

FEM based Estimation of Biological Interaction Using a Cantilever Array Sensor

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Abstract: Nanofabrication processes like atomic layer deposition aim to create thin films of one molecule thickness. These ultra thin films find their applications in microcircuits and in biological interaction estimation. Here, we employ nano fabricated cantilever array sensors for detecting a specific disease causing antigen in the blood serum. Each cantilever is coated on its top surface with a antibody specific to antigen which is to be detected, where antibody is immobilized using a Au-SH linkage. The model has an array of five nanorods made up of gold on which Immunoglobulin G (IgG) is immobilized, IgG is one of the most abundant proteins in human serum with normal levels between 8-17 mg/ml in adult human blood. IgG is important for our defence against microorganisms and the molecules are produced by B lymphocytes as a part of our adaptive immune response. When an antigen of attomolar concentration sits on the cantilever beam it creates a load in the order of femto newtons. Simulation of such interactions in Comsol Multiphysics results in the cantilever displacement to larger extents. Especially the free end is deflected more which is a direct measure of amount of the antigen –antibody interactions, results of which are comparable with the practically obtained results.

Keywords: cantilever array, antigen-antibody interaction, immunoglobulin G.

1. Introduction

Microcantilevers were first designed and fabricated for use as force sensors, possessing an extremely high force sensitivity, in the Pico newton (pN) range also. Availability of inexpensive, mass-produced cantilevers also triggered applications other than imaging, where cantilevers act as physical, chemical, and biological sensors. These early observations later lead to the development of a unique family of mechanical sensors with numerous new applications in physical, chemical and biological sensing. The deflection of a cantilever can be due

to number of processes such as molecular adsorption, thermal effects, electric and magnetic fields, and fluid flow. Adsorption-induced deflections are attributed to changes in the surface free energy and are observed only when a differential adsorption occurs between the cantilever surfaces. Depending on the mode of operation, several methods for reading the movement of a cantilever have been developed. These readout techniques can be applied to a single cantilever or to an array of cantilevers. In this method, the two surfaces of the cantilever have different characteristics because of which the target molecules preferentially get adsorbed to one of the surfaces (Fig. 1). This difference in characteristics can be achieved by depositing probe molecules preferentially on one of the surfaces. The intermolecular interaction of the biomaterial on this surface generates surface stresses on one side of the cantilever which are good enough to bend the cantilever by a detectable magnitude. The deflection of the cantilever can be measured by sensing the change in resistance of a piezoelectric material embedded on the surface of the nanocantilever or sensing the deflection of a laser beam reflected from the nanocantilever surface. Arrays of microcantilevers have also been used in bio-applications for greater reliability and accuracy. Here the net differential signal from the array of nanocantilevers is the sensor signal

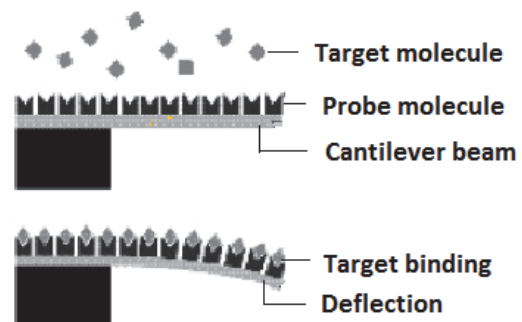


Figure 1. Principle of nano-cantilever-based biosensing involving the measurement of effects of surface-stress changes. [2]

1.1 Nano Cantilevers based chemical adsorption

When the molecular adsorption is confined to a single surface of a nanocantilever, the molecular interactions can be studied by: (i) noting a shift in the resonance frequency and (ii) monitoring the cantilever bending. The latter offers an advantage over other acoustic sensors (QCM, SAW) by providing an additional measurable physical quantity: the surface stress caused by the forces involved in the adsorption process. In molecular recognition experiments using an array of cantilevers, adsorption enhancement is achieved by coating each cantilever sensor with a different sensitive layer allowing the array-device to operate as an artificial chemical nose.

