Simulation of Droplet-based Microfluidic Lab-on-a-Chip Applications

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Abstract Miniaturization of biological and chemical assays in lab-on-a-chip systems is a highly topical field of research. Droplet-based microfluidic chips are types of these miniaturized systems. They expand the capability of assays with special features that are unreached by traditional workflows. In particular, small sample volumes, independent separated reaction units, high throughput, automation and parallelization of assays are prominent features of droplet-based microfluidic devices. Full custom centric design of droplet-based microfluidic lab-on-a-chip technology implicates a high system integration level and design complexity. Therefore advanced development methodologies are needed, comparable with the methods in electronic design automation. Our design and simulation toolkit meets these requirements for an agile and low-risk development of custom lab-on-a-chip devices. The system simulation approach enables a fast and precise prediction of complex microfluidic networks. This fact is confirmed by reference and benchmark experiments. The results show that the simulation correctly reproduces the experimental measurements.

Keywords: droplet-based microfluidics, micofluidic design automation, system simulation, lab-on-a-chip technology

1. Introduction

Lab-on-a-chip technology aims to bring complex laboratory workflows into small and portable devices that can be used independently from the laboratory infrastructure.

It enables for assays in the field of life sciences all the advantages of miniaturization (Mitchell 2001, Gulati 2009). Key features are small sample and reagent volumes, low cost and less laboratory space, high throughput of samples, potential for point of care diagnostics and the high automation of repetitive assay steps. Taking the advantage of these features depends on a fast, reliable and low-risk way for lab-on-a-chip development. The result of this process has to meet the functional and non functional requirements of the prospective user.

Full custom centric design in droplet-based lab-on-a-chip systems means the integration and functional combination of dedicated microfluidic operation units for sample droplet generation, dosing, mixing, merging or aliquoting into an application specific lab-on-a-chip system (Henkel 2004). To cope with the increasing complexity of development (Thorsten 2002), robust methodologies and processes developed by modern engineering into microfluidic development. Up to now iterative development is the most common approach in lab-on-a-chip technology. Considering, that a single development cycle takes about three months. Reducing the number of required iterations becomes a key issue for efficient development of lab-on-a-chip systems for custom process protocols.

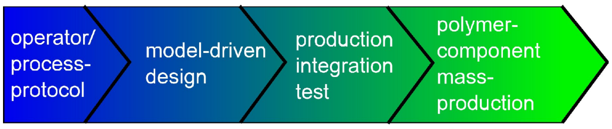


Fig. 1. Development cycle for microfluidic lab-on-a-chip systems

The solution is to serialize lab-on-a-chip development into well-defined tasks as shown in fig. 1 with milestones and decision points after each. At the end of each task must be decided, weather an additional development turn for the preceding tasks is required or if the next task can be started.

Primary tasks like the requirement analysis and the implementation of the process protocol demand special attention. Failure in an early stage of development entails huge effort later. Efficient computer assistance tools like CAD tools for microfluidic bio chips can prevent this waste of resources (Chakrabarty 2005).

Currently there are some CAD tools for microfluidics available. Toolkits based on computational fluid dynamics (CFD) are useful for all kinds of microfluidic design and simulation tasks (Glatzel 2008). The disadvantage of this technique is the huge consumption of resources in time and processing power. Also, the usability of this approach is limited to small systems like single operation units, while the simulation of complex lab-on-a-chip systems is not practicable.

In this paper we report on implementation and validation of a software toolkit for systems simulation of droplet-based microfludic networks. Therefore a microfluidic chip device, that behaves like a state machine is prepared. The operation characteristics are measured and compared with simulation results. Conformity of simulation and experimental results indicates correct operation of the toolkit.

2. Droplet-based microfluidics

The main field of application for the presented fluid network simulation toolkit “FluNet” is to assist the development process for droplet-based lab-on-a-chip systems.

