A Phototransistor-Based High-Sensitivity Biosensing System Using 650-nm Light

Yu-Wei Chang, Yu-Ting Tai, Yang-Tung Huang, *Member, IEEE*, and Yuh-Shyong Yang

***Abstract*—A miniature optical biosensing system based on a PMOS phototransistor and absorption photometry is proposed. The phototransistor was manufactured in a standard 0.35-μm CMOS process, and it exhibited a responsivity higher than 1000 A/W for 650-nm light. For biochemical applications, the $\mathrm{TMB/H_2O_2/HRP}$ method was adopted as a useful basis in our system. A sample volume of only 10 μl was required to be dropped on the slide above the phototransistor. Experimental results demonstrated that a high sensitivity of 2.5 $\mu\mathrm{A/pM}$ was achieved, and the minimum HRP concentration successfully detected was 2.7 pM. This detection limit is three orders of magnitude better than that of a lately reported silicon biosensor, and is even comparable to that of a commercial spectrophotometer.**

***Index Terms*—Biomedical transducers, medical diagnosis, photodetectors, phototransistors.**

I. Introduction

A new generation of biochemical detection is expected worldwide owing to the rapid progress of biotechnology and microelectronics in the last decade. Among various kinds of detection methods, the detection of optical properties changed by chemical reactions is a competent approach to examine various important biological molecules [1], [2].

Horseradish peroxidase (HRP) is a popular enzyme that has been widely used in biochemical applications. While an antibody is used to recognize a target protein of interest, HRP can be conjugated to the antibody to serve as a label for determining the amount of the target [3]. HRP is usually used combining with hydrogen peroxide $(\mathrm{H_2O_2})$ to oxidize an added substrate that is luminescent or chromogenic. Reported optical methods for HRP detection include the $\mathrm{luminol/H_2O_2/HRP}$ reaction [4], the $\mathrm{ABTS/H_2O_2/HRP}$ reaction [5], and the $\mathrm{TMB/H_2O_2/HRP}$ reaction [6]. The end products of these reactions can either emit or absorb the light of specific wavelengths for analysis.

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For commercial instruments, a photomultiplier tube usually serves as the optical sensor in a spectrophotometer. Despite its high sensitivity, the spectrophotometer has limited applications in home care instruments because of its bulky size, high cost, and high voltage (about 1000 V) [7]. Since the development of a portable, reliable, inexpensive, and convenient biosensor is the most important niche in the health care industry [8], the silicon-based biosensing system with advantages of low cost and high throughput has become an attractive candidate for personalized diagnostic kits.

A conventional CMOS photodiode can easily be formed by utilizing a single $\mathrm{N^+/P_{well}}$, $\mathrm{P^+/N_{well}}$, or $\mathrm{N_{well}/P_{sub}}$ junction, but the responsivity is low [9]. Avalanche multiplication is a way to bring an internal current gain. However, the demand for high bias voltage also limits its use in many applications. Circumventing this difficulty, a CMOS phototransistor could have a current amplification under moderate bias through the internal transistor action [10].

In this research, a high-sensitivity phototransistor manufactured in a standard CMOS technology is proposed. Based on this phototransistor, a miniature optical biosensing system is developed. The optoelectronic measurements and biochemical experiments are also presented.

II. Principles and Methods

A. Biochemical Reaction

The $\mathrm{TMB/H_2O_2/HRP}$ reaction is adopted as the basis for biomedical applications in our system. TMB stands for $3,3',5,5'$-tetra-methyl-benzidine. It is neither mutagenic nor carcinogenic [11] and is a widely used reagent for ELISA (enzyme-linked immuno-sorbent assay).