1.2 Nano Cantilevers based biosensing

Nano-fabricated cantilever sensors with selective coatings for target immobilization are ideal candidates for biosensing applications. The mass resolution obtained with cantilevers in air is in the Pico and the femtogram range. Mass changes on the cantilever can accurately be determined by running the instrument in static mode and dynamic mode.

In Static mode (fig 2), deflection/bending of the cantilever is due to surface stress changes on the cantilever that occur because of the analyte interaction.

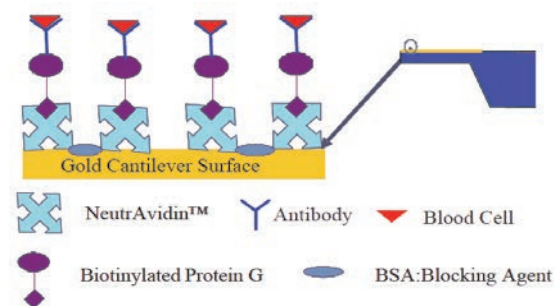


Figure 2. Representation of antibody antigen interaction (Different layers applied to cantilever surface).[1]

In dynamic mode (fig 3), the cantilever is oscillated at its resonance frequency. The resonance frequency changes with the mass load where the cantilevers are actuated at their resonance frequency. If additional mass is

adsorbed onto the cantilever surface, the resonance frequency will be shifted to lower frequencies. These shifts allow calculation of the mass change. There are few published reports about nano cantilever biosensors operating in dynamic mode capable of the detection of microorganisms. The obtained results showed high nano cantilever sensitivity in dynamic mode.

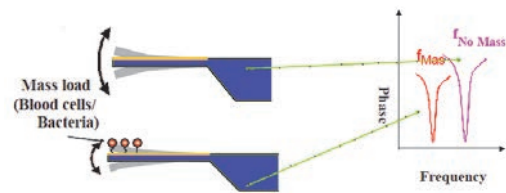


Figure 3 . Representation of frequency phase relationship.[1]

2. Theory

With the notable exception of the sensor, the majority of rapid detection systems employ whole antibodies (monoclonal and polyclonal), and increasingly smaller antigen-binding antibody fragments obtained through enzymatic engineering and combinatorial biology display technologies for recognition and quantification of target analytes.

Antibody recognition elements make use of the high sensitivity and specificity of biomolecular antibody-antigen interactions. There exists a large body of published literature on the subject of generating antibody fragments using enzymatic engineering of whole antibody molecules. Hereby the use of combinatorial biology based library systems (e.g., phage display) for the selection of reduced size antibody fragments with specific affinity to analyte targets of interest. Such smallest antibody are used for detecting the complementary antigen. Examples of such minimal size binders are the antigen-binding fragment (Fab, ~50 kDa), the single chain antigen binding fragment (scFv, ~25 kDa), and the single domain antibody fragment (sdAb/VHH). The sdAbs found in camelid and some shark species are unique and are also the smallest known antigen binding antibody fragments (~12–14 kDa).

3. Structure Design

In this study, a 3D design of a cantilever array sensor for biosensing application was proposed and analyzed using a FEM based simulation. Generally cantilever arrays have their potential use in the field of microcircuits and the bio-functionalized arrays like the ELISA test(Enzyme Linked Immuno Sorbent Assay) systems. This system has all its dimensions in the nanometer range, and is capable of sensing even the attomolar concentration of the antigen binding to the antibody. The mass change due to the interaction is estimated with the maximum displacement occurring at the free end. Here the antigen molecular mass is taken as approximately 150Kda.

4. COMSOL Multiphysics Analysis

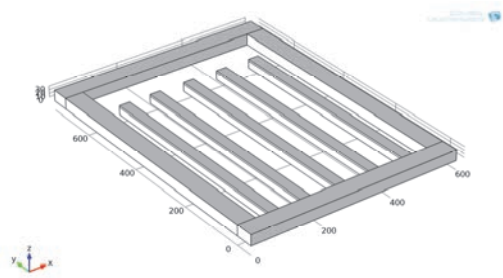


Figure 4. A 3-D model of a NEMS based cantilever array sensor .

