

PurpleDrop: A Digital Microfluidics-Based Platform for Hybrid Molecular-Electronics Applications

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Abstract—Molecular manipulation and analysis are the cornerstone of life sciences. With the recent advances in molecular data storage and computing, it has become an increasingly exciting and viable alternative for the post-CMOS scaling era. Widespread use of molecular manipulation/analysis and data storage/computing requires a scalable and low-cost platform for hybrid molecular-electronics systems. This enables us to build on the best of what molecular and electronics systems can offer. In this article, we present PurpleDrop, a full-stack digital microfluidic platform for hybrid molecular-electronic systems in multiple domains, and focus on DNA data storage as a use case. We describe its design principles and relevant aspects such as closed-loop operation

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with computer vision and capacitive sensing, on-board magnetic bead extraction, and polymerase chain reaction, among other primitives. Importantly, we emphasize the ability to express and execute protocols and computation that include molecular and computational components.

THE SEMICONDUCTOR INDUSTRY has seen a profound trend in miniaturization driven by scientific and technological advancements that have enabled us to generate, manipulate, and store massive amounts of data. However, as we approach the physical limits of conventional storage and computing hardware, alternative approaches must be investigated.

DNA data storage systems are a prime example of molecule-based systems capable of achieving far greater information density (10^{18} bytes/mm³) and stability (thousands of years) than conventional magnetic, optical, and solid state storage technologies. DNA is also likely to have eternal format relevance, owing to interest in DNA for health and biotechnology applications.

Molecular systems uniquely require macro-level physical storage and manipulation, e.g., mixing, splitting, heating, or diluting various molecules in solution. At present, these operations are most commonly manually performed by humans in laboratories. Automation is key if we are to develop sophisticated and robust hybrid molecular-electronic systems¹ that combine the best features of electronic computing with the benefits of molecular density and stability.

We have developed a digital microfluidic (DMF) automation platform, *PurpleDrop*, in response to this need and demonstrated its potential in hybrid molecular-electronic systems. *PurpleDrop* aims to 1) minimize cost and hardware complexity, 2) maximize scalability, and 3) maximize modularity. Furthermore, *PurpleDrop* is designed with a larger system in mind: we have designed a complementary software stack to make the most of DMF's flexibility without sacrificing reliability.

In this article, we present the *PurpleDrop* platform and demonstrate its application in molecular computing. Using data storage as a case study, we first provide background on modern DNA data storage and present the need for future automation and scale. Building on

previous work demonstrating DNA data storage with microfluidic retrieval via *PurpleDrop*,² we now turn our attention to automating subsequent steps in the DNA data storage pipeline.

Finally, we provide a detailed description of the *PurpleDrop* platform and explain the functional considerations that informed its design.

HYBRID MOLECULAR-ELECTRONIC SYSTEMS

Electronic systems are well understood, and can perform a wide variety of tasks in extremely specific and controllable ways. Molecular systems, however, offer remarkable advantages in performance and energy efficiency.

In the molecular domain, computation occurs in the same solution in which data-containing molecules reside, making it possible to perform

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many molecular computational processes in parallel. Performing an operation on a few molecules takes the same amount of time as for trillions of molecules,

with the caveat that some operations could take hours to complete. Given these qualities, molecular systems are particularly well suited for applications with large data sets that can handle the high latency that comes along with the high throughput.

In the case of DNA data storage, combining the extreme density and parallelism of the molecular domain with the precision of electronic systems could yield a more efficient and robust system than either domain in isolation.

