10.48047/jocaaa.2024.33.06.59

Study of Design-Time Verification and Security Evaluation Techniques for Microfluidic Biochips

MS. JAYA GUPTA
Assistant Professor, Department of Computer Science and Engineering, Dronacharya Group of Institutions, Greater Noida, Uttar Pradesh
jaya.gupta@gnindia.dronacharya.info

Mr. NAVEEN
Assistant Professor, Department of Computer Science and Engineering, Dronacharya College of Engineering, Gurugram, Haryana
naveen.rathi@ggnindia.dronacharya.info

Abstract

Microfluidic biochips are used in biochemical laboratory assays and protocols, such as Lab-On-a-Chips (LoC), for rapid and accurate disease diagnosis. Current LoCs are built on Continuous-flow MicroFluidic (CMF) biochips and Digital MicroFluidic Biochips (DMFB), with the Micro-Electrode-Dot-Array (MEDA) architecture providing additional flexibility in droplet movement. This thesis aims to design an efficient routing solution for MEDA architecture, demonstrating how it shortens assay completion time by utilizing more flexibility for droplet movement. The study also focuses on developing an exact method for droplet routing on MEDA architecture; utilizing all MEDA features and ensuring minimal time routing solutions. The study examines security assessment and analysis of microfluidic biochips, specifically actuation sequences, to ensure error-free operation. Error recovery techniques are discussed; including probabilistic timed automata (PTA) based techniques and adaptive online methods. The thesis focuses on a droplet routing framework that utilizes MEDA architecture's advantages over traditional EWOD biochips, ensuring accurate droplet routing and minimizing assay completion time.

Keywords: Lab-On-a-Chips, Micro-Electrode-Dot-Array, Probabilistic timed automata, Digital MicroFluidic Biochips

Introduction

Microfluidic biochips are being advocated for on-chip implementation of various biochemical laboratory assays and protocols, such as Lab-On-a-Chips (LoC). These technologies offer a low-cost platform for reducing healthcare costs, providing point-of-care health services, and managing bio-terrorism threats [1].

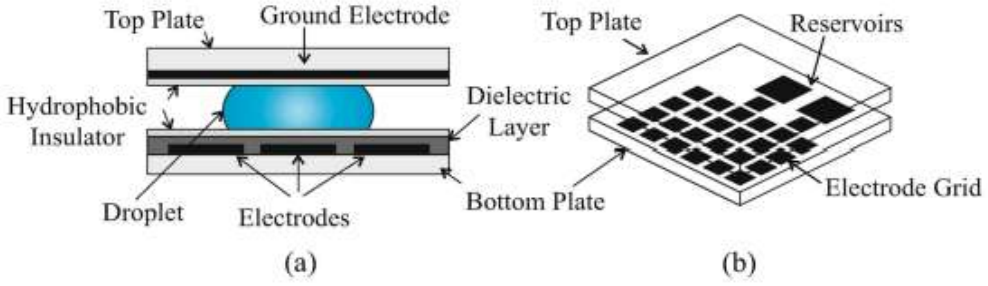


Fig.-1 A side view of the DMFB, where droplet is sandwiched between two plates

10.48047/jocaaa.2024.33.06.59

They are particularly useful for rapid and accurate diagnosis of diseases like malaria, HIV/AIDS, and neglected tropical diseases. An LoC implements one or more biochemical laboratory protocols or assays on a single chip, simplifying cumbersome laboratory procedures. Biochips offer advantages such as low sample and reagent consumption, less error likelihood, high throughput, and high sensitivity. Research in this new discipline of nano-biotechnology requires interdisciplinary integration of biomedical electronics, biochemistry, in-vitro diagnostics, computer-aided design, optimization, and microelectronic technology [2-5].

