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Master Thesis

Valves for Microfluidic Devices

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**Göteborg, Sweden
2007**

Abstract

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1. Introduction

1.1 Brief history of microfluidics

In interfacing biology and microtechnology, microfluidics has become one of the major theme. By definition microfluidics is a technology, which manipulates small amounts of liquids (from 10^{-9} to 10^{-18} litres) in micron size channels and containers, which are forming so called “Labs on a Chip”¹. Initially, microfluidics appeared in different chromatography systems, like GPC, HPLC, CE. Presently microfluidics has become a subject of interest in many academic laboratories, with the intention to reduce the amount of reagents and sample in chemical and biological analysis, also providing massive parallelization and new physical phenomena that are not accessible in macroscopic labs. However, microfluidics has not yet become a widespread technology in consumer products.

However scientific and technological areas where microfluidics could bring significant breakthroughs in the future are, first of all, chemical analysis, biology and medicine. Even though microfluidic is still in its infancy, we can already hopefully draw parallels to the miniaturization process in the field of microelectronics, which has been a crucial milestone in technological progress during 20th, due to the drastic developments in data processing and communication technology (Figure 1.1-1). Nevertheless, firsts ancestors of nowadays computers were much different from modern laptops and pocket sized PDAs, which are results of fifty year of exponentially progressing miniaturization of electronics (it is known as Moore’s law).

Before becoming wide spread any new technology meets challenges to overcome. In microfluidics one of the difficult choices is material of device. Typical microfabrication is based on a silicon substrate, which is expensive and requires complex facilities to process. Since the areas of microfluidic chips are generally larger, than microelectronics ones, the high price of such a device could hinder commercialization. As an alternative, cheaper polymeric materials (PDMS) are gaining interest. In all cases, control of mixing and fluid flows as well as interfacing to micro- and macroscopic parts (“world-to-chip problem”) are important issues and have still a long way to go. The thesis presented here covers studies of microvalves, which are useful when designing chips, where fast and precise liquid manipulation is needed.

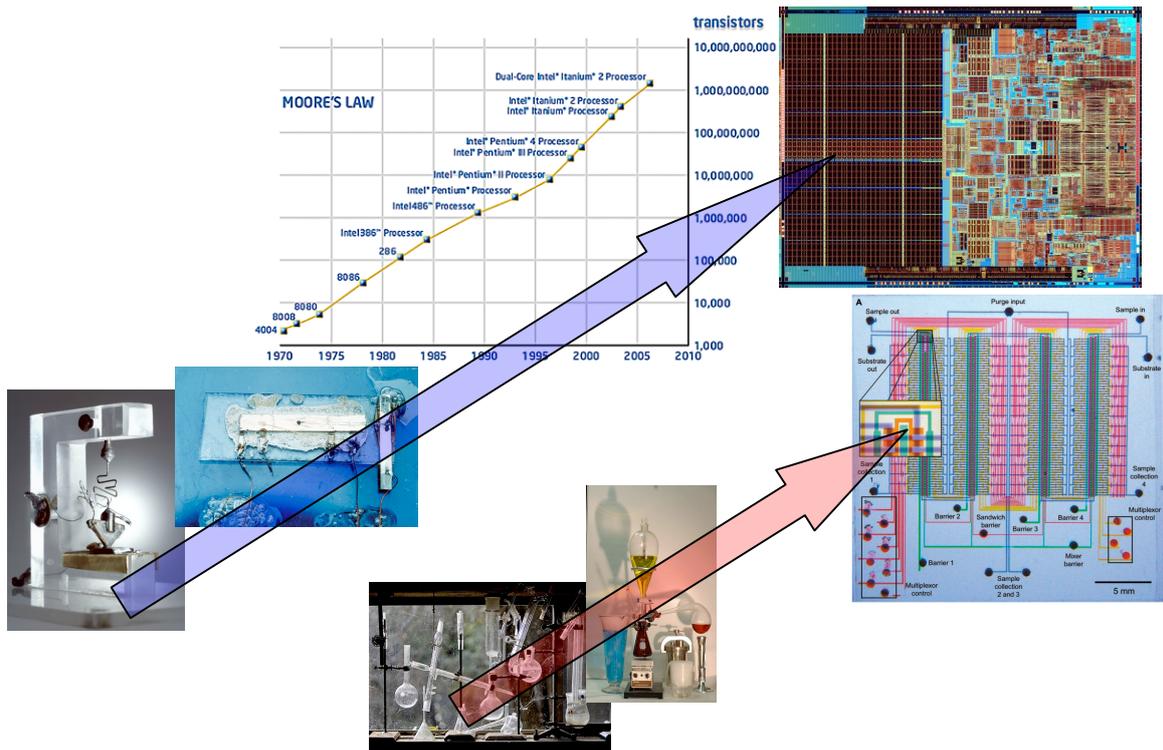


Figure 1.1-1. Microelectronics versus microfluidics. From transistor via first integrated circuit (IC) to modern day's dual core processor (top). From classical (flasks and tubes based) chemistry lab to a microfluidic "Lab on a chip" – a large-scale integration in microfluidic design (Thorsen *et al*²) (bottom)

1.2 Fluid physics at micrometer scale

Like microelectronics meeting quantum effects while going smaller in dimensions of structures, microfluidics also encounters new phenomena, not observable in macroscopic experiments. Sometimes these phenomena may open new doors to technological solutions unreachable so far, as it may provide a challenge another time. In this chapter I give a short overview over fluid physics and its implications for micrometer-scale channels.

1.2.1 Basic principles. The Navier-Stokes equation^{3; 4}

A Newton law that describes inertia of motion ($F=ma$) is one of the foundations of classical mechanics. An analogous formula, called the Navier-Stokes equation, exists for fluidic systems. In order to derive the Navier-Stokes equation we must make an assumption that fluid motion is describable as a continuous velocity (or flow) field (Figure 1.2-1). It means that the Navier-Stokes equation does not concern discrete molecules and has its limits of applicability. Other parameters like pressure, density etc. are also assumed to be continuous and differentiable. The starting point of derivation lies on the most basic principles of physics, which are different conservation laws, like mass, momentum and energy. While imagining an arbitrary volume Ω bounded by a surface $\partial\Omega$, Reynold's transport theorem says that any change of some intensive physical property X over time inside the volume Ω (first term of equation 1.2.1) must be equal to the loss or gain through the surface $\partial\Omega$ (second term), added everything produced or consumed inside (third term).

$$\frac{d}{dt} \int_{\Omega} X d\Omega + \int_{\partial\Omega} X \mathbf{v} \cdot \mathbf{n} d\partial\Omega + \int_{\Omega} Q_X d\Omega = 0 \quad (1.2.1)$$

Q_X is denoting the production rate in the volume Ω . Using Leibniz' rule and the divergence theorem, we can rewrite the first and second terms respectively.

$$\int_{\Omega} \frac{\partial X}{\partial t} d\Omega + \int_{\Omega} \text{div} \left(X \mathbf{v} \right) d\Omega + \int_{\Omega} Q_X d\Omega = 0 \Rightarrow \int_{\Omega} \left(\frac{\partial X}{\partial t} + \text{div} \left(X \mathbf{v} \right) + Q_X \right) d\Omega = 0 \quad (1.2.2)$$

Since the equation must hold for any arbitrary volume, it requires that the integrand is zero.

$$\frac{\partial X}{\partial t} + \text{div} \left(X \mathbf{v} \right) + Q_X = 0 \quad (1.2.3)$$

Firstly we take into account conservation of mass. Since mass cannot be generated arbitrarily, $Q_X=0$.

$$\frac{\partial \rho}{\partial t} + \text{div} \left(\rho \mathbf{v} \right) = 0 \quad (1.2.4)$$

In the case, where the fluid is incompressible (the density is constant) the equation is reduced to $\text{div} \left(\mathbf{v} \right) = 0$. The second important conservation is that of momentum, which must be preserved in all directions independently (ρv_i is the momentum density in one direction). The rate of momentum change corresponds to the force acting on the fluid ($dp/dt = F$).

$$\frac{\partial}{\partial t} \left(\rho v_i \right) + \text{div} \left(\rho v_i \mathbf{v} \right) + Q_{xi} = 0 \quad (1.2.5)$$

↓

$$\frac{\partial \rho}{\partial t} v_i + \rho \frac{\partial v_i}{\partial t} + \underbrace{\text{grad} \left(\rho v_i \right) \cdot \mathbf{v}}_{=\rho \mathbf{v} \cdot \text{grad} \left(v_i \right) + v_i \mathbf{v} \cdot \text{grad} \left(\rho \right)} + \rho v_i \text{div} \left(\mathbf{v} \right) = f_i \quad (1.2.6)$$

$$\Downarrow$$

$$v_i \left(\frac{\partial \rho}{\partial t} + \text{div}(\rho \mathbf{v}) \right) + \rho \left(\frac{\partial v_i}{\partial t} + \mathbf{v} \cdot \text{grad}(v_i) \right) = f_i \quad (1.2.7)$$

The forces acting on the fluid may be of different origin. For clarification we can divide them into two groups. The first is represented by the divergence of the stress tensor inside the fluid $\text{div} \underline{\underline{\sigma}}$. All other forces are summed into $\underline{\underline{F}}$ (gravitational force, for example).

$$\rho \left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \text{grad}(\mathbf{v}) \right) = \text{div}(\underline{\underline{\sigma}}) + \underline{\underline{F}} \quad (1.2.8)$$

Tensor components can be divided into shear (τ) and normal (σ) stresses

$$\sigma_{ij} = \begin{pmatrix} \sigma_{xx} & 0 & 0 \\ 0 & \sigma_{yy} & 0 \\ 0 & 0 & \sigma_{zz} \end{pmatrix} + \begin{pmatrix} 0 & \tau_{xy} & \tau_{xz} \\ \tau_{yx} & 0 & \tau_{yz} \\ \tau_{zx} & \tau_{zy} & 0 \end{pmatrix} \quad (1.2.9)$$

Pressure, for example, is a typical “normal” stress, while viscous drag contains both normal and shear components. Thus, the stress tensor is describing both hydrostatic pressure and viscous drag phenomena. For practical purposes the most crucial simplification is the incompressible Newtonian fluid. Newtonian fluids have the following properties: firstly, the stress is linearly dependent on strain, secondly the fluid is isotropic and, finally, the fluid at rest is described by $\text{div} \underline{\underline{\sigma}} = -\text{grad}(p)$, which means it cannot have any shear stress, only hydrostatic pressure. For Newtonian fluids the viscous force is given by the Laplacian $\eta \nabla^2 \mathbf{v}$, which implies that the viscosity acts as a diffusion of momentum. We obtain thus a Navier-Stokes equation for Newtonian fluids:

$$\boxed{\rho \left(\frac{\partial \mathbf{v}}{\partial t} + \mathbf{v} \cdot \text{grad}(\mathbf{v}) \right) = \text{div} \underline{\underline{\sigma}} + \underline{\underline{F}} = -\text{grad}(p) + \eta \nabla^2 \mathbf{v} + \underline{\underline{F}}} \quad (1.2.10)$$

Consequently, the left side in the equation stands for the inertial term. Term $\rho \frac{\partial \mathbf{v}}{\partial t}$ describes the time dependent change of the flow field (unsteady acceleration), whereas $\mathbf{v} \cdot \text{grad}(\mathbf{v})$ describes the momentum change of a fluid volume moving spatially along the flow-lines. For instance, when a fluid stream is flowing from a wider tube into a narrower one, the velocity of the fluid increases, even though the flow pattern is steady. This component of the equation is also called convective acceleration and it turns the equation into a nonlinear partial differential equation, making it unsolvable in almost every practical situation. Also, the nonlinearity is responsible for chaotic behavior and turbulence.

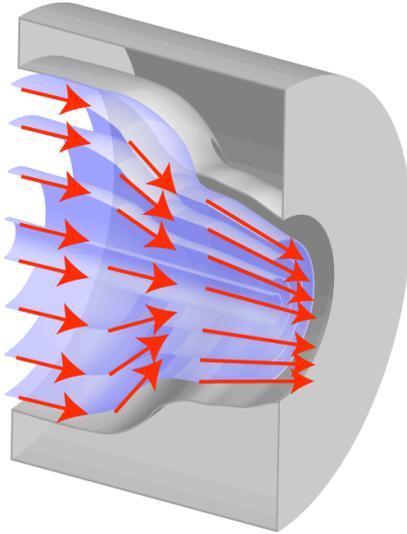


Figure 1.2-1. Flow field of fluid. Fluid can experience two types of acceleration. In-time acceleration and in-space acceleration. In-time acceleration means that fluid flow is accelerating, when in-space acceleration may exist also in case of stationary flow. For example, when the flow channel becomes narrower, the flow speed must increase to maintain the same flow rate. It indicates that the velocity of a fluid particle increases spatially.