This NEMS based cantilever array sensor (figure 4)was modeled and simulated using COMSOL Multiphysics 4.1 - MEMS module.

Materials: The cantilever array were made of gold(Au). The block encapsulating the cantilever array was made by an insulating material i.e. silicon nitride (Si_3N_4). The surface of the gold is coated with a material(fab-protein) with thiol end group.

Physics Used: The Solid Mechanics physics in terms of boundary load was applied to the required boundaries. The displacement corresponding to the binding antigen (load i.e. pressure or force) tend to create a specific mass change. The main advantage of this NEMS based cantilever array sensor is that even a small quantity of test sample (in nano liters) is enough for estimating the probability of the disease incurred to the patient.

5. Domain equations

In physics, resonance is the tendency of a system to oscillate at maximum amplitude at certain frequencies, known as the system's resonance frequencies (or resonant frequencies). At these frequencies, even small periodic driving forces can produce large amplitude vibrations, because the system stores vibrational energy.

$$f_n = \frac{\lambda_n^2}{2\pi \sqrt{12}} \frac{t}{L^2} \sqrt{\frac{E}{\rho (1 - \nu^2)}} \quad (n= 0,1,2,\dots)$$

where,

- f_n = Resonant frequency
- λ_n^2 = dimensionless nth mode eigen value
- ν = Poisson ratio
- ρ = Density
- t = thickness of the cantilever
- E = effective young's modulus
- L = length of the cantilever

The mass sensitivity (S_m) of a cantilever is defined as the change in frequency divided by the mass load. It can be experimentally calculated by the following equation

$$S_m = -\frac{\Delta f}{\Delta m}$$

where,

- S_m = Mass sensitivity
- Δf = Change in frequency
- Δm = Mass load

Calculating the mass sensitivity theoretically depends on how the mass is loaded onto the cantilever surface. If the mass is loaded at the tip of the cantilever the mass sensitivity (S_m) is given as

$$S_m = \frac{\Delta f_n}{\Delta m} \cong \frac{1}{2} \frac{f_n}{M_e}$$

$$M_e = 0.236Lwt\rho$$

where,

- M_e = Effective mass

As can be seen from above equation the mass sensitivity is fundamentally depended on the material properties of the cantilever. It can be seen from above equation that the mass

sensitivity increases with decreasing cantilever size.

Molecular weight of anti IgG is approximately 150 KDa., ie. 24.906×10^{-20} g. The equivalent force experienced on the cantilever surface per mole concentration is calculated using the formula $F = ma$ (N).

6. Results and discussions:

The resonant frequency of the nano cantilever, changes as the analyte to be sensed attaches to the nano cantilevers (figure 9). The change in the resonance frequency depends on the mass of the analyte and its concentration in the sample volume.

However, this method is not very efficient in liquid phase due to the damping effect of the liquid. Alternatively, nano-cantilevers can be employed as surface stress sensors. This is useful in liquid phase environments. Most of the biomolecules are available in liquid phase environments hence this method turns out to be more effective for monitoring the binding of biomolecules. [1]

S.No	No of molecules	Force applied (nN)	Displacement (nm)
1.	10	24.407	28.731
2.	15	36.611	42.990
3.	20	48.814	57.383
4.	25	61.018	71.564
5.	30	73.220	85.266
6.	35	85.425	99.5

Table 1. Displacement obtained for various concentration of analyte.

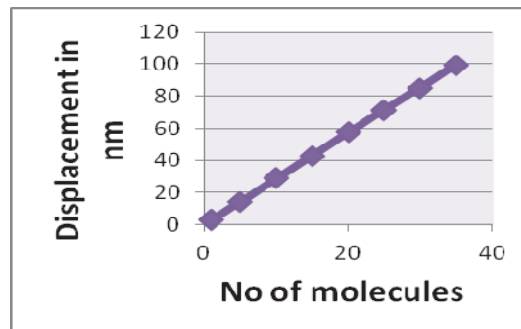


Figure 5. Displacement of the cantilever array with respect to number of molecules interacted with the surface.

