CMOS image sensor-based implantable glucose sensor using glucose-responsive fluorescent hydrogel

Takashi Tokuda,1,* Masayuki Takahashi,2 Kazuhiro Uejima,1 Keita Masuda,1 Toshikazu Kawamura,1 Yasumi Ohta,1 Mayumi Motoyama,1 Toshikiko Noda,1 Kiyotaka Sasagawa,1 Teru Okitsu,3 Shoji Takeuchi,3 and Jun Ohta1

1Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma, Nara 630-0192, Japan
2TERUMO Co., R&D Headquarters, 1500 Inokuchi, Nakai-machi, Ashigarakami-gun, Kanagawa 259-0151, Japan
3Institute of Industrial Science, University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan
*tokuda@ms.naist.jp

Abstract: A CMOS image sensor-based implantable glucose sensor based on an optical-sensing scheme is proposed and experimentally verified. A glucose-responsive fluorescent hydrogel is used as the mediator in the measurement scheme. The wired implantable glucose sensor was realized by integrating a CMOS image sensor, hydrogel, UV light emitting diodes, and an optical filter on a flexible polyimide substrate. Feasibility of the glucose sensor was verified by both in vitro and in vivo experiments.

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1. Introduction

Diabetes mellitus is one of the most common metabolic diseases all over the world, especially in advanced nations. The main symptom of the disease is high blood-glucose level, which results in complications such as kidney failure and diabetic retinopathy. For diabetic patients, controlling the blood glucose to prevent complications is the most important daily healthcare activity.

Currently, self-monitoring of blood glucose (SMBG) is widely used for daily checks by the diabetic patients [1]. To perform the SMBG measurement, a prick (typically at a fingertip) is made using a lancet device and a small amount of blood is sampled. The blood glucose is measured by a portable sensing device with a disposable sensor chip. Because blood sampling is required for measurements, SMBG cannot be used for the continuous monitoring of blood glucose in daily life. Although SMBG is an effective solution for monitoring diabetes, there is a demand for more frequent glucose measurements.

For some medical treatments, a glucose monitoring system with an indwelling sensor chip is used [1, 2]. The technology is called the continuous glucose monitoring system (CGMS). However, the life of a CGMS system is less than a week, and it cannot be used regularly in normal life because of its invasiveness and the risk of infection.

A small-sized, wireless, fully implantable glucose-sensing device is an ideal solution for continuous glucose monitoring with minimal invasiveness and infection risk. Many groups have been working on the development of such an implantable glucose-monitoring device.

In this work, we propose a CMOS image sensor-based implantable glucose sensor that is based on the previously reported technology of implantable CMOS image sensors. We use an optical sensing scheme in the sensor, which is another technical uniqueness of the proposed technology. Almost all the current glucose sensing products (SMBG and CGMS) and many next-generation glucose sensors under development use electrochemical glucose-sensing schemes using enzymes such as glucose oxidase (GOD) or glucose dehydrogenase (GDH) [3–6]. We adopt an alternative glucose measurement scheme, which is a fluorescence-based method that uses a glucose-responsive fluorescent hydrogel.

Concept, design, and fabrication of the CMOS image sensor-based implantable glucose sensor are described. Functional verification results from both in vitro (out-of-body) and in vivo (in-living-body) experiments are presented to show the feasibility of the proposed glucose monitoring technology.

2. Concept of the CMOS image sensor-based implantable glucose sensor

Figure 1 shows the concept of the CMOS image sensor-based implantable glucose-sensing system. The sensing system consists of a wireless fully implantable sensor device and an external controller. We drive the implant wirelessly from the external controller. Since the device will be fully implanted under the skin, the infection risk is significantly smaller than the current CGMS. In this work, we focus on a wired implantable glucose sensor for in vitro and in vivo experiments.

