

(Supplementary Information)

Sensitivity Enhancement of **Dynamic Mode Microcantilever
by Stress Inducer and Mass Inducer to Detect PSA at Low
Picogram Level**

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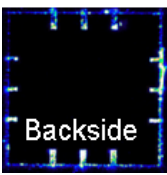
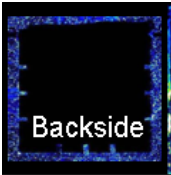



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1. Surface Functionalization of Microcantilever Surface and Modification of Silica Particles

Table S-1. Nonspecific binding control of silica nanoparticles according to the surface treatment of the cantilever and the silica nanoparticles.

BSA treatment of cantilever surface	BSA	No treatment	BSA
Surface modification of silica particle	BSA	PEG2000	PEG2000
			
			

2. Equation Derivation

- Relationship between resonant frequency shift and surface stress.

For the quantitative analysis of generated surface stress on the **dynamic mode microcantilever** surface, we derived an equation that can be used to calculate surface stress quantity from the resonant frequency shift value.

Let us consider a microcantilever, consisting of multiple layers including a

piezoelectric layer, with the surface stress, τ . The nanomechanical dynamic motion of the microcantilever can be described by the equation of motion, as follows:

$$\frac{\partial^2 M(x,t)}{\partial x^2} + \frac{\partial}{\partial x} \left[N(x) \frac{\partial w(x,t)}{\partial x} \right] + \rho A \frac{\partial^2 w(x,t)}{\partial t^2} = 0 \quad \text{for } 0 \leq x \leq l \quad [1]$$

where $M(x,t)$ is the bending moment, $N(x)$ is the axial loading induced by the surface stress, i.e. $N(x) = \tau x$, $w(x,t)$ is the deflection, ρ is the effective density, A is the cross-sectional area, and l is the length of a microcantilever. The elasticity theory provides the bending moment $M(x,t)$ as follows:

$$M(x,t) = \left[\sum_{k=1}^N E_k I_k \right] \frac{\partial^2 w(x,t)}{\partial x^2} \equiv \xi \frac{\partial^2 w(x,t)}{\partial x^2} \quad [2]$$

where E_k is the Young's modulus for the k -th layer, I_k is the moment of inertia for the k -th layer, N is the total number of layers in the microcantilever, and ξ is the effective bending modulus ($\xi = 6.49 \times 10^{-12} \text{ N}\cdot\text{m}^2$). From equation [1] and [2], the equation of motion for the motion of the microcantilever is

$$\xi \frac{\partial^2 w(x,t)}{\partial x^2} + \frac{\partial}{\partial x} \left[\tau x \frac{\partial w(x,t)}{\partial x} \right] + \rho A \frac{\partial^2 w(x,t)}{\partial t^2} = 0 \quad \text{for } 0 \leq x \leq l \quad [3]$$

The harmonic oscillation of the deflection, represented as $w(x,t) = u(x) \exp(i\omega t)$, allows one to construct the eigenvalue problem, given as

$$\wp u(x) = \rho A \omega^2 u(x) \quad \text{for } 0 \leq x \leq l \quad [4]$$

where ω is the resonant frequency, $u(x)$ is its corresponding eigen-mode, and the differential operator \wp is defined as

$$\wp = \xi \frac{d^4}{dx^4} + \frac{d}{dx} \left(\tau x \frac{d}{dx} \right) \quad [5]$$

We use the Ritz method (*), which assumes the eigen-mode in the form of

$$u(x) = \sum_{i=1}^n u_i \psi_i(x) \equiv \mathbf{u} \cdot \boldsymbol{\Psi} \quad [6]$$

in which \mathbf{u} is the vector whose component u_i is the constant to be determined, and $\boldsymbol{\Psi}$ is the vector whose component $\psi_i(x)$ is the polynomial basis function such as $\psi_i(x) = (x/l)^{i+1}$ for $i=1, \dots, n$, where n is the degree of polynomial. From equations [4]-[6], one can construct the numerical eigenvalue problem, as follows.

$$\mathbf{K}\mathbf{u} = \omega^2 \mathbf{M}\mathbf{u} \quad [7]$$

where \mathbf{K} is the stiffness matrix and \mathbf{M} is the mass matrix, given as

$$K_{ij} = \int_0^l \left[\xi \psi_i''(x) \psi_j''(x) + \tau x \psi_i'(x) \psi_j'(x) \right] dx$$

$$M_{ij} = \rho A \int_0^l \psi_i(x) \psi_j(x) dx$$

The numerical implementation of the eigenvalue problem given as equation [7] provides the resonant frequency ω as a function of the surface stress τ , in the following form:

$$\varpi_i \equiv \frac{\omega_i}{\omega_i^0} = \left[1 + \frac{\bar{\tau}}{\alpha} \right]^{\frac{1}{2}} \quad [8]$$

where ϖ_i is the dimensionless resonant frequency defined as the ratio of the resonant frequency ω_i with regard to the reference resonant frequency, ω_i^0 , which is the resonant frequency without any surface stress, i.e. $\omega_i^0 = (\mu_i/l)^2 \sqrt{\xi/(\rho A)}$, where the parameter μ_i satisfies the transcendental equation, such as $\cos \mu_i \cosh \mu_i + 1 = 0$, $\bar{\tau}$ is the dimensionless surface stress defined as $\bar{\tau} = \tau/\tau_0$ with the normalization factor $\tau_0 = \xi/l^3$, and the parameter $\alpha = \pi^2/2$.

