PROGRAMMABLE MAGNETIC ROBOT (PROMAGBOT) FOR AUTOMATED NUCLEIC ACID EXTRACTION AT THE POINT OF NEED

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ABSTRACT

For numerous diseases across the globe, nucleic acid testing remains the clinical standard. However, there is a need for these methods to be field deployable. Recent improvements in optical detection and isothermal amplification have pushed POCT devices closer to laboratory standards. However, sample preparation remains a bottleneck that has not seen improvements. Here we present an automated device using paramagnetic beads for nucleic acid extraction in under 10 minutes. Linking our device with microfluidics creates a system that is fully automated, user-friendly, ultra-portable, notably accessible, and sample adaptable.

KEYWORDS

POCT; Electromagnet; Magneto-fluidic; Nucleic Acid Extraction; Sample Preparation

INTRODUCTION

Nucleic acid testing (NAT) remains the clinical standard for identifying and quantifying infectious diseases[1]. However, the laboratory procedures for these methods require long waiting periods, trained staff, and expensive hardware to analyze the testing results [2]. Point-of-care (POC) devices introduce NAT in low-resource regions by simplifying the steps into compact and portable form-factors [2, 3]. However, recent developments have improved amplification and detection methods to be better suited in low-resource areas[4-6]. While necessary, complete NAT contains a bottleneck for point-of-need testing: sample preparation. Standard methods require extensive manual processes, laboratory devices, toxic chemicals, and trained professionals. These issues severely limit the scope of POC devices when providing quality NAT. Numerous sample preparation systems for laboratories, lab-on-chip systems, and some POC devices depend on silica columns, membranes, or organic solvents. An alternative, silica coated paramagnetic beads (PMBs), offer a solution by eliminating the need for noxious chemicals and simplifying device complexity.

Devices that take advantage of PMBs for POCT applications include bead control through permanent magnets, electromagnet (EM) controls, EM actuated magnets, centrifuge style rotation, automated pipetting, and fluid flow methods [7, 8]. While effectively integrating PMBs into their system, these devices still lack practical applications to field deployment and are only capable of single style extraction. This gap creates the need for a fully automated, user-friendly, ultra-portable, notably accessible, and sample adaptable device system.

This work demonstrates a programmable magnetic robot (ProMagBot) capable of automated nucleic acid extraction. The entire ProMagBot system can be utilized in areas of low resources for nucleic acid extraction of multiple sample mediums in under 10 minutes.

RESULTS & DISCUSSION

ProMagBot Overall Device Design

As seen in **Figure 1a,** the ProMagBot system is composed of multiple parts: (from top to bottom) the top casing, camera, light guide, user interfacing features, the Raspberry Pi 4, a spacer and viewing window, the electromagnetic PCB, the bottom casing, microfluidic cartridge, and the compact battery. Overall, the project can be divided into three subcategories: A computer vision system (**Figure 1b**), microfluidic cartridge (**Figure 1c**), and an electromagnetic PCB paired with a permanent magnet (**Figure 1d**). Locating a permanent magnet above electromagnetic coils allows this device to employ a novel magnet-on-top (Mag-On-Top) approach to magneto-microfluidic separation of nucleic acids. Computer-aided tracking of the permanent magnet within the electromagnetic coil array provides rigidity to the magnet pathway. It allows for redundancies in motion that are lacking in other forms of magnet actuation in POCT devices.

User interaction with the device follows the steps highlighted in **Figure 2**. First, the user collects the sample off chip and transfers it to a collection tube. Using a one hundred µL pipette, the user transfers the lysate mixture into the microfluidic cartridge. Then, the user can power on the device. Once on, the device will initiate a bootup sequence, and when ready, indication LEDs will flash. The device will run for 8-10 minutes. The device will signal when the extraction is complete using the indicator lights. Then, the user removes the cartridge from the device. Lastly, the eluted nucleic acids can be removed from the cartridge.

