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Probing biomolecular interaction forces using an anharmonic acoustic technique for selective detection of bacterial spores

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Abstract
Receptor-based detection of pathogens often suffers from non-specific interactions, and as most detection techniques cannot distinguish between affinities of interactions, false positive responses remain a plaguing reality. Here, we report an anharmonic acoustic based method of detection that addresses the inherent weakness of current ligand dependant assays. Spores of Bacillus subtilis (Bacillus anthracis simulant) were immobilized on a thickness-shear mode AT-cut quartz crystal functionalized with anti-spore antibody and the sensor was driven by a pure sinusoidal oscillation at increasing amplitude. Biomolecular interaction forces between the coupled spores and the accelerating surface caused a nonlinear modulation of the acoustic response of the crystal. In particular, the deviation in the third harmonic of the transduced electrical response versus oscillation amplitude of the sensor (signal) was found to be significant. Signals from the specifically-bound spores were clearly distinguishable in shape from those of the physisorbed streptavidin-coated polystyrene microbeads. The analytical model presented here enables estimation of the biomolecular interaction forces from the measured response. Thus, probing biomolecular interaction forces using the described technique can quantitatively detect pathogens and distinguish specific from non-specific interactions, with potential applicability to rapid point-of-care detection. This also
serves as a potential tool for rapid force-spectroscopy, affinity-based biomolecular screening and mapping of molecular interaction networks.

**Keywords:** nonlinear acoustics; anharmonic interaction; label-free detection; force spectroscopy; bacillus subtilis

1. Introduction
Established biochemical methods for pathogen detection are primarily based on the detection of specific nucleotide sequences within the pathogen genome or on the detection of pathogen-specific surface epitopes using specific receptors (antibodies, peptides or aptamers). The most widely used methods in the two categories are the nucleic acid-based assays, such as polymerase chain reaction (PCR), and the antibody-based assays, such as enzyme-linked immunosorbent assays (ELISA). Although offering high sensitivity, and in some cases high selectivity, these methods can require time-consuming and skill-demanding sample preparation. Moreover, being label-dependent, they are confined to specialised laboratories with appropriate interrogation equipment. However, in clinical diagnosis, food and environmental monitoring, and detection of biowarfare pathogens, there is an increasing demand for rapid and easy-to-use detection platforms that can be implemented at the point-of-care (POC).

Labelled antibody-based tests, such as dip-stick method(Dewey et al. 1989) and lateral flow devices (LFD)(Lane et al. 2007), are relatively fast and easy-to-use but provide only qualitative or semi-quantitative data at limited sensitivity(Skottrup et al. 2008). Immunosensors based on platforms such as surface plasmon resonance (SPR)(Hoa et al. 2007), quartz crystal microbalance (QCM)(Cooper and Singleton 2007) and cantilever-based sensors(Waggoner and Craighead 2007), provide rapid and easy detection with high sensitivity and quantification wherein the binding of receptors (immobilized on the immunosensor surface) with pathogens via pathogen-specific surface epitopes results in a direct measurable signal without the requirement for labels. Yet, an inherent shortcoming in most sensing techniques used here is the inability to differentiate between specific (target) and non-specific (non-target) interactions, the latter resulting from the formation of non-specific bonds that, though weaker, cannot be easily dissociated. The resulting false positive responses can lead to misdiagnosis, consequent mistreatment (clinical) and false alarms (biosecurity).
Here we report a technique based on nonlinear acoustics that can be used to sense biomolecular interaction forces and hence distinguish the target from non-targets in an analyte. Acoustic nonlinearity has been used in non-destructive testing for measurement of strength of adhesive bonds (Fassbender and Arnold 1996). The underlying concept here is that binding forces in the soft interface layer are nonlinear and result in a nonlinear modulation of transmitted or reflected ultrasonic waves that can be analysed to measure the binding forces (Hirsekorn 2001). Similarly, surface-coupled streptavidin-coated polystyrene microbeads (SCPM) on thickness-shear mode (TSM) quartz crystal in air result in a significant enhancement to its nonlinear response (Ghosh et al. 2010). We employ this principle for the first time to investigate the interaction forces at the biological interface in an immunosensor. By studying the nonlinear acoustic response from a TSM quartz crystal with functionalized specific antibodies, we can sensitively detect \textit{Bacillus subtilis} spores and distinguish them from physisorbed SCPM.

