Concept and simulation of micromixers and aliquoting for PCR systems

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Abstract

In this paper, different concepts for mixing sample and master mix by means of simulation are discussed. The main focus was on mixers with a low dead volume, because of their relevance to PCR. In addition, a concept for aliquoting the mixed solution was elaborated and simulated. The simulation showed very good mixing with serpentine- and tesla-mixers at flow rates of 1-16 µL/s. Furthermore, a uniform distribution with aliquoting could be achieved, while a slight dependence between flow rate and pressure was observed. In the further course, these concepts are to be combined and tested on a microfluidic chip. This can then be integrated into the workflow of existing PCR protocols.

1. Introduction

Normally, sample preparation for PCR instruments is carried out with trained personnel. This includes the dosing of a mastermix with a biological sample with subsequent mixing, as well as the division into several sample vessels. To reduce the workload, approaches of fully automated PCR systems are pursued, which in most cases can only be used in proprietary thermal cyclers. To tackle this problem a “System on a Chip” can be a solution. In this case the chip takes the workload of mixing the biological sample and the distribution of such into sample vessels. Mixing the sample evenly is a key factor for reproducible results with PCR systems. Therefor different kinds of fluidic mixing principles are discussed in the next chapter.

2. Theoretical background

2.1. Fluidic mixing principles

Micromixers can be divided into planar and three-dimensional systems. Three-dimensional mixers often allow better mixing because turbulence can be introduced in a further spatial axis perpendicular to the direction of flow. However, these must be machined on both sides during manufacturing. This presents challenges in manufacturing as well as in joining technology. The three-dimensional mixers generally additionally have a larger dead volume than planar mixers.

For this reason, this paper focuses on planar mixers (Figure 1) and explains their most important principles in the following. The most intuitive mixers are so-called serpentine structures (or meander structures). In the process, the near-edge flows penetrate the middle flow area at edges through a stall. A further development of this was presented by Lee et al. (Lee et al. 2011). In this mixer, there are finer channels through the meander structures. They function as "shortcuts". These channels are supposed to fill with the help of the capillary effect and release the channel contents with the occurrence of a flow at the other end of the channel. For this to work successfully, the channels for the "shortcuts" must be much thinner than the main channels. Otherwise, most of the flow would go through the "shortcuts" in the main flow direction.

Another important principle is found in split-and-recombine mixers (SAR for short). Here the flow is repeatedly split and combined, resulting in collisions. The resulting turbulence leads to mixing. SAR mixers can be further divided into symmetrical (Tran-Minh et al. 2014) and asymmetrical mixers (Razavi Bazaz et al. 2020) . However, due to their parallel channels, most SAR mixers have a rather high dead volume, which makes system emptying via air supply through an inlet considerably more difficult. A special type of SAR mixer is the Tesla mixer. It stands out due to its better mixing properties and the numerous investigations in studies. The design of this asymmetric mixer allows the recirculation of liquid components into the downstream mainstream. Thus, it achieves a similarly high degree of mixing in the wider Reynolds range as the serpentine structures.

Finally, there are planar micromixers that work with obstacles. In these, bodies such as prisms or cylinders stand in the channels (Bhagat et al. 2007) . Each obstacle acts as a kind of SAR mixer and can occupy all or part of the height of the fluidics. However, the small obstructions relative to the channel make fabrication difficult, as they could easily tear or break off during casting or milling. Therefore, this type is more suitable for lithographic manufacturing.

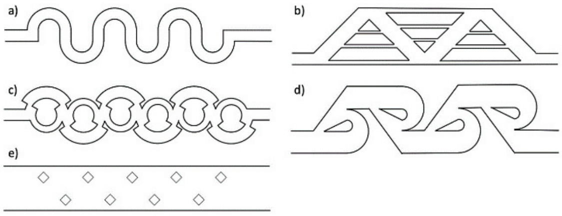

Figure 1: a) serpentine, b) serpentine with shortcuts, c) asymmetrical SAR, d) tesla SAR, e) obstacles

2.2. Flow distribution

For multiple determination of samples or redirection to other areas of the microfluidic chip, flow splitting is necessary. For this purpose, Y- or T-crossings are usually used to achieve symmetrical splitting. Splitting's according to this principle are only possible in fractions of size 2-n. To achieve other fractions, other techniques must be used. Commonly used for this are planar manifolds, in which the fluid is directed consecutively into branches. However, the partitioning in these systems is highly dependent on the fluid properties and on the flow velocity. Another alternative is offered by three-dimensional manifolds, such as those offered by Darwin Microfluidics. Here, a liquid flow entering from above is distributed to several outlets arranged in a circle. However, this principle is hardly integrable on microfluidic chips since no impulse along the xy-plane may occur for precise distribution.

