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Bioluminometry by CMOS-based active pixel photodiode array with accurate background noise compensation

Yoshiaki Yazawa, Masao Kamahori[1] and Hideki Kambara[1]

Hitachi, Ltd., Advanced Research Laboratory
[1]Hitachi, Ltd., Central Research Laboratory
1-280, Higashi-Koigakubo Kokubunji-shi, Tokyo 185-8601, Japan
Phone: +81-42-323-1111 E-mail:yyazawa@rd.hitachi.co.jp

1. Introduction

Bioluminescence is increasingly being used for a variety of analysis in the chemical, biological and medicine fields [1, 2]. By this technique, DNA sequencing and SNP typing can be done with simple equipments through this technique compared to widely used methods such as fluorescent detection and electrophoresis, because neither excitation light sources nor sophisticated optics are required. This report describes a CMOS-based active pixel photodiode array [3] for bioluminometry are described. As for addressing pixels in a photodiode array, the random access concept, rather than sequential access, is desirable when flexible signal processing is required [4]. The array has 30-pixel photodiodes, any two of which are randomly addressable at the same time by two independent decoders. One is for a target sample, and the other is for a reference selection. We used the array in a bioluminometric assay to quantify the amount of ATP and this revealed a linear response for molecular range of 50 amol-200 fmol due to accurate background compensation by the simultaneous double addressing architecture.

2. Experimental and Results

The architecture of photodiode array

The system architecture for the array is in Fig.1 and it has 30 pixels and two independent decoders. The pixels consist of photodiodes with a large active sensing area of 2 x 2 mm, corresponding to the shape of the sample reaction well to achieve high light-collection efficiency. Photodiodes are operated in a charge-integration mode, and hence the captured number of photons is converted to the change in cathode voltage. Each pixel is an active cell with a source-follower MOS transistor that transfers the signal voltage of the photodiode to the output terminal. Each pixel has also a reset MOS transistor and two selector MOS transistors controlled by an s-decoder to select the target-sample-pixel and a c-decoder to select the control pixel. Two arbitrary wells on the sample plate can be selected as a target and a control. This is useful in highly accurate back-ground compensation, which is especially important in measuring bioluminescence that is inherently accompanied by a considerable level of back-ground light emission. Figure 2 is a photograph of the fabricated photodiode array in its ceramic package.

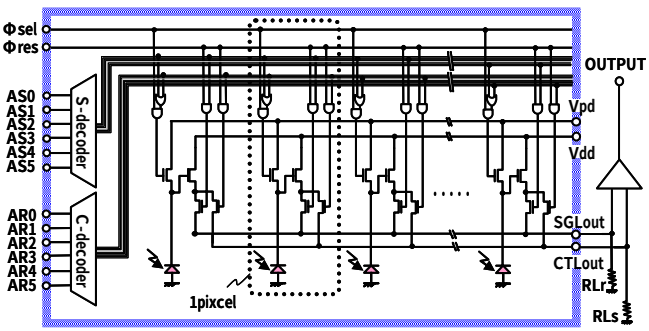

Fig. 1 General architecture of photodiode array.

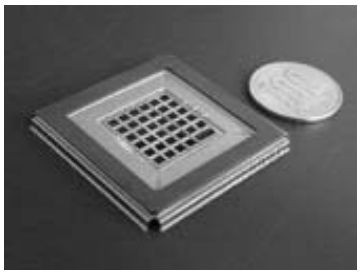

Fig. 2 Photograph of packaged chip.

Bioluminescence measurement

When ATP (adenosine triphosphate) acts on luciferin in the presence of luciferase, bioluminescence emission occurs. Through this reaction, expressed as follows, the amount of ATP can be quantified with simple measuring apparatus consisting of photodetectors, which is expressed as follows.

$$ATP + luciferin + O_2 \xrightarrow{luciferase} AMP + PPi + oxyluciferin + CO_2 + h\nu \quad (1)$$

For example, DNA sequences or SNPs can be analyzed using this reaction [1, 2]. When an extension reaction occurs on single strand DNA producing complementary DNA, inorganic pyrophosphate (PPi), which is transformed into ATP under the presence of APS (adenosine phosphosulfate) and ATP sulfurylase, is released.

$$APS + Pyrophosphate \xrightarrow{ATP\ sulfurylase} ATP + SO_2^{2-} \quad (2)$$

The DNA sequence can be analyzed by measuring the transformed ATP. In our experiment, the amount of ATP measured with the photodiode array is described for common ground DNA analysis.

