Extending the Single-Target Droplet Generation Method CoDOS to Multi-Target Synthesis

Yusuke Igarashi
Ritsumeikan Univ.
octo@ngc.is.ritsumei.ac.jp

Shigeru Yamashita
Ritsumeikan Univ.
ger@fc.ritsumei.ac.jp

Abstract— This paper presents an enhanced method based on CoDOS, a droplet generation algorithm for DMFB biochips, to efficiently generate multiple droplet types. By optimizing droplet generation, our approach reduces reagent usage and operational costs. Compared to manual synthesis and dilution, our method offers improved automation and cost-effectiveness. Experimental results demonstrate approximately a 1% improvement in droplet efficiency.

I. Introduction

In recent years, research and development of biochips for biochemical experiments has been actively progressing. Biochemical experiments involve processes such as droplet mixing and dilution, which account for a significant portion of the cost and time when performed manually. One of the key objectives of biochip research is to reduce such costs by replacing manual operations with automated ones [1][2].

This study focuses on a type of biochip known as a Digital Microfluidic Biochip (DMFB), which is capable of performing 1:1 mixing and dilution operations. DMFBs have been successfully applied in a variety of fields, including clinical diagnostics and immunoassays [3][4][5].

To support droplet generation on DMFBs, various algorithms have been developed. This paper focuses on one such algorithm, CoDOS [6], which was originally designed to generate a single type of droplet at a specified node. CoDOS reduces the number of droplets used, their cost, and the number of waste droplets by identifying and sharing common operations during droplet generation.

Although CoDOS was designed for single-target droplet generation, it can also be applied to multi-target droplet generation. However, existing approaches for multi-target use are essentially extensions of the original single-target algorithm and lack sufficient optimization.

In this paper, we propose an improved algorithm for generating multiple types of droplets by enhancing the scheduling of shared operations. This enhancement results in a reduction in both the number of droplets used and the associated costs. Compared to existing methods, our approach achieves an improvement of approximately 1% [7].

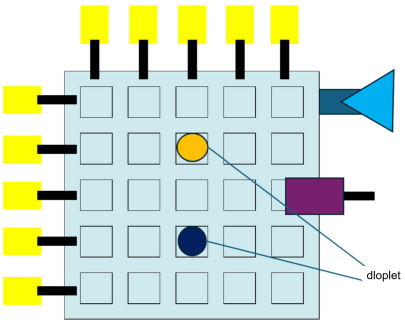

Fig. 1. Structure of DMFB

II. Background Knowledge

A. Digital Microfluidic Biochip (DMFB)

DMFB is a type of biochip that performs droplet dilution and generation using electrodes arranged in a two-dimensional array and electrostatic actuation. The structure of DMFB is shown in Figure 1. Droplets are inserted from a designated location, and operations such as dilution, splitting, and mixing are performed on the two-dimensional array [7].

In DMFB, droplet mixing is performed only in a 1:1 ratio. The result of the mixing is represented as a concentration vector, defined as $CV_n \equiv < v_1, v_2, \ldots, v_i >$. When CV_x and CV_y are mixed, the resulting droplet concentration CV_z is calculated as $z_i = \frac{x_i + y_i}{2}$. Additionally, since each dilution operation mixes one droplet of each type, two droplets of the same concentration are generated in a single operation [7][9][11].

Furthermore, for any $CV_n \equiv < v_i >$, when the number of droplet types being mixed is N, it always holds that $\sum_{i=1}^{N} v_i = 2^d$.

