Electronic Supplementary Material (ESI) for RSC Advances.


**Supplementary Information**

# A new methodology for optical biosensing with drop-casting fabrication of sensor chips and irradiation/detection of a single laser beam

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## Reproducibility of the glucose detection

Temporal changes of reflection intensities were repeatedly measured at the same condition to evaluate the reproducibility of the glucose detection shown in Figure 5. A 10 μL drop of glucose (100 and 10 μM) solutions was injected in each hole on the sensor chip and a green laser beam (2 mW) was focused on the surface. The temporal change of the reflected laser intensity was measured by a photomultiplier and the output voltage was recorded at a sampling rate of 50 Hz. Figure S1 shows three curves of temporal changes measured at same glucose concentrations. The curves taken at 100 μM glucose concentration show larger changes in a shorter irradiation time than those at 10 μM concentration, indicating that a higher glucose concentration gives a larger morphological change of the film surface in a shorter time. However shapes of four curves measured at the same concentration are not completely same. This corresponds to the morphological difference among four protrusions produced at same glucose concentrations as shown in Figure 4 and causes error bars in Figure 5. Local inhomogeneity of the drop-cast film may be attributed to this morphological difference. The reproducibility could be improved by the further optimization of the film preparation.

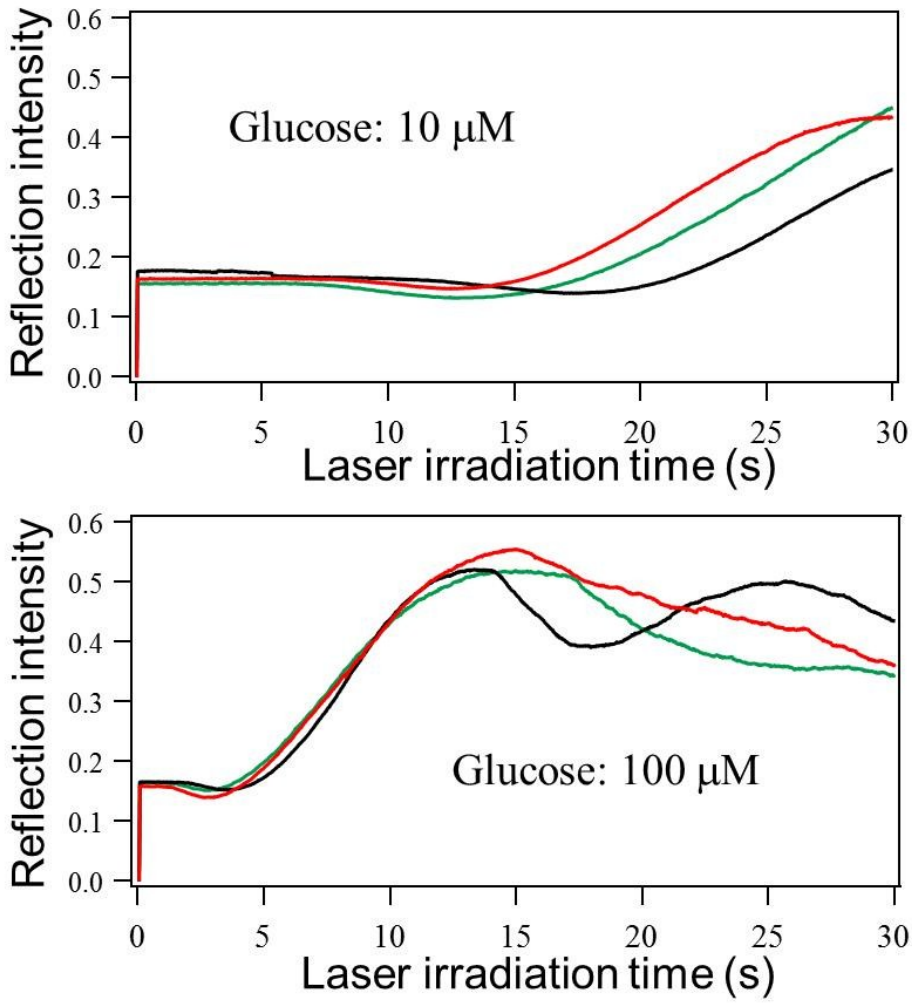


Figure S1

Temporal changes of the reflection intensity of the focused green laser beam at (a) 10 μM and (b) 100 μM glucose concentrations. The measurements were performed three times at the same concentrations. The sensing spot was changed and fresh glucose solution was applied for each measurement to evaluate the reproducibility.

**Laser wavelength dependence of the glucose detection**

The polymerization of oPD induced by focusing a laser beam is responsible for the change of the laser reflection intensity. We tested the laser wavelength dependence of the glucose detection by using blue (473 nm), green (532 nm), and red (632.8 nm: He-Ne laser, Coherent 31-2066-000) lasers. Specifications of the blue and green lasers are described in the main text. The intensity of each laser was optimized in order to give the highest sensitivity (blue: 0.1 mW, green: 2 mW, red: 2 mW at the laser focus). In this experiment, 20 μL of glucose solution was added to the same amount of enzyme solution, which was prepared by dissolving GOD and HRP in the citrate buffer. After incubation for 1 min, 20 μL of 1 mM o-PD was mixed in it. 10 μL of the mixed solution was dropped on a glass plate, and the laser beam was focused on it. Figure S2(a)-(c) show temporal variations of the reflection intensities of different laser beams. Reflection curves depended on the glucose concentration in all cases. Peak times vs glucose concentrations are shown in Figure S2(d). The detectable range of the glucose concentration was different. The red laser beam gives the poorest sensitivity for the glucose detection. The limit of detection with the blue laser beam was better than that with the red one, but slightly worth than that with the green one. The first peak of the reflection intensity of the green laser appeared in a short time as compared to those of other laser beams at the same glucose concentration, meaning that the green laser beam is suitable for the fast and sensitive detection. This dependence of the laser wavelength is discussed as follows.