Now, let us construct the relationship between the resonant frequency shift and the surface stress induced by the antigen-antigen interactions. We assume that the

microcantilever exerts no surface stress when there are no antigen-antibody interactions on the microcantilever surface. Then, assuming that the microcantilever begins to exert the surface stress when it is immersed into the antigen solution, such that there are antigen-antibody interactions on the microcantilever surface, the surface stress generated in the microcantilever as the result of antigen-antibody interactions is:

$$\tau = \alpha\tau_0(\omega^2 - 1) \quad [9]$$

Equation [9] enables one to calculate the surface stress induced by antigen-antibody interactions from the measurement of the resonant frequencies prior to and following the antigen-antibody interactions on the surface of the microcantilever.

- Theoretical resonant frequency shift from induced mass

When the polyclonal antibody-conjugated nanoparticles interacted specifically at the antigen on the microcantilever surface, the resonant frequency of the microcantilever was shifted into a lower frequency range due to the induced mass of the nanoparticles. In order to calculate the theoretical mass from the resonant frequency shift value, we utilized the well-known equation,

$$f_0 = \frac{\lambda_1}{2\pi} \sqrt{\frac{k}{m}} \quad [10]$$

in which, λ_1 is the eigenvalue of the 1st resonance mode, m is the mass of the cantilever structure, and k is the spring constant. Assume that the resonant frequency was shifted only by the induced mass of the nanoparticle. That is, the spring constant change did not occur in the nanoparticle induction. After the specific interaction between polyclonal antibody-conjugated nanoparticles and antigen on the

microcantilever surface, the resonant frequency is given as:

$$f_1 = \frac{\lambda_1}{2\pi} \sqrt{\frac{k}{m + \Delta m}} \quad [11]$$

where, Δm is quantity of the induced mass.

When the polyclonal antibody-conjugated nanoparticles interacted specifically with the antigen on the microcantilever surface, we assessed the numbers of nanoparticles via the SEM image (**Figure S-1**). We then calculated the mass of the specifically bound nanoparticles on the microcantilever surface. **In the picogram level, it is hard for the silica particles to form the uniform and dense film on the microcantilever. However, they are well-dispersive on the whole surface of microcantilever from the base part to the tip part. So, the resonant frequency shifts generated by the silica nanoparticles were calculated by the homogeneous film of the same weight as the total silica particles bound on the surface.** Finally, the theoretical resonant frequency shift value could be estimated, as in the following equation,

$$\Delta fr = fr_1 - fr_0 = \frac{\lambda_1}{2\pi} \left(\sqrt{\frac{k}{m + \Delta m}} - \sqrt{\frac{k}{m}} \right) \quad [12]$$

Equation [12] enables us to estimate the theoretical resonant frequency quantity caused by the induced mass of nanoparticles from the calculation of the SEM image on the microcantilever surface (**Table S-2**).

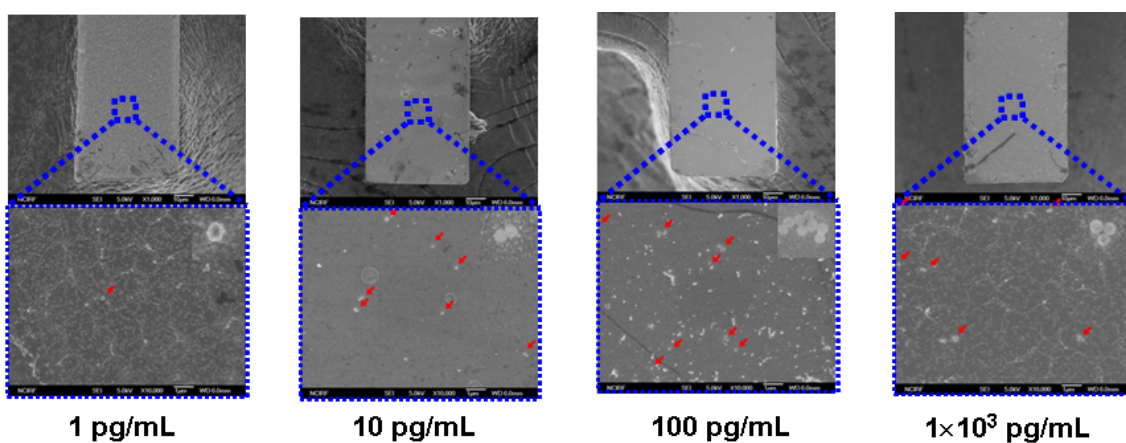


Figure S-1. Field emission-scanning electron microscope (FE-SEM) images after the secondary immunoassay by silica nanoparticle (140 nm) binding.

Table S-2. Resonant frequency shifts calculated from the mass of silica nanoparticles bound on the cantilever surface.

Diameter (nm)	140
Volume of Single Silica Nanoparticle (m ³)	1.44E-21
Mass of Single Silica Nanoparticle (g)	2.87E-15

Concentration of PSA (pg/mL)	No. of Silica NPs	Total Mass (g)	Effective Mass (0.236 × total mass, g)	Resonant Frequency Shift (Hz)
1	8523	2.45E-12	0.58E-12	765
10	22160	6.36E-11	1.57E-12	1485
100	24149	6.94E-11	1.64E-12	2435
1000	30778	8.84E-11	2.07E-12	2595