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# A wireless biosensor using microfabricated phage-interfaced magnetoelastic particles

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**Abstract**

A micro-scale, free standing, wireless biosensor has been developed using magnetoelastic particles composed of an amorphous iron–boron binary alloy. Upon the application of an external magnetic field, these particles exhibit a characteristic resonance frequency, determined by their size and mass, due to the phenomena of magnetoelasticity. The particles are produced using the microelectronic fabrication techniques of photolithography and physical vapor deposition (sputtering). The biosensor is formed by coating the magnetoelastic particle with a thin layer of gold and immobilizing a biomolecular recognition element (bacteriophage) on the surfaces. Bacteriophage genetically engineered to bind *Bacillus anthracis* spores was used in this set of experiments as the detection probe. Once these targeted spores come into contact with the biosensor, the phage will bind selectively with only that pathogen, thereby increasing the particle's mass and causing a shift in the resonance frequency. Due to the magnetic nature of the sensing platform, this resonance frequency shift may be detected remotely by a wireless scanning device, presenting a distinct advantage over other techniques. A good correlation between the actual number of spores bound to the sensors and the calculated attached mass, based upon resonance frequency shifts, was obtained from the experiments.


*Keywords:* Magnetoelastic; Magnetostrictive; Biosensor; Sputtering; Bacteriophage; *Bacillus anthracis*; Spores

## 1. Introduction

As part of the ongoing effort to secure the safety of our food supply, as well as to guard against the possibility of bioterrorism, much research has been focused on developing detection techniques that are not only accurate, fast, and inexpensive, but can also offer detection remotely of sealed containers and packages. Towards these goals, immunoassay-based, label-free biosensing systems have proven to be among the most promising methods for microorganism detection [1–3]. Immunoassay-based biosensors, such as acoustic wave biosensors, are composed of a transducer, which converts biological signals of target–receptor interaction into electrical or other measurable signals, and a bioprobe that possesses affinity for a target pathogen. Various types of acoustic wave devices have been fabricated and used for biological detection in combination with immobilization techniques of biological recognition probes [4,5]. These acoustic wave sensor platforms provide the capability of real-time detection and several other advantages, such as high sensitivity, simplicity, and low cost [3,6,7].

Pathogen detection at very low concentrations, down to the ultimate goal of single cell or spore detection, has been a key challenge in the biosensing area. Substantial progress has been made in the application of silicon-based and piezoelectric-based cantilevers and microcantilevers due to their high sensitivities [8]. The ability to detect about 200 bacterial cells [9] and 60 fungal spores [10] using functionalized cantilevers has been reported. Additional studies have shown that as few as 50 spores can be detected in water by cantilevers using thermal noise as the excitation source [11]. Compared with traditional microcantilevers, magnetoelastic-based microcantilevers have been demonstrated to have a better overall sensitivity and a quality

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factor ($Q$-value) due to the material's properties and wireless actuation mode [12,13].

The potential of a free-standing, magnetoelastic strip as the basis of a remote sensor has been demonstrated for the detection of changes in various physical properties [14], as well as the presence of specific biological species [15–17]. This method offers real-time detection of targeted pathogens in various liquid media and, since it is wireless, no direct contact between the sensor and the measuring device is required. A key issue with this technique, however, has been sensitivity. As is the case with microcantilevers, sensitivity will increase as the size of the sensor is decreased [13]. In order to directly detect the presence of a small number of spores or cells (e.g. on the order of $10^2$ or less), the sensor must theoretically have a maximum dimension in the range of $10^2$ microns. Traditionally, such sensor platforms have been fabricated by the mechanical polishing and dicing of commercially available amorphous alloy ribbons, which are then coated with a gold layer for biocompatibility [18]. This method has led to problems in dimensional repeatability (e.g. edge defects) and surface quality (e.g. poor gold layer adhesion). Additionally, it has proven to be extremely difficult to make samples of the required size with a mechanical dicing technique. The goal of this research is to solve the above problems by employing techniques found in microelectronic fabrication (specifically photolithography and physical vapor deposition) to produce magnetoelastic sensor platforms of desired size and shape with excellent dimensional control and surface quality. Functionality of the sensors in detection of *Bacillus anthracis* spores was explored using a landscape phage recognition interface.