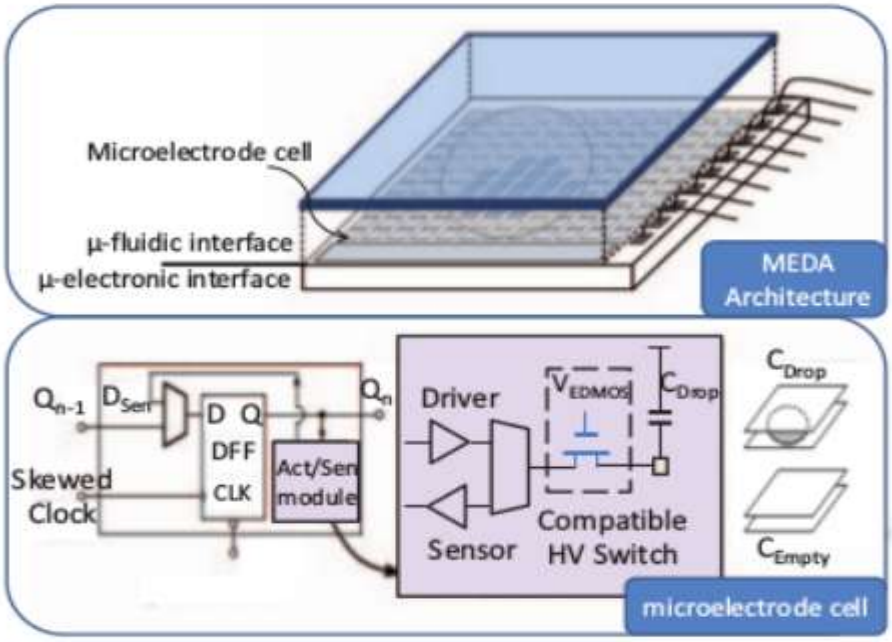


Fig.-2 An illustration of MEDA architecture and the micro-electrode cell

Current LoCs are built based on two mechanisms of fluid flow: Continuous-flow MicroFluidic (CMF) biochips and Digital MicroFluidic Biochips (DMFB). CMF-biochips support a variety of fluidic, chemical, and biological operations on-chip and provide a powerful mixing mechanism. DMF biochips use the principle of Electro-Wetting-On-Dielectric (EWOD) to transport droplets from one electrode to another on the grid. A new DMFB architecture, the Micro-Electrode-Dot-Array (MEDA), has been proposed, providing additional flexibility in droplet movement, droplet reshaping, split/merge capability, in-place sensing, and the capability to manipulate variable-size fractional droplets while performing fluidic operations [6-8].

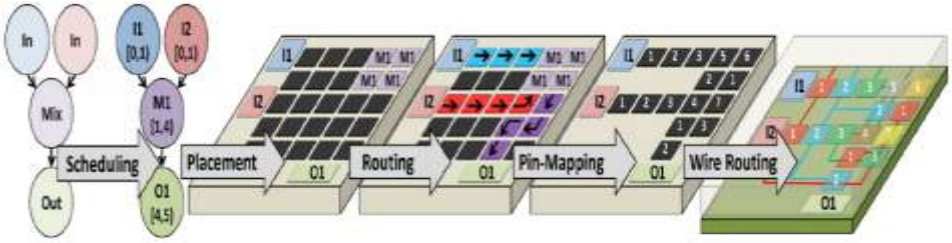


Fig.-3 Synthesis cycle in Digital Microfluidic Biochips

The development of technologies to improve the health of people in poor regions is a major

10.48047/jocaaa.2024.33.06.59

challenge in science and engineering, with over 1 billion people lacking healthcare services. Microfluidics has shown significant promise in this direction by miniaturizing protocol assays needed for disease diagnosis and reaching out far and wide. Research activities on microfluidic systems are multidisciplinary in nature, with significant development in the area of Computer-Aided Design (CAD) automation for microfluidic biochips. The main motivation for this thesis is to design a more efficient routing solution for MEDA architecture using all features and demonstrate how it shortens assay completion time by utilizing more flexibilities for droplet movement. Additionally, the thesis aims to set up a validation framework for microfluidic protocol descriptions, ensuring that the initial input is correct before synthesizing the protocol. This includes balancing objectives such as bioassay completion time, interference-free routing of droplets, waste and reactant optimization, and heat minimization [9-11].