In droplet-based microfluidics each droplet represents an individual sample, that needs to be processed according to a given laboratory workflow. This workflow can be described as a sequence of unit operations. For each unit operation like dosing, mixing, splitting or merging droplet based microfluidics is providing a microfluidic operation unit (Haeberle 2007). The laboratory workflow is now implemented by appropriate interconnecting these operation units. An overview on recently available operation units is given in fig. 2. All these operation units are controlled by pressure driven flow and implemented as self operating or self controlling units. That means – they perform their operation without additional external actuation or control dependent only on process conditions and pressure drop at the different ports of the unit. Process characteristics of an operation can be controlled by geometrical scaling of the unit (Henkel 2004, Malsch 2008, Ackermann 2007). Pressure driven droplet based microfluidic networks are inherently difficult to integrate and scale. The flow characteristics at a distinct point are governed by parameters that vary along the flow path. At any location the droplet dynamics are globally coupled. This means almost all droplets influence each other. In combination with the nonlinear equations describing the droplet displacement (Schindler 2008), it generates a high grade of complexity for the estimation of the expected behavior. Our CAD-toolkit is primarily designed for setup, systems simulation and interactive optimization of pressure driven droplet-based microfluidic lab-on-a-chip devices. The selected software architecture takes care on portability and thus, the toolkit can be easily adapted to other microfluidic platforms with low effort.

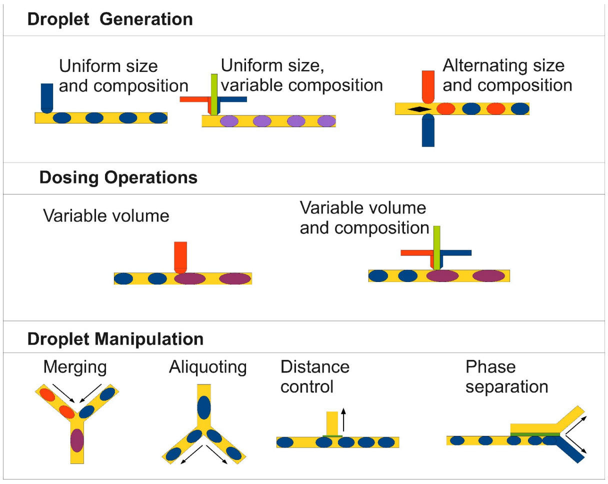


Fig. 2. Symbolic representations of available and interoperable operation units for droplet generation, dosing and droplet manipulation.

3. Microfluidic networks

Comparable to large scale integration in electronic design automation (EDA), a fast behavioral simulation for droplet-based microfluidic systems is necessary to prevail in the face of their raising complexity by establishing microfluidic design automation. Therefore, a system simulation approach for droplet-based microfluidic networks has been

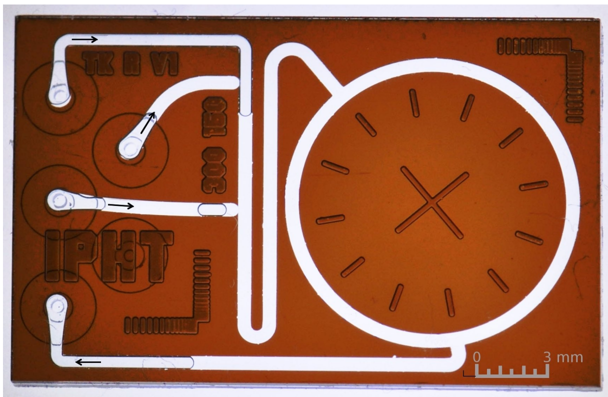


Fig. 3. A microfluidic chip fabricated in all-glass technology for benchmarking the simulation toolkit. Dependent on the particular system state the droplet choose either the short or the longer branch of the loop.

developed. The example chip shown in fig. 3 implements a self-regulated channel network. It is used in our paper to explain the simulation approach and as a benchmark for the capabilities of the simulation toolkit. The chip is based on similar designs previously reported in literature (Link 2004, Fuerstmann 2007).

A combination of microfluidic functional units on a chip as shown in fig. 3 basically represents a network of channels, intersections and interfaces, thus a network of interconnected basic operation units. Hence, every operation unit has a representative counterpart in the abstract microfluidic network, mapping its functionality as well as geometrical and physical parameters. The corresponding microfluidic network map of the chip is shown in fig. 4.

The microfluidic network specifies only the necessary information to understand the behavior of a microfluidic design and to derive the fabrication layout. The full information, like the final placement of the operation units is defined in the additional documentation. Our toolkit provides tools to set up these networks (fig. 5).

