



# Lipoarabinomannan-based tuberculosis diagnosis using a fiber cavity ring down biosensor

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**Abstract:** Despite existing for millennia, tuberculosis (TB) remains a persistent global health challenge. A significant obstacle in controlling TB spread is the need for a rapid, portable, sensitive, and accurate diagnostic test. Currently, sputum culture stands as a benchmark test for TB diagnosis. Although highly reliable, it necessitates advanced laboratory facilities and involves considerable testing time. In this context, we present a rapid, portable, and cost-effective optical fiber sensor designed to measure lipoarabinomannan (LAM), a TB biomarker found in patients' urine samples. Our sensing approach is based on the applications of phase shift-cavity ringdown spectroscopy (PS-CRDS) to an optical fiber cavity created by two fiber Bragg gratings. A tapered fiber is spliced inside the optical cavity to serve as the sensing head. We functionalize the tapered fiber surface with anti-LAM antigen CS-35 through a unique chemistry, creating a strong affinity for LAM molecules. We measure the phase difference between the cavity transmission and the reference modulating signal at the cavity output. The measured phase is directly proportional to the injected LAM concentrations in aqueous solutions over the sensing head. Our demonstrated sensor provides a detection limit of 10 pg/mL and a sensitivity of 0.026°/pg/mL. This sensor holds promise for numerous applications in the healthcare sector, particularly in low-resource settings.

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## 1. Introduction

Tuberculosis (TB) is one of the deadliest global health threats, causing over 1.4 million deaths and 10 million new cases yearly, with a disproportionate burden on low and middle-income countries [1]. Currently, sputum culture is the gold standard test for TB detection [2], but this method entails a protracted turnaround time of four to eight weeks. Moreover, it necessitates highly trained laboratory personnel and resource-intensive laboratory environments to execute multiple processing steps. Patients must endure multiple visits to diagnostic centers, presenting a substantial challenge, particularly in remote and low-resource regions.

One of the significant WHO objectives to fight TB is the development of a rapid, sensitive, specific, portable, and cost-effective diagnostic TB sensor [3]. In recent years, various optical and non-optical sensors have emerged for TB detection in human breath, blood, sputum, and urine [4–8]. For instance, nucleic acid amplification tests (NAATs) like GeneXpert and its variants employ patient sputum for rapid TB diagnosis. Nevertheless, these systems, though increasingly prevalent in TB diagnostics, still need to achieve WHO-recommended sensitivity, specificity, and cost-effectiveness standards [9]. In contrast, a Raman spectroscopy-based TB diagnostic

system has recently demonstrated the requisite sensitivity, specificity, and portability, although cost remains a limiting factor [10].

Furthermore, there is a pressing need to monitor the disease state once treatment commences to optimize medications. Monitoring the treatment response in NAAT-based systems is challenging [9], while Raman-based systems, integrated with AI, have shown limited capabilities [10]. Therefore, time-consuming culture tests remain the primary method for assessing treatment response.

Sputum-based diagnostic systems face various challenges, including sample variations among patients and difficulties in obtaining sputum samples from children and elderly individuals. Consequently, non-sputum-based tests are highly sought, with researchers exploring potential TB biomarkers in blood, breath, and urine. Among these media, urine is appealing due to its easy collection from children and the elderly and a lower risk of infection during collection.

Lipoarabinomannan (LAM), a glycolipid found in the cell walls of TB-causing mycobacteria, has emerged as a promising urine-based TB biomarker, first demonstrated in the early 2000s [11]. Subsequently, researchers have developed various LAM detection assays for TB diagnosis. Notably, the AlereLAM test, while cost-effective, needs to improve in terms of desired sensitivity. However, the Fujifilm TB LAM test appears as a promising solution, particularly for children and individuals with HIV, aiming to enhance the sensitivity of AlereLAM. Additionally, innovative approaches, such as nanotechnology and GC/MS methods, show promise in augmenting LAM detection sensitivity. Nevertheless, challenges persist in achieving the desired levels of sensitivity, accuracy, and cost-effectiveness as recommended by the WHO [12,13].