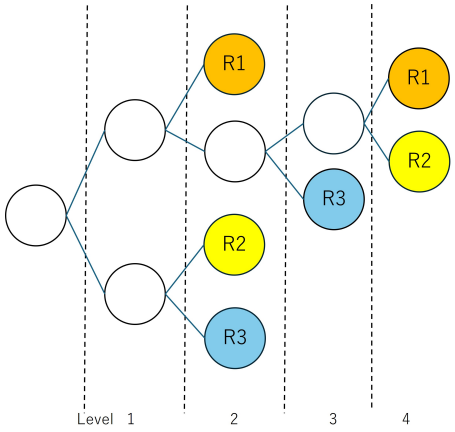

Fig. 2. Dilution graph generated by BS

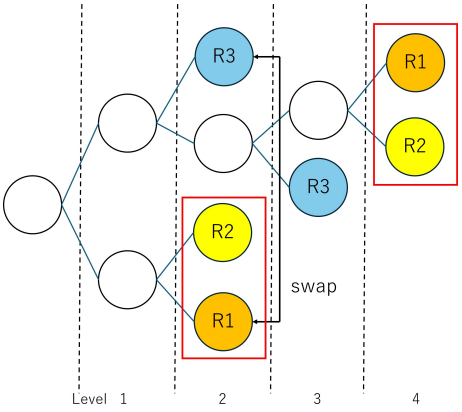

Fig. 3. Rearrangement of the dilution graph into a shareable form

B. *CoDOS (Common Dilution Operation Sharing Algorithm)*

This section explains CoDOS (Common Dilution Operation Sharing Algorithm), which forms the foundation of the method proposed in this paper. CoDOS is one of the algorithms for biochips that generate a single target droplet by mixing multiple types of droplets. In the following, we refer to generating a single type of droplet as the **single-target** case and generating multiple types as the **multi-target** case.

As an example, let the target concentration vector be $CV_n \equiv< 5, 5, 6 >$. CoDOS first uses the bit-scanning method (BS), an existing method [8][10]. In BS, each element of the concentration vector is converted into a fraction with the total sum $5 + 5 + 6 = 16 = 2^4$ as the denominator. These fractions are then converted into binary. In this case, $\frac{5}{2^4}, \frac{5}{2^4}, \frac{6}{2^4}$ are converted into binary as $0.0101_2, 0.0101_2, 0.0110_2$. A dilution tree is then generated, with the first digit after the decimal point representing level 1. A dilution tree schematically represents the actual operations performed on the biochip. The generated dilution tree is shown in Figure 2.

The next step is to search for common parts in the dilution graph generated by this process. First, identical combinations of nodes at different levels are explored. In this example, nodes R_1 and R_2 exist at levels 2 and 4. Sharing this part can reduce the number of droplets used. Figure 3 shows how the arrangement of nodes within the same level is modified so that the mixing of nodes R_1 and R_2 occurs at two locations. Table I shows how the performance is improved by this sharing operation. Improvements are observed in all aspects: the number of droplets used, the number of waste droplets, and the number of operations.

The arrangement where the droplet generated by mixing nodes R_1 and R_2 is represented as node $R_{(1,2)}$ is shown in Figure 4.

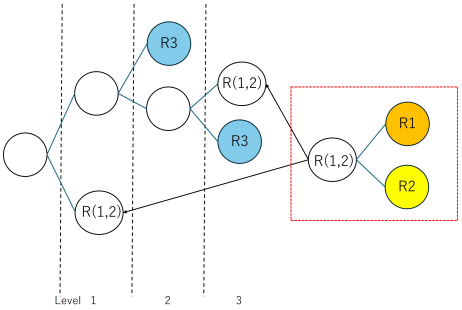

Fig. 4. Rearranged dilution graph into a shareable form

There are also cases where CoDOS can be applied multiple times. The example in Figure 5 shows an operation where the mixing portion of R_2 and R_3 is shared as $R_{(2,3)}$. This example still allows further application of CoDOS. Mixing portions of R_1 and $R_{(2,3)}$ exist at levels 2 and 3 respectively, which can be shared. The dilution graph after applying CoDOS again, where the mixed droplet is represented as node $R_{(1,(2,3))}$, is shown in Figure 6.

TABLE I
EXAMPLE OF IMPROVEMENT THROUGH CODOS APPLICATION

	BS	CoDOS
Number of droplets used	6	4
Number of waste droplets	5	3
Number of operations	5	4