Security threats are important concerns in microfluidic executions today, as attackers can tamper a verified synthesized actuation sequence while it is loaded in the grid memory. The presence of a malicious droplet on the way of any valid droplet on the assay can tamper the execution of the bioassay. The motivation behind this work is to assess a synthesized actuation sequence against an attack model and check whether the malicious attack can tamper a given actuation sequence. This thesis focuses on the development of an exact method for droplet routing on MEDA architecture, which exploits all MEDA features and ensures minimal time routing solutions. The framework works in two steps: pre-synthesis verification, which verifies the informal description of a bio-chemical protocol given by biologists or chemists before its synthesis, and post-synthesis verification, which verifies the synthesized actuation sequence before its execution on the grid [12-14].

The thesis also introduces a security assessment mechanism to examine a given synthesized actuation sequence against malicious attacks before execution. Additionally, a debugging framework is developed to root cause the source of erroneous outputs at the end of execution of a correct bio-assay. The problem statement involves determining a route for each droplet of the assay from the source position to the corresponding target position, avoiding blockages and violating fluidic constraints. The solution methodology explores classical routing methods and uses a symbolic modeling approach to find the solution. The method is implemented in Java and uses Yices as the constraint solver. The thesis also presents a verification framework for end-to-end verification for digital microfluidics, working in two phases: pre-synthesis and post-synthesis. The pre-synthesis verification framework verifies the correctness of the given description of the assay before synthesis, while the post-synthesis verification tool verifies a microfluidic actuation sequence generated out of a MEDA synthesis tool with respect to different erroneous situations possible on the chip [15-18].

The study focuses on the security assessment and analysis of microfluidic biochips, specifically actuation sequences. It aims to identify pre-conditions on droplets, grid resources, and grid locations to ensure error-free operation. The analysis begins with the initial configuration of the biochip and continues until an erroneous translation or the end of the assay is reached. The study also examines trustworthiness assessment of synthesized actuation sequences against maliciously dispensed droplets [19-20].

The assessment method checks whether the route of a hypothetical malicious droplet can

10.48047/jocaaa.2024.33.06.59

interfere with any existing droplet on the assay. The main idea behind this method is to model all possible routes of the malicious droplet, checking for intersection between all possible routes of the malicious droplet and the routes of the existing droplets. The study also addresses root-causing execution errors on microfluidic executions. The method produces a set of suspected locations and operations that may be possible origins of the error. The debug methodology discards operations irrelevant to the erroneous outputs and computes a symbolic expression of the set of operations that can directly/indirectly affect the erroneous outputs. The method is shown to significantly reduce the size of the suspect erroneous region. The thesis is organized into several chapters, including preliminaries, routing problems on MEDA, verification methods, security threats on microfluidic executions, debug methodologies, and future directions. The study provides valuable insights into the security assessment and analysis of microfluidic biochips [21-23].

Literature Review

Microfluidic biochips have become a powerful and reliable emerging technology used in biotechnology applications such as chemical synthesis, disease diagnosis, and drug discovery. These biochips consist of a two-dimensional grid with electrodes and reservoirs, actuated by different voltages. The synthesis process consists of three levels: architectural, physical, and chip. The architectural level consists of scheduling and resource binding of biochemical operations, while the physical level involves on-chip droplet routing and cross contamination avoidance techniques. The chip-level synthesis step maps control pins to electrodes and attempts pin-count minimization. To prevent placement or routing failures, authors propose a heuristic approach for one-pass synthesis for DMFB. Digital microfluidic biochips have matured significantly over the past decade, with MEDA biochips being an extension of the conventional EWOD-based DMFB architecture. MEDA biochips provide new features on the chip, such as high-level synthesis methods, ILP-based reservoir-replacement methods, operation-variation-aware module placement algorithms, and adaptive routing strategy-based synthesis frameworks. These advancements offer new features for efficient reaction synthesis and monitoring [24-25].