One potential avenue toward developing a viable LAM detection test for point-of-care TB diagnosis involves using optical biosensors. However, research in the realm of LAM photonics biosensors remains limited. In a notable work, researchers employed a Mach-Zehnder interferometer on a chip, coupled with a broadband source, spectral filters, and a CMOS camera, to establish a correlation between LAM concentration and spectral shifts. The work demonstrated a detection limit of 475 pg/mL [14].

In the present work, we demonstrate the first application of fiber cavity ring down spectroscopy for LAM detection toward TB diagnosis. We build a fiber cavity using fiber Bragg gratings at 1550 nm with a tapered fiber as a sensing head. The tapered fiber is functionalized with monoclonal antibodies employing a novel chemical functionalization protocol for LAM binding. Using the phase shift-cavity ring down spectroscopy (PS-CRDS) principle, we show the LAM detection limit of 10 pg/mL in aqueous solutions.

We now describe the rest of the paper. Section 2 describes various materials and methods in the work, including tapered fiber fabrication, chemical functionalization, and experimental setup. Section 3 provides results and related discussions, followed by concluding remarks in Section 4.

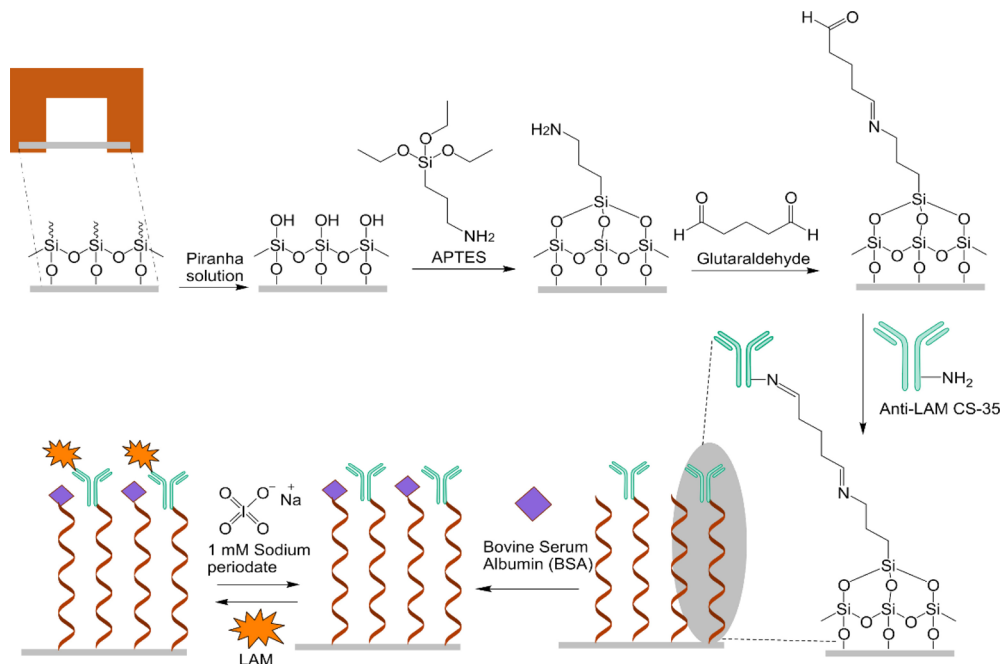
## **2. Materials and methods**

### *2.1. Sensing head*

Tapered fibers are employed as sensing heads in our work. Tapered fibers are engineered thin optical fibers that allow a portion of the light to interact with the surrounding analyte. We fabricate tapered fibers using computer-controlled motorized stages to pull an SMF-28 fiber while heating it with a flame. One end of the fiber is attached to a 1550 nm laser source, while the other end is connected to an oscilloscope to monitor the tapered fiber's quality during the fabrication process. The fabricated tapered fiber is then carefully removed from the tapering setup by attaching it to a U-shaped copper-clad printed circuit board with nichrome soldering. The soldering is necessary because glues or epoxies can be damaged by harsh chemicals like the piranha solution used in the following surface functionalization steps [15].

