Generic Sample Preparation for Different Microfluidic Platforms

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***Abstract*—Sample preparation plays a crucial role in several medical applications. Microfluidic devices or *Labs-on-Chips* (LoCs) got established as a suitable solution to realize this task in a miniaturized, integrated, and automatic fashion. Over the years, a variety of different microfluidic platforms emerged, which all have their respective pros and cons. Accordingly, numerous approaches for sample preparation have been proposed—each specialized on a single platform only. In this work, we propose an idea towards a generic sample preparation approach which will generalize the constraints of the different microfluidic platforms and, by this, will provide a platform-independent sample preparation method. This will allow designers to quickly check what existing platform is most suitable for the considered task and to easily support upcoming and future microfluidic platforms as well. We illustrate the applicability of the proposed method with examples for various platforms.**

***Index Terms*—Microfluidic, Sample preparation, Generic, Lab-on-Chip.**

I. Introduction

Sample preparation, i.e., the dilution and/or mixing of fluids in certain ratios, plays a crucial role in point-of-care (PoC) clinical diagnosis [1], [2]. For example, real-time RT-PCR, which recently gained further interest for screening COVID-19, always requires sample preparation as a pre-processing step [3], [4]. Before, sample preparation already constituted a major step in several procedures for clinical diagnostics, DNA analysis, drug design, and gene sequencing [1], [5]. While originally executed in explicit labs requiring substantial manual labor and bulky equipment, nowadays microfluidic devices (often also known as *Labs-on-Chips* or LoCs) got established as a low-cost and high-throughput solution to conduct sample preparation in a miniaturized, integrated, and automatic fashion on a single chip.

In fact, over the years, a variety of different microfluidic platforms have been proposed. For example:

- *Digital Microfludic Biochips* (DMFB, [6]), which manipulate droplets of nano/pico-liter volume on a 2D electrical grid. This type of chips performs basic microfluidic operations by applying a sequence of time-varying voltage values to the individual electrodes.
- *Micro-Electrode-Dot-Array Biochips* (MEDA, [7]), which are basically an extended form of DMFBs and realize different microfluidic operations with a group of micro-electrodes (rather than individual electrodes). By this, more advanced operations (such as mixing of droplets with different volumes, more flexible shapes, etc.) become possible.
- *Continuous Flow Microfludic Biochips* (CFMB, [8]), which perform microfluidic operations by employing a *flow layer*, i.e., a set of microchannels through which the considered fluids are pumped through, and a *control layer*, i.e., a set of microvalves controlled by by external air pressure to control the fluid flow in the flow layer.
- *Droplet-based Microfluidic Networks* (DMN, [9]), where the considered fludids are encapsulated in terms of droplets (as in DMFBs and MEDA), put into another (immiscible) fluid which works as carrier for the droplets, and then pumped through a set of microchannels (as in CFMBs). The different microfluidic operations are then realized by different modules which can be addressed by utilizing a passive routing concept which does not require dedicated electrodes or valves but completely relies on the microfluidic resistance of the channels and droplets.

Having these platforms, a main challenge for designers is how to properly realize a sample preparation procedure on them so that the desired sample, i.e., a droplet or fluid-mixture with a certain *Concentration Factor* (CF) is generated and, at the same time, further objectives such as minimizing the number of applied operations or the required samples are addressed. In order to tackle this challenge, numerous automatic solutions have been presented over the past years, e.g., for DMFB biochips [10]–[15], CFMB biochips [16]–[19], and MEDA biochips [14], [20].

All these approaches have in common that they basically address the same design task, but eventually differ in their specifics; namely, in exploiting the potential and obeying the restrictions of the respectively considered platform. This state-of-the-art constitutes severe shortcomings since (1) designers aiming to determine the best platform for realizing a particular sample need access to all the resulting tools for all available platforms and (2) new sample preparation methods have to frequently be (re-)developed once another platform (such as DMN) shall be considered.

In this paper, we propose an idea towards a platform-independent (generic) sample preparation approach for breaking the "vicious circle" of constantly (re-)developing sample preparation methods for each microfluidic platform. The core of the proposed solution rests on the following idea: Rather than addressing the respective potential and restrictions explicitly (using a dedicated strategy as done in previous work), we generalize them in terms of generic constraints.