Microfluidic Cartridge Design

For the ProMagBot system, a three-layered microfluidic cartridge is utilized to separate, contain, and encompass the extraction solutions. This cartridge is designed to bind the PMBs assay to nucleic acids in the lysis chamber. In part one of this step, the lysis buffer adds detergents and surfactants to the sample to break down cells, bacteria, and viral particles (**Figure 3i**). In part two, the binding buffer adds chaotropic salts and decreases the pH of the solution to an ideal condition for nucleic acids to bind with the silica PMBs. The permanent magnet located vertically above the cartridge will help in this mixing process before dragging the nucleic acid-bead complexes (NABC) through the first of the oil valves into the washing chamber. In this chamber, the solution gradually increases pH. It includes other non-toxic chemicals to help remove unwanted proteins and salts (**Figure 3ii**). The magbot will encourage mixing and then move the NABC to the elution chamber. Here the pH level of the solution is significantly higher than the starting solution to reverse the binding process. In a basic environment, the nucleic acids dissociate from the PMBs, which can then be removed via a magnet (**Figure 3iii**). The remaining solution in the elution chamber is a concentrated batch of nucleic acids ready for extraction by the user.

Electromagnetic PCB Design

One advantage of the ProMagBot system is running off a compact battery. The ProMagBot device contains Groups and Blocks of electromagnetic coils that are actuated in series to accomplish this. First, this creates stepwise coils that can be used sequentially to move a magnet in one direction or another. Second, this significantly reduces the complexity of the device's electrical components by removing the need for a multiplexer. Using six MOSFET switches, the device contains 4 directions of motion (±x,±y). In **Figure 4a**, the coil layout is visualized for both the X and Y directions.

In either direction, a Block containing three separate coils also belongs to a unique Group. Blocks repeat seven times in both X and Y directions, while Groups remain linked together through the PCB. When one Group activates, all coils within that Group and Block

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activate as well. In this manner, the permanent magnet position is defined as: ($X_{i,j}$, $Y_{m,n}$), where X signifies the X-direction Blocks and Groups, *i* ranges from 1 to 7 (# of repeated Blocks), and *j* represents the Group number from 1 to 3. Similarly, Y signifies the Y-direction Blocks and Groups, where *m* represents the Block number from 1 to 7, and *n* demonstrates the Group number from 1 to 3. For any given moment, along any given pathway, the magbot is defined in both the X and Y direction by the Block and Group that last attracted it.

Figure 4b demonstrates how the permanent magnet moves by stepwise motion. In these images, the magbot starts aligned with coils X-Group-2 and Y-Group-2. The previous Y-Direction coil switches off, and the next coil powers on to move the magnet one step down. Ergo, in Step-1, X-Group-2 remains on, the Y-Group changes, and Group-3 is now powered. Likewise, for Steps 2 & 3, the X-Group coils are manipulated identically to move the magnet along the X-Direction. In Step-3, it is critical to note that the magnet moves upward to realign with Y-Group-2. By these methods, the power efficiency of the ProMagBot is kept to a minimum. It allows the device to remain equipment-free and deliverable to low-resource areas because of its internal battery.

Computer Vision-based Control

One drawback to our Block and Group setup of electromagnetic coils is that there are no internal referencing mechanisms to ensure that the magbot is located where the control program assumes. To solve this problem, we have implemented a computer vision algorithm to control the magbot. The algorithm analyzes the magbot position after each motion step and compares that against the preplanned pathway points. Three unique pathways were defined, demonstrating that the algorithm can integrate with the EM coils. The magbot sequentially moves to each defined point by the computer vision program. These pathways in **Figures 5a, b, and c**, exaggerate movement in the four cardinal directions. From these demonstrations, it is clear to visualize that the magbot is capable of movement in any direction and has the potential to follow any programmable pathway.