## 2.2. Surface functionalization

The various steps involved in surface functionalization are outlined in the schematic diagram presented in Fig. 1. The process begins by activating optical fibers using a piranha solution ( $\text{H}_2\text{SO}_4$ :  $\text{H}_2\text{O}_2$  7:3), which generates a reactive functional group on the surface. Subsequently, the fibers undergo treatment with APTES, facilitating the installation of amino groups on their surface. The next stage involves generating surface aldehydic groups through the use of glutaraldehyde. This compound reacts with the amino groups of the antibody, enabling the functionalization of the monoclonal anti-mycobacterium tuberculosis LAM on the fiber surface. Specifically, the monoclonal anti-mycobacterium tuberculosis LAM, clone CS-35 (NR-13811), can bind specifically with the LAM. In the following sections, we elaborate on our tapered fibers functionalization protocol details.



**Fig. 1.** The schematic illustrates the surface functionalization process of a tapered fiber, comprising four key steps: amino functionalization, aldehyde functionalization, immobilization of the CS-35 antibody, and application of BSA blockers to reduce nonspecific binding.

### 2.2.1. Materials

Concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ), hydrogen peroxide 30% ( $\text{H}_2\text{O}_2$ ), acetone, deionized water, 3-aminopropyl triethoxy silane (APTES), ethanol, acetic acid, glutaraldehyde, monoclonal anti-Mycobacterium tuberculosis LAM Clone CS-35 (NR-13811), purified lipoarabinomannan LAM H37Rv (NR-14848). The protocols for cleaning and activation followed by the functionalization of tapered fiber with APTES, glutaraldehyde, and antibody monoclonal anti-Mycobacterium tuberculosis LAM were adapted from the related protocols reported in our earlier works and the other relevant literature [16–21].

### 2.2.2. Cleaning and activation treatment of tapered fibers

Tapered fibers are immersed in acetone for 10 minutes and subsequently air-dried. The activation of the cleaned surface is accomplished using a piranha solution. A fresh piranha solution is prepared by pipetting out 70 mL of concentrated sulfuric acid and mixing it with 30 mL of hydrogen peroxide. The fibers are carefully placed in a petri dish, and the piranha solution is gently poured over them until fully covered. The tapered fiber is left in the acidic solution for one hour, followed by meticulous washing with deionized water until the water reaches a neutral pH.

### 2.2.3. Amino functionalization on tapered fibers

The first step in the functionalization process involves creating amino groups on the surface of the tapered fiber. This procedure includes preparing a 1% APTES solution, utilizing ethanol and acetic acid as a solvent in a volume ratio of 5:2. In a falcon tube, 49.5 mL of the prepared solvent is combined with 0.5 mL of 3-aminopropyl triethoxy silane. The solution is thoroughly mixed on a vortex machine to ensure homogeneity. Subsequently, the activated tapered fibers are immersed in petri dishes containing the 1% APTES solution. The fibers are left in the solution for 10 minutes and then washed with ethanol. Finally, the functionalized fibers are positioned in an oven at 100°C for 1 hour.

### 2.2.4. Aldehyde functionalization on tapered fibers

During this functionalization step, aldehyde functional groups are introduced onto the surface of the tapered fiber. To achieve this, a 1% aqueous glutaraldehyde solution is prepared using deionized water. The initial 50% glutaraldehyde solution is diluted to 1% by pipetting 1 mL glutaraldehyde and combining it with 49 mL deionized water. The mixture is homogenized by shaking on a vortex machine for a few minutes. Next, the APTES-functionalized fibers are immersed in the glutaraldehyde solution for 20 minutes at room temperature. Finally, the fibers are washed with deionized water to complete the process.

