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Advancements and Challenges in Digital Microfluidic Biochip Applications: A Comprehensive Survey

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Abstract

Early 1990s, the field of Lab-on-Chip (LoC) has undergone a significant amount of development. The primary goal is to build full biological and chemical laboratories on the surface of silicon or another polymer chip which is driven by technological applications. The latest version of lab-on-a-chip is Digital Micro Fluidic Biochips (DMFB) could run complicated biochemical lab assays (bioprotocols) on a tiny device that fluid samples can be automatically and analyzed with high precision. The DMFB performs various operations on the solution using electrical actuation. These operations include mixing, separating, merging, and washing. Thus, Sample preparation plays a vital role in the DMFB. The process of creating solutions with a desired volume that has been predetermined is known as sample preparation. In most cases, this is accomplished by carrying out a series of activities involving the mixing of several chemical solutions with a specific ratio of volume to total volume. DMFB used these methods for healthcare diagnosis, DNA testing, drug creation, gene sequencing, and more. To test, it only needs a small number of samples and chemicals. The time it took to get the results was 10 times faster than in normal labs. Many algorithms and techniques have been proposed in earlier research. This paper provides a comprehensive analysis of various algorithmic challenges encountered during the implementation of biochemical protocols on a DMFB. We will pay specific attention to a few sample preparations techniques, such as mixing trees, scheduling algorithms, and waste awareness.

1. Introduction

Throughout the course of human history, infectious illnesses with pandemic potential have repeatedly emerged and spread, posing several problems for public health [1]. The only way to prevent the virus from being passed on to humans is to develop improved ways of detecting and identifying illnesses, which should be made available to the general public for reasons of public safety [2]. Mobile testing, also called Point-Of-Care Testing, is a simple test. It gives quick medical diagnostic information where the patient is being treated. This kind of testing is really important for many reasons, like preventing diseases, avoiding food poisoning, & monitoring health in hospitals or clinics. In traditional labs, preparing samples can take a lot of time and requires complex tools. This adds to the hassle. On the other hand, some tests involve expensive reagents and may take blood from newborns. Not to mention collecting DNA from crime scenes too. When using the traditional approach, more reagents are required, and more samples are used for each individual test. so, they failed to analyze the disease with few samples. Thus Lab-on-Chip (LoC) has been established with low-cost and high-throughput solution. [3].

An emerging field of interdisciplinary study known as "Lab-on-Chip (LoC)" aims to reduce the rising healthcare expenses associated with the rapid diagnosis of several diseases, such as cancer, DNA analysis, HIV, and so on. A LoC typically implements a bioprotocol on a single chip with a surface area of a few square centimeters, offering quick and inexpensive diagnostic solutions for a range of medical applications [4]**.** An array of tiny test sites

(microarrays) arranged in a specific pattern on a solid substrate makes up a biochip. For greater throughput and speed, this enables a number of tests to be run concurrently. By simultaneously evaluating a panel of pertinent tests in a single sample, the biochip is used to build a patient profile. Success in this new area will depend on the development of algorithmic micro fluidics and design automation tools specific to LoCs.

