Portable sample processing for molecular assays: application to Zika virus diagnostics

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Due to the Covid-19 pandemic, we have seen how important is to implement quick laboratory diagnostic for early therapeutic intervention and infection surveillance. However, in certain places as low income or far away from big cities, it difficult to establish a diagnostic and that has shown the need to implement point of care platforms that can be portable and easy to use outside of centralized laboratory.

For viral infection like SARS or Zika, a clinical diagnostic approach involves viral RNA extraction from human biological samples followed by PCR assay targeting virus-specific nucleic acid. Viral RNA is routinely extracted using commercially available solid-phase RNA extraction kits that are often supplied by a handful of major manufacturers who vertically integrate these kits with their proprietary instruments and essential plastic wares (Han et al., 2021). As is shown here, these systems can be very prohibitive with the consequence of limiting the diagnostics and surveillance.

In this regard, Narahari et al. (2022) have developed a portable platform for RNA extraction and amplification prior to detection of viral target of interest using a digital microfluidic (DMF) platform and a custom hardware design with open-source software for controlling the hardware (fig. 1).

The workflow of the system as is shown in fig. 1 start with a sample of plasma (orange) potentially containing virus is lysed (denoted pre-processing or 'P') and RNA (red traces) is reversibly captured on magnetic beads (gray circles). The magnetic beads are then processed on DMF using the Zed Box, which includes a motorized magnet and built-in thermal cycling system, which enables automated RNA extraction and clean up (denoted 'E'), as well as Zika RNA amplification (denoted 'A') via a programmable protocol. The amplified product is then assayed using a one-pot, paper-based, colorimetric, cell free protein expression system (denoted 'C') revealing a colour change from yellow (negative) to deep red (positive).

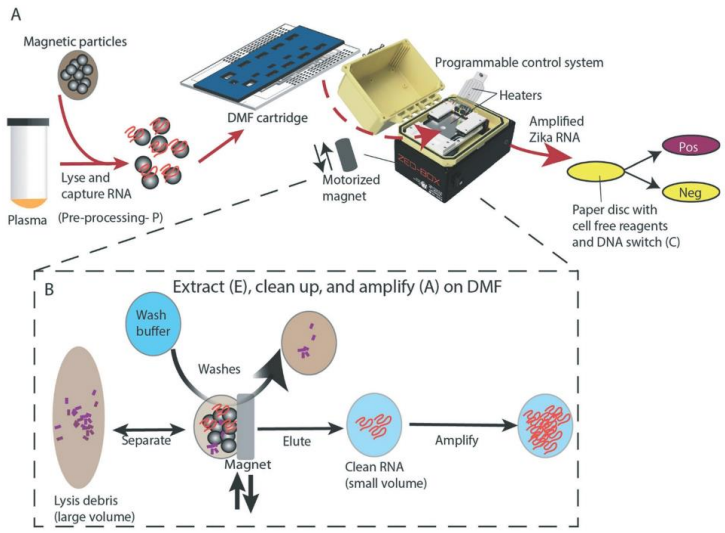

Fig. 1 Processing sample system workflow, only the extraction and amplification are done in the DMF cartridge. The workflow introduced here involves five major steps: 1) sample lysis and reversible RNA capture on magnetic beads, 2) RNA extraction, 3) RNA clean-up, 4) isothermal amplification of Zika RNA by nucleic acid sequence-based amplification (NASBA), and 5) detection using the toehold switch-based Zika virus sensors, a concept that was established in previous work by Pardee et al. (2016).

The choice of the device was because the authors are trying to develop a solution for a process normally ignored in the development of new point of care devices. According to the authors own words “while portable diagnostic sensors have (with good reason) attracted great attention, the pre-processing steps required upstream of the sensor are often ignored, leading some to label them the “forgotten beginning” of portable diagnostics” (Narahari et al., 2022).

The major novelty of the new system is to allow for droplet-in-air amplification using humidified cartridges that do not require oil fillers or solvent injection to counter droplet evaporation at elevated temperatures. The system is therefore straightforward to use and amenable to implementation in real-world settings where users may lack experience with operating microfluidic devices (Narahari et al., 2022).

What is Digital microfluidics

Digital microfluidics is terminology normally reserved to microfluidics chips that use discrete fluid droplets that are manipulated on the surface of an array of electrodes coated with a hydrophobic insulator. This enables on-demand manipulation of individual, low-volume droplets on an array of electrodes, using active methods that use electro field forces (dielectrophoresis (DEP) and electrowetting on dielectric (EWOD)) for generation of droplets. Compared to passive method that used immiscible liquids and the chip geometry to form droplets, active methods generate droplets at low frequencies and the droplets are usually small (nL to pL volumes), however, for applications that do not require high throughput, active methods are enough to perform assay with clinical relevance.