The oxidation of TMB by $\mathrm{H_2O_2}$ with HRP can be easily observed for qualitative analysis. The reactant solution is visually light green, whereas the soluble end product is deep blue. After the addition of sulfuric acid $(\mathrm{H_2SO_4})$ to the media, this reaction would be stopped and result in a yellow product [12]. The process can be expressed as [13]

$$\mathrm{TMB} \xrightarrow{\mathrm{H_2O_2+HRP}} \mathrm{TMB^+} \xrightarrow{\mathrm{H_2SO_4}} \mathrm{TMB^{2+}}. \quad (1)$$

B. Absorption Photometry

For quantitative analysis, the analyte concentration can be evaluated by detecting the optical absorbance of the solution. The absorption spectra obtained from a standard spectrophotometer (Hitachi U-3310) show that the reactant TMB has an absorption peak at 280 nm, whereas the blue product $\mathrm{TMB^+}$

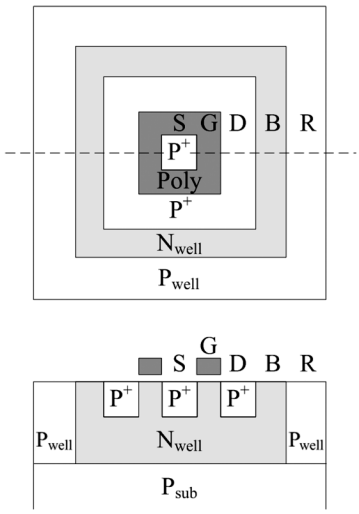

Fig. 1. The top view and cross section of the PMOS phototransistor.

has absorption peaks at 370 and 650 nm. With the presence of H_2SO_4, the yielded yellow product TMB^{2+} has an absorption peak at 450 nm.

The absorbance A of the materials is described by the Beer–Lambert law [2]

$$A = -\log\left(\frac{I}{I_0}\right) = \epsilon_\lambda \cdot l \cdot c \Longrightarrow A \propto c \tag{2}$$

where I_0 and I, respectively, denote the initial light intensity and the light intensity after passing through the material, ϵ_λ is the wavelength-dependent molar absorptivity in units of $\mathrm{l \cdot mol^{-1} \cdot cm^{-1}}$, l is the path length in units of cm, and c is the concentration of absorbing species in the material in units of M $(1\ \mathrm{M} = 1\ \mathrm{mol \cdot l^{-1}})$. When the light source and the path length are fixed, the absorbance would be proportional to the analyte concentration. Therefore, detecting the optical signals modulated by the biochemical reactions is an efficient way to quantitate the analyte of interest.

Regarding a commercial spectrophotometer, the used sample volume and the path length are about 1 ml and 1 cm, respectively. Referring to (2), when a shorter path length is used, the change in the absorbance becomes smaller, which makes the detection of concentration more difficult. For a miniature system, in order to further reduce the sample volume as well as the path length, the development of a high-sensitivity optical sensor is of great importance.

C. PMOS Phototransistor

The top view and cross section of the proposed PMOS phototransistor are shown in Fig. 1. The P^+ source placed in the center is surrounded by the polysilicon gate and the P^+ drain, while the N_{well} bulk (B) is enclosed by the P_{well} ring (R). It can be regarded as a PMOS with a photodiode connected across the bulk (B) and the square ring (R).

The PMOS phototransistor leaves the N_{well} bulk floating and uses the N_{well}/P_{sub} junction diode for the optical access [14]. The photogenerated carriers swept into the N_{well} would change the bulk potential, and thereby modulate the threshold voltagem as well as the output drain current.

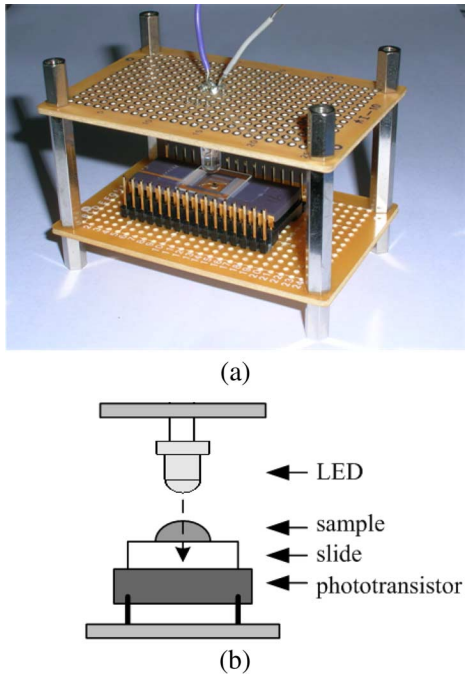

Fig. 2. The phototransistor-based biosensing system: (a) photogragh and (b) illustrated diagram.

