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Ultrahigh-sensitivity label-free singlemode- tapered no core-singlemode fiber immunosensor for *Listeria monocytogenes* detection

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Abstract

A challenge for optical fiber biosensor is to achieve ultrahigh sensitivity with narrow full width at half maximum (FWHM) of the spectrum. To address this challenge, an ultrahigh-sensitivity microfiber interferometer fiber ring laser (FRL) biosensor is proposed and investigated for *Listeria monocytogenes (L. monocytogenes)* detection. The fiber biosensor is composed of a singlemode- tapered no core-singlemode (STNS) fiber configuration, which is functionalized with the anti-*L. monocytogenes* antibodies. An Erbium Doped Fiber Amplifier is applied to the sensor to excite laser and thus reduce the FWHM of the spectrum, which significantly improved the limit of detection (LoD). The proposed STNS FRL biosensor has excellent reproducibility, specificity and sensitivity for *L. monocytogenes*. The developed STNS FRL biosensor can directly detect *L. monocytogenes* cells with LoD as low as 1.0 cell/mL, indicating the capability for detecting single cell of *L. monocytogenes*. Real lettuce and milk samples have been tested and test result in lettuce and milk samples has deviations within ±30% from that of Phosphate-buffered saline (PBS) for *L. monocytogenes* concentrations vary from 10^1 to 10^3 cells/mL(g). The developed STNS FRL biosensor has ultrahigh sensitivity, good stability, reproducibility, and specificity, which has potential applications in diseases/medical diagnostics.

Key words: Fiber optic sensor, fiber ring laser, *Listeria monocytogenes*, foodborne pathogens.

1. Introduction

Listeria monocytogenes (*L. monocytogenes*), as one of the most common Gram-positive foodborne intracellular pathogens, is a rod-shape bacteria (diameter ~ 0.5 μm × 2.0 μm) and the causative agent of Listeriosis which is a systemic disease [1-3]. The clinical symptoms caused by Listeriosis infection are mainly sepsis, meningitis, mononucleosis, osteomyelitis, myocarditis, spontaneous abortion or even death [4-6]. The mortality rate of Listeriosis among immunocompromised patients, pregnancies, cancer patients and old person is as high up to 30%. Even at low infectious doses, they may be infected because of lower immunity [7-8]. *L. monocytogenes* has three significant characteristics: wide distribution, large living environment plasticity and large adaptability. *L. monocytogenes* is often parasitic in soil, water, plants, animals (such as poultry, insects, fish, birds) and foods, including milk, vegetables, and meat [9]. Moreover, *L. monocytogenes*, as a typical cold-tolerant bacteria, can survive at a temperature of 2 °C - 42 °C and can grow and multiply in the refrigerator for a long time which become a potential hazard to products [10]. Therefore, it is very likely that the food in the refrigerator will be contaminated. In addition, *L. monocytogenes* is adapted to both acidic, alkaline and salty environmental conditions [11-12]. The *L. monocytogenes* in food poses hidden danger to human safety. In USA, the zero-tolerance policy is formulated regarding the *L. monocytogenes* content in food [13]. Therefore, to reduce the risk of diseases induced by *L. monocytogenes*, a rapid and precise detection is required before consuming the contaminated food [14]. The common *L. monocytogenes* detection method is bacteria culture method, which usually takes 1-5 days to culture the *L. monocytogenes* and require well-trained professionals for the test [15-16]. Recently, some rapid detection techniques, for example, polymerase chain reaction [17], enzyme-linked immunosorbent assay [18] and flow cytometry methods [19] have been developed. However, these methods have several significant shortcomings such as relatively poor LoD, high costs and relying on expensive equipment.

