SAND2016-10698C

PORTABLE CENTRIFUGAL MICROFLUIDIC PLATFORM FOR NUCLEIC ACID DETECTION

20th International Conference on Miniaturized Systems for Chemistry and Life Sciences, µTAS 2016 Conference
Christopher R. Phaneuf, Yooli K. Light, Huu M. Tran, Anup K. Singh, Chung-Yan Koh
Sandia National Laboratories, Livermore, CA USA

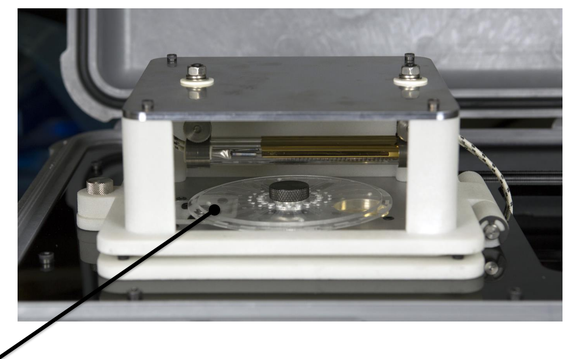
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

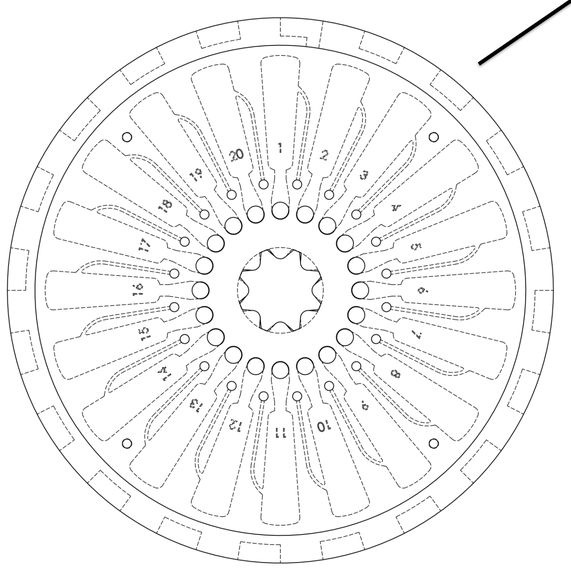
ABSTRACT

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.

DEVICE

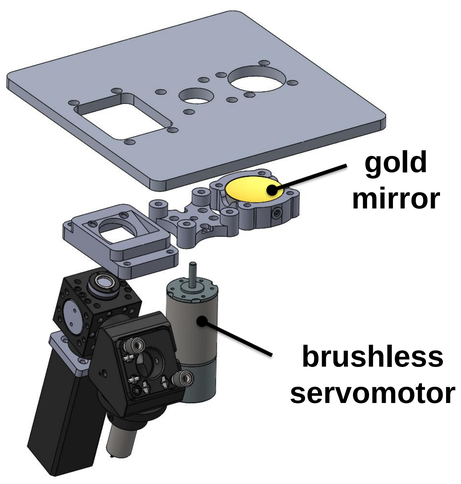
The heating system is based on a custom carbon filament medium-wave infrared emitter mounted in a hinged enclosure in order to swivel the heater into position over the disc when ready for operation. While the disc rotates at 100 RPM, the heater is powered at 28W to achieve uniform heating to 65°C. These components, along with a power supply and control electronics, are housed in a Pelican case.



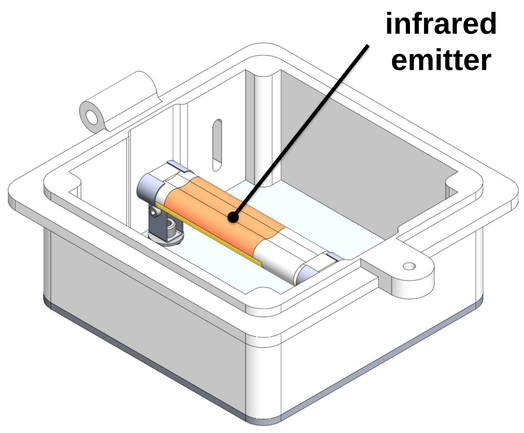


PMMA + PSA disc
10 µL chambers

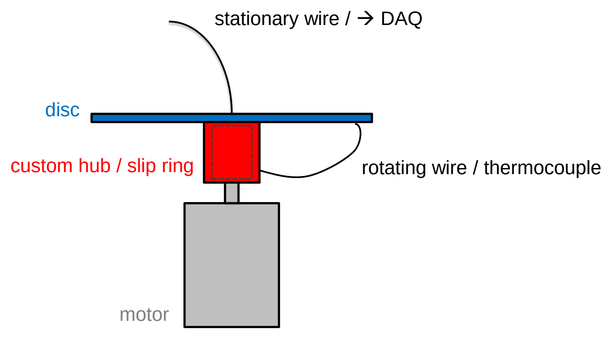
The microfluidic disc has an outer diameter of 89 mm and is composed of three layers: the top and bottom are made from 1.5 mm thick cast PMMA and fabricated using aCO_2 laser cutter. The middle layer is made from 125 µm thick double-sided, pressure-sensitive adhesive (PSA) and define the channels. Inlet ports are 1.5 mm diameter; channel length (*radial*) is 25 mm.