CONCLUSION

To summarize, our ProMagBot system can extract nucleic acids in under 10 minutes while also remaining well suited for low-resource areas. Silica-coated paramagnetic beads (PMBs) are used within the lab-on-chip cartridge to bind nucleic acids after sample lysis. The cartridge contains the chemical chambers and volumes needed, encloses all liquids, and separates the solutions using oil valves. These features create a stable and robust cartridge that is portable, easy to handle, and user-friendly. The ProMagBot device completes the nucleic acid extraction by pulling the PMBs through oil valves into washing and elution chambers that purify the nucleic acids. When the magbot moves away from the central microfluidic channel, the PMBs are relieved from the magnetic field and then passively precipitate. Thus, increasing the mixing inside the chamber compared to other devices. The device utilizes a 2D array of EM coils for magbot movement in X and Y directions. Using the 2D EM coils coupled to a computer vision control algorithm, the pathway for movement is adaptable and programmable for the magbot (ProMagBot). While the EM coils provide movement in X and Y directions, the computer vision algorithm adds tracking redundancy to movement. Also, power consumption is minimized, and multiple extraction runs operate off one compact battery. Overall, the ProMagBot device is a highly streamlined setup capable of nucleic acid extraction. ProMagBot minimizes user interference, increases usability, broadens accessibility, expands convenience, and embraces accommodation.

MATERIALS & METHODS

Microfluidic Cartridge

Inside the microfluidic cartridge, there are four separate components. The three main chambers house the lysate mixed and loaded from the user, 80 (µL) of washing buffer, and 30 (µL) of elution buffer (Invitrogen ChargeSwitch Kit). The last remaining component is mineral oil (80 µL x2) that separates the chambers, sourced from Sigma Aldrich. The cartridge itself is composed of three stacked layers: base, channel spacer, and cover. These layers are 1/32”, 1/16”, and 1/32” thick polymethyl methacrylate (PMMA) purchased from E-Plastics.com and bonded together using a chemical solvent. These layers were designed using PTC Creo and then laser-cut using a VLS3.60DT machine from Universal Laser Systems.

ProMagBot device and EM control board

All components of the ProMagBot device mount within a 3D printed case made of ABS plastic sourced from MakerBot.com. The case, spacers, and viewing windows were modeled using PTC Creo. The magnetic robot is a permanent magnet, 0.25” in diameter, with a magnet density of N50. All electronic components such as the indication LEDs, pushbuttons, switches, or connectors were purchased from Digikey.com. The Zippy Compact battery and connectors to match were purchased from HobbyKing.com. The Raspberry Pi 4 and Pi Camera module v2 were purchased from Canakit.com. The EM PCB was custom designed using Autodesk Eagle and then fabricated by OshPark.com.

Computer Vision module

Computer vision and magnetic robot tracking were created using a Raspberry Pi camera module v2 paired with a Raspberry Pi 4. The module mounts 3.5 in. away from the magnetic robot and stage. The stage illuminates from light created by two 120-degree wide-angle LEDs inside a light guide (Digikey.com). The image capturing and magnetic robot detection automatically operates by a custom Python script on the Pi 4 using built-in and OpenCV libraries.

Data analysis method and statistics

All statistical analysis was computed using MATLAB R2020. Data processing was managed within MATLAB, with plots generated as well.