1.2.2 Peculiarities at small scales. The Reynolds number.

In order to facilitate the understanding how changing of the system parameters affect it, we can rewrite the initial Navier-Stokes equation to a dimensionless form⁵. In order to do so, we substitute the variables

$$(x^*, y^*, z^*) = \frac{1}{l}(x, y, z), \quad \mathbf{v}^* = \frac{\mathbf{v}}{v}, \quad p^* = \frac{p}{\rho v^2}, \quad t^* = \frac{tv}{l} \quad (1.2.11)$$

where l and v are characteristic length and velocity scales of the system and ρ is the density of the fluid. Applying this substitution to the Navier-Stokes equation results in a dimensionless form:

$$\frac{\partial \mathbf{v}^*}{\partial t^*} + \mathbf{v}^* \cdot \mathbf{grad}^*(\mathbf{v}^*) = -\mathbf{grad}^*(p^*) + \underbrace{\left(\frac{\eta}{l^2 \rho}\right)}_{\frac{1}{\text{Re}}} \nabla^{*2} \mathbf{v}^* + \underbrace{\left(\frac{gl}{v^2}\right)}_{\frac{1}{\text{Fr}^2}} \mathbf{F} \quad (1.2.12)$$

The solution of the equation is depending on two dimensionless parameter called Reynolds number $\text{Re} = \rho vl / \eta$ and Froude number $\text{Fr} = v / \sqrt{gl}$. When flow channels are becoming smaller, the Re number decreases, indicating that viscous forces start to dominate over inertial ones, allowing us to discard nonlinear terms, resulting in Stokes laminar flow equation:

$$\rho \frac{\partial \mathbf{v}}{\partial t} = \text{div} \boldsymbol{\sigma} + \mathbf{F} = -\mathbf{grad}(p) + \eta \nabla^2 \mathbf{v} + \mathbf{F} \quad (1.2.13)$$

It also shows that low-Reynolds number flows do not have turbulences. This is one of the most significant phenomena arising in fluidic systems on small size scale making them behave counter intuitively to our experience with high-Reynolds number in everyday life (Figure 1.2-2). On one hand, as a consequence of laminar flow, mixing in microfluidics becomes much slower, while on the other hand, it can be done in a controllable manner not possible on large scales. Applications of this phenomenon are reviewed in the following chapter.

$$\text{Re} = \frac{|\text{Inertial forces}|}{|\text{Viscous forces}|} \quad (1.2.14)$$

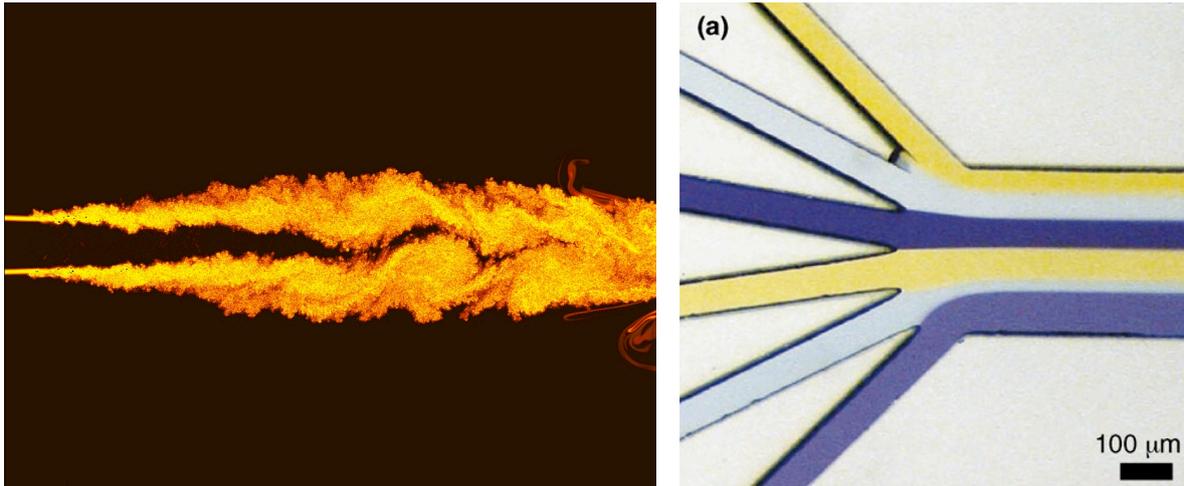


Figure 1.2-2. Turbulent flow (Left) *versus* laminar flow (Right). Turbulent flow is chaotic, laminar flow instead has well predictable behavior – side-by-side flowing streams are mixed only by diffusion. (Images: J. Schulze *et al* Universität München and D. B. Weibel *et al* ⁶)

1.2.3 Fluid flow in microchannels

Poiseuille flow and Taylor dispersion⁷

Poiseuille or just pressure-driven flow is most familiar from the macroscopic world. Hydrostatic pressure pushes viscous liquid through pipes, exactly like in domestic water systems. If the two main forces, hydrostatic pressure and viscous drag, are canceling each other, the result will be a stationary stream. When assuming a simple circular tube (radius R) and laminar flow, the fluid velocity is zero at the tube wall and maximum at the center, resulting in a parabolic flow profile (Figure 1.2-3)

$$v_r = U \left(1 - \frac{r^2}{R^2} \right) \quad (1.2.15)$$

When imagining a narrow plug of fluid inserted into such a tube (Figure 1.2-3 **A**) laminar Poiseuille flow would stretch it into a parabolic shape (**B**). After a while diffusion smears the parabola into a homogeneous plug again (**C**). It has been shown that with time the concentration distribution of the substance in the initial plug evolves into Gaussian distribution with width w

$$w \propto \sqrt{\frac{v^2 l^2}{D} t} \quad (1.2.16)$$

Actually, simple diffusion would also led to Gaussian broadening, which indicates that it is effectively enhanced diffusion along the direction of flow. Consequently, the diffusivity along the direction of the flow would be

$$D_{\text{AlongFlow}} \propto \frac{v^2 l^2}{D} = Pe^2 D \quad (1.2.17)$$

Where D is the molecular diffusivity and Pe is the Péclet number (see following chapter) This enhanced mixing process is called Taylor dispersion.

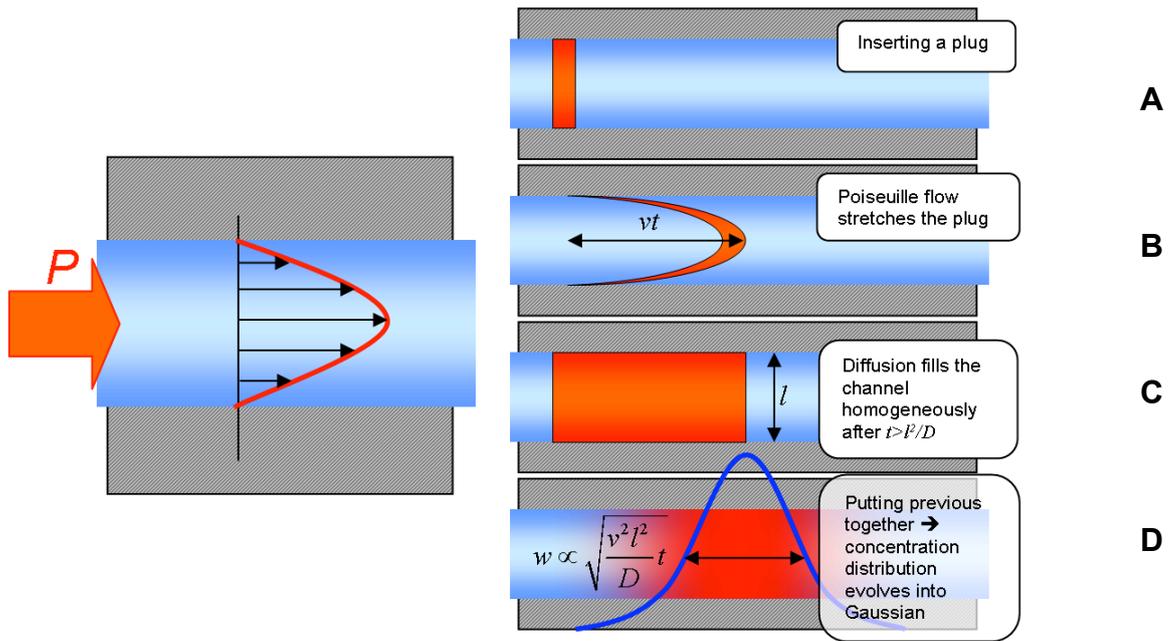


Figure 1.2-3. Poiseuille flow and Taylor dispersion.

Electroosmotic flow⁸

In contrast, electroosmotic flow (EOF) has a totally different type of flow mechanism, which becomes observable when the surface-to-volume ratio increases. Due to the involved chemical processes, contact areas between two different media (channel wall and liquid) may have a thin double-charge interfacial layer. For example, when silicon (or plasma treated PDMS), which is tailored by silanol groups on the surface (Si-OH) is brought into contact with water solution (pH>3), the silanol groups behave as acid and donate protons to water. Thus, the channel surface obtains a small negative charge while the thin water layer carries a positive one. When applying voltage, the charged water layer starts to move and if the channel is small enough, it drags the rest of the liquid with it (Figure 1.2-4). The phenomenon is called Electroosmotic flow (EOF) and finds a practical application in capillary electrophoresis (CE), which is extensively used as a method to separate biomolecules.

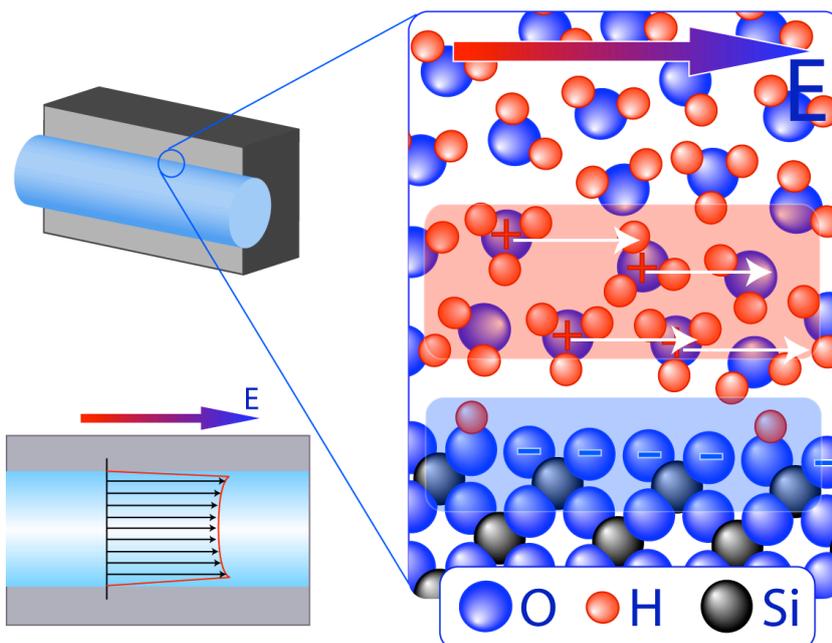


Figure 1.2-4. Electroosmotic flow

1.2.3 Some other characteristics of fluid behavior⁷

Besides the Reynolds number, a variety of dimensionless ratios exists that characterize the competition between different phenomena, the most commonly used ones are summarized in following table

Table 1.2-1. Dimensionless values describing motion phenomena

Re	Reynolds	$\rho v l / \eta$	Inertial/Viscous
Pe	Péclet	$v l / D$	Convection/Diffusion
Ca	Capillary	$\eta v / \gamma$ γ - surface tension	Viscous/Interfacial
Ra	Rayleigh	$v_{Buoyancy} l / D$ $v_{Buoyancy}$ - flow due to the density differences	Buoyant convection/Diffusion
Kn	Knudsen	λ / l λ - mean free bath	Molecular/Continuous

The Péclet number

The Péclet number shows the significance of diffusion compared to the flow. A high Pe implies that diffusion is not very substantial. Since microfluidics is used to manipulate and analyze different chemical processes, the understanding and controlling mixing of liquid ingredients has great importance. When $Pe \gg 1$, the mixing is Taylor dispersion mediated, when $Pe \ll 1$, diffusion dominates.

Importance of surface

The capillary number Ca describes the properties of a surface. In the case of laminar flow, two different fluid streams should flow along each other and mix only diffusively (Like on figure 1.2-2). However the picture might be different when fluids are immiscible. Surface tension can start to affect the flow and because of a Rayleigh-Plateau instability (fluids are trying to minimize the surface to volume ratio), the stream may break and form droplets. This phenomenon is used to generate monodispersed droplets in microfluidic devices. However surface tension exists also between liquid and the device walls. Since surface forces can be manipulated by various means, like chemical reaction on surface, temperature gradient etc. the fluid droplet can be driven. From a practical point of view, the most interesting method to change surface tension is probably electro-wetting (Figure 1.2-5)

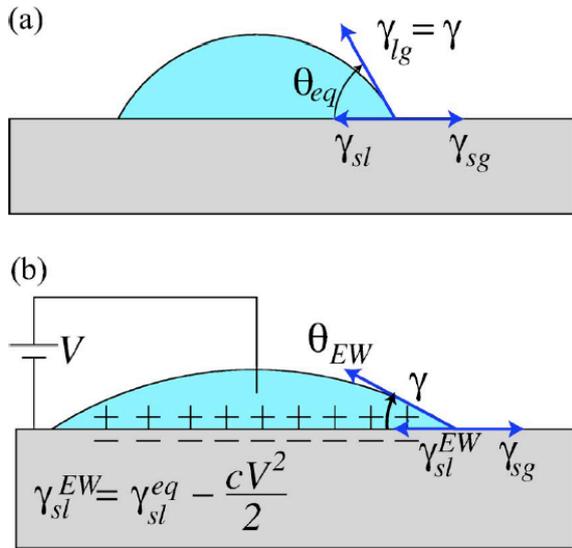


Figure 1.2-5. Electro-wetting. (Figure: Squires *et al*⁷)

When applying a voltage between liquid and surface, the interface acts as a capacitor. In order to decrease the total electrostatic energy, the capacitance must be maximized, which drives the droplet to increase its contact area with the surface, which, in other words, means increased wetting and decrease in contact angle.

$$\cos \theta = \cos \theta_0 + \frac{cV^2}{2\gamma_{liq-gas}} \quad (1.2.18)$$

where c is capacitance of interface per unit area

$$c \propto \epsilon / \lambda_{Debye}$$

Buoyancy

When liquids with different densities (either due to the composition or temperature gradient) are brought together, gravitation tries to minimize the energy by placing the denser liquid below the lighter one. These buoyant forces are balanced by viscous drag, which yields a characteristic velocity scale for buoyant flow.

$$v_{buoyant} \propto \frac{\Delta\rho g l^2}{\eta} \quad (1.2.19)$$

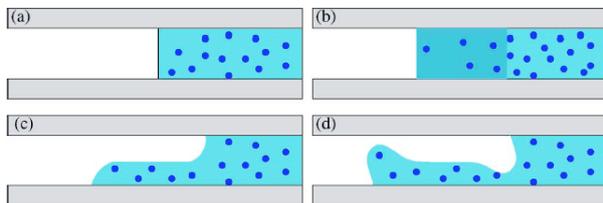


Figure 1.2-6. Buoyant flows. (a) initial interface between two fluids. (b) diffusion dominated interface spreading ($Ra \ll 1$). No buoyant effects. (c) Advection dominated interface spreading ($Ra \gg 1$) (d) as (c) but inertia has greater importance (Figure: Squires *et al*⁷)

The ratio of buoyant flow and diffusive flow is called Rayleigh number Ra and, in microfluidics, it appears that Ra can be significantly smaller than in macroscopic systems, which finds application in protein crystallization, for example.