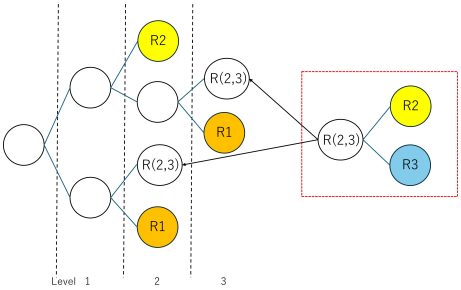

Fig. 5. Example graph where CoDOS can be applied multiple times

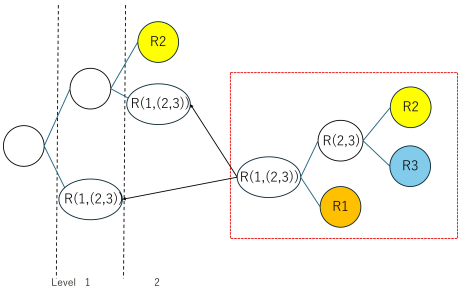

Fig. 6. Graph after multiple CoDOS applications

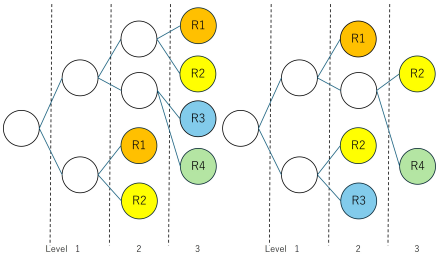

Fig. 7. Example of a graph to which the proposed method is applied

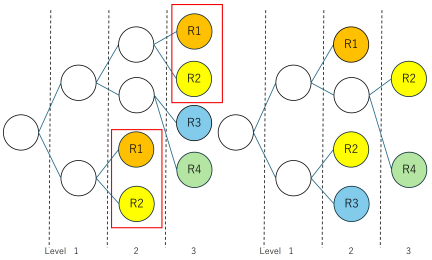

Fig. 8. Shareable portion using existing method

III. Application of CoDOS to Multi-Target Cases

A. Sharing Order in Existing Methods

In Chapter 2, CoDOS was described as an algorithm for single-target cases, but it can also be applied to multi-target scenarios. A multi-target case can be handled by treating each target as a single-target dilution tree and performing operations individually. In existing methods, a dilution tree is generated for each target concentration, and CoDOS is applied to each of them. After generating the optimized dilution graph for each target, shared portions across all graphs are explored, and CoDOS is applied accordingly. As an example, we use the dilution graphs for droplets R_1, R_2, R_3, and R_4 shown in Figure 7. When CoDOS is applied using the existing method, the portion enclosed in red boxes in Figure 8 becomes a shareable section. Once this part is shared, there are no further shareable sections in each individual graph, so the operation ends.

B. Changing the Sharing Order of Identical Portions

In the existing method, exploration and sharing are performed within each individual dilution graph first, followed by exploration across all graphs. However, it is considered that more optimal sharing can be achieved by exploring shareable portions across all dilution graphs from the beginning. Therefore, we propose an algorithm that enables such operations.

As an example, we show the sharing between droplets R_1 and R_2. The candidate locations for sharing are shown in Figure 9. When there are more than three candidates, priority is given to the location with the highest number of droplets at the same level. If the number of droplets is also the same, priority is given to the lower-level location. According to this strategy, the resulting dilution tree after one sharing operation is shown in Figure 10. In this example, there is another shareable section, as shown in Figure 11. Sharing is repeated in this way until no more shareable sections remain.

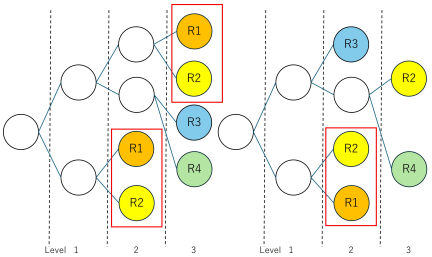

Fig. 9. Candidate shareable portions

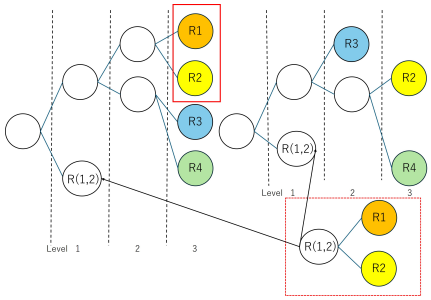

Fig. 10. Dilution tree after one sharing operation

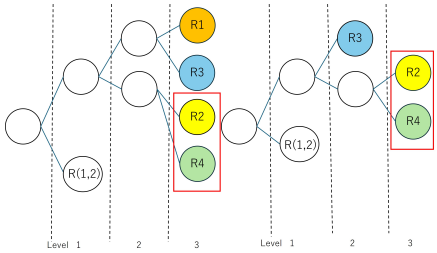

Fig. 11. Second candidate shareable portion

TABLE II
EXPERIMENTAL RESULTS FOR 5 DROPLET TYPES

	Number of Droplets	Cost
$n = 4$	99.9434%	99.2702%
$n = 5$	99.9572%	99.1211%
$n = 7$	99.8159%	98.9589%
$n = 10$	99.5660%	98.9943%

TABLE III
EXPERIMENTAL RESULTS FOR 7 DROPLET TYPES

	Number of Droplets	Cost
$n = 4$	99.8843%	99.2238%
$n = 5$	99.7932%	98.9854%
$n = 7$	99.7818%	98.8658%
$n = 10$	99.3351%	98.6443%

IV. Experimental Results and Discussion

In this section, we present and discuss the experimental results.