### 2.2.5. Antibody immobilization on tapered fibers

Finally, the antibody is immobilized on the tapered fibers. The selected antibody, serving as a counterpart to the LAM (lipoarabinomannan) antigen, is the monoclonal anti-*Mycobacterium tuberculosis* LAM, Clone CS-35 (NR-13811), provided by BEI resources. The tapered optical fibers are immersed in the antibody solution (150 mg/mL) overnight at 4°C. The conjugation mediates the immobilization of the antibody on the surface between the amino group present in the anti-LAM CS-35 antibody and the aldehyde groups on the surface of the tapered fiber. Subsequently, BSA blocking is performed by submerging the optical fiber in a 0.5% BSA solution for 15 minutes at room temperature. This step with BSA helps prevent any nonspecific binding of biomolecules on the substrate surface.

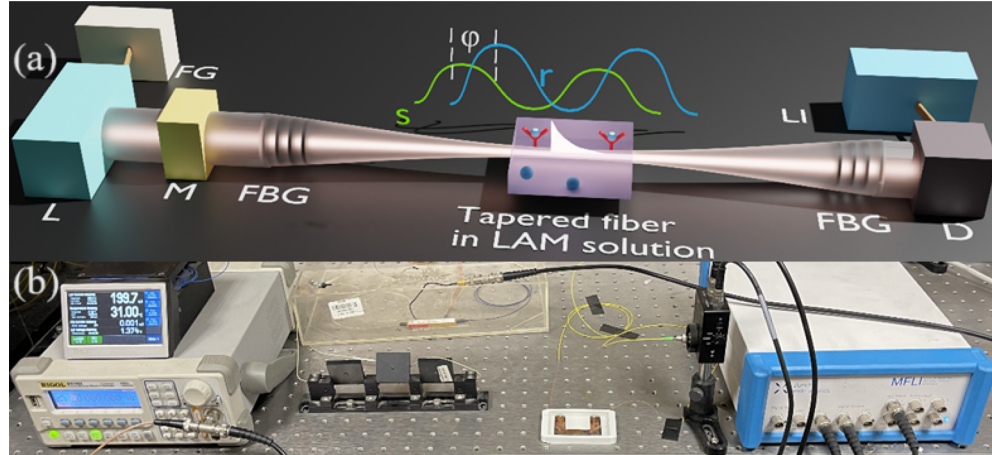
## 2.3. Measurement principle and experimental setup

We employ the principle of phase shift-cavity ring down spectroscopy (PS-CRDS), which involves measuring the phase shift,  $\varphi$ , of a sinusoidally modulated laser at the cavity output [22,23]. The phase shift,  $\varphi$ , is measured with respect to the sinusoid phase of the reference modulating signal and is related to the cavity ring down time,  $\tau$ , via the following equation:

$$\tan \varphi = \omega \tau, \quad (1)$$

where  $\omega$  is the amplitude modulation frequency. Figure 2 shows the schematic diagram of our sensor that uses a fiber cavity for PS-CRDS measurements. The detailed experimental setup and measurement procedure are explained in our earlier work [15]. Briefly, we construct an optical fiber cavity of length 38 cm by splicing functionalized tapered fiber of waist diameter

$\approx 10.2 \mu\text{m}$  in between two fiber Bragg gratings (FBGs). The tapered fiber waist diameter can impact the sensor performance. As the waist diameter decreases, more optical mode leaks out of the tapered fiber into the surroundings, resulting in increased cavity losses, i.e., the cavity ring down time decreases. However, there is a delicate balance between the cavity ring down time and the mode's interaction with the sample for optimum sensitivity. For our current work, we find that tapered fiber around a  $10 \mu\text{m}$  waist diameter produces optimum results.