1.3 Applications of microfluidics in chemistry and biology

In the following sub-chapter I am giving some examples describing practical applications of microfluidics.

1.3.1 Applications in structural biology

The power of microfluidics lies not just in smaller scales, but also in new and unusual physics. Here I describe two cases, where microfluidic circuits are utilized for studying proteins structure and folding. Both are phenomena of great interest for structural biologists.

Protein structure^{9; 10}

Proteins are the central building blocks for the machinery of life. Elucidating proteins structure may help to understand their functioning and also malfunctioning, the latter being the reason for various diseases. Proteins are the targets for drugs, and knowledge about their structure greatly facilitates the design of new pharmaceuticals, which otherwise is done by large scale screening. The main method for decoding protein structure is using different kinds of x-ray diffraction experiments, which however need high quality crystalline samples of material. But obtaining crystals of large and most interesting membrane protein complexes is anything but a simple task. Currently there is no approach to predict exact conditions for the crystal growth. Common procedure is going through laborious trial-and-error work and the usage of expensive robots. And even worse, purified protein samples for crystallization are mostly available only in mg quantities. This is a true challenge. One method for crystal growth is free interface diffusion (FID). Conventional FID is done in capillaries, where two liquids are brought to contact and left to mix diffusively. Nevertheless it is complicated to achieve a good liquids interface. The main problem is buoyancy driven convection, which disturbs the slow diffusive mixing.

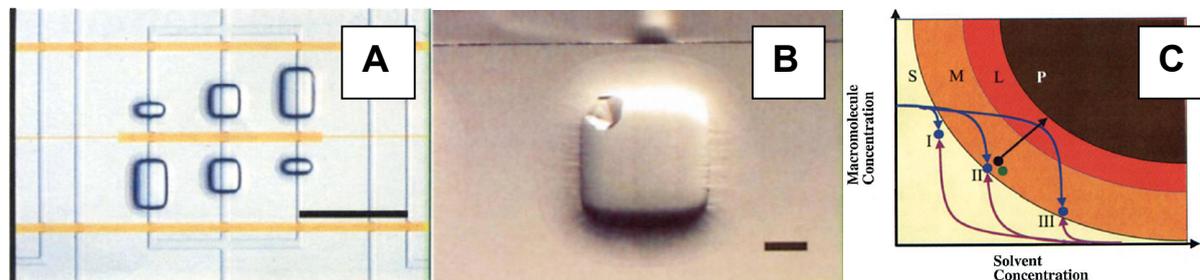


Figure 1.3-1. Protein crystallization in a microfluidic chip. (A) Reactors coupled diffusively together through the valve (yellow) gated channel. (B) Glucose isomerase crystal. (C) Crystallization conditions. Due to diffusion, the concentration passes many points in phase-space. (Images: Hansen *et al*)

Buoyancy is eliminated if the capillary is in vertical position, so that the dense liquid is underneath the light one. However, there is a danger that growing crystals are sinking down from the interface. A solution for the problems might come from microfluidics. In microchannels where $Ra \ll 1$ (see chapter 1.2) the viscosity suppresses buoyant flow and the liquid interface is significantly easier to set up and maintain. The possibility for massively parallel experiments in a single chip and very small sample volumes are making microfluidics an ideal choice for protein crystallization studies. Hansen *et al* are describing a simple PDMS microfluidic chip, which can simultaneously perform 144 FID crystallization trials, each using only 20nL of sample. Concentration profiles and kinetics can be precisely controlled by different reactor and channel geometries (Figure 1.3-1). Loading of these reservoirs takes advantage of PDMS gas permeability, which means applying pressure push gas into PDMS and fills even dead-end channels. Microreactors can be connected and isolated with simple pneumatic membrane valves in PDMS. This method has shown to be useful in crystallizing protein complexes that are hard to crystallize with other methods. It has been noticed that some

crystals grow faster using this method as compare dto other more conventional techniques (24h versus 1 week).

Protein folding¹¹

The process how a protein chain collapses and finally folds into a defined globule is currently not well understood. It is not easy to observe this process. Some intermediate steps and partly folded states have been captured by time resolved SAXS, but lately it has been thought that coupling the experiments to microfluidic mixing device could facilitate this study. For example, Pollack *et al* studied the collapse of Bovine beeta-lactoglobuline (BLG, a milk protein). At first BLG is mixed with urea, a denaturation agent. When the narrow laminar stream of denaturated BLG is surrounded by buffer solution, the concentration of small urea molecules will drop rapidly in the central stream and initiate the collapse of the protein chain (Figure 1.3-2). Thus, the rapid and controlled concentration change can be used to study the process of protein folding.

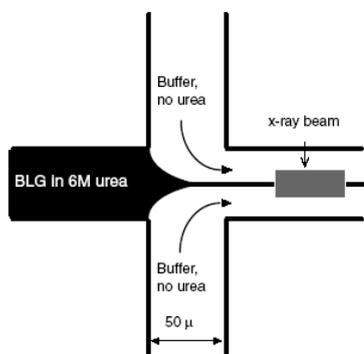


Figure 1.3-2. Time resolved collapse of a protein chain. Black is a stream of unfolded BLG protein chains in denaturation agent. When the BLG stream meets the buffer solutions, the denaturation agent diffuses out of the stream, initiating the folding process. After 6ms ~ 20% of protein is compact, reaching the final, native, state needs seconds (Figure: Pollack *et al*)

1.3.2 Applications in chemical biology

Chemotaxis^{6, 12}

Chemotaxis is a cell migration in a gradient of molecules called chemoattractants. It has a crucial role in embryogenesis, wound healing and cancer growth. In order to study this phenomenon a method to generate and hold precise gradient patterns is really beneficial. However it is hard to achieve a constant linear concentration gradient in macroscopic systems. Fast and controllable gradient generation becomes possible with laminar flows in microfluidics.

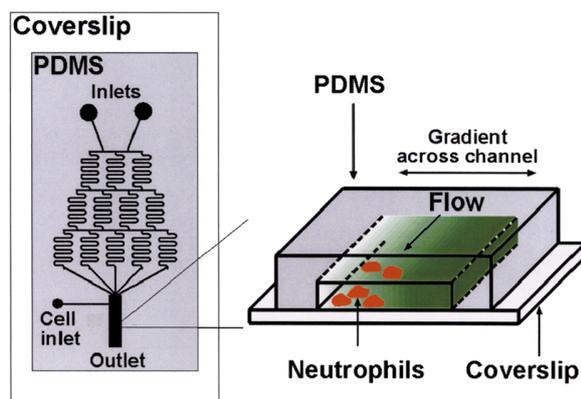


Figure 1.3-3. Studying neutrophils mobility in complex and linear gradients of interleukin-8 (Figure: Jeon *et al*)

1.3.3 Applications in organic synthesis

Radio labeled imaging probes¹³

Microreactors are holding a promise to improve the efficiency of syntheses of sensitive compounds, for example ^{18}F -labeled imaging probes for positron emission tomography. The problem with these compounds is the half-life of ^{18}F , which is only 110min, which means a rapid synthesis of doze is necessary. Lee et al have shown a design of a microfluidic chip that is capable to perform 5-step synthesis of 2-deoxy-2- ^{18}F -fluoro-D-glucose. The full length of the synthesis is 14min, yielding over 90% purity. Usage of microfluidics in the synthesis of pharmaceutical and other high quality chemicals might be an interesting future prospective.

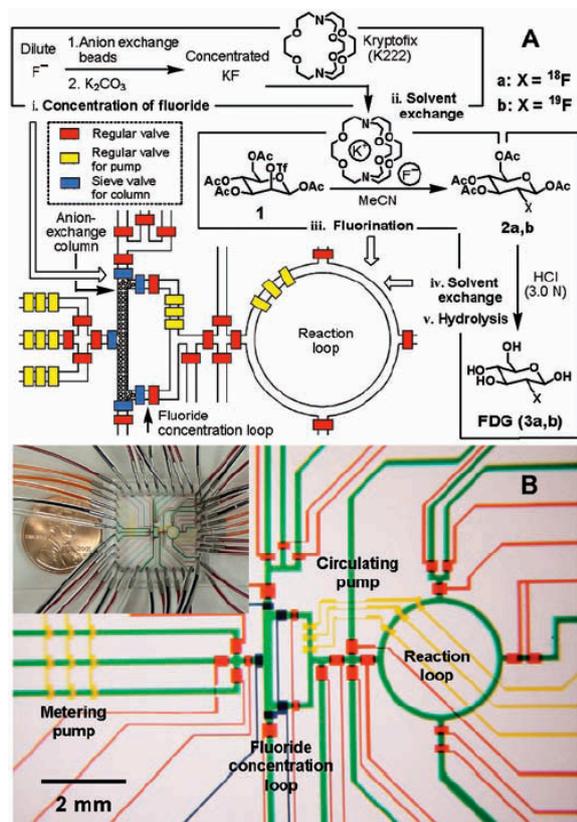


Figure 1.3-4. Synthesis of 2-deoxy-2- ^{18}F -fluoro-D-glucose. The procedure contains: fluoride concentration, water evaporation, radiofluorination, solvent exchange, hydrolytic deprotection. The chip has rotary pumps for mixing, affinity column to concentrate ionic solution. Solvent evaporation occurs through PDMS, which is moderately permeable for different vapours. (water and MeCN is used here) (Figure: Lee *et al*)

1.3.4 Applications in cell manipulation

Controlling bacteria population¹⁴

Current techniques allow to cultivate only a tiny fraction of all bacteria species. In order to improve the precise control of growth conditions, microfluidics with an active feed-back can be used. A lab on a chip can incubate cells in microreactors, clean, dilute and wash, to avoid biofilm formation, which can be a severe problem in microdevices. The cell density can be tested by sensing signaling molecules. As a result it is possible to study growth dynamics, chemical effectors, collect materials that cells are emitting, etc. It is an excellent method for chemical genetics and pharmaceutical studies.

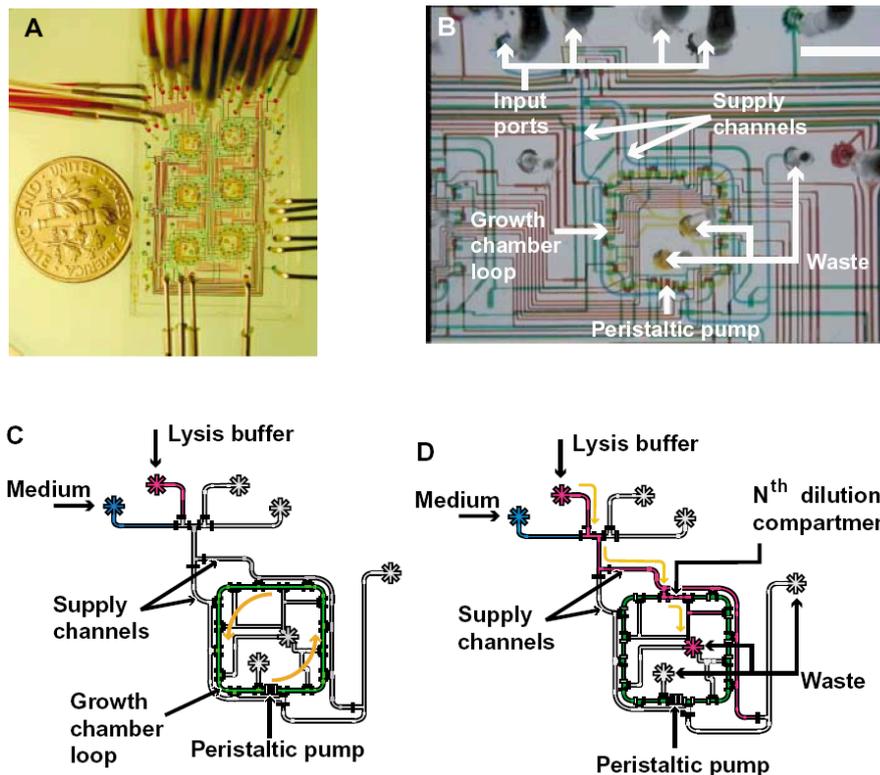


Figure 1.3-5. Bacteria population control in a microchemostat (Figure: Balagadde *et al*)

Positioning small molecules in a cell¹⁵

We can also take advantage that microfluidic devices can be made in the same size range as living cells. It is possible to use laminar flow to expose different parts of cell to different chemicals. For example, Takayama *et al* used it to study mitochondria migration in cells. First they labeled mitochondria on one side green and on another red, which made it possible to observe how mitochondria were mixing. To understand cell's physiology, it is important spatially regulated signaling, cellular communication and other aspects of microscopic structure to metabolism.

Chemical cytometry on pL scale¹⁶

Cytometry is an analysis of the chemical content of a cell. Using a microfluidic device it is possible to perform the entire process in a single chip, which can handle cells, lyse them to derive the chemical content and separate derived compounds with capillary electrophoresis. In ref. 16, detection was performed with laser induced fluorescence.

Other opportunities in microbiology¹⁷

Understand cells organization in multicellular colonies, how they communicate and finally differentiate into various phenotypes. As well as collectiveness it is crucial to understand the behavior of single cell. Also the development of analytical tools is bringing so far mostly qualitative biology more into the league of exact sciences like physics. Microtechnology can be used to replica mold structures of various polymers, micro-contact print different liquids, micro-mould, etc. Microfluidics can be used to pattern cells on surfaces, etc. encapsulate cells into polymer matrixes. One of the hot-topic is related to PCR (polymer chain reaction) in microfluidics.

1.4 Microfabrication techniques

In the following chapter i give an overview of microfabrication techniques¹⁸, with main emphasis on procedures used in our research project.

1.4.1 Photolithographic process

Photolithography (In Greek lithos - stone, graphein - write) is a technology of writing patterns onto the surfaces using light. In many ways it is similar to regular photography. First use of lithography dates back to 1822, when Nicéphor Niépce in France used sunlight exposed bitumen. Sunlight made bitumen harder to dissolve in lavender oil. In the middle of the 20th century, photolithography found its place in manufacturing integrated circuits, it has been the foundation of miniaturization science (both in electronics and other fields), having a great significance for modern technology. All information technology rely on microelectronics and optoelectronic communication. Microfabricated MEMS are also used in sensors, optics, manipulators and many other applications. The classical microfabrication is based on photolithography. The entire process consists of the deposition of a polymeric photoresist film, which is exposed through a photomask to UV radiation. UV radiation causes a chemical reaction that alters the solubility of the polymer film. This property is later used in a development step to selectively remove or preserve the exposed areas (positive or negative photoresist). The remaining polymer structure acts as a mask for further chemical treatment. The etching process removes material from uncovered areas. Etching processes are divided into wet and dry etching. In wet etching, the substrate is treated in solution, but in dry etching gasses and plasma are used instead. The resist film can be also used to deposit materials. After deposition of a new layer the resist can be dissolved, which means everything deposited on it will be removed. This is called a lift-off process and its very beneficial in-case of materials that are hard to etch, such as noble metals.