Droplet routing is a crucial step in bio-assay synthesis, and previous research has focused on routing methods for EWOD-based digital microfludic biochips (DMEB) and MEDA. Some previous works have used the A∗ algorithm, a two-stage DMFB routing algorithm, bypassibility for droplets, the BioRoute algorithm, an entropy-based algorithm, and an exact method for droplet routing. For conventional DMFB, the A∗ algorithm routes droplets sequentially by selecting the highest prioritized droplet first. The BioRoute algorithm divides the routing problem into global and detailed routing, solving them consecutively. However, these heuristic approaches cannot guarantee minimality of routes. For MEDA, several routing algorithms have been proposed, but they leave room for improvement. Some work proposes a technique to approximate droplet routing time between source and target positions, while others propose A*-based approaches for droplet routing on MEDA architectures [26].

10.48047/jocaaa.2024.33.06.59

Methodology

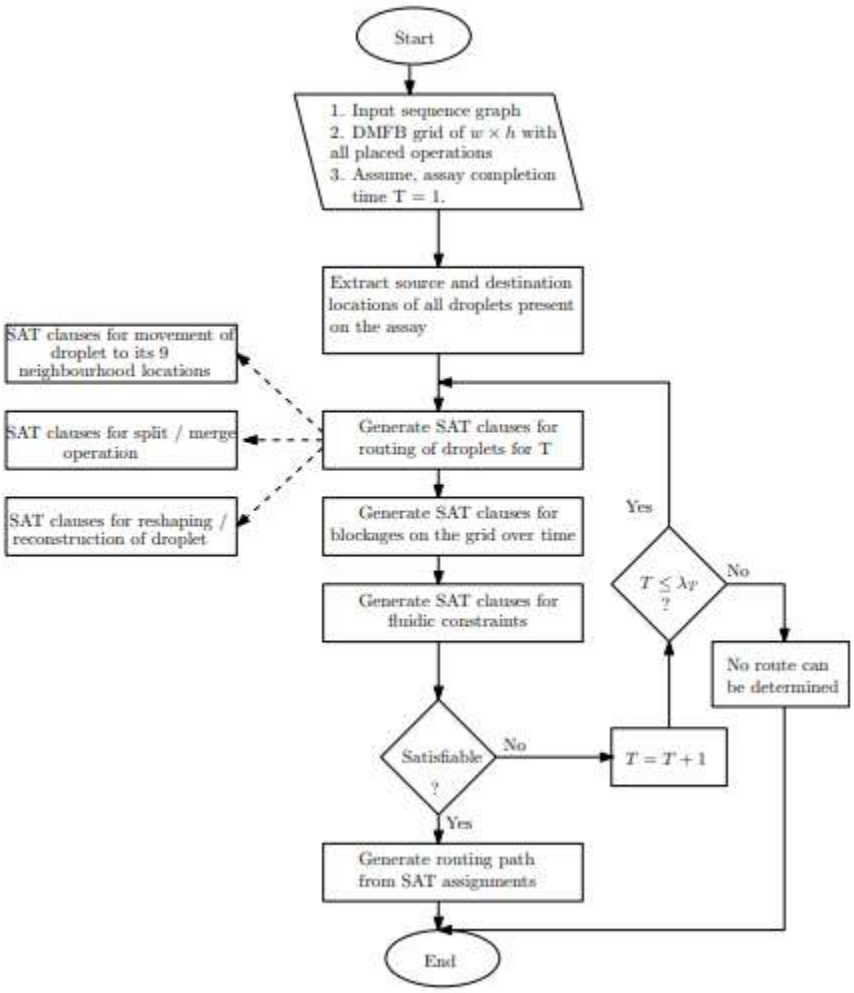


Fig.-3 Work flow

In this study, we suggest a droplet routing paradigm that aims to take advantage of the extra benefits that MEDA biochips offer over their conventional EWOD equivalents. Our formulation and experiments demonstrate that this framework typically results in similar or superior routes and, consequently, assay completion times. Our methodology is based on a discrete optimization technique that