PRESSURE PREDICTIONS FOR LAB-ON-A-CHIP OPERATIONS USING A MICROFLUIDIC NETWORK SOLVER AND FLUIGENT PX

Reliable operation of lab-on-a-chip systems depends on user-friendly, precise, and predictable fluid management tailored to particular sub-tasks of the microfluidic process protocol and their required sample fluids[1]. **Pressure-driven flow control**, where the controlled pressure pushes the liquid of the reservoir, provides excellent flow control for a wide range of flow rates with a response time of few milliseconds

We report on the use of Fluigent products, and more specifically the **Fluigent PX**, for delivering highly precise predicted pressures with an excellent response time using a microfluidic network solver developed by Böke et al. The benefits of this approach are demonstrated by creating multi-component laminar co-flow and the creation of droplets with variable composition.

This case study was made possible thanks to Julia Sophie Böke, Daniel Kraus, and Thomas Henkel from the Leibniz Institute of Photonic Technology in Jena, Germany. For more information, visit their team website.

Introduction

Over the last decade, complex microfluidic flow protocols have been implemented, allowing to integrate several fluidic steps into a single device . It is becoming more common to see analytical devices that include reagents mixing, droplet or particle generation (with analytes such as cells encapsulated within them), and analysis. As a result, the performance requirements of microfluidic devices are increasing, and reliable control and prediction of flow rate and operating pressure are crucial. For automating microfluidic workflows, "Microfluidic design automation" (MDA) tools have been developed[1]. They permit the prediction of the required pressures for achieving the desired flow rates for a specific microfluidic chip. It relies on the relation between the pressure difference, flow rate, and the hydrodynamic resistance, which are analogous to a voltage difference, current, and electrical resistance respectively. If the pressure and resistance of the system are known, the flow rate can be determined. The definition of resistive networks permits one to predict the flow rate within the microfluidic network (MFN). This provides precise data on the transport of fluids, which allows one to achieve co-flows or droplet generation in an effortless manner.

In this case study, we report the use of Fluigent products for the development of a microfluidic platform where fluids are controlled using an MFN solver (the software is called mfnSolver) for fast and precise prediction of pressure settings for steady-state operation conditions and gaining insights into on-chip pressure drop and flow characteristics. The microfluidic automated operations are demonstrated by creating laminar co-flow of up to four components, and droplets with variable composition in different regimes.

Materials & methods

1) Microfluidic flow control

- Fluigent PX-1 pressure-based flow controllers, 1 bar. The quality of set pressure and displayed pressure is illustrated below.
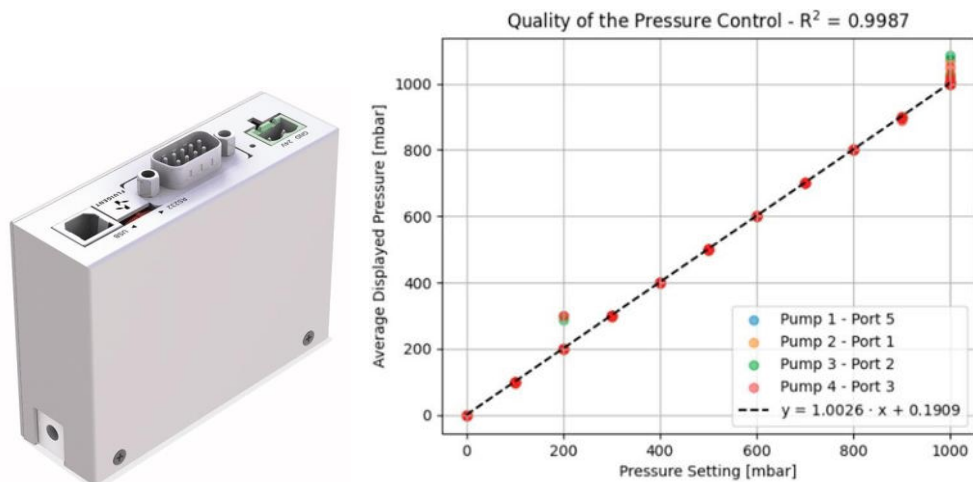

