Development of Automated Detection and Wireless Reporting for a Handheld Point-of-Care Test

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***Abstract*—Second-generation rapid diagnostic tests, e.g. Lab-on-a-Chip (LoC) molecular diagnostics can help decentralize medical diagnostics for infectious diseases and provide far wider coverage than existing methods. Furthermore, they can provide results far more quickly, enabling early diagnosis and prevention of epidemics. However, to ensure a short turnaround time in testing, a compact, ideally low-cost, automated readout method is required. Preferably, the readout should also have integrated means of communication for automatic report of the results. We demonstrate a readout solution based on an ESP12F microcontroller board to read results from a LoC device demonstrated in our previous works. The readout solution relies on an LED-photodiode setup to read the results of immunochromatographic test strips. We evaluated the device by comparing readouts of positive and negative strips. During the evaluation, the device prototype was run from batteries and transmitted results via Wi-Fi to a PC, where a MATLAB application processed, displayed and saved results.**

Keywords—Lab-on-a-Chip, Point-of-Care, Automated readout, NAAT

I. Introduction

According to the World Health Organization (WHO), mortality rates due to infectious diseases have significantly decreased since 1990, in comparison with other causes [1]. However, a WHO report also indicated that 84% of all health hazards between 2001-2013 were due to infectious disease outbreaks [2]. Early diagnosis is essential to preventing epidemics, but diseases exist that have incubation periods of several days before the onset of symptoms, during which time the infection spreads. It was particularly evident during the novel coronavirus (COVID-19) outbreak of 2020 that widespread testing was vital to halting the spread of the infection [3]. Testing should be as decentralized and as fast as possible, yet most rapid diagnostic tests (RDT) are unreliable for the detection of asymptomatic hosts, as they rely on immunoassays, which require an immune response to be positive [4]. The gold standard for the detection of pathogens is DNA amplification [5], which on the other hand is more challenging to implement in a portable device than an immunoassay. One of the key technical challenges is the detection of results. The other challenge, which affects all RDTs is reporting results. The primary advantage to Point-of-Care rapid testing is that it can be done anywhere. However, in the case of epidemic prevention, this advantage is lost if results are not reported to the authorities. Therefore, an ideal RDT must be capable of automatically processing and reporting test results.

Some market-available RDTs exist that are capable of automated readout of results, for instance the Clearblue Digital Pregnancy Test [6] can interpret results from an immunoassay automatically, ruling out human errors during a readout. Several home healthcare monitoring devices, such as glucometers, are equipped with Bluetooth communication to synchronize data with a smartphone and/or a cloud-based data storage [7]. Lab-on-a-Chip (LoC) devices offer a higher level of complexity than typical RDTs, and can implement more complex diagnostic tests, such as DNA amplification assays or ELISA (enzyme-linked immunosorbent assays) tests complete with sample preparation. Palm-sized, USB-powered devices have been demonstrated, which were capable of automatically detecting HIV-1 viral RNA [8]. A handheld ELISA test was also demonstrated for HIV detection, which was capable of automatic detection and had a wireless transceiver for cellular communication to send results to a remote database. In a previous paper, we surveyed the communication technologies behind medical Internet of Things devices and existing Point-of-Care applications [9]. Despite major advances in automated RDTs, second-generation RDTs, and in particular, LoC nucleic acid amplification tests (NAATs), have not yet been reported with automated, wireless reporting of results. While wireless communication holds security risks that we cannot cover in this paper, the advantages are clear: it offers a higher degree of flexibility; it requires no physical contact and enables a larger distance between the test device and the data collection device(s), which are decisive advantages in epidemic prevention.

In our previous works, we focused on thermal engineering aspects and a basic implementation of an instrument-free LoC DNA amplification test [10]–[12]. In this paper, we demonstrate a system for automated detection and wireless transmission of results from an instrument-free, handheld, LoC NAAT. We discuss the development of the readout board and verify its function by detecting template DNA inside the 3D printed LoC cartridge of our previously reported diagnostic test platform.