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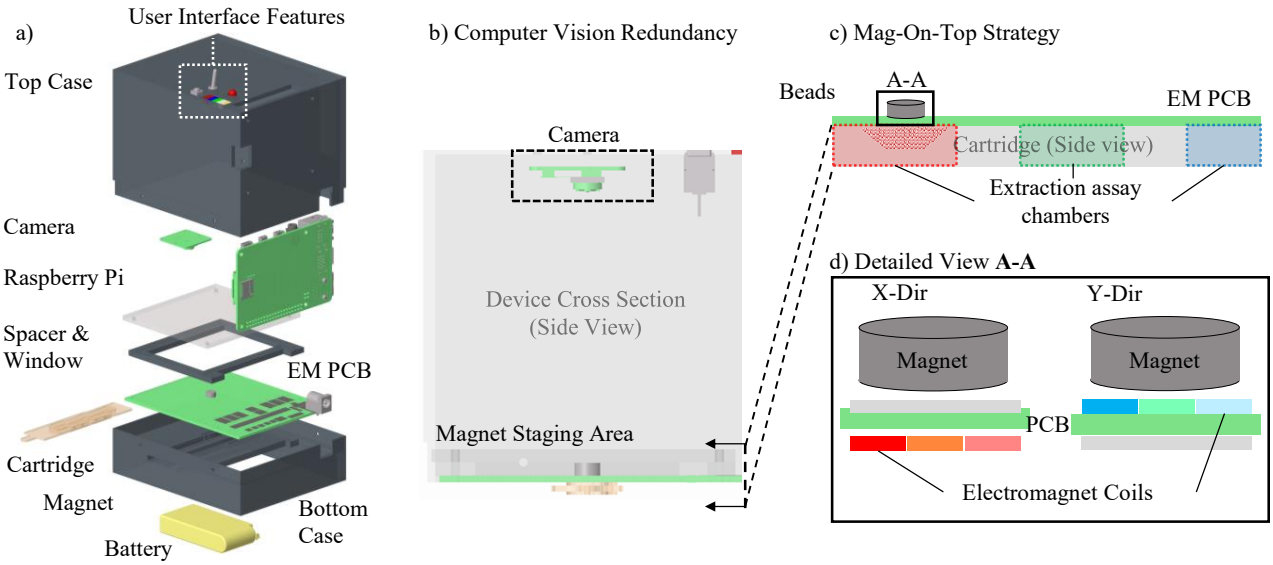

Figure 1. Modeling images of the overall device schematic and the associated internal subsections. a) 3D modeled view of the device highlight all internal components. b) device cross section that demonstrates the geometry setup for the computer vision module and staging area. c) Side view of the microfluidic cartridge to establish the physical relationship between the magnetic robot, PCB, and PMBs. d) Detailed view of the specific geometry of the EM PCB where coils are located on top and bottom of the board.

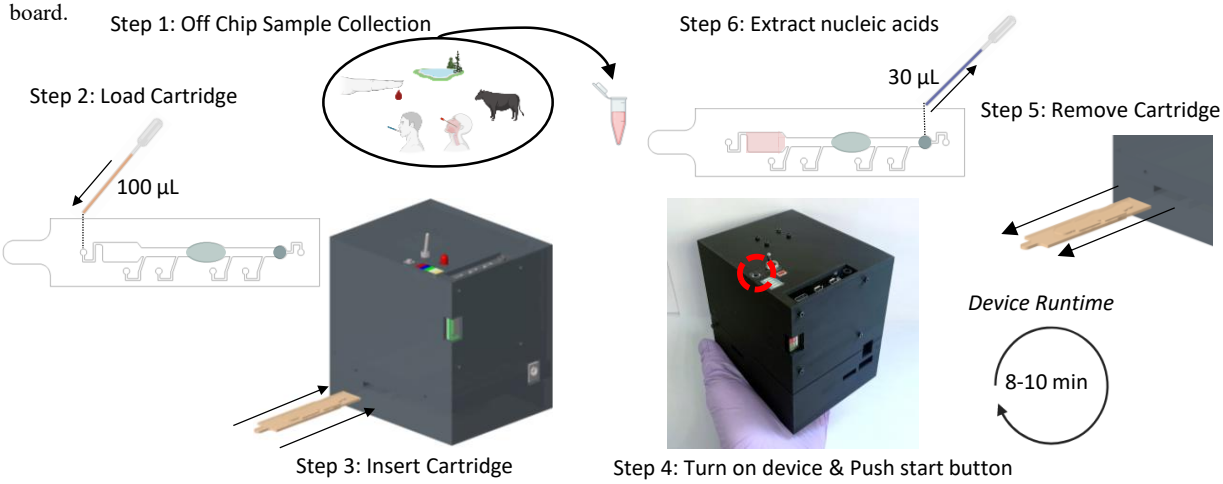

Figure 2. Device workflow from start to finish highlighting users input of sample collection to an output of eluted nucleic acids. Any manually required steps are straight forward and keep the device user-friendly.