- Reservoirs: Fluiwell-4C 2 mL vials screwed to its rack for pressure connection
- Flow sensors: Fluigent Flow Unit M (0-80 µL/min)
- Flow control software: Fluigent OxyGEN software

2) Microfluidic chip

The flow-focusing unit (FFU) of the Leibniz-IPHT PDG2 Droplet Generator Chip (PDG2). The PDG2 chip is a glass chip design that permits either the creation of laminar co-flow for up to four components, or droplets with variable composition in different regimes (dripping, transition, jetting) from up to three components. It consists of 4 inlets (P1, P2, P3, P5) and 1 outlet (P4)[1].

Dimensions: Channel width: 165 µm before the flow focusing region, 200 µm at the observation chamber, and 150 µm otherwise - Channel height: 100 µm.
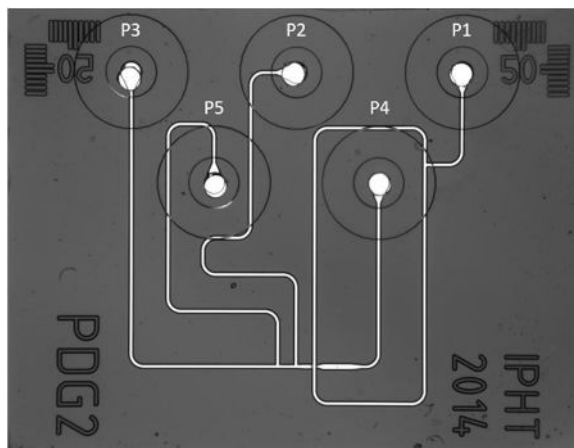
For droplet generation, a surface treatment is performed with PlusOne Repel-Silane ES (a solution of Dimethyldichlorosilane (2% w/v) in Octamethylcyclotetrasiloxane).

3) Buffer and Reagents

- Dye solutions: 2 mmol/L Bromophenol blue solution (Sigma-Aldrich) for which 13 mg of the sodium salt dye was dissolved in 10 mL sodium phosphate buffer - 4.9 mmol/L Orange G solution containing 22 mg disodium salt dye in a 10 mL buffer
- Surfactant for droplet generation: Fluigent dSurf (2% surfactant in Novec™ 7500)

4) mnfSolver

The mfnSolver developed by Böke et al. provides the pressure and flow rate at all defined nodes and edges of the microfluidic chip[1]. For a given network, the solution can be obtained manually or semi-automatically using computer algebra systems. As explained previously, it is based on Kirchhoff's first law. Similarly to the relation between voltage, current and electrical resistance, a relation between pressure difference, flow rate, and hydrodynamic resistance exists, with the following equation:

$$R = -\frac{\Delta p}{Q}$$

A user defines the flow rates along arbitrary edges of the microfluidic network, and the solver calculates the required pressure settings at the Input-Output's to achieve these flow rates. One of the main benefits of the developed simulation is its ability to solve a complete microfluidic system for inline prediction of the required pressure settings within less than 200 ms[1].
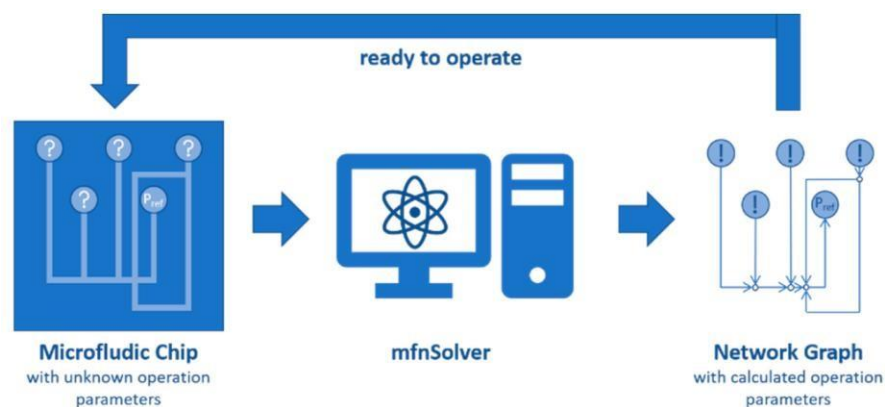
Figure 1: Schematic representation of the solver concept and its purpose[1]

Finally, the mfnSolver can be utilized to design and optimize microfluidic components, predict network performance, and automate the operation of devices based on pressure-driven fluid management.