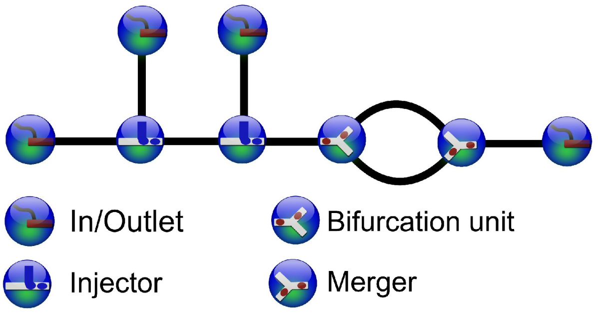


Fig. 4. The microfluidic network map is an abstraction of the chip form fig. 3 to the level of interconnected operation units.

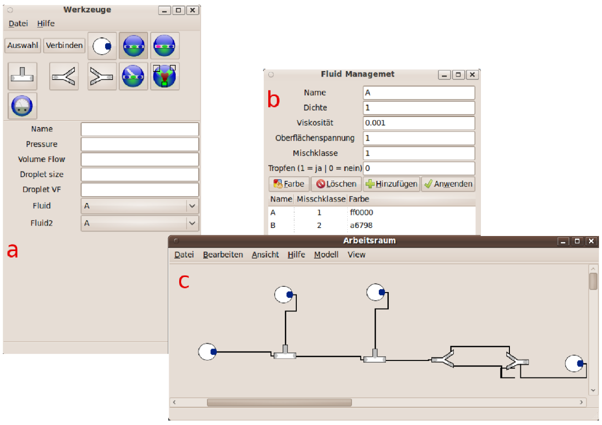
Every single basic operation unit has a parameter set by which it is completely characterized. These parameters have to be specified when the user inserts the equivalent symbol on the workspace to assure that all requirements for every operation unit are met. A simple plausibility test guarantees the correct syntax of the microfluidic network by checking that all operation units are connected and all parameters are set. For the analysis of the requirements it is important to have a clear and unambiguous communication between the engineer and the prospective user to ensure the correct functionality of the developed chip. The microfluidic network can be used to discuss the functionality and behavior. In summary, a microfluidic network formalizes the requirements for the lab-on-a-chip system. This kind of information organization is the best input for a computer simulation.

3.1 Organization of microfluidic network

A software representation of a microfluidic network is a consistent model. It supports the functionality of basic operation units and the transport of fluids in the channel. Implemented rules for the behavior of the basic operation units and the transport characteristics of the channels were deduced from experimental data and CFD simulations.

In the software model, operation units define the behavior of fluids at channel crossings. The functional rules of these units describe the behavior of fluids passing them. So the implementation of operation units defines the changes in the flow and the path of the droplets.

The total volume of the fluids is allocated in the channels connecting the operation units, they have to realize the transport of fluids. No change in the order of fluid segments in the channel happens during the transport. It is only a simple transport in one way dependent on the pressure gradient. Therefore channels implement the transport model of the droplet-based microfluidics.



Fluid A

Fluid2 A

Name A

Dichte 1

Viskosität 0.001

Oberflächenspannung 1

Mischklasse 1

Tropfen (1 = ja | 0 = nein) 0

Name	Misschklasse	Farbe
A	1	ff0000
B	2	a6798

Fig. 5. CAD Tool for the development of droplet-based microfluidic applications
Window **a** is the tool selection. It contains the parameter set for the selected operation unit.
Window **b** describes the properties of the fluids used by the simulation system.
Window **c** is the design workspace. This is the place where the operation units are combined to form the microfluidic network.

4. Simulation

The aim of the simulation is to determine the behavior of droplets in a microfluidic network that is derived from an application specific lab-on-a-chip device concept. Pressure drop and volume flow are the main state variables from which the droplet displacement and the fluid sequence in the channels can be calculated by taking into account the hydrodynamic parameters. With respect to the implemented microfluidic organization based on functional nodes that are linked by connectors its overall behavior can be calculated with some general rules. Kirchoff described these rules for electronic circuits with the electric current as transport variable. The other value is the force which drives the movement of the electrons, the electric tension. Both values are connected through the electric resistance. In analogy there is the volume flow, the pressure drop and the fluid resistance in microfluidics. (Angelescu 2011) The rules of Kirchoff laws allow to calculate the distribution of current and electric tension in the network with respect to the resistance on each node. Considering the resistive behavior of the nodes this leads to a system of differential equations.

