

Nanomechanical biosensing with immunomagnetic separation

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We report a biosensing method that combines immunomagnetic separation and nanomechanical detection. In this method, same magnetic beads that are used to “fish” biomolecules from complex mixtures enable deflection of a cantilever structure upon excitation by an oscillating magnetic field. Biotin-coated magnetic beads were used to capture and separate streptavidin from serum. Streptavidin loaded magnetic beads were exposed to a differential cantilever system whose sensing arm was functionalized with biotin. The magnetic force applied on the streptavidin-beads resulted in differential cantilever deflections that could be detected down to $0.26 \text{ \AA}_{\text{rms}}$ in air. © 2010 American Institute of Physics. [doi:10.1063/1.3489356]

Over the past decade, researchers have developed and reported on numerous cantilever-based biosensing methods.^{1–3} In a typical detection experiment, a cantilever is functionalized with receptor molecules against targets that are to be detected. Binding of targets induces either static deflections¹ or changes in resonance frequency due to mass loading.³ The advantages of cantilever biosensors include high sensitivity, scalability due to microfabrication (both in terms of size and number), multiplexing, and possibility of integration with other lab-on-chip devices. However, it is challenging to operate cantilevers in biologically significant and complex mixtures such as blood, serum, and saliva. Some problems associated with these mixtures are viscosity of medium, nonspecific binding of other biomolecules, and possible interference with the cantilever operation (especially for optical measurements). Hence, cantilever-based biosensing techniques are most effective when performed in clear buffer solutions, heavily diluted mixtures, and water.^{2,4–6} Target capturing in complex media is an important limitation for cantilever-based biosensing. Herein we present a method that effectively bypasses the need to directly expose complex mixtures to the cantilever surface. Target molecules are “fished” from complex mixtures using magnetic beads, which are then suspended in friendly buffer solutions before exposure to the cantilever surface.

Magnetic nanospheres or microspheres or “beads” are widely used for biological target capturing and separation.^{7,8} Various sensing methods integrated with magnetic beads such as optical diffraction gratings, microfluidic electrochemical systems, biobarcode assays, and nanowires have been reported.^{9–12}

In a previous work we showed that subangstrom level deflections of $500\text{-}\mu\text{m}$ -long cantilevers can be detected when oscillating magnetic fields excite paramagnetic or superparamagnetic beads that are bound on the cantilever surfaces.¹³ An oscillating magnetic field results in cantilever actuation at frequencies where $1/f$ -type flicker noise is minimal and sub-angstrom level thermomechanical noise is dominant. As a result, extremely small deflections can be detected. In this

mode of operation, the excitation is neither static nor resonant; it is at a frequency in between the two.¹³

Herein we demonstrate the combination of cantilever-based biosensing with immunomagnetic separation to isolate target biomolecules from a complex biological mixture (50% newborn bovine serum), circumventing the need to directly expose the complex mixture to the cantilevers. In this detection system, the magnetic beads simultaneously serve following two purposes: (1) as immunomagnetic capture agents and (2) as nodes on cantilever for magnetic force induction.

A differential cantilever pair with adjacent sensing and reference arms was fabricated with the following parameters: $\rho=2900 \text{ kg/m}^3$, $E=200 \text{ GPa}$, $L=250 \text{ }\mu\text{m}$, $w=50 \text{ }\mu\text{m}$, $t=0.5 \text{ }\mu\text{m}$, and $Q=9$ in air.¹⁴