Partial results

1) Microfluidic system implementation

The generic mfnSolver is applied to the PDG2 chip geometry for MFN analysis and calculation of initial experimental pressure parameters to set flow rates, guaranteeing steady operation using Python and the necessary numerical library[1]. The hydrodynamic resistance is calculated based on the fabrication mask, and the Kirchhoff-graph of the network of the system consisting of the microfluidic pressure pumps, and tubing connected to the chip, is also implemented in the mfnSolver. The output prediction for a standard flow rate of 0.16 µL/min is shown in figure 2. Note that other sets of values were obtained using different standard flow rates. The solver provides the predicted pressure, which is used as the input for pressure-driven operation during the experimental validation. The software implementation is repeated for predicting values for multi-component droplet generation (figure 2).
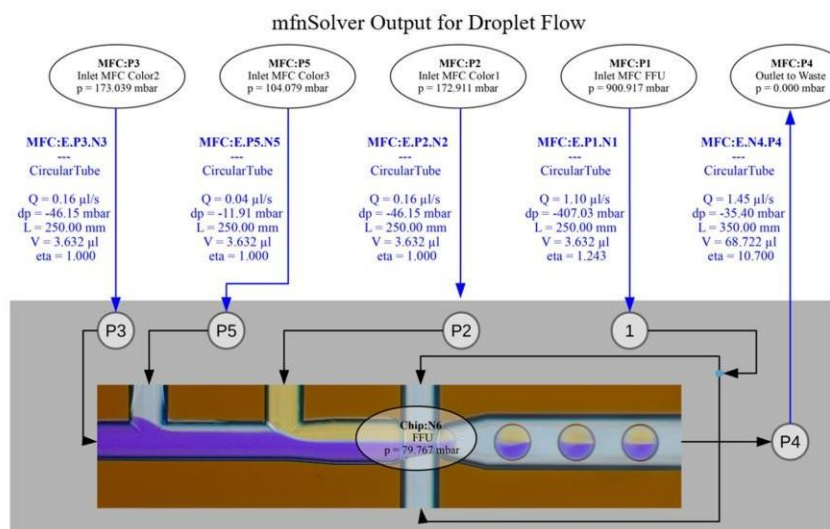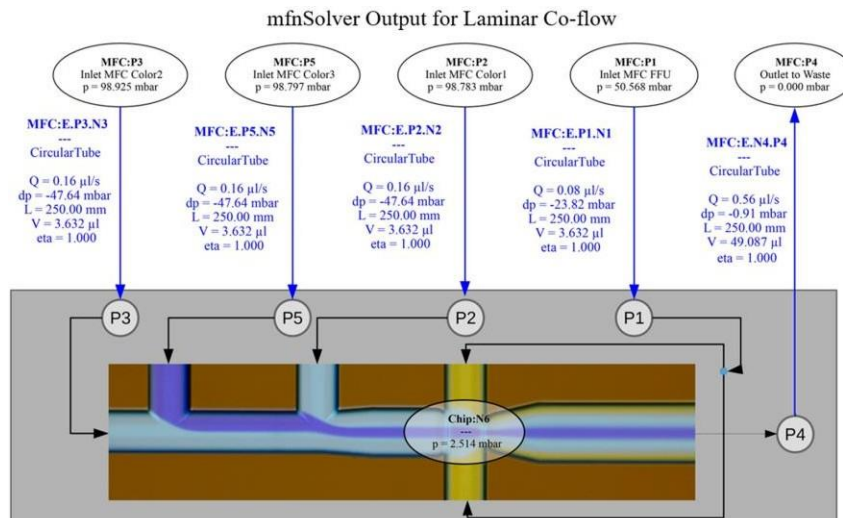
Figure 2: mfnSolver output with predicted pressure settings for the feedlines to create laminar coflow (top) and multi component droplet flow (bottom) in the PDG2 chip device[1]