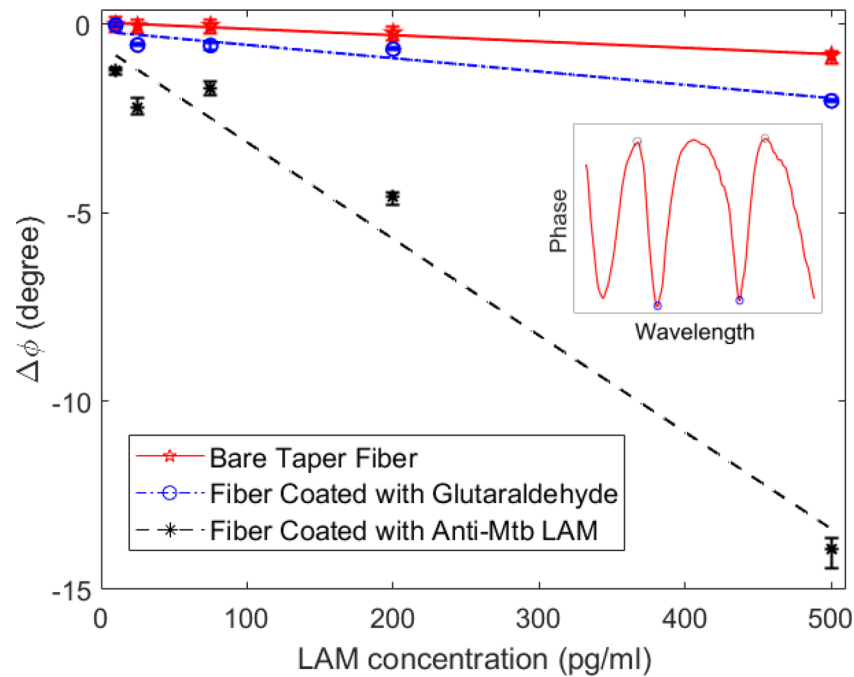
**Fig. 2.** Experimental setup. (a) Schematics L-Laser, FG-Function generator, M- Modulator, FGB-Fiber Bragg grating, LI: Lock-in amplifier, D-Detector. 's' represents the signal at the detector, and 'r' denotes the reference modulating signal from FG. The signal 's' undergoes a phase shift,  $\phi$ , with respect to the reference signal 'r' due to LAM binding events on the functionalized tapered fiber. (b) Physical experimental setup

We place the functionalized tapered fiber assembly into a home-built fluidic cell to probe the sample effectively. We use an external Mach-Zehnder modulator to amplitude modulate a 1550 nm continuous wave laser diode with a 4 MHz and 2 V<sub>pp</sub> sinusoidal signal. Simultaneously, we current-modulate the source laser using a triangular wave (10 Hz, 50 mV<sub>pp</sub>) provided by a function generator. The PS-CRDS measurements at the cavity resonances are then recorded using a photodetector and a lock-in amplifier. The PS-CRDS measurements provide a phase shift,  $\phi$ , with respect to a reference amplitude modulating signal. As the LAM binding on the functionalized tapered fiber leads to increased cavity losses, the ring-down time,  $\tau$ , decreases, consequently causing a reduction in the phase shift,  $\phi$ , as predicted by Eq. (1).

### 3. Results

We conduct experiments at three stages to evaluate the sensor's performance. In the first stage, we test a bare tapered fiber, i.e., a fiber without any surface functionalization. We first immerse the tapered fiber in DI water and record the average phase shift values,  $\phi_w$ , from 50–55 data points of absolute phase shift dips (Fig. 3 inset). Each absolute phase shift is determined by the corresponding phase dip size [15]. Then, we submerge the tapered fiber in a LAM aqueous solution (starting from low concentrations) and measure the phase shift value,  $\phi_s$ . Our measurand,  $\Delta\phi = \phi_s - \phi_w$ , denotes the difference between the phase shifts of LAM solution and DI water, as represented by red data points in Fig. 3. We repeat the same procedure to record the difference in phase shifts for different aqueous LAM concentrations as denoted by red data points in Fig. 3.

