PORTABLE CENTRIFUGAL MICROFLUIDIC PLATFORM FOR NUCLEIC ACID DETECTION

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ABSTRACT

The threats of disease outbreaks and bioterrorism demand field-deployable technology capable of rapid, sensitive, and accurate diagnosis. In order to address such public health concerns, we present a portable centrifugal microfluidic platform and demonstrate sensitive detection of *E. coli* down to single digit starting copies using isothermal amplification via loop-mediated isothermal amplification (LAMP). The platform, which is composed of a compact optical system for laser induced fluorescence (LIF) detection, a quiet brushless motor, and an efficient non-contact heater, offers an easy-to-use system capable of performing sensitive pathogen screening in a lab-free environment.

KEYWORDS: centrifugal, heat transfer, isothermal, nucleic acid test, LAMP, portable

INTRODUCTION

The technology presented here leverages previously reported developments of microfluidic systems designed at Sandia National Labs for novel immunoassays [1,2]. Towards maturing this technology, a heating system was incorporated to enable nucleic acids tests using techniques such as LAMP. Past implementations of heated centrifugal systems typically suffer from complicated and unreliable approaches such as the use of a slip ring for electrically interfacing with the rotating device as required for more traditional heating elements [3,4]. Slip rings introduce greater complexity, limit the available rotational speeds, and have finite lifetimes due to wearing down of their brushes. Other published heating methods include induction heating [5], which offers a non-contact solution but requires extensive circuitry and on-disc electrodes, increasing cost and complexity of disc fabrication. Infrared laser heating has been demonstrated but is only suitable for small targets and contributes to increased disc complexity by requiring an embedded metal plate to achieve high temperatures via indirect heating of the sample [6]. Thermoelectric heating, commonly used for PCR thermocyclers, has been implemented but requires additional moving parts, such as a linear actuator [7], to bring the disc into contact with the heat source. The non-contact heating system presented here provides temporally stable and spatially uniform temperature control with a simple, reliable set of components.

EXPERIMENTAL

The platform is primarily composed of three subsystems: 1) optical system for LIF, 2) rotary control system, and 3) non-contact heating system. The optical system consists of standard elements, including a laser diode module, PMT, and excitation and emission filters. The rotary control system is built around a brushless DC servomotor with an absolute encoder (Faulhaber, 2232S012BX4AES-4096). Optical switches interact with markings on the disc for home positioning. The heating system is based on a custom carbon filament medium-wave infrared emitter (Heraeus) mounted in a hinged enclosure in order to swivel the heater into position over the disc when ready for operation (Fig. 1). While the disc rotates at 100 RPM, the heater is powered at 28W to achieve uniform heating to 65°C. These components, along with a power supply and control electronics, are housed in a Pelican case.

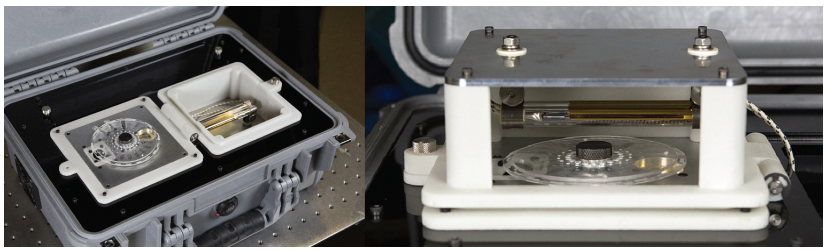

Figure 1: The microfluidic platform in shown in both the open (left) and closed (right) positions. The bottom image shows a special version of the heater enclosure featuring a window for observation via infrared camera. The carbon filament infrared emitter can be seen positioned over the disc when in the closed position.

RESULTS AND DISCUSSION

Once calibrated for open loop temperature control, the platform was tested by amplifying a heat-killed *E. coli* O157:H7 target using a LAMP reaction with QUASR chemistry [8]. With a 10x serial dilution of the target DNA from 10^4 cells/µL to ~1 cell/µL, sets of 10 µL reactions were run in triplicate for each template concentration along with a negative template control (NTC). The disc was heated to 65°C for 45 min, allowed to cool, and fluorescence was measured. Successful detection over the range of dilutions was observed (Fig. 2).

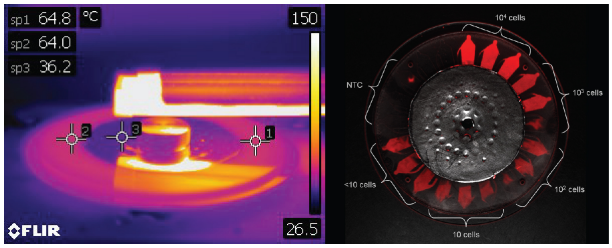

Figure 2: An infrared camera was used to monitor disc temperature for calibration of the temperature control system (left). Detection of E. coli was confirmed by measuring fluorescence on a gel imager (right).

CONCLUSION

Having demonstrated the basic functionality of this platform, future applications include hybrid immunoassay-LAMP screening and real-time detection of targets including Dengue, Zika, and Ebola.

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