

## Surface analysis of SH-SAW immunosensors using displacement penetration effect into specimens

粘性侵入効果を用いた SH-SAW 免疫センサによる表面解析

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### 1. Introduction

Shear horizontal surface acoustic wave, SH-SAW, based sensor simultaneously measures multiple physical quantities of liquid specimens, such as density, viscosity, permittivity, and conductivity. Taking advantages of these features, SH-SAW sensors have been applied to wide varieties of sensing applications. An SH-SAW biosensor is a form of SH-SAW sensor applications. The SH-SAW biosensor detects immunoreactions happen on its sensing surface as velocity and/or amplitude change of SH-SAW. Besides, the SH-SAW biosensor has sensing property determined by depth of sensing surface, which is called a viscous penetration depth. The displacement penetration, denoted  $\delta$ , is determined by the viscosity,  $\eta_{liq}$ , and density,  $\rho_{liq}$ , of the specimen and angular frequency of sensor device,  $\omega$ , which is expressed as eq. 1.

$$\delta = \sqrt{\frac{2\eta_{liq}}{\rho_{liq}\omega}} \quad (1)$$

In the previous SH-SAW biosensor studies, structures of measuring objects have not been taken into consideration. In other words, mass of measuring objects has been treated as concentrated into a limited volume on the sensor surface. However, since antigens and antibodies actually have three-dimensional structures, adding vertical information into evaluation is considered to contribute in more precise immunoreaction analyses. In order to clarify sensing characteristics of SH-SAW biosensors in height direction, in this report, we evaluate a correlation between SH-SAW biosensor response and displacement penetration by measuring various concentration of gold nanoparticles which are diluted with different viscosity buffers.

### 2. Methods

In this evaluation, we measured various concentrations of gold nanoparticles by using SH-SAW sensor device operating at 250MHz. Also,

each gold nanoparticles were diluted with different concentrations of glycerol to compare displacement penetration effects by viscosity difference. Responses of SH-SAW biosensor were recorded as velocity and amplitude change generated between standards and each specimen.

#### 2.1. Gold nanoparticle preparation procedure

In this evaluation, we used commercially available 20nm gold nanoparticle (BBI Solutions, Cat.#: EM. GC20). Initial concentration of gold nanoparticle is labeled OD-1, fundamental optical density. Four groups of containers filled with 10 milliliters of OD-1 were centrifuged at 16,500G for 15 minutes. Obtained supernatant of OD-1 in the centrifuge process was removed from the containers. Four different concentration of 200 microliters glycerol aqueous solutions, 0, 10, 20, and 30 wt%, were added into each group of container, obtained OD-50 a fifty times concentrated sample having different viscosity. Obtained OD-50 samples were divided into two containers in half. One group of containers were stored in a shelf as OD-50 samples. The others were diluted with 100 microliters of glycerol aqueous solutions, above mentioned yet, obtained OD25 samples. This dilution process was repeated five times to obtain series concentration of gold nanoparticles OD-0, OD-1.56, OD-3.13, OD-6.25, OD-12.5, OD-25, and OD-50, having different viscosity. Here, OD-0 means glycerol aqueous solutions itself.

#### 2.2. Measurement procedure

SH-SAW biosensor response was measured for each OD number series belonging glycerol aqueous concentration. Measured velocity change and amplitude change were recorded as a reference of OD-0 measured value. Measurements were performed in ascending OD-number order, velocity change and amplitude change data were recorded 60 seconds after each sample applied onto sensing surface of the SH-SAW biosensor.

### 3. Results

Measured velocity change of SH-SAW is increased with the OD number at each glycerol aqueous concentration. Also, a rate of velocity increasing is risen with glycerol aqueous concentration. In order to analyze these measured results, the perturbation theory analysis was applied to an assumed texture structure  $S_m$  which is formed as concentration of floating gold nanoparticles, **Fig. 1**. Velocity change of area  $S$  is calculated as the sum of velocity changes in the areas  $S_m$  and  $S_l$ , independently calculated using eqs. 2 and 3. Coefficient  $A$  was determined by normalized at OD-50 results. Both measured and calculated results are shown in **Fig. 2**.

