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Laser Micromechanical Sensor for Measurement of the Mass of Bacterial Cell Colonies

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Abstract—We presented results of applying an adaptive interferometry technique, based on dynamic holograms recorded in photorefractive crystal, to accurately determine the mass of biofilm bacteria colonies by the measuring of microcantilever frequency shift. During the experimental testing, biofilms of *Escherichia coli* were grown on the surface of a cantilever previously coated with a BaF_2 film. The proposed experimental setup is completely non-contact due to using an optical method of excitation and detection of cantilever oscillations. Based on the measured shift in the resonant frequency, the mass of the biofilm grown on the cantilever was calculated to be $(2.072 \pm 0.003) \times 10^{-9}$ gram.

Keywords: laser biosensor, adaptive interferometer, mass measurement, resonant microweighing, biofilm, *Escherichia coli*

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INTRODUCTION

In recent years, the field of biosensing has experienced substantial advancements, driven by the need for highly sensitive, rapid, and cost-effective diagnostic tools [1]. Among the various technologies being explored, resonant micromechanical biosensors have emerged as a promising solution, leveraging the unique properties of micro-scale mechanical systems to detect biological interactions with exceptional precision [2]. Cantilevers, as fundamental components of micro-electromechanical systems (MEMS), possess intrinsic advantages such as high sensitivity, scalability, and the ability to operate in diverse environments [3]. These tiny mechanical beams can be engineered to vibrate at specific resonant frequencies, which shift in response to biological interactions occurring on their surfaces. By functionalizing cantilevers with target-specific receptors, it is possible to achieve real-time detection [4] of a wide range of biomolecules, including proteins, nucleic acids, and pathogens, making them valuable in fields such as medical diagnostics, environmental monitoring, and food safety [5].

The development of resonant cantilever biosensors involves a multidisciplinary approach, integrating principles from physics, chemistry, biology, and engineering. Advances in microfabrication techniques have enabled the production of cantilevers with precise geometries and material properties, enhancing their sensitivity and specificity [6]. Furthermore, innovations in surface chemistry have facilitated the selective binding of analytes, improving the accuracy of detection. In this paper, we demonstrate the effectiveness of applying the principles of adaptive interferometry to construct laser biosensors [7, 8]. The use of an adaptive interferometer [9] for detecting oscillations of micro-oscillators (cantilevers) allows recording oscillations with an amplitude of less than a nanometer with a poorly reflective or complex surface shape [10]. Moreover, dimensions of the oscillator can be smaller than the size of the light beam. In this paper, a fully optical non-contact system for measuring biofilm mass using the adaptive interferometry method is proposed.

EXPERIMENTAL SETUP AND METHODS

The biosensor was developed using a silicon cantilever, commonly employed in atomic force microscopy, as the primary sensing element. Figure 1 illus-

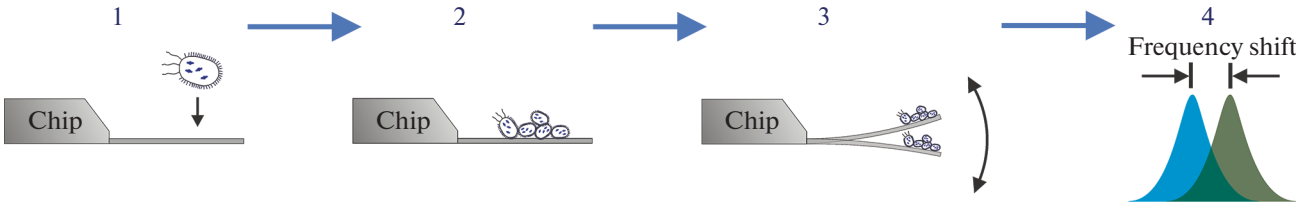

Fig. 1. Main steps for measuring biofilm mass by resonant microweighing. Step 1: Attach the cell culture to the cantilever surface. Step 2: Allow cell proliferation to form a biofilm. Step 3: Install the cantilever into the measurement setup, excite it, and record its oscillation frequency. Step 4: Determine the biofilm mass from the frequency shift observed with and without the biofilm.

