A Pulse System with Cascade Amplifier for Detection of *Bacillus Anthracis* Spores on Micron-Scale, Phage-Immobilized Magnetoelastic Biosensors

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Recently, free-standing phage-based magnetoelastic (ME) sensors have been investigated as a wireless detection method for real-time pathogen monitoring. The ME biosensor is comprised of a ME resonator which is an amorphous alloy, and a bio-molecular recognition element that binds specifically with a target pathogen. Under an alternating magnetic field, the ME biosensor results in an oscillating shape change with a characteristic resonant frequency. Once contact with specific target pathogen, an increase mass in the resonator will result in a decrease of the resonant frequency. Currently, the most common technique for characterizing these sensors is a network analyzer with an s-parameter adapter to sweep the excitation frequency over a fixed range and monitor the output for maximum signal amplitude. This method is good for sensors with large size. For small sensors, the sweep rate of frequency must be very slow to allow the system to observe the sensor output, which becomes difficult to detect the accurate frequency in a short time.

In previous work, Shen *et al.* demonstrated that a pulse system with time domain is another method to test the ME sensors' resonance frequency. Their system allowed fast, accurate detection on 1 mm ME sensors. In order to further develop this method, herein, we optimized it to investigate the detection limit of micro-level ME sensors. Figure 1 shows a block diagram. In the system, a pulse wave will be generated to excite the sensor; a pick-up coil will sense the oscillation and convert it to an electrical signal. Based on the acquired damping oscillating signal, the weight change of sensor can be calculated. In order to address the noise and interference problems, cascade amplifications with band-pass filtering have been implemented, which significantly increase the clarity of the signal.

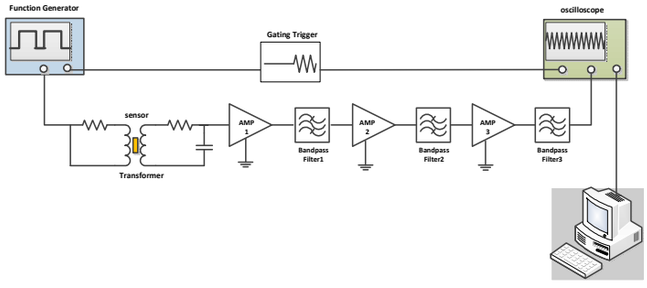

Figure 1 a block diagram of the optimized pulse system

Fabrication of the ME Biosensor Strip-shaped ME resonator of size 200 x 40 x 15 µm were fabricated by dicing METGLAS® 2826MB alloy ribbon. Then the sensor platforms were cleaned in acetone and ethanol ultrasonically. The cleaned resonators were then subjected to a thermal anneal in a vacuum oven at 200 ^{o}C for 2 hours in a vacuum (>10^{-3} torr) to remove residual stresses. Chromium and then gold were sputtered onto all sides of the resonators. JRB7 phage was immobilized on the ME sensor surface by direct physical adsorption. The ME sensors with JRB7 phage were measured before depositing spores as the blank sensor to eliminate the detection errors.

Test 200 µm ME biosensor under pulse system Figure 2 shows a 200 µm sensor's oscilloscope waveforms of (1) sensor "ring-down", (2) function generator. The measured waveform in the oscilloscope shows that the signal has been amplified to 100mV amplitude.

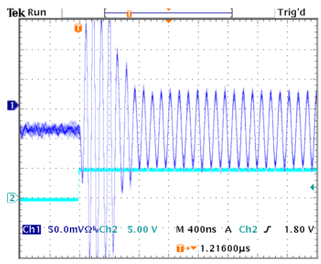

Figure 2 a 200 µm sensor's oscilloscope waveforms of (1) sensor "ring-down", (2) function generator.

Deposition of *Bacillus Anthracis* Spores on 200 µm ME biosensors Each sensor platform was placed under the optical microscopy. Diluted spore solutions containing 10^{2} spores/ml was used to deposit on the sensor platform by a capillary tube with a diameter of 1 micron. Figure 3 show the SEM image with countable spores on individual sensor platform.

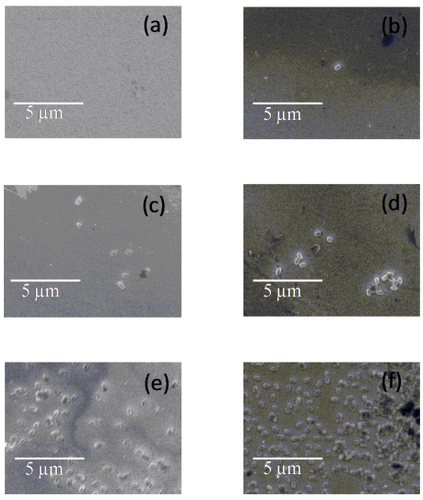

Figure 3 SEM image with countable spores on individual sensor platform

The mass sensitivity of the ME sensor was studied in pulse system. Table 1 shows resonance frequency shift and spore counted for each individual sensors. The average S_m from experiment is 6.69 Hz/pg, which is in a good agreement with the theoretical value 7Hz/pg.

ME sensor	Length (µm)	Initial f_0 (MHz)	Final f_0 (MHz)	Δf (KHz)	Δm (pg)	Spore count
1	190	11.7623	11.7615	0.26	42	21
2	192	11.5564	11.5554	0.45	64	32
3	196	11.3205	11.3194	0.59	88	44
4	198	11.2062	11.2048	0.68	106	53
5	201	11.0123	11.0109	0.83	124	62
6	203	10.8235	10.8220	0.98	154	78
7	205	10.6163	10.6145	1.23	178	89
8	209	10.5658	10.5640	1.33	186	93
9	210	10.5157	10.5132	1.98	290	145
10	211	10.4268	10.4241	2.11	310	155
Average Sm (Hz/pg)	6.69					
Standard deviation	0.34					