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Label-free, real-time and multiplex detection of *Mycobacterium tuberculosis* based on silicon photonic microring sensors and asymmetric isothermal amplification technique (SPMS-AIA)

Qing Liu[a,*], Boon Kiat Lennon Lim[a], Swee Yin Lim[a], Wen Ying Tang[b], Zhonghua Gu[a], Jaehoon Chung[a], Mi Kyoung Park[a], Timothy Barkham[b]

[a] *Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), 2 Fusionopolis Way, #08-02, Innovis, Singapore 138634, Singapore*
[b] *Department of Laboratory Medicine, Tan Tock Seng Hospital (TTSH), 11 Jalan Tan Tock Seng, Singapore, 308433, Singapore*

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ABSTRACT

Rapid diagnosis of *Mycobacterium tuberculosis* complex (MTBC) is crucial for the control and treatment of tuberculosis. Currently available diagnostic tools are unable to meet clinical needs, especially in resource limited settings, as they suffer from being time-consuming, costly, insensitive, or dependant on sophisticated instruments and well trained personnel. An optical diagnostic platform (SPMS-AIA) consisting of the silicon photonic microring sensor and the asymmetric isothermal amplification technique was developed to detect MTBC in a rapid (one hour), isothermal, label-free and real-time manner. The performance of the SPMS-AIA platform was assessed by detecting two MTBC specific biomarkers, IS*6110* and IS*1081*. Detection limits were as low as 3.2 copies for IS*6110* and 12 copies for *IS1081* respectively when tested with *Mycobacterium tuberculosis* H37Rv genomic DNA. An evaluation for detection of IS*6110* in clinical sputa showed that the SPMS-AIA platform performed as well as the established market leader GeneXpert MTB/RIF platform. Of 26 MTBC culture-positive and smear-positive specimens, 23 were found positive by the SPMS-AIA platform (89% sensitivity). Of 47 MTBC culture-positive and smear-negative specimens, 34 were positive by the SPMS-AIA platform (72% sensitivity): this is similar to the sensitivity of 70% by the GeneXpert MTB/RIF platform. The versatility of the SPMS-AIA platform was demonstrated by multiplex detection of IS*6110* and IS*1081* in 10 clinical specimens. The SPMS-AIA platform shows great potential for development into a point-of-care (POC) diagnostic tool for use in resource limited settings.

1. Introduction

According to World Health Organization (WHO), tuberculosis (TB) is one of the leading infectious diseases causing deaths in adults [1]. In 2015 alone, the estimated number of new TB cases was up to 10.4 million and the death toll from TB was 1.8 million [1]. TB is an air-borne infectious disease mainly affecting the lungs. It is caused by a closely related complex of bacteria known as *Mycobacterium tuberculosis* complex (MTBC) [1]. Clinical diagnosis is difficult as the symptoms, including cough, fever, and weight-loss, are common in other pulmonary diseases, both infectious and non-infectious. These include emphysema, lung cancer and other bacterial and fungal infections. Therefore, rapid identification of MTBC is critical for the effective control of the spread of TB, reduction of inappropriate use of antibiotics, and implementation of tailored treatment modalities [2].

Conventional TB diagnostic tools include sputum smear microscopy and culture [3–5]. While sputum smear microscopy is relatively simple, inexpensive and rapid, it performs poorly with an average case detection rate of 30–60% and even lower in TB/HIV co-infection cases [3,4,6]. Culture remains the gold standard for TB diagnosis with higher sensitivity and specificity [5]. However, it is time-consuming, expensive, laborious, and requires well trained personnel, adequate laboratory equipment and strict bio-safety measures. A variety of nucleic acid amplification (NAA) diagnostic tools have been commercially available and widely adopted for almost two decades [7,8]. Among the NAA tools, the Xpert MTB/RIF platform has received much attention and was first recommended by WHO in 2010 as part of its policy to rise to the challenge of global TB care and control [9]. However, current NAA tools also suffer weaknesses. In addition to the variable and unsatisfactory performance in sputum smear-negative TB cases [8,10,11], all the existing

* Corresponding author.
E-mail address: findliuqing@hotmail.com (Q. Liu).