Fiber optic sensor has evanescent field at the interface between the fiber sensor and surrounding material, which has been widely used for biosensing applications [20]. The optical fiber sensor candidate structure includes optical fiber interferometers [21-22], fiber gratings [23], Sagnac interferometers [24], Fabry-Perot cavity [25], microfiber resonator [26] and surface plasmon resonator [27]. Among these techniques, optical fiber interferometer has advantage of ease fabrication and high sensitivity. However, the optical fiber interferometer suffers disadvantage of relative wide spectrum bandwidth, which has significant negative impact on the limit of detection (LoD) of the sensor. For example, Kumar and Chen *et al* proposed an STNS sensor, which has high refractive index (RI) sensitivity and functionalized this sensor for human chorionic gonadotropin and inactivated *Staphylococcus aureus* detection [28-29]. Although the sensor has high sensitivity, the FWHM of the spectrum is relatively large (~1 nm), which leads to a high LoD. Fiber ring laser (FRL) wavelength demodulation technology, as one of the most promising technologies, is an ideal solution to the above problem, which has high signal-to-noise (SNR), narrow bandwidth, and thus significantly improved the LoD of the sensor. For example, Liu

et al experimentally demonstrated a singlemode-claddingless-singlemode (SCS) FRL sensor with FWHM of 0.03 nm [30]. Sun *et al* experimentally developed dual-wavelength FRL based on a combination of FBG and an SCS fiber multimode interferometer for measuring both temperature and liquid level simultaneously [31]. Gonzalez *et al* presented an FRL based temperature sensor combined with an FBG and a Mach–Zehnder interferometer with temperature sensitivity of about 18.8 pm/°C [32]. In this paper, an ultrahigh sensitivity tapered STNS fiber biosensor is firstly embedded into an Erbium Doped Fiber Amplifier (EDFA, EDFA-PA-45-B, SN: 181117-11 1530-1565 nm) FRL system for *L. monocytogenes* detection. Without sacrificing sensitivity of the STNS fiber biosensor, the LoD is significantly improved due to the narrow FWHM of the FRL. Experimental results show that the STNS FRL biosensor can directly detect *L. monocytogenes* sample with concentration as low as 1.0 cell/mL, indicating the capability for detecting single cell of *L. monocytogenes*, the best reported for direct detecting *L. monocytogenes* samples using optical fiber sensor structures so far. Real lettuce and milk samples contaminated by *L. monocytogenes* were tested using the developed STNS FRL sensor, where 4 cells could be detected by the developed biosensor.

2. Materials and methods

2.1. FRL sensing system and principle

Fig. 1(a) (i) illustrates a diagram of the experimental STNS FRL sensor system for *L. monocytogenes* detection. Fig.1 (a) (ii) shows a typical measured spectral response of the STNS sensor (without FRL, dashed red) and the STNS FRL (solid black). LoD is the main parameter to evaluate the performance of a sensor device, which can be calculated as the ratio of the sensitivity (S) and the quantitative resolution (R) of the sensor [33]. The detailed description can be found in the supporting document 1.1.

