Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Demonstration of bioimaging using bipolar electrochemical microscopy improved in spatial resolution

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We compared the experimental result shown in Fig. 5B to a result of mathematical simulation of oxygen concentration gradient, in order to assure that the ECL disappearance under the living spheroid in Fig. 5B are not only due to oxygen diffusion inhibition, but also due to the respiratory activity of living cells, even if the size of the living spheroid were larger than that of the dead spheroid shown in Fig. 5A. The diameters of the dead spheroid and the living spheroid were estimated to be 210 µm and 259 µm, respectively from Fig. 5A using Image J software[1-2]. We conducted a mathematical simulation of oxygen diffusion using COMSOL Multiphysics (COMSOL AB, Sweden) when two spherical substances with diameters of 210 µm and 259 µm were put on a substrate spaced by 2513 µm. In this simulation, the two spherical materials were assumed to be oxygen impermeable. By this simulation, the case where both spheroids have no respiratory activity and the elimination of ECL is caused only by oxygen diffusion inhibition was reproduced in the calculation. In the simulation, oxygen concentration in the bulk was set to 0.209 mM, oxygen concentration on the bottom was set to 4.93×10^{-3} mM, and the oxygen diffusion coefficient was set to 2.1×10^{-9} m^2/s. The employed value of oxygen concentration on the bottom (4.93×10^{-3} mM) was calculated from the simulation by COMSOL Multiphysics, under the condition that electrodes with a diameter of 8 µm are arrayed at intervals of 40.8 µm.

Fig. S1 shows the result of the simulation of the oxygen concentration gradient on the bottom. Fig. S1A shows the simulation well reproduces the inhibition of oxygen concentration gradient by the oxygen-impermeable spherical substances. Fig. S1B is a one-line plot of oxygen concentration gradient on a line connecting two contact points of the spherical substances to the bottom (Fig. S1A white dotted line).

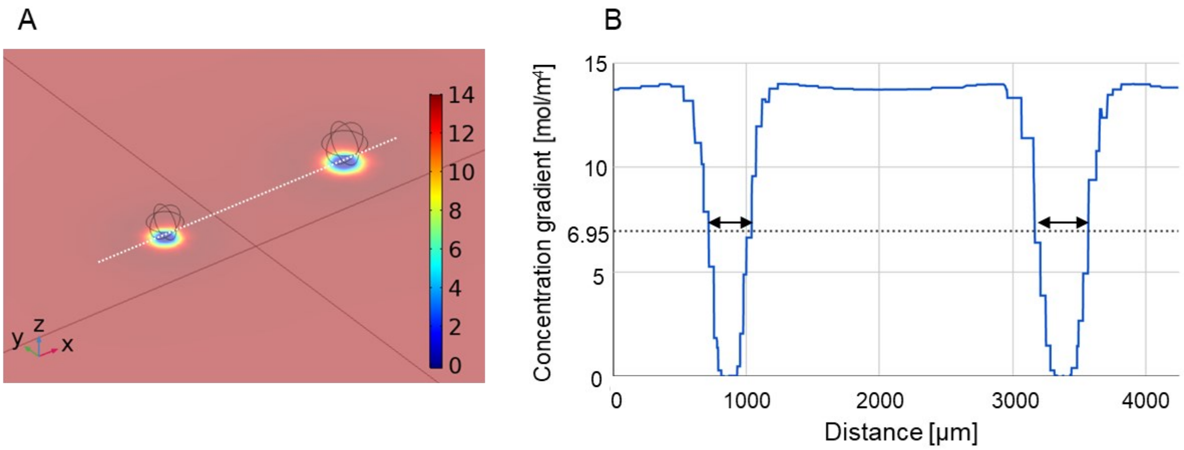

Fig. S1 A) Simulated oxygen concentration gradient at the bottom on which two oxygen-impermeable spherical substances with diameters of 210 µm and 259 µm were put as dead spheroids. B) One-line plot of oxygen concentration gradient (blue line) at the white dotted line on Fig. A. The baseline (black dotted line) is the middle value between the maximum and minimum concentration gradient values. This simulation represents only the oxygen diffusion inhibition effect; therefore, the respiratory consumption effect is not considered.

