device with a $Ta_2O_5/SiO_2/Si$ structure and developed an immobilization method of biomaterial using the self-assembled monolayer (SAM) method. We compared the sensing characteristics of the fabricated LAPS device for penicillin with those of Si_3N_4 and Ta_2O_5 films. Also, we investigated the possibile application to a bioimage sensor using the fabricated LAPS.

2. Sensing Principle of LAPS

An important family of chemical sensors on silicon consists of an electrolyte/insulator/silicon (EIS) structure.[10] Figure 1(a) shows a schematic diagram of LAPS with an EIS structure. An alternative light of 10 kHz frequency is illuminated onto the LAPS device and a DC voltage is applied to the electrolytes. An inversion layer is turned by a DC bias in the LAPS device and then the charges in the inversion and insulator layers are modulated by the electron hole pair to make an AC photocurrent.[10] In the accumulation state, there is no photocurrent because there is no electric field on the surface of silicon. Therefore, a curve of AC photocurrent vs DC bias voltage shows large changes between the accumulation state with no photocurrent and the inversion state with the maximum photocurrent.[10] The photocurrent curve of the potential change on the interface between the sensing membrane and the electrolytes tends to shift along the bias voltage axis, and it can be realized by calculating an inflection point on the curve as shown in Fig. 1(b). That is, ψ_p is the surface potential of silicon at the inflection point and second differential point, and the voltage (V_p) at the inflection point given by eq. (1).[10]

$$V_p = V_{FB} + \psi_p + \frac{\sqrt{2\varepsilon_s q N_{dop} \psi_p}}{C_{ins}} \tag{1}$$

(a)



(b)

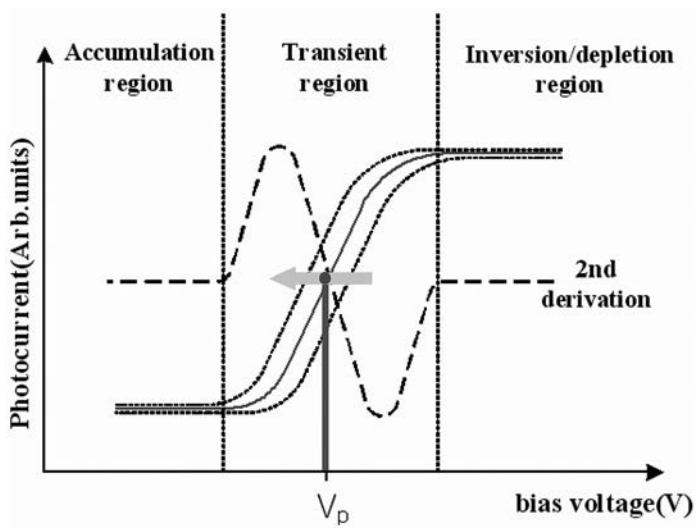


Fig. 1. Schematic diagram of detecting system and graph of photocurrent vs bias voltage. (a) Schematic diagram of LAPS system. (b) Relationship of photocurrent vs bias voltage.

Here, C_{ins} is the capacitance of the insulator, N_{dop} is the doping concentration of the silicon, and ε_s is the dielectric constant of silicon. In other words, V_{FB} makes V_p change because ψ_p

is constant. Flat-band voltage (V_{FB}) in EIS structures depends on the surface potential on the interface between the electrolytes and the insulator. Consequently, the photocurrent curve tends to shift along the bias voltage axis according to the changes in ion concentration, and it is expressed by the changes in voltage at the inflection point of the photocurrent curve. Therefore, the sensitivity of the ion is defined by the eq. (2).

$$\frac{\partial \psi_0(\textit{Ion concentration})}{\partial\, \textit{Ion concentration}} = \frac{\partial V_p}{\partial\, \textit{Ion concentration}} \tag{2}$$

Equation (2) shows the sensitivity and potential difference to changes in ion concentration on the interface between the electrolytes and the sensing membrane, and is very important because it influences signal amplitude and operational characteristics in LAPS.

3. Experiment

3.1 *Device fabrication and measurement system*

Figure 2 shows the fabrication process for the LAPS devices. First, to get the LAPS devices with $Si_3N_4/SiO_2/Si$ and $Ta_2O_5/SiO_2/Si$ structures, oxide (500 Å) and Si_3N_4 layers were grown by thermal oxidation and the LPCVD method on a p-type silicon wafer, respectively. The back side of the LAPS device was etched to 250 μm thickness using the silicon anisotropic etching (TMAH etching) method. After silicon anisotropic etching, the Si_3N_4/SiO_2 layer was removed. Then, two different sensing membranes were deposited on the oxide layer (300 Å), namely, is Si_3N_4 (by LPCVD, 800 Å), and Ta_2O_5 (by RF sputtering, 800 Å). Finally, gold was evaporated onto the back side of the electrode of the devices except for the region illuminated by a light source.