The output drain current I_D of a MOSFET operated in the saturation region can be expressed as

$$I_D = \frac{1}{2}\frac{W}{L}\mu C_{\mathrm{ox}}(V_{GS} - V_T)^2\left(1 + \frac{V_{DS}}{V_A}\right) \tag{3}$$

where W, L, μ, C_{ox}, V_{GS}, V_T, V_{DS}, and V_A are the channel width, the channel length, the carrier mobility, the oxide capacitance per unit area, the gate-source voltage, the threshold voltage, the drain-source voltage, and the early voltage, respectively [10]. The shift amount of the threshold voltage due to the bulk charge effect is given by

$$\Delta V_T = \frac{\sqrt{2\varepsilon_s q N_B}}{C_{\mathrm{ox}}}\left(\sqrt{2\psi_B + V_{SB}} - \sqrt{2\psi_B}\right) \tag{4}$$

where ε_s, q, N_B, and ψ_B are the permittivity of silicon, the unit electric charge, the doping concentration of the bulk, and the bulk Fermi level from the intrinsic Fermi level, respectively; V_{SB} is the source-bulk voltage resulting from the optical access.

Using the proposed layout style, the sensing area of the outer ring photodiode is enlarged, so as to cause a larger shift amount of the threshold voltage. The channel length is also smaller than that of a conventional PMOS with the same area. Since the output drain current is inversely proportional to the channel length, a larger photocurrent response could be expected.

D. Biosensing System Setup

The proposed PMOS phototransistor with area of $100\ \mu\mathrm{m} \times 100\ \mu\mathrm{m}$ was manufactured using the TSMC (Taiwan Semiconductor Manufacturing Company) 0.35-μm standard CMOS technology. The photograph and the illustrated diagram of the whole biosensing system are shown in Fig. 2, and its dimensions are about $7\ \mathrm{cm\ (length)} \times 5\ \mathrm{cm\ (width)} \times 5\ \mathrm{cm\ (height)}$. A red LED (Centenary 31134) is used as the light source, and

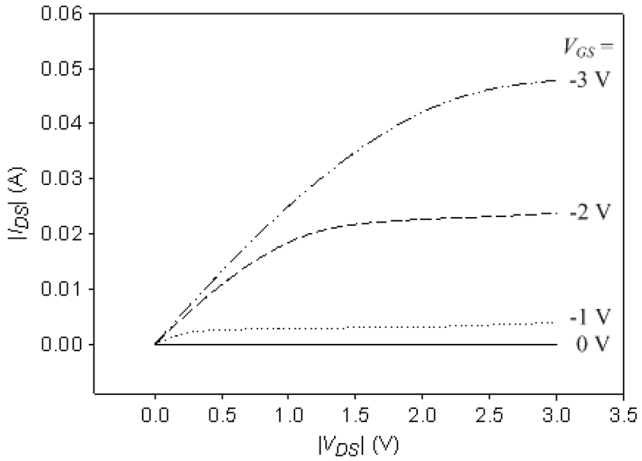

Fig. 3. The measured $I-V$ characteristics of the PMOS phototransistor.

a slide is placed above the PMOS phototransistor to hold the biochemical sample. A sample volume of only 10 μl is required for the detection. The path length l through the sample is about 0.1 cm.

The light traveling through the sample is modulated and then detected by the PMOS phototransistor, which converts the modulated optical signals into current signals. The current signals are measured using a precision semiconductor parameter analyzer (HP 4156A), which is connected to a personal computer via a general purpose interface bus (GPIB). An application software (Interactive Characterization Software, ICS) is used for data acquisition.

E. Reagent Preparation

TMB liquid substrate system, HRP-streptavidin, and phosphate buffered saline (PBS) powders were purchased from Sigma. Other chemical reagents were of analytical grade and used without further purification.

The HRP stock solutions (1 $\mu g/\mu l$) were prepared in a PBS buffer and stored at -20 °C. The stock enzyme solutions were melted in an ice bath just before use and diluted with double distilled water ($\mathrm{ddH_2O}$). The reactions were performed at 25 °C.

III. Experimental Results

A. Device Characteristics

While the proposed PMOS phototransistor was without illumination, the measured $I-V$ characteristics are shown in Fig. 3. The bias conditions were as follows: the source was grounded, the drain voltage swept from 0 to -3 V, the gate voltage stepped from 0 to -3 V, the bulk was floating, and the ring voltage was fixed at -3 V. It can be seen that for $V_{DS} = -3$ V, the phototransistor was operated in the saturation region. The larger $|V_{GS}|$ was, the larger $|I_{DS}|$ was.