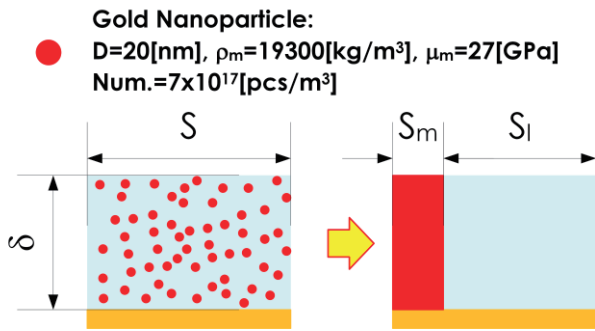


Fig. 1 Texture structure assumption for perturbation theory analysis.

$$\left(\frac{\Delta V}{V}\right)_m = -A\left(\rho_m - \frac{\mu_m}{V^2}\right)\delta \frac{S_m}{S} \quad (2)$$

$$\left(\frac{\Delta V}{V}\right)_l = -A\left(\rho_l - \frac{\mu_l}{V^2}\right)\delta \frac{S_l}{S} \quad (3)$$

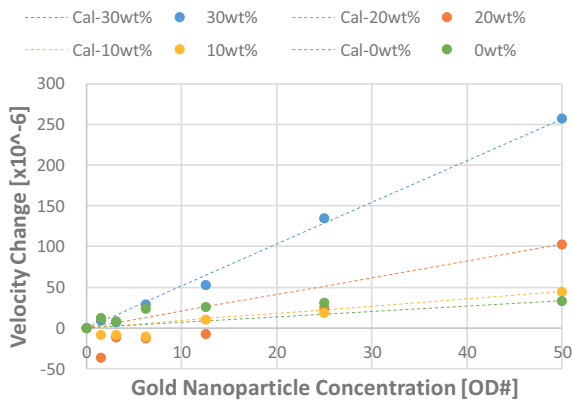


Fig. 2 Measured and calculated velocity change in various gold nanoparticle concentration in different glycerol aqueous concentration.

### 4. Discussions

The velocity increasing ratio risen accompanying with glycerol concentrating, it can be found in Fig. 2, is considered to be reflected displacement penetration effects. Regarding velocity change in the evaluation, perturbation analysis described measured results well. On the other hand, the perturbation theory describes no amplitude change observed under density and viscosity change of specimen. However, a series of measured results shows that amplitude was changed in accordance with velocity. **Fig. 3** shows velocity and amplitude change obtained in the measurements. In a series of OD-number change measurement, glycerol concentration kept constant, the amplitude increment is considered to be brought by unknown phenomena. To clarify this mechanism, theoretical analyses are required.

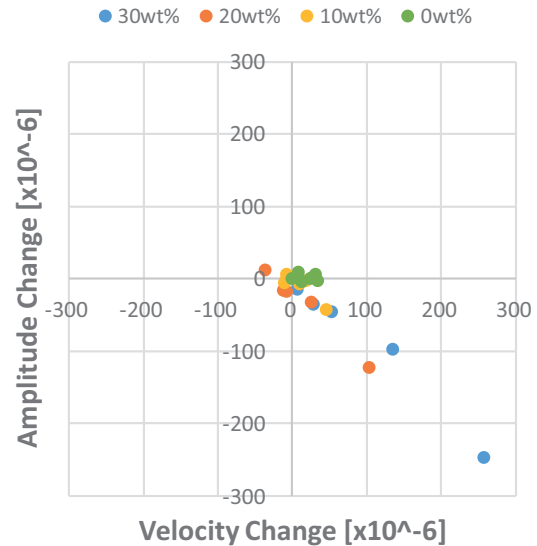


Fig. 3 Correlation between velocity and amplitude in gold nanoparticle concentration evaluation in different glycerol aqueous concentration.

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