Archival DNA Data Storage

Figure 1 shows a DNA data storage system. Such systems encode binary data into DNA sequences of nucleotides (A, C, G, and T) that are

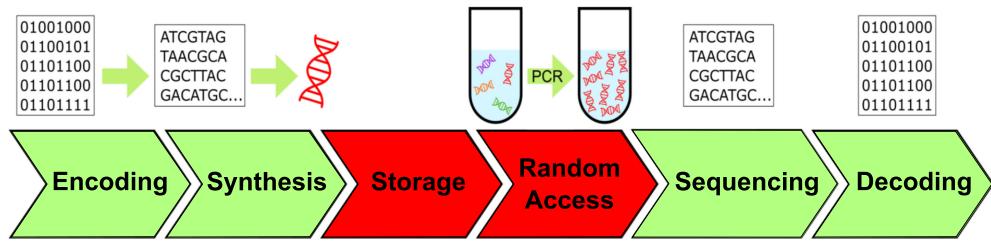


Figure 1. DNA data storage workflow. Digital data is encoded into a nucleotide sequence used to synthesize DNA “files.” DNA files can be stored in pools containing many files if they are tagged with unique identifiers (corresponding to PCR primers). To recover the original digital data, random access selectively retrieves and amplifies only the file(s) of interest from the pool. The selected files are then sequenced and decoded. A notable gap between synthesis and sequencing exists, and automation of this workflow requires an efficient solution for the physical storage and retrieval of DNA data.

translated into manufactured molecules (or oligonucleotides) by a chemical process and stored in molecular pools. To recover the stored information, the DNA molecules must be sequenced (read) and decoded back into digital data. Previous work in DNA data storage has covered synthesis, encoding and decoding, DNA preservation, random access protocols, and sequencing technologies.

However, achieving high density data storage in practice will require an automated mechanism for physical storage, organization, and retrieval of files in molecular form, a need illustrated by the gap in the modern DNA data storage pipeline depicted in Figure 1.

Physical Storage and Random Access

The manner in which DNA data is physically stored in a system is an essential consideration in how it is retrieved. DNA data must be in solution for sample preparation and sequencing steps, but storing DNA in liquid form requires separate vessels that reduce information density. DNA can be easily isolated and more densely stored when dehydrated, and multiple file pools can be stored in close proximity if dehydrated onto a substrate such as glass.²

To “write” DNA files to a storage substrate, samples must be deposited in a spatially isolated arrangement, treated with preservatives, and dehydrated onto the surface for indefinite storage. The “read” process involves rehydrating a sample with water, performing random access via polymerase chain reaction (PCR) (or other molecular methods), and preparing the sample

for sequencing via PCR and other heat dependent operations.

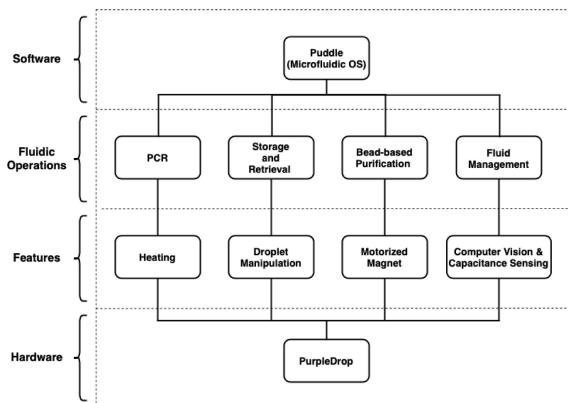
Random access allows specific data to be retrieved without wastefully sequencing an entire pool of data. DNA files are tagged during synthesis with unique addresses (short strands of DNA corresponding to specific files) that enable retrieval of only those strands. Larger addresses use more nucleotides in the strand, but allow more data to be stored in a pool.

Storing DNA in physically isolated pools enables information density gains because physically isolated pools can share the same molecular address space. This leads to a hierarchical addressing scheme where the address of a file encodes both the physical location of the pool and molecular address within that pool.²

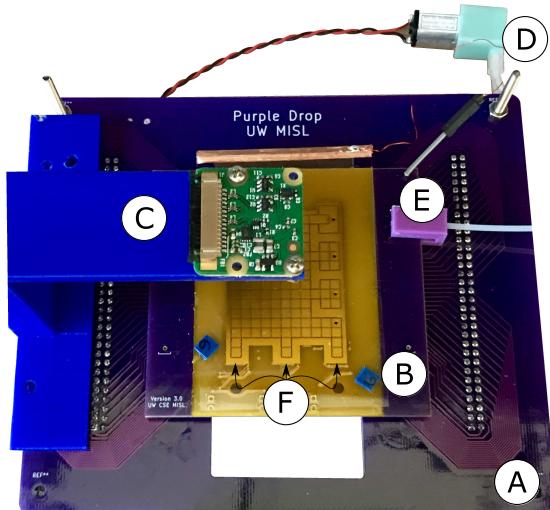
Molecular Random Access Methods

Random access can be implemented in various ways in molecular systems.