By combining the newly fabricated cantilevers with immunomagnetic bead-based separation, we detected a model protein, streptavidin, from a model complex mixture, bovine serum. A schematic of the detection system is shown in Fig. 1. The sensing arm of the gold-coated cantilever was functionalized with biotin-coupled bovine serum albumin (BSA) [Pierce, 2.5 mg/ml, in phosphate buffered saline (PBS)] using a nanojet nozzle (MicroFab Tech). Five 1 nl droplets were sequentially deposited to the cantilever surface for functionalization. Next, the entire sensor structure (both cantilevers) was dipped into BSA (Sigma, 10 mg/ml, in PBS) solution for 30 min in order to passivate the reference cantilever and also to prevent nonspecific binding to any possible blank areas on the sensing cantilever. Cantilevers were washed with PBS before bead exposure. Different concentrations (0.28, 0.37, 1.89, 5.68, 17, 37.8 and 114 nM) of streptavidin (Pierce) were added to 50% newborn bovine serum in PBS (Invitrogen) to form a model complex mixture (5 ml). $25 \text{ }\mu\text{l}$ of biotin coupled magnetic beads (diameter $=1.5 \text{ }\mu\text{m}$, 5×10^8 particles/ml Bangs Laboratory) were added to the complex medium. After 25 min of shaking, streptavidin-loaded magnetic beads were separated from the complex mixture using a permanent magnet and washed vigorously with a PBS solution. Streptavidin-loaded magnetic beads were resuspended in a relatively low volume ($200 \text{ }\mu\text{l}$) of PBS and exposed to both cantilevers for 15 min. Cantilevers were washed with PBS and deionized water, and then dried with nitrogen for measurements and imaging. The

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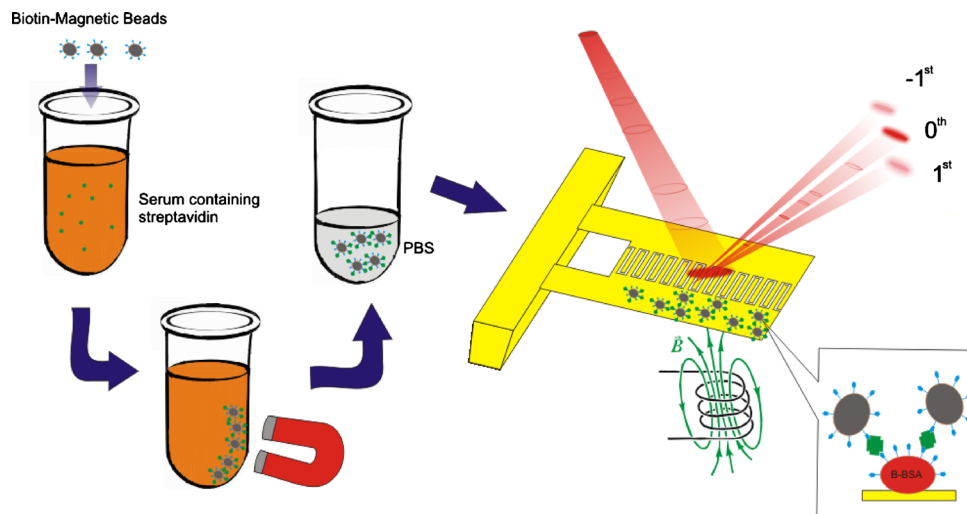


FIG. 1. (Color online) Schematic of the sensing system. Target molecules are captured and separated from 50% serum using coated magnetic particles. Target loaded magnetic particles are washed, diluted, and exposed to cantilever. Presence of magnetic beads on the sensing arm causes a differential deflection relative to the reference arm upon applying a magnetic field. The differential motion is detected directly using interferometry.

streptavidin–biotin interaction caused more beads to bind on the surface of the sensing arm than the adjacent reference cantilever. An alternating magnetic field from an electromagnet resulted in relative bending of the sensing arm with respect to the reference arm. Interferometry was used to measure this differential cantilever deflection.^{4,13}

Before measuring deflections, cantilevers were first examined under a bright-field microscope. Figure 2 shows some example cantilevers that bear different numbers of beads, due to different streptavidin concentrations that the beads were exposed to. Also shown in Fig. 2 is the result of a control experiment where no streptavidin was present in the sample solution. In the case of no streptavidin, few beads bound nonspecifically to both sensing arm (left) and control arm (right) demonstrating the importance of simultaneous differential detection. Also shown in Fig. 2 is another experiment that demonstrates an advantage of magnetic-induced deflection versus imaging. Here, only the sensing arm of the cantilever was intentionally and nonspecifically coated with nonmagnetic, silicon oxide beads (diameter=165 nm).