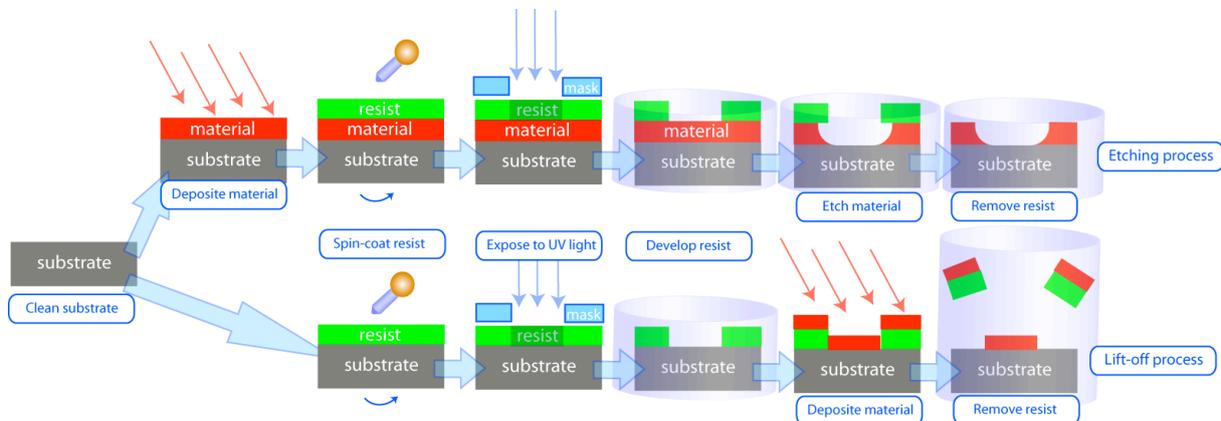


Figure 1.4-1. The photolithographic process

1.4.2 Materials

Microfabrication has been developed as a tool for microelectronics, which focuses on the construction of electronics circuits on small scales. Due to the origin most materials in microtechnology are inorganic (doped Si, GaAs, oxides, metals, etc.). Organic polymers have been used only as photoresists. More lately, microfabrication has been spreading to and mixing with other fields than electronics and the chemical and structural variety of materials has greatly increased too. Here we bring examples of some most common materials and techniques used in microtechnology to form structures!

Hard materials

Most common materials in microfabrication are silicon (Si) and silicon oxide (SiO₂).

Si: Silicon wafers used in process labs are typically cut from large silicon single crystal, which have been grown using Czochralski or Float-zone method. Both of them are starting from a single crystal seed and by slowly cooling molten Si (mp. 1414°C) enabling relaxation of atoms into highly ordered crystal. It implies also that Si wafers have special crystalline orientation. Besides orientation, important characteristic of Si is conductivity type. For electronics purposes Si is doped to have n- and p- regions. Dopants are introduced with diffusion process at high temperatures. Exact doping concentration profiles are quite well reproducible characteristics, which depends on process parameters (like time, temperature, concentration etc)

SiO₂: Silicon oxide is another important part in silicon processing. When exposed to oxygen Si oxidizes and forms a thin oxide layer. This oxide is first of all a good electrical insulator, but also barrier for diffusion, etching and other processes. Silicon oxide can be grown in various ways - most commons are thermal oxidation and CVD.

Glass: Glass is amorphous silicate. Glass is particularly interesting for bio chip applications, since its transparent, which allows to combine chips with optical instruments like spectrometers or microscopes. In order to fabricate structures into glass, glass can be etched with a solution of HF (HF:HNO₃:H₂O volume ratio 20:14:66)¹⁹, which unfortunately is an inconvenient chemical to handle, because of toxicity and corrosiveness. Conventional resist materials are not sufficiently stable to protect glass against HF, which means masking has to be utilized using a sacrificial metal layers of chromium and gold. To form closed structures, like microfluidic channels in glass, different glass layers can be bonded using high temperature treatment (440°C for 0.5h).

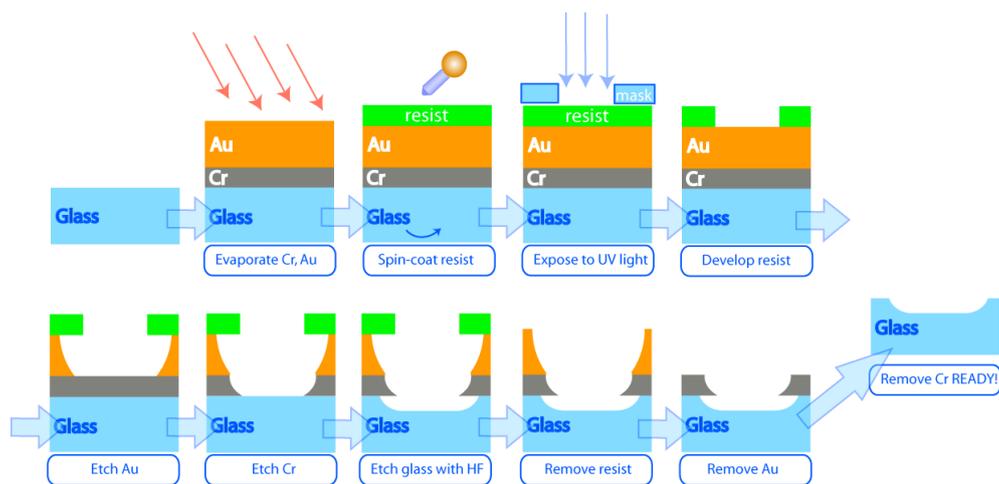


Figure 1.4-2. Fabricating structures into glass

Metals

Metals are mostly used in microtechnology to make electrically conductive wires. They can be deposited by evaporation, sputtering or using electrochemistry. To form structures, either a lift-off or etching process can be used.

Polymers

Most common polymers in microfabrication are different photoresists (and also e-beam resists). Resists can be patterned using an exposure technique, which alters the chemical structure of the material so it would or wouldn't dissolve later in developer. Photoresists are more profoundly introduced in section 1.4.3. Another way to deposit polymers has its origin in soft lithography, where replica molding has been used to obtain polymer structures. This technique is further described in section 1.4.8

1.4.3 Photoresists

Photoresists are the key components in photolithographic pattern transfer. When exposed to UV light, photoresists experience a chemical change, which makes removal rates for exposed and unexposed areas different. After development, the mask pattern will be transferred onto the resist. Radiation can induce changes in different chemical properties of photoresists:

- Change in molecular weight
 - Cross-linking (increase in molecular weight, harder to dissolve)
 - Chain scission (decrease in molecular weight, easier to dissolve)
- Reactivity change (exposed areas react differently in further treatments)
- Polarity change (turn hydrophilic compound to hydrophobic or vice versa, change in solubility)

By composition, resists are typically containing the following parts:

- Polymer solution (base resin, changes with exposure)
- Sensitizer (controls photochemical reaction)
- Casting solvent (needed for coating)

If the resist is without sensitizer it is called single-component resist, whereas resists with sensitizer are called two-component resists. In the following, I describe shortly the main characteristics, chemical principles and usages of resists.

Sensitivity of photoresist

The sensitivity of a resist is describing which radiation dose is needed to cause sufficient chemical change. Sensitivity is wavelength dependent. It is important that a resist is insensitive to normal cleanroom light (stable to handle) and maximally sensitive to the exposure light (UV). Sensitivity can be described in many ways: intrinsic sensitivity or photochemical quantum efficiency Φ is the ratio of photo induced reaction events to the total number of absorbed photons at a given wavelength.

$$\Phi = \frac{\text{Photo events}}{\text{Number of absorbed photons}} \quad (1.4.1)$$

The G-value, another characteristic parameter, is showing the number of scissions or cross-links per 100eV absorbed energy. (Examples: $G(\text{PMMA})=1.3$, $\Phi(\text{PMMA})=0.02$, $\Phi(\text{DQN})=0.2\dots 0.3$)

Contrast of photoresist

Contrast is a measure of a resists response to dose variation. If the contrast is high, then already slightly under-exposed resist behaves as completely unexposed, which actually allows more vertical side-walls of resist windows. In other words, there is always some scattering under the mask edges near features, but if resist is irresponsive to this small illumination (High contrast resist) side walls are more vertical. For example, in positive resists the contrast γ is describing the ratio of the dose needed for complete resist removal after development (D_P) and the dose at which some resist starts to develop (D_{P0}). Below dose D_{P0} thickness does not vary considerably.

$$\gamma = \left(\log \frac{D_P}{D_{P0}} \right)^{-1} \quad (1.4.2)$$

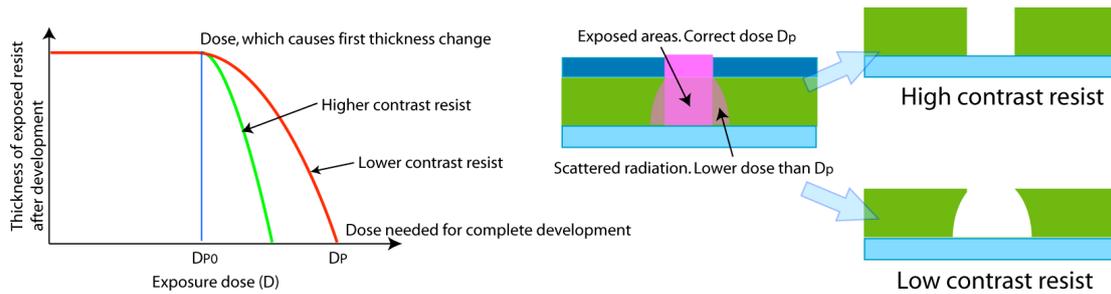


Figure 1.4-3. Illustration of resist contrast and effect to side walls of resist windows

Positive resists

In a positive resist, exposure causes weakening of the material, so that development will remove all irradiated areas. Most common classes of positive resists are based on poly(methylmethacrylate) known as PMMA or photoactive diazoquinone esters (DQN).

PMMA is a single-component resist. Exposure to deep UV radiation (DUV) induces chain scission, which makes the polymer more soluble. Maximum sensitivity of PMMA is at 220nm. Above 240nm the polymer is insensitive to radiation, but it can be also used as x-ray and e-beam resist. However PMMA is not very sensitive, requiring a dose around 250mJ/cm². PMMA can be improved by adding sensitizer (a sensitivity 150 mJ/cm² can be reached)

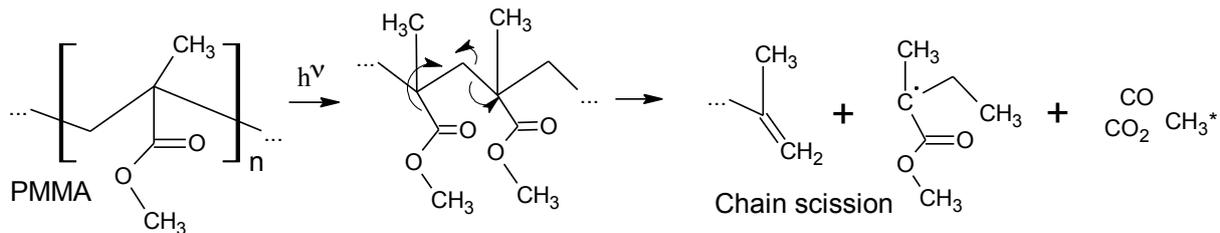


Figure 1.4-3. Structure and photo-induced chain scission reaction in PMMA resist

DQN is a two component resist made of phenolic novolak resin, which intrinsically is soluble in alkaline aqueous solutions. Another constituent of resist is a photoactive diazoquinone ester (20-50%), which forms a complex with the phenolic -OH groups of the base polymer and render it insoluble (the ester acts as a solubility inhibitor). When irradiated, the diazo group is cleaved and a rearrangement reaction turns the compound into carboxylic acid, which is hydrophilic and also does not bind to novolak resin, allowing removal in basic aqueous development. The sensitizer also enables more homogeneous exposure. A sensitized resist has a high absorption of UV light, but during the exposure the sensitizer is bleaching and allowing reaction to proceed deeper into resist layer. This fact in combination with spectroscopy can be used to estimate the proper exposure time and dose. DQN resists are prevalent in near UV lithography (Hg lines 365, 405, 435nm), but they are not useful in DUV due to the strong unbleachable absorption below 300nm, which does not allow uniform exposure.

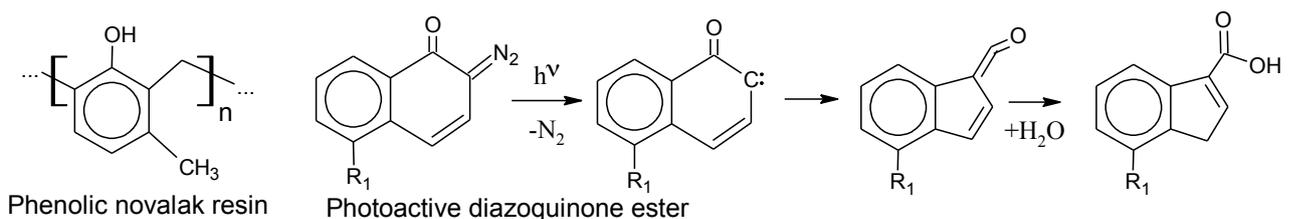


Figure 1.4-4. Composition and photochemical mechanism of DQN resists.

Negative resists

In negative resists exposure causes hardening of material for further development. Mostly it is due to the cross-linking of polymer, which makes them insoluble. Probably the most well known negative resist is SU8, developed by IBM.