PCR is widely used in molecular biology to rapidly make many copies of specific DNA samples in solution. It involves mixing a number of reagents, including enzymes, small sequences of single-stranded DNA called primers, individual nucleotides, and the DNA to be copied, and controlling their temperature, typically done by an instrument called a thermocycler. When the temperature is raised, DNA “melts,” and the two sides of its double helix come apart. As the solution cools, primers attach to the single-stranded sides of the DNA, creating double-stranded sections of DNA. This enables enzymes to also attach at the double-stranded to single-stranded



(a)



(b)

Figure 2. (a) PurpleDrop's modular features enable fluidic operations essential to hybrid molecular-electronic systems such as automated DNA data storage. (PurpleDrop in a hybrid molecular-electronic system.) (b) PurpleDrop is comprised of two main PCBs: a parent board that houses electronic components (A), and a removable child board that contains the electrode surface (B). Peripheral hardware systems include three on-board heaters (F), a computer vision system consisting of a camera on 3-D printed mount (C), and a fluid input-output system powered by miniature peristaltic pumps (D) that pump fluids onto and off the board via capillary tube ports (E). A motorized magnet (not pictured) is mounted on the underside of the electrode board. (The PurpleDrop DMF device.)

transitions and perform a copy of the DNA by adding complementary individual nucleotides as it traverses the molecule. In DNA data storage systems, molecules representing a file are tagged with a unique primer target site (address). *PCR-based random access* selects specific DNA files from a pool (molecular random access) using the unique primers associated to those files, after the pool is physically retrieved from a library containing multiple DNA pools (physical coordinate random access).

Separation-based purification methods such as magnetic bead wash can also be used to accomplish random access. In this modality, magnetic beads coated with DNA strands that selectively bind to a specific file in the pool are used to extract files via separation and washing. Purified samples are amplified via PCR and sequenced.

At present, retrieval, purification, and amplification steps are typically performed by humans and expensive, bulky thermocyclers. Streamlined, scalable DNA data storage will require fluid handling solutions for integrated molecular processing steps such as file retrieval and PCR.

PURPLEDROP: A SIMPLE, FLEXIBLE DMF DEVICE

Microfluidic technology has potential applications in a broad range of areas such as synthetic biology, medical diagnostics, automated experimentation, and molecular computing. PurpleDrop was inspired by its DNA data storage use case, but designed with a greater vision in mind: a full-stack microfluidic platform that is cost effective, reliable, and capable enough to be the foundation of scalable computer systems with molecular components.

The PurpleDrop hardware, shown in Figure 2(b), and complementary software stack, Puddle,³ manage design tradeoffs by keeping hardware costs low and compensating with sophisticated software. PurpleDrop is comprised of consumer materials, and can be assembled outside of a clean room, with an assembled form factor of approximately 110 × 140 × 100 mm.

PurpleDrop's modular features, depicted in Figure 2(a), can be combined in numerous ways to enable operations such as those required for DNA data storage. Together, PurpleDrop and

Puddle provide a platform that could allow users to easily combine computation and fluidic operations to implement hybrid molecular-electronic systems.

Microfluidics Background

Microfluidic devices can perform chemical and biological protocols at smaller scales, lower cost, and higher precision than humans.^{4,5} These devices all center around the manipulation of small fluid volumes, but different approaches offer different tradeoffs among size, cost, flexibility, and reliability.