is incrementally resolved with constraint solvers. The routing problem's discrete parameters are modeled and addressed in order to achieve this. Due to its continuous nature, velocity has been excluded from our optimization considerations. All droplets are assumed to have the same uniform velocity by our framework. At the conclusion of the execution, our framework offers an actuation sequence for the specified biochemical experiment. Prior to being executed, the generated actuation sequence must be validated. In the following chapter, we go over the verification procedure. In conclusion, droplet routing is an essential step in bio-assay synthesis, and previous research has focused on routing methods for EWOD-based DMEB and MEDA. Future research should focus on improving routing time and incorporating diagonal movement with droplets' horizontal and vertical movements. The MEDA architecture has been studied for its features such as splitting-merging and reshaping droplets, but none of these

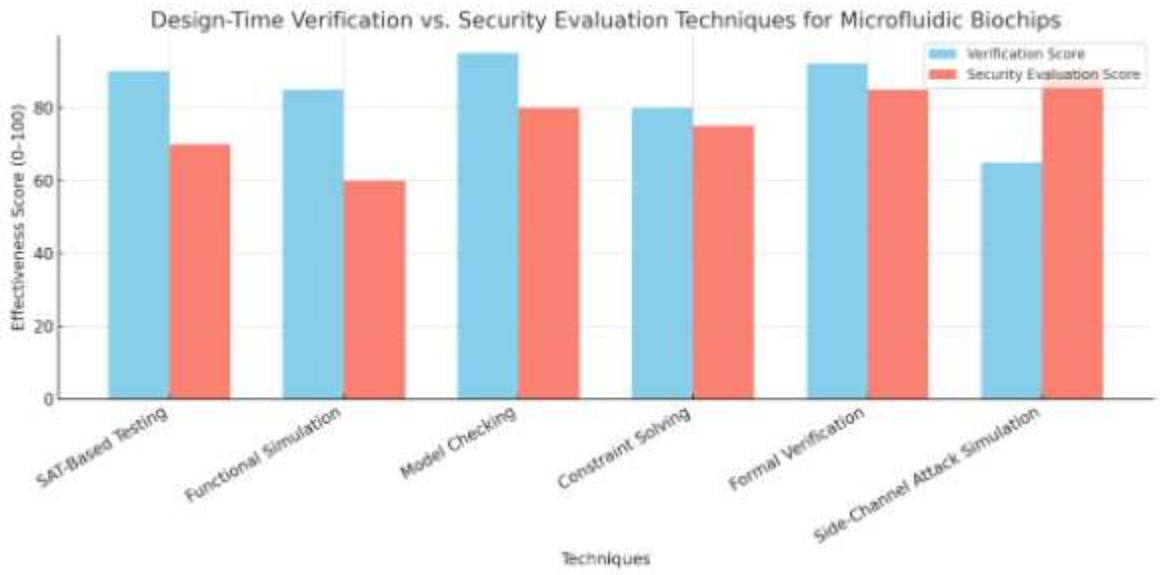
10.48047/jocaaa.2024.33.06.59

research works provide an exact solution for the routing problem. Previous research has attempted to formalize the expression and translation steps involved in bioassay synthesis, such as the SimBiosys tool, Biocoder, Bioscript, Compiler, and Aquarium. However, these methods can lead to wastage of assay reagents and biochip execution cycles for errors that are detectable at compile time [26].