II. Materials and Methods

A. Electronics design

To make an affordable readout module, an ESP12F board was used (Ai-Thinker, Shenzen, China), which is a low-cost board with an integrated ESP8266 MCU (microcontroller unit) and Wi-Fi with a full TCP/IP stack that allowed communication using serial commands. Since the readout target was a set of immunochromatographic (lateral flow) strips in a LoC device, we decided to implement a contactless optical readout. We used high-intensity and highly focused red LEDs (Kingbright KPBDA-3020SURKCGKC-PF 3.0 x 2.0 mm Right Angle SMD LED) for illumination, and wide-area photodiodes (BPW34) to transform the readout into an intensity measurement. This infrared (IR) photodiode worked on a wide wavelength range of 430-1100 nm, thus it was also capable of visible and UV detections with a practical ±60 degrees of aperture (half sensitivity angle was ±65 degrees). The LEDs were finely oriented and distanced to illuminate the readout zones. To reduce the number of components, we used a single photodiode per strip, placing it between the control and the test line (Fig. 3/B). Furthermore, to improve the contrast, the LEDs were tubed to block as much of the secondary emission lobes as possible. The beam diameter was set to the size of the readout zone (~3.3 mm for both lines), which was close to the almost squared beam projection available by the LEDs at their planned disposition (~3x3 mm). For the LED, various wavelengths were tested in the IR and UV ranges, but red worked the best. This was likely due to the plasmon resonance of the specific gold nanoparticles. This resonance is responsible for the red-coloring of the lines and their density in the strip, for the different response of the strip material (uncolored) to the different wavelengths of the spectrum, for the optical characteristics of the transparent plastic film of the readout window, for the high brightness of the LED, and for the quality of the LED beam.

The ESP MCU had an operating voltage of 3.3 V, whereas the PIC had an operating range of 2-5.5 V. Therefore, a single switching power regulator was used for the electrical circuit, using two AAA batteries as the power source. The ESP MCU had an average current consumption of a few tens of mA, peaking at a few hundred mA only for Wi-Fi transmission and heavy processing. Thus, it was enough to use a capacitor to avoid strong power line voltage changes, which could generate ESP failures. Since the ESP8266 had only a single ADC channel, we had to use another low-cost MCU (PIC12F675, by Microchip Technology, Chandler, AZ, USA) for LEDs switching, the photodiode readout, and the transmission of the data encoded in a string format with serial communication back to the ESP MCU. The PIC MCU had a negligible power consumption, and the LED power consumption was reduced by alternating between the two sensed lines of each strip and only turning the LED on until a stable readout was acquired. We also considered designs with no transistors, in which the PIC itself would drive the LEDs. This setup worked, but due to the limited output current of the PIC, we preferred to add two driving transistors, one per couple of LEDs and increase the LED currents. Due to tuning needs, the first prototype was made with relatively long wires between the PIC and the ESP MCU. In this setup, we saw a number of errors in the transmitted data. After a compact prototype board was made, the error rate dropped to negligible rates. The final schematic is shown split between Fig. 1 and Fig. 2.

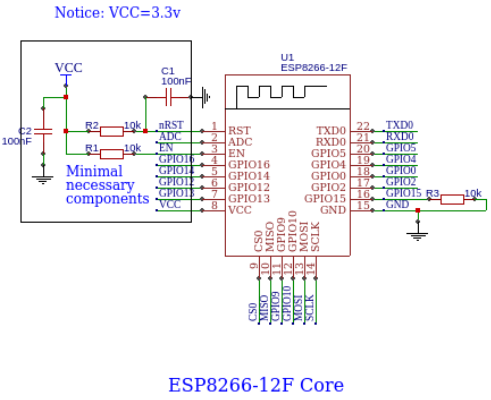

Fig. 1. ESP12F Wi-Fi microcontroller schematic with its minimal necessay components to work in this project. Pin RXD0 (serial input) is connected with Tx of Fig. 2.

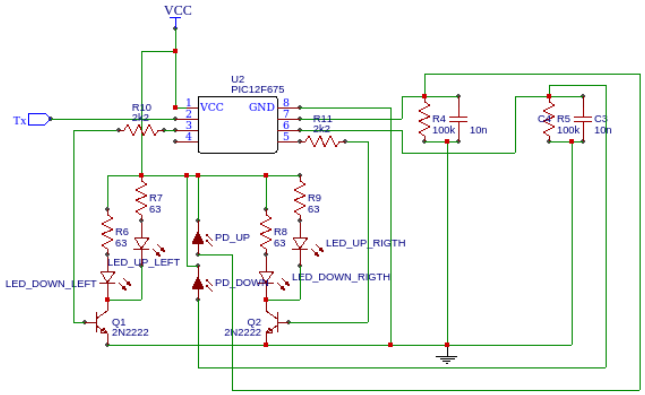

Fig. 2. PIC microcontroller schematic with photodiodes (with their inverse polarization components), and the LEDs (with their control and limiting transistors and resistors).

B. Experimental setup

Most first-generation Point-of-Care rapid diagnostic tests (RDT) rely on immunochromatographic assays, commonly implementing an enzyme-linked immunosorbent assay (ELISA) [4]. However, even second-generation RDTs, such as portable DNA amplification tests, can rely on immunochromatography to visualize DNA amplicons on lateral flow strips. For this analysis, we chose the non-instrumented nucleic acid amplification (NINAAT) platform that relies on lateral flow visualization [13].