Channel-based devices are manufactured ahead-of-time as a set of channels designed to meet the needs of a particular protocol. These devices can offer some flexibility by including configurable valves,^{6,7} but their design is largely fixed-function. On the other hand, liquid handling robots feature mechanical arms or gantries that hold pipettes. The robots can be programmatically controlled, offering a great deal of flexibility, but they are large and expensive, costing from many thousands to hundreds of thousands of dollars.

A more recent technology, DMF moves droplets around a grid of electrodes using a phenomenon called electrowetting on dielectric (EWOD).^{4,5} In EWOD DMF, droplets sit on a substrate material that is patterned with electrodes, insulated by a dielectric layer, and coated with a hydrophobic layer. The dielectric layer prevents electrolysis of droplets and the hydrophobic layer reduces the surface energy in contact with the droplets, making them easier to move. Some devices have a second plate with a hydrophobic coating added on top, effectively sandwiching the droplets between the two plates. The top plate typically consists of a continuous ground electrode made from conductive, transparent indium tin oxide glass.

Applying voltages to electrodes changes the wettability of droplets on the surface. To move a droplet, an electrode adjacent to it is activated while the electrode directly beneath it is deactivated, generating an electrowetting force that pulls the droplet onto the active electrode. This process is repeated to move droplets along any path of adjacent electrodes.

DMF devices can be highly programmable, offering flexibility at small size and low cost. These features are highly desirable features in the molecular computing space, where automation and parallelization are essential.

Key PurpleDrop Features and Operations

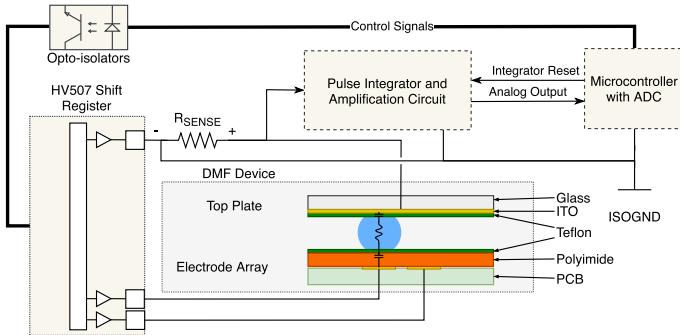
PurpleDrop is designed to support several operations required by automated DNA data storage while navigating tradeoffs among cost, reliability, and scalability.

Moving Droplets Electrode substrates are commonly made from silicon or glass making them smooth and level, but requiring costly clean room processes. Printed circuit boards (PCBs) can also be used as cheaper, more durable substrates, sacrificing some smoothness.⁸ PCBs can also accommodate multiple electrical wiring layers, increasing addressability, while glass and silicon substrates (outside of expensive integrated circuit-style fabrication) are typically limited to one layer with conduction lines patterned onto the surface.

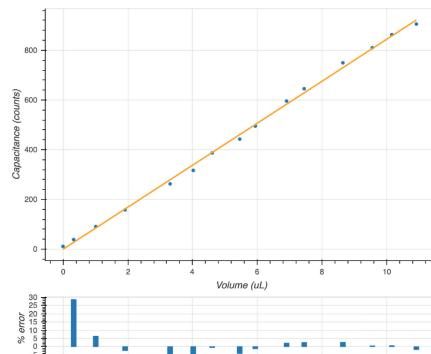
PurpleDrop follows OpenDrop's⁹ design with a removable "child" electrode board that consists of a two layer PCB with an array of 127 electrodes. The parent PCB contains the electrical components. Child electrode boards can be easily recoated or replaced upon wear or contamination with minimal cost and effort.

The dielectric and hydrophobic layers have similarly significant influence over performance. PurpleDrop's electrode PCB is coated with a 25.4- μm -thick polyimide tape dielectric layer (Caplinq, PIT0.5S-UT/100), with a top coat of Teflon AF 1600 (spin-coated at 98 xg for 60 s, DuPont/Chemours) for hydrophobicity. We chose polyimide tape over more traditional DMF dielectric materials such as Parylene due to its advantages in cost, dielectric strength, durability, and fabrication complexity. Polyimide tape is inexpensive, costing about \$0.07 per electrode board before scale. In contrast, Parylene C costs approximately \$100 per board.