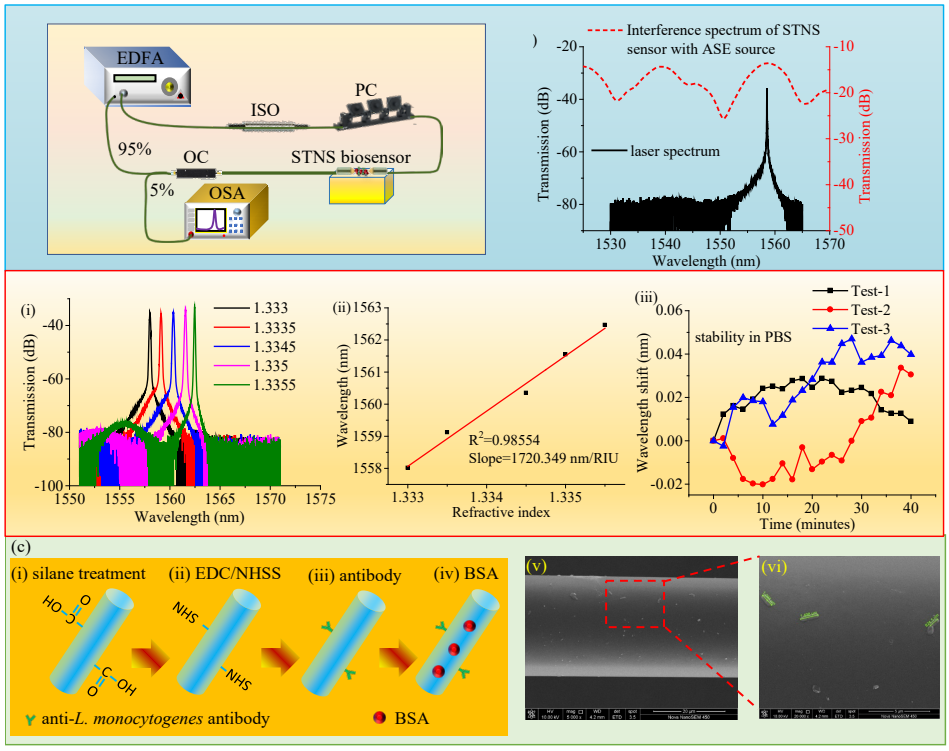

Fig. 1. (a) System device diagram: (i) the experimental setup; (ii) spectra of an STNS sensor with and without FRL; (b) The STNS FRL sensor (10.32 μm taper waist diameter): (i) spectral response and (ii) RI sensitivity in the RI range from 1.333 to 1.3355; (iii) stability test in Phosphate-buffered saline (PBS) buffer for 40 minutes; and (c) STNS functionalization: (i) silane treatment to create carboxyl group; (ii) EDC (1-(3-Dimethylaminopropyl)-3-ethylcar-bodiimide hydrochloride) /NHSS (hydroxy-2,5-dioxopyrolidine-3-sulfonicacid sodium salt treatment to create N-hydroxysuccinimide (NHS) active ester); (iii) immobilization of anti-*L. monocytogenes* antibodies; (iv) Bovine serum albumin (BSA) treatment to block unbind sites; (v) scanning electron microscope (SEM) image of the STNS bind with *L. monocytogenes* and (vi) amplified *L. monocytogenes* bind to the STNS fiber sensor.

$$\mathrm{LoD} = \frac{R}{S} = \frac{3\sigma}{S} = \frac{3\sqrt{\sigma^2_{ampl\ -noise} + \sigma^2_{temp\ -induced} + \sigma^2_{spect\ -res}}}{S} \quad (1)$$

$$\sigma_{ampl\ -noise} \approx \frac{FWHM}{4.5\times(SNR)^{0.25}} \quad (2)$$

$$\sigma_{spect\ -res} = \frac{R_W}{2\sqrt{3}} \quad (3)$$

$$\sigma_{temp\ -induced} = 10\ fm \quad (4)$$

2.2. The STNS FRL fiber sensor for RI measurement

Fig. 1(b) (i)shows the spectral responses with high RI sensitivity of 1720.35 nm/RIU in Fig. 1(b)(ii). Fig. 1 (b) (iii) shows the STNS FRL sensor has good stability (±0.035 nm) over 40 minutes in PBS buffer. The detailed content can be found in the supporting document 1.2.

2.3. Functionalization of the STNS FRL sensor

Fig.1(c) (i-iv) shows the functional process. The detailed content can be found in the supporting document 1.3.

2.4. The preparation of *L. monocytogenes* in milk and Lettuce samples

Pasteurized milk and lettuce sample were spiked using different concentrations (2.3×10^2 - 2.3×10^4 cells/mL) of *L. monocytogenes*, with the support of immunomagnetic separation (IMS) technology for separating the target *L. monocytogenes* from milk and lettuce sample. The detailed content can be found in the supporting document 1.5, 1.6 and 1.7.

2.5. Detection of *L. monocytogenes*

The functionalized STNS sensor is placed inside the container for different concentrations of *L. monocytogenes* collected from PBS, milk and lettuce solutions. Fig. 1c (v-vi) shows SEM images of the functionalized STNS fiber senor bind with *L. monocytogenes* (the rod-shape). The detailed content can be found in the supporting document 1.8.

3. Results and Discussions

3.1. *L. monocytogenes* in PBS buffer

The STNS FRL biosensor (immobilized with 200 μg/mL anti-*L. monocytogenes* antibody) was firstly immersed into 1.0 cell/mL *L. monocytogenes* solution diluted with PBS buffer (400 μL). Fig. 2(a) shows the summarized measurement result. The experimental result shows that in the first 8 tests (each test last 80 minutes), no obvious wavelength shift was observed. At the nineth test, a wavelength shift of 0.2 nm has been observed, which is although small, but observable compared to