Micro fluidics refers to the study and application of small fluid quantities. Several types of micro fluidic-based biochips have been existing in recent years. These are: 1. A paper-based microfluidics device is a kind of microfluidics made from paper or similar porous materials. It helps in handling tiny volumes of fluids using capillary action. This method dates back to litmus paper, which has served as a valuable platform for various analytical experiments [5,6]. These devices offer several benefits. They don't require pumps or extra equipment to work. Moreover, they are generally cheaper to produce and easier to dispose of compared to metal and plastic alternatives [7]. One notable application of these devices is urinalysis. They can measure protein and glucose levels in urine through colorimetric assays [8]. However, not everything about paper-based microfluidic devices is perfect. One limitation involves the uneven concentrations of reagents or mixed results in the test areas. Additionally, the shelf life of reagents on these devices is quite short. When stored on paper media, many kinds of reagents, including enzymes, tend to lose their biological activity fairly quickly. This decline can significantly impact the precision and accuracy of the tests [7]. 2. Continuous Flow Micro Fluidic Biochip (CFMB) is the flow without disrupting continuity; micro fluidics is the control of liquid flow through artificially created micro channels. Fluid flow is created by internal processes like electric, magnetic, or capillary forces as well as external sources like micro pumps (such peristaltic or syringe pumps).[9] The consumption of reagents and samples can be decreased to nano liter levels as compared to conventional laboratory equipment and methods, considerably lowering the cost of the experiment [10]. As a result, CFMBs have been extensively used in a variety of biochemical experiments, including DNA analysis, pharmacological sample preparation, clinical diagnosis, and cell culture [11]. In these CFMB, the flow of liquid is managed by micro-pumps, micro-valves, electro-kinetics, and electro-osmosis. However, the variety and practicability of these biochips are limited due to the presence of channels that are permanently engraved and sophisticated external support [12]. 3. One of the most innovative technologies is a fully software-Programmable Micro fluidic Device (PMD). Assays can be implemented using simple software programmes, and PMDs are made to be used in a variety of situations. Each chamber in PMDs is encircled by four valves, and the generic design of these devices is made up of a network of intersecting channels that are each independently controlled by a valve. Additionally, the channel network model can be viewed as a mesh. [13] Adaptive enzymatic assays, fixed-pH reactions, automatic optimization of PCR settings, feedback-driven directed evolution, and intricate procedure sequences are examples of applications that can benefit from software-programmable micro fluidic devices [14]. 4. On the other hand, Digital Micro Fluidic Biochips (DMFB) utilizes electrical actuation to control discrete droplets of nano liter-sized reactant fluids on a two-dimensional electrode array (dispensing, navigation, merging, mixing, splitting, washing, and sensing). DMFB have recently achieved widespread use in the development of Loc applications.[15]

DMFBs are excellent for flash chemistry applications since rapid responses are seen when using volumes at the nano liter scale. Digital Micro Fluidic Biochips are excellent choices for point-of-care and near-patient testing due to the reduced size of DMFBs, which also contributes to their portability. It has the potential to be an effective tool for molecular point-of-care diagnostics [16]. DMFB has demonstrated its efficacy in a wide variety of biochemical applications, including protein crystallization, DNA sequencing, advanced diagnostics and medications, clinical diagnostics, chemical synthesis, and biological analysis.

Currently available DMFBs are: it was recommended to use a DMFB to find Plasmodium parasites (Malaria) in human blood. The recommended chip's architecture is particularly suitable to recognize malaria. The advantages of a miniaturized device include portability, ease of use & faster detection compared to traditional microscopy methods [17]. For example, there is a biochip can effectively identify glucose levels in the bloodstream. This makes it very useful for people with diabetes. By using this biochip, it can replace the old method of glucose measurement that relies on a spectrophotometer due to its rapid analysis time (less than 60 seconds) & fewer reagents needed [18]. Moreover, biochips can detect trinitrotoluene (TNT) toxicity in water and find explosives in both water & soil [19]. Also, DMFBs have shown their usefulness in applications related to genetic engineering.

Many biochips are proposed for use in the polymerase chain reaction (PCR) [20], which plays an important role in modern biology by amplifying DNA fragments. Tissue engineering and drug discovery are two of the other growing uses for biochips [21]. Screening is a method used to check infants for genetic disorders that, if not addressed promptly after birth, might cause irreparable organ damage. A DMFB only needs a small portion of the sample and reagent amounts required by traditional procedures to screen for Pompe and Fabry disorders [22]. The time to result was 10 times quicker than when using normal methods since the incubation period was cut down to less than 2 hours [23].

The following outlines the structure of this review paper: Section 2 provides a brief overview of current techniques that utilize the Mixing Tree for automated sample preparation workflows. Alongside, this section discusses suitable scheduling algorithms, plus it also highlights strategies for managing waste droplets effectively. Moving on to Section 3, this part details the detection methods that are accessible for DMFB. Finally, Section 4 wraps things up with some concluding remarks.

2. Methods for Sample preparation

The process of mixing two or more biological fluid reagents in specific ratios is called sample preparation. It's a key step needed in many bioprotocols. This step combines fluids while considering their concentration factors (CFs). Sample preparation involves phases like dilution & mixing. These phases are now often automated and integrated on-chip. This helps achieve high-throughput applications, allowing for a variety of solution concentrations. Lately, there have been several reports discussing various algorithms for the automatic dilution & mixing of fluids in the literature.