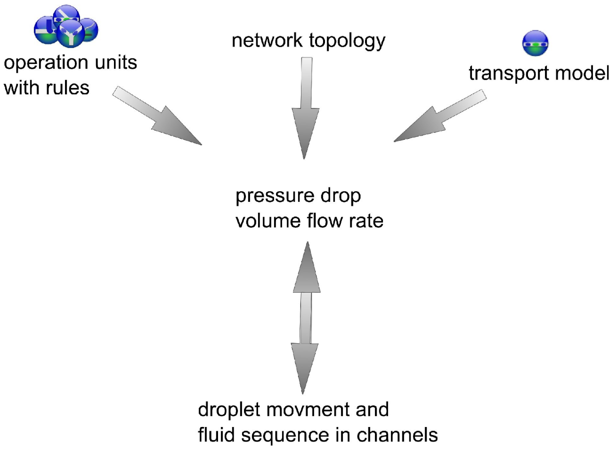


Fig. 6. Overview of the simulation process. It shows how the boundary conditions of the simulation and the state variables are linked with the virtual fluid administration.

4.1 Simulation algorithm

To avoid the solution of complex differential equations, an algorithm is implemented which is based on the modified nodal analysis (Ho 1975). This algorithm calculates electric current and electric tension in a network. It deals with nonlinear electric resistances and energy storages based on the Kirchoff laws. So it is adequate for the calculation of microfluidic networks.
Yet the similarities between electric and microfluidic networks already ends at the homogeneity of the transported matter. In droplet-based microfluidics the transported matter is an inhomogeneous stream of droplets separated by another fluid. Many adjustments have to be considered to transfer the methodologies from the electronic design automation. An overview of the algorithm provides fig. 6. The network representation on which the simulation is based depicts a further abstraction of the microfluidic network. Its general topology is preserved, but it contains some important differences. The functional elements and the droplets are mapped to nodes. Additionally, each droplet in a channel represents a separate node in the simulation network, because of their huge influence on the hydrodynamic resistance calculation (Schindler 2008, Labrot 2009).
A central ingredient of the calculation algorithm is the transport model describing the correlation between pressure distribution,

volume flow rate and the hydrodynamic resistance in the channels. It was derived from experimental data, obtained by measurement of pressure throughput characteristics in test channels with systematic variation of droplet distance and flow rate. The pressure drop in a channel with a given volume flow rate depends on the count of droplets, the distances between them, the geometry of the channel and the used fluids.

To obtain a mathematical form suitable for the solution algorithm, the simulation network is converted to a matrix representation. This matrix is based on the application of the Kirchoff's circuit law as a system of equations. The values for resistance are calculated with the help of the transport model and the application of operation unit rules.

4.2 Flow simulation

Due to the inhomogeneity of the transported matter, it is necessary to know the duration during which the volume flow and pressure drop are constant. Events like the creation of a new droplet or a droplet passing a functional node require a new calculation of the physical variables on each node in the simulation network. Thus, prior to droplet moment all channels and functional elements are tested for such events to determine the next time step.

Based on this, new positions for each segment in each channel are calculated. Through the previously determined length of the time step it is impossible that a droplet will pass an operation unit during two events. After the movement in the channels the simulation algorithm process the operation unit rules for fluid administration. These rules describe the change of the segment sequence by the operation units.

Operation units with more than two ports need rules on how to distribute the amount of fluids based on their functionality. Therefor each operation unit has its own set of rules.

After the movement of the segments a new calculation is necessary. The old values for pressure drop and volume flow are good start values for the next calculation.

4.3 Simulation efficiency

Basically the calculation time for a simulation is influenced by the number of droplets in the system, the formation rate of droplets and the amount of operation units in the network. For a given observation time the simulation time also dependents on the volume flow since the number of events that have to processes are determined by it.

The simulation is fast enough to enable the engineer to test a micro channel network design within minutes what otherwise would require days or weeks. This improvement of the development procedure leads a way to a faster, low-risk and more reliable development. It decreases the main risk of development not to meet the requirements with the product. This means a waste of time and resources can be avoided by using a simulation-assisted development process. The simulation during on early design stages gives certainty, because their results can be often compared with the requirements. These facts prove the practical importance of the development toolkit.

5. Validation

The combination of the transport model, the simulation algorithm and the virtual droplet administration is validated with an experiment.

5.1 Methodology

A circular micro channel system with two T-shaped junctions for inflow and outflow of droplets is used for the benchmark as microfluidic state machine. The system was previously described for investigation of droplet transport characteristics (Link 2004, Fuerstmann 2007, Labrot 2009). The flow rate in each of the branches depends on the number and distance of the droplets inside. A droplet, arriving at the loop is directed to the branch with the higher flow rate. So, transport of droplets continuously changes the system state. These changes are measured by digital imaging and compared with the simulated system evolution. The assumption that the droplet count in the branches of the loop, the droplet size, the droplet spacing, the channel geometry and the volume flow influences the the hydrodynamic resistance can be verified with the benchmark chip.