Figure 3 demonstrates the dependence of bead number difference on streptavidin concentration. For each sensor, a “differential number of beads,” i.e., the difference between number of beads on the sensing arm and the control arm was counted using an imaging algorithm written in MATLAB. For

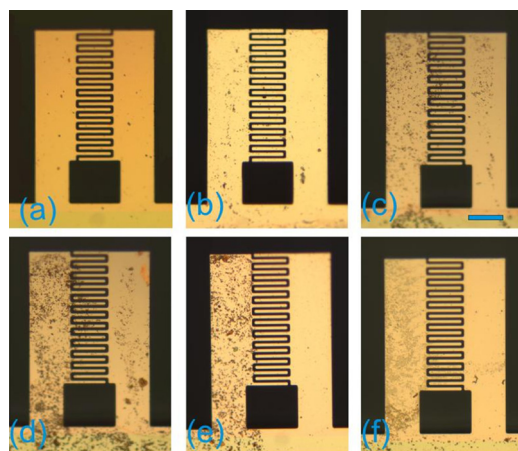


FIG. 2. (Color online) Optical micrographs of some of the cantilevers with different streptavidin concentrations, including control experiments. (a) control (0 streptavidin), (b) 0.37 nM, (c) 17 nM, (d) 37.8 nM, (e) 114 nM, and (f) silicon oxide beads. Scale bar=50 μm .

each concentration six different sensors were considered each having a reference and a sensor cantilever with an area of $250 \times 50 \mu\text{m}^2$. The plot in Fig. 3 demonstrates the averages and standard deviations. Imaging the surface of the cantilever with silicon oxide beads revealed 847 beads which is equivalent to 5 nM of streptavidin, i.e., a false signal since the silicon oxide beads bear no streptavidin. On the other hand, deflecting cantilevers by means of a magnetic field should inherently suppress this problem since nonmagnetic beads (or by the same token any other nonmagnetic contaminants) are not expected to produce a significant deflection signal.

To deflect the cantilevers that have magnetic beads on them, a sinusoidal input signal (2333 Hz, 20 V_{pp}) was used to drive an electromagnet made in house. Note that frequency of input signal is high enough to operate cantilever in the region where $1/f$ type noise is no longer significant and only thermomechanical noise is dominant.¹³ This excitation frequency is also lower than the observed resonant frequency of the cantilever (~ 7 kHz). A laser beam (632.8 nm wavelength) was used to illuminate the diffraction grating, i.e., the interdigital fingers of the sensing and control arms. A photodiode was used to measure the intensity of zeroth (I_0) order diffraction mode that represents the relative motion between the two cantilevers.^{15,16}

The power spectral density (PSD) of the output signal was recorded by a computer with LABVIEW (National Instru-

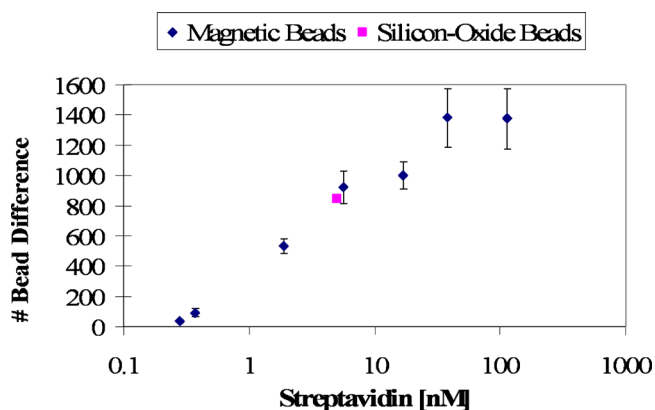


FIG. 3. (Color online) Variation in differential bead number with the streptavidin concentration. The counting was performed on six different cantilevers for each concentration.