We illustrate the idea with examples showing that the characteristics of the different microfluidic operations (namely, dispensing, mixing, splitting) can be generalized in terms of constraints such as supported fluid volumes for dispensing

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and mixing, granularity, constraints on mixing and splitting, etc. This could allow for the development of a platform-independent sample preparation method which, eventually, could generate results for a particular platform by just properly instantiating those generic constraints based on the characteristics of the chosen platform.

The remainder of this paper is structured as follows: Section II reviews the basics on sample preparation and the microfluidic operations which are required for realizing sample preparation. Based on that, Section III provides the main ideas and concepts towards generic sample preparation as proposed in this work. Finally, Section IV concludes this paper.

II. Sample Preparation and Required Operations

A. Sample Preparation

Sample preparation (e.g., dilution) is the task of mixing a specific amount of raw sample and buffer fluids to generate a mixture of a desired *CF*. The *CF* ($0 \leq CF \leq 1$) indicates the amount of sample x to buffer y fluids and can either be written as ratio CF $= x : y$ with ($x, y \in \mathbb{N}$) or as decimal value with CF $= \frac{x}{x+y}$. Here, we assume that the *CF* of a raw sample (buffer) is 1 (0). However, the correct mixing ratio of sample and buffer fluids for a desired *CF* can often not be applied directly due to particular platform-depended constrains (e.g., fixed droplet size, minimum/maximum mixture volumes, etc.). This can be compensated by conducting a series of mix-split operations, eventually realizing the desired CF while also considering the constraints. These operations can be described as a directed acyclic graph, known as *sequencing graph* (see, e.g., Fig. 2, which is discussed later in more detail).

Generally, a mix operation requires to merge two substances with different *CFs* of C_1 and C_2 and a volume ratio of $V_1 : V_2$, resulting in a new substance with *CF* = $\frac{C_1 \times V_1 + C_2 \times V_2}{V_1 + V_2}$. By splitting and mixing this new substance with additional sample or buffer fluids multiple times, the desired *CF* can eventually be realized. Note that the depth d of the *sequencing graph* is determined by a user-specified error-tolerance limit ϵ ($0 \leq \epsilon < 1$) that indicates the accuracy of the desired *CF*.

B. Microfluidic Operations Required for Sample Preparation

In order to realize a particular *CF* on a microfluidic biochip, a certain sequence of microfluidic operations needs to be conducted on the respective platforms. More precisely, the respectively required fluids (either in form of droplets or as continuous fluid) need to be *dispensed and moved* inside the platform, need to be *mixed* with each other, and need to be separated (splitted). All platforms reviewed above (i.e., DMFB, MEDA, CFMB, and DMN) support these operations—albeit in different fashions. Fig. 1 summarizes the characteristics of the different implementations. More precisely:

DMFBs perform all these operations with droplets of a fixed size (e.g., 1X volume). Hence, for DFMBs the volume ratio for mixing two droplets is always $V_1 : V_2 = 1 : 1$, resulting in a 2X-volume mixture droplet with *CF* = $\frac{C_1 + C_2}{2}$. Furthermore, this platform can only split the mixture droplet into two equal droplets, i.e. with a volume of 1X.

MEDA biochips extend the above concept and perform more flexible operations by allowing droplet volumes that are integral multiples of the minimum droplet volume (e.g., 1X volume) which depends on the size of the micro-electrodes. More precisely, MEDA biochips are able to generate integral