The measuring system consisted of a potentiostat, a light source, a reactive chamber and signal detection parts, such as an xyz stage control. An 830-nm laser diode was used as a modulated light source (1–10 kHz), and a bias voltage was applied in the range from –2 to 2 V. The measuring system was simplified using LabVIEW software to handle the complex signal processing.

3.2 *Fabrication of sensing membrane*

Penicillinase was immobilized on the sensing membrane using the SAM method. Figure 3 shows a schematic diagram of the reaction. First, to clean and produce a hydroxide layer on the sensing surfaces of the LAPS devices, the surfaces were immersed in a piranha solution (H_2SO_4:H_2O_2=7:3), rinsed with H_2O, and immersed in 0.5 M NaOH followed by 0.1 M HCl to neutralize the surface.[11] Then, the sensing surfaces were immersed in a 2.5% (v/v) APTMS solution that was made using unhydrous ethanol, to make a stable coupling layer, for 30 min in an N_2 chamber. As the result, the amine terminal substrate coupled with the GA on the basis of an amide reaction. Then, the amine-terminated substrate was immersed in a 0.3 M-GA methanol solution with 0.1 M NHS and 0.4 M EDC for 3 h. These reactions created an active surface with carboxylic acid terminal groups. For a carboxylic acid terminal substrate, the substrate was immersed in an aqueous

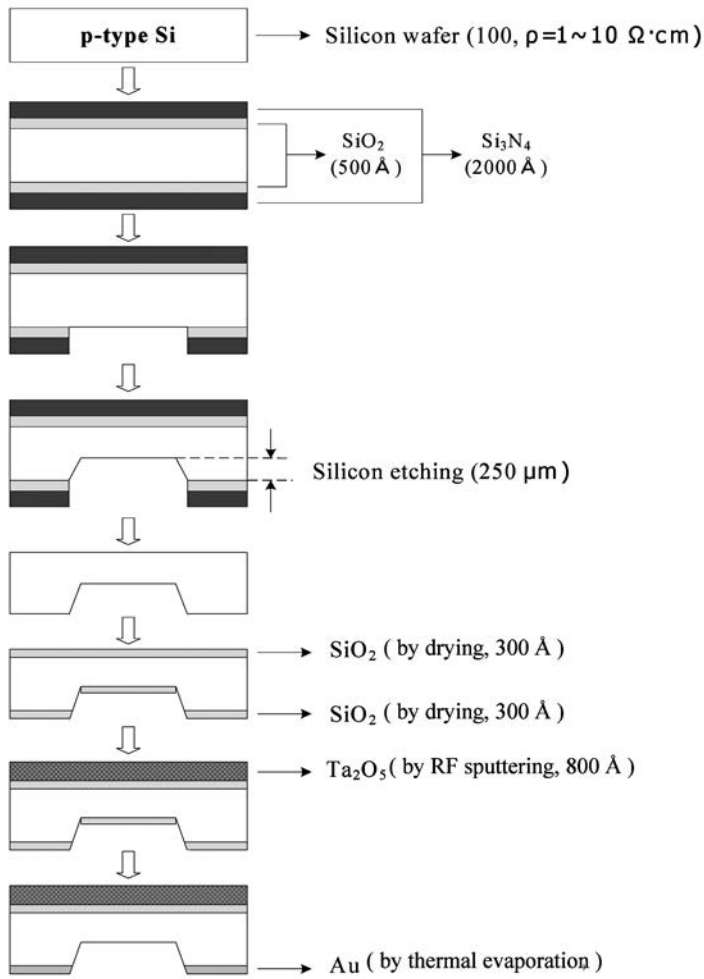


Fig. 2. Fabrication process of LAPS devices.

solution with 0.1 M NHS and 0.4 M EDC for 2 h, and rinsed with H_2O. Finally, the substrate was immersed in a 0.5 mg/ml penicillinase PBS buffer (pH 7.4) for 15 h. Penicillin G solution was made by adding penicillin G (potassium salt, 1600 unit/mg, Aldrich) to a Tris buffer solution, pH 7.