A. Evaluation Method

The number of targets was fixed at 2, while the number of droplet types and the maximum level were varied. Experiments were conducted with 5 and 7 types of droplets. The droplets were weighted by cost: {1, 2, 4, 8, 16} for 5 types and {1, 2, 4, 8, 16, 32, 64} for 7 types. The levels were varied across four patterns: $n = 4, 5, 7, 10$. For each condition, 10,000 concentration vectors were randomly generated, and the algorithm was executed on each. The evaluation metric is the average performance ratio of the proposed method compared to the existing method, which is set as 100%. The percentage value indicates how much of the original cost or droplet count remains using the proposed method.

B. Experimental Results

The experimental results are shown in Tables II and III.

C. Discussion

The improvements were limited to at most around 1%, and thus were not significant. In terms of cost, better results may be expected when diluting extremely expensive reagents compared to solvents. However, there was almost no noticeable change in the number of droplets used. Additionally, a trend was observed where increasing the maximum level yielded slightly better results.

V. Conclusion

In this thesis, we proposed an improved method for multi-target droplet generation using CoDOS by modifying the procedure for identifying shareable parts within dilution graphs, aiming to reduce the number of droplets and the associated cost. As a result, we found that although a slight reduction is possible, the improvement was minimal. The reductions in droplet count and cost were limited to around 1% at most, indicating that the proposed method did not achieve significant improvement over existing methods.

As discussed, while there may be potential for cost improvement in cases where there is a large disparity in droplet costs—such as when diluting expensive reagents with solvents—there was no significant difference observed in the droplet count. As a future direction, although further improvement of CoDOS itself may be challenging, we believe that CoDOS has structural flexibility and potential for integration. Therefore, we aim to explore enhancements through combinations with other techniques.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Number JP20H04160 and JSPS Bilateral Program Number PJSBP120247717.

References

[1] P. R. C. Gascoyne and J. V. Vykoukal, "Dielectrophoresis-based sample handling in general-purpose programmable diagnostic instruments," Proceedings of the IEEE, vol. 92, no. 1, 2004, pp. 22–42.

[2] Rajesh Kolluri, JV Phani Kumar, and Sumanta Pyne. Heterogeneous earliest finish time based scheduling for digital microfluidic biochips. In BIODEVICES, pp. 175–182, 2019.

[3] V. Srinivasan, V. K. Pamula, and R. B. Fair, "An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids," Lab on a Chip, vol. 4, no. 4, pp. 310–315, May 2004.

[4] T.-Y. Ho, J. Zeng, and K. Chakrabarty, "Digital microfluidic biochips: A vision for functional diversity and more than Moore," in Proc. IEEE/ACM ICCAD, 2010, pp. 578–585.

[5] D. Chatterjee, A. J. Ytterberg, S. U. Son, J. A. Loo, and R. L. Garrell, "Integration of protein processing steps on a droplet microfluidics platform for MALDI-MS analysis," Analytical Chemistry, vol. 82, no. 5, pp. 2095–2101, Mar. 2010.

[6] Chia-Hung Liu, Hao-Han Chang, Tung-Che Liang, Juinn-Dar Huang .Sample preparation for many-reactant bioassay on DMFBs using common dilution operation sharing,2013 IEEE/ACM International Conference on Computer-Aided Design (ICCAD)

[7] Rupam Bhattacharya, Pranab Roy, Hafizur Rahaman.Homogeneous droplet routing in DMFB: An enhanced technique for high performance bioassay implementation.Integration Volume 60, January 2018, Pages 74-91

[8] Y.-L. Hsieh, T.-Y. Ho, and K. Chakrabarty, "On-chip biochemical sample preparation using digital microfluidics," in Proc. IEEE BioCAS,2011, pp. 297–300.

[9] H. Ren, V. Srinivasan, and R. B. Fair, "Design and testing of an interpolating mixing architecture for electrowetting-based droplet-onchip chemical dilution," in Proc. IEEE TRANSDUCERS, 2003, pp. 619–622.

[10] W. Thies, J. P. Urbanski, T. Thorsen, and S.Amarasinghe, "Abstractionlayers for scalable microfluidic biocomputing," Natural Computing, vol.7, no. 2, pp. 255–275, May 2008.

[11] P. Paik, V. K. Pamula, and R. B. Fair, "Rapid droplet mixers for digital microfluidic systems," Lab on a Chip, vol. 3, no. 4, pp. 253–259, Sep. 2003.