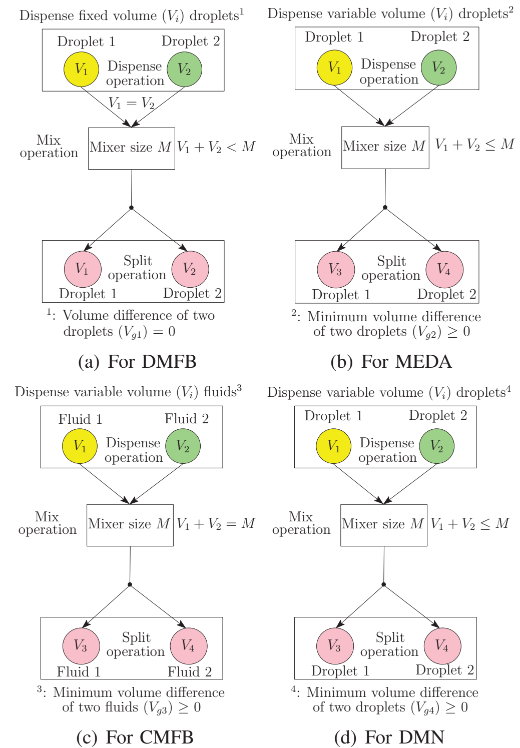


Fig. 1. Characteristics of fluidic operations ($V_{g3} > V_{g2} > V_{g4}$)

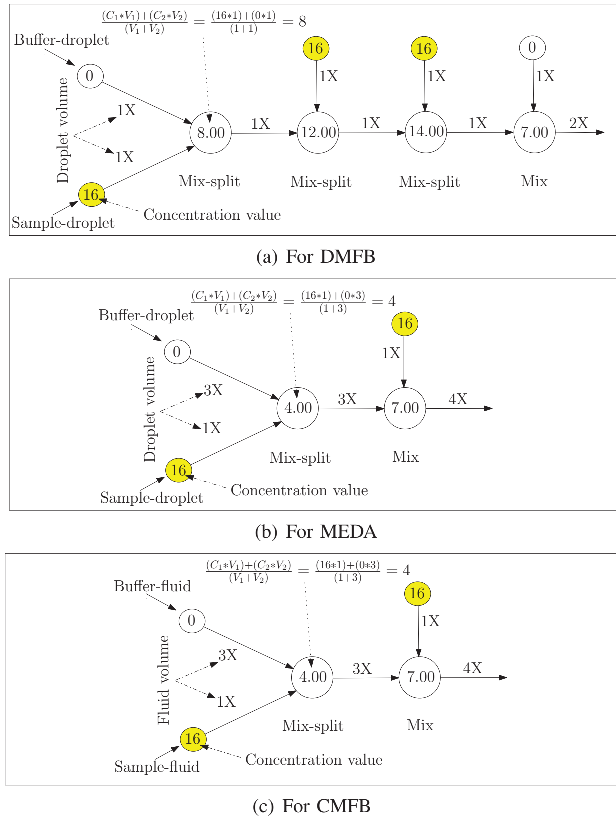
(e.g., 1X, 2X, 3X, $\cdots$) as well as fractional volume droplets ($<$ 1X) since the size of the MEDA-microelectrodes can be much (e.g., 16 times) smaller than DMFB-electrodes [14]. Thus, it allows an arbitrary volume ratio for the mix operation, i.e., $V_1 : V_2$ with $(V_1, V_2 \in \mathbb{N} \mid V_1, V_2 \geq 1)$. However, the mixing operation is constrained in the size of the resulting droplet, which has to be smaller than a certain mixer size M, i.e., $V_1 + V_2 < M$.

CFMB biochips allow only integral volume fluids (e.g., 1X, 2X, 3X, $\cdots$) and can also exploit several arbitrary volume ratios for the mix operation. However, since the mixing operation of CFMB chips is performed by using a specially designed N-segmented rotary mixer [17], [18] with a given size M, the total volume of the two fluids must add up to this value, i.e., $V_1 + V_2 = M$.

DMN biochips are able to generate droplet volumes of arbitrary sizes as long as they are in a specific minimum and maximum range, which enables DMN chips to perform mixing operations in a much more flexible way compared to other biochips (e.g., DMFB, MEDA, CFMB). Similar to MEDA chips, the combined volumes of the mixed droplets also have to be smaller than the mixer size M, i.e., $V_1 + V_2 \leq M$.

These different implementations of course need to be considered when realizing a sample preparation procedure on one of these platforms.

