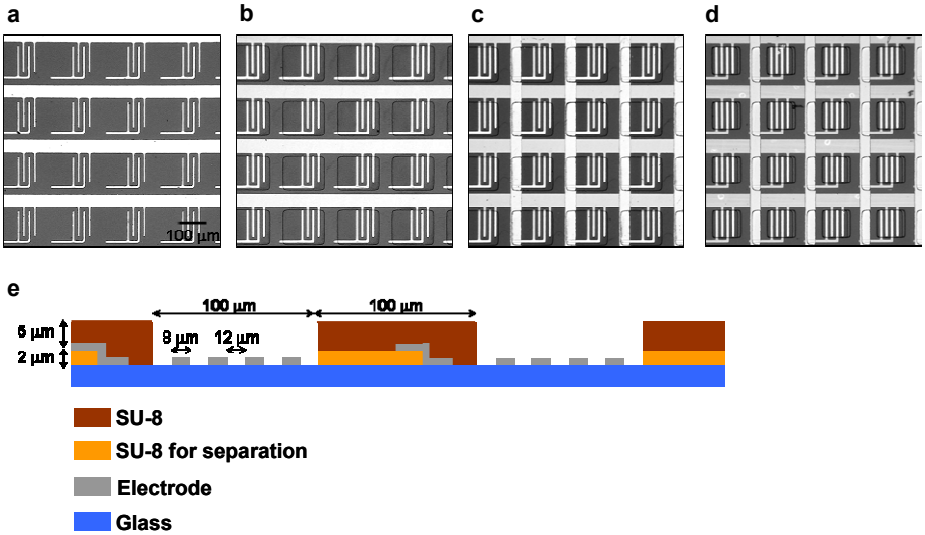
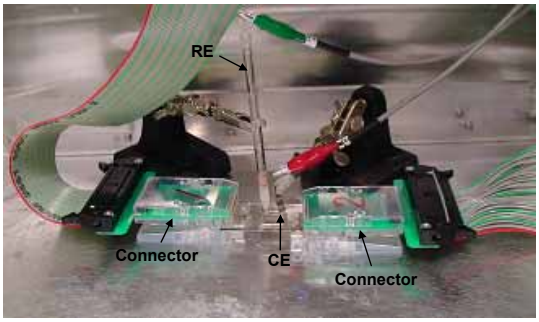
Supporting Information Figures



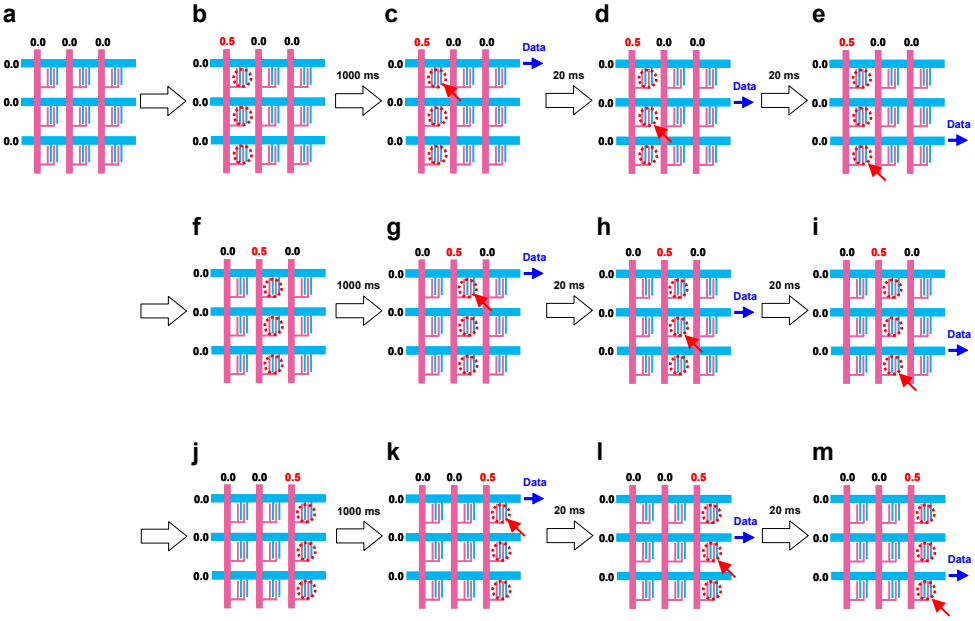
Supporting Information Figure 1

(a-d) Image of the device at each stage of the fabrication process shown in Fig. 2. (e) Illustration of the microwell cross-section.