the background noise (±0.035 nm) within PBS. This is possibly because the concentration of *L. monocytogenes* (1.0 cell /mL) is so low that there is no (or very low number) *L. monocytogenes* in the test sample (400 μL) or no *L. monocytogenes* were captured by the anti-*L. monocytogenes* antibody on the surface of the STNS sensor in the first 8 test. In the nineth test, a *L. monocytogenes* was captured by the anti-*L. monocytogenes* antibody and thus resulting in a wavelength shift. It is noted that the wavelength shift is continuous in the first 2 minutes, and then stabilized. This is possibly because the binding process is a dynamic process [34], which will take some time to fully attach the bacteria onto the surface of the fiber, particularly when the size of bacteria used in the experiments is relatively large (in the order of micrometer). The same STNS FRL biosensor was then immersed into *L. monocytogenes* solution with concentration of 10 cells/mL and a wavelength shift of 0.6 nm/mL was observed. Thus, all further studies were carried out using *L. monocytogenes* samples with concentration staring from 10 cells/mL.

The concentration of anti-*L. monocytogenes* antibody will have impact on the sensing performance of the STNS sensor and thus two groups of identical STNS FRL biosensors (fabricated with same parameters, 5 sensors for each group) were studied, which were functionalized with 100 μg/mL and 200 μg/mL of anti-*L. monocytogenes* antibody. Each functionalized sensor was then immersed into different concentrations (10^1, 10^2, 10^3, 10^4 and 10^5 cells/mL, from low to high concentration in sequence) of *L. monocytogenes* solutions. Between each measurement, the STNS sensor was washed with PBS for 20 minutes. Fig. 2(b) gives the measured spectrum of the STNS FRL biosensor (functionalized with 200 μg/mL anti-*L. monocytogenes* antibody) at different time. Fig. 2(c) summarized the peak wavelength shifts vs. time at different *L. monocytogenes* concentrations (10^1, 10^2, 10^3, 10^4 and 10^5 cells/mL).

It was clearly seen from Fig. 2(c), for both samples functionalized with different concentrations (100 and 200 μg/mL) of anti-*L. monocytogenes* antibody, the peak wavelength undergoes redshift monotonically vs. time. This reveals the dynamic binding process of *L. monocytogenes* onto the STNS biosensor. As the concentration of *L. monocytogenes* increases, the value of wavelength shift increases monotonically. It can be seen that the peak wavelength shift is observed mainly in the initial 12 minutes and then stabilized. During the binding process, the peak wavelength shift experiences an exponential variation over time, which agrees with the kinetic characters of the immune behavior [35]. Fig. 2(d) summarized the peak wavelength shift of the biosensor in different concentrations of *L. monocytogenes*, demonstrating that the STNS FRL biosensor immobilized with a 200 μg/mL concentration of anti-*L. monocytogenes* antibody has a larger peak wavelength variation and thus a higher sensitivity than that of functionalized with lower concentration anti-*L. monocytogenes* antibody (100 μg/mL). This is possibly because that higher concentration of anti-*L. monocytogenes* antibody has better capability to bind *L. monocytogenes* and thus more *L. monocytogenes* were captured for the same concentration of the *L. monocytogenes*, particular in relatively high concentration of *L. monocytogenes* solutions. However when the concentration of *L. monocytogenes* is higher than 10^3 cells/mL, the wavelength shift rate decreases, which is possibly due to the saturation of the anti-*L. monocytogenes* antibody immobilized on the STNS fiber sensor surface. Reproducibility tests were carried out by using five different STNS fiber biosensors (same fabrication parameters) to do measurements for

the same concentration of *L. monocytogenes*. Results in Fig. 2(d) demonstrate that the STNS FRL biosensor has very good reproducibility. When the concentration of *L. monocytogenes* is 10 cells/mL, the average wavelength shift of the STNS sensor functionalized with 200 μg/mL anti-*L. monocytogenes* antibody is 0.78 nm among the five measurements with FWHM ~0.28 nm and SNR ~45 dB. It is noted that the FWHM of the sensor immobilized with anti-*L. monocytogenes* antibody is 0.28 nm, which is a little bit larger than that of 0.15 nm without functionalization, but significantly lower than that of STNS sensor without FRL (3.8 nm).