The evolving landscape of Digital Microfluidic Biochips (DMFB) faces new threats due to security attacks and malicious manipulations. Recent work has identified several alarming backdoors in the design life cycle that can be compromised by an attacker, leading to undesirable consequences. Attacks can be administered in various ways, such as modifying the actuation sequence, manipulating the mixer cycle times, inserting additional mix/mov operations, lengthening droplet movements, or making device-level modifications [27].

Software checkers can detect actuation tampering by crosschecking the actuation sequence before loading it to the DMFB, but they cannot detect hardware fault detection attacks. An error recovery method based on checkpoints is proposed, which involves inserting checkpoints at several locations on the bio-chemical assays and examining the droplet for errors. However, rollback recovery method is not helpful for error recovery due to the shortage of physical-aware systems and control software. Error correcting methods for cyberphysical integration have been proposed [28].

Analysis Report



This plotted graph compares the efficacy of different methods for microfluidic biochips in terms of Design-Time Verification and Security Evaluation. Please let me know if you would like additional approaches or data, or if you would prefer a different visualization (such as a line chart or radar map). This summary discusses various error recovery techniques and their applications in various fields. Some methods aim to identify erroneous regions online during

10.48047/jocaaa.2024.33.06.59

error occurrences in execution and propose re-synthesis of these regions. Error-correcting methods are based on dictionary systems, which store a complete list of errors and their corresponding recovery techniques [29]. A dynamical error recovery technique during sample preparation is also proposed. A cyber-physical control algorithm is proposed to rectify dynamically detected hard and soft errors during assay execution. Watermarking techniques for bio-protocol IP protection are also proposed. Defects in flow-based micro-fluidic chips are studied, and fluidic constraint violation checking is addressed using synthesis tools. MEDA architecture has advanced sensing technology, allowing errors to be evaluated quantitatively. An efficient Probabilistic Timed Automata (PTA) based technique for error recovery in MEDA architecture is proposed, which integrates multiple local error recovery flows to obtain global error recovery flows. An adaptive online method for error recovery is proposed using the flexible and sophisticated features of MEDA architecture. A security mechanism is proposed to validate assay execution, and a defensive technique is proposed to guarantee the correctness of the bioassay implementation [30].

Conclusion

This thesis focuses on a droplet routing framework that utilizes MEDA architecture's advantages over traditional EWOD biochips, ensuring accurate droplet routing and minimizing assay completion time. The framework uses a satisfaction encoding to solve the routing problem incrementally. The second chapter validates bio-chemical protocol descriptions before and after synthesizing them. A pre-synthesis verification framework validates the correctness of the assay description, while a post-synthesis verification tool provides a correctness proof. The security assessment framework examines the correct synthesized actuation sequence against malicious attacks. The framework models the malicious attack as the dispense of a malicious droplet on the grid during execution, checking if the malicious droplet can enter the route of any valid droplet.
The final chapter proposes an error debugging methodology to isolate the root cause of an error or malicious attack on a given synthesized actuation sequence. The framework takes the synthesized actuation sequence and erroneous outputs as input and provides a set of operations as the probable source of malicious attack. The method assumes a single source of malicious attack, but may influence multiple outputs. If run-time observations of sensors are available, the source of the attack location can be determined by comparing sensor logs of the correct assay and the compromised one.This thesis focuses on designing methods for analysis of issues in the Micro-Electronic Devices (MEDA) context using symbolic methods. Symbolic encodings offer advantages over explicit representation methods, as they can scale to large-sized assay descriptions on complex grids. Future work aims to model velocity dynamics and explore better routing strategies by optimizing droplet velocities and discrete routes. Advanced techniques are needed to secure microfluidic reactions against the latest attacks, such as hardware Trojans, Denial-of-Service, and over-production. Future work will explore other microfluidic platforms, such as Programmable Microfluidic Devices (PMD) and Paper-based Microfluidic architecture. PMD is an advanced CMF biochip that can implement a controlled mix-split sequence, allowing for homogeneity of mixing and programmability on a single LoC architecture. The Paper-based microfluidic (PB-DMFB) architecture uses micro-PADs for bio-analysis, which requires a small volume of fluids and has no external supporting devices or electric power sources. It is cost-effective, easy-to-use, disposable, and compatible with most medical/biomedical applications. Cyberphysical based DMFB integrates software-based control, fluid handling operations, and