While the phototransistor was illuminated, the output drain current was modulated. For $V_{DS} = -3$ V, the measured photocurrent responses under various illumination intensities of 650-nm light are shown in Fig. 4(a). The photocurrent response divided by illumination intensity can give the responsivity, and the results are shown in Fig. 4(b). For $V_{GS} = -2$ V and -3 V, the phototransistor exhibited a responsivity higher than 1000

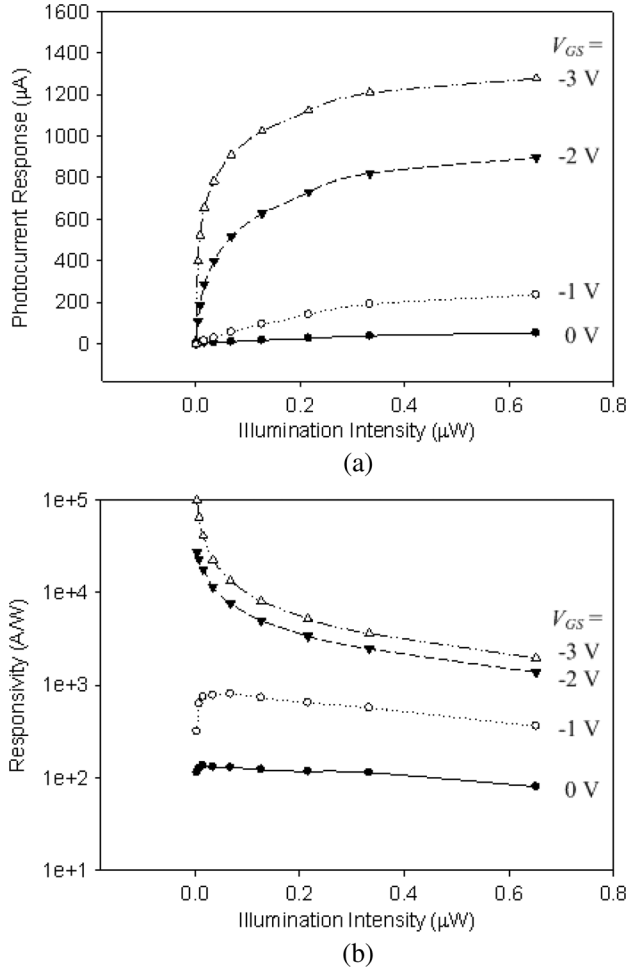

Fig. 4. The optoelectronic characteristics of the PMOS phototransistor for $V_{DS} = -3$ V with various V_{GS}: (a) photocurrent response versus illumination intensities and (b) responsivity versus illumination intensities.

A/W. The responsivity is 2000 folds higher than that of a traditional silicon P/N junction photodiode (about 0.5 A/W) [7].

Even for very small bias voltages such as $V_{DS} = -0.1$ V and $V_{GS} = 0$ V, the phototransistor can successfully detected the change of illumination intensity and exhibited a responsivity of about 50 A/W.

Two measurements with an interval of four months were performed, and the device dark current were observed to examine the device stability. For $V_{DS} = -3$ V and $V_{GS} = -2$ V, the drift amount of dark current after four months was 29.2μA, which is much smaller than the amplitude of photocurrent response (at least hundreds μA). For $V_{DS} = -0.1$ V and $V_{GS} = 0$ V, the drift amount of dark current after four months was 0.75 nA, which is also much smaller than the amplitude of photocurrent response (at least hundreds nA). Hence the stability of the proposed PMOS phototransistor could be acceptable.