Furthermore, in previous design iterations using Parylene C (5 μm applied via vapor deposition), we faced significant challenges with the gaps between electrodes interfering with droplet movement. The copper electrodes are patterned



(a)



(b)

Figure 3. (a) Block diagram of capacitance sensing circuitry: An analog integrator circuit and microcontroller are used to measure the total charge transferred while selected electrodes are charged and (b) the measured capacitance plotted for varying droplet sizes.

with 1oz of copper, which corresponds to a height of approximately 1.4 mil above the FR4. Droplets frequently snagged on electrode edges when transitioning between electrodes as a result, resulting in droplet fragmentation/immobilization. In contrast to conformal coatings like Parylene, the polyimide tape forms a relatively level surface that covers the gaps in between electrodes and allows droplets to smoothly transition between electrodes.

Fluid Management We have implemented computer vision and capacitance sensing subsystems that provide dynamic droplet tracking, volume detection, and error correction to enhance reliability.

Volume sensing and droplet tracking provide critical information about the number and size of droplets on the board. This allows a higher level system like Puddle to perform fluid resource management by automatically placing and routing droplets. Furthermore, these features enable dynamic error correction where the system validates that the operations occurring on the board match those executed by the software.

The computer vision system³ consists of a Raspberry Pi camera on a 3-D-printed mount. Droplets are detected based on color.

The presence of a conductive fluid between an electrode and the top-plate increases the capacitance between the two conductors, with the amount of the increase depending on the area covered by the fluid. The capacitance sensing system measures this capacitance to detect the

presence of a droplet and measure its volume. The PurpleDrop electrodes and top-plate are switched between the high voltage supply (V_{HV}) and 0 V at 500 Hz to generate an ac actuation voltage. During each cycle, the total charge transfer is measured while the active electrodes are driven from 0 V to V_{HV} , by integrating the voltage across a sense resistor connected between the top-plate and its driver. This provides capacitance measurement at a rate of 500 Hz, which is significantly faster than a typical camera and image processing. Figure 3(a) shows a diagram of the capacitance measurement system.

In order to measure the relationship of capacitance to drop volume, a drop of water was placed on PurpleDrop and slowly enlarged by adding approximately 0.5 μ L at a time. At each step, a camera was used to measure the area covered by the drop, and capacitance was recorded. Volume was calculated using the known height of the gap between the top-plate and the electrode board. Results for this test are shown in Figure 3(b).

Capacitance sensing has several advantages over the computer vision system: lower latency, lower computational load, and robustness to lighting changes. Using capacitance sensing in conjunction with computer vision could lead to more robust and precise sensing than either system alone.

Polymerase Chain Reaction Filler fluids such as silicone oil are commonly used in two-plate DMF devices to reduce droplet resistance,

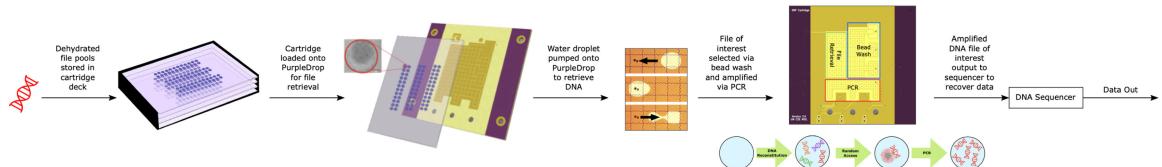


Figure 4. DNA data storage pipeline with microfluidic storage, retrieval, and sequencing preparation. Digital data is encoded into nucleotide sequences and synthesized into DNA. DNA files are stored in spatially isolated pools of dehydrated DNA, organized into a “library” of pools. Each library is contained on a glass cartridge with a unique ID, and these cartridges are stored in a deck. An address system mapping files to specific libraries, pools, and PCR primers is used to locate files. Cartridges are loaded onto PurpleDrop for microfluidic file retrieval with magnetic-bead-based random access. Retrieved files are amplified via PCR and output to a sequencer for data recovery.