2. Theoretical basis

Supplementary Figure 1 illustrates a spore attached to the sensor via a biomolecular tether, modeled as a spring. The high relative motion between the spore and the sensor surface at resonance causes significant strain in the tether, which causes forces to act on it. These forces are transmitted onto the sensor. The horizontal component of the transmitted force ($F_{tx}$) is only significant here as only the horizontal shear forces are transduced into charge due to piezoelectric effect of TSM quartz. Hence, $F_{tx}$ modifies the shear forces of the bulk quartz and modulates the electrical response of the sensor. Since the force component $F_{tx}$ is nonlinear due to the intrinsic characteristic of the biomolecular tether and the inclination angle of the spring to the surface (Ghosh et al. 2010), the modulated electric response is also nonlinear (anharmonic) with significant increase in the amplitude of the higher Fourier harmonic components (of drive frequency $f$) in the electrical response. Since the nonlinearity in $F_{tx}$ is largely symmetrical, the electrical response comprises predominantly of odd harmonics. Hence, the modulation causes deviation in only the odd harmonic components of $f$. The relative deviation in the first harmonic ($f$) is negligible and in practice cannot be reliably extracted from the linear response and drive signal feedthrough. Among the higher odd harmonics, we chose to measure the shift in amplitude of the third harmonic ($3f$) as the amplitude decreases with increase in the harmonic number.
3. Experimental approach
We chose TSM AT-cut quartz as the sensing platform since its use in QCM sensors is well established and the TSM has good quality factor in liquid (approximately 2000 in our case). Following immobilisation of B. subtilis spores by anti-spore IgG (as detailed in the Methods section), the sensor was driven with a pure sinusoidal voltage of frequency close to its fundamental resonant frequency ($f = 14.3 \text{MHz}$) with linearly increasing amplitude for 2 min.

4. Results and discussion
Measurements were taken before and after the incubation of spores as described in the Methods section. The increase in the amplitude of $3f$ response (current) was found to be significant after incubation of spores (Fig. 1a) given the number of spores captured on the sensor (Sensor 1) was approximately 6400, as observed using an optical microscope. This is equivalent in weight to 9.6 ng, using the experimentally determined value of 1.5 pg as the weight of a spore(McCormick and Halvorson 1964). For similar measurements from a sensor (Sensor 2) with physisorbed SCPM of similar size as the spores (~1 µm), the amplitude of $3f$ response was also found to increase after physisorption of beads.

4.1 Selectivity
The increase discussed above was however much less significant given the comparatively higher population of the SCPM used (30 µL of $2 \times 10^8$ beads/ml) (Fig. 1b). In particular, the graph of deviation in the amplitude of $3f$ response against the oscillation amplitude (signal) was strikingly different for the two cases (Fig. 1c-d). Lower signal intensity and increased noise was measured from SCPM coated sensors. Since, as explained before, the interaction forces at the biological interface generate this signal, this characteristic difference can be explained by weaker antibody-SCPM interaction forces than antibody-spore interaction forces. Moreover, the signal from the sensor with SCPM reduced on successive scans but the signal from the sensor with spores was statistically reproducible from successive scans. This can be explained since physisorbed SCPM decouple from the surface at higher oscillation amplitudes as expected whereas the specifically captured spores do not. This hypothesis is confirmed
by the increase in quality factor of the sensor with SCPM after the 3f response measurement scan. This also demonstrates a capability of self-cleaning the sensor surface of non-specific interactions. Similar characteristics to that observed with SCPM on anti-spore antibody were also found with *B. subtilis* spores on a non-specific (goat IgG) antibody (data not shown).