For our experimental analysis, we used a 3D printed scale model of the Lab-on-a-Chip (LoC) cartridge detailed in our previous work [12]. 3D printing was done using an Envisiontec Perfactory 4 DLP (digital light projection) printer (Envisiontec Perfactory 4, Envisiontec GmbH). The cartridge was cold-laminated on top and bottom with clear plastic foil (Greiner MTP sealers, Greiner Holding AG). The cartridge was designed to hold 2 lateral flow strips. Each strip had 4 readout zones (one control and one test zone on each strip). The readout electronics were attached to the cartridge as shown in Fig. 3/A. The readout zones (one control and one test on each strip) were aligned with their respective LED at approximately 8 mm distance and the photodiode in the middle at approximately 10 mm distance (Fig. 3/B.). In this pilot study, the readout electronics were taped on top of the

LoC cartridge, and the whole setup was covered with a cardboard box to block out any external light. However, the enclosure design detailed in [12] can also accommodate the readout electronics with minimal changes (Fig. 4/A.) and will be used in the future. Two AAA alkaline batteries, using a step-up converter (based on MT3608, Xi'an Aerosemi Technology Co., Ltd., Shenzhen, China) to upregulate the input voltage to a stable 3.3V, powered the readout electronics board.

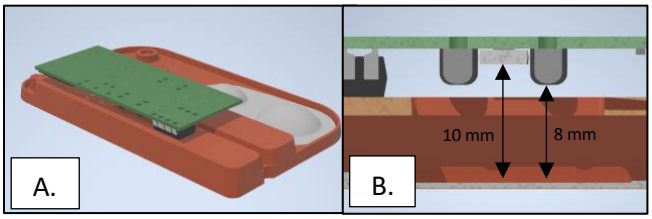

Fig. 3. Experimental setup: (A.) The 3D printed scale model of the Lab-on-a-Chip cartridge, (B.) the cross-sectional view of the readout zone (the LED was 8 mm, the photodiode was 10 mm from the plane of the lateral flow strip).

Tests were conducted with concentrated Neisseria Gonorrhea template DNA and custom-made lateral flow strips provided by SelfDiagnostics Deutschland GmbH. For each test with positive samples and each readout zone, 50µl DNA was diluted in 50µl ultrapure water. Afterwards it was pipetted onto the lateral flow strips located in the 3D printed LoC cartridge. Negative samples only contained 100µl ultrapure water.

For testing, a MATLAB application was written (using MATLAB 2019b). The application was compiled into a standalone executable for Windows 10 using the MATLAB compiler. After connecting to the readout electronics via WLAN, the MATLAB application would query the board via its IP address, collect and format the data from the board and fill up the fields in the GUI (graphical user interface) window shown in Fig. 4/B. After the measurement, the application would export formatted raw data into a MS Excel file. The MATLAB application was run on a PC with a Core i5-7300U CPU and 8 GB RAM but is possible to run as a standalone application on any PC capable of running Windows 10 and MATLAB runtime. In the future, we plan to provide a smartphone application to the end-user, but for testing, using a PC was more convenient.

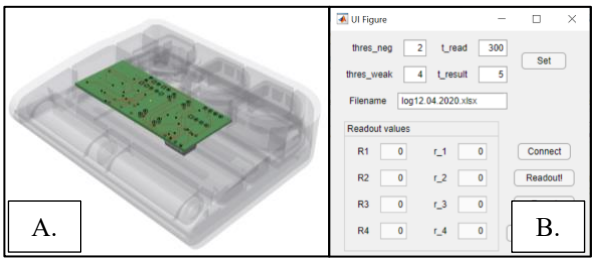

Fig. 4. End-user application concept: (A.) The standalone MATLAB application developed for data collection, formatting and exporting to Excel. (B.) The position of the electronics board in a modified version of prototype assembly reported in our previous work [12]. The board is designed to run off the two AAA alkaline batteries integrated in the device.

III. Results and Discussion

A. Electronics testing

The PIC microcontroller executed four ADC readout per check cycle, two readouts in each photodiode (one illuminated by the left LED and one by the right LED). The PIC microcode executes a first cycle of four readouts at boot-up for self-calibration, which values were compared with the following readouts. The readouts were then subtracted from the initial conditions, allowing the comparison between white and colored strip. The 4 boot-up readouts are packed together with the most recent 4 readouts and continuously sent to the EPS01 Wi-Fi module that will make them available through the Wi-Fi connection to the MATLAB application.