4. Results and Discussion

4.1 *pH response of LAPS device*

The curves of the photocurrent characteristic according to pH changes in the fabricated LAPS devices using Si_3N_4 and Ta_2O_5 sensing membranes are shown in Fig. 4. As the potential of the sensing surfaces changed with a variation in ion concentration, the applied

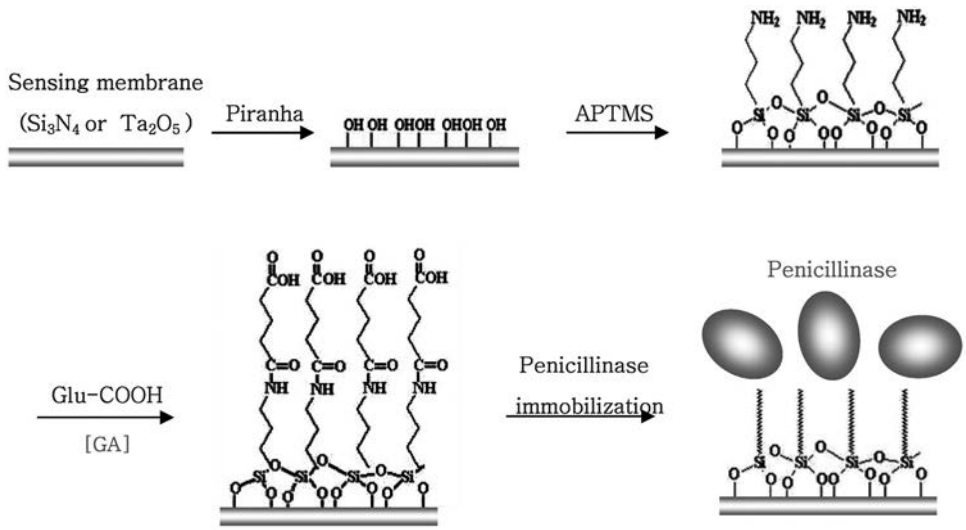


Fig. 3. Schematic diagram of penicillinase immobilization.

DC bias voltage was also changed to maintain the same electric field in the semiconductors. Consequently, the photocurrent curve moved along the bias voltage axis, as shown in Fig. 4. The sensitivities of the fabricated LAPS devices with Si_3N_4 and Ta_2O_5 films were ±54 mV/pH and ±59 mV/pH, respectively.

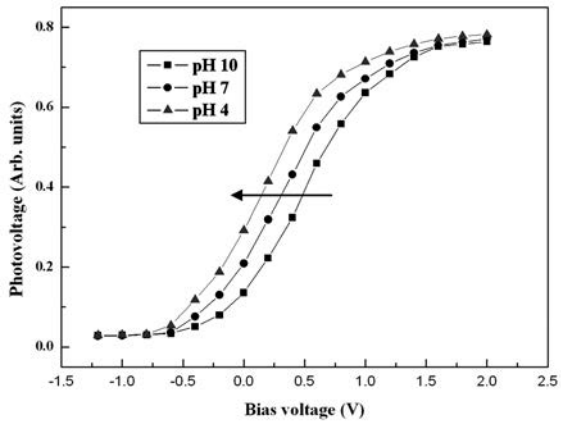
4.2 *Response characteristics to penicillin and image detection*

We could confirm the immobilization state of penicillinase on the sensing membrane using AFM images. Figures 5 and 6 show 1 × 1 μm AFM images for each state of the SAM on the Si_3N_4 and Ta_2O_5 surfaces. In Figs. 5 and 6, the RMS roughnesses of the SAMs with penicillinase were higher than those with –NH2, –COOH. Therefore, we can recognize the immobilization of a penicillin-sensing membrane on Si_3N_4 and Ta_2O_5 surfaces.

Figure 7 shows the photocurrent curves of the LAPS devices with the Si_3N_4 and Ta_2O_5 sensing membranes resulting from the enzyme reaction. The change in the concentration of hydrogen ions created by the enzyme reaction induced a variation in surface potential. Therefore, the photocurrent curves shifted according to an increase in the penicillin concentration in the range from 0.1 to 10 mM. Then, the characteristic responses of the LAPSs with the Si_3N_4 and Ta_2O_5 sensing membranes were compared. The measured sensitivity of the sensor that had a Si_3N_4 surface was 60 mV/decade and that of the sensor that hatd a Ta_2O_5 surface was 74 mV/decade, as seen in Fig. 8.