DMF Cartridge and ZED box characteristics

According to Narahari et al. (2022), all parts that form the DMF cartridge were designed using AutoCAD software. The bottom plates were manufactured from 3 in × 3 in chromium-coated glass substrates via UV photolithography and wet etching. The electrode array included 58 square interdigitated driving electrodes (2.8 mm × 2.8 mm), 5 loading electrodes (four measuring 2.8 mm × 5.6 mm, and one measuring 5 mm × 4 mm), 8 buffer reservoir electrodes (11 mm × 6.5 mm), 10 dispensing electrodes (2.8 mm × 5.6 mm), and 3 extraction lane reservoir electrodes (7 mm × 6.4 mm), each connected by a conductive trace to a contact pad on the edges. Top plates were composed of a rigid, visually transparent acrylic substrate (1.5 mm thick) interfaced with a flexible PET-ITO electrical grounding layer (MITO-60-125, 60 Ω sq.in.$^{-1}$, 125 µ m thick). To fabricate each top plate, acrylic substrates were laser-cut using a Hobby Series Full Spectrum, 40 W CO_2 benchtop laser cutter into 8 cm × 5 cm pieces, punctuated with three kinds of rectangular windows (through-holes), including eight ~ 5.5 mm × 4 mm reservoir windows, four ~ 3 mm × 4 mm and one ~ 5 mm × 2.5 mm loading window, and two 7 mm × 6.5 mm extraction windows (fig. 2A).

The Zed Box that controls the hardware operations has its own PCB designed in KiCad and it hosts a microcontroller (ATmega328P, Microchip Technology Inc.), two high-power transistors (SQJA60EP-T1_GE3, Vishay Siliconix) for controlling peripheral heating units, two low-power transistors (DMG2302U-7, Diodes Incorporated) for controlling peripheral cooling fans and LEDs, a stepper driver (A4988, Pololu) for controlling the actuation of the pelletizing magnet, and a temperature/humidity sensor (HIH6030-021-001, Honeywell) for logging the external temperature and relative humidity of the environment (fig. 2B).

The Zed Box was designed to be controlled by a host computer running the open-source DMF control software Microdrop (https://microfluidics.utoronto.ca/dropbot/). A custom plugin for Microdrop was written in Python which permitted the user to program and control all the DMF operations as well as the states and timings of each of the peripheral devices in the instrument from within the Microdrop user interface. When used in DMF operations, a completed DMF cartridge was connected to a custom manifold that permitted Zed-Box controlled pogo-pins to interface with the chromium contact traces on the bottom plate of the cartridge.

Also, a custom GUI (operating in MicroDrop) was written to guide the user step-by-step through the extraction and amplification process described here, including prompts for all sample and reagent loading and collection steps. In each loading step, the user pipettes a reagent into the appropriate window while the relevant electrode (under the window) is actuated, pulling the fluid under the relevant flap.

Finally, an external tube heater was developed for use as an ancillary system to the DMF platform, for thermal lysis of virus particles. Briefly, a part was designed to hold a single 600 µL Eppendorf centrifuge tube in Fusion360 and 3D printed in High Temp Resin using the stereolithographic 3D printer Form 2 (Formlabs). A 20 cm resistive heating wire (5Ω, 32-gauge Clapton coil, GiniHomer LifeMods) was looped around the 3D printed part, and an NTC thermistor (10 kΩ, MC65F103A, Amphenol Advanced Sensors) was inserted at the bottom of the part to provide temperature feedback. The tube heater module was controlled by the Zed Box and the computer along with the other peripheral units (fig. 2C).