B. Biochemical Detection

The system setup is illustrated in Fig. 2. A sample volume of only 10 μl was required to be dropped on the slide above the phototransistor. For the experiments performed using 650-nm light with different HRP concentrations, the measured photocurrent responses for $V_{DS} = -3$ V with various V_{GS} are shown in

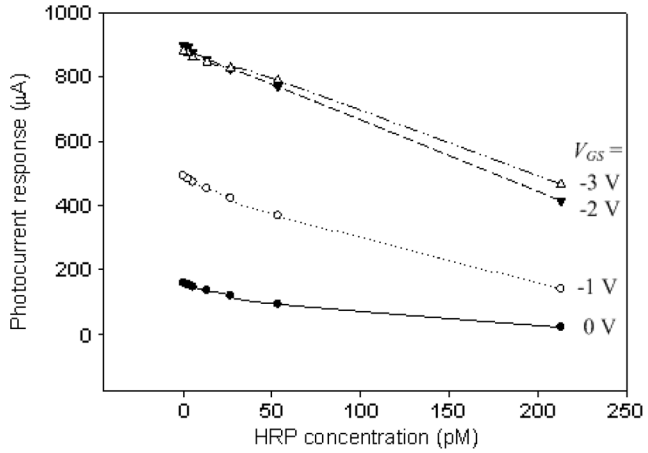

Fig. 5. The photocurrent response versus HRP concentration for $V_{DS} = -3$ V with various V_{GS}.

TABLE I
THE STATISTICAL INFORMATION OF THE PHOTOCURRENT RESPONSE $I_{\rm ph}$ AND THE ABSORBANCE A FOR $V_{DS} = -3$ V AND $V_{GS} = -2$ V UNDER VARIOUS HRP CONCENTRATION

	$I_{\rm ph}$ (μA)		Absorbance	
Conc. (pM)	mean	SD	mean	SD
buffer	901.0	1.140	-0.004	0.002
2.7	893.2	2.510	0.017	0.005
5.3	879.2	1.140	0.054	0.002
13.3	857.4	1.643	0.113	0.004
26.6	826.8	1.924	0.195	0.004
53.2	770.8	1.304	0.346	0.003
218.8	414.8	3.194	1.302	0.007
darkroom	0.0	1.342	2.417	0.003

(Conc.: concentration, SD: standard deviation)

Fig. 5. The used HRP concentration values were 0.0, 2.7, 5.3, 13.3, 26.6, 53.2, and 212.8 pM, respectively. Each data point is the average of five measurement results.

For $V_{DS} = -3$ V and $V_{GS} = -2$ V, the highest sensitivity of 2.5 μA/pM was achieved, and the linear relationship ranged from 2.7 to 212.8 pM with the coefficient of determination $R^2 = 0.9990$ calculated by an application software (Microsoft Excel). The statistical information of the photocurrent response $I_{\rm ph}$ is summarized in Table I. For the buffer solution, the $I_{\rm ph}$ mean is 901.0 μA and the standard deviation is 1.140 μA. For the 2.7-pM solution, the $I_{\rm ph}$ mean is 893.2 μA and the standard deviation is 2.510 μA. Since the difference in the $I_{\rm ph}$ mean is much larger than the standard deviation, the experimental results indicated that the minimum HRP concentration successfully detected was 2.7 pM. This detection limit is three orders of magnitude better than that of a lately reported silicon biosensor (2.4 nM) [15], and is even comparable to the limit obtained from a commercial spectrophotometer (Hitachi U-3310). The required sample volume of our system is also smaller.

For $V_{GS} = 0$ V, the phototransistor can sill be used for biochemical detection, and the measured $I - V$ characteristics under various HRP concentrations are shown in Fig. 6, with an arrow indicating the trend of increasing concentration. When the HRP concentration is increasing, the color of the end product become darker, which implies that more light will be absorbed, and thus the output drain current become smaller and closer to the condition in a darkroom. Even for very small bias voltages

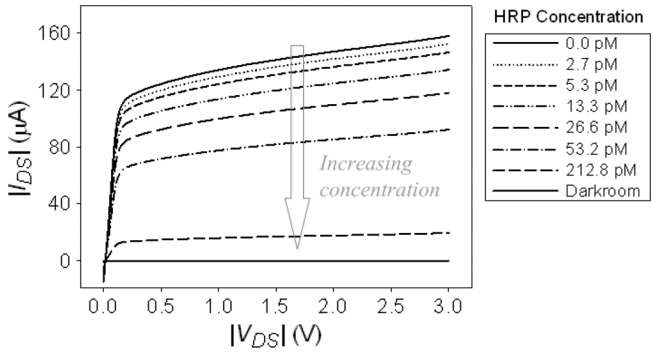

Fig. 6. The measured $I - V$ characteristics for $V_{GS} = 0$ V under various HRP concentrations.

