

Micro-Weighing Based Biosensor with Adaptive Interferometry [†]

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Abstract: In this work, an adaptive holographic interferometer was implemented for the measurement of a micromechanical oscillator frequency. A silicon micro-cantilever mounted on a piezoquartz plate was used as the sensing element. Out-of-plane vibrations of the cantilever were excited using a sinusoidal electrical signal. The cantilever vibrations were measured with the adaptive interferometer using two waves coupling in a semi-insulating photorefractive CdTe:V crystal. In the experiment, the mass of absorbed molecules of bovine serum albumin (BSA) was measured at various concentrations of water solution. The biosensor demonstrated the ability to measure the concentration of BSA in water solutions with a concentration of 0.2 mg/mL. The result shows the possibility of using adaptive interferometry to detect the vibration of micromechanical sensors and the potential prospects for building biosensors based on them.

Keywords: biosensor; micromechanical oscillator; laser biosensor; holographic interferometry; mass sensor; adaptive interferometer

1. Introduction

Until recently, the most widely used type of biosensors was sensors based on fluorescent labels. These sensors are low cost for a large number of samples and do not require expensive devices to measure concentrations of biomolecules [1]. However, this detection method has a number of disadvantages. Considerable time is required to attach the label to the object of study. Often, labels bind to bodies or antibodies in a random manner, and, depending on the binding site, labels can affect the functional part of the protein, reducing its chemical activity. In view of such an influence on the object of study, the use of this method for studying the dynamics of chemical and biological processes is difficult or impossible. In addition, new markers and labeling methods are being developed for each biological object separately. This contributed to the development of a wide variety of “label free” detection systems that do not use labels that have equal or higher sensitivity than marker detection methods [2].

Some of the most commonly used biosensors include quartz resonators, surface plasmon resonance biosensors, whispering gallery mode resonators (WGM biosensors), and micro- and nanoelectromechanical systems [3–5]. These biosensors typically require a sensor area that is limited to tens or hundreds of microns in size. However, decreasing the size of the sensor often results in decreased sensitivity. On the other hand, biosensors that utilize micromechanical oscillators in a dynamic mode can actually increase in sensitivity with decreasing sensor size. In dynamic mode, changes in mass due to object adsorption onto the surface of the micro-oscillator can be measured through the frequency of natural oscillations. This method of mass measurement is known as resonant microbalance. Currently, sensors utilizing resonant microbalance technology can accurately measure mass changes down to several attograms [6].



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2. Experimental Setup and Methods

The micro-mechanical biosensor was created using an AFM silicon cantilever, which served as the main element of the biosensor. The cantilever had dimensions of $125 \times 30 \times 2.5 \mu\text{m}^3$ and was mounted on a positioning system. To excite vibrations, the cantilever was fixed on a piezoquartz plate and subjected to a sinusoidal electrical signal with an amplitude of up to 80 mV and varying frequency, supplied from an electrical signal generator. The vibrations of the cantilever were measured using a holographic adaptive interferometer [7,8], as shown in Figure 1. The interferometer object wave was reflected from the cantilever and propagated through a semi-insulating photorefractive CdTe:V crystal, where it was mixed with the reference wave. The wave coupling ensured phase-to-intensity transformation in the object wave, and changes in the intensity were recorded using a photodetector connected to an electrical signal spectrum analyzer that was synchronized with the electrical signal generator. Thus, the feedback spectra of the micromechanical sensor were recorded.

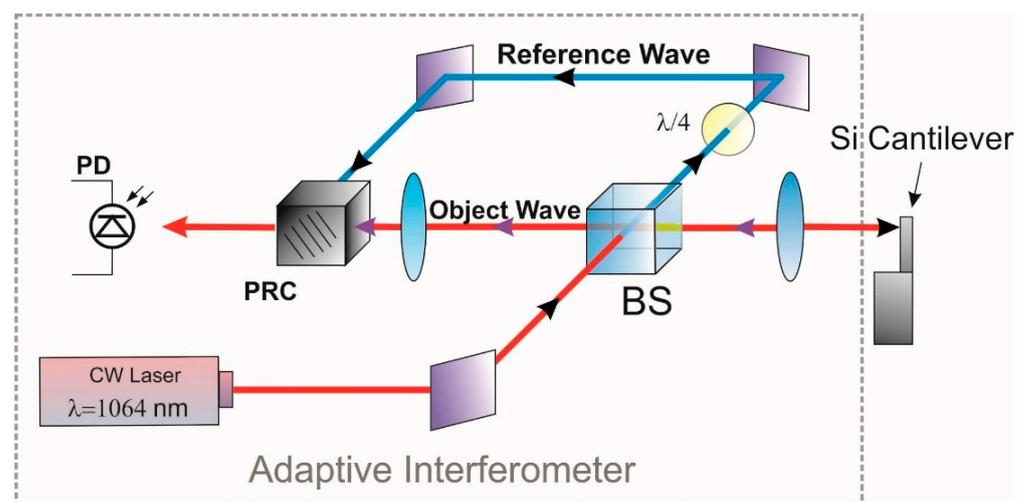


Figure 1. Scheme of the experimental setup. BS—beam splitter; PRC—photorefractive crystal; PD—photodetector; $\lambda/4$ —quarter wave plate.