2.1. Mixing Algorithm

Optimizing sample preparation involves three key factors: the use of essential reactants, the total number of dilution steps, & the amount of waste generated. It's important to consider expensive reagents and valuable materials. For instance, tiny amounts of bio samples like a baby's blood or DNA from a crime scene can significantly affect overall costs. Additionally, the number of dilution procedures usually correlates with the time taken for sample prep. When time is limited, especially in urgent clinical scenarios, a lengthy preparation process can be quite problematic. Each moment counts in emergencies. Finally, a large volume of trash makes it extremely difficult to treat waste in subsequent steps. Due to the limited number of waste reservoirs that can be found on a DMFB, this might also increase the preparation time. So, when preparing samples, it is certainly essential to take into account the three minimization objectives listed above [36].

2.2. Mixing Tree

The BioStream protocol language and the Fluidic ISA are two novel abstraction layers for micro fluidic biocomputers that were presented by William Thiesis et al. All hardware supporting a certain fluidic ISA can use the protocols described in BioStream. They construct two essentially different micro fluidic devices that enable the execution of the identical BioStream code to show this portability [9]. A mixing tree with longer dilution or mixing sub trees and fewer numbers of different leaf nodes is what Sudip Roy et al. presented as an algorithm for automatically preparing solutions of multiple fluids. When compared to the bit scanning approach, this decreases the time it takes for droplets to move from various reservoirs at the chip boundary to the on-chip mixers. Additionally, this approach lowers the possibility of cross-contamination between the droplet routes of various fluids, which lowers the expense and energy required for the control of wash droplets [37]. A reagent-saving mixing technique for biological samples with various targets was suggested by Yi Ling Hiesh et al. The first mixing algorithm of its sort with the goal of reducing reagent consumption was proposed. The simultaneous preparation of several target concentrations was also ensured. It decreases the time required to prepare samples as well as the quantity of reagent and waste droplets [19]. Srijan Kumar and colleagues introduce a mixing algorithm that minimizes the size of the mixing tree by removing common subtrees under permutation at the same level. This innovative method stands out when compared to current techniques. It successfully decreases the total number of mix-split stages, reduces waste droplets, & minimizes the number of mixer modules required for completing tasks efficiently [19]. In another study, Chia-Hung Liu and his team present a new method called

Common Dilution Operation Sharing (CODOS). The goal here is to cut down on costs or the amount of reactant needed for preparing samples with many reactants. It starts by creating a recipe matrix tailored to the desired concentration. Then, it finds & merges the rectangles that offer the most significant savings in reactants through an iterative process. Notably, CODOS uniquely addresses cost minimization by considering each reactant's price individually [38].

2.3. Scheduling Algorithm

To schedule operations for DMFB, which is a tough NP-Complete problem, kolluri Rajesh and colleagues proposed a hybrid approach. This method combines the Artificial Bee Colony (ABC) technique with Generalized N-point crossover (GNX)-based scheduling. They found that it provided better assay completion times compared to previous algorithms. It also needed less time to run. Meanwhile, Daniel Grissom introduced a resource-constrained method known as Force-Directed List Scheduling (FDLS) focused on Digital Micro Fluidic Biochips (DMFB). The results showed that FDLS has clear advantages over List Scheduling (LS) and Path Scheduling (PS). These two heuristics were previously the most effective for DMFBs. Moreover, FDLS proved to be competitive with longer-running systems for DMFB scheduling that use genetic algorithms for iterative improvement. According to Abhimanyu Yadav et al., a very effective Integer Linear Programming (ILP)-based solution for scheduling activities while taking into account dynamic reconfigurability has been proposed. A case involving an operation with numerous successors, which is required in various bioassays, such as sample preparation applications cannot be dealt with directly by the approach. A preliminary experiment shows that the suggested ILP formulation can successfully discover the optimal scheduling for real-world sample preparation applications in an acceptable amount of time, even though our ILP formulation appears to be a little more complicated than the previous one [41]. Daniel Grissom et al., Path Scheduler (PS) [19] explores DAG, path by path. That is, it schedules all nodes along the path, unlike LS and FDLS. PS reduces on-chip storage of droplets, which annihilates resource blocking by employing two types of priorities to order operations for scheduling. PS is faster than LS and FDLS. It only works on trees/forests and fails in scheduling other types of DAGs [42]. Andrew J. R. et al. developed a hybrid priority scheduling algorithm solution that is specifically suited to digital micro fluidics and has the ability to produce schedules that are almost optimal in most cases in a relatively short amount of time.. Additionally, we recommend using programmable detectors to further enhance system performance [43].