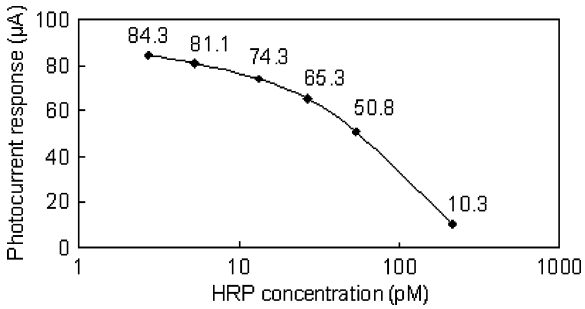

Fig. 7. The photocurrent response versus HRP concentration for $V_{DS} = -0.1$ V and $V_{GS} = 0$ V.

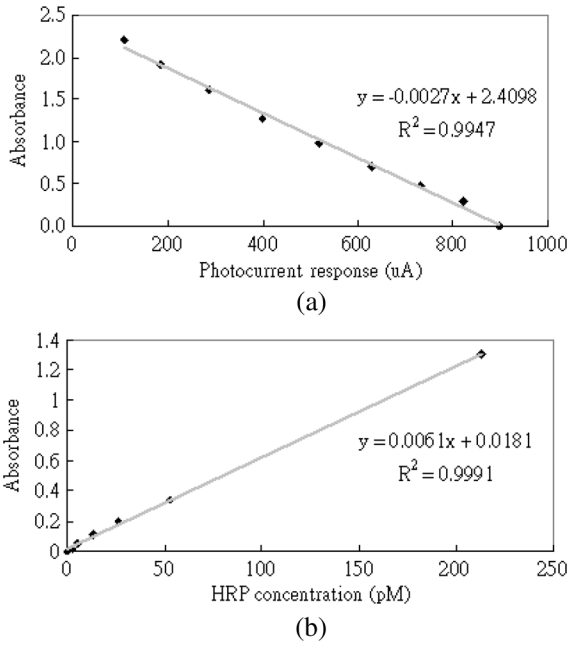

Fig. 8. The corresponding absorbance versus: (a) photocurrent response and (b) HRP concentration.

such as $V_{DS} = -0.1$ V, the experimental results shown in Fig. 7 also demonstrated a detection limit of 2.7 pM. The difference in $I_{\rm ph}$ mean between the buffer and 2.7-pM solution is 3.1 μA, which is much larger than the standard deviation of 0.1 μA.

To investigate the corresponding absorbances for various HRP concentration solutions, first, the relationship between the absorbance and the photocurrent response can be obtained by combining (2) and Fig. 4(a), and the results are shown in Fig. 8(a).

Then, combining Figs. 5 and 8(a) gives the relationship between the absorbance and HRP concentration. For $V_{DS} = -3$ V and $V_{GS} = -2$ V, the obtained results show that the linear detection range was from 2.7 pM to 212.8 pM with $R^2 = 0.9991$, which is depicted in Fig. 8(b). The statistical information of the absorbance A is summarized in Table I.

IV. Conclusion

Based on absorption photometry and a PMOS phototransistor manufactured in a standard CMOS technology, a high-sensitivity biosensing system has been presented. The whole system was assembled into a compact prototype, and the $\mathrm{TMB}/\mathrm{H_2O_2}/\mathrm{HRP}$ reaction was adopted as the basis for biomedical applications in our system. This system exhibited a high detection capability of 2.5 $\mu\mathrm{A}/\mathrm{pM}$, a great detection limit of 2.7 pM, a large linear detection range from 2.7 to 212.8 pM, and a low required sample volume of 10 μl. Therefore, the miniature CMOS optical biosensing system has great potential toward a practical home care instrument for personalized clinical diagnosis.

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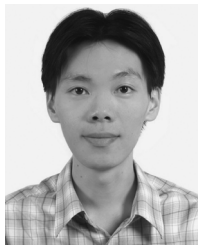

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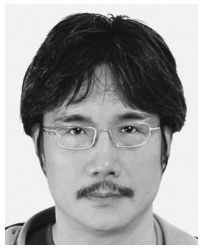

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