3. Material and Methods

In the beginning, a literature search was carried out on existing micromixers and their mixing efficiency. After evaluating the systems according to mixing efficiency, production possibilities and dead volume, individual systems were selected for further review. Based on the criteria mentioned, the decision was made for meander structures without and with "shortcuts" as well as Tesla mixers. To obtain the most meaningful simulation results possible, a tapered Y-junction was chosen as the fluid inlet in a 1:3 ratio. In this way, proportionality between channel widths and the corresponding volumetric flow can be achieved. The original channels are parallel to each other and have a length of 1 mm, so that the flow is as laminar as possible at the beginning.

For the sake of comparability, a uniform channel depth of 0.3 mm was chosen. The mixer outlet should have a width of 0.6 mm. To avoid pressure differences and speed changes, the sum of parallel channel widths should also be 0.6 mm. Due to manufacturing constraints, it was determined that channels should have a minimum width of 0.1 mm with minimum inner radii of 0.05 mm. An outer radius of at least 0.025 mm was chosen to limit the simulation time. With a sharp edge, on the other hand, grid refinement would not lead to simplification of the problem.

Two approaches can be chosen to third a flow in planar microfluidic systems. One of the two is instantaneous thirding, which will not be discussed further here as it is assumed to be highly momentum dependent. The other approach uses two consecutive Y-crossings. At the first one, a division in the ratio 2:1 is to take place. The second crossing should then halve the larger current again. The Simulations were solved using SolidWorks Flow Simulation from Dassault Systèmes.

4. Results and Discussion

Planar micromixers were chosen to mix a master mixture with a biological sample. This requires a less complex manufacturing and joining technique than three-dimensional mixers. Based on this, serpentine mixers with and without diffusers as well as a Tesla mixer were designed and simulated. The simulations were carried out with total volumetric flows rates of 1 µL/s and 16 µL/s. In Figure 2 it can be seen that the liquid moves through the mixer in a largely laminar manner and that no or only slight turbulence occurs due to the stall at the edges. At the higher speed, there are still visually recognisable differences at the outlet. They correspond to concentrations of about 0.248 and 0.252. For the quantitative evaluation, the variance-based mixing index MV was determined. This shows very good mixing. Therefore, the more sensitive, standard deviation-based mixing index MS was used for further evaluation of the mixing. This also shows very good, almost complete mixing at the selected flow velocities. mixing. Figure 3 shows a lower mixing rate than Figure 2. The fluid simulation Figure 4 show a clear backflow of the fluid around the drop-shaped