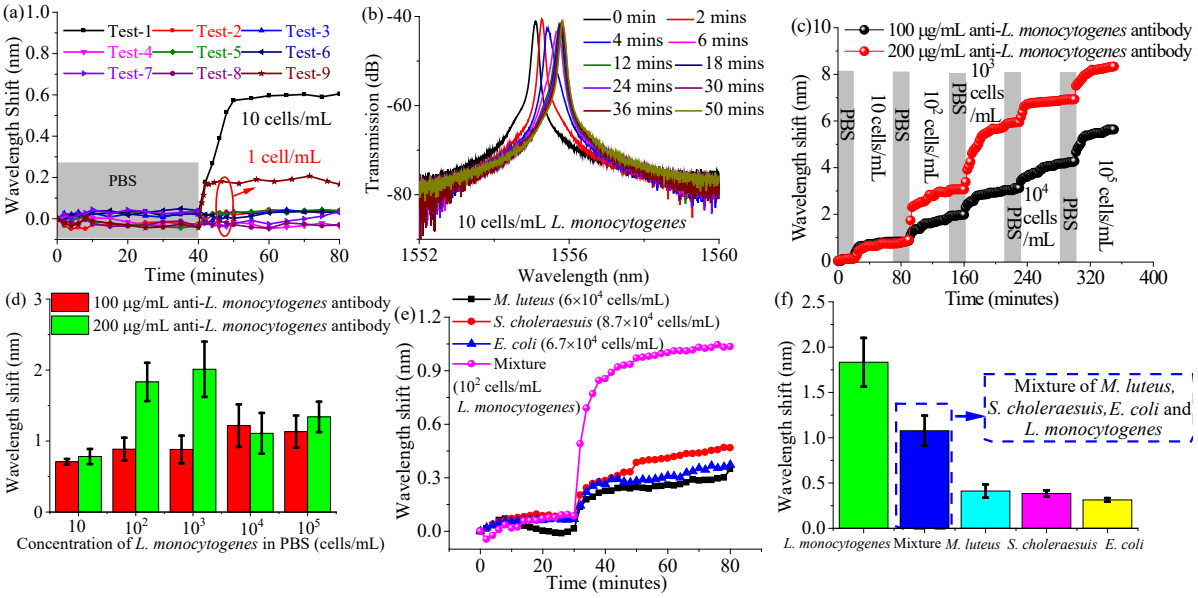

Fig. 2. (a) Summarized wavelength shift of the STNS FRL sensor immobilized with 200 μg/mL anti-*L. monocytogenes* antibody vs. time by immersing the biosensor into 1.0 cell/mL and 10 cells/mL *L. monocytogenes*; (b) Measured spectra for detecting 10 cells/mL *L. monocytogenes*; (c) Comparison of different immobilization concentrations (100 μg/mL, 200 μg/mL) for *L. monocytogenes* detection; summarized wavelength shift vs. concentration of *L. monocytogenes* for the STNS FRL biosensor (d) Reproducibility tests - summarized wavelength shift in different concentrations of *L. monocytogenes* solution; (e) Specificity test of wavelength shift vs. time in *M. luteus*, *S. choleraesuis*, *E. coli* and mixture of *M. luteus* (6×10^4 cells/mL), *S. choleraesuis* (8.7×10^4 cells/mL), *E. coli.* (6.7×10^4 cells/mL) and *L. monocytogenes* (10^2 cells/mL); (f) Reproducibility tests - summarized wavelength shift in different bacteria solutions.

The specificity of the STNS FRL sensor was studied by immersing the identical sensor under the same preparation parameters (functionalized with 200 μg/mL anti-*L. monocytogenes* antibody) into four different types of bacteria for three times, namely 6×10^4 cells/mL *Micrococcus luteus* (*M. luteus*), 8.7×10^4 cells/mL *Salmonella choleraesuis* (*S. choleraesuis*), 6.7×10^4 cells/mL *E. coli* and mixed solution of *M. luteus* (6×10^4 cells/mL), *S. choleraesuis* (8.7×10^4 cells/mL), *E. coli.* (6.7×10^4 cells/mL) and *L. monocytogenes* (10^2 cells/mL). Experimental results are shown in Fig. 2(e-f). The peak wavelength shifts of identical sensors in *L. monocytogenes* solution were observed to be far greater than the *M. luteus*, *S. choleraesuis* and *E. coli*, showing that the specificity and reproducibility of the STNS FRL sensor are very good. The wavelength shift in mixture is smaller than that in PBS solution, which is possibly due to the block of binding between anti-*L. monocytogenes* antibody and *L. monocytogenes* by other bacteria.