**Figure 1** The electrical response of the sensor (current) measured at 3f or three times the drive frequency, *f*. (a) Amplitude of 3f response from a sensor (Sensor 1) with approximately 6400 *Bacillus subtilis* spores specifically captured on it. (b) Amplitude of 3f response from a sensor (Sensor 2) with streptavidin-coated polystyrene beads (SCPM) physisorbed on it using a 30 µL solution of 2×10^8 beads/ml. (c,d) Deviation in amplitude of 3f response from the respective sensors.

In order to test the relative contribution of specific and non-specific interactions to sensor response, the normal wash step following a 9 min immobilization of approximately 30 µL 1×10^5 cfu/ml spores was omitted. As expected, the first scan from this sensor (Sensor 3) was similar to the case with SCPM (**Fig. 2**) with loosely or non-specifically bound spores also contributing to the signal. The successive decrease in signal and signal noise in the second and third scans provided confirmation of self-
cleaning of the sensor surface and that the signal from latter scans resulted predominantly from specifically captured spores.

**Figure 2** The deviation in amplitude of 3f response (current) for a sensor (Sensor 3) where the normal wash step following a 9 min immobilization of approximately $1 \times 10^5$ cfu/ml spores was omitted.

### 4.2 Force-spectroscopy

The dynamics of a spore coupled to the oscillating TSM quartz is modeled as a rigid microsphere attached to a surface oscillating in-plane as described in the Supplementary Section S1. Based on the force-extension graphs reported in AFM studies (Linke and Grutzner 2008), the characteristic force ($F$) versus extension ($s$) function of a biomolecular linker tethering the spore to the surface is assumed as follows.

$$F(s) = [a_1 \sinh(s/b_1) + a_2 \sinh^3(s/b_2)] \exp[-(cs + d)^n]$$

The parameters in the function are initially guessed and the differential equations describing the spore dynamics, as illustrated in the Supplementary Section S1, are solved to compute the signal. The functional parameters are altered to fit this computed signal with the experimentally observed signal. The resulting fit for the sensor with 6400 spores **(Fig. 3a)** was obtained for the following values of the coefficients of $F(s)$ in eq 1: $a_1 = 420$; $b_1 = 20$; $a_2 = 600$; $b_2 = 20$; $c = 1/22$; $d = 0.25$; $n=7$. The resulting
force-extension function is presented in Figure 3b along with the operating force and extension in the linkers.

Figure 3 (a) Deviation in $3f$ current versus sensor oscillation amplitude (referred to as signal) from model and experiment with Sensor 2 having 6400 bound spores, and (b) characteristic force-extension function of the linker binding the spore to the surface estimated from model along with the operating force-extension.

It may be noted from Figure 3b that under the drive parameters of the experiment (frequency and amplitude range) the maximum operating extension remains less than the unbinding threshold and the maximum force in the linker less than the unbinding force. Hence, no unbinding of specifically bound spores occurs. This confirms the observation of reproducibility of signals from successive scans. Thus, by comparing the signal estimated from the model with experimental observations and determining the force-extension function, it is possible to estimate the peak force and the maximum extension corresponding to unbinding of the linker.

The estimated peak force is comparable with that reported in the AFM experiments with single molecules of titin (Linke and Grutzner 2008). The signals for
two other concentrations of spores estimated using the determined function $F(s)$ closely matched the experimental observations (Fig. 4). The small difference for 2200 spores can be attributed to relatively lower signal-to-noise ratio. The difference for 10000 spores may be attributed to a few spores being loosely or non-specifically bound to the surface. Also, the measured response is found to be nearly proportional to the number of spores captured on the surface.

![Graph](image)

**Figure 4** Signal corresponding to 20 nm of oscillation amplitude for three different spore concentrations from experiments and model.

An appropriate surface design with knowledge and control of $N_l$ should allow more accurate estimation of $F(s)$. Thus, using the model it is possible to reproduce and predict the experimental observation, and also determine the characteristic force-extension function of the biomolecular tether binding the spore to the surface, particularly the peak force and maximum extension at unbinding. Hence, the presented anharmonic acoustic technique can be potentially employed for force-spectroscopic studies.