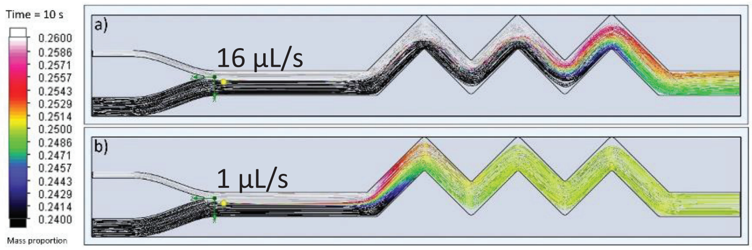

Figure 2: Serpentine mixer with different volumetric flow rates

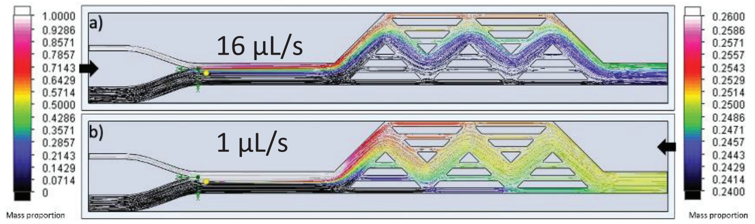

Figure 3: Serpentine mixer with 0.15 mm passageways

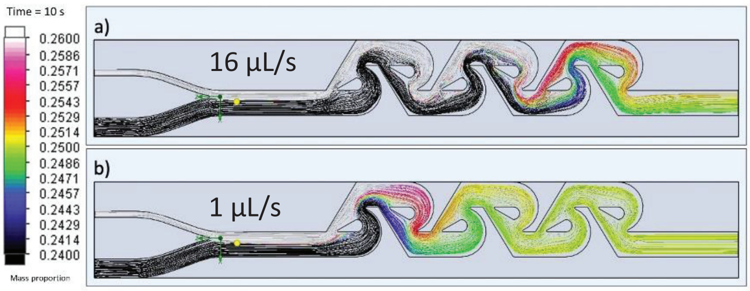

Figure 4: Tesla mixer after a 10 s timeframe

obstacle. This is accompanied by the successively increasing mixing of the liquid, which can be seen at both flow velocities.

	Mixing Index 16 µL/s	Mixing Standard Deviation Coefficient 16 µL/s	Mixing Index 1 µL/s	Mixing Standard Deviation Coefficient 1 µL/s
Serpentine mixer	0.9967	0.56 %	0.9999	0.00004 %
Serpentine mixer with passageway	0.7623	42 %	0.9998	0.03 %
Tesla mixer	0.9982	0.1 %	0.9999	0.0001 %

Table 1: Mixing Index and Mixing Standard Deviation Coefficient for the chosen micromixers.

Figure 5 shows the pressure drop. This could be determined by the simulation. The pressure drops evenly over the flow splitter to the normal pressure at the outlet by about 2300 Pa. This pressure drop is the result of molecular interactions between the water and the wall (adhesion) and the surface tension of the water. There is a slight dependence on the flow velocity. As expected in advance, the ratio of the splitting is 1:1 in most of the flow velocity range. As the velocity increases, the influence of the momentum increases. For this reason, the flow is higher in the outer channel.

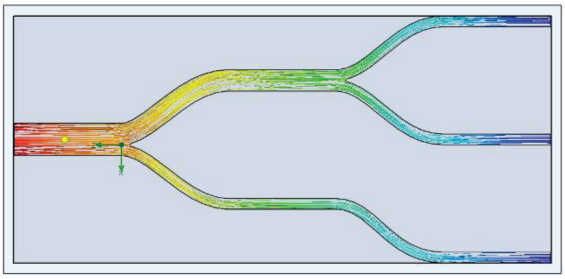

Figure 5: Simulated pressure drop of the Flow Splitter

5. Outlook

Since interactions can occur between the individual system components simulated so far, a simulation of the entire system is essential before production. For this, the system must be designed and simulated before an evaluation with adjustments can be made. Once the simulation results are satisfactory, the system can be manufactured and tested in the laboratory setup.

Literature

Bhagat, Ali Asgar S.; Peterson, Erik T. K.; Papautsky, Ian (2007): A passive planar micromixer with obstructions for mixing at low Reynolds numbers. In: *J. Micromech. Microeng.* 17 (5), S. 1017–1024. DOI: 10.1088/0960-1317/17/5/023.

Lee, Chia-Yen; Chang, Chin-Lung; Wang, Yao-Nan; Fu, Lung-Ming (2011): Microfluidic mixing: a review. In: *International journal of molecular sciences* 12 (5), S. 3263–3287. DOI: 10.3390/ijms12053263.

Razavi Bazaz, Sajad; Amiri, Hoseyn A.; Vasilescu, Steven; Abouei Mehrizi, Ali; Jin, Dayong; Miansari, Morteza; Ebrahimi Warkiani, Majid (2020): Obstacle-free planar hybrid micromixer with low pressure drop. In: *Microfluid Nanofluid* 24 (8). DOI: 10.1007/s10404-020-02367-x.

Tran-Minh, Nhut; Dong, Tao; Karlsen, Frank (2014): An efficient passive planar micromixer with ellipse-like micropillars for continuous mixing of human blood. In: *Computer methods and programs in biomedicine* 117 (1), S. 20–29. DOI: 10.1016/j.cmpb.2014.05.007.