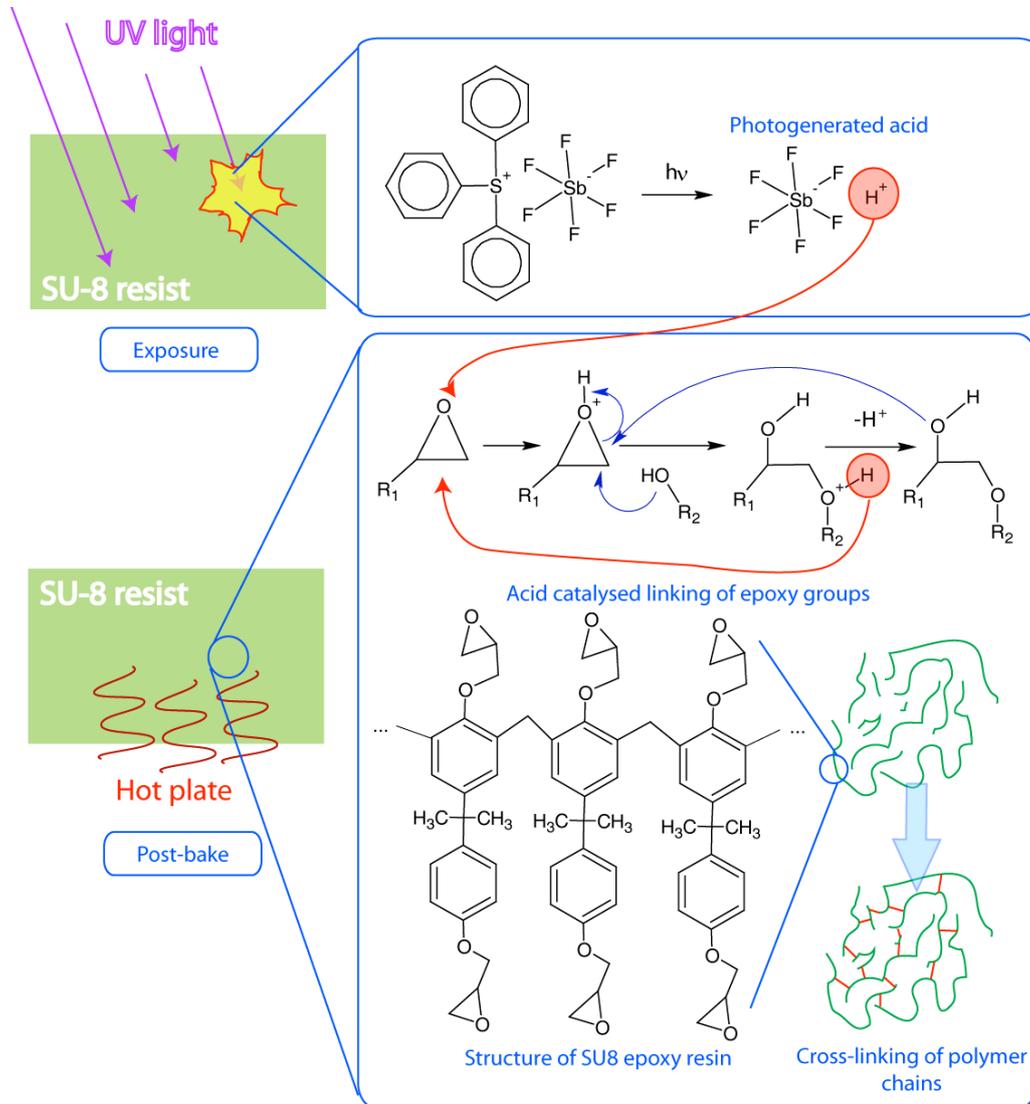


Figure 1.4-5. Chemical cross-linking principle of the SU8 resist.

SU8 is exploiting a chemical amplification, which means that UV light produces a catalytic photoproduct (in SU8, onium salts that produce a Lewis acid)²⁰. One generated acid molecule can catalyze thousands of reaction events, that increase the sensitivity of the resist (amplifies). This catalyzed cross-linking takes place actually during the after-exposure-bake, which means that baking time and temperature are crucial parameters of the process. With SU8, sensitivity values of $5\text{mJ}/\text{cm}^2$ or better are accessible²¹. SU8 is an excellent resist for various microtechnology applications. It exhibit high thermal and chemical stability, excellent sensitivity, resolution and aspect ratio. Very thick resist layers ($\sim 500\mu\text{m}$) can be coated in a single run. SU8 is a permanent resist, applicable for micromachining, molds etc. because stripping of hardened SU8 is still problematic.

Dual-tone resists and image reversal

Dual-tone means that resist can be used as a positive or a negative resist, depending on the developer or post-exposure treatments. The chemical mechanisms responsible for the duality can

be quite different. For example, tBOC-containing resists are based on a photogenerated acid (like SU-8), which catalyzes the deprotection of phenolic -OH group. It turns the initially hydrophobic polymer into hydrophilic one. Which tone the resist will be, would depend on the developer. Non-polar solvents remove unexposed resist (acting as a negative photoresist), but polar solvents dissolve the exposed part of the resist (acting thus as a positive photoresist). Image reversal can also rely on post-exposure treatments. For example in DQN resists (like the Shipley 1800 series) irradiation turns the diazoquinone ester into a carboxylic acid, but when treated with imidazole, the carboxylic acid group will be cleaved and the resulting molecule is highly resistant to development and for further exposure. When now exposed again with UV light without a mask, the diazoquinone in previously unexposed regions will be converted to a solution promoting carboxylic acid, whereas all previously exposed areas are staying insoluble, which means after development a negative image is obtained. Rohm and Haas has taken the DQN resist as a base for a dual-tone resist, adding a small amount of melamine based cross-linker. Post-exposure heating causes carboxylic acid groups to catalyze a cross-linking reaction, which turns the exposed areas insoluble and results in a negative structure.

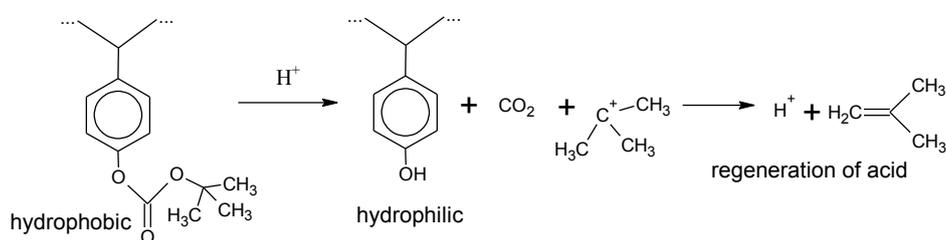


Figure 1.4-6. tBOC-based resists.

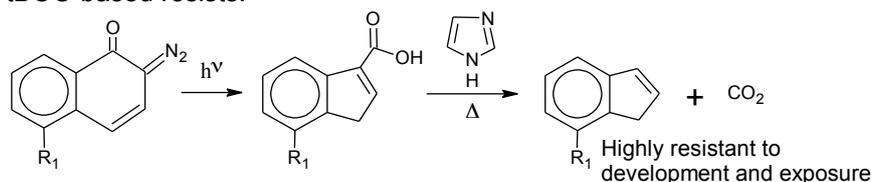


Figure 1.4-7. Shipley 1800 as a dual-tone resist.

Resist adhesion

In order to fabricate good quality structures, resist adhesion to wafers has great significance. Positive resists are especially prone to poor adhesion. The first reason for poor adhesion can be humidity, which implies that substrates should be carefully dried. Oxide covered surfaces (Also Si, which is always covered by a thin native oxide layer) are absorbing water molecules. When covered with photoresist, it can bind to the water layer, which weakens the resist bonding considerably. More hydrophobic substrates generally adhere resists better. For that purpose wafers can be primed with hexamethyldisilazane (HDMS). It will cover oxide surfaces with thin layer of strongly bound methyl groups, which renders the surface more hydrophobic and enhances resist adhesion.

Resist profile

For different purposes different resist profiles are favorable. For example, lift-off process need an undercut to avoid complete coverage with metal, which would not allow lift-off solvents reaching the polymer. Some micromachining applications instead need precisely vertical side walls, etc. In general, positive resist yield a vertical or overcut profile. Positive resist overcut is usually the result of a too low dose, and undercut of a too high dose with back scattering. Negative resists instead have more likely a vertical or undercut profile. When using positive resists for lift-off, often multilayers are needed (a separate undercut or lift-off layer)

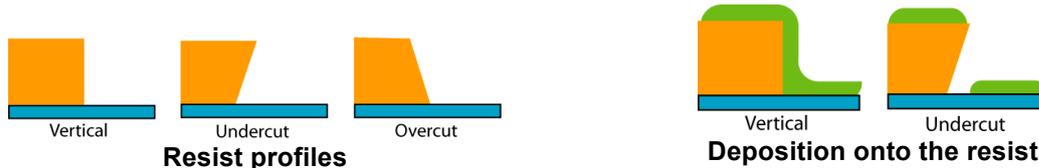


Figure 1.4-8. Different resist profiles

Choice of resist

Positive resists are generally more expensive but have better contrast and smaller feature size, better step coverage, lower swelling in developer, and good thermal stability. However they have a smaller development process window, lower sensitivity and lower Si adhesion.

Negative resists are cheaper; they have wide development process window (they are insensitive to over-development), higher sensitivity and better wet chemistry resistance (stable in alkaline and acidic solutions). They do not have good plasma etch resistance and display higher swelling in developer. Industrial positive resists are more favorable since they are developed in aqueous solutions whereas negative resists are mostly developed in organic ones, which are more problematic from the environmental and safety point of view.

1.4.4 Spin-coating

Spin-coating is a simple fluid flow based technique to cover substrates with thin uniform films. It is a common method to prepare polymer films. In photolithography, spin-coating is the prevalent method for creating thin uniform photoresist layers. Since the homogeneous thickness of resist is crucial to have even development times and thus precision of geometries, spin-coating is one of the most critical process steps in photolithography. A spin-coating process can be divided into the following parts:

1. Substrate mounting to a vacuum chuck, which holds it during the spinning
2. Polymer solution dispensing onto the substrate
3. Spinning of the substrate to distribute the solution evenly, excess solution is expelled over the edge. When a uniform thickness is obtained, viscous flow causes the film to become thinner and thinner
4. Baking to remove the solvent and obtain a solid film

The theoretical model of spin-coating can be rather complex and contain various aspect, some insight can be obtained when using the simplified approach described already in 1958 by Emislie et al ²². We can consider that thinning is caused by viscous flow on the surface so that centrifugal and viscous forces are canceling each other.

$$-\eta \frac{\partial^2 v}{\partial z^2} = \rho \omega^2 r \quad (1.4.3)$$

When assuming that the film is initially uniform, the equation of thinning would give us

$$h = \frac{h_0}{\sqrt{1 + \frac{4\rho\omega^2 h_0^2}{3\eta} t}} \quad (1.4.4)$$

It is easy to see that the process is depending on viscosity and density, which means evaporation of the solvent is of utmost importance. Thus, in order to have good reproducibility of film thickness, it is important to control the dispensed volume, the exact spin-speed-and-time relation and environmental characteristics like temperature and humidity which are affecting solvent evaporation. More generally, the empirical equation of thickness can be written as²³

$$h = KC^\beta \eta^\gamma \omega^\alpha t^x \quad (1.4.5)$$

Where C is the polymer concentration; η is the viscosity; K , β , γ , α and x are empirical constants. In case of absent evaporation, $\alpha=-1$ and $t=-1/2$, for constant evaporation $\alpha=-2/3$, for a variable evaporation rate $\alpha\approx-1/2^{24}$. However, in practice calibrated spinning recipes and thickness curves are mostly used.

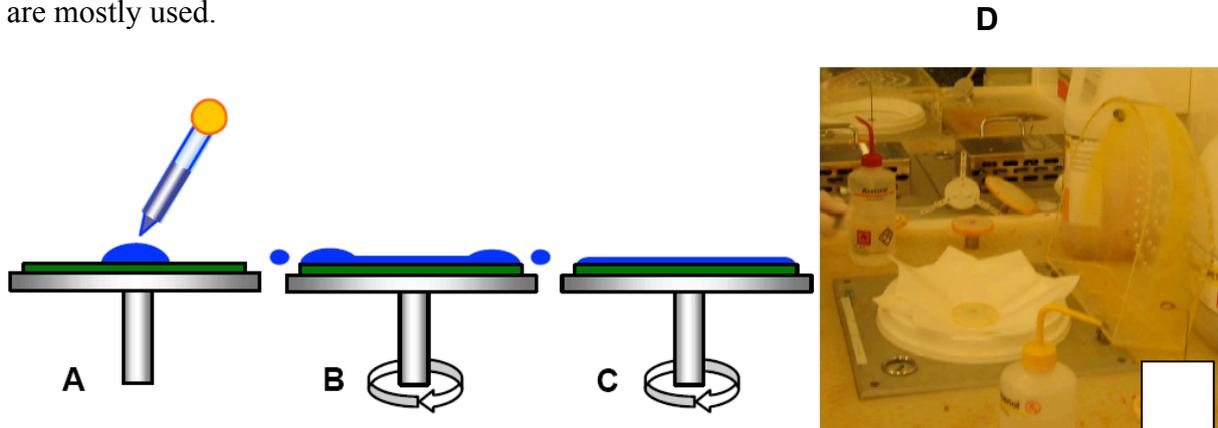


Figure 1.4-8. Spin-coating process. Dispensing of solution (A). Distribution (B). Uniform thinning (C). Spin-coater in Chalmers clean room facility MC2 (D)

Edge-bed removal

During spinning, the centrifugal force is pushing a liquid solution towards the edge of the substrate, where it is heaped up and finally expelled over the edge. Nevertheless after spinning some higher fillet is left on the edge. This unevenness would not allow tight mask contact and thus obstruct obtaining maximum resolution in contact lithography. In order to remove troublesome edge-beds, the following method can be used: some solvent (like acetone) is dispensed through a narrow syringe needle to the edge of a fast spinning wafer²⁵. The solvent dissolves the polymer and it will thus be removed. The strong centrifugal forces do not allow the solvent to flow inwards.