### 4.3 Proportionality with concentration

The detection is quantitative. The oscillations of the spores are synchronous and hence the shear forces arising from them are additive. Moreover, since the 30 µL spore solution covers a small region at the centre of the quartz crystal, the oscillation amplitudes experienced by the spores are approximately the same. This explains (and is
supported by) the experimental observation that the signal at any oscillation amplitude is approximately proportional to the number of spores specifically captured on the surface (Fig. 4).

This proportionality relationship does not hold with the concentration of spore solution since the percentage of spores successfully captured on the surface decreases with higher concentrations, possibly due to greater steric hindrance. **Supplementary Figure 2** presents the number of spores captured as a function of spore concentration as observed in our experiments. This is consistent with previously reported observations. (Dhayal et al. 2006)

### 4.4 Sensitivity and detection limit

The detection is sensitive. The signal-to-noise ratio (SNR) observed with 6400 spores (~7.7) indicate that the approximate detection limit to be 430 spores (assuming the detection limit corresponds to a SNR of 2). Put into context, extrapolations from animal data suggest that the human LD$_{50}$ (lethal dose adequate to kill 50% of exposed individuals) is 2500 to 55000 inhaled *B. anthracis* spores. (Inglesby et al. 2002) The quantitative model predicts that the sensitivity can be further enhanced by optimizing the drive frequency and amplitude. **Figure 5a** shows that the deviation in 3$f$ current as a function of oscillation amplitude (signal) increases with increase in drive frequency.
According to the estimates, the signal increases with drive frequency as \( f^{1.916} \) (Supplementary Information Figure 3), e.g. a five-fold increase in frequency gives twenty-two-fold increase in signal. However, the peak force and extension threshold corresponding to unbinding is also reached at lower oscillation amplitudes (Fig. 5a,b). While the extension of the linker is below unbinding limits at 14.3 MHz of drive, the peak force and extension threshold for unbinding is reached at 71.5 MHz of drive (in the figure, the signal for 14.3 MHz drive has been enhanced by 1% for clarity of presentation). Hence, the maximum drive amplitude should be appropriately decreased for higher drive frequencies to avoid unbinding of spores, which is important to maintain reproducibility of signal over successive scans.

Moreover, the percentage of spores captured from a given sample may be improved by use of alternative ligands, further optimisation of immobilisation conditions, or employing a smart incubation technique, such as employing magnetic
beads in a flow cell. (Kang-Yi et al., 2010) All these factors should improve the overall sensitivity of the detection system and could potentially result in the detection of single spores. Improved incubation techniques should also reduce the incubation time and hence decrease the overall response time of the sensor (currently ~15 min including incubation and detection).

5. Comparison with other POC immunosensing methods

A nonlinear acoustic biosensor based on the described anharmonic detection technique (ADT) would have distinct advantages compared to existing POC immunosensors for a number of reasons.

First, since the signal is based on the force of interaction between the sensor and the coupled particles, specific and non-specific interactions can be distinguished using the shape of the signal profile for varying drive amplitude. The fabrication of a reliable reference sensor, as utilised in some resonant frequency shift based platforms (such as the QCM and cantilever) involves the challenge of maintaining both sensors at the same conditions (of surface, environment and analyte). Alternatively, use of a flow-through sample delivery procedure instead of static addition to reduce non-specific binding needs optimisation of flow rate, since larger pathogens, such as bacterial spores, may get washed away without having a chance to bind to antibodies. (Lee et al. 2005) Extensive washing to get rid of non-specific binding often result in loss of receptor from the surface decreasing surface bio-activity (Cass and Ligler 1998; Hao et al. 2009). In comparison, the ADT operating at high oscillation amplitudes results in the unbinding and removal of weaker interactions and confirms the same from reproducibility of successive scans and decrease in overall noise. It may be mentioned here that a rupture event scanning (REVS) method was described to detect pathogens from the abrupt changes in the third harmonic response of a quartz crystal due to reported ‘rupture’ of pathogens at high oscillation amplitudes. (Cooper et al. 2001) The capability for differentiation with respect to non-specific binding was claimed here since ‘rupture’ of loosely bound particles was expected to occur at lower drive voltages. However, a thorough mechanistic analysis for qualitative and quantitative understanding of the signal is missing and failures to reproduce ‘rupture’ of specific bonds have been reported. (Edvardsson et al. 2005) Furthermore, as our experiments reveal, some non-specific interactions may unbind at higher amplitudes or in the second scan. Hence, it
could be challenging to separate the REVS signals from specific and non-specific interactions.

