Evaluation of antigen-antibody reaction on suspended graphene by optical interferometry
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INTRODUCTION
Graphene-based chemical and biological sensors are received considerable attention because of its excellent mechanical and electrical properties such as ultrathin film, high Young’s modulus, and high electron mobility. In particular, the individual molecule of CO₂ has been detected on a bilayer graphene bridge using resistivity change by molecular absorption [1]. Molecular selectivity is necessary to apply suspended graphene as a sensor. However, chemical functionalization on the bridge graphene for selective molecular detection is difficult because the liquid trapped in the gap of the structure breaks easily the suspended graphene by surface tension force. To solve this problem, we have previously fabricated a suspended graphene with drum structure by using low-pressure dry-transfer technique [2]. The cavity sealed structure by the suspended graphene allows wet process for chemical functionalization [3]. In this study, we evaluated antigen-antibody reaction on the suspended graphene, which deformed upward by Coulomb repulsive force of adsorbed antigen. We also demonstrated real time and label-free molecular detection on the suspended graphene by using optical interferometry in liquid.

DEVICE FABRICATION
Fig.1 shows the fabrication process of suspended graphene by using low-pressure dry-transfer technique. Polymethyleneacrylate (PMMA) was coated on chemical vapor deposited graphene on a Cu foil and pasted polydimethylsiloxane (PDMS) to handle the PMMA/graphene/Cu sheet. Second, Cu was etched with FeCl₃ solution. After drying, PDMS/PMMA/graphene put on a Si substrate with pre-patterned cavities and heated on a hotplate at 150 °C in a vacuum chamber of 1.0 atm gauge pressure. After removing PMMA by NMP-based solvent at 60 °C, the graphene chip was dried by supercritical CO₂ drying to avoid break of the suspended graphene.

EVALUATION BY OPTICAL INTERFEROMETRY
Fig. 2 shows the suspended graphene developed by dry-transfer process. Optical microscopy image is found to be blue color which is attributed optical interference of the nanocavity caused by slight reflection of the suspended graphene [4]. Fig. 3 shows the reflection spectrum of the suspended graphene measured by microscope spectroscopy. Optical interference property is evaluated by using the following equation [5]:

\[
R = \frac{\sqrt{r_g r_s} (1 - \sqrt{2 r_g r_s}) \cos \frac{4 \pi d}{\lambda} + (\sqrt{r_g r_s} + \sqrt{r_g r_s})^2}{1 - 2 \sqrt{r_g r_s} \cos \frac{4 \pi d}{\lambda}}
\]  

(1)

where \(R\) is reflectance, \(\lambda\) is wavelength, \(d\) is a gap length between the suspended graphene and the Si substrate, \(r_g\) and \(r_s\) are reflectance, \(t_g\) and \(t_s\) are transmittance of the suspended graphene and the Si substrate, respectively. As a result of calculation using the equation (1) with the gap length of 375 nm, it is in good agreement with the measurement result as shown in Fig. 3. The result suggests that the gap length under the suspended graphene can be evaluated by optical interferometry.

MOLECULAR DETECTION EXPERIMENT
For antibody immobilization on the suspended graphene surface, we used the noncovalent functionalization using 1-pyrenebutanoic acid succinimidyl ester (PBSE). First, the graphene chip was soaked in PBSE solution with a concentration of 1 mg/mL for 60 minutes. After that, the graphene chip was rinsed with phosphate buffered saline (PBS). Second, the graphene chip was soaked in anti-bovine serum albumin (BSA) antibody solution with a concentration of 100 µg/mL for 60 minutes. Then, the antibody modified graphene chip rinsed with PBS. Finally, antibody modified graphene chip was soaked in BSA antigen solution and human serum albumin (HSA) antigen solution with a concentration 10 µg/mL, respectively. Fig. 5 shows the change in interference spectrum in each solution measured by optical interferometry. The peak position shifts from the initial position by 34 nm in the BSA antigen solution and by 19 nm in the HSA antigen solution. In addition, interference color of the suspended graphene changes from yellow to red in BSA antigen solution. From the obtained spectrum shift, the change in the gap length is calculated by using the equation (1), and the gap length increases by 18 nm and 9 nm in BSA antigen solution and HSA antigen solution, respectively. BSA treated suspended graphene is much deformed, which is attributed that a large number of molecules is immobilized. These results suggest that the BSA antigen is selectively adsorbed on the suspended graphene surface by antigen-antibody reaction and deformed upward by Coulomb repulsive force. In conclusion, we demonstrated selective molecular detection by evaluation of antigen-antibody reaction on suspended graphene using optical interferometry.

REFERENCES
Fig. 1 Process overview of low-pressure dry-transfer of CVD graphene.

Fig. 2 (a) Optical microscope image and (b) scanning electron microscopy image of suspended graphene with drum structure.

Fig. 3 Typical reflection spectrum of suspended graphene with nanocavity.

Fig. 4 Spectral shift in (a) BSA antigen solution and (b) HSA antigen solution.

Fig. 5 Displacement of suspended graphene caused by molecular adsorption.