lower the driving voltage requirements, and slow evaporation;⁴ however, fluid media complicate certain operations. Inputting or removing droplets from the device is much simpler in an air medium, where introducing unwanted air bubbles is not a concern. We found that using an air medium was also advantageous for thermocycling protocols like PCR. When using a silicone oil medium (Clearco PSF-2cSt), we observed bubbles beginning to form around droplets at the heating site around 80°C, with significant bubbling and fragmentation of the droplet above 90°C. This made it impossible to move droplets away from the heaters for the remaining steps in the protocol. We decided instead to operate in air and utilize the computer vision and fluid input/output systems to provide automated droplet replenishment.³

PurpleDrop’s fluid input/output system is driven by small peristaltic pumps (Takasago Fluidic Systems), each fitted with a capillary tube that serves as the interface between the pump and the electrode surface. The other port of the pump is fitted with tubing that can be coupled to arbitrary reservoirs or devices.

PurpleDrop has three heaters used for thermocycling, and compensates for the increased droplet evaporation in air by using the computer vision system (capacitance sensing could also be used) to monitor droplet volumes and provide real-time feedback to Puddle. When droplets fall below a threshold volume, a replenishment water droplet is pumped onto the board and carried to the reaction site. This process could extend the number of cycles of PCR possible indefinitely.

Bead Extraction The magnetic-bead-based random access implementation discussed earlier is well matched with PurpleDrop’s DNA data storage architecture and hardware. PurpleDrop is equipped with a motorized magnet that facilitates magnetic bead wash. A small servo motor mounted near the underside of the electrode board moves a magnet underneath droplets containing magnetic beads. Once in place, the magnet causes the beads to aggregate at the bottom of the droplet. With the magnet held in place, the supernatant droplet (i.e., the fluid without the beads being held by the magnet) is moved away via electrowetting until complete separation from the pellet [see Figure 6(a)]. After separation, washing and resuspension steps complete the purification process.

Implementing Hybrid Molecular-Electronic Primitives With PurpleDrop

To demonstrate how PurpleDrop can automate DNA data storage and random access, we implemented several key operations: file storage and retrieval in a DNA library, magnetic-bead-based random access for file selection from a pool, and file amplification for sequencing preparation.

DNA Data Storage and Retrieval We previously evaluated PurpleDrop as a file retrieval mechanism in a high density DNA data storage library.² Libraries consist of dehydrated pools of DNA files organized in arrays on the droplet-facing sides of PurpleDrop’s glass top plates, referred to as cartridges when containing DNA data (see Figure 4). Water droplets are used to retrieve and prepare files for sequencing, as depicted in Figure 5.

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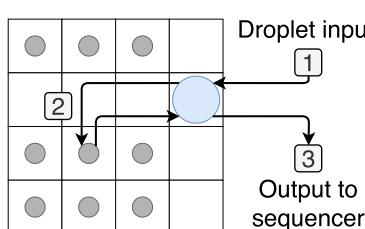
def dna_lookup(key):
    spot = SPOTS[key]
    d = input(...)
    d.mix_at(spot)
    sleep(60 * seconds)

    output("sequencer", d)