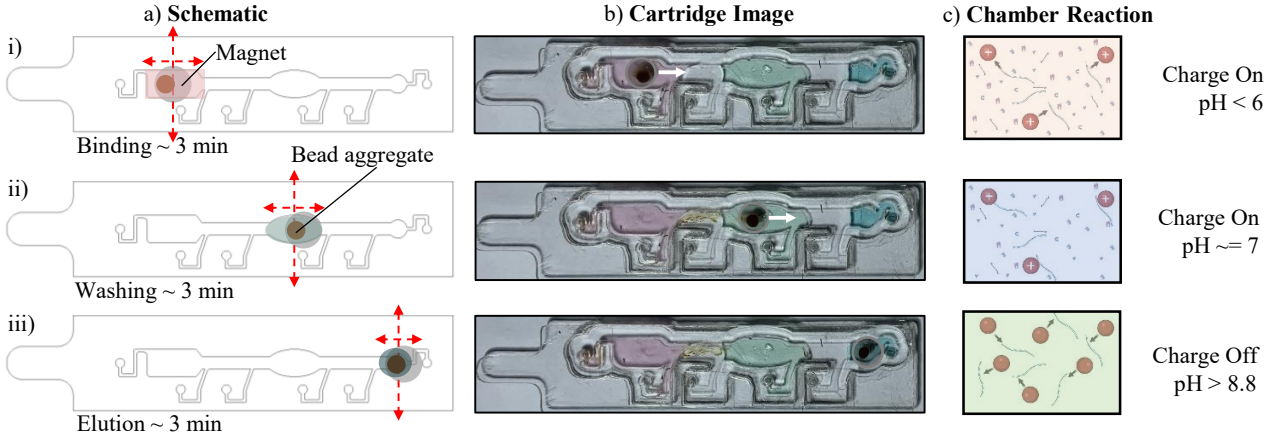

Figure 3. Schematic overview of the magnet pathway during microfluidic cartridge extraction. a) Illustrated pathway that the magnetic robot will follow during each mixing chamber. b) Captured images of the microfluidic cartridge with beads inside that are moved along by manual magnetic robot movement. c) Chemical reaction diagrams of the charge switchable beads. i, ii, and iii) correspond to the PMBs and magnetic robot aligned with the three solution chambers for lysing, washing, and elution.