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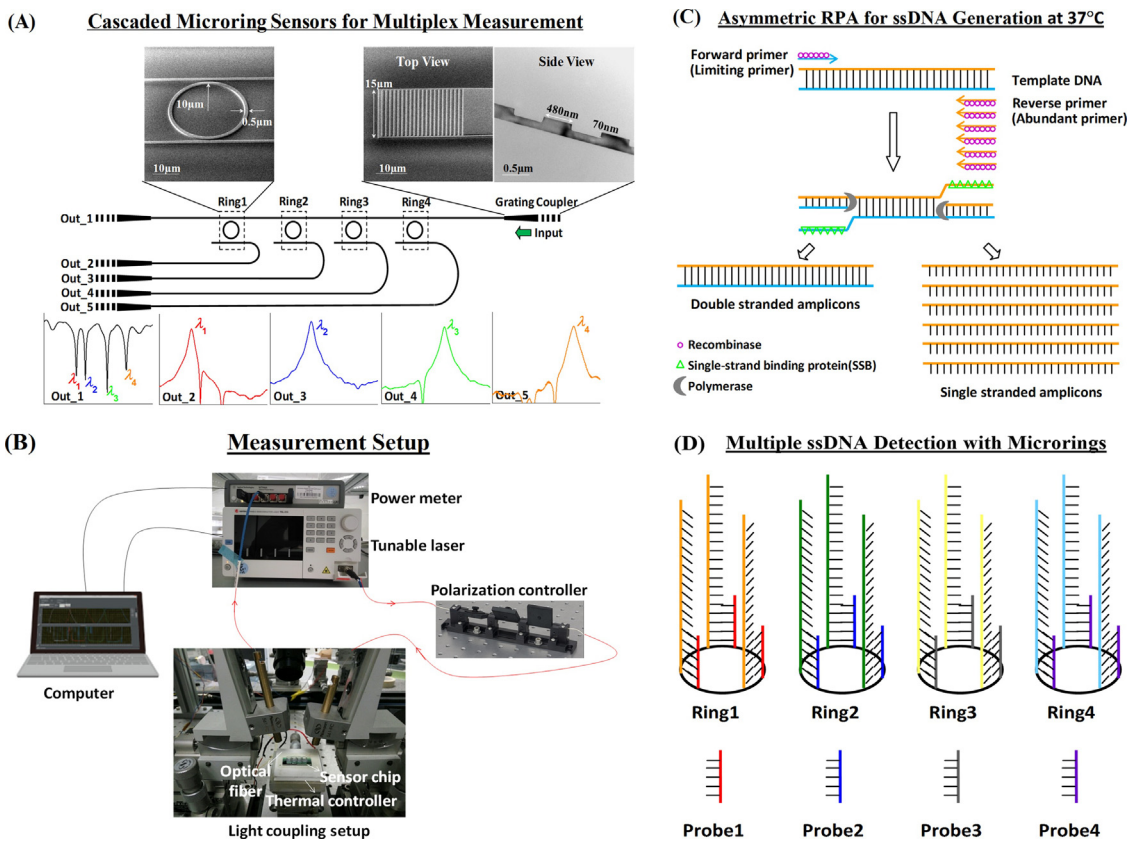

Fig. 1. (A) Optical resonance wavelength shift measurement: the four cascaded microring sensors for multi-sample measurement. (B) Optical setup for the measurement. (C) Target multiplication: asymmetric RPA process for single-stranded DNA generation at 37 °C. (D) Specific interaction with target molecules: multiplex ssDNA-probe binding on the microring sensors.

NAA tools require sophisticated laboratory infrastructure, instrumentation and special operation and maintenance, resulting in NAA tests being costly and restricting their use to high income countries [7]. Regions and countries with a high burden of TB and TB-HIV co-infections are usually resource limited settings; they lack equipment or financial resources to implement currently available NAA diagnostics [12,13]. In this context, there is still great demand for the development of new diagnostic tools which combine good and robust performance with simplicity and cost-effectiveness.

Sensors based on photonic devices have emerged as a promising technology for various biosensing applications [14]. In particular, silicon-on-insulator (SOI) microring resonator based photonic sensors have recently been widely investigated and have attracted interest because they are compact, robust and highly sensitive. The fabrication technique is compatible with complementary metal-oxide-semiconductor (CMOS) fabrication processes, enabling mass production of low-cost disposable sensing chips. SOI microring sensors have been reported in many studies for protein, miRNA and DNA detection [15–17]. Previously, an isothermal solid-phase amplification/detection (ISAD) platform based on a photonic sensor was developed by our group for TB detection [18]. However, the ISAD platform lacked a multiplexing capability.

In this study we developed an optical diagnostic platform (SPMS-AIA) based on the silicon photonics microring sensor coupled with the asymmetric isothermal amplification technique, which was a modified version of the recombinase polymerase amplification (RPA) technique [19,20] and specifically designed for this platform. The silicon microring sensor operated at the TM (transverse-magnetic) polarized light achieved 3 times higher sensitivity than the widely used ones operated at the TE (transverse-electric) polarized light. Moreover, the four microring sensors were cascaded to enable measurement of four samples simultaneously. The asymmetric isothermal amplification technique was proved to be able to specifically and efficiently generate single-stranded DNA products of the two TB biomarkers for sensor detection individually or in a multiplex manner. The diagnostic usefulness of the SPMS-AIA platform was demonstrated by detection of a single MTBC biomarker (IS*6110*) as well as multiplex detection of both biomarkers (IS*6110* and IS*1081*) in clinical sputum specimens, both of which were achieved in a rapid, label-free and real-time manner. To the best of our knowledge, this is the first study reporting the isothermal multiplex detection of TB biomarkers with the optical sensor.

2. Experimental

2.1. Sensor design, fabrication and measurement

The layout of each microring sensor array is shown in Fig. 1(A) which consists of four cascaded microrings that are coupled with one common input waveguide (called “through” channel). Each microring has a corresponding output channel (called “drop” channel). As shown in Fig. 1(A), the band-rejection spectrum at the “through” channel output (output_1) has four resonance wavelengths, each corresponding to one of the four microring sensors. By measuring the resonance wavelength shift of these four resonance wavelengths, the binding process on these four microring sensors can be monitored simultaneously. The position of the resonance wavelength of each band-pass spectrum at the four “drop” channel outputs (Output_2-5) is the same as that in the band-rejection spectrum at Output_1. By measuring the resonance wavelength shift at each “drop” channel, one sensor can be monitored at one time. The

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