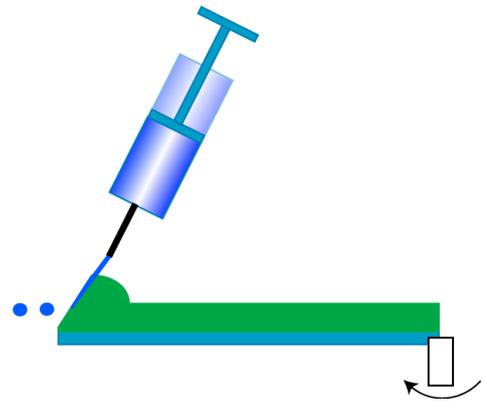


Figure 1.4-9. Principle of edge-bed removal. Partially removing photoresist on the edge of a spinning wafer with narrow jet of solvent from a syringe.

1.4.5 Alignment and exposure

To selectively expose parts of a photoresist layer, photomasks are used. The typical photomask consists of a substrate material which is transparent to radiation and has an opaque layer to shadow areas that should remain unexposed. For near UV light, regular glass can be used as a photomask substrate, whereas deep UV (DUV) requires the more expensive quartz. The absorbing layer is usually a Cr film (around 800Å), which is patterned using e-beam lithography. Lower resolution masks can be also fabricated using cheap transparency films and high resolution printing ($\sim 2\mu\text{m}$). Masks can have different polarities; light field masks have transparent background and opaque features, whereas in dark field mask it is inverted to opaque background with transparent structures.

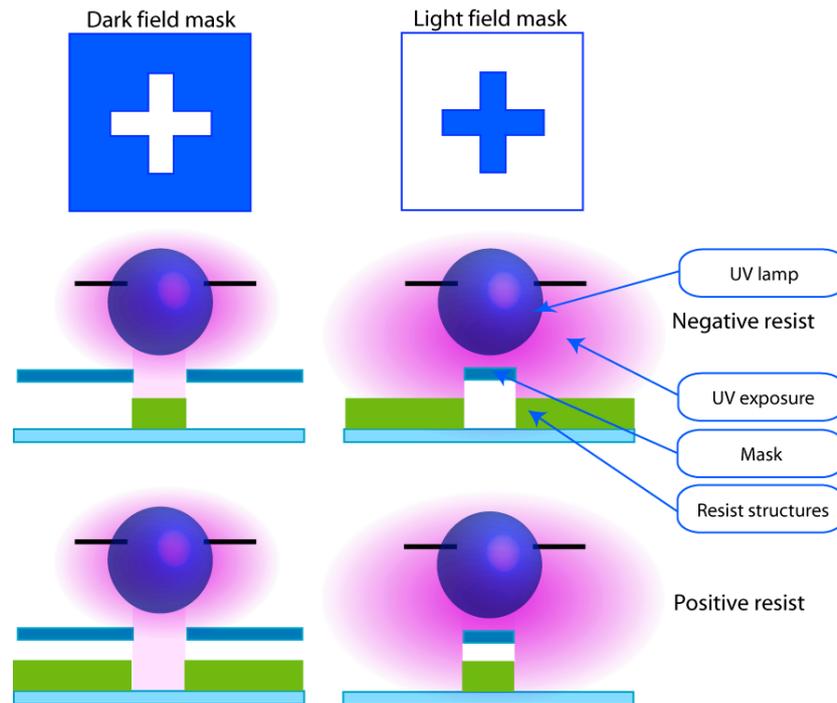


Figure 1.4-8. Dark and light field masks and obtained resist structures with negative and positive resists. The figure does not reflect the actual fabrication procedure (structures are forming after development, not during exposure)

The image-to-resist transfer methods in optical lithography are divided into

- Projection photolithography
- Proximity photolithography (or soft contact)
- Contact photolithography (or hard contact)

Projection photolithography is in principle very similar to the diapositive projector, where a mask image is projected with the help of an objective system. This technique has several advantages, like lack of contact between wafer and mask, which increases the lifetime of the mask, and the possibility for optical reduction. On the other hand, the high quality optics needed is expensive and at shorter wavelengths absorption in the optics becomes more and more significant, which finally requires that extreme UV projectors must use reflective optics instead of refractive ones. The resolution of projection lithography R is given by

$$R = \frac{k\lambda}{NA} \quad (1.4.6)$$

Where λ is the wavelength of light, NA is the numerical aperture of the optics (usually NA is in range 0.16...0.6) and k is an experimental parameter. Another important characteristics of projection system is the depth of focus (DOF) which characterize in how vast range sharp image is obtained. It is important that DOF is larger than the thickness of the resist.

$$DOF = \frac{k\lambda}{NA^2} \quad (1.4.7)$$

In proximity photolithography the mask is placed in the direct vicinity of the resist and when exposed to a parallel beam of UV, light the shadow of the mask is cast on to the resist. Diffraction of light limits the resolution of this technique.

$$R = b_{\min} = \frac{3}{2} \sqrt{\lambda \left(s + \frac{z}{2} \right)} \quad (1.4.8)$$

Where s is gap between resist and mask, and z is thickness of photoresist.

In the case where the gap between mask and resist is zero, the technique is called contact photolithography. In this case the resolution is going to be

$$R = b_{\min} = \frac{3}{2} \sqrt{\lambda \frac{z}{2}} \quad (1.4.9)$$

Hardware needed for contact lithography is easier and cheaper, which makes it a more accessible technique for academic users. But due to fast mask aging it is not so commonly used in industrial processes.

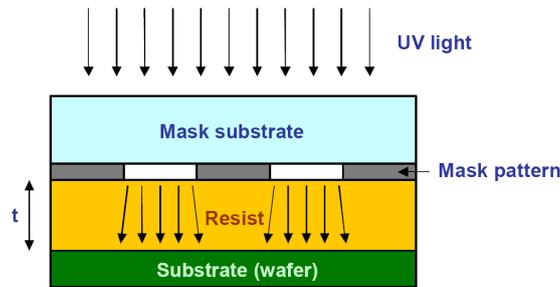


Figure 1.4-9. Contact photolithography

Exposure dose and time

The important parameter of exposure is the dose of radiation, which causes proper chemical reactions in the resist.

$$Dose \left(\frac{J}{cm^2} \right) = Intensity \left(\frac{W}{cm^2} \right) \cdot time(s) \quad (1.4.10)$$

Advances in photolithography

Conventional photolithography is restricted by the wavelength of light. It means that either principally new techniques or shorter wavelength light sources are needed to obtain smaller structures. Examples could be extreme UV (EUV, 10-14nm) and x-ray. Problem with EUV is the strong absorption, which mean reflective optics and vacuum environment is needed. X-ray could not be readily projected at all, which mean only 1:1 shadows are possible, however X-ray can work without vacuum environment. X-ray radiation is exploited also in LIGA (*Lithographie Galvanoformung, Abformung*) technique to build high precision metal molds for injection molding or embossing plastics. A typical resist used in x-ray lithography is PMMA (not very sensitive 2000-6500mJ/cm²). Generally x-ray and e-beam properties of resists are fairly similar. Gaining the maximum from photolithography also involves various improvements of resist, planarization of surfaces, antireflective coating (to have more homogeneous resist exposure and avoid standing waves and scattering), and mask improvements (like phase shift masks and grey-tone masks)

1.4.6 Deposition

In order to form structures, material layers are either added or removed. Adding is usually called deposition, whereas removing is etching or lift-off. Deposition of photoresist or polymers by using spin-coating was covered in chapter 1.4.4, but there is vast variety of other deposition techniques, like bonding, casting, CVD, droplet delivery system, electrochemical deposition, ion cluster deposition, laser phase deposition, liquid phase epitaxy, molecular beam epitaxy, screen printing, spray pyrolysis, sputter deposition, thermal evaporation and many others. In the next section I describe two of them more thoroughly, since they are used in the project.

Thermal evaporation

Thermal evaporation is very classical method for depositing metal layers. Essentially it is boiling or sublimating heated material to substrate in vacuum. Vacuum is necessary for atoms, which have been escaped from the source boat, to have free way to fly and land on substrate. High pressure would mean scattering before reaching substrate and also high contamination. For good quality layers the vacuum should be as high as possible (10^{-5} Torr or lower and oxygen partial pressure less than 10^{-8} Torr, to avoid oxidation). To heat substrate for evaporation there are several techniques. Most simple is a resistive heating, which mean source metal is placed on tungsten boat or filament, which is heated till high temperatures by passing current through. The problem of resistive heating is contamination due to the some evaporation from boat as well. Another widely used method is heating by electron beam (e-beam). In this case high intensity electron beam is directed with magnets to source and the current cause's heating. Cooled source by itself act as boat as well, which mean there is no contamination. However hitting substrate with high-energy electrons causes X-ray radiation which may damage a substrate. Heating can be done also by RF induction or with high power lasers the last one can have perfect characteristics, but has more expensive setup. Things to keep in mind are that low-pressure evaporation causes atoms to leave source and fly directly to substrate, which means if there is some obstacle in the middle of way, a shadow will be cast. Also growth rate of metal layer is inversely proportional to the source to substrate distance, which means there is a slight difference in the growth rates in different regions of substrate, if needed substrate rotation should be used to avoid positional difference. In order to control thickness quartz crystal based deposition monitors are used. The deposited metal causes slight change in the resonance frequency of crystal oscillator, which is used to deduce thickness of deposited layer.

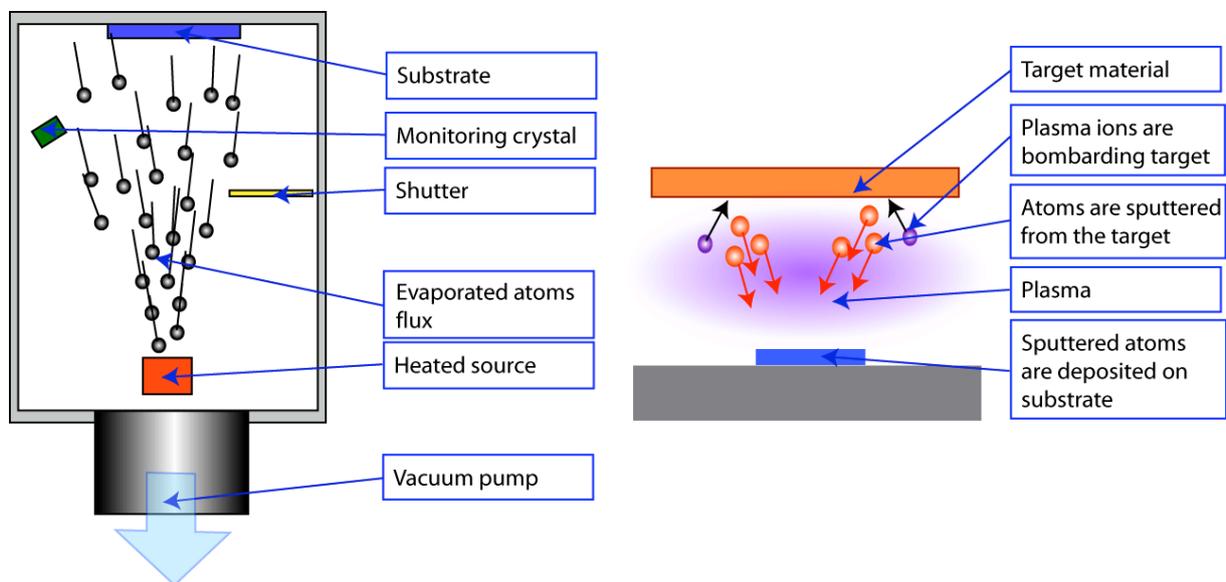


Figure 1.4-10. Principles of thermal evaporation (left) and sputtering (right)

Sputtering

In case of sputtering deposition, inert gas plasma ions (like Ar^+) are employed to physically bombard source and kick out atoms or clusters of atoms (usually 0.1 .. 20 atoms per ion), which will be deposited on substrate. Sputtering possesses several advantages over thermal evaporation, like wider materials choice (sputtering can be used to deposit composite alloys, which would not maintain their composition in evaporation or materials that are hard to evaporate). Also sputtered layers tend to have better surface adhesion and uniformity. Since the sputtering occurs at much

higher gas pressures, there is much less shadow effect, since sputtered particles can scatter around corners. Disadvantages compare to evaporation are maximum achievable growth rate which is much lower and general complexity of setup. Sputtering process can be controlled by several parameters, like: gas pressure, flow rate, substrate temperature, sputter power and bias voltage. Plasma ion energy has optimum range for maximum sputter rate, after which ion implantation process starts to dominate gradually. Highest sputter rates are possible with magnetron sputtering (up to 1 μ m/min).

1.4.7 Etching

Etching techniques are used to remove materials. By the type these techniques are divided into wet and dry etching. Wet etching occurs in solution that reacts with materials and gradually dissolves it, whereas dry etching is performed with gasses or plasma.

Dry etching

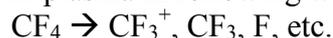
Dry etching by itself encompasses vast variety of technique, which have different mechanisms and material removal properties. Most of the techniques have plasma involved in one or another way. Widely we can divide these methods into chemical, physical or combined.

Chemical etching means that reactive gas molecules are landing on the surface of material that is wanted to etch, react with surface atoms and form a volatile compound, which separates from surface and diffuses into bulk gas that is gradually exchange and pumped away. This kind of etching is generally isotropic, which means it does not have preferred direction and it is also selective for material, which means we can choose a proper gas that react only with substrate but not with the masking layer for example.

Physical etching instead is based on momentum transfer effect. High energy (speed) plasma ions are hitting substrate and collision expels pieces of material (sputter), like a billiard ball. In this case removal can be highly anisotropic since ions are bombarding from certain angle, but on the other hand method is not very selective which means it removes all type of materials.

To have benefits of both mechanisms, like good selectivity and high removal rates together with directionality (avoid feature broadening) methods can be combined into chemically assisted ion-beam etching. In means that reactive gas is fed together with bombardment of unreactive ions. Removal rate can greatly exceed both of the components alone, while still maintaining good selectivity and anisotropy.

The difference between these techniques is made essentially by the plasma conditions and gas pressure. When gas pressure is high ($10^{-1} \dots 10^1$ Torr), the collision rate between ions and molecules is high, which means they do not acquire high energy or directionality. In this case plasma is more essential as a mean to produce very reactive species, both neutral and ions. For example, tetrafluoromethane molecules can be cleaved in plasma in following way:



Physical sputtering instead works in much lower pressures (like 10^{-4} Torr). It implies that ions are accelerated a long way, they obtain high energy and their motion is more directional.