10.48047/jocaaa.2024.33.06.59

reaction outcome detection. Fluidic operations need to be executed accurately for accurate analysis of biochemical reactions. Authors propose an operation-interdependency-aware synthesis method that uses multiple clock cycles to overcome uncertainties in the completion times of mixing operations. Future work will consider all fluid handling operations when examining physical design of biochips under uncertainties.

References

1. Java development tool kit, https://www.oracle.com/technetwork/java/javase/downloads/index.html, 2018.
2. Ali, S. S., Ibrahim, M., Sinanoglu, O., Chakrabarty, K., and Karri, R. Security assessment of cyberphysical digital microfluidic biochips. IEEE/ACM transactions on computational biology and Bioinformatics 13, 3 (2015), 445–458.
3. Ali, S. S., Ibrahim, M., Sinanoglu, O., Chakrabarty, K., and Karri, R. Microfluidic encryption of on-chip biochemical assays. In 2016 IEEE Biomedical Circuits and Systems Conference (BioCAS) (2016), IEEE, pp. 152–155.
4. Alistar, M., and Pop, P. Towards droplet size-aware biochemical application compilation for am-ewod biochips. In Design, Test, Integration and Packaging of MEMS/MOEMS (DTIP), 2015 Symposium on (2015), IEEE, pp. 1–6.
5. AbdelTawab, A.M., Abdelhalim, M.B. and Habib, S.E.D., 2022. Moving Object Detection over Wireless Visual Sensor Networks using Spectral Dual Mode Background Subtraction. International Journal of Advanced Computer Science and Applications, 13(1).
6. Akilan, T., Wu, Q.J. and Yang, Y., 2018. Fusion-based foreground enhancement for background subtraction using multivariate multi-model Gaussian distribution. Information Sciences, 430, pp.414-431.
7. Alipour, P. and Shahbahrami, A., 2022, February. An adaptive background subtraction approach based on frame differences in video surveillance. In 2022 International Conference on Machine Vision and Image Processing (MVIP) (pp. 1- 5). IEEE.
8. Aung, S.S. and War, N., 2019, November. Foreground objects segmentation in videos with improved codebook model. In 2019 International Conference on Advanced Information Technologies (ICAIT) (pp. 161-166). IEEE.
9. Bera, N., Majumder, S., and Bhattacharya, B. B. Simulation-based method for optimum microfluidic sample dilution using weighted mix-split of droplets. IET Comput. Digit. Tech. 10, 3 (2016), 119–127.
10. Barnard, K., 2022. Background Subtraction: Theory and Practice. Springer Nature.
11. Belagiannis, V., Schubert, F., Navab, N. and Ilic, S., 2020, October. Segmentation based particle filtering for real-time 2d object tracking. In European Conference on Computer Vision (pp. 842-855). Springer, Berlin, Heidelberg.
12. Bohringer, K. Towards optimal strategies for moving droplets in digital microfluidic systems. In IEEE International Conference on Robotics and Automation, 2004. Proceedings. ICRA'04. 2004 (2004), vol. 2, IEEE, pp. 1468–1474.
13. Bourouis, S., Pawar, Y. and Bouguila, N., 2021. Entropy-based variational scheme with component splitting for the efficient learning of gamma mixtures. Sensors, 22(1), p.186.
14. Çelik, Y., Altun, M. and Güneş, M., 2017, September. Color based moving object tracking with an active camera using motion information. In 2017 International Artificial Intelligence and Data Processing Symposium (IDAP) (pp. 1-4). IEEE.
15. Chandrakar, R., Raja, R., Miri, R., Sinha, U., Kushwaha, A.K.S. and Raja, H., 2022. Enhanced the moving object detection and object tracking for traffic surveillance using RBF-FDLNN and CBF algorithm. Expert Systems with Applications, 191, p.116306.
16. Chen, F., Wang, X., Zhao, Y., Lv, S. and Niu, X., 2022. Visual object tracking: A survey. Computer Vision and Image Understanding, 222, p.103508.
17. Chen, Z., Wang, R., Zhang, Z., Wang, H. and Xu, L., 2019. Background–foreground interaction for moving object detection in dynamic scenes. Information Sciences, 483, pp.65-81.
18. Comaniciu, D. and Meer, P., 2002. Mean shift: A robust approach toward feature space analysis. IEEE Transactions on pattern analysis and machine intelligence, 24(5), pp.603-619.
19. Dong, Y. and DeSouza, G.N., 2011. Adaptive learning of multi-subspace for foreground detection under illumination changes. Computer Vision and Image Understanding, 115(1), pp.31-49.