2.4. Waste Aware

Designing DMF biochips involves some tricky challenges especially when it comes to mapping lab-bench methods into them. One of the main issues is figuring out how to automate the process of diluting a biochemical sample to a specific concentration factor. This needs to happen in only a few mix/split stages while using as little sample fluid as possible. Nowadays, biochips are becoming much smaller, capable of handling tiny picoliter volumes of discrete fluid droplets. These droplets are stored in even smaller nanoliter volume reservoirs on the chip. Thus, avoiding waste in a bioassay is very important. To optimize usage of sample materials & costly reagents, we should keep waste droplet formation to a bare minimum. Also, reducing the number of waste reservoirs on the chip can help cut down costs. Processing those waste droplets can be quite labor-intensive. So, minimizing their quantity is essential for efficiency and effectiveness in biochip design. Reducing sample and trash droplets also reduces the transportation load for on chip routing [44]. Sudip Roy & his team introduced a technique that automates the dilution & mixing of samples and reagents. They also designed an layout suited for on-chip applications. This method reduces waste significantly, which means less need for large amounts of samples, reagents, & buffer solutions. Additionally, the technology minimizes the loss of samples and buffers by always using the border CFs that approach the target concentration in a dynamic way. Similarly, Bhargab B. B. and colleagues developed an Improved Dilution/Mixing Algorithm (IDMA). This algorithm makes the most of intermediate droplets produced during dilution, thus cutting down on both sample and reagent use while also reducing waste creation. It aims for a specific concentration factor with a granularity of 1/2n and runs in O(n) steps. In another study, Juinn-Dar Huang et al. introduced a multitarget sample preparation approach designed to lower the usage of reactants & decrease waste for digital microfluidic biochips. Their system takes full advantage of waste recycling & shares intermediate droplets effectively. The results demonstrated that their waste recycling

algorithm outperforms current advanced multitarget sample preparation methods, reducing waste & operation counts by 48% and 37%, respectively.

3. Detection

Due to the prolonged incubation period and the massive number of asymptomatic carriers of this disease, controlling in the pandemic period is difficult [47]. The conventional approaches to molecular laboratory analysis, however, are frequently labour- and time-intensive, difficult, and time-consuming. The typical diagnostic laboratory is actually composed of three distinct areas: a sample processing area, a reactant preparation area, and a testing area. In order to keep aerosol contamination at bay, the laboratory must be operated by trained personnel [48]. Therefore, especially in light of the outbreak and spread of a global epidemic, rapid, portable, and practical diagnostic tools have taken the lead. Devices like these can be found anywhere, from large, central facilities in highly specialized labs to the homes of individual patients. M. tuberculosis and 16 NTM isolates may be quickly identified using a newly developed biochip method, according to L. Zhu et al. The platform's tools along with the microarray parts have been assessed independently before. This was done for detecting serious cases of respiratory syndrome. Compared to older biochemical methods, this biochip technique is easier to use & gives results much quicker. A new nucleic acid detection system, brought about by Siyi Hu and colleagues, uses DMF technology. It combines fast nucleic acid amplification with real-time monitoring the amplified products & automatic nucleic acid extraction. In this system, the use of DMFB leverages automatic droplet control together with functional components, creating a full, automated nucleic acid extraction & molecular diagnostic system. Their study showed that the DNA extraction on-chip was as effective as off-chip methods. Moreover, they found that it could be automated & done in less time.Rapid identification of infectious diseases is made possible by the detection results, particularly in light of the present worldwide pandemic outbreak.

4. Conclusion

In this review article, we offer a broad look at the newest developments in digital microfluidic biochips (DMFBs). Over the last few years, there has been a significant increase in aimed at solving the difficulties involved in sample preparation on DMFBs. Many ideas have come up to tackle these problems, but only a handful have shown real success in reducing these issues. Most of these challenges require the use of graph-theoretic methods & optimization techniques. In addition, there are still many unresolved problems in important areas like sample preparation, chip design, and testing. These ongoing challenges highlight the urgent need for more research & development to push forward the capabilities and applications of DMFB technology.

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