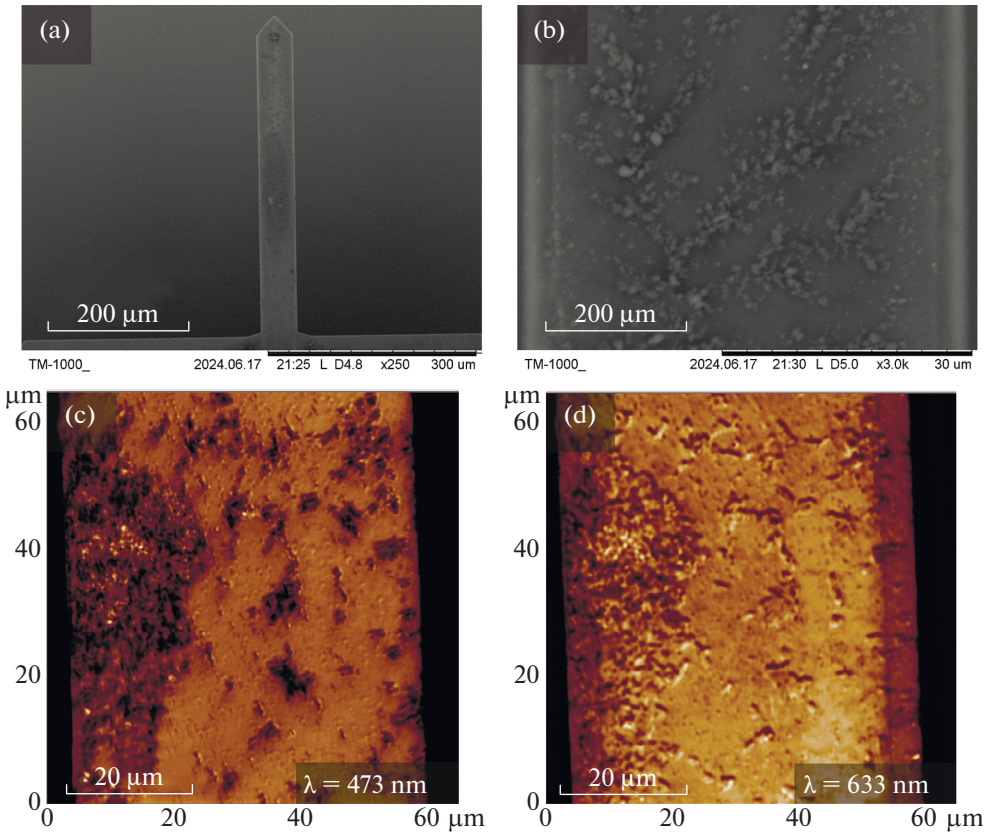

Fig. 2. Images of the cantilever after formation on the surface of the Escherichia coli biofilm. (a), (b) images obtained in a Hitachi TM1000 scanning electron microscope; (c), (d) mapping performed in an NT-MDT NTEGRA Spectra II Raman spectrometer at a laser wavelength of 473 nm (c) and 633 nm (d).

trates the principle and main steps involved in measuring biofilm mass. Which consists of measuring the difference in the resonant frequency of the cantilever oscillations before and after biofilm attachment.

The cantilever's natural oscillations are induced using a pulsed Nd:YAG laser, characterized by a pulse energy of 0.3 mJ, a duration of 5 ns, and a repetition rate of 20 Hz. Using a pulsed laser to excite the cantilever oscillations ensures high accuracy in determining its resonant frequency. The oscillations are detected by an adaptive holographic interferometer, where the object wave, reflected from the cantilever, interacts with the reference wave after passing through a semiconductor photorefractive CdTe:V crystal. This two-wave interaction transforms the phase variations into intensity changes of the object wave, which are then recorded by a photodetector and analyzed with an oscilloscope. The adaptive nature of the holographic interferometer is particularly beneficial in mitigating the effects of temperature fluctuations, mechanical vibrations, and other environmental noise. The system's ability to continuously adapt ensures consistent measurement accuracy.

Prior to the experiment, cantilevers with dimensions of 450 × 45 × 2 μm were pre-treated with an ion beam for 5 minutes, followed by the deposition of a

100 nm thick barium fluoride (BaF_2) film in a vacuum chamber on one surface. *Escherichia coli* (*E. coli*) biofilms were cultured on the surface of microcantilevers immersed in LB culture medium supplemented with 5 μg/mL nitrofurantoin. To simulate liquid flow, a Rocker-Shaker MR-12 was used, tilting the test tubes at a 6-degree angle with a frequency of 17 cycles per minute for 20 hours. The microcantilevers were securely positioned in custom holders designed to fit 1 mL test tubes. Following biofilm formation, the cantilevers were rinsed with distilled water to remove any planktonic bacteria. After drying, the cantilevers were mounted in an adaptive holographic interferometer to assess their oscillation frequency.

EXPERIMENTAL RESULTS

To monitor the attachment of bacteria to the cantilever surface, we used an atomic force microscope. Additionally, a surface study was conducted using a Raman spectrometer, mapping the cantilever surface at wavelengths of 473 nm and 633 nm (Fig. 2). The structure corresponding to a growing biofilm is clearly visible in Fig. 2. Analysis of these images indicates that *E. coli* formed a well-connected film to the cantilever, with discernible foci of bacterial colony attachment.

The frequency of the cantilever's natural oscillations was determined by accumulating signals for 30 s, followed by a Fourier transformation of the resulting signal using MATLAB software. The Fourier spectrum was then approximated by a Lorentz function in OriginPro software to identify the central frequency at the resonance peak. The cantilever oscillation frequency was 11,291.9 ± 8.1 Hz before bacterial attachment and 10.795.2 ± 6.2 Hz after attachment. Based on the measured frequency shift, the biofilm mass was calculated using COMSOL Multiphysics software [11], resulting in a mass of $(2.072 \pm 0.003) \times 10^{-9}$ g.

CONCLUSIONS

In this work, a method of resonant microweighing was employed to measure the mass of biofilms formed by *E. coli* bacteria. The oscillations of the cantilever were measured using an adaptive holographic interferometer. The *E. coli* biofilm was cultivated on the cantilever surface for 20 hours under liquid flow conditions induced by a shaker. The measured mass of the biofilm was $(2.072 \pm 0.003) \times 10^{-9}$ g. The formation of the biofilm was verified using a scanning electron microscope and Raman spectrometer, which confirmed its attachment to the cantilever.

It is noteworthy that the proposed method for measuring biofilm mass is completely contactless, allowing for the measurement of biofilm mass on a cantilever in both air and liquid environments. This makes the proposed biosensor promising for the online monitoring of cell colony states.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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