Figure 3 illustrates the experimental setup for the ATP assay. The ATP solution is instilled with a capillary dispenser into the reaction well that has a diameter of 2 mm and contains 4 µl of solution including luciferin and

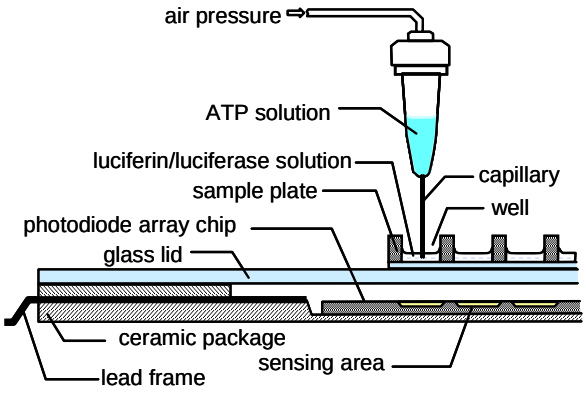

Fig. 3 Schematic setup for ATP assay.

luciferase. The amount of dispensed ATP is precisely controlled to 0.05 µl-accuracy by air pressure and the time this pressure is applied. The sample plate is simply placed on the glass lid of the photodiode array, which does not need any light collecting optics. As the detector is located just 3.1mm under the sample solution, hence an optical coupling efficiency of 3% is achieved. The transient behavior of bioluminescence reaction is dependent on the luciferase concentration. The time resolved light signals for different luciferase concentration are shown in Fig. 4. Since

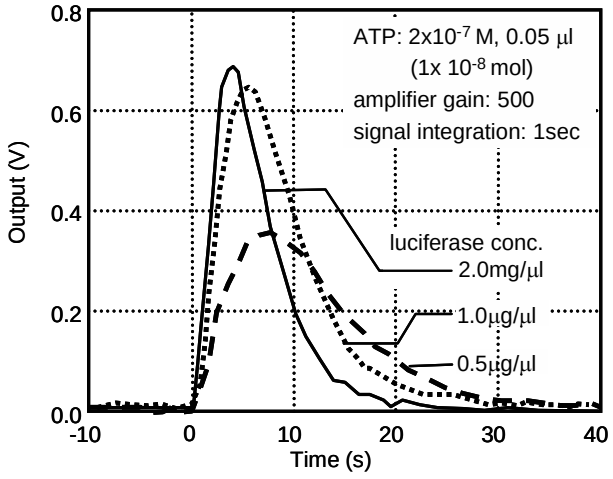

Fig. 4 Dependence of photodiode output on time for dispensing ATP to luciferin/luciferase solution.

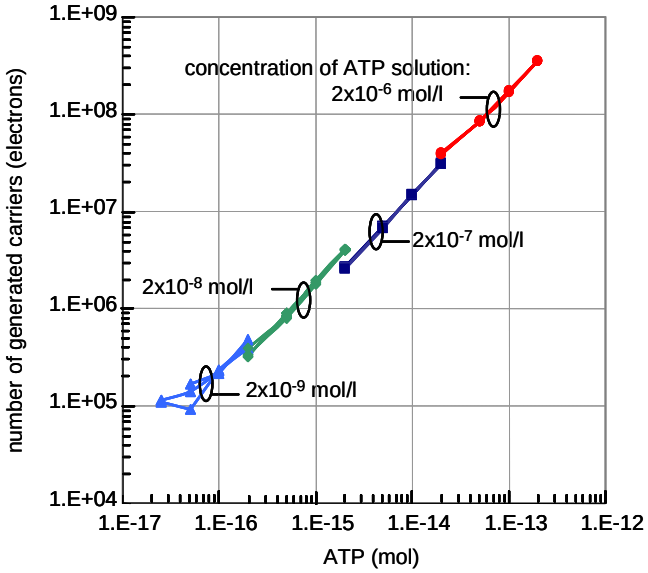

Fig. 5 Dependence of generated carriers on numbers of ATP molecules.

the peak widths are about 10 s, emission profiles can be traced well by setting the integration time at 1 s. Figure 5 shows the dependence of generated carriers on the number of ATP molecules. ATP solutions at four concentrations were used and dispensed to the reaction well by changing the volume of the solution. There was a linear response over a molecule ATP range that was almost four orders of magnitude i.e., 50 fmol-200 fmol. The measurement accuracy was about 50 fmol probably resulting from reset-switching noise, rather than thermal noise which was estimated to be $4x10^4$ electrons due to large photodiode capacitance.

3. Conclusions

We developed a photodiode array with a simultaneous double pixel addressing architecture, and we used this in a bioluminometrical assay to quantify the amount of ATP and this revealed the minimum number of detectable molecules to be 50 amol and a linearity range of 50 amol-200 fmol due to accurate background compensation.

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