The stability of the designed immunosensor in storage has been evaluated. Each time 6-7 fiber biosensors with the same fabrication and functionalization parameters were fabricated. The fiber biosensors not used in the same day will be stored in a fridge at 4 ℃. Our study shows that after 5-6 days, there is no significant performance degradation for *L. monocytogenes* detection.

Table 1 compares the performance between our developed STNS FRL biosensor and those of recently published *L. monocytogenes* detection methods. As can be seen from this table, our proposed STNS FRL biosensor has the best LoD as low as 1.0 cell/mL using equation (1) and the measured result in 10 cells/mL solution. The experimental results in 1.0 cell/mL *L. monocytogenes* solution verified that the STNS FRL biosensor can detect single colony of *L. monocytogenes*, which is the lowest LoD reported so far.

Table 1 Summarized published methods for detecting *L. monocytogenes*

Methods	LoD	Response time	Reference
Integrated Mach-Zehnder Interferometer optical biosensors	10^5 CFU/mL	NA	[38]
Aptamer-fibre-optic sensor	10^3 CFU/mL	NA	[39]
Multiplex fiber optic sensor	10^3 CFU/mL	less than 24 h	[40]
Optical biosensor	10^2 CFU/mL	30 min	[41]
Sandwich-type electrochemical immunosensor	6 CFU/mL	60 min	[42]
Fluorescence aptasensor	8 CFU/mL	NA	[43]
magnetic relaxation DNA biosensor	10^2 CFU/mL	approximate 2 h	[44]
Surface Plasmon Resonance	1.78×10^4 CFU/mL	30 min	[45]
multiplex PCR assay	10^2 CFU/mL	within 1.5 hours	[46]
fluorescence immunoassay	10^2 CFU/mL	12 h	[47]
Competitive annealing mediated isothermal amplification novel nucleic acid-based detection technology	10^2 CFU/mL	less than 2 days	[48]
A Cell-Based Biosensor System	4 CFU/mL	24 h enrichment	[49]
self-stable precipitation polymerization method	10^4 CFU/mL	10 min	[50]
fluorescence method	10 CFU/mL	NA	[51]
colorimetric assay	2.17×10^2 CFU/mL	incubated at 35 °C for 15–18 hours	[52]
STNS FRL biosensor	**1.0 cell/mL**	**< 30 min**	**Proposed method in this paper**

3.2. *L. monocytogenes* in milk sample and lettuce sample

Fig. 3(a) demonstrates the wavelength shifts of the STNS FRL biosensor immobilized with 200 μg/mL anti-*L. monocytogenes* antibody at different concentration of *L. monocytogenes* in milk sample with volume of 400 μL. The STNS

FRL biosensor has similar wavelength shift trends in PBS buffer with good stability. When the *L. monocytogenes* concentration increased to 10^1, 10^2, and 10^3 cells/mL, obvious wavelength shifts can be observed. The tests of *L. monocytogenes* in milk sample were conducted for three times using three identical STNS FRL sensors functionalized with 200 µg/mL anti-*L. monocytogenes* antibody and the results exhibited that the STNS FRL biosensor has very good reproducibility in the *L. monocytogenes* in milk samples. The measured average wavelength shifts of the sensor with FWHM ~0.36 nm and SNR ~45 dB for the *L. monocytogenes* with concentration of 10^1, 10^2, and 10^3 cells/mL are 0.91 nm, 1.35 nm, and 1.52 nm respectively, corresponding to measurement deviation of 17%, -26% and -24% from that of PBS solutions, respectively.

L. monocytogenes is a common food-borne pathogen, which is ubiquitous in lettuce. Therefore, the lettuce samples are applied to actual detection for experimental research. The STNS FRL biosensors functionally modified with 200 µg/mL anti-*L. monocytogenes* antibody were immersed in *L. monocytogenes* in lettuce with concentration of 10^1, 10^2, and 10^3 cells/g placed in a V-groove with a volume of 400 µL whose wavelength move toward long wavelength direction exhibited in Fig. 3(b) with FWHM ~0.38 nm and SNR ~45 dB. The STNS FRL biosensor has good reproducibility and corresponding average wavelength shift is 1.02 nm, 1.44 nm and 1.6 nm respectively, corresponding to a deviation of 31%, -20%, -21% from that of PBS solutions, respectively. The results have been summarized in Table 2.