Supporting Information Figure 2

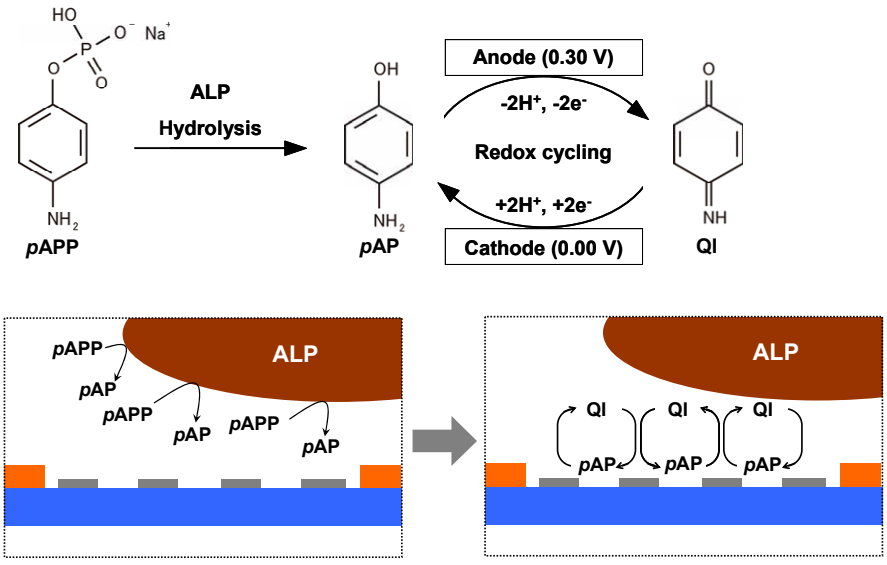
Clip connectors were attached to the device and the sample solution was introduced onto the device. The Ag/AgCl reference electrode and the counter electrode were inserted into the sample solution.



Supporting Information Figure 3

Scheme for imaging based on electrochemical reaction of FMA. This figure shows the procedure to detect electrochemical responses at the 3×3 crossing points. (**a**) All electrodes were held at 0.00 V. (**b**) The first column electrode was stepped from 0.00 to 0.50 V. FMA was oxidized at the first column electrode, and the oxidation product (FMA^+) was reduced back to FMA at the row electrodes. The red dotted circles indicate the areas where redox cycling was induced. (**c**) After waiting 1000 ms for stabilization of the current, the reduction current at the first row electrode was acquired. The red arrow indicates the detection point. (**d**) After waiting 20 ms for switching the electrodes with the multiplexer, the reduction current at the second row electrode was acquired. The electrochemical signals at the first column electrode were sequentially acquired (**b-e**). (**f**) After measuring the responses at the first column electrode, the first column electrode was stepped back from 0.50 to 0.00 V and the second electrode was stepped from 0.00 to 0.50 V. The electrochemical signals at the sensor points of the second column electrode were sequentially acquired (**g-i**). The electrochemical signals at the sensor points of the third column electrode were sequentially acquired (**j-m**). 32×32 crossing points with IDAs were formed at the device and electrochemical signals from 1024 points were obtained using this measurement process. The change in the current compared with the background signal was considered as the current response at the sensor microwells. The time required to obtain all 1024 response data was within 1 min for FMA.

In this study, *p*AP produced from *p*APP by ALP catalysis was also detected for acquiring the images of ALP using the system (Supplementary Figure 4). For measuring *p*AP, the column electrode was stepped from 0.00 to 0.30 V, the waiting time for stabilization of the current was 2000 ms, and the waiting time for switching the electrodes with the multiplexer was 200 ms. The time required to obtain all 1024 response data was within 4.5 min for *p*AP.



Supporting Information Figure 4

For electrochemical imaging of enzyme activity, 4.0 mM *p*APP in pH 9.0 HEPES buffer solution was introduced into the device. The ALP/BSA aggregate (approximately 1-mm diameter) was added and floated on the solution. *p*APP was catalytically hydrolyzed by ALP to yield *p*AP, which oxidized at the anode (+0.30 V). The oxidation product, QI, is reduced back to *p*AP at the cathode (0.00 V) and diffused to the anode to be oxidized. The redox cycling between the anode and cathode amplifies the electrochemical signal from *p*AP produced by ALP.