As mentioned in the previous section, most of the glucose-sensing technologies use enzyme-based electrochemical methods. The key components in electrochemical measurements are enzymes like GOD or GDH. Although they are not consumed in the sensing reaction, the performance of the sensors gradually degrade over the period of a few days or weeks.
In this work, we use the optical-sensing scheme that uses a glucose-responsive fluorescent hydrogel. Using the glucose-responsive fluorescent dye [7], Takeuchi et al. developed implantable hydrogel microbeads [8] and a hydrogel fiber [9]. The hydrogel shows fluorescence in the blue-green light region. Since the hydrogel has a phenylboronic acid structure that selectively interacts with glucose, the fluorescence intensity increases with increase in the glucose concentration [8]. They implanted the hydrogel in the ears of a rat and experimentally verified the fluorescence-based glucose sensing with an external light source and an external photosensor [8]. They used UV light with a peak wavelength of 405 nm for excitation, and the peak emission wavelength was 488 nm [8]. They also reported that the glucose sensing function was functional for at least 140 days after implantation. The results suggest that the glucose-responsive fluorescent hydrogel is a promising alternative to the enzymes. Because 140 days is still not sufficiently long for a long-term implant, they are working on further lifetime evaluation and realizing functional improvements in the glucose-responsive fluorescent hydrogel. An evaluation of the long-term biocompatibility and toxicity is also underway.

![Fig. 1. Concept of the CMOS-based implantable glucose-sensing system using glucose-responsive fluorescent hydrogel.](image)

In this work, we integrate the hydrogel with the light emitting diodes (LEDs) (excitation light sources) with an implantable CMOS image sensor (fluorescence sensor) in a miniaturized implantable device. The combination of the excitation light source, fluorescent hydrogel, and sensor enables glucose measurement in living tissue without an external light source or a detection device. We expected an improvement in the measurement stability, because the relative position of the excitation light source, fluorescent gel, and sensor are kept stable, unlike the earlier case where the excitation and the observation was done using external devices. With this architecture, we also expect a reduction in the UV exposure on tissue. Because the wavelength of the excitation UV is around 400 nm, which is slightly shorter than visible light, its risk is limited. However, to reduce the risk of UV exposure, the exposure time should be as short as possible. We can operate the UV LED synchronously to the CMOS sensor. Moreover, because our CMOS image sensor can complete a single-frame measurement within 100 ms, the UV exposure time can be set to less than 100 ms for each measurement.

We have previously developed implantable CMOS image sensors for biomedical observation platforms, especially for functional and molecular imaging of the brain [10–12]. In this work, we use the same kind of sensor to detect the fluorescence of the glucose-responsive hydrogel. The detailed sensor design, packaging structure and process is presented in the next section.
3. Design and fabrication of the CMOS image sensor-based implantable glucose sensor

3.1 Design of the implantable CMOS image sensor for glucose sensing

The core of the implantable glucose sensor is the CMOS image sensor that detects fluorescence from the glucose-responsive fluorescent hydrogel. We designed a small, dedicated CMOS image sensor using the 0.35 µm-rule 2-poly, 4-metal standard CMOS technology.

Figure 2 shows (a) layout, (b) block diagram, and (c) schematic of the pixels of the implantable CMOS image sensor used in the implantable glucose sensor. Table 1 lists the sensor specifications. Pixel size is 7.5 µm × 7.5 µm, and the sensor has a 30 × 60 (or 90) pixel array. The size of the sensor is 320 µm × 790 µm (or 1125 µm). The sensor is operated by four wires, three input wires (VDD, GND CLK) and one output wire (VOUT). The sensor was designed for wired operation with DC power supply between VDD and GND, and a driving clock (CLK). The pixel values are streamed out of the output as an analog voltage, VOUT. VOUT is transferred to an external control board, and converted into digital values using a commercially available analog-to-digital converter chip and recorded by a PC. The voltage range of the sensor output is approximately 1 V, while typical noise levels are greater than 1 mV. Therefore, to prevent quantization noise, we require an ADC with a 10-bit resolution.

