Dual-mode stress and mass measurements with chemical and biochemical microcantilever sensor arrays

Konrad Nieradka\textsuperscript{a} *, Katarzyna Kapczyńska\textsuperscript{b}, Jacek Rybka\textsuperscript{b}, Piotr Grabiec\textsuperscript{c}, Teodor Gotszalk\textsuperscript{b}

\textsuperscript{a}Faculty of Microsystem Electronics and Photonics, Wroclaw University of Technology, Wroclaw 50-372, Poland
\textsuperscript{b}Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw 53-114, Poland
\textsuperscript{c}Division of Silicon Microsystem and Nanostructure Technology, Institute of Electron Technology, Warsaw 02-668, Poland

Abstract

In this work, we present dual-mode measurements of chemical and biochemical microcantilever sensor arrays. Multiple microcantilevers are monitored simultaneously in our homemade nanomechanical analyzer. A single stationary optical beam is used to read bending of the microcantilevers and to record their thermal noise. Added mass is calculated from measured thermal noise resonance frequency shift and surface stress is assessed based on static bending. We present experimental data on self-assembling monolayers growth, receptors immobilization, and target molecule detection on microcantilevers. Crosstalk between both measurement modes is possible. We show experimental proofs for such crosstalk and discuss its nature.

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1. Introduction

The microcantilever sensors allow insight into nano-scale phenomena, which are beyond reach of many conventional analytical methods. They translate physical and chemical interactions of their functional surfaces with environment into changes of their mechanical properties and can be operated in two distinctive modes. In static mode, interactions between molecules on the sensor’s surface cause the sensor to bend. This bending $\Delta z$ can be translated into surface stress difference $\Delta \sigma$, which is a measure of

* Corresponding author. Tel.: +48-71-320-40-75; fax: +48-71-328-35-04.
E-mail address: konrad.nieradka@pwr.wroc.pl.
intermolecular interactions. This transduction mechanism is described by Stoney’s formula, Eq. (1). In dynamic mode, molecules, microbes, and other species adsorbing or desorbing from the sensor’s surface change its effective mass $m_{eff}$ as described by Eq. (2), and thereby change the resonance frequencies $f_r$ of its vibration modes. Tracking of sensor’s resonances allows monitoring adsorbed mass.

$$\Delta z = \frac{3(1-\nu)}{E} \frac{l^2}{t^2} \Delta \sigma$$  \hspace{1cm} (1)

$$f_r = \frac{1}{2\pi} \sqrt{\frac{k}{m_{eff}}}$$  \hspace{1cm} (2)

In equations (1) and (2), $l$ and $t$ stand for microcantilever’s length and thickness, respectively, $E$ for Young modulus, $\nu$ for Poisson ratio, and $k$ for microcantilever’s spring constant. Eq. (2) in particular shows that under certain conditions, both modes may interfere with each other. Additional stress introduced to the microcantilever’s surface may alter the spring constant, and thus give a false readout of reduced mass loading. This can be expected a significant factor in case of the softest microcantilevers, dedicated to static mode sensing. In such case, both phenomena should be monitored simultaneously.

2. Experimental setup

In our work, we present dual-mode measurements of microcantilever sensors. We use our homemade nanomechanical analyzer allowing simultaneous measurement of up to 8 microcantilevers with expanded beam deflection (EBD) method that we proposed in Ref. [1]. Resonance frequencies of several flexural and torsional modes for each microcantilever are measured by fitting Lorentz-curves to their thermal noise spectra, a method described in Ref. [2]. The schematic diagram and a photo of developed system are shown in Fig. 1.

Individual microcantilevers are covered with functional layers by immersing them in microcapillaries, as shown in Fig. 2. Growth of self-assembling monolayers (SAMs) and binding of biomolecules are studied by preparing pairs of reference and sensing microcantilevers and exposing them to target substances. Depending on the choice of those layers, the functionalization procedure and the microcantilever stiffness, the resulting sensor may be more useful in one of these modes or both modes may complement each other. Inadequate functionalization, on the other hand, may lead to misinterpretation of results.

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![Schematic diagram and a photo of developed microcantilever array sensors readout system](image)

Fig. 1. Schematic diagram and a photo of developed microcantilever array sensors readout system. I-V stands for current-to-voltage converters, Arith for analog arithmetic circuits, ADC for analog to digital converters, FFT for fast Fourier transform, Avg for averaging, and L-M for Levenberg-Marquardt algorithm.
3. Results and discussion

To demonstrate how surface stress may affect mass measurement, we have functionalized one microcantilever with poly(ethylene glycol) methyl ether thiol (mPEG) self-assembling monolayer to serve as a reference sensor. The other cantilever has been exposed to mPEG solution during measurement. The resulting surface stress and mass readout signals are shown in Fig. 3. Significant surface stress increase occurring upon self-assembling monolayer growth stiffens the microcantilever and misleadingly suggests desorption of molecules.

We have prepared a pair of sensing and reference microcantilevers for binding of avidin proteins. The reference microcantilever had its both surfaces covered with SAMs terminated with mPEG molecules. The same termination has been applied to one side of the sensing microcantilever, while the other side has been terminated with biotin molecules. Fig. 4 shows the results of subsequent expositions to avidin and biotinylated antibodies. Both sensors give significant false response in mass readout due to varying density and viscosity of the media, but the differential signal is free of this effect. The stiffening effect is not so pronounced due to smaller surface stress generated in further distance from the gold surface.

Fig. 3. Simultaneous measurement of static bending and resonance frequency shift of a 2-cantilever array during functionalization with mPEG thiol self-assembling monolayer. Minute dynamic mode signals are emphasized by plotting 20-point moving average on top of them. Strong surface stress induced by SAM on such soft cantilever (30 mN/m) causes significant crosstalk to the resonance measurement, falsely suggesting decreasing bound mass.
Fig. 4. Dual-mode measurement of avidin binding to a biotinylated cantilever and subsequent binding of a biotinylated antibody do immobilized avidin. Minute dynamic mode signals are emphasized by plotting 20-point moving average on top of them. Little crosstalk is observed.

4. Conclusions

We have demonstrated dual-mode measurements of microcantilever sensor arrays applied as biosensors. We have used this technique to monitor the self-assembly process, receptor immobilization, and protein detection. The results show the importance of proper selection of cantilevers and their functional layers in relation to possible crosstalk between surface stress and mass signals.

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