After all to describe plasma there is needed a variety of parameters: composition, density, energy, bias which in plasma machines are regulated by the base pressure, electrical power, bias (higher bias yield higher etch rates) and gas flow rates (unit sccm – standart cm^3/min). Flow rate should be such that at least 10 times more gas is provided than there is needed for etch (Utilization factor $U < 0.1$) Also there are several designs for generating plasmas with different properties. Besides

others a critical issue in plasma devices is the homogeneity (same conditions must be maintained all over the substrate).

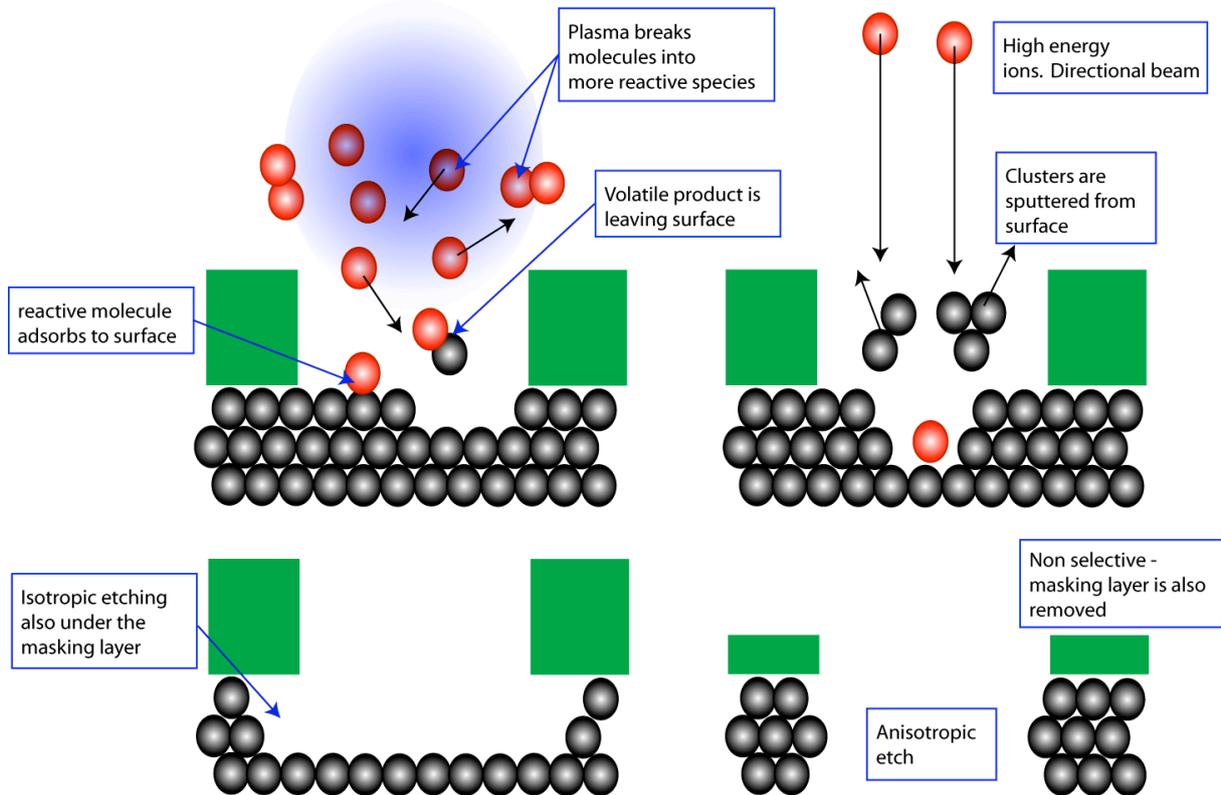


Figure 1.4-10. Chemical and physical dry etching. Figure is illustrative kind.

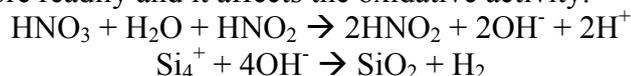
Wet etching

In wet etching technique solid material is removed by the reaction with liquid phase etchant (either in solution bath or spray device). Usually wet etching is isotropic unless the substrate is monocrystalline (for example: Si wafer), then etching can also occur in particular crystalline direction. In following, we bring a few examples about most common wet etching methods:

Glass and SiO₂:

Wet etching of SiO₂ and glass (glass in common sense is also silica with various additives), is carried out by different HF solutions. During the reaction HF turns SiO₂ into water soluble H₂SiF₆, which then leave into solution phase. A typical example of glass etch solution is HF:HNO₃:H₂O vol. ratio 20:14:66. Pure SiO₂ layers are etched either by HF (48%) (rate 20-2000nm/min) or buffered HF (BHF) which is water solution of HF:NH₄F (100-500nm/min).

Si: Microfabrication consumes extensively mono-crystalline silicon wafers, which can be etched isotropically (same etch rate in every direction) or anisotropically (some direction is favored). Isotropic etchants are usually based on acids, like HF/HNO₃/CH₃COOH (HNA). They are used at room temperature or slightly above and the mechanism behind the etching starts with oxidation of Si into SiO₂. The oxidizing agent in mixture mentioned above is HNO₃, but other oxidizers are also possible and CH₃COOH serves as a solvent, which could be replaced also by water, but in water nitric acid dissociates more readily and it affects the oxidative activity.



HF acid dissolves SiO₂ as described above. Since etching is heterogeneous reaction the total rate can be limited by the chemical reaction itself or by the transport of reactant and product on the surface. The reaction limited process is depending strongly on temperature while diffusion limited

process is less temperature dependent instead it is more affected by agitation. Since agitation is much harder to reproduce the reaction limited processes are more favorable. However typically isotropic etching is more diffusion limited. For example HF:HNO₃:CH₃COOH (vol ratio 5:10:16) at room temperature etches 4-20µm/min. The rate dependence in each individual composition can be seen from iso-etch curves. Besides rate, important characteristic is smoothness of resulting surface. For instance some mixtures are roughening surfaces (reaction controlled. When concentration of HF is high and HNO₃ low) while other can polish (diffusion controlled. When concentration of HF is low and HNO₃ is high). In the similar way temperature is affecting mechanisms; at higher temperatures reactions are more diffusion limited, which cause smoother surfaces, while at lower temperature reaction limiting step yield more rough surface. As the oxidation of Si is charge transfer reaction, holes on surface can be also provided by positive bias on Si wafer instead of using HNO₃. It allows carrying out etching under milder conditions. Also due to the charge transfer role isotropic etching is depending on dopant type and concentration. Highly doped Si can be etched much faster than slight doped one. A crucial aspect in isotropic etching is masking, common photoresists can only shortly withstand so aggressive reactants, which mean sacrificial masking layers made of Au/Cr or Si₃N₄ are needed.

Another class of etching procedures for monocrystalline Si is anisotropic etching. These reactions are favoring some crystalline directions making possible to design sidewalls with special orientations. Etchants are typically alkaline (KOH, NaOH, NH₄OH and organic bases). These processes are reaction limited, which means they are strongly depending on temperature and not very much on agitation; also they are much less sensitive to doping. The problem of anisotropic etching is speed which is much slower (in the range 1µm/min) and need higher temperature (80°C and more). Different solutions have different sidewall angles. KOH solutions are fairly aggressive, while organic alternative EDP is toxic. In case of anisotropic etching SiO₂ can be used as a sacrificial masking layer. The ratios of etching rates Si:SiO₂ in case of KOH and EDP are 400:1 and 5000:1, which shows good selectivity.

Au: Gold is used both as a good material for electrical connection wires as well as for sacrificial masking layer for more aggressive wet chemical treatments. Typical etching solutions are *aqua regia* (3:1 vol conc. HCl:HNO₃), which gives etch rate 25-50µm/min or milder iodine based etchant (4g KI, 1g I₂, 40mL H₂O), which etches 0.5-1µm/min.

Cr: Chromium is essential as surface adhesion layer between silicon and metals, but also material used for making photomasks. For Cr etch variety of etchants are possible, like HCl:CeSO₄ (sat. solution) (1:9) or HCl:glycerine (1:1). Both of them etch in the range of 80nm/min.

1.4.8 Soft lithography techniques^{17; 26}

Conventional photolithography is based on optical projection of image to the resist. The resolution of this process is limited by the wavelength of light. Even though force of miniaturization has pushed wavelengths of extreme UV light into the range around 13nm, these systems are remaining very expensive and inaccessible for many users. Soft lithography instead uses elastomeric stamp, which allows patterning of self-assembling monolayers (SAMs), unsensitized polymers, colloidal materials, sol-gel materials, organic and inorganic salts and biological macromolecules. The ultimate feature size can be in the same range as with common photolithography (100nm). But fundamentally soft lithography is not limited by wavelength of light; it can be much less expensive alternative to photolithography; in many cases would not need even clean room and also could provide novel opportunities, like patterning non-planar surfaces (microcoils, stents, gratings etc.) which is hardly possible with conventional photolithography. When going into details, the name soft lithography, in fact, encompasses several different techniques, like:

- Microcontact printing (μ CP)
- Replica molding (REM)
- Microtransfer molding (μ TM)
- Micromolding in capillaries (MIMIC)
- Solvent-assisted micromolding (SAMIM)

The central element in all these techniques is soft elastomeric stamp, which gives also a name “soft lithography”. Besides, in contrast to classical photolithographic fabrication, that is orientated to process hard inorganic materials, soft lithography deals mostly with organic substances.

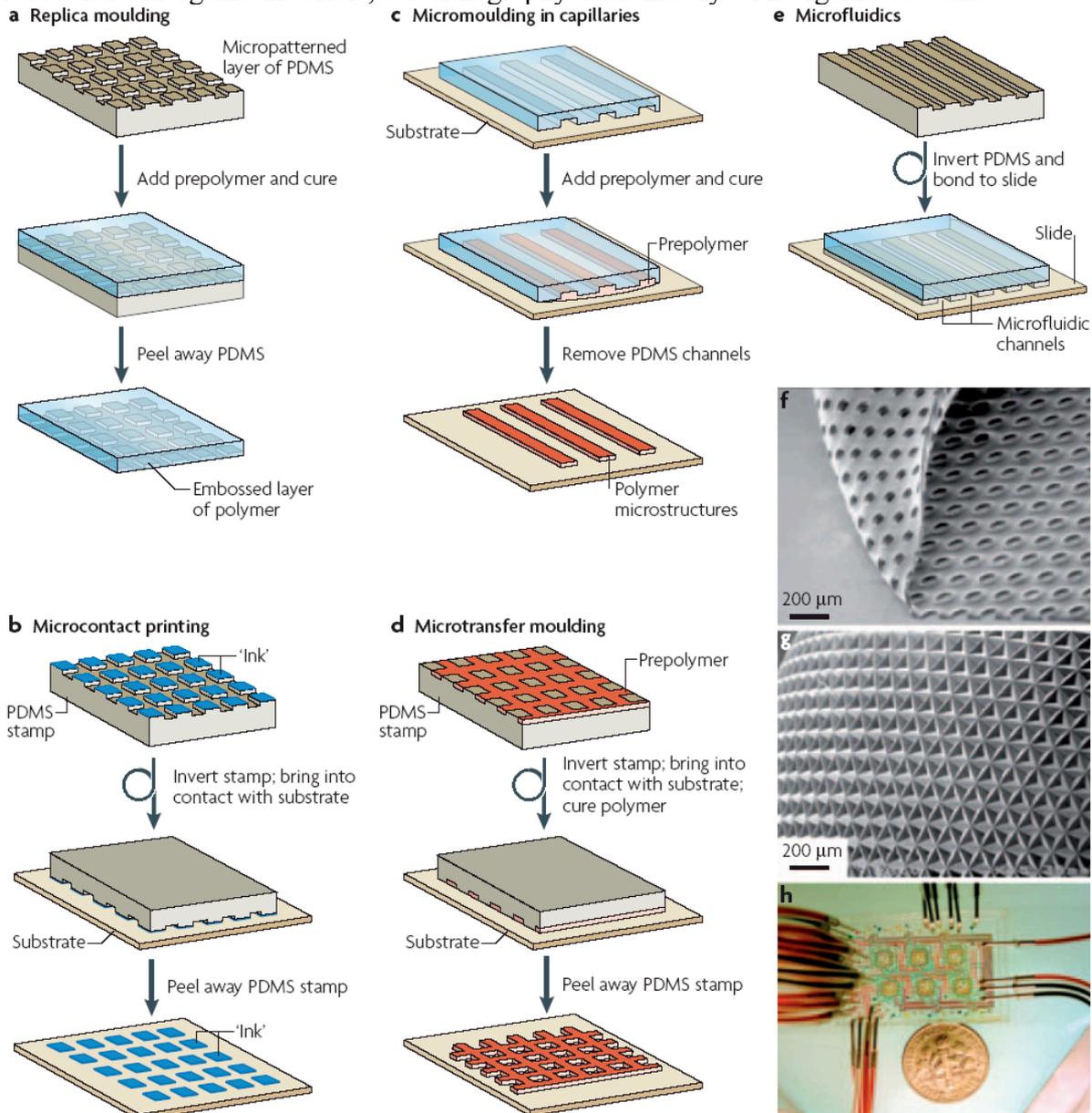


Figure 1.4-10. Soft lithography techniques (Figure by Weibel *et al*¹⁷).

Microcontact Printing (μ CP)

Microcontact printing is a tool for patterning self-assembled monolayers (SAM). Self-assembly is a process of spontaneous organization of molecules into structures, which properties are defined by the structure of building blocks. Usually SAMs are prepared by the exposure of substrate to ligand containing molecules, either in vapor phase or in solution. Most typical substrate ligand combinations are:

- Au substrate + sulfur compounds (thiols, sulfides, disulfides)
- Ag, Cu, Dp, GaAs, InP substrate + thiols (R-SH)
- SiO₂, glass substrate + R-SiCl₃, R-Si(OR')₃
- Metal oxides + carboxylic acids

In μ CP the elastomeric stamp is covered with self-assembling “ink” molecules and then they can be transferred to the substrate. Resolution of SAMs obtained with μ CP could be as good as 35nm and speed of formation 0.3s for thiol SAMs on Au surface. We can selectively alter different surface properties, for example hydrophilic and hydrophobic regions or functional groups for bonding further functionalities etc. SAM patterns can be used as an ultra thin resist for wet etching, to alter wetting and nucleation properties of surface, which is useful in growing different materials (eg. selective nucleation in CVD, liquid polymer wetting). In biological context μ CP can be exploited to locate proteins and determining locations for cell adhesion (Maybe future analytical tools are based on arrays of cells). Various coin metals (like Au, Ag, Cu) can be patterned by thiols, which may act as a protective resists in wet etching. Despite this technique still generates too many defects for advanced microelectronics fabrication, it is useful for sensor, solar cells, MEMS, optical fabrication.