![Diagram of the implantable CMOS image sensor.](image)
Table 1. Specifications of the CMOS image sensor

| Specification            | Value                                      |
|--------------------------|--------------------------------------------|
| Device process           | 0.35 µm 2-poly, 4-metals standard CMOS    |
| Sensor size              | 320 µm × 790 µm (or 320 µm × 1125 µm)      |
| Pixel size               | 7.5 µm × 7.5 µm                            |
| Array size               | 30 × 60 (or 30 × 90)                       |
| Pixel circuitry          | 3-Tr Active Pixel Sensor (APS)             |
| Operating voltage        | 3.3 V                                      |

As shown in Fig. 2(c), the pixel circuitry is a conventional active pixel sensor (APS) [13] that is commonly used in image sensors designed for standard CMOS processes. The APS operation is generally described as an accumulation measurement, in which the photoelectrons generated by incident photons are accumulated in a specific exposure (photocarrier integration) period [13]. The light intensity was measured as follows. Prior to starting the light detection, a 3.3-V (VDD) voltage was applied on Φ_RST to turn on the reset transistor M₁ and V_PD; thus, the potential of the N-region (cathode) of the pixel photodiode (PD) is increased to V_RST. Then, M₁ was opened with Φ_RST = low (0 V) to start the “exposure.” During the exposure, photo-generated electrons are accumulated in the N-region of the PD, resulting in a drop in V_PD. After a specific exposure period, we can read out V_PD via three series-connected source followers (column and horizontal readouts, and the output buffer shown in Fig. 2(c)) to determine the integrated light intensity. The vertical and horizontal scanners in Fig. 2(b) generate the digital pulses Φ_X_SEL and Φ_Y_SEL shown in Fig. 2(c). To capture an image, we select one row and readout pixel values in the row. After all the pixel values in the selected row were read out, we reset the row, and select the next row for readout. All the pixels perform the accumulation of photo-generated electrons during the other rows are accessed for readout and reset. This operating sequence gives once-per-frame resetting with equal exposure time, but start and end timings are different between rows, which is called as “rolling-shutter” operation. The pulses Φ_X_SEL and Φ_Y_SEL are used to select the pixels one by one to be read out. By adjusting the exposure period, we can change the sensitivity. In general, a greater exposure period gives a higher sensitivity, but it trades-off against the frame rate of the image sensor. The sensing performances of our active pixel sensor circuit were presented in our previous publication [14].

As is the nature of a conventional CMOS image sensor, the sensitivity curve of the present sensor covers near-UV, visible, and near-IR wavelength regions. Unlike consumer or most scientific and industrial imaging applications, the glucose-sensing scheme presented in this work does not require high frame rate. Approximately 10 fps is adequate. Furthermore, neither a high sensitivity nor a wide dynamic range is necessary. In most cases, we can increase the fluorescence intensity by using a higher excitation intensity to compensate for the limited sensitivity of the CMOS image sensor. The most important factor that determines the sensing performance of the fluorescence-based glucose sensor is the suppression ratio of the on-chip filter used to eliminate the scattered excitation light. This is described in the next subsection.

3.2 Device structures for in vitro and in vivo functional verification

In the proposed glucose-sensing scheme, when the glucose-responsive fluorescence gel is excited with UV light (typically λ ~400 nm), it emits blue-green fluorescence with a peak wavelength of 488 nm. To realize this measurement scheme in a single device, we assembled the core hardware of the sensor as shown in Fig. 3(a). The implantable CMOS image sensor and the excitation light sources (UV LEDs) were integrated on a flexible polyimide substrate.
We used a commercially available LED chip with the following characteristics; size of 300 µm square, peak wavelength of 400 nm, total emission power of 10 mW with a drive current of 20 mA. A UV-cut, long-path film filter was attached to the pixel array on the CMOS image sensor to eliminate the scattered excitation light.

The LED chips were bonded on the polyimide flexible substrate next to the CMOS image sensor along the long side of the sensor, as shown in Fig. 3(a). The alignment of the LEDs causes a nonuniform illumination pattern, resulting in nonuniform fluorescence from the glucose-responsive hydrogel. However, this nonuniformity provides a significant advantage for the proposed glucose sensor. As same as conventional image sensors, the CMOS image sensor used in the present work has a limited dynamic range. If light intensity is too high, the pixels show saturation. The nonuniform LED illumination causes a variation in excitation intensity along the long side of the CMOS image sensor for the sensor structure shown in Fig. 3(a). We can choose the pixels so that the glucose-responsive fluorescent hydrogel provides an appropriate signal range that is sufficient for fluorescence evaluation, but not enough to saturate.