Fig. S2A is the experimental result re-posted from Fig. 5B. We made one-line plot of ECL intensity along with a yellow line on Fig. S2A using Image J software[1-2] (Fig. S2B). The noisy plot was obtained because the ECL was occurred only on the dispersed electrodes.

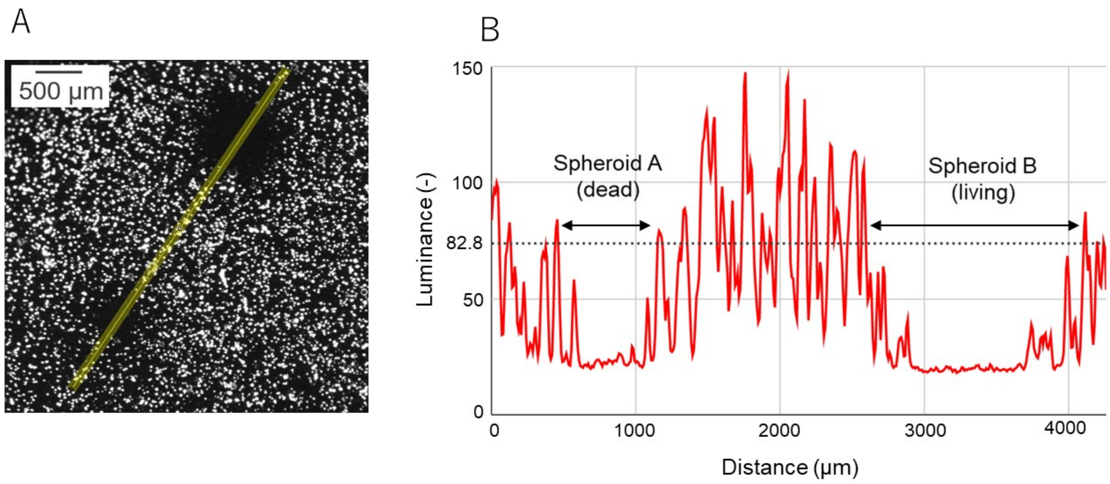

Fig. S2 A) Experimental result of ECL under the cBPE array with live (top right)/dead (lower left) spheroids (re-posted from Fig. 5B). B) One-line plot of the ECL intensity along with the yellow line of Fig. S2A. The baseline (black dotted line) is the middle value between the maximum and minimum luminance intensities.

To compare the simulated result (representing only the oxygen diffusion inhibition effect) to the experimental result (one spheroid having respiratory activity), we summarized the result of the simulation and experimental results using the baselines of Figures S1 and S2 to evaluate the width of oxygen diffusion inhibition and ECL elimination. The effect of inhibiting oxygen diffusion by spheres of different sizes and the effect of reducing ECL signals by dead/living cell spheroids and are shown using ratio and difference values. The difference (also ratio) between Spheroid A and Spheroid B in the ECL elimination width (experimental) is large compared to the size difference (ratio) and the oxygen diffusion inhibition width difference (ratio). This result clearly shows that spheroid B is consuming oxygen by respiration, resulting in a wider ECL elimination width in experimental condition.

Table S1 Comparison of the decrease of ECL signal in Fig. 5B and the simulated effect of oxygen diffusion inhibition of the spheres in different size.

	Spheroid A (µm)	Spheroid B (µm)	**Ratio (-) (B/A)**	**Difference (µm) (B-A)**
Diameter of the spheroid	210	259	**1.233**	**49.0**
Oxygen diffusion inhibition width (simulated)	327.7	403.9	**1.233**	**76.2**
ECL elimination width (experimental)	857.1 (Dead)	1526.8 (Living)	**1.781**	**669.7**

References
1. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012.
2. Schneider, C.A., Rasband, W.S., Eliceiri, K.W. "NIH Image to ImageJ: 25 years of image analysis". Nature Methods 9, 671-675