2) Experimental validation

For validating the predicted flow parameters, Fluigent flow rate sensors are integrated into the feedline between the tubing to monitor the flow rate during the experiment. A standard flow rate of 0.16 µL/s has been chosen for the model. Within each set, the flow rate through one channel is changed in five steps from 0.08 µL/s to 1.28 µL/s, while the remaining ones are set constant to the chosen standard flow rate as input for the model[1]. The pressure of a port is increased from 50 mbar to 800 mbar, according to the mfnSolver calculations for laminar co-flow. Each pressure setting is kept constant for 10 s. A recording of 90 s per pressure setting is performed to demonstrate flow stability of the laminar co-flow. The flow pattern was steady during all experiments.
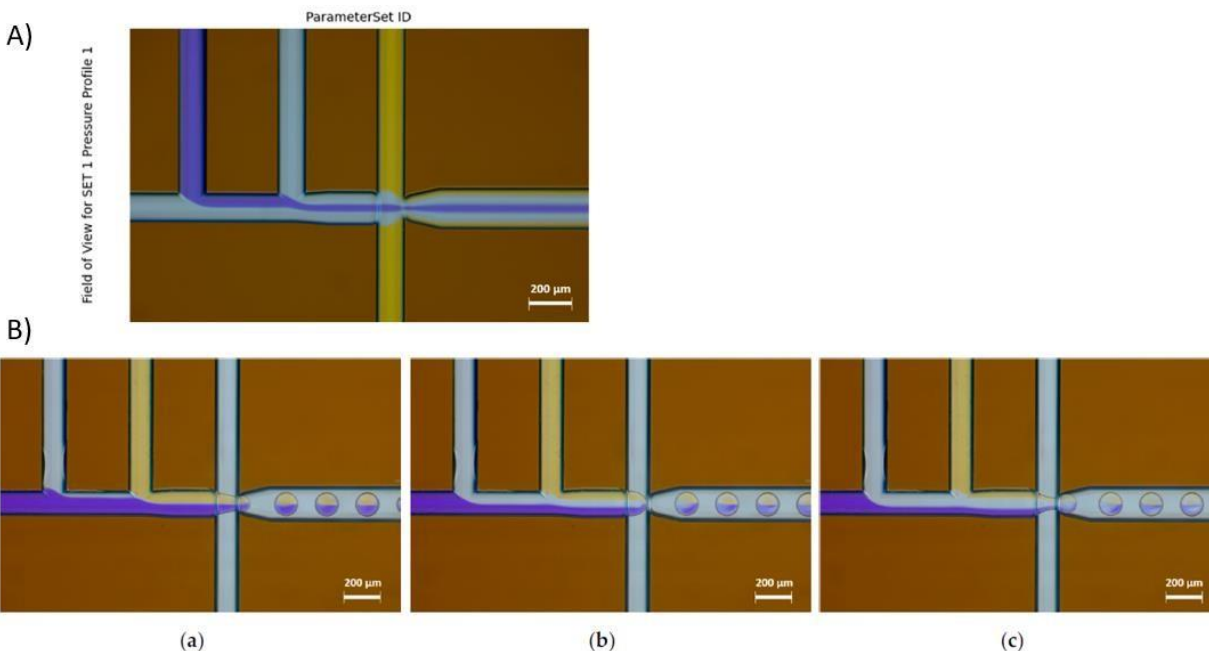
Figure 3: Experimental field of view A) Laminar flow, for a defined set of pressure parameters, with port P1 containing the orange dye solution fed with 50 mbar while all remaining ports are operated at the standard pressure of 100 mbar B) droplet-flow. A laminar flow pattern is created as the dispersed phase. The input pressure of the continuous phase channel at is kept constant at 900 mbar, while pressures from other channels vary according to the targeted flow rate scenario ((a), (b) or (c))
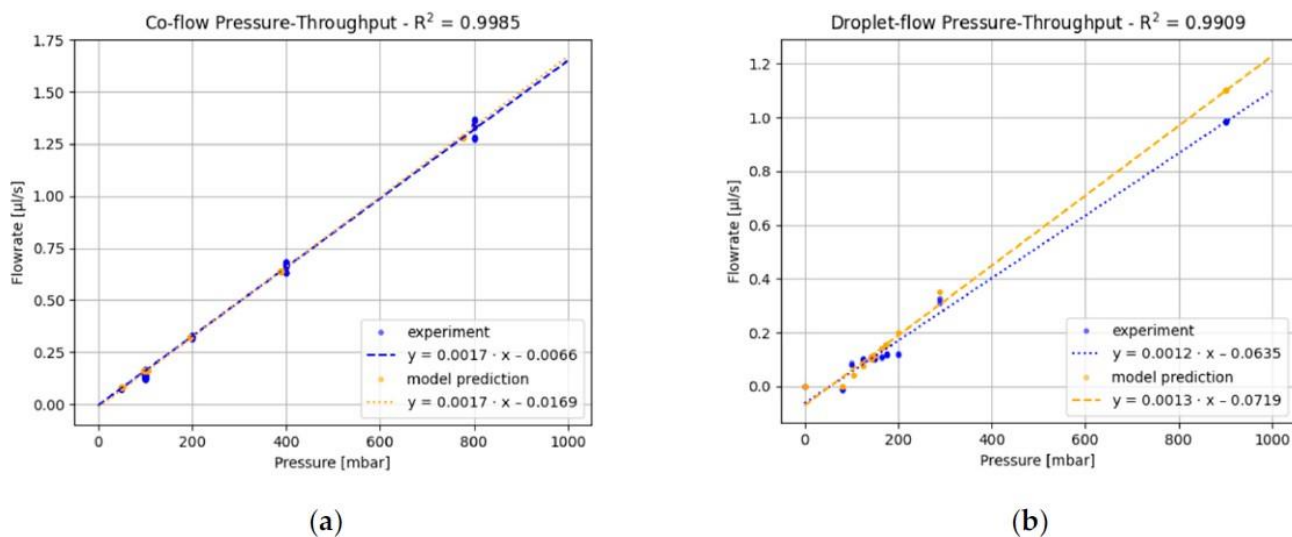