When a droplet moves through a branch, it increases its hydrodynamic resistance. The pressure over both branches is equal and the sum of the volume flows in the loop is constant. Hence, the change of hydrodynamic resistance results a change of volume flow in both branches of the loop. A higher flow rate is the result of a smaller hydrodynamic resistance. The change in droplet velocity visualizes the variations of the hydrodynamic resistance with respect to the current parameters. Each decision of a droplet for on branch modulates the speed of the droplets in both branches. Therefore, the microfluidic network is in itself a relative unstable system. Small differences in the flow conditions will lead to large changes in the behavior of the droplet sequences.

During the validation experiments the velocity of the droplets is examined as an indicator for the volume flow rate. This is an indirect method to measure the hydrodynamic resistance. The speed of the aqueous droplets that have been colored by a dye is measured with a charge coupled device (CCD) camera and the resulting images are processed with a custom image processing tool based on OpenCV. Obtained are the velocities of all droplets and the droplet count in each channel. The results are shown in fig. 7 upper diagram.

A microfluidic network of the same chip used for the experiments is simulated and compared to the experimental results.

To set up the simulation all geometrical and hydrodynamic parameters are adapted to the experimental setting. The results of the simulation are shown in fig. 7 lower diagram.

A loop with a asymmetric loop branches is used for the validation experiment. This fact defines the path of the droplet even if the same count of droplets were in the branches. The experiment and the simulation starts with an empty loop and tat is filled up with droplets.

Both approaches result in a constant periodic sequence, as is shown in fig. 7.

5.2 Results

Touchstone of the network simulation algorithm is its ability to handle complex network architectures with integrated fluidic loops correctly. The experimental verification leads to the result shown in fig. 7. It reflects general effects of droplet-based flow in channels. Change of the droplet count results in a change of the measured or calculated volume flow.

As discussed, the volume flow in both channels represents the relation between droplet count and hydrodynamic resistance. Therefore changes in the hydrodynamic resistance are reflected by changes in volume flow. This can be seen in the diagrams on fig. 7. A high droplet counts raise the hydrodynamic resistance in the channel. An incoming droplet increases the hydrodynamic resistance. As a result the volume flow in the affected channel drops (for example at 5.1s in the experiment). As seen in the diagram the volume flow rate in the other branch increases accordingly. In the experiment the volume flow rate change happens immediately after the change in droplet count. The influence of fluid inertia is smaller than the temporal resolution of the experiment. The simulation results reflect this. As can be seen in direct comparison in fig. 7. the trailing or raising edges of droplet count and volume flow rate change almost synchronously both in the experiment and the simulation. The behavior of the volume flow rate as a result of the changes in droplet count is the same in both diagrams. This implicates the conformity of the simulation and the experiment.

The droplet count represents the droplet sequence in the channels and thus representing the behavior of the system. So the almost equal droplet count graphs for the simulation and the experiment also indicate the accordance between them.

The comparison between the simulation and the experiment exposes the nearly identical behavior of both. The differences in droplet timing entering or leaving the loop is based on the high sensitivity to small differences in the experimental settings. The experimental