Second, ADT in principle allows direct detection of pathogens without any intermediate processing step. In comparison, while this is usually not a problem with resonant frequency shift based sensors, SPR is not sensitive to pathogens since the size of most pathogens is greater than the effective penetration depth of evanescent field of most SPR sensors (approximately 150 nm in Biacore sensors (GE Healthcare Bio-Sciences AB 2008)). Hence, intermediate steps are often implemented using sandwich assays (Bokken et al. 2003) or subtractive inhibition assays (Leonard et al. 2004).

Third, the initial sensitivity demonstrated is comparable with the best reported results with QCM and SPR, but as the prior discussion suggests, detection of single pathogens could be realized without the necessity for miniaturization.

Fourth, ADT for the first time shows the promise of a rapid force-spectroscopic technique that simultaneously averages over multiple biomolecules. In comparison, the conventional methods, such as the Atomic Force Microscopy (AFM), measure single molecules at a time, requiring time-consuming multiple measurements for statistically averaged data.

6. Conclusion
The anharmonic detection technique (ADT) described here opens a new paradigm of biological detection. This technique discriminates between specific and non-specific interactions and has an additional level of selectivity over the efficacy of the receptor. Moreover, this enables quantitative measurement with high sensitivity with the potential for the method to be extended to the detection of a single bacterial spore. This also allows for a rapid, easy-to-use and cost-effective detection technique implementable on an entirely electronic portable platform for POC applications. Due to its ability to determine force-extension characteristics of molecular linkers, ADT also lends itself to applications in molecular force spectroscopy. The feasibility for detection of pathogens and cells from environmental isolates, and further applications to biomolecular characterization and mapping of molecular interaction networks using ADT warrants further investigation.
Materials and Methods

Spore preparation and storage. Vegetative cultures of *B. subtilis* 168 (A. Moir) were maintained on nutrient agar plates (oxoid) incubated at 37°C. Sporulation was induced by inoculating 400 ml supplemented nutrient broth (SNB) with 1 ml of a mid-log-phase culture. SNB medium consists of the following (per litre): Difco nutrient broth, 8.0 g; glucose, 1.0 g; KCl, 1.0 g; MgSO₄·7H₂O, 246 mg; CaCl₂·2H₂O, 147 mg; MnCl₂·4H₂O, 4 mg; and FeSO₄·7H₂O, 0.3 mg. The pH was adjusted to 7.2 prior to autoclaving. Cultures were incubated (37°C, 225 rpm) for 72 h. Spores were separated from vegetative debris and lysed material by repeated cycles of centrifugation and resuspension in ice-cold water (15,000 g for 10 min, 4°C). Purified spore preparations contained greater than 98% phase bright spores as determined by phase-contrast microscopy and were stored on ice (optical density at 600 nm [OD₆₀₀] of 100), protected from light.

Antibody functionalization of the quartz crystals. The gold electrodes of the quartz crystals (XL 1050, procured from Lap-Tech Inc., Bowmanville, Ontario, Canada) were cleaned successively with acetone, isopropyl alcohol (IPA) and ultra pure ethanol, and immediately placed in a petridish of 1 mM ethanolic solution of 16-Mercaptohexadecanoic acid (MHDA) (448303-1G, procured from Sigma-Aldrich) and left overnight for formation of a self-assembled monolayer (SAM). The next day, the crystal surface was carefully washed in ethanol and treated with a mixture of aqueous solutions of 0.4 M EDC and 0.1 M NHS for 5 min. After washing the mixture with HEPES (7.4 pH), a 40 µL drop of 670 µg/ml anti-spore antibody (rabbit IgG, procured from Thermo Scientific, PA1-7203) solution in acetate buffer (4.5 pH) was placed on the thiol-functionalized gold electrode for 30 min. The antibody functionalized crystals were then carefully washed with HEPES solution to remove any non-specifically bound antibody.