Figure S3 shows an absorption spectrum of 2,3-diaminophenazine (DAP). After the addition of glucose on the chip, DAP molecules are produced in the solution due to HRP enzyme reaction. The laser reaction is initiated by the light absorption of DAP. From this point of view, the blue laser beam should induce the most rapid and largest morphological change. However the red laser beam gave the best result as shown in Figure S2. This suggests that the absorption band of the laser irradiated area shifts to the longer wavelength region due to the elongation of the π-conjugation by the polymerization. This is supported by the fluorescence spectral change in Figure 3. The light absorption by the polymerized moiety is important for the morphological change.

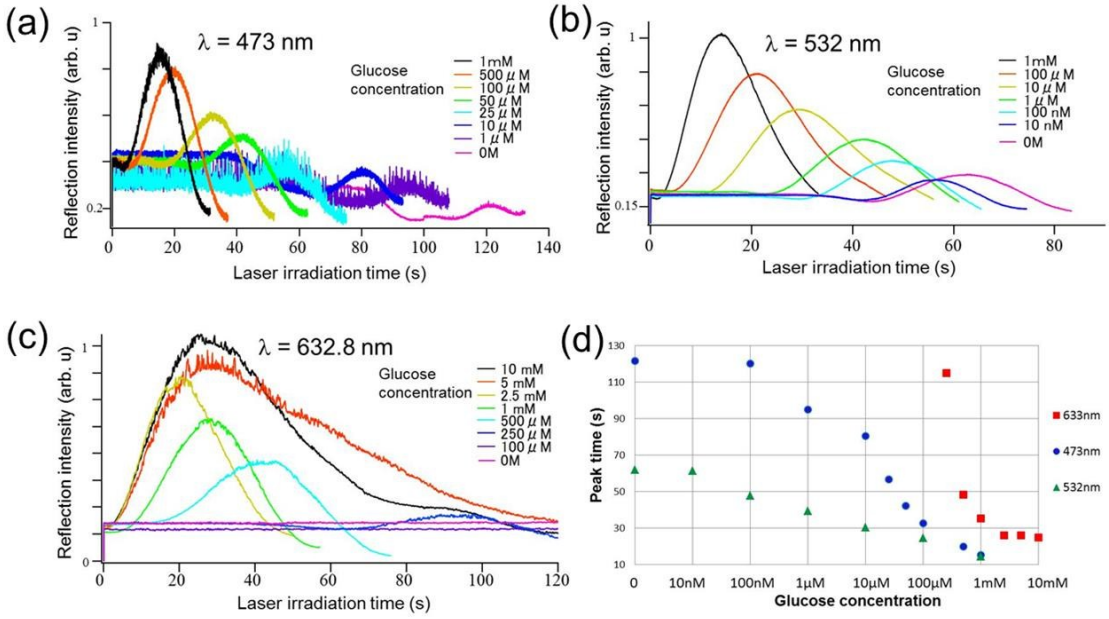


Figure S2

Temporal changes of the reflection intensity of focused laser beams from sensing spots. Laser wavelengths are (a) 473 nm, (b) 532 nm, and (c) 632.8 nm. (d) Peak times of temporal variation curves vs glucose concentrations evaluated by using laser beams with different wavelengths (circle: 473 nm, triangle: 532 nm, square: 632.8 nm).

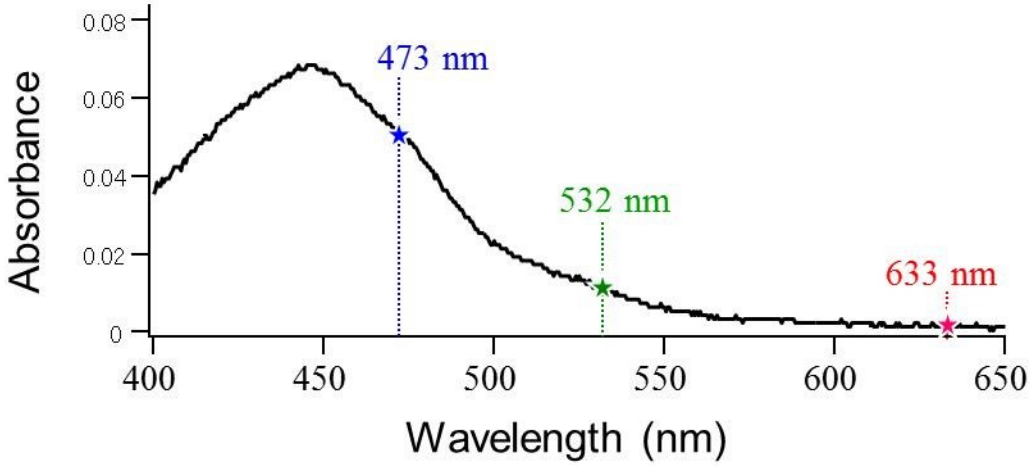


Figure S3

Absorption spectrum of 2,3-diaminophenazine produced in an o-PD solution.