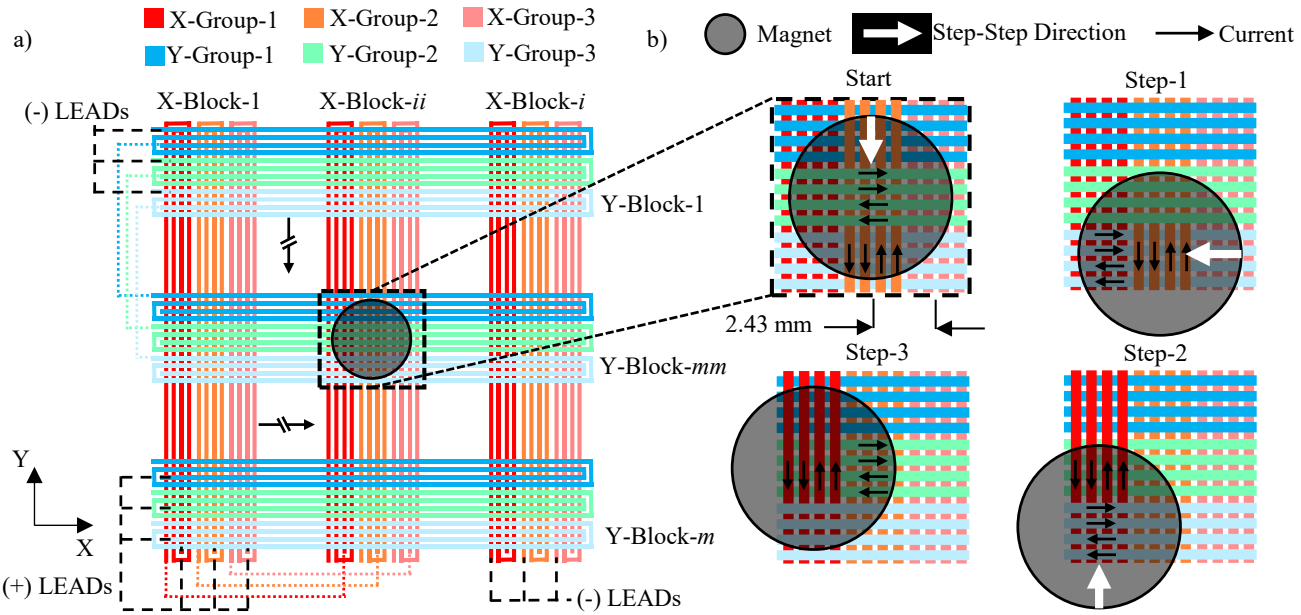

Figure 3. Overall schematic of the electromagnetic coils that appear on the ProMagBot PCB. a) Electromagnetic coil diagram highlighting the coil geometry on the PCB board. b) Enhanced view of the magnetic robot interaction with current induced coils. Shown from Start to Step-3, the magnetic robot can be moved up, down, left, right or vice versa.

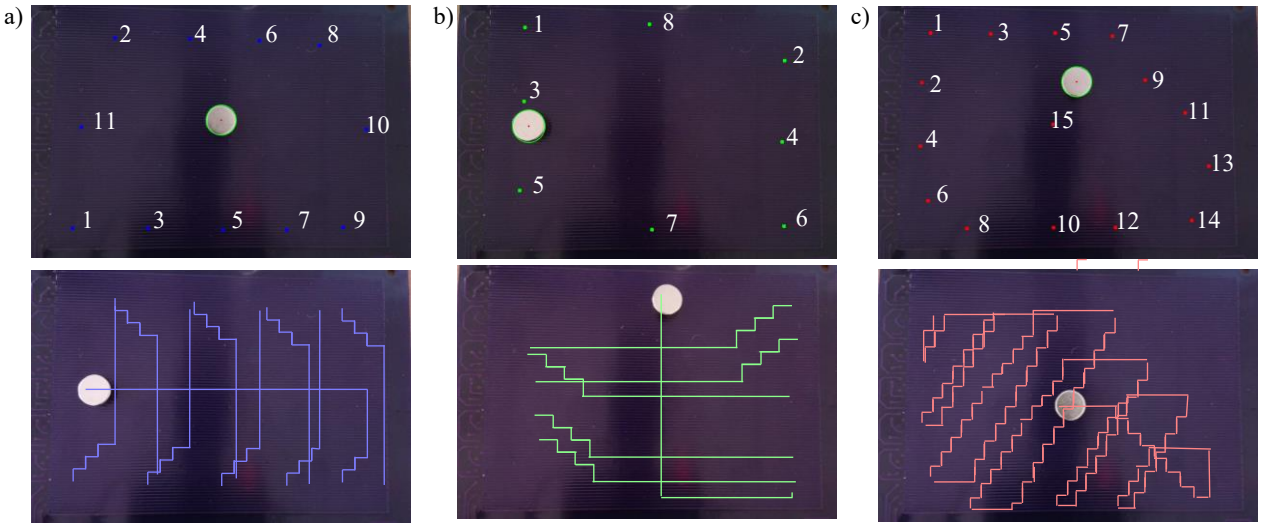

Figure 4. Pictures from device camera representing potential pathways for the magnetic robot to travel. a) ,b) and c) all show images of the magnetic robot traveling along various sinusoidal pathways.

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