Capturing the spores on the crystals. Three spore concentrations were prepared in acetate buffer - 1×10⁵, 1×10⁶ and 1×10⁷ cfu/ml. A 30 µL drop of the spore solution was placed on the antibody-functionalized electrode for 9 min. This was followed with a wash of acetate buffer to remove the non-specifically bound spores. The number of spores on the sensor was counted under an optical microscope.
**Adsorbing microbeads on the crystals.** A 7.4 pH solution of streptavidin-coated polystyrene microbeads (SCPM) of diameter 0.97 µm (procured from Bangs Laboratories Inc., CP01F/8963) was prepared in phosphate buffer saline (PBS). The concentration of the solution prepared was $2 \times 10^8$ beads/ml. A 30 µL drop of the microbeads solution was placed on the antibody-functionalized electrode for 60 min for the physisorption to take place. The sensor was not washed after this step.

**Taking the measurements.** A 8 µL drop of PBS was placed on the sensor for taking the measurements, to ensure that the antibody layer was never dry. This was followed for all cases with or without the spores and the microbeads. The sensor was covered to control the evaporation of the drop during scan and a fresh drop of 8 µL was replaced before any successive scan. This was to ensure that there was no significant variation in the quality factor or resonant frequency over a scan due to possible evaporation of the drop and from one scan to the next. Employing a flow cell will help both in maintaining the humidity and temperature over a scan and next.

After placing the drop, the sensor was driven close to its fundamental resonant frequency $f$ by a pure sinusoidal electrical signal using a 33220A Agilent function generator. The drive voltage was linearly increased from 0.07 $V$ rms to 12 $V$ rms in 2 min. The higher harmonics in the drive signal were substantially attenuated by a low pass filter. The transduced electrical signal (response) was received by an SR844 lock-in amplifier (Stanford Research Systems) that recorded the in-phase and quadrature (vector) components at $3f$ i.e. the third harmonic or three times the drive frequency. A passive frequency tripler was used to generate the reference signal for the lock-in. Two quadrature receivers were additionally employed to detect the voltage and the quartz output current at $f$ (drive frequency).

The amplitude of oscillation ($a$) of the sensor was computed using the relationship $a = V_d k_v Q$, where $V_d$ is the drive voltage, $Q$ is the quality factor of the oscillator and $k_v$ is the electromechanical coupling constant, which for an AT-cut TSM quartz oscillator is 1.4 pm/V (Borovsky et al. 2000).
Acknowledgement
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References
Anthrax as a biological weapon, 2002 - Updated recommendations for management. 
Figure Captions

Figure 1 The electrical response of the sensor (current) measured at 3f or three times the drive frequency, f. (a) Amplitude of 3f response from a sensor (Sensor 1) with approximately 6400 Bacillus subtilis spores specifically captured on it. (b) Amplitude of 3f response from a sensor (Sensor 2) with streptavidin-coated polystyrene beads (SCPM) physisorbed on it using a 30 μL solution of 2×10^8 beads/ml. (c,d) Deviation in amplitude of 3f response from the respective sensors.

Figure 2 The deviation in amplitude of 3f response (current) for a sensor (Sensor 3) where the normal wash step following a 9 min immobilization of approximately 1×10^5 cfu/ml spores was omitted.

Figure 3 (a) Deviation in 3f current versus sensor oscillation amplitude (referred to as signal) from model and experiment with Sensor 2 having 6400 bound spores, and (b) characteristic force-extension function of the linker binding the spore to the surface estimated from model along with the operating force-extension.

Figure 4 Signal corresponding to 20 nm of oscillation amplitude for three different spore concentrations from experiments and model.

