Bioimaging Using LSI-based Amperometric Biosensing System with 400- Electrodes

Kumi Y. Inoue,[*ab] Kosuke Ino,[b] Mustafa Şen,[b] Masanori Nakano,[b] Kosuke Takara,[b] Reyushi Kubo,[b] Masahki Matsudaira,[a] Atsushi Suda,[c] Ryota Kunikata,[c] Hitoshi Shiku,[abd] and Tomokazu Matsue[abd]

[a]Micro System Integration Center (μSIC), Tohoku University, 519-1176 Aramaki-Aza-Aoba, Aoba-ku, Sendai 980-0845, Japan

[b]Graduate School of Environmental Studies, Tohoku University, 6-6-11 Aramaki-Aza-Aoba, Aoba-ku, Sendai 980-8579, Japan

[c]Japan Aviation Electronics Industry, Limited, 3-1-1, Musashino, Akishima-shi, Tokyo 196-8555, Japan

[d]The World Premier International Research Center Advanced Institute for Materials Research (WPI-AIMR), Tohoku University, 2-1-1 Katahira, Aoba, Sendai 980-8579, Japan

We have developed an LSI-based amperometric sensor called “Bio-LSI” with 400 measurement points as a platform for electrochemical bio-imaging and multi-point biosensing.[1)] The system is comprised of a 10.4 mm ×10.4 mm CMOS sensor chip with 20×20 unit cells, an external circuit box, a control unit for data acquisition, and a DC power box (Fig. 1A-E). Each unit cell of the chip contains an operational amplifier with a switched-capacitor type I-V converter for in-pixel signal amplification (Fig. 1F). We successfully realized a wide dynamic range from ±1 pA to ±100 nA with a well-organized circuit design and operating software. The spacial resolution is 250 μm and the temporal resolution is 18-125 ms/400 points, which depends on the desired current detection range. The coefficient of variance of the current for 400 points is within 5%. An improved version of Bio-LSI (2G-Bio-LSI) has a mode-selective function, which can individually specify each mode of 400-electrodes from V1, V2, electrometer, or off mode. This system enables complex measurements and operations.

We demonstrated some real-time imaging using Bio-LSI[2)]. One is a demonstration to detect a respiration activity of HepG2-cell spheroids using Bio-LSI. Living spheroids and formaldehyde-fixed spheroids (negative control) were put on a Bio-LSI chip filled with ERAM-2 buffer (Research Institute for the Functional Peptides, Japan) (Fig. 2A). Then, we simultaneously stepped the potential of 400-platinum electrodes on the Bio-LSI from +0.10 V to −0.50 V vs. Ag/AgCl at t=0 s and monitored currents generated by reductions of dissolved oxygen on the electrodes. Figure 2B shows a color map indicating current changes from t=−5 s to 10 s. Figure 2C shows amperograms at electrodes under the living (L1-3) and fixed (D1, 2) spheroids. As shown in these figures, the oxygen concentrations under the living spheroids were lower than that under fixed spheroids because of respirations of the living cells. This result indicates a promising use of the Bio-LSI as a device for drug screening on tailor-made medicine.

Another demonstration is an imaging using a mode-selective function of 2G-Bio-LSI. A 2G-Bio-LSI chip was covered with 3 mL of 2.0 mM ferrocenemethanol (FcOH). First, 0.00 V vs Ag/AgCl was applied to all 400 electrodes for 5 min. Then, the potential of electrodes indicating white in Fig 3A was stepped to + 0.50 V to oxidize the FcOH, while that indicating blue remained 0.00 V. Figure 3B and C show redox currents on 400 electrodes after 1 s and 50 s from the potential step, respectively. We successfully imaged that the oxidized FcOH at the electrodes applied 0.50 V was gradually diffused and reduced at the electrodes applied 0.00 V.

We are now under development of practical application of Bio-LSI. Our Bio-LSI is a promising tool for a wide range of analytical fields, including diagnostics, environmental measurements, and basic biochemistry.

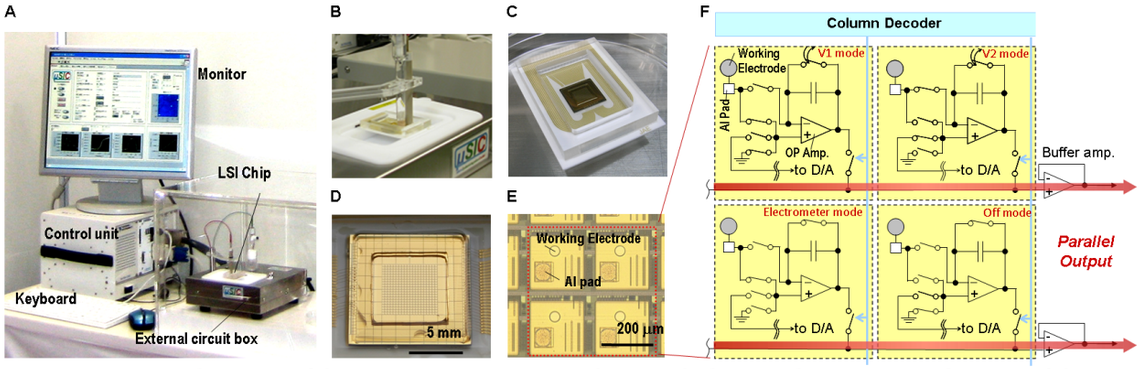

Figure 1. (A) Complete view of the Bio-LSI system. (B-E) Photograph showing the experimental setting of the Bio-LSI chip unit with counter and reference electrodes. (C) Bio-LSI chip unit with acryl well to introduce liquid samples. (D) Bio-LSI mounting on unit chip and insulated with PDMS . (E) Microgram of unit cells. (F) Circuit diagram of unit cells.

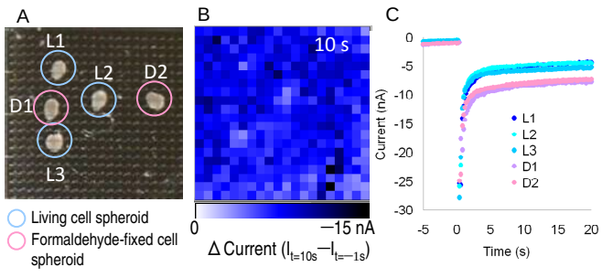

Figure 2. Respiration activity of living (○) and formaldehyde-fixed (○) HepG2 cell spheroids was detected by chrono-amperometry. The potential of platinum working electrodes was stepped from +0.10 V to −0.50 V vs. Ag/AgCl at 0 s.

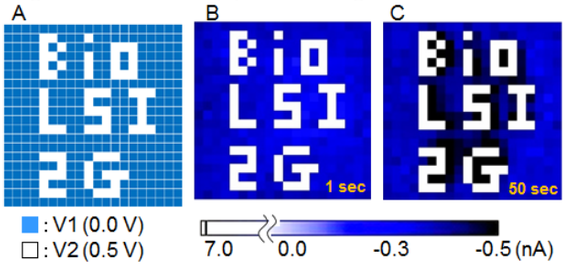

Figure 3. Demonstration of mode-select function (V1 mode and V2 mode) of Bio-LSI. Redox molecule was 2.0 mM ferrocenemethanol.

References 1) Kumi Y. Inoue, *et al.*, Lab Chip 12 (2012) 3481. 2) Mustafa Sen, *et. al.*, Biosens. Bioelectron., in press