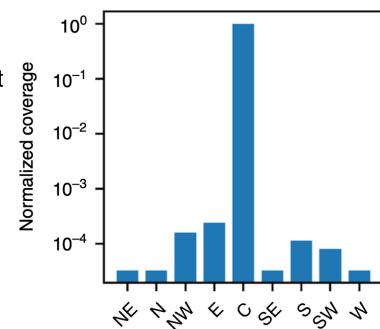
    data = get_data()
    seq = process(data)
    return seq

```

(a)



(b)



(c)

Figure 5. Puddle code snippet (a) and associated diagram (b) depicting DNA file location and retrieval via PurpleDrop. File locations are given in cardinal coordinates with C referring to the central file in the configuration shown. A key maps each file to a physical location in library and a PCR primer. Retrieved files can be output directly to a sequencer. (c) Sequencing results from the work by Amin *et al.*² show successful recovery of the retrieved central file. (a) Puddle retrieval code. (b) DNA file retrieval. (c) Sequencing coverage of each discovered file in cardinal coordinates (log scale).

Using this architecture, we demonstrated that it is possible to store dehydrated files in DNA on PurpleDrop (with an estimated storage density of 50 TB per 50- × 50-mm cartridge), and successfully retrieve and recover the files without contamination. This storage scheme allows for

physical isolation, enabling pools to share the same addressing space and increasing the storage capacity of the system. Microfluidic retrieval provides the needed actuation for accessing and transporting data, with the added benefit of introducing near-data processing opportunities.

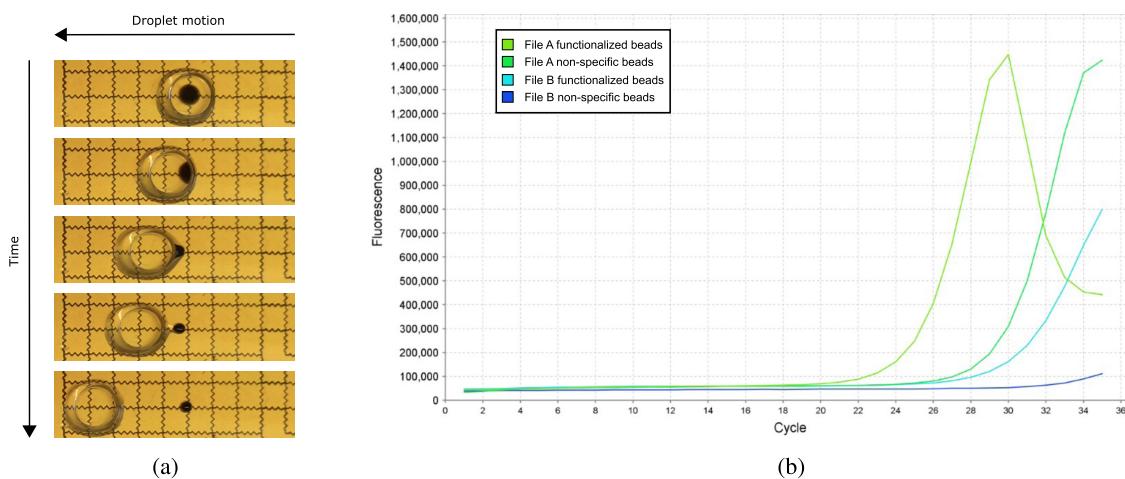


Figure 6. (a) Motorized magnet is moved underneath the electrode board and positioned under the droplet containing DNA and functionalized magnetic beads. The magnetic beads are pulled out of solution with specific DNA attached and the supernatant droplet is moved away. (b) Retrieval with file A-specific beads results in qPCR amplification of File A that takes place in fewer cycles than for file B and the control groups. We attribute the latter to primer-dimer formation during qPCR. PCR amplification on PurpleDrop could be calibrated to amplify only file A by limiting the number of cycles to 30, i.e., after amplification of file A and before amplification of file B. (a) Magnetic bead wash on PurpleDrop. (b) Quantitative PCR (qPCR) amplification for magnetic-bead-based random access.

For DNA data storage to become a reality outside of the research laboratory, scalable physical storage with automated file retrieval, as well as automated file preparation for sequencing are imperative. We envision PurpleDrop filling the gap in the data pipeline between DNA synthesis and sequencing, as illustrated in Figure 1. In addition to file retrieval, random access, PCR, and other sequencing preparation steps are promising candidates for DMF automation.

Magnetic-Bead-Based Random Access

To demonstrate magnetic-bead-based random access on PurpleDrop, we synthesized a pool containing two unique DNA files, files A and B, in equal concentrations. Magnetic beads (Invitrogen, 65601) functionalized with (i.e., attached to) primers for file A were synthesized to selectively extract file A from the pool containing both files via bead wash. Control beads functionalized with a dummy primer (i.e., coated with a primer not found in the pool to reduce nonspecific binding that can occur with uncoated beads) were used to confirm selectivity.