Example 1. *Let's assume a target-*CF = $\frac{7}{16}$ *should be generated. Then, Fig. 2(a), Fig. 2(b), and Fig. 2(c) show the respectively resulting sequence of operations (in terms of a sequencing graph) as generated by dedicated sample preparation methods for DMFBs (namely* BS *[10]), for MEDA (namely* FacDA *[20]), and for CFMB (namely* FloSPA *[18]),*



(a) For DMFB

(b) For MEDA

(c) For CMFB

Fig. 2. Sequencing graphs generated using dedicated approaches

respectively.[1] *As it can be observed, all solutions follow the characteristics of the implementations of the respectively required operations. For simplicity, in this paper, only the numerator part of all* CFs *are shown in all figures.*

III. Towards Generic Sample Preparation

As reviewed in the introduction, numerous automatic methods for sample preparation have been proposed over the past years [10]–[20]. Each of them focused on one dedicated platform only and a method for one platform almost always is not applicable for another platform or does not utilize the full potential of the platform. Obviously, this limits the flexibility of the currently available solutions.

In this section, we propose the general idea towards a generic sample preparation method. To this end, we acknowledge that differences in the respective implementations of the microfluidic operations needed for sample preparation exists (as discussed above by means of Fig. 1), but also see the potential to generalize the respective characteristics. More precisely, for facilitating the generic idea, the proposed method generalizes different existing constraints in a broader fashion and selects an appropriate setup (as required) from the following set of constraints.

1) Constraints on the Dispensing Operation:

- *Volume:* Provide the minimal and maximal volume of a generated droplet/fluid, by specifying the lower and upper bound variable lb_{dv} and ub_{dv}, respectively. If only one volume can be generated (e.g., in DMFB) these values are equal.
- *Granularity:* Provide the volume granularity V_g, which is the minimal volumetric difference of two *un-equal* droplets/fluids. For CFMB (MEDA), this value would be 1X (< 1X) since they allow integral (fractional) volumes of fluids (droplets). For DMN, this value would be much smaller than MEDA, since arbitrary droplet volumes can be generated between the range of lb_{dv} and ub_{dv}. The value is not important for DMFB because only one volume can be generated.

2) Constraints on the Mixing Operation:

- *Volume:* Provide the minimal lb_{md} and maximal ub_{md} volume of the combined mixture droplet/fluid. For DMFB, these values always correspond to 2X, while for the other platforms these values depend on the size of the mixer component. Similar to the DMFB platform, these values become equal for CFMB, since they are always mixed inside a mixer with a certain size. The volume of the mixture droplet/fluid (t_{mv}) should be within the range [lb_{md}, ub_{md}].
- *Unequal mixing:* Define if it is possible to mix droplets of un-equal sizes. This can be provided by a variable U_{mix}, which is supposed to be set to *false* for DMFB, since they always have a volume mixing ratio of $V_1 : V_2 = 1 : 1$. In contrast, this variable is supposed to be set to *true* for the other platforms, since they allow to mix droplets of variable sizes.

3) Constraints on the Split Operation:

- *Unequal splitting:* Define if it is possible to split droplets into two unequal volumes and provide the minimum (lb_{cd}) and maximum (ub_{cd}) volume of the child droplets/fluids if necessary. For DMFB, only equal splitting of droplets is possible and, thus, both lb_{cd} and ub_{cd} are always 1X. Instead MEDA, CFMB, and DMN also allow unequal splitting, but the two equations lb_{cd} = lb_{dv} and ub_{cd} = ub_{dv} - lb_{dv} always hold, due to the previous constraints.

Overall, setting all the constraints/variables as introduced above allows the designer to select a setup for sample preparation which is inspired by a specific microfluidic platform (e.g., DMFB, MEDA, or any other). Then, the set of instantiated constraints can be passed to a corresponding generic sample preparation method which will generate the desired result for the chosen/instantiated platform. Thus, the proposed method allows the user to perform design explorations on how the desired concentration ratio can be realized on different platforms.

Example 2. *Let's assume again that a target-*CF = $\frac{7}{16}$ *should be generated. If DMFB is considered as platform, then the constraints listed above are initialized as follows:*

- Constraints on the Dispensing Operation: *The minimum (lb_{dv}) and maximum (ub_{dv}) volume is initialized with 1X and the granularity V_g is set to 0 since DMFBs do not support variable size droplets.*
- Constraints on the Mixing Operation: *The minimum (lb_{md}) and maximum (ub_{md}) volume supported by the mixer is set to 2X and U_{mix} is set to* false*, since DMFBs do not allow to mix unequal droplet volumes.*

[1]Note that, to the best of our knowledge, no automatic sample preparation methods for DMN exist yet.