In the case of a LAPS bioimage sensor, an image is detected by scanning light pixel by

(a)



(b)

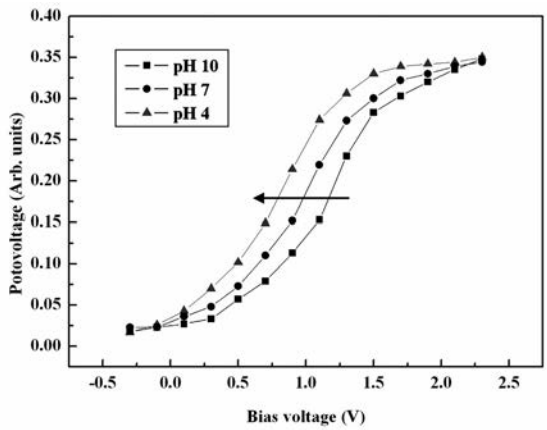


Fig. 4. pH vs photocurrent curves (a) Si_3N_4 membrane, (b) Ta_2O_5 membrane.

pixel on the back side of the sensor. If laser light and a fixed DC bias voltage are applied to the sensor, a photocurrent, including light position information, is generated.[5] Therefore, the visual image is represented by the composition of the photocurrent at each pixel. In order to obtain a high resolution image, we etched the back side of the LAPS device. To define the image pattern, a pattern should be formed on Ta_2O_5 surface using a photoresist. A standard pH solution was used as an electrolyte supply. Figure 9 shows an obtained image that took 10 min to measure 128 × 128 pixels over a 1 × 1 cm area.

5. Conclusions

In this study, we fabricated penicillin-sensitive LAPS devices with Si_3N_4 and Ta_2O_5 films and investigated their possible application to a bioimage sensor. Penicillinase was immobilized on Si_3N_4 and Ta_2O_5 surfaces by the SAM method. Then, we compared the

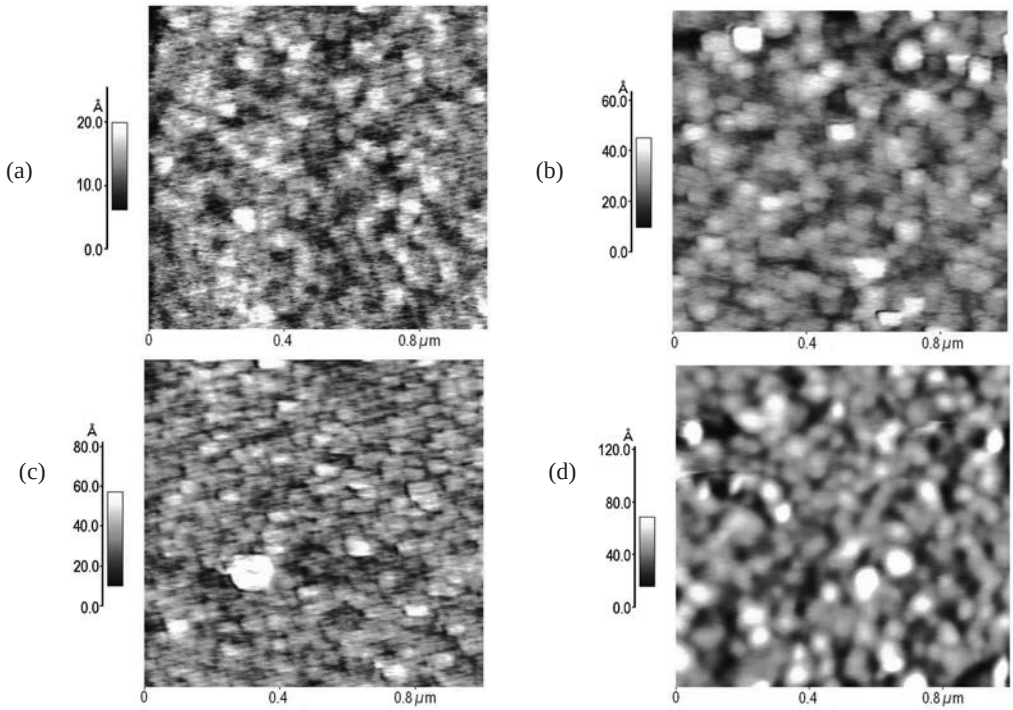


Fig. 5. AFM images of immobilized penicillinase on Si_3N_4 thin film. (a) Piranha-treated Si_3N_4. (b) APTMS monolayeron Si_3N_4. (c) GA monolayer on APTMS. (d) Immobilized penicillinase on GA.