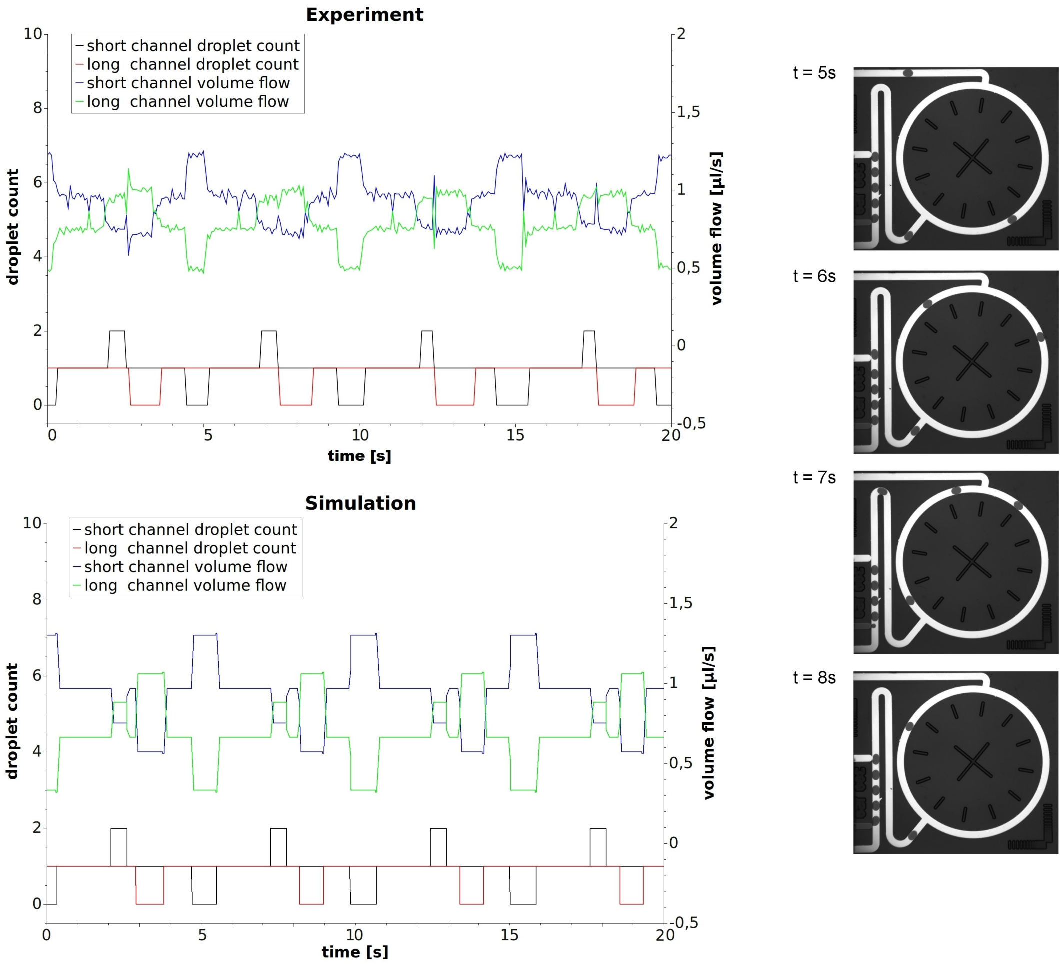


Fig. 7. Comparison between experiment results (upper diagram) and simulation results(lower diagram). The pictures on the right side show the generated droplet patterns in the experiment.

uncertainties can lead to a minimal delay in the droplet timing for a split second. In nearly every setting this will lead to a different behavior of the whole system. The droplet count in a channel changes the droplet velocities in both branches. In consequence the choice of the droplets influence the moment of the next leaving droplet, which changes the system behavior again. The simulation results were exactly reproduced for each periodical droplet pattern. The experimental results vary sometimes but only in a small amount around the uncertainty of experimental data. So the simulation algorithm, the underlying models and the droplet administration were validated.

5. Conclusion

The simulation algorithm has been presented and successfully applied. The developed simulation network aggregates functional nodes, interconnected by virtual channels. Droplets are administrated in their spatial and temporal context including changes in volume, composition and fluid parameters caused by droplet processing at operation units.

Functional nodes in the microfluidic networks map the behavior and functionality of microfluidic operation units. In contrast to common simulation tools the toolkit does not implement the finite element method. A simulation speed is reached which enables new agile and low-risk development methodologies. The toolkit integrates seamless in the development procedure.

The simulation results for the selected benchmark system conform with the measured data. So, the toolkit is able to model even systems correctly, that respond very sensitive on parameter changes. The simulation itself takes only a few seconds. So interactive optimization of a lab-on-a-chip design becomes possible. The results can be visualized and discussed with the customer to verify the correct implementation of the given protocol already at the design task. By conventional development, this step becomes part of the initiation process and requires fabrication of prototypes. Risks and uncertainty in the development are reduced by using the toolkit, because the development toolkit prevents early failure. The number of development cycles for the most time consuming and expensive prototyping stage can be significantly reduced. Efficiency in lab-on-a-chip development is increased, the development risks are reduced. Moreover, the toolkit – and the planned components for user interaction and design assistants take care on consistency and completeness of the process description and the availability of all of the required parameters and fluid properties. This makes the toolkit to a valuable component in microfluidic design automation (MDA) similar to the design tools used in EDA.

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