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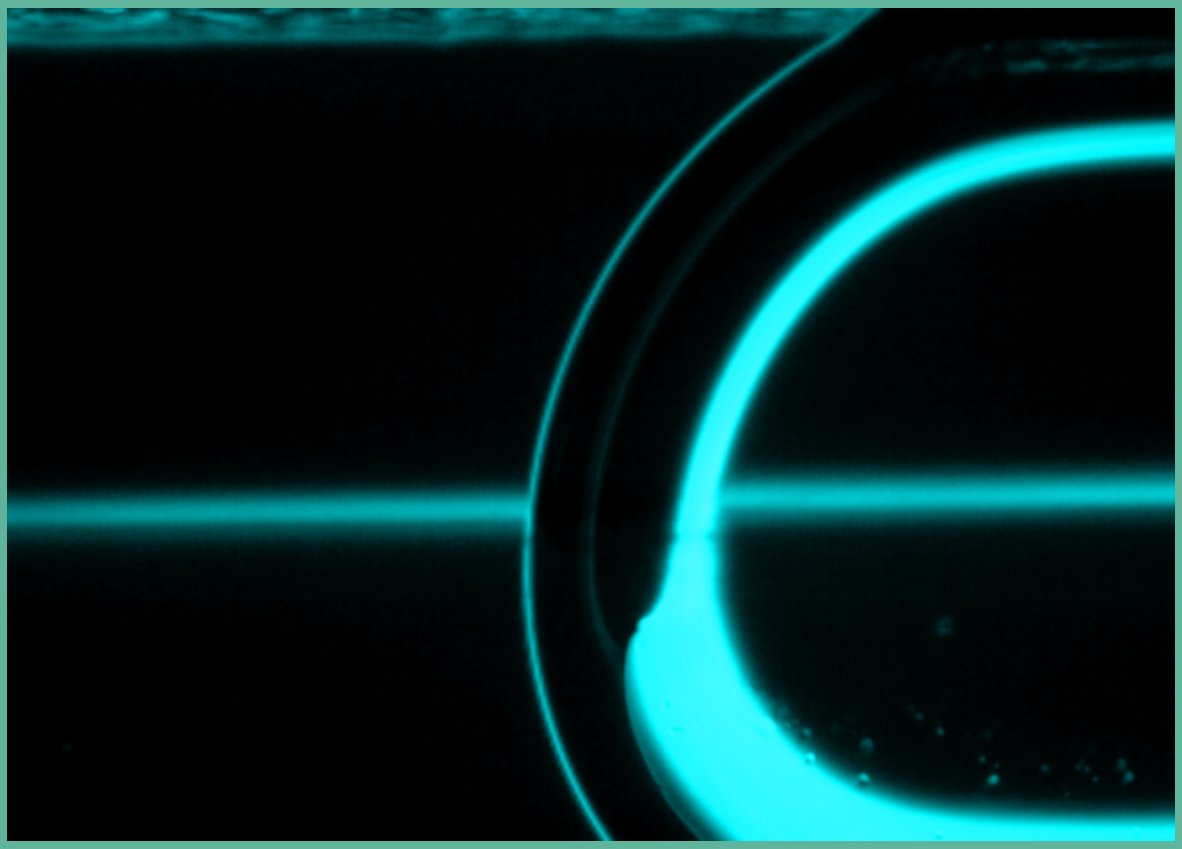
Flow Control in Microfluidics: Understand and Implement an Optimal Pressure-Driven Flow Control



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Contents

1 Preface

There are many guides concentrated on the manufacturing of microstructuring, microfludic chips or computational methods for simulating microchannels, e.g. based on the method of finite elements (FEM) or smooth particles with detailed calculations. This guide is not about such things. We want to share some basic knowledge on different types of the flow control in Microfluidics as well as provide some basic theoretical information, which can be favourable while planning or optimising experimental setup.

You need this guide if:

If you work with flow control in Microfluidics and trying to solve problems efficiently. The flow control should be fast, precise and gentle.