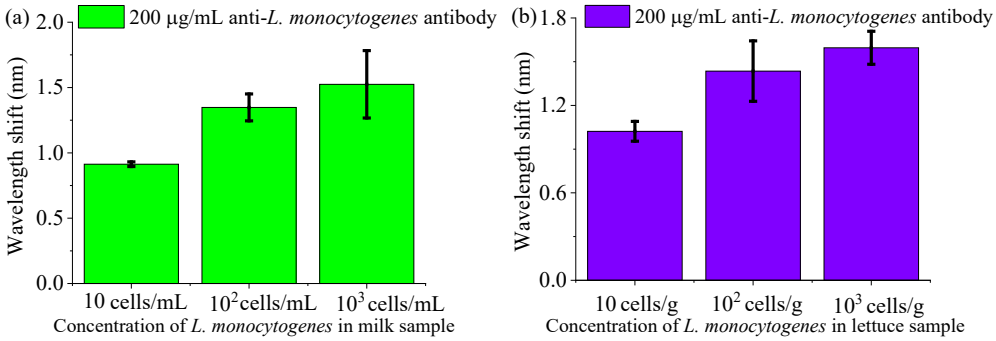

Fig. 3. Wavelength shift of the STNS FRL sensor functionalized with 200 µg/mL anti-*L. monocytogenes* antibody for detection of *L. monocytogenes* (a) In milk sample with concentration of 10^1, 10^2, and 10^3 cells/mL and (b) In lettuce sample with concentration of 10^1, 10^2 and 10^3 cells/g.

Table 2 Comparison of wavelength shift in different *L. monocytogenes* sample solution

L. monocytogenes in different solution		10 cells/mL or g Wavelength shift (nm)	10^2 cells/mL or g Wavelength shift (nm)	10^3 cells/mL or g Wavelength shift (nm)
PBS		0.78±0.11	1.83±0.27	2.01±0.39
Milk		0.91±0.02	1.35±0.1	1.52±0.26
Lettuce		1.02±0.07	1.44±0.21	1.6±0.11
Deviation from PBS	Milk	17%	26%	-24%
	Lettuce	31%	-21%	-20%

Table 2 shows that for both the milk and lettuce samples the deviations for the lower concentration level, 10 cells/mL, are positive, however for higher concentration level (>100 cells/mL), the deviations are negative. This is possible because that there are matrix interferences in the sample predominates compared to *L. monocytogenes* in PBS solution. The signal enhancement in lower concentration is caused by nonspecific binding of matrix interferences in the sample predominates. While the signal change value decreases at higher concentrations, possibly due to matrix interference with *L. monocytogenes*, which blocks the specific binding to the optical fiber and thus reduces the specific binding signal.

4. Conclusion

In conclusion, a novel ultrahigh sensitive label-free STNS FRL biosensor is proposed experimentally investigated for measurement of *L. monocytogenes*. The STNS FRL biosensor is functionalized with anti-*L. monocytogenes* antibody, which can specifically bind with analyte *L. monocytogenes*. Experimentally detection of *L. monocytogenes* in PBS buffer has been studied using an STNS FRL sensor with radius of 5.16 μm, immobilized with 200 μg/mL anti-*L. monocytogenes* antibody, which has a measured LoD of 1.0 cell/mL, without any enrich of *L. monocytogenes*. Specificity test has been studied by immersing the developed biosensor into four different types of bacteria, namely *M. luteus*, *S. choleraesuis*, *E. coli* and mixed solution of *M. luteus*, *S. choleraesuis*, *E. coli* and *L. monocytogenes*, and the experimental results show that the developed biosensor has very good specificity. The *L. monocytogenes* in contaminated milk and lettuce was also detected using the above developed STNS FRL biosensor. The tested results reasonably agree well with the calibrated result in PBS with deviations below ±30% for concentration of *L. monocytogenes* varies from 10^1 to 10^3 cells/mL(g). The developed STNS FRL biosensor has ultrahigh sensitivity, good stability, reproducibility and specificity, and thus for other applications such as in diseases/medical diagnostics.

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