The interferometer used a continuous laser with a wavelength of 1064 nm. The cantilever was coated with a 20 nm thick gold film via electron beam evaporation to improve the reflection of the object beam of the interferometer, thereby increasing the signal-to-noise ratio of the measuring system. The spectrum recorded using the electrical signal spectrum analyzer is shown in Figure 2a, and a resonant peak is clearly visible (Figure 2b), corresponding to the frequency of natural oscillations of the cantilever. To determine the oscillation frequency, the spectrum was processed in OriginPro software via approximating the resonance peak with a Gaussian curve. The measured resonance frequency of the cantilever used in this work was $253,442 \pm 23$ Hz.

In this work, the operation of the biosensor has been tested for the detection of BSA molecules (bovine serum albumin) absorbed on a gold layer deposited in vacuum. The gold layer was activated previously with a solution of NHS-EDC (N-hydroxysuccinimide + 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide). The cantilevers were then placed in a flow cuvette with a volume of 4 mL filled with a solution of Na₂PO₄ with a concentration of 0.013 M/mL and pH = 8.6. The cell was installed in the object beam of an adaptive interferometer. Binding of molecules to the cantilever leads to a change in its resonance frequency, which is measured using the adaptive interferometer. The mass of the attached molecules and the concentration of the solution can be determined via changes in the resonance frequency.

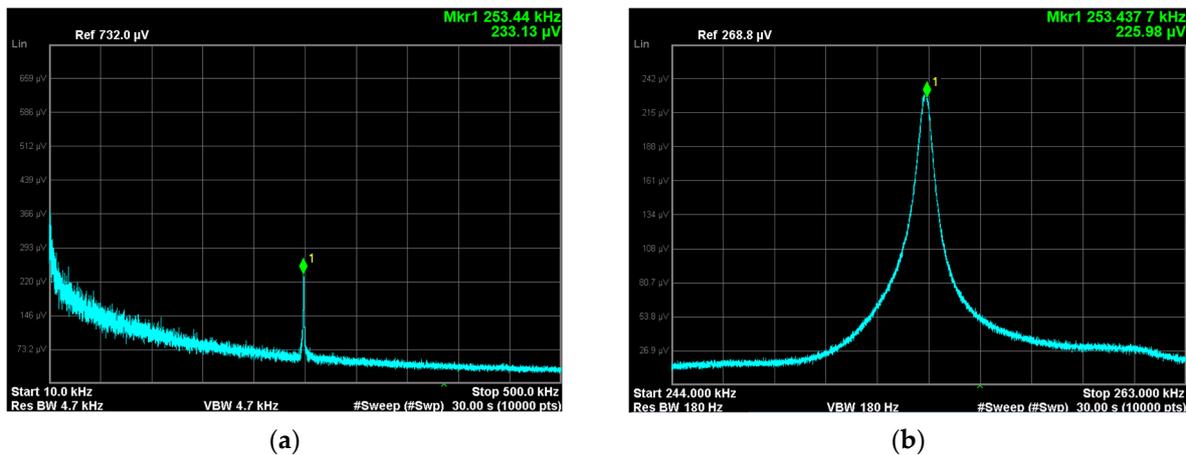


Figure 2. Spectrum recorded with an electrical signal spectrum analyzer: (a) spectrum in a wide frequency range with a well-defined resonant peak; (b) resonance peak corresponding to the natural frequency of the cantilever.

3. Experimental Results

The oscillation frequency of the cantilever was measured before and after transferring it to the liquid cell. The frequency decreased from $253,442 \pm 23$ to $98,561 \pm 41$ kHz, which is consistent with the model described in paper [9]. It should be noted that transferring the cantilever to a liquid not only decreases the resonant frequency, but also reduces the quality factor of oscillations by about 100 times. However, detecting vibrations in water results in a significant decrease in the intrinsic noise of the measuring system. Therefore, despite the decrease in the quality factor of oscillations, there was no observable decrease in accuracy for determining the oscillation frequency of the cantilever. To test the operation of the biosensor, a BSA solution with a concentration of 10 mg/mL was introduced into the cuvette. The attachment of BSA molecules resulted in a shift in the resonant frequency of the cantilever of 2049 Hz. Taking into account the error in determining the resonant frequency, the minimum detectable concentration of BSA in this experiment was 0.2 mg/mL.

The accuracy of determining the resonant frequency can be improved through increasing the amplitude of the signal applied to the piezoceramic plate that excites the cantilever oscillations. It has been experimentally established that a five-fold increase in signal amplitude is possible. Further increases in signal amplitude lead to cantilever damage, and long-term operation with an increased amplitude can cause a decrease in the frequency of resonant oscillations of the cantilever, likely due to the growth of internal defects. This can ultimately lead to cantilever damage.

4. Conclusions

The results show that the biosensor is capable of detecting the mass and concentration of absorbed BSA molecules in a water solution. The attachment of BSA molecules is performed through activating the surface of the silicon cantilever with a solution of NHS-EDC. This study indicates that adaptive interferometry can be used to detect the vibration of micromechanical sensors and shows potential prospects for building biosensors based on them.

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