The CMOS image sensor needs to detect the fluorescence from the hydrogel to measure the glucose concentration. Since the pixel can detect both the UV excitation and blue-green fluorescence, we integrated an on-chip filter to eliminate the scattered UV excitation light. We used a commercially available blue-cut film filter (60% transmission at 522 nm, and less than 1% transmission at 488 nm). The film filter was cut to fit the pixel array and bonded onto the CMOS image sensor.

Fig. 3. Structure of (a) the core hardware, and the devices for (b) basic in vitro, (c) semi-chronic in vitro, and (d) in vivo functional verification.
Using the same core hardware (Fig. 3(a)), we fabricated three kinds of CMOS image sensor-based glucose-sensors: two for \textit{in vitro} functional evaluations and the other for \textit{in vivo} experimental verifications (Fig. 3(b), 3(c), and 3(d), respectively). As shown in Fig. 3(b), we used a simple, open-structured sensor for the basic \textit{in vitro} functional evaluation. A preformed 3 mm \times 5 mm \times 1.5 mm block of the glucose-responsive fluorescent hydrogel was attached on the core hardware. A 30 \times 90 version of the image sensor was used for this experimental phase. After the basic functionality was confirmed using the device (see Fig. 3(b)), we attached a stainless-steel tube to keep the glucose-responsive fluorescent hydrogel on the CMOS image sensor. To verify the glucose monitoring functionality in semi-chronic \textit{in vitro} experiments, we used the device packaging shown in Fig. 3(c) because of the more stable placement of the sensor device in the experimental solution. For the \textit{in vivo} experimental verification, we assembled the sensor device with a polyimide outer tube with slits (see Fig. 3(d)). This sensor device was designed to be used in an ear of rat, like the experiments performed in Ref. 8 and 9. A 30 \times 60 version of the image sensor was used for the sensor devices shown in Fig. 3(c) and 3(d).

In the \textit{in vivo} experiments, glucose in subcutaneous interstitial fluid was measured to estimate the blood glucose. The stainless-steel inner tube was used as the structural element, and the polyimide outer tube was used to keep the glucose-responsive fluorescent hydrogel within the sensor. 125 \, \mu m wide and 1.4 mm long slits were made at 500 \, \mu m intervals on the polyimide outer tube to introduce glucose to the glucose-responsive fluorescent hydrogel. In order to fill the fluorescent hydrogel in the tube structure, we injected a monomer solution and polymerized the hydrogel in the assembled sensor device.

4. Functional evaluation of the implantable glucose sensor
4.1 Basic sensing properties evaluated in acute \textit{in vitro} experiments

At first, the basic glucose measurement capability of the proposed implantable glucose-sensor was characterized in acute \textit{in vitro} experiments. The sensor shown in Fig. 3(b) was dipped in a saline solution, and the glucose concentration of the solution was increased from 0 to 250 mg/dL.

Fig. 4. Images taken during the \textit{in vitro} glucose measurement experiment. Images were taken in W/B, and shown in pseudo-color. Line profiles are also shown. A 30 \times 90 version of the image sensor was used.
The glucose-responsive fluorescent hydrogel was illuminated by the UV excitation light and fluorescence images filtered by on-chip optical filter were taken by the CMOS image sensor. Figure 4 shows the typical images obtained from the acute in vitro experiments. Line profiles in the images are also shown in Fig. 4, and the axes are the same for the four profiles. Since the UV LEDs for excitation were placed next to the shorter sides of the pixel array, the intensity detected near the shorter sides was greater than that detected at the center.

We can choose one or more pixels for measurement, as indicated in Fig. 4. Figure 5 shows the detected light intensity as a function of the glucose concentration. The positions of the pixels are shown in Fig. 4.

Near the shorter sides, the pixel saturates and no information is obtained about glucose concentration (see the plot for pixel 5). The output values for pixels 1–4 show a linear increase with the glucose concentration. The rate of increase (coefficient) depends on the position of the pixel. The pixels at the center of the array (pixels 2, 3, 4) show a smaller rate of increase in comparison to pixel 1, which is near the saturated area. These plots show the feasibility of the proposed CMOS image sensor-based implantable glucose sensor.