The Wi-Fi microcontroller was set in Access Point mode; such a modality generates an ad-hoc Wi-Fi password protected network, to which the end-user device connects automatically. The connection was secured using WPA2 (Wi-Fi Protected Access). The end-user device could be a smartphone with a specifically designed app, or as in the current developing case, a PC. The end-user device, once it is connected to the Wi-Fi network, should provide instructions to the user about the correct use of the test and then provide real-time test results. The end-user device can retrieve data strings containing the readouts simply downloading a local network resource available at a specific local address; such a data string is updated each half second.

The data string (*e.g.*, "Hello! C=008 R1=059 R2=069 R3=055 R4=068 r1=059 r2=069 r3=055 r4=068 B") has space separated fields. It is composed by a header data which starts the string ("Hello!"), a sequential counter to recognize updates ("C=008"), the boot-up readout values necessary as initial reference ("Rn=xyz", n=[1,4]), the current/updated readout values ("rn=xyz" , n=[1,4]), and a footer data which ends the string ("B"). All numerical values are bytes (0-255), those bytes corresponding to readouts are to be intended as relative intensity measurements made by the ADC.

B. Readout test

For the readout test, the setup described under Section II.B. was used. Before the test, 3 positive and 2 negative samples were mixed together in 1.5 ml Eppendorf tubes and stored at room temperature until the test. During each test, the board was under powered on conditions and connected to the MATLAB application running on the PC. For each test, a new set of lateral flow strips were placed in the LoC cartridge and the bottom foil was replaced. A 5-minute readout was started in the MATLAB application, and then the DNA sample was pipetted onto the collection pad of the lateral flow strip. After each readout, the application formatted and exported the raw data to a MS Excel file. The results for each zone were calculated from the difference between the first readout value (after starting the board up, *i.e.*, a fresh white background) and the current readout value. To calculate the result, the average of the last 2 minutes were calculated. This was done to ensure that the strip had fully run before the readout, and no false red zones remained. On a lateral flow strip, positive signal means a clear red line, which is the same color as a running strip. A negative signal means the native white color of the strip. Test results shown in Fig. 5 indicate a clear difference between positive and negative signals in all four zones. A logarithmic scale was used for the plot for easy

visual comparison. For the positive results, the average signal intensity was 58.85 ± 19.87 (a.u.), whereas for the negative results, the average was 0.27 ± 0.47 (a.u.).

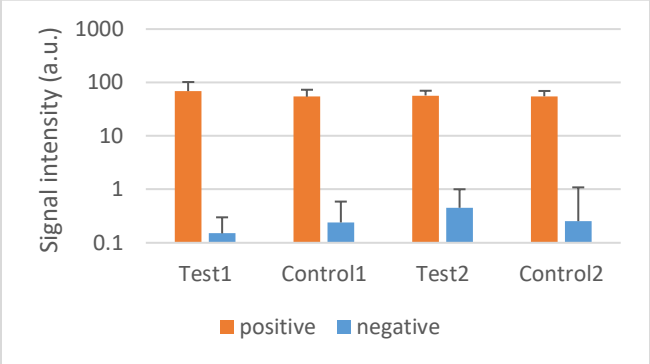

Fig. 5. Readout test results indicated a clear difference between negative and positive signals in all four readout zones.

Please note that this pilot study only yielded a proof-of-concept test result, and further testing under various lighting conditions and target DNA concentrations is necessary to evaluate the system performance. In this test, pure template DNA was used in a high concentration, which produced a well-defined and strong positive signal. On the end-user side, however, DNA concentration and thus signal intensity and sample purity can greatly vary.

IV. Conclusions

Summarily, we developed an automated digital readout module for our previously demonstrated LoC device, which relied on two lateral flow strips to visualize results. The developed readout module was a compact, low-cost and low-power circuit board built around an ESP12F board with an ESP8266 MCU. The module had built-in Wi-Fi connectivity, and was wirelessly connected to a PC with a MATLAB application for result interpretation and storage. The readout electronics consisted of a set of two red LEDs and a photodiode for each lateral flow strip in the LoC device. The readout was controlled and results transmitted to the ESP MCU by an additional PIC12F675 MCU. We tested the board with a 3D printed LoC prototype cartridge and two lateral flow strips. We tested and compared readout results for 3 positive and 2 negative strips. This proof-of-concept experiment demonstrated that the system was capable of differentiating between positive (~60 a.u. intensity) and negative (<1 a.u. intensity) results. However, future investigations should target testing weak positives and difficult readout scenarios, such as under direct sunlight. It also needs to be investigated what improvements the device enclosure needs to make it suitable for housing the readout module. In addition, there are several potential future improvements for the board itself, such as: further miniaturization, additional software functions, smartphone application, Cyclic Redundancy Check (CRC) code to discard corrupted chunks of data after communication, and correction code to remove transmission errors.

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