In the second stage, we use a functionalized tapered fiber. The functionalization strategy employed in this study to install different chemical functionalities on the surface of the tapered fiber is illustrated in Fig. 1. In the first step, the tapered fibers undergo a thorough cleaning process



**Fig. 3.** The experimental results depict the sensor response for three types of tapered fibers. The slope of the line is highest for the fiber functionalized with the CS-35 antibody, while the fiber without any functionalization produces a minimal response. This successfully demonstrates the correct fiber chemical functionalization and accurate functioning of the sensor.

to eliminate any dust particles and debris that may lead to measurement errors. A thorough washing with acetone is employed to remove any organic contaminants adsorbed on the fiber surface. The fiber is then treated with a strong Piranha solution, which is a strong oxidizing agent. Treatment with Piranha solution helps further cleaning the fiber as well as enhance the number of the surface silanol groups. The surface silanol groups are employed to functionalize the fiber surface with APTES through the silanization process. This resulted in the functionalization of fiber surface with amino groups. In the subsequent functionalization step, the surface amino groups react with glutaraldehyde to achieve fiber surfaces with aldehyde groups installed. Each glutaraldehyde molecule has two aldehyde groups. One of the aldehyde groups reacts with the surface amino groups of the APTES functionalized fibers through a condensation reaction producing imine linkage, while the second aldehyde group is displayed on the surface for further functionalization. We first conduct control experiments to exclude any contribution of surface aldehyde towards the binding of LAM to bare fiber surface and fiber surface functionalized with aldehyde groups. For this, we first immerse the cleaned bare or aldehyde groups functionalized tapered fiber in DI water to record the reference phase shift. We then immerse the fiber in the aqueous LAM solution for 15 minutes to ensure maximum interaction with LAM and measure the phase shift. After that, we treat the fiber with a 1 mM solution of sodium periodate for 15 minutes to detach the LAM molecules from the fiber. We then repeat the same procedure for different aqueous LAM concentrations, and the results are shown by blue data points in Fig. 3.

In the third stage, we use a fully functionalized tapered fiber with LAM antibodies (Fig. 1). The LAM antibody is covalently conjugated to the surface of aldehyde groups of functionalized tapered fibers. The LAM antibody is conjugated to the surface via imine linkages formed between

the amino groups of the antibody molecule and the aldehyde groups present on the surface of the glutaraldehyde-functionalized tapered fiber. With the LAM antibody functionalized fibers in hand, we repeat the same measurement procedure described in the second stage, and the results are shown with black data points in Fig. 3.

The results show that the non-functionalized clean bare tapered fibers and tapered fiber functionalized with glutaraldehyde produce very low responses compared to LAM antibodies functionalized fiber, which confirms that the surface functionalization protocol and sensing measurements are working correctly. These results also show that the functionalized sensing head has a high specificity for LAM. We can infer that the sensor's sensitivity is  $0.026^\circ/\text{pg/mL}$ , as determined from the linear fit of the black data points associated with the Anti-Mtb LAM functionalized tapered fiber.

We establish 10 pg/ml as our detection limit, reflecting the minimum LAM concentration detected by our sensor, observed as the initial data point in Fig. 3. The noise in our measurements arises from various sources, including system noise and mechanical stress noise in the tapered fiber induced by solution pressure [24]. To mitigate the noise impact, we conduct ten measurements for each aqueous LAM concentration, as depicted by the error bars in Fig. 3, derived from the maximum and minimum values among these ten measurements. For the fiber functionalized with Anti-Mtb LAM, the standard deviation values for the respective five data points of aqueous LAM concentrations [10, 25, 75, 200, 500] pg/mL are [0.0570, 0.1583, 0.1181, 0.1080, 0.2447] degrees. The mean phase values, accompanied by 95% confidence intervals, are  $[-1.23 \pm 0.0353, -2.19 \pm 0.0981, -1.71 \pm 0.0732, -4.56 \pm 0.0669, -13.91 \pm 0.1516]$  degrees. Notably, the phase shift reading for the 10 pg/ml LAM concentration stands at -1.23 degrees, approximately seven times higher than its corresponding  $3\sigma$  (where  $\sigma$  denotes standard deviation) and one and a half times higher than the maximum  $3\sigma$  value observed in the 500 pg/ml reading.