![Graph showing sensor output levels for pixels 1–5 as a function of glucose concentration. The LEDs were operated with 1 mA.](image)

In addition to the measurement functionality, Fig. 5 shows the advantage of the proposed glucose sensing method using the CMOS image sensor. As shown in Fig. 5, the sensor has a limited dynamic range (a value range of 0–1200), and the measurement depends on the pixel position. Even without changing the excitation intensity or the exposure period for the APS operation, we can perform glucose measurements at multiple sensitivities. For small glucose-sensitivity, it is reasonable to use a pixel value in the high-sensitivity area, which is the pixel near the “saturation border.” To measure a wide dynamic range of glucose concentration, the central part of the pixel array can be used.

In this work, we used the image sensor to understand the distribution of the fluorescence from the gel. Based on the results shown in Figs. 4 and 5, a line sensor can be used for fluorescence detection in the proposed glucose sensing scheme. Furthermore, there is another option to use a single or limited number of pixels for fluorescence measurements. The reduction in the number of pixels leads to a reduction in the power consumption. However, such single-pixel architecture requires more controlled LED and sensor operation to maintain the fluorescence signal within a suitable range. We believe that a linear sensor is the most suitable sensor type for the next step.

4.2 Evaluation of glucose-monitoring capability in semi-chronic in vitro experiments

After gaining a better understanding of the positional dependence of the fluorescence intensity and basic behavior of the CMOS-based implantable glucose sensor, we performed...
semi-chronic *in vitro* experimental evaluations. To keep the glucose-responsive fluorescent hydrogel on the sensor, we used the device packaging shown in Fig. 3(c). We installed the sensor core hardware in a cell-like structure made with plastic, and filled it with the fluorescent hydrogel. The sensor was stored in saline solution without glucose at 36°C. We performed an *in vitro* glucose monitoring experiment in which we increased and decreased the glucose concentration at intervals of two weeks.

Figure 6 shows a typical glucose-monitoring trace obtained in a semi-chronic *in vitro* experiment. The sensor was dipped in saline solution, and the glucose concentration was increased from 0 to 300 mg/dL in 50 mg/dL steps, and then decreased to 0 deg in 100 mg/dL steps. As shown in Fig. 6, the sensor output traces the glucose concentration with a transition time of 15-20 min while both increasing and decreasing the concentration. This trace suggests that the sensor has the capability to detect changes in the glucose concentration whether it is increasing or decreasing. We also confirmed that the drift of the sensor output is as small as 69/h in the sensor output value, which corresponds to an error of approximately 2.8 mg/dL per hour. As mentioned in subsection 4.4, we consider that the proposed implantable monitoring device should be used with periodic calibration using SMBG, and the present result is acceptable as an indication of the performance of the prototype device.

**Fig. 6.** Typical glucose-monitoring trace obtained in the semi-chronic *in vitro* experiment.

**Fig. 7.** (a) Sensitivity curves for glucose-monitoring trials in the semi-chronic *in vitro* experiments, and (b) sensitivity changes due to operation and storage in saline at 36°C.
Figure 7(a) shows the sensitivity curves (relationship between sensor output and glucose concentration) for the monitoring trials in the semi-chronic experiments. We performed the experiments for up to 47 days. Figure 7(b) shows the sensitivity, which was estimated from the inclinations of the plots in Fig. 7(a). The sensitivity shows fluctuations of 16% (in standard deviation) between the trials. We consider this mismatch to be due to the positional and shape instability of the glucose-responsive fluorescent hydrogel, and this should be improved in future works.

We confirmed that the sensor works correctly after operating for 47 days, and we observed no significant decrease in the sensitivity. This result is consistent with the semi-chronic in vivo evaluation for the glucose-responsive fluorescent hydrogel [9]. The present result suggests that there was no significant performance deterioration due to CMOS sensor integration, and we expect that the lifespan of the CMOS-based implantable glucose sensor will be determined by the life of the glucose-responsive fluorescent hydrogel.