Replica Molding (REM)

Replica molding allows one to fabricate 3D structures out of wide variety of materials. The procedure is starting by making a master structure, which is done with conventionally nanomanufacturing techniques. Then the master is treated to reduce surface adhesion, followed by the casting of liquid elastomer precursor (usually PDMS). When elastomer is cured it can be peeled off from master and inverted replica is ready (Inverted means that where in master are elevated structures there in replica are cavities and vice versa).

This replica structures can be directly used as microfluidic channels, for example, or in next moldings when structures are filled with some materials and then left to cure. Since PDMS is rather inert elastic material, with low surface energy it has excellent release properties from variety of materials. Another benefit of PDMS molds is elasticity that allows structures to be transferred to curvy surfaces (complex optical systems for example). Also slight shrinkages and drawings are possible. By using REM structures in the range of 30nm with 5nm precision could be made in organic materials.

Microtransfer Molding (μ TM)

When replica molded PDMS stamp/mold has been made as described above it can be used in microtransfer molding. Mold is filled with liquid material and excess is removed mechanically or by blowing with gas flow. Then it placed into contact with surface and left to cure, after which PDMS mold is removed leaving structures on surface. It can be used even for building 3D microstructures by adding layer to each other. μ TM can have applications in optics (eg. gratings, polymeric waveguides), tissue engineering etc. It can be used to cover quickly large areas with microstructures. If microtransfer molded (μ TM) structures are wanted to use as etching mask usually some thin film of material between structures should be removed with plasma.

Micromolding in Capillaries (MIMIC)

Also in MIMIC elastomeric PDMS mold is exploited. It is placed against substrate surface and channels are formed. The open ends of channels are placed into liquid, which will be spontaneously sucked into the channels by capillary forces. Material from the solution can be deposited (thermal or UV curing of polymer or deposition of colloids, salts etc.). After deposition elastomer mold will be removed. As previous ones, this method is also suitable for non-planar surfaces. The only serious restriction of MIMIC is need for hydraulic network between channels, that they could

be filled. Gas from closed end of capillaries can escape usually by diffusing into PDMS. The rate of filling is depending on the interfacial free energy and viscosity of liquid.

Solvent-Assisted Micromolding (SAMIM)

SAMIMs is a similar technique to replica molding. The elastomeric stamp is wetted with solvent that is good for dissolving or swelling substrate polymer. When the stamp is pressed against substrate, solvent is softening it and structure is embossed into substrate. Solvent evaporates by diffusing through PDMS and will be thus removed.

General problems of soft lithography are distortions, quality of patterns, which is not compatible with microelectronics fabrication, but still beneficial in other fields.

1.5 PDMS

PDMS (polydimethylsiloxane) is widely used organic silicon-based polymer, which is well known because of its mechanical properties²⁷. The backbone of PDMS is flexible making loosely entangled chains viscoelastic, which means that during long flow times it acts as a viscous liquid while during short times as an elastic solid. When cross-linked, PDMS chains form silicone rubber with excellent elastomeric properties. PDMS has received a lot of attention as a material suitable for fabricating micro devices for biotechnology and chemistry. Cheap PDMS (50 times cheaper than monocrystalline silicon) combine a lot of favorable properties⁸. First of all, the low temperature curable PDMS structures can be prepared by using simple replica molding technique and different layers are sealed reversible or irreversible on developer choice.

Since it is soft material the procedures are much more forgiving and many cases even do not need cleanroom facility. The softness also makes mechanically moving components, like valves and pumps, more tolerant to particle containing liquids, like cells. Furthermore the reversible deformability of PDMS makes it favorable in all membrane actuators (simple valves and pumps). In visible and near-UV (below 280nm) region transparent PDMS is suitable for optical visualization and spectroscopy to monitor the interior of the device. From biological point of view, it is important that PDMS is non-toxic and could be used for cell cultivation and in-vivo devices. PDMS is also penetrable for air, which is enough to supply cell cultures with necessary oxygen and fill dead-end channels. These advantages have made PDMS a one of the most prominent material in microfluidics.

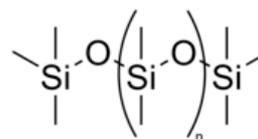
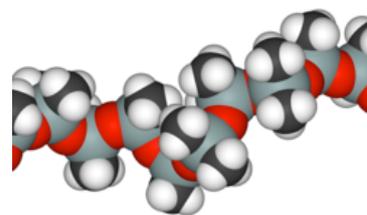


Figure 1.5-1. Basic structure of PDMS.

1.5.1 Curing chemistry of PDMS

Most typically used starting material for preparing cross-linked silicone rubbers is Sylgard 184 elastomer kit (by Dow Corning), which contains two parts of liquid chemicals, one called base and another curing agent²⁸. After mixing of these two (suggested wt ratio 10:1), curing process starts, which cross-links prepolymers found in both components and consequently turns liquid into rubber. The main chemical constituent of base mixture is dimethylvinyl-terminated dimethyl siloxane (cas: 68083-19-2, more than 60 wt% of base), whereas curing agent is made of dimethyl, methylhydrogen siloxane (cas: 68037-59-2, 40-70 wt% of curing agent)²⁹. In the presence of platinum catalyst hydrosilylation reaction cross-links methylhydrogen siloxane unit (from curing agent) and vinyl ending of base polymers, turning short liquid polymer chains into elastomeric network³⁰. Pt catalyst is homogeneous type Pt complex (or in some cases colloidal, especially when Pt(0) is formed³¹). Examples of hydrosilylation catalysts are $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}/i\text{PrOH}$ (Speier's catalyst) or $\text{Pt}\{(\text{CH}_2=\text{CH})(\text{CH}_3)_2\text{SiOSi}(\text{CH}_3)_2-(\text{CH}=\text{CH}_2)\}$ ³² (Karstedt catalyst³³, Pt complex with coordinated double bonds), but there are many others as well. Dow Corning does not refer in their datasheets particular catalyst they are using. The time needed for curing after mixing is depending on temperature. For Sylgard 184 the recommended curing times are 48h at room temperature, 45min at 100°C, 20min at 125°C, 10min at 150°C. During the curing PDMS material also shrinks slightly ~1%²⁶. In conclusion, PDMS fabrication using Sylgard 184 elastomer kit is relatively easy and foolproof. Neither uncured nor cured PDMS possess hazardous properties for humans. Some sulfur and phosphorous containing compounds, amines etc. can poison the catalyst and cause inhibition of curing process.

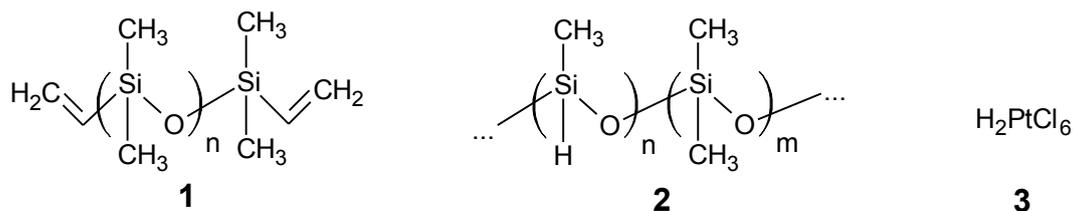


Figure 1.5-2. Basic components of curable PDMS elastomer (eg. Sylgard 184 kit). Dimethylvinyl-terminated dimethyl siloxane (1). Dimethyl, methylhydrogen siloxane (2). Some Pt catalyst (For example: Speier's catalyst (3)).

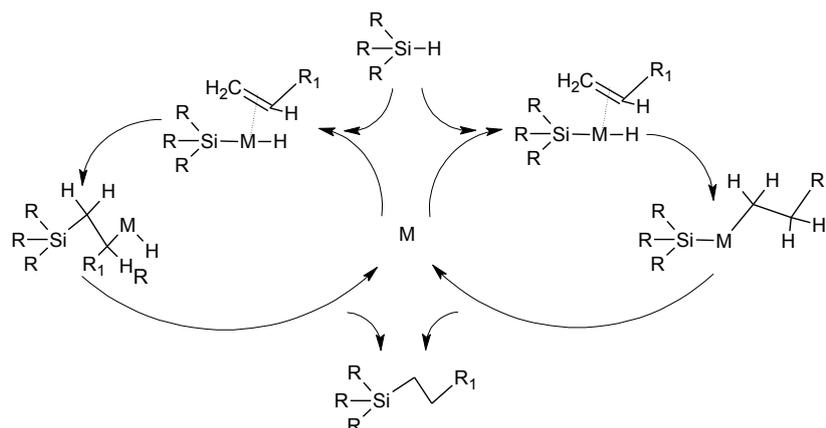


Figure 1.5-3. Transition metal (M) catalyzed hydrosilylation reaction³⁰. Catalytic cycle has two alternative routes, where both are beginning with oxidative addition of hydrosilane ($(R)_3\text{-Si-H}$), followed by migratory insertion of coordinated alkene either into M-H bond (hydrometalation) or into M-Si bond (silylmethallation). Final step is reductive elimination, which liberate product and catalyst.

1.5.2 Surface treatments and bonding of PDMS

After preparation, as described above, the PDMS surface is hydrophobic. In order to alter surface properties, PDMS can be exposed to oxygen plasma⁸. Reactive oxygen radicals are attacking methyl groups (Si-CH_3) and substitute them by silanol groups (Si-OH), which render the surface hydrophilic. However hydrophilic surface in air is unstable, silanol groups are migrating into bulk and condense (to reduce surface free energy), which return surface to hydrophobic state in roughly 30min. Surface can be stabilized when kept in contact with some polar solvent. At aqueous environments where $\text{pH} > 3$, silanol group dissociate into Si-O^- and H^+ , forming a negative surface charge. If positive surface charge is desirable PDMS must be coated by positive polymer, like polybrene. Various chemical modifications of PDMS are possible through condensation reaction between silanol and silyl chloride, hydroxyl and carboxyl groups.

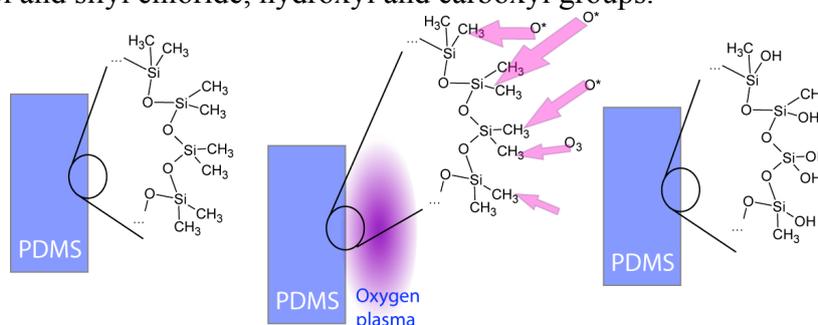


Figure 1.5-4. Surface treatment of PDMS with oxygen plasma.

Essential from microfabrication point of view is possibility for strong covalent bonding between slabs of PDMS or PDMS and glass, which makes possible to seal replica molded microfluidic

devices, for example. Bonding occurs due to the condensation reaction between silanol groups, when plasma treated surface are brought together.

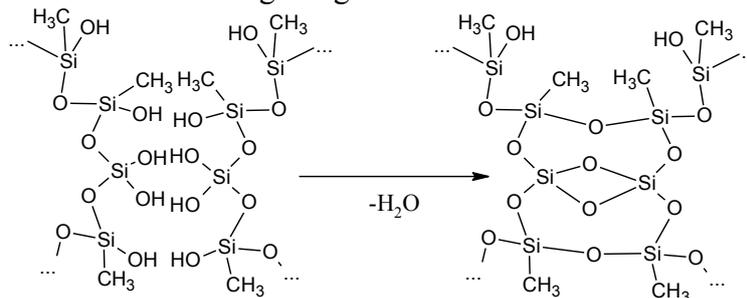


Figure 1.5-5. Covalent bonding between plasma treated PDMS surfaces (condensation of silanol groups).

Some authors have also used UV-ozone treatment for activating PDMS surface³⁴. Basic surface oxidation principle behind UV-ozone treatment is same as in case of plasma, described above.

For aiming good adhesion, plasma treatment has great significance and as revealed in studies, the quality of bonding is depending strongly on plasma parameters^{35; 36}. In general, good bonding was obtained when using high reactor pressures, low plasma power and short times. Using low pressures causes more anisotropic bombardment of high energy ions, which may broke backbone of PDMS and thus weaken the surface layer. Also too deep oxidation (with high power and long time) of surface change PDMS into brittle silica which weakens interface area. Thus optimum plasma power and time exist (~ 20W for 20s), which yields best bond strength and minimum contact angle of surface. (Untreated PDMS, contact angle is 109deg while minimum contact angle after plasma treatment is less than 5deg). It has been also observed that PDMS surfaces are aging, which mean that plasma treatment cannot activate them anymore, also it was found that aged PDMS surfaces can be recovered by dipping material into solution of HCl for ~5-10min³⁵.

1.5.3 Chemical compatibility of PDMS

When using these systems as bioreactors attention must be paid for interactions between PDMS and solution. PDMS can be used mostly for water based solutions, since organic solvents like chloroform, ether, THF etc. will diffuse into PDMS and swell it. Lee *et al* have made thorough study of PDMS compatibility with various solvents³⁷.

According to their study:

- PDMS is not swelled in: nitromethane, DMSO, ethylene glycol, glycerol, water.
- PDMS is very slightly swelled (~5% or less) in: dimethyl carbonate, acetone, pyridine, NMP, acetonitrile, phenol, ethanol, propylene alcohol, methanol.
- PDMS is extensively swelled (5-30%) in: ethyl acetate, benzene, chloroform, 2-butanone, chlorobenzene, DCM, dioxane, propanol, tert-butyl alcohol.
- PDMS is severely swelled (>30%) in: pentane, hexane, heptane, triethyl amine, ether, cyclohexane, xylene, toluene, THF