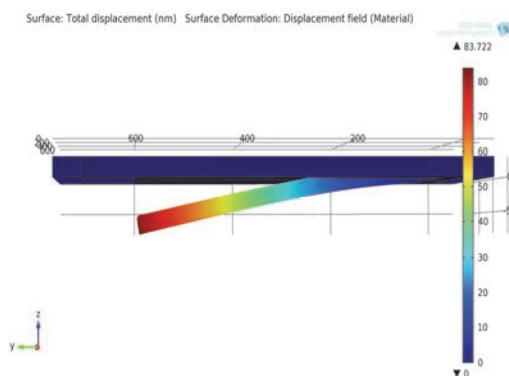


Figure 6. Displacement of the cantilever array in 2-D view due to the interaction.

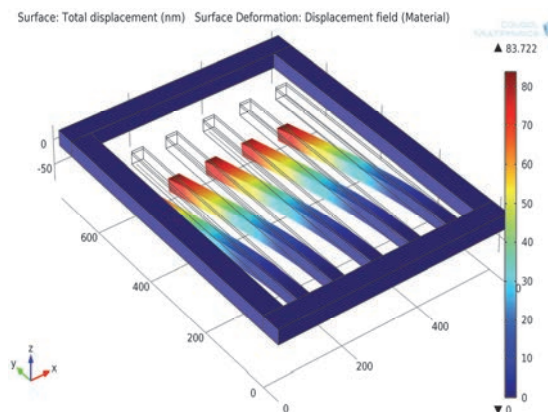


Figure 7. Displacement of the cantilever array in 3-D view due to the target analyte interaction.

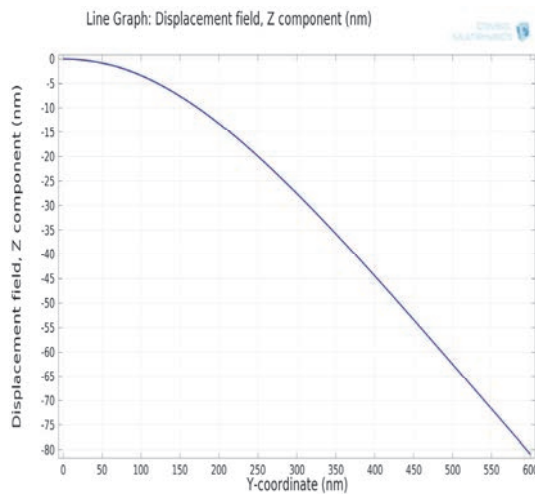


Figure 8. Maximum deflection of the cantilever from its free position.

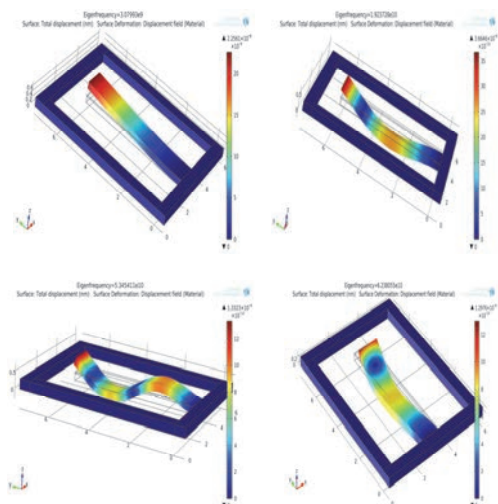


Figure 9. Modes of vibration of a cantilever beam (dynamic mode) from its free position.[3]

The simulated results show that a single molecule interaction with the surface creates maximum displacement of the cantilever. Also, it is observed that depending on the amount of antigen on the cantilever surface, the frequency gets shifted to lower frequencies .

7. Conclusions

The main aim of this simulation study is to develop more accurate real time cantilever array sensors, especially for use in the medical field.

The cantilever array sensor has potential advantages like its sensitivity towards a small concentration, selectivity towards an analyte, specificity on the target molecule, and repeatability characteristics. These prove the cantilever array sensor to be a versatile tool in various fields. Our simulation gives a gist of how a NEMS based cantilever array sensor can be used as diagnostic tool.

8. References

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