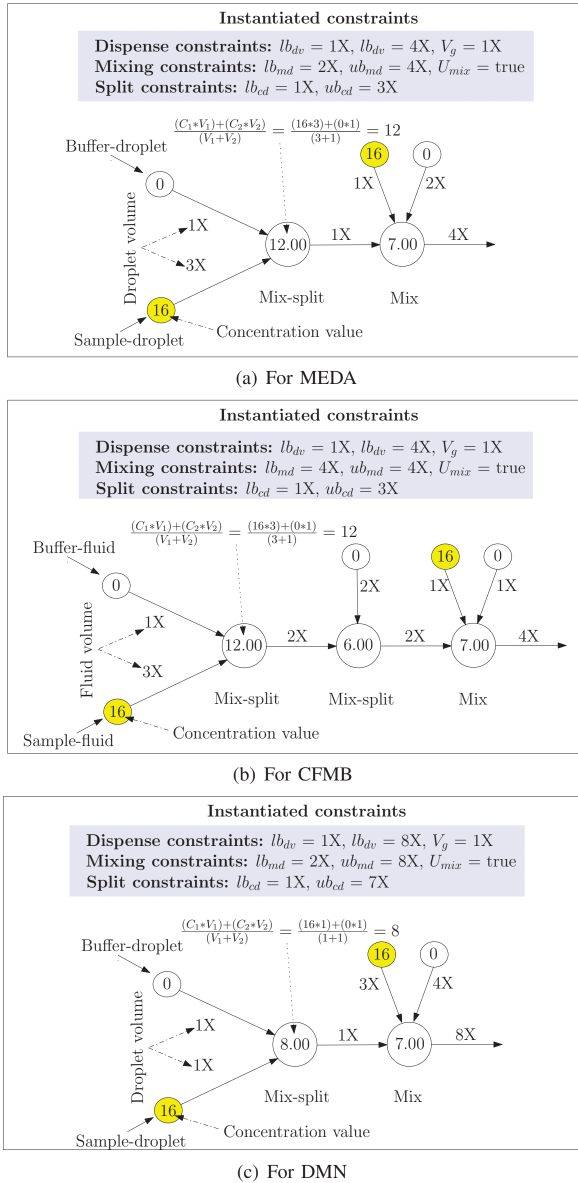


Fig. 3. Sequencing graphs generated using the proposed generic approach

- Constraints on the Split Operation: *The minimum* (lb_{cd}) *and maximum* (ub_{cd}) *volume of the child droplets are defined by 1X since DMFBs only support equal splitting.*

Doing a sample preparation with such constraints eventually will lead to a solution as shown in Fig. 2(a), i.e., exactly the same result is obtained by considering the characteristics of DMFBs in a dedicated fashion and using an instantiation of the generic constraint proposed above.

In a similar fashion, results for MEDA and CFMBs can be generated using an instantiation of the constraints as sketched in the top of Fig. 3(a) and Fig. 3(b), respectively. This leads to sequencing graphs as shown in the bottom of Fig. 3(a) and Fig. 3(b) which, although not exactly identical, are rather close to the results obtained by the dedicated approaches shown before in Fig. 2(b) and Fig. 2(c).

Moreover, the use of generic constraints even allows to generate a sequence graph for DMN, for which no solution has been proposed thus far. This can be accomplished by instantiating the constraints as shown in the top of Fig. 3(c) – leading to the result as shown in the bottom of Fig. 3(c).

IV. Conclusions

In this paper, we proposed the idea of a generic method for sample preparation for different microfluidic platforms. By this, we aim to break the "vicious circle" of constantly (re-)developing sample preparation methods for each and every platform and plan to provide designers with a method to quickly check what platform is most suited for the desired task. To this end, we generalized the characteristics of the different microfluidic operations needed for sample preparation and formulated them in terms of generic constraints to be instantiated for the respectively considered platform. The proposed idea has been illustrated by means of examples. Future work obviously includes the implementation and evaluation of the proposed idea.

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