Figure 5 (a) Dependence of signal on drive frequency from model for a sensor with 6400 bound spores, and (b) Operating force-extension for different drive frequencies from model.
Fig. 1
Fig. 2
Fig. 4

- **Experiment**
- **Model**

Δ3F current (μA) vs. No. of captured spores (cfu)
Fig. 5

(a) Δ3F current (μA) vs. Amplitude of oscillation (nm)
- Red: 14.3 MHz drive
- Green: 42.9 MHz drive
- Blue: 71.5 MHz drive

(b) Force (pN) vs. Extension (nm)
- Green: Characteristic Force-extension
- Red: Force-extension 14.3 MHz drive
- Blue: Force-extension 71.5 MHz drive
Supplementary Information for “Probing biomolecular interaction forces using an anharmonic acoustic technique for selective detection of bacterial spores”

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S1. Quantitative model

The model of a rigid microsphere attached to a surface oscillating in-plane described by (Ghosh et al. 2011) is adapted and employed here to simulate the experiments with the spores. Supplementary Figure 1 shows the model of a spore bound to the sensor surface via the surface protein-antibody-PEG-thiol linker modeled as a spring.

Supplementary Figure 1 Spore attached to the sensor surface via a biomolecular tether (modeled here as a spring).
The differential equations describing the dynamics of the spore as illustrated in Supplementary Figure 1 is given by

$$
m_x \ddot{X}(t) = F_{tx} - \gamma \dot{X}(t) - \lambda (X(t) - V_x(t)) = F_{tx} - (\gamma + \lambda) \dot{X}(t) + \lambda V_x(t)$$
$$m_y \ddot{Y}(t) = F_{ty} - (\gamma + \beta) \dot{Y}(t)$$

Here $V_x(t)$ is the velocity of the surface; $m_x$ and $m_y$ are the effective masses of the spore along X and Y directions; $\gamma$ is the coefficient of Stokes’ viscous resistance from bulk liquid; $\lambda$ and $\beta$ are the coefficients of viscous drag at the spore-surface interface due to liquid motion in the X and Y directions respectively.

The biomolecular tether is assumed to comprise $N_l$ linkers and the characteristic force-extension function of a linker is assumed as follows, based on the force versus extension graphs reported in AFM studies (Linke and Grutzner 2008).

$$F(s) = [a_1 \sinh(s / b_1) + a_2 \sinh^3(s / b_2)] \exp[-(cs + d)s]$$

Here $s$ is the extension of the tether. The coefficients of this function are initially guessed and the horizontal shear force on the sensor due to the spore ($F_{tx}$) is expressed as

$$F_{tx} = F_i \cos \phi + F_a \sin \phi = -N_l F(s) \cos \phi + (-k_a N_l \cot \phi) \sin \phi$$
$$= -N_l [F(s) \cos \phi + k_a \cos \phi]$$

Here, $F_i$ and $F_a$ model respectively the force along the tether (due to its deformation) and the force perpendicular to the tether (due to repulsion from close proximity of the surface) as illustrated in Supplementary Figure 1. Using this $F_{tx}$, the differential equations in (2) were solved numerically using Wolfram Mathematica 7.0 for 6400 spores, assuming diameter of 1 $\mu$m. The evaluated velocity and acceleration vectors are then used to compute $F_{tx}$ from Eq. 1 $F_{tx} = m_x \ddot{X}(t) + (\gamma + \lambda) \dot{X}(t) - \lambda V_x(t)$. The third Fourier harmonic of $F_{tx}$ is then computed by solving the following integration numerically.
\[
F_{t \times 3f} = \frac{\omega}{\pi} \int_0^T F_t \exp(3j\omega t)dt
\]  

Here \( j \) is the imaginary unit and \( T = 2\pi/\omega \) is the period of oscillation. \( F_{t \times 3f} \) is multiplied by the appropriate force-to-charge conversion factor for AT-cut quartz (Ward 1992) and by an enhancement factor for proximity to third overtone resonance. The resulting electric charge is differentiated with respect to time (multiplied by \( 3\omega \)) to compute the 3f current offset on background (deviation from baseline). This deviation in 3f current versus oscillation amplitude (signal) is compared and fitted with the experimentally observed graph using trial and error.

\textbf{Figure 2} The percentage of number of spores successfully captured on the surface decreases with the concentration of the spore solution possibly because of increasing steric hindrance.
Figure 3 Enhancement of signal \((s)\) with increase in drive frequency \((f)\). Gradient of the log-log graph stands for the index of the power, \(n\), in the relationship \(s \propto f^n\).

References