All reagents were loaded onto the electrode board, and the magnetic beads were mixed and incubated with the file pool. The magnet was then used to extract the beads from the droplet as described in the “Key PurpleDrop Features and Operations” section. Following separation, multiple washing steps with 80% ethanol were performed to remove any unbound molecules from the pelleted beads. Finally, resuspension buffer was used to elute and output the purified sample. Quantitative PCR (qPCR) was used to assess random access performance [see Figure 6 (b)]. qPCR of the PurpleDrop-purified sample showed amplification of file A, with some amplification of file B appearing to occur multiple cycles later. We attribute the latter to primer-dimer formation during qPCR. Primer-dimers are common PCR byproducts that form when primers attach to each other and undergo enzymatic strand extension. These strands are generally much

shorter than file strands, and can be detected by measuring the strand lengths of the qPCR reaction products. The control bead experiments showed comparable late-cycle amplification of both files, which confirms that no file selection took place. These results suggest that PurpleDrop could be used to locate and selectively retrieve files stored in high density libraries.

PCR PurpleDrop is well positioned to automate protocols such as PCR at miniaturized scales that reduce reagent consumption, equipment

cost, and human involvement. Using the automated replenishment approach described in “Key PurpleDrop Features and Operations” section, we demonstrated the first fully automated execution of PCR with replenishment in air.³ We have also demonstrated basic sequencing preparation steps on PurpleDrop, with automated output to a sequencer through the fluidic input/output system.³ PurpleDrop’s versatile features enable a diverse set of fluidic operations that can be combined to implement automated DNA storage and retrieval in a low-cost, scalable, and efficient way.

DISCUSSION

We have presented PurpleDrop, a DMF platform designed with hybrid-molecular systems in mind. PurpleDrop provides necessary features for implementing these systems, i.e., storing, retrieving, and manipulating DNA, and integrates with computing systems that can provide higher level control. We expect this combination of programmability and biological primitives to be critical for future hybrid-molecular systems.

While this work focused on data storage, we have designed PurpleDrop to capture the flexibility and accessibility of DMF technology in a general way. Combined with future work, PurpleDrop and other DMF devices could impact a diverse range of domains from diagnostics to high throughput experimentation.

ACKNOWLEDGMENTS

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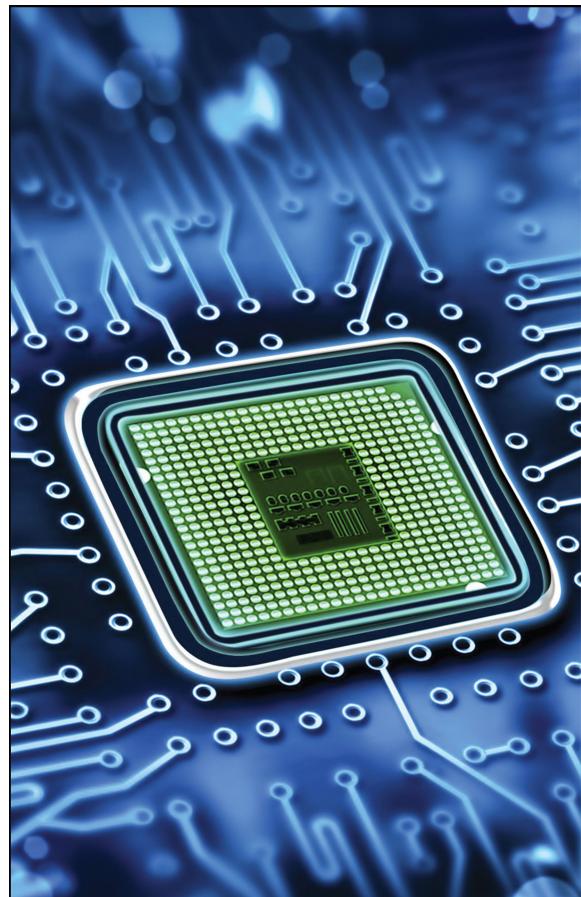
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