Figure 4: Correlation of pressure settings, predicted by the mfnSolver, with experimental results for the co-flow and droplet models[1]

The comparison of the pressure-throughput characteristics predicted for laminar co-flow and the experimental parameters of the pressure control and the feedline flow sensors are shown in Figure 4. We can conclude that the presented model based on the Kirchhoff nodal analysis can predict the overall pressure-throughput of the laminar co-flow scenario. Thus, it can be used to provide pressure-driven flow-control in the feedlines to the microfluidic chip[1]. For droplet generation, a laminar co-flow pattern of the

three sample fluids is created as the dispersed phase. The flow-focusing unit is now functionalized as a droplet generator. The total flow rate of the dispersed phase and the continuous phase is kept constant, while the color pattern of the laminar co-flow is varied, as illustrated in Figure 3 B). The comparison of the pressure-throughput characteristics predicted for droplet flow and the experimental parameters are shown in Figure 4.

Conclusion

As microfluidic applications are becoming increasingly complex, flow automation is highly advantageous as it usually allows for more reliable and easier lab-on-a-chip operations. We here reported a solver for a reliable tool for fast and precise prediction of pressure settings for steady-state operation conditions and getting insights into on-chip pressure drop and flow characteristics. Fluigent products – more specifically Fluigent PX – were decisive for implementing flows with excellent precision and high response time.

References

1. Böke, J. S., Kraus, D. & Henkel, T. Microfluidic Network Simulations Enable On-Demand Prediction of Control Parameters for Operating Lab-on-a-Chip-Devices. Processes **9**, 1320 (2021).