## 2. Materials and methods

### 2.1. Principle of detection

The phenomenon of magnetoelasticity occurs when, upon the application of an external magnetic field, a material experiences a shape change due to the superposition of its internal magnetic moments due to spin–orbital coupling [19]. If this field is alternating ("AC") and the piece of material is planar (i.e. its thickness is much less than its length and width), then the fundamental resonance frequency of the longitudinal vibration caused by magnetostriction is described by Liang et al. [20]:

$$f_0 = \sqrt{\frac{E}{\rho(1-\nu)}}\frac{1}{2L} \tag{1}$$

where $E$, $\rho$, $\nu$, and $L$ represent the elastic modulus, density, Poisson's ratio, and the length, respectively. Additionally, this resonance frequency will be affected by the sensor's mass load as described by Grimes et al. [14]:

$$\Delta f = -\frac{f_0}{2}\frac{\Delta m}{M} \tag{2}$$

where $\Delta f$ is the change in resonance frequency, $\Delta m$ is the change in the sensor's mass due to the attached load, and $M$ is the original mass of the sensor. Note that the negative sign indicates that the frequency will shift to a lower value upon an increase in the sensor's mass load. Also, this relation assumes a load that is evenly distributed over the surface of the sensor.

The principle by which the property of magnetoelasticity may be utilized as a biosensing platform is shown schematically in Fig. 1. First, the magnetoelastic particle (MEP) is coated with a gold layer to enable biocompatibility with protein molecules such that they may be attached onto the surface. Next, a biomolecular recognition element (either bacteriophage or antibody) is immobilized onto the gold surface, enabling the capture of a single targeted pathogenic species. At this point, the fundamental resonance frequency is measured in order to completely characterize the biosensor prior to exposure to target containing solutions. This is accomplished by applying an AC magnetic field to set up resonance, as well as a DC biasing magnetic field for amplification of the signal. Then, when the sensor is exposed to the targeted bacteria or spores, they bind to the sensor, thereby increasing its mass and lowering its resonance frequency. Also, based on the magnitude of the frequency shift, the approximate number of attached spores or cells may be calculated using Eq. (2) (assuming an approximate mass of each spore or cell).

### 2.2. Magnetoelastic platform material

An amorphous binary alloy of iron–boron was chosen as the basis for the sensor platform material for several reasons. Primarily, it is a much-studied magnetoelastic system that easily forms the amorphous phase with boron content equal to or greater than the eutectic composition of 16–17 at.% [21,22]. Also, as is the case with other metallic glasses, its soft magnetic properties, moderately high level of magnetostriction ($\lambda_s$), and low magneto-crystalline anisotropy combine to result in a very high magneto–mechanical coupling efficiency ($k_{33}$), making it better suited to high frequency sensor applications, as opposed to the giant magnetostrictive compounds, which are better suited for application in mechanical stress sensors and high-power acoustic devices [23,24]. Finally, and practically, since it is only a two-component system, fabrication is relatively simple and it is much easier to achieve the desired composition using physical vapor deposition (or "sputtering") than would be the case with a multi-component system, such as those commonly employed in commercially available amorphous metal ribbons.

### 2.3. Sensor platform fabrication

The sensor platform fabrication process is shown schematically in Fig. 2. Fabrication of the sensor platform begins by coating a 100 mm plain silicon test wafer with a layer of chromium, and then gold, each at a thickness of 30–40 nm. This is accomplished using a Denton Vacuum Discovery-18$^{\text{TM}}$ magnetron sputtering system, which employs three cathodes (each holding a 3-in. diameter disk-shaped target) aimed off-axis at a circular, rotating substrate platform, along with DC and RF power supplies. The gold layer is needed to adhere the next deposited film (also gold) to the wafer, while the chromium merely serves to act as a bond between the silicon and the