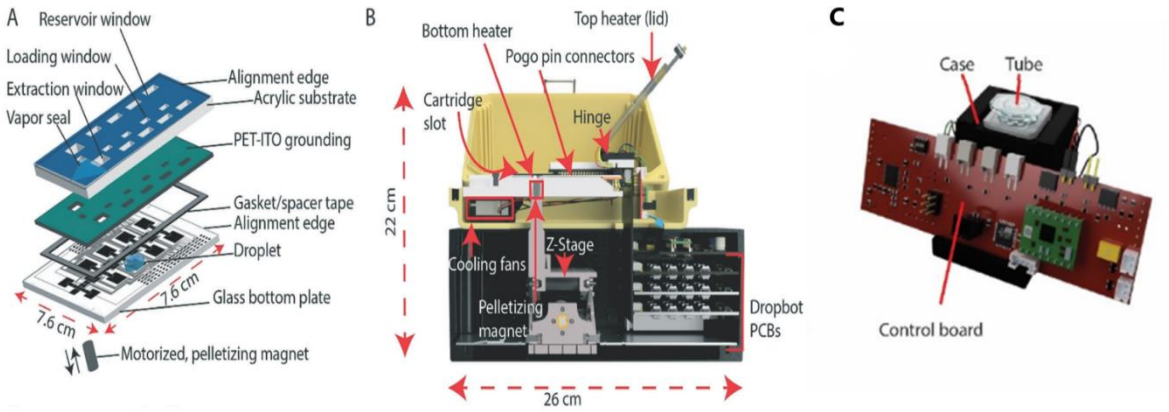

Fig. 2 A) Schematic showing an exploded view of the DMF cartridge, which comprises a rigid, acrylic substrate (gray) with laser-cut windows for reagent and sample loading, a peelable vapor seal (blue), a flexible, PET-ITO electrical grounding layer (green), a gasket and gap-height spacer (gray), and a glass bottom plate. A liquid droplet (blue disc) is sandwiched between the bottom plate and the PET-ITO substrate. A motorized, pelletizing magnet is located under the bottom plate within the control hardware. B) B) Cross-sectional view of the Zed Box control hardware, showing the placement of the two resistive heaters, one of two cooling fans, a pelletizing magnet mounted on a motorized z -stage, and control electronics (Dropbot PCBs). The DMF cartridge is loaded into the box, and interfaces with the control electronics via pogo-pin connectors. When in use, the top heater is lowered so the cartridge is in conformal contact with both heaters. C) Rendered image of the ancillary tube-heater, a 3D printed case (black, PLA, Ultimaker) with dimensions 41x40x53 mm with an embedded heating element (not shown) holds a 600 µL Eppendorf centrifuge tube (gray) and is mounted to the control board (red), monitored by a laptop computer running a Jupyter Notebook script.

Context and state of the art

Only regarding with digital microfluidics, recently The Gaudi Labs (known for selling open-source equipment like PocketPCR low-cost USB powered PCR thermocycler) have developed an open

source OpenDrop digital microfluidics platform. The device uses electro-wetting technology to control small droplets of liquids. According to the seller, the device could have broad potential applications in lab on a chip device for automating processes of digital biology. For OpenDrop, until the moment, there is not a reported similar workflow like the develop by Narahari et al (2022), however, the last iteration of the prototype OpenDrop V4 include peltier elements for heating and cooling, electromagnets for magnetic beads or optical sensors for reaction measurements (OpenDrop V4 from GaudiLabs, 2020), which could be leading to similar workflow in the future.

Regarding to extraction and amplification is common to have microfluidics systems that are easier to fabricate and control as paper microfluidics or low-cost 3D printed microfluidic devices. Kadimisetty et al. (2018) developed a simple, inexpensive, disposable, fully 3D printed microfluidic reactor array that was capable of carrying out extraction, concentration and isothermal amplification of nucleic acids in a variety of body fluids, being able to detect samples with *P. falciparum* and *N. meningitidis*. This device compared to the DMF Cartridge and ZED box is easier to manage, however, it is limited to only to that application, on the contrary, the DMF Cartridge and ZED box offers more modular design which can be applied to other diagnostics as been shown before in hemagglutination assays (Sklavounos et al, 2021).

Evaluation of openness of the design project

One of the points more critical was the paper's documentation. Although the authors have shown before systems that are open hardware and software, the state of the documentation is not clear enough to follow and be able to determine the state of the project (whether it is an open hardware or not). At one point, the authors said that the system can be used with open software, nevertheless, it appears that the documentation is not actualized for this system. Also, there is not a documentation that allows you to replicate the system, which can indicate that the hardware is not an open-source project.

Potential application

The core of the device is completely associated to biological assay like molecular detection, however, it could also be used in interdisciplinary research like in chemical synthesis, which normally certain compound is costly and only required in very tiny amounts.

Finally, even if the system addresses an overlooked area of laboratory automation and on-site testing like sample processing prior to analysis, the prototype still needs some improvements like removing manual steps, integrate complete workflow on the chip, right now the detection that is one of the most important points in a diagnostic assay is done in a separate way (off the chip), becoming a weak point if the real intention is to implement a real lab on chip, also the chip need to be contained in a larger electrode array for multiple samples and all controls that can be run in parallel.

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