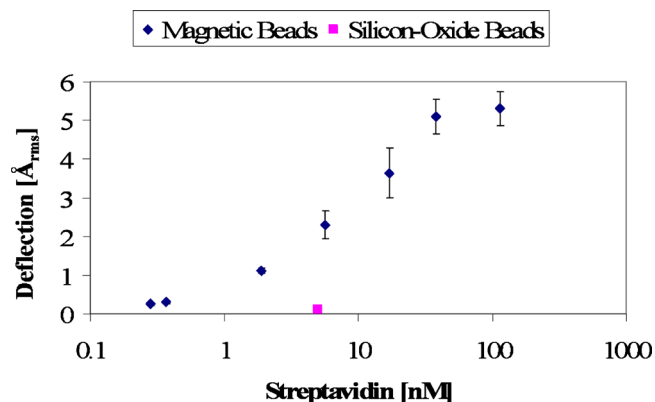


FIG. 4. (Color online) Variation in differential cantilever deflection with streptavidin concentration.

ments) software. The corresponding cantilever deflection was calculated from the PSDs over a 1 Hz bandwidth. Figure 4 illustrates the dependence of the differential cantilever deflection on the concentration of streptavidin. In case of the control experiment (no streptavidin) and the experiment with silicon oxide beads, $\sim 0.1 \text{ \AA}_{\text{rms}}$ deflection was observed which is close to thermomechanical noise level of the cantilevers ($0.07 \text{ \AA}_{\text{rms}}$) at 2333 Hz. Theoretically, for a thermomechanically limited cantilever system,¹³ four beads located at the tip of the cantilever can be detected using magnetic actuation in the thermomechanically limited low noise region using our setup.¹⁴ Our minimum detectable signal ($0.26 \text{ \AA}_{\text{rms}}$) that results from about 50 beads is reasonable since (1) the beads are distributed over the cantilever surface and are not located exactly at the tip and (2) the deflection observed due to 50 beads is larger than the thermomechanical noise level.

As the concentration of streptavidin increased from 0.28 to 114 nM, more beads bound to cantilever surface resulting in a corresponding increase in the differential cantilever deflection. The minimum detectable concentration was 0.28 nM, beyond which the number of beads on the cantilever surface was not sufficient to actuate the cantilever. In the current case, the beads and the cantilever surface are both functionalized with the same receptor molecules. This increases the possibility of a target molecule being sandwiched between two beads, hence lowering the possibility of bead binding to the surface. We expect that lower concentrations can be detected with other biomolecular targets for which the beads and sensor surfaces are generally functionalized with different capture molecules. Also, a full chemical optimization of functionalization steps (concentrations, types, and/or incubation times of receptors, targets, and beads) should lead to detecting lower concentrations.

In conclusion, we have demonstrated a biomolecular sensing scheme by combining bead-based immunomagnetic separation and nanomechanical biosensing. Combination of immunomagnetic separation and nanomechanical biosensing introduces some important advantages. (1) Target molecules are separated from complex biological mixtures *before* exposure to the sensor surface, greatly reducing nonspecific binding effects. (2) Magnetic separation allows resuspension of target molecules in smaller volumes which constitutes an effective increase in concentration and increases the possibility of target capturing on the cantilever surface. (3) Magnetic beads captured on the cantilever surface by means of biomol-

ecular recognition inherently allows the application of an external magnetic force which deflects the cantilevers differentially without needing any additional fluorescent or radio-labels. (4) Combining immunomagnetic separation with nanomechanical sensors completely uncouples the primary target separation/preparation from the detection process as follows: e.g., target molecules in a blood sample can be separated by magnetic beads and resuspended in a friendly buffer, allowing storage of both the rest of the blood sample and the target-bound beads for future use (the cantilever detection no longer needs to involve the original sample). Moreover, this method allows detecting extremely small deflections by exciting the cantilever at a specific frequency in a relatively low-noise region and hence avoiding $1/f$ -type low frequency noise. In addition to *a priori* separation of target molecules from complex fluids, the system offers an inherent line of defense against nonspecific binding since only magnetic beads contribute significantly to cantilever deflection. Stray entities such as salt residues, dirt, or other nonmagnetic particles do not cause a significant cantilever signal. These nonspecific entities can constitute a significant background in direct bright-field imaging unless high-resolution microscopes are used in conjunction with sophisticated imaging algorithms that can utilize various optical characteristics of these particles. The method is also fluorescence or radio label-free and possesses array capability. Our future goal is to use this method to detect clinically significant biomolecules from bodily fluids in a multiplexed fashion by utilizing an array of cantilevers functionalized against various target molecules.

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