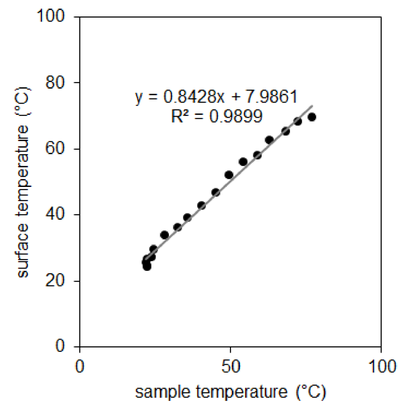


optical system

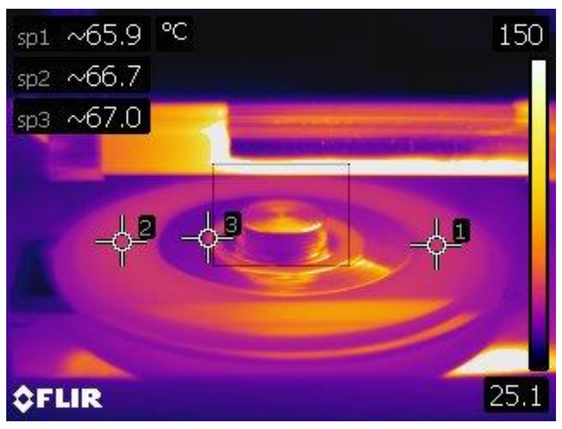


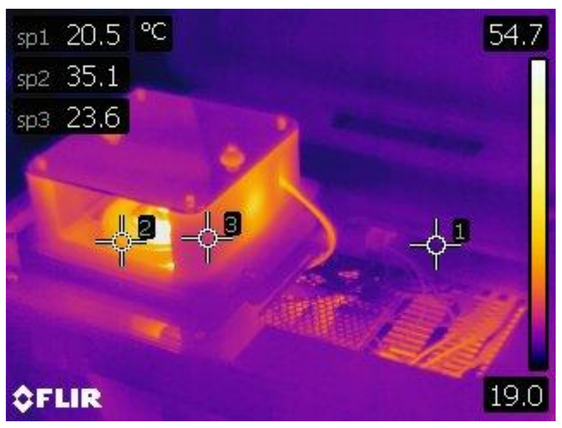
CALIBRATION





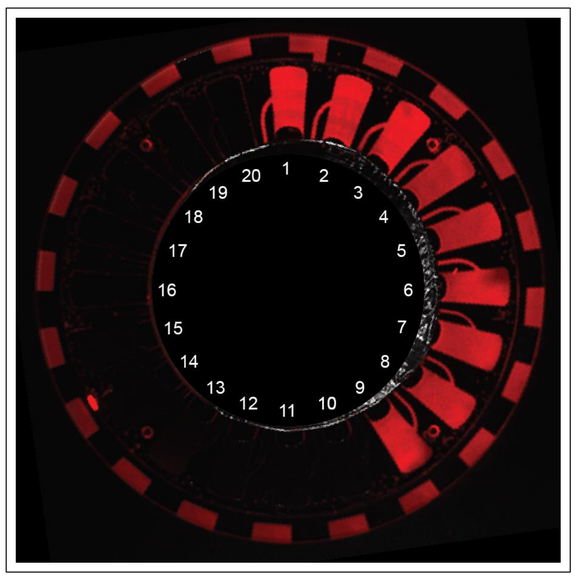
Calibration of the heating system was performed by first fabricating a disc with a micro-thermocouple embedded in one of the reaction chambers. This thermocouple was connected to a custom hub with a built-in slip ring, allowing the thermocouple to rotate with the disc during heating while the output wiring remained stationary. In parallel with the thermocouple measurement, an infrared camera was positioned above the disc to measure the top surface temperature of the disc. Temperatures collected from the top surface of the disc were correlated with true sample temperatures measured using the embedded thermocouple. This correlation was then used for open loop operation of the disc in all future experiments, requiring only a simple infrared camera measurement to confirm setpoints.





DETECTION

The calibrated heating system was tested by amplifying a heat-killed E. coli O157:H7 target (KPL, Cat. No. 50-95-90) using a LAMP reaction with Cy5-labeled primers targeting the stx1 gene. With a 10x serial dilution of the target DNA from 10^3 cells/µL to 10 cell/µL, sets of 10 µL reaction were run in triplicate for each template concentration along with negative template controls (NTC) and negative controls with Listeria template. The disc was heated to 65°C, incubated for 45 min then convectively cooled with a high speed spin. Successful detection over the range of dilutions was observed.



chamber	sample
1	LAMP, Cy5, *E coli*, 1000 cells
2	LAMP, Cy5, *E coli*, 1000 cells
3	LAMP, Cy5, *E coli*, 1000 cells
4	LAMP, Cy5, *E coli*, 100 cells
5	LAMP, Cy5, *E coli*, 100 cells
6	LAMP, Cy5, *E coli*, 100 cells
7	LAMP, Cy5, *E coli*, 10 cells
8	LAMP, Cy5, *E coli*, 10 cells
9	LAMP, Cy5, *E coli*, 10 cells
10	LAMP, Cy5, *E coli*, NTC
11	LAMP, Cy5, *E coli*, NTC
12	LAMP, Cy5, *E coli*, NTC
13	LAMP, Cy5, *E coli*, Listeria NC
14	LAMP, Cy5, *E coli*, Listeria NC
15	LAMP, Cy5, *E coli*, Listeria NC
16	water
17	water
18	water
19	water
20	water

ACKNOWLEDGEMENTS

This work was funded by the Laboratory Directed Research & Development Program at Sandia National Laboratories under project 165676. Sandia National Laboratories is a multi-mission laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

National Nuclear Security Administration
U.S. DEPARTMENT OF ENERGY