#### 4. Conclusions

Our work showcases the pioneering use of a phase shift cavity ringdown spectroscopy in conjunction with fiber cavities toward rapid TB diagnostics. Our experiments demonstrate that we can detect LAM as a TB biomarker at concentrations as low as 10 pg/mL in aqueous solutions, which provides almost fifty times improvement compared to the previous work conducted in urine samples [14]. Compared to conventional and earlier LAM sensors, our demonstrated sensor method offers significant advantages, including higher sensitivities, quicker detection, reduced costs, minimal sample preparation requirements, simplified system complexity, fewer fabrication difficulties, and enhanced potential for portability. Table 1 shows a comparison of our sensor with previous works on the detection of LAM as a TB diagnostics biomarker.

**Table 1. A comparison of various LAM detection schemes**

| Sensor        | Detection limit  | Platform    | Portability | Reference    |
|---------------|------------------|-------------|-------------|--------------|
| GC/MS         | 3000-28000 pg/mL | Non-Optical | No          | [25]         |
| ELISA         | 1000 pg/mL       | Non-Optical | Somewhat    | [26]         |
| Uri-TB        | 50 pg/mL         | Non-Optical | Somewhat    | [26]         |
| MZI chip      | 475 pg/mL        | Optical     | Yes         | [14]         |
| Fiber PS-CRDS | 10 pg/mL         | Optical     | Yes         | Current Work |

Considering the existing challenges within the current landscape of tuberculosis (TB) diagnostics, particularly in resource-limited settings that necessitate expensive laboratory facilities and skilled personnel for time-intensive patient testing, our demonstrated optical fiber sensor holds promise for advancing toward a point-of-care device. This sensor has the potential to

efficiently monitor TB progression or gauge medication response in patients by correlating the concentration of LAM with the disease.

We envision a system where only tapered fiber serves as a disposable element of the sensor while the remaining components remain the same. Despite this, the overall system retains its portability, aligning with our eventual goal of creating a point-of-care TB sensor. The disposable fiber is not only easily replaceable but also cost-effective, priced at just a few US cents. In contrast, the rest of the system incurs a one-time cost of less than \$4000 as per our first lab prototype. The costs are expected to be reduced significantly in subsequent sensor iterations. This cost is substantially lower than the prevailing infrastructure for well-established tuberculosis diagnostics, which typically costs hundreds to millions of dollars.

Unlike established sputum-based approaches, our sensor operates by detecting the urine biomarker, offering several advantages, such as minimizing cross-contamination and facilitating sample collection from vulnerable populations such as children, the elderly, or immunocompromised individuals. Nevertheless, working with urine samples poses specific challenges that may impact the sensor's LAM detection. These challenges include variations in sample pH, the presence of salts and concentrated inorganic ions, and the potential adsorption of urine components on the walls of the sample container, which could interfere with LAM detection [27].

There are several potential avenues for enhancing the current research work in the future. These avenues encompass conducting tests with urine samples, addressing the challenges mentioned above with urine samples, implementing temperature control for the fiber Bragg gratings (FBGs) and the fluidic cell, and refining and optimizing various parameters for tapering the fibers to increase the sensor's sensitivity. We also plan to explore improving TB diagnosis using our sensor, aiming to detect multiple TB biomarkers in urine [28] and potentially incorporating machine learning approaches [29] for enhanced accuracy. We foresee that the sensor setup we have demonstrated, with modifications to the surface chemistry protocol, could evolve into an affordable, real-time, and dependable optical sensor applicable across a wide range of sectors, such as food safety, healthcare, environmental monitoring, and agriculture, especially in resource-constrained environments.

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**Data availability.** Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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