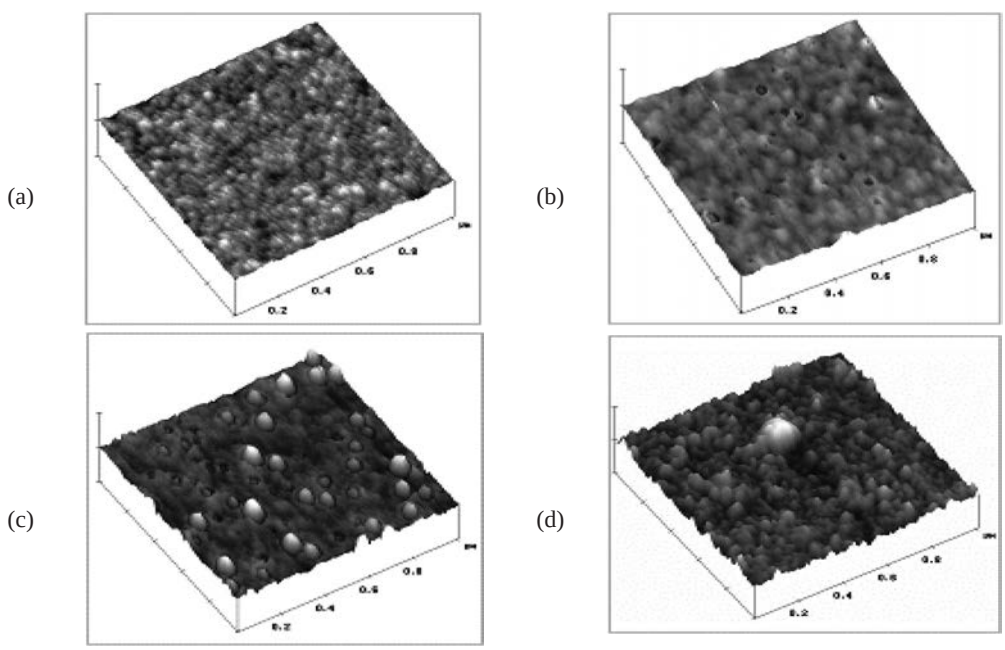
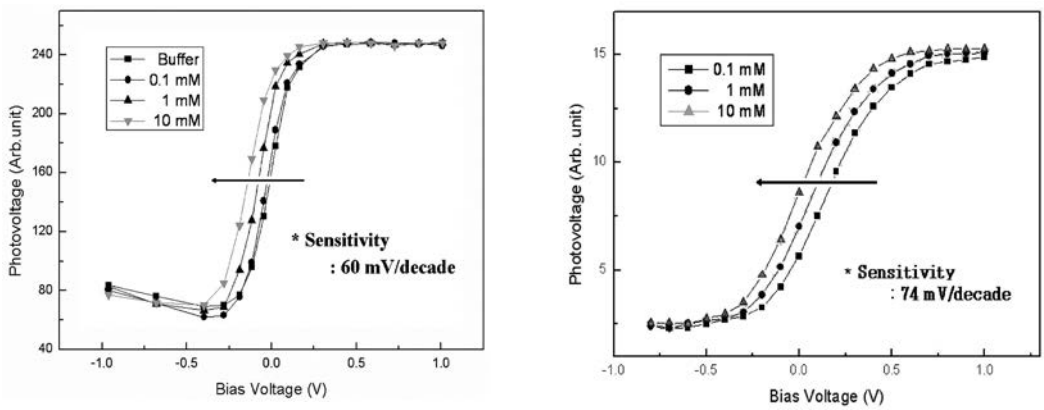


Fig. 6. AFM images of immobilized penicillinase on Ta_2O_5 thin film. (a) Piranha-treated Ta_2O_5 (b) APTMS monolayer on Ta_2O_5 (c) GA monolayer on APTMS. (d) Immobilized penicillinase on GA.



(a) (b)

Fig. 7. Characteristic responses according to penicillin concentration. (a) Si_3N_4 sensing membrane. (b) Ta_2O_5 sensing membrane.