4.3 Setup and experimental procedure for an acute in vivo glucose sensing experiment

We performed an acute in vivo functional verification using a rat. As described in subsection 3.2, we assembled the core hardware and the glucose-responsive fluorescent hydrogel in a tube structure (see Fig. 3(d)).

We implanted the sensor under the skin on the rat's ear. The position of the sensor was the same as that used in the Ref. 8, and 9. We used a similar insertion procedure for the proposed CMOS-based glucose-sensor as that for the fiber-shaped fluorescent hydrogel [9].

Fig. 8(a) shows the rat's ear with the implanted glucose sensor, and Fig. 8(b) shows the experimental procedure.

The animal experiment consisted of two parts: device implantation and functional evaluation. The device implantation and the functional evaluation were performed on the same day. The functional evaluation experiment was performed about an hour after the implant surgery. During the functional evaluation, the rat was anesthetized with Isoflurane and the glucose concentration in the interstitial fluid was measured with the proposed glucose sensor. The blood glucose measured using a commercially available SMBG device every 5 minutes was used as control data.

An intraperitoneal injection of glucose solution and an intraperitoneal injection of insulin 40 min after the glucose injection were performed as glucose-changing events. We expected that the blood glucose of the rat would show a steep increase because of the injected glucose followed by a marked decrease because of the insulin. After the experiment, the rat was appropriately sacrificed without causing unnecessary pain. All the animal experiments were performed following the guidelines of The University of Tokyo.
4.4 Results of the in vivo glucose sensing experiment

Figure 9 shows the results of the in vivo experiment. The round marks in Fig. 9 show the glucose concentration measured by the proposed CMOS-based implantable glucose sensor. The circles show the fluorescence intensity measured at the center \((x = 15, y = 30)\) of the CMOS image sensor with a LED current of 3 mA. The square marks show the blood glucose measured using the conventional SMBG device. Currently, the sensor measures the glucose level of the interstitial solution, and not the blood glucose, which is the same as CGMS technology. Therefore, we have to calibrate the sensor output with the blood glucose level measured by SMBG (or some other blood glucose measurement technology). In Fig. 9, the plot of the sensor output was scaled to be the same in the vertical direction as that of the blood glucose measured by SMBG. As shown in Fig. 9, an increase and a decrease of the glucose level caused by the intraperitoneal injections of glucose and insulin were observed. The results show the capability of the proposed implantable glucose sensor to measure the glucose level in a living body.

However, Fig. 9 indicates an issue with the proposed implantable glucose sensor system. A time-lag of 60 min was observed between the measured results for the proposed sensor and the SMBG device. The time lag is partly due to the nature of the glucose level change in the interstitial fluid. It is reported that the glucose level of the interstitial fluid reflects the blood glucose level with a lag of 5–15 min (typical) [15]. The measured lag of 60 min is longer than the typical transition lag in the glucose level in the interstitial fluid. We suspect i) delay in the diffusion of the blood glucose into the glucose-responsive fluorescence hydrogel, ii) limited opening area of the polyimide outer tube, and iii) placement of the measurement face as possible reasons of the greater time lag.

In order to solve the time lag issue, we are currently modifying the device structure and the application protocol. We will reduce the hydrogel thickness, increase the opening area of the polyimide tube, and alter sensor placement in the body.

In addition, the plot for the proposed sensor in Fig. 9 has lacked regions around 5min and 25min due to an operation error in the sensor driving system. It is an instrumental issue to be overcome in future revisions.
5. Conclusions

A novel implantable glucose-sensor was proposed and experimentally verified. A small-sized implantable CMOS image sensor with integrated UV LEDs and optical long-path filter was used to measure the fluorescence of glucose-responsive hydrogel. We fabricated glucose sensors for in vitro and in vivo functional characterization. The glucose sensing capability was experimentally verified by the in vitro experiments. We also successfully performed an acute in vivo glucose monitoring by implanting the sensor in a rat's ear tissue. The capability of the CMOS-based implantable glucose sensor was experimentally verified. It was also seen that a lag of 60 min was observed in the in vivo operation, which should be resolved in the future work. We are also currently working on the development of a CMOS line sensor-based glucose sensor.

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