What is the guide about:

- Flow control in Microfluidics, particularly comparison of different flow control methods

- Basic principles of pneumatic and hydrostatic pressure and Microfluidics

- How to evaluate and thus choose the best method of flow control for a specific microfluidic setup

- How to implement pressure-driven flow control and to use additional tools for determining the volume flow rate

- How to divide pressure using four different pressure channels to a higher number of microfluidic outlets / inlets

- How to handle air bubbles in microfluidics setups

What can you learn in this guide:

- Comparison of different flow control methods in Microfluidics

- Use of pressure driven flow control and and determining the volume flow rate

- Evaluating different influencing factors on the flow control

- Preventing difficulties of the flow control and adjusting the setups

What isn't the guide about:

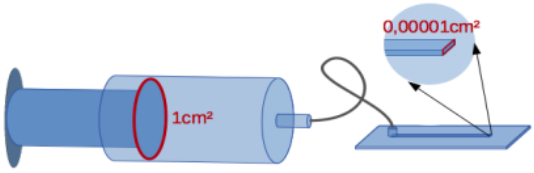
- Production of microfluidic chips

- Hydrodynamic simulations

Enjoy the reading and write us if you have any additional questions:

contact@biophysical-tools.de

2 Pressure-driven and Volume-driven Flow Control in Microfluidics



Since decades syringe and peristaltic pumps belong to biological and chemical laboratories. Thus, it was comprehensible that they were the first choice for the use with microfluidic devices, i.e. at a microscale. Still, in the hydrodynamical view both instruments for the volume-driven flow control have a number of bottlenecks which make them not favourable for the use at a microscale.

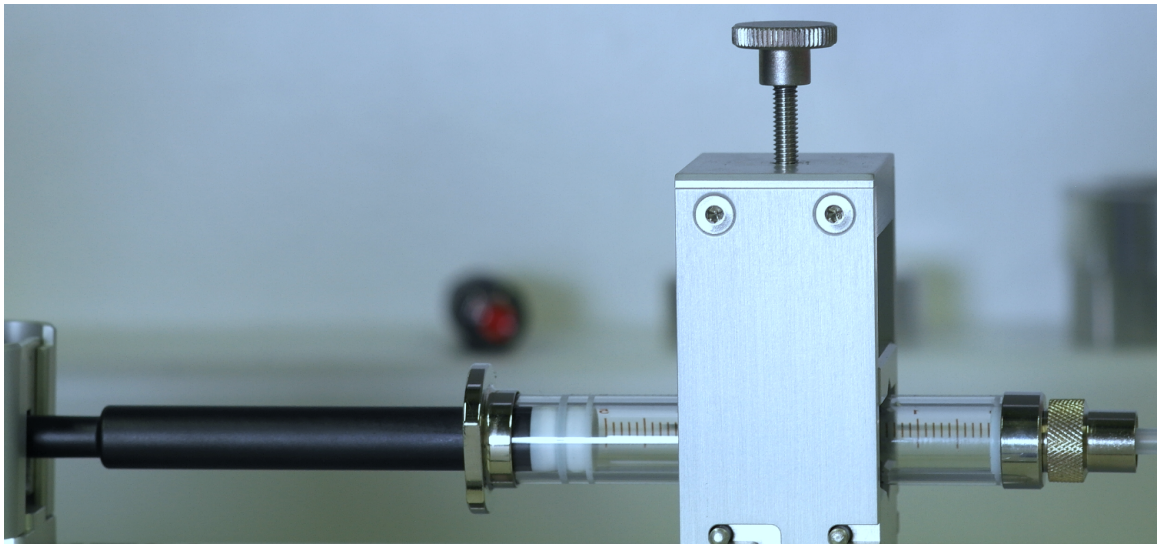
For instance, **syringe pumps** have considerable limits on the side of the liquid column. If a syringe with a cross-sectional area of A = 1 cm2 is connected to a typical microchannel of 10x100?m=0.00001xA cross-section, this results in a 100,000-fold amplification of the flow velocity in the microchannel. Probably, this disadvantage can be overcome by usage of highly precise syringe pumps in the market, but still there will be more difficulties, such as refilling the volume during the experiment, which has necessarily to be interrupted for it.

In the **peristaltic pump** the volume is displaced by peristaltic tube squeezing. This makes it difficult to determine the exact perfusion volume, since it is dependent on the tube material, which again varies depending on the temperature and wear. This approach is associated with some difficulties at a microscale. Furthermore, the flow is pulsating and cells or other objects under observation can be damaged during the squeezing process.

Another way to control the flow in the microfluidic channel is to use the **pressure**. The easiest way is the hydrostatic pressure whereas 100 mbar correspond to 1 m water column. Still, minor air fluctuations or changes of the atmospheric pressure or vibrations will impact the experiment.

Starting in 2004, pressure-driven flow controllers are used in Microfluidics. They allow to control the flow in a microchannel very fast, gently, precisely and not prone to vibrations. Also refilling of fluids without interrupting the experiments is possible. For this approach it is necessary to think in pressure, not in volume in order to control the flow in the microchannel. The flow rate can then be determined with flow meters, calculated or calibrated, e.g. by weighing.