10.48047/jocaaa.2024.33.06.59

20. Fattahi, S. and Sojoudi, S., 2020. Exact guarantees on the absence of spurious local minima for non-negative rank-1 robust principal component analysis. Journal of Machine Learning Research, arXiv preprint arXiv:1812.11466.
21. Garcia-Garcia, B., Bouwmans, T., Sehairi, K. and Zahzah, E.H., 2022. Real-Time Implementations of Background Subtraction for IoT Applications. In Computer Vision and Internet of Things (pp. 263-286). Chapman and Hall/CRC.
22. Gemignani, G. and Rozza, A., 2016. A robust approach for the background subtraction based on multi-layered self-organizing maps. IEEE transactions on image processing, 25(11), pp.5239-5251.
23. Goyette, N., Jodoin, P.M., Porikli, F., Konrad, J. and Ishwar, P., 2012, June. Changedetection. net: A new change detection benchmark dataset. In 2012 IEEE computer society conference on computer vision and pattern recognition workshops (pp. 1-8). IEEE.
24. Han, G., Wang, J. and Cai, X., 2017. Background subtraction based on modified online robust principal component analysis. International Journal of Machine Learning and Cybernetics, 8(6), pp.1839-1852.
25. Huang, R., Pedoeem, J. and Chen, C., 2018, December. YOLO-LITE: a real-time object detection algorithm optimized for non-GPU computers. In 2018 IEEE International Conference on Big Data (Big Data) (pp. 2503-2510). IEEE.
26. Keuper, M., Tang, S., Andres, B., Brox, T. and Schiele, B., 2018. Motion segmentation & multiple object tracking by correlation co-clustering. IEEE transactions on pattern analysis and machine intelligence, 42(1), pp.140-153.
27. Chakrabarty, K., and Su, F. Digital microfluidic biochips: synthesis, testing, and reconfiguration techniques. CRC Press, 2006.
28. Chen, Y.-H., Hsu, C.-L., Tsai, L.-C., Huang, T.-W., and Ho, T.-Y. A reliability-oriented placement algorithm for reconfigurable digital microfluidic biochips using 3-d deferred decision making technique. IEEE Trans. on CAD 32, 8 (2013), 1151–1162.
29. Cho, M., and Pan, D. Z. A high-performance droplet routing algorithm for digital microfluidic biochips. IEEE Transactions on Computer-Aided Design of Integrated Circuits and Systems 27, 10 (2008), 1714–1724.
30. Chung, W.-C., Cheng, P.-Y., Li, Z., and Ho, T.-Y. Module placement under completiontime uncertainty in micro-electrode-dot-array digital microfluidic biochips. IEEE Transactions on Multi-Scale Computing Systems 4, 4 (2018), 811–821.