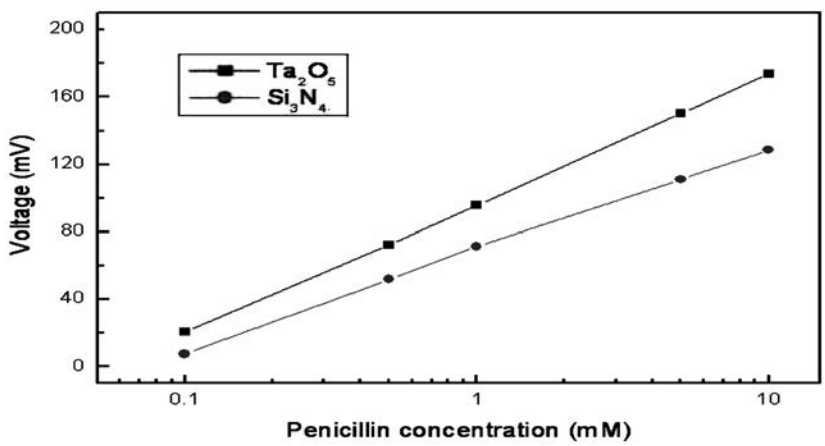


Fig. 8. Characteristic graphs of LAPSs with Si_3N_4 and Ta_20_5 sensing membranes.

characteristic responses according to the penicillin concentrations of the penicillin-sensitive LAPS devices with Si_3N_4 and Ta_2O_5 films. The measured sensitivity of the sensor that had a Si_3N_4 surface was 60 mV/decade and that of the sensor that had a Ta_2O_5 surface was 74 mV/decade. As a result, it was confirmed that the sensitivity of the Ta_2O_5 sensing

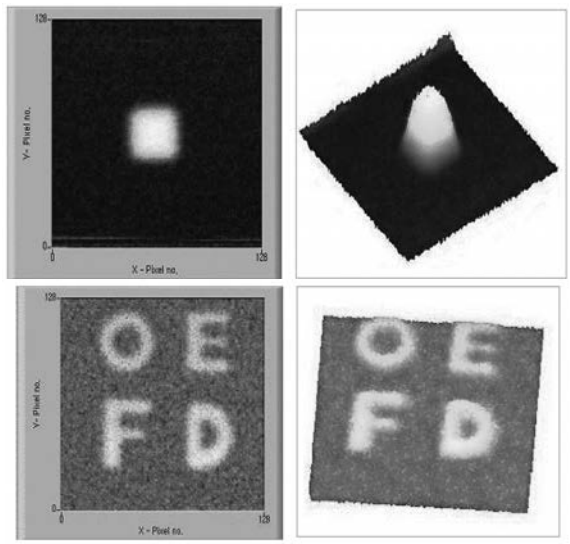


Fig. 9. Image characteristic of the LAPS devices using etched wafers.

surface is better than that of the Si_3N_4 surface. Also, we detected an image with a resolution of 128 × 128 pixels over a 1 × 1 cm area.

Therefore, the proposed LAPS-type sensor is expected to have useful applications in biochemical, environmental, military and other various fields.

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References

1 J. J. Caras: Anal. Chem. **52** (1980) 1935.
2 D.G. Hafeman, J. W. Parce and H. M. McConnel: Science **240** (1988) 1182.
3 G. Gehring, D. L. Patterson and S. Tu: Anal. Biochem. **258** (1998) 293.
4 C. Ercole, M. Del Gallo, M. Pantalone, S. Santucci, L. Mosiello, C. Laconi and A. Lepidi: Sens. Actuators, B **83** (2002) 48.
5 S. K. Bae, I. Y. Park, S. W. Kang and J. H. Cho: IEICE Trans. Electron E **86** (2003) 1109.
6 L. Bousse and S. Mostarshed: Sens. Actuators, B **17** (1994) 157.
7 Y. Ermolenko, T. Yoshinobu, Y. Mourzina, K. Furuichi, H. Iwasaki, Y. Vlasov and M. J. Schoning: Chemical Sensors **17** Supplement B (2001) 66.
8 S. Inoue, M. Nakao, T. Yoshinobu and H. Iwasaki: Sens. Actuators, B **32** (1996) 23.
9 Y. Ermolenko, T. Yoshinobu, Y. Mourzina, S. Levichev, K. Furuichi, Y. Vlasov, M. J. Schoning and H. Iwasaki: Sens. Actuators, B **85** (2002) 79.
10 L. Bousse, J. C. Owicki and J. W. Parce: Chemical Sensor Technology **4** (1992) 145.
11 Z. Q. Wei, C. Wang and C. L. Bai: Surface Science **467** (2000) 185.