3 Methods of Flow Control



Pressure and flow rate are the main physical parameters which describe flow in microfluidic channels and devices. They are not independent from each other, but related through the flow resistance depending on the channel design and fluid properties quite similar to voltage and current in electrical circuits.

Pressure is defined as force per area and we distinguish hydraulic and pneumatic pressure. These quantities are studied in the fields of Hydraulics and Pneumatics and all together in Hydrodynamics. Hydraulic pressure describes the exerted pressure transmitted by an incompressible medium as a liquid onto an immersed object or a boundary, e. g. water on biological cells or oil phase droplets in multi-phase fluids. Pneumatic pressure, on the other hand, exerts force by means of a compressible medium, e. g. air, on an object or a boundary. Compressible media act like a spring and can store energy. This energy can be recuperated later allowing for smoothing out unwanted pressure pulses. Pneumatic pressure can be transformed into hydraulic pressure by using a free water-air interface in a closed vessel carrying over the energy storage property to the liquid.

Displacement of liquids and gases can be realised by essentially two approaches: volume-driven or pressure driven flow. They rely on the properties of the conjugated quantities "pressure" and "volume" as known from thermodynamics. Both methods have very different characteristics - especially scaling - and one can choose one of them to control fluids.

Volume-driven control relies on the volume as a means of fluid movement control, requiring a closed and leakage-free system. They are best realised with liquids, since liquids essentially keep their volume mostly independent of pressure (incompressible). In microfluidics water and oil are the most common representatives. Pressure is generated by piston displacement in a syringe and exerted onto a downstream channel system which has to be tightly closed with exception of the waste outlet, of course. Here, pressure is not controlled and often not monitored. The scaling of this approach is poor, e. g. a minute piston displacement can result in a very large (up to 6 orders) increase in hydraulic pressure in an attached microfluidic device.

Further elasticity in the tubing and materials, leaks or air bubbles may reduce considerably responsiveness degrading device reliability and safety. In larger hydraulic systems sudden changes in volume flow may result in large pressure shocks ("hydraulic surge" or "water hammer") or create strong shear forces damaging sensitive parts of devices, such as valves and small microfluidic structures or embedded objects like biological cells.

Pressure-driven control, on the other hand, is best realised with compressible gasses, such as air or inert gasses as nitrogen. Pneumatic pressure is typically generated by a pressure and/or vacuum control system fuelling a pressure storage recipient. The pressurised gas can subsequently drive liquid flow in microfluidic devices by connecting them to the liquid in the storage recipient. Sudden changes in flow rates will not cause pressure spikes, even if the gas source delivers pulsating pressure, since gas absorbs excessive forces and pulses by means of it?s compressibility. As the liquid in the recipient is in contact to gas atmosphere, the system is not anymore closed as beforehand. Therefore pressure driven systems are usually ?open systems?. The displaced volume is not controlled and often not monitored. As pneumatic-driven systems do not get in direct contact with the handling fluid any kind of contamination is prevented and subsequent cleaning of the device is unnecessary. It is advantageous that back pressure is absorbed by gas compression and quickly eliminated by pressure stabilisation. By design, pressure-driven systems hence are more reliable, safer and require minimal maintenance. Thus, pneumatic pressure-driven systems are ideal for microfluidic channels and devices.

4 Flow Meters and Pressure-driven Flow Control in Microfluidics

When working with pressure-driven flow control systems in Microfluidics, we are working with pressure, not with volumetric flow rates. However, in some cases it is necessary or helpful to know the volumetric flow rate of the fluid in the microchannel. For example, in order to follow a specific protocol for a cell culture, stoichiometry in the microchannel or just to monitor the consumption of precious reagents. In such cases there are different approaches to determine the flow rate in the channel. In general, you can calculate it by weighing the droplets at specific pressure values and thus calibrate the system. This is the most precise method, however, cumbersome. Or you can use commercially available flow meters.

The available flow meters in the market are usually integrated upstream of the microchannel. However, the measured flow rate is not precisely equivalent to the actual volumetric flow rate, since the method is indirect, e.g. based on the temperature or Coriolis force. It makes the calibration before use mandatory. Furthermore, the accuracy of the currently available flow meters in the μl range has to be optimised significantly in order to obtain reliable results.

The liquid flow meters for the Microfluidics use different approaches. The most known ones use two principles:

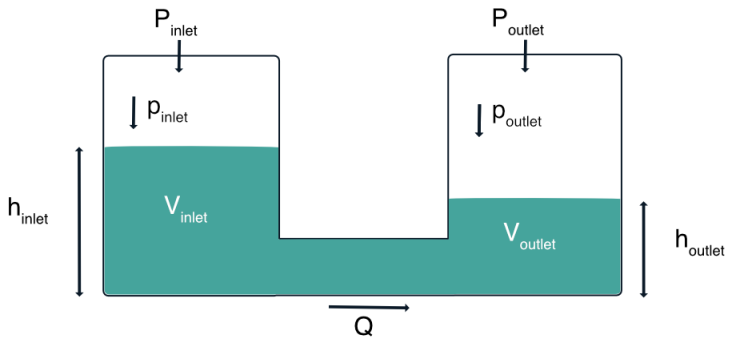
- temperature-based flow measurement

- Coriolis force-based flow measurement

For the temperature-based measurement a tiny amount of heat is introduced to the fluid. The temperature differentials, which are monitored by sensors up- and downstream, provide information on the actual flow. Still, in this case you have to consider the physical properties of the used fluid, e.g. specific heat capacity of the fluid.

Coriolis flow meters, which are widely used in industry, are also available for the mass flow measurement in Microfluidics. They usually are based on u-shaped pipes. The principle of these flow meters is based on the measurement of the phase shift of the steady vibration at different locations along the pipe, which, however, emerges through the incoming fluid into the system. For constant density fluids, the volumetric flow rate can be directly determined from the measured mass flow rate of the sensor. However, this approach is difficult for fluids with variable density, e.g. compressibility, droplets, bubbles, and is prone to external vibrations or movements.

5 Hydrostatic and Pneumatic Pressure in Microfluidics



Our atmosphere applies pressure on all surfaces, liquids can also do so. In case of the atmosphere the weight of the entire gas above is pushing downwards due to gravity creating an isotropic pressure to generate a quite impressive pressure at sea level equivalent to approx. a mass of 1 kg pushing on 1 cm^2. Liquids, however, have a much higher density than air, approx. 1000-fold, and a much more rapid pressure raise with depth is fact. The pressure of a liquid due to its weight is termed hydrostatic pressure.

The physical origin can be illustrated by a cylinder (or any other shape) placed on a flat surface A. The cylinder with a certain mass and gravitational force F will apply a pressure p onto the bottom interface with given area: $p = F/A$. This holds true for solid, liquid and gaseous matter as well. When substituting the force term with the material properties of a liquid, e.g. water, and the geometric properties of the hypothetical cylinder, the hydrostatic pressure will be $p = h \cdot \rho \cdot g$, whereas h is the height of the liquid column, ρ ("rho") the liquid density and the gravitational acceleration. Thus, the hydrostatic pressure does not depend on the size of the cross-sectional area of the liquid column, but only on the height of the liquid column and the density of the liquid. In fact, the hydrostatic pressure is also indifferent to the shape of the hypothetical column.

When working with pressure-driven microfluidic systems, the hydrostatic pressure should always be considered, no matter how small the liquid quantities are. For instance, a one-meter-high water column is already generating a hydrostatic pressure of approx. 10 kPa, or 100 mbars, being quite significant in Microfluidics. Thus, when driving fluids through microfluidic devices, the liquid levels in microfluidic devices can vary considerably in height which is often neglected. Liquid reservoirs of tall and narrow shape appear to be especially prone to systematic pressure deviations. Other common sources of experiment errors are long microfluidic tubings transferring fluids upwards and downwards and even worse if still being partially filled with air. Here, the applied pressure at the source may deviate strongly from the received pressure at the microfluidic device. Once all tubings are filled with liquid and the micro-channel is placed approx. at the same height as the reservoirs and tubings are reduced to minimum, this phenomenon reduces strongly. How to explain this?

Responsible for this is the **"hydrostatic drift"**. It can be understood and calculated as follows:

The flow rate Q is proportional to the applied pressure (P) difference plus the hydrostatic pressure (p) difference:

$$Q \sim \Delta P = P_{inlet} - P_{outlet} + p_{inlet} - p_{outlet}$$

The flow resistance R determines the resulting flow rate:

$$Q = \frac{1}{R} \cdot \Delta P$$

Flowing liquid changes the liquid levels in the outlet-reservoir with liquid volume $V_{outlet}(t)$:

$$\frac{dV_{outlet}(t)}{dt} = Q(t)$$

With constant reservoir cross section A, note that $V_{inlet}(t) + V_{outlet}(t)$, $h_{inlet}(t) + h_{outlet}(t)$ and also $p_{inlet}(t) + p_{outlet}(t) = p_{const}$ remain constant, if the total amount of liquid remains unchanged during this experiment. Hence, the level in the reservoirs changes with volume $V_{oulet}(t) = A \cdot h_{outlet}(t)$ accordingly:

$$\frac{dV_{outlet}(t)}{dt} = A \cdot \frac{dh_{outlet}(t)}{dt} == \frac{1}{R} \cdot \Delta P(t) = \frac{1}{R}(P_{inlet} - P_{outlet}) + + \frac{1}{R}(p_{inlet}(t) - p_{outlet}(t))$$

Hence:

$$\frac{A}{\rho \cdot g} \cdot \frac{dp_{outlet}(t)}{dt} = \frac{1}{R}(P_{inlet} - P_{outlet}) + \frac{1}{R}(p_{const} - 2 \cdot p_{outlet}(t))$$

which is an inhomogeneous ordinary differential equation of 1st order with an exponential solution rising or decaying with the time scale $\frac{A \cdot R}{2\rho \cdot g}$.

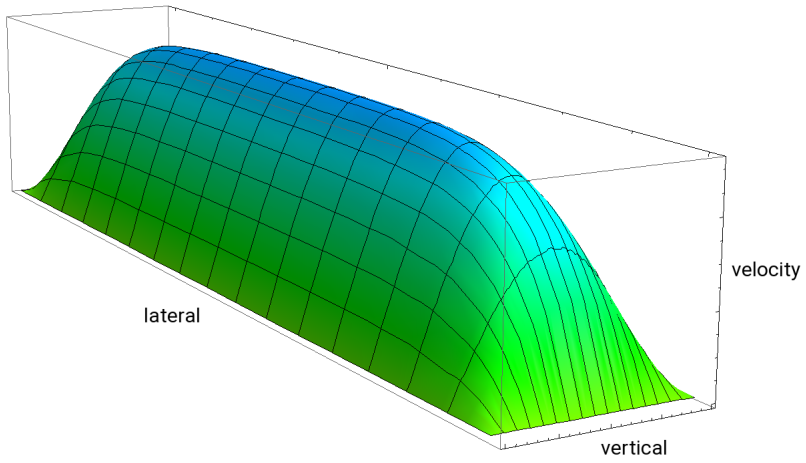
We conclude that less tall reservoirs of larger cross section, or higher hydrodynamic flow-channel resistances reduce the hydrostatic drift.

If the flow rate should remain constant over a long period of time, hydrostatic drift compensation is recommended: The applied pressures have to be conducted such that the right side of the differential equation remains constant. The P2CS has a build-in function to accomplish this compensation automatically, with the function-setup:

set:hystatic_pressure
set:hystatic_pressure:timescale

On the other hand, the hydrostatic pressure can also be useful as an excellent source of (quasi-)static pressure for experimental setups. As illustrated, a one-meter large water column can generate significant pressures at the lower end. When doing experiments which require constant pressures with minimal liquid transfer (such that the liquid level of the column will not significantly decrease), water columns present an excellent and inexpensive tool for microfluidic and mesofluidic applications.

6 Flow Rates and Profiles in Micro- and Meso-Channels



Friction appears in relative motion between solid bodies, between fluids in a pipe and its wall, but also far from boundaries inside flowing fluids between layers of same velocities (lamina). This has deep implications on the way the fluid behaves. The flow through a pipe or channel can be discriminated in **turbulent and laminar flow**, which depends on the size of the channel, the flow speed and the properties of the liquid. That means: The larger the size of the channel, the higher the flow speed and the lower the viscosity, the more likely the flow develops turbulence which is described by the Reynolds number Re:

$$\mathrm{Re} = \frac{\bar{u}\,\rho\,L}{\eta}$$

with the (average) flow speed $\bar{u}$, the channel length L, the density ρ and the dynamic viscosity η of the fluid. The characteristic dimension is a length scale of the system determining the overall behaviour of the fluid - in our case it can be the lateral or vertical channel size. Re relates flow momentum density $\bar{u} \cdot \rho$ (inertia) to the viscosity. Turbulence starts at $\mathrm{Re} \gtrsim 2000...3000$, a number hard to reach in micro-channels because of the very small L and ρ, fortunately. Hence, flow is laminar in our case and friction dominates the flow properties. Friction has averaging smoothing properties, sharp edges in the velocity field $v_x(x, y, z)$ get rounded very quickly. Viscosity is the macroscopic consequence of friction. Diffusion in fluids acts in a way to minimise shear stress and large gradients, however, this is less possible near the channel walls, since they do not move *a priori*.

The consequence is that the friction is strongest right at the boundaries, the resulting shear stress τ_w is measured by the viscosity constant and the flow speed gradient, e.g. at wall sections parallel to the y-axis:

$$\tau_\mathrm{w} = \eta\,\frac{\partial v_x}{\partial z}\ .$$

In **laminar flow** the averaging tendency of diffusion has as consequence that the boundary layer reaches more and more into the flow and eventually dominates the velocity gradients in the whole micro-channel. So the shear stress τ_w distributes eventually smoothly over the entire channel, hence we drop the index "w" standing for "wall". τ also can be interpreted as the momentum flux across the lamina from the bulk towards the boundaries. This means indeed, that the moving fluid pulls the tubing towards its flow direction (a garden hose stretches out, once water is flowing through).

If we look at the change rate of this shear stress in the fluid across the channel, we obtain a number, which is surprisingly constant at any point. This is the externally applied pressure gradient along the channel which makes the fluid moving. We now understand the inner mechanics of the governing equation of a stationary flow against a pressure gradient in x-direction in a tube of arbitrary shape, which reads:

$$\frac{\partial^2 v_z}{\partial y^2} + \frac{\partial^2 v_z}{\partial z^2} = \frac{1}{\eta}\frac{\partial p}{\partial x} \ .$$

Note that with vanishing pressure gradient (right side) of very large viscosities this equation approaches the diffusion equation where any movement is suppressed by the boundary condition (disappearing velocity at the boundaries). This equation is a reduced Navier-Stokes equation.

For time-dependent laminar flow the left side just needs to be extended by $-\rho/\eta \cdot \partial v_x/\partial t$.

The most common channel geometries for microfluidic applications are cylindrical or rectangular. For **cylindrical shapes** the solution is a parabolic velocity profile u_C:

$$u_C\,(r) = u_0 \left(1 - \left(\frac{r}{R}\right)^2\right)$$

with the maximum velocity in the center of the tubing $u_0 = -R^2\,(\partial p/\partial x)/(4\,\eta)$, the radius of the tubing R and the distance from the center line of the tubing r. The negative sign indicates that the flow is oriented inverse to pressure gradients, i.e. from high to low pressures. Integration gives the total volume flow rate: $Q = \pi R^4/(8\eta) \cdot \Delta p/\Delta x$, the famous Hagen-Poiseuille law.

For **rectangular channel profiles** ranging from $-w/2 < y < +w/2$ and $-h/2 < z < +h/2$ with width w and height $h \leq w$, the velocity profile can be obtained by solving the partial differential equation with the method of separation of variables, which can be found in the literature.[1] Now the solution consists of a series of entangled hyperbolic and trigonometric functions depending on the inverse aspect ratio w/h and y/h, z/h. It is useful to note that for small aspect ratios the lateral profile is nearly entirely flat whereas the vertical profile remains curved. This is plausible, since going to the extreme case of an infinitely wide channel, the horizontal profile becomes perfectly flat. This observation hints at a way to minimise the dispersion of small samples dissolved in a flowing carrier fluid.

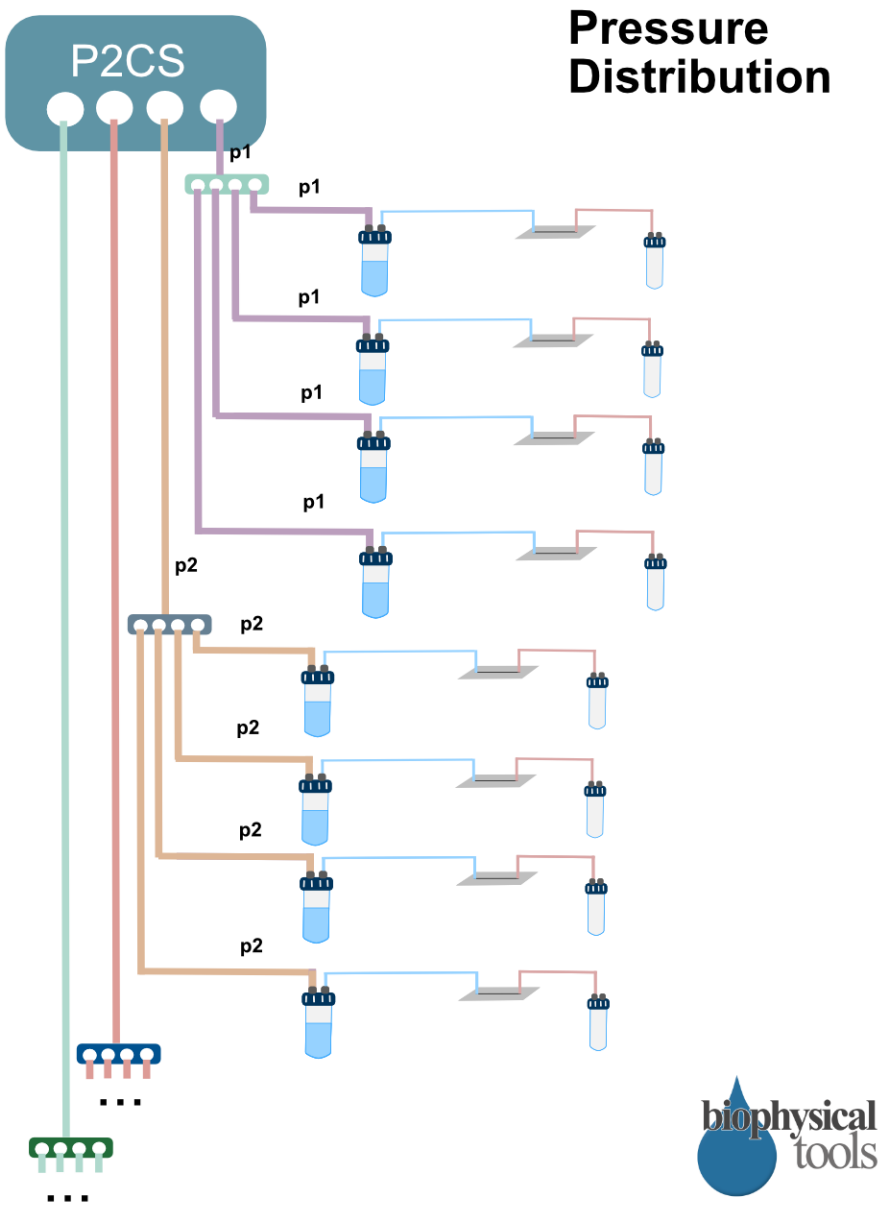
A very good approximation for the total volume flow rate for the whole range from close to unity to very small aspect ratios h/w is given by[2]

$$Q = \frac{833\,h^3\,w - 523\,h^4 \tanh(\pi/2 \cdot w/h)}{\eta}\ \frac{\Delta p}{\Delta x} \ .$$

On the website of Biophysical Tools you can find a calculator for the total volume flow rate and the related pressure in cylindrical and rectangular channels. We invite you to explore different value sets and different geometries.

[1] Bastian E. Rapp, Microfluidics: Modeling, Mechanics, and Mathematics (https://doi.org/10.1016/C2012-0-02230-2)
[2] C. Fütterer, Biophysical Tools GmbH

7 Pressure Distribution while Using Pressure-driven Flow Control in Microfluidics and Mesofluidics - Application Note



While utilising pressure-driven flow control, typically users connect each microfluidic port to one port of a flow control system, one-to-one. That means: You need as many pressure channels of a flow control system as many inlets/outlets you have. But usually there is no need for many different pressure levels in an experiment, rather two - four pressure values are sufficient. For instance, in parallel experiments with several chips - as in high-throughput platforms, setups are often based on distribution of the same pressure in 4, 8 or more channels. This can be done with manifolds without additional pressure channels of a flow control system. A more sophisticated solution is to equip such manifolds with valves or to rely on different switches, which will be discussed in another Application Note in more details.

A manifold distributes one pressure channel into several pressure channels, whereby the pressure in all channels is basically the same. If the output pressure of the pressure-driven flow controller is changed, the pressures in the pressure channels follow it practically instantaneously. However, as the pneumatic volume is increased by additional channels, the dynamics slows down slightly, i.e. the pressure in the system follows the set pressure, which is determined by the user, with a slight delay.

For most applications such a delay is neglectable. It generally lies in the range of fractions of a second. For manifolds with many branches a faster dynamics can be realised - e.g. with P^2CS Plus, which can regulate pressures in larger volumes ("mesofluidics"). Besides, for this purpose P^2CS has its own control parameter that allows the control to be kept free of oscillations and overshooting even for larger pneumatic volumes.

However, a number of experiments or procedures require reliable switching on or off pressures and flows. In such setups manifolds should be equipped with switches. That is what Fluid Switch ("FS") does - as an extension to the flow controller P^2CS. It allows both - pneumatic and hydraulic channels - to be switched at submillisecond speed. Each channel can be switched completely independently of the other channels. This allows to prevent parasitic flows due to drift, nucleation or reactions. Switching is directly controlled by the P^2CS firmware and is real-time, i.e. each valve is controlled with microsecond precision, so that even ultra-fast flow changes can be achieved precisely and reproducibly.

8 Why should You not Use High Pressures for Pressure-driven Flow Control in Microfluidics

Reason:

Pneumatic pressures become dangerous and pneumatic flow control looses some of its advantages.

Explanation:

Due to the high compressibility, gasses do behave as a spring. You may experience this by doing an experiment: Take a (plastic) syringe and close the outlet firmly with a finger or a cap. Then press the piston into the syringe as much as you can and observe the traveling distance. Then release the piston again while keeping the outlet closed. Repeat the experiment with a water-filled syringe. The distance should be much less now. In case of air the piston jumps swiftly back in its initial position on piston release. In case of water, the effect is much less or absent.

In case of handling errors, e.g. with setups working at 5 bars or more, the compressed air may discharge on your lab bench like an air gun. Precious or aggressive liquids may get spilled over large distances. Connectors become projectiles and may travel more than 10 metres.

There are some additional side effects, too. If expanding gas through a small aperture (?nozzle?) the system is getting cooled considerably as an adiabatic expansion takes place.

For very high pressures syringe pumps seem to be the better choice for most experiments since liquids are not able to store much energy when compressed. If adiabatic expansion cooling is desired, you are fine with high pneumatic pressures, of course.

However, pneumatic flow control is the best choice at low pressures.

Numbers:

The compressibility of fluids is $\kappa = -\frac{1}{V}\frac{dV}{dp}$. The compressed volume is represented as V at a given pressure p. As the closed volume decreases when increasing pressure, the sign is negative.

At a straight channel with cross section area A the volume is changed by a solid or liquid piston moving alongside the x-axis. Initially its position is at L_x, hence, the initial volume is $V_0 = AL_x$. If moving the piston, the volume changes according to

$$dV = A\,dx$$

With this expression the compressibility can now be transformed to

$$\kappa = -\frac{1}{L_x}\frac{dx}{dp} \text{ or } dx = -\kappa L_x\,dp$$

Our goal is to calculate the energy stored in compressed fluid - gas or liquid. The energy is "force times distance":

$$E = \int F\,dx$$

where F is the force applied to the piston.

Now we are able to obtain the stored energy:

$E = \int pA\,dx$ where p is the pressure applied to the piston of cross section A.

Now we would need a relation $p(x)$. Replacement of dx gives us a simpler path to the result:

$$E = -\kappa AL_x \int p\,dp$$

The stored energy E is proportional to the compressibility κ.

Values for the compressibility are known:

$$\kappa_{air} \approx 10000Pa^{-1}$$

$$\kappa_{water} \approx 0.5Pa^{-1}$$

The ratio is $\kappa_{air}/\kappa_{water} \approx 20000$!

We discover that air can store 20000 times more elastic energy than water supposing the same pressures.

9 Avoiding Air Bubbles in Microfluidic Setups While Using Pressure-Driven Flow Control

Air bubbles are annoying in microfluidic setups, since they can disturb or even hinder an experiment. Even after a careful initial flushing of the whole system with liquids, air bubbles can occur because of drastic change of flow rates and pressures, nucleation of air in liquids, e.g. when using unfavorable combination of inner diameters between the single modules of the system.

Further, air bubbles can temporally increase the flow resistance, thus, the flow rate may drop. They can also delay changes of flow rate due to compressibility.

At such small length scales, the surface tension of water-air-interfaces is a relevant factor. Air bubbles can occur in various ways. Assuming that all connectors are airtight, the most common cause is that air and/or air bubbles were already in the system at the beginning. To get rid of dead volume air bubbles, the whole system should be flushed with (degassed) water or medium first in all channels. This typically removes more than 90% of air bubble-related issues in most microfluidic systems. Air bubbles may still stick in transitory elements such as connectors or in hydrophobic tubings. Finger snipping against the respective components may quickly solve the problem, otherwise a disassembly can be necessary.

The two other causes of air bubbles are the small air diffusivity through tubing (practically relevant for many very long tubings) and the pressure-depending air solubility of water (relevant when applying pressure drops or strong pressure gradients in the system). For those two cases, degassing or use of bubble traps can be helpful.

Thus, to avoid air bubbles you can:

- flush the whole setup beforehand

- degas fluids

- consider the changes of the inner diameters between single modules to avoid a pressure drop within the system

- double-check the system on tightness

- use bubble traps (if absolutely necessary)

10 About Us

Biophysical Tools GmbH is your competent partner for Microfluidics – starting with the experiment planning up to its realisation. We are experts in ultra-precise and -fast flow control of fluids (liquids and gases) in Microfluidics and Mesofluidics, which is the focus of our both leading product groups, and in automation and miniaturisation of experiment protocols where fluidics plays a role.

Our goal is to provide highly precise standards for quantitive measurements in Biophysics, Biochemistry, Biology and Medicine. In addition, you benefit from our optional Open Source solution for the system control, for you can develop and adapt it on your own. We integrate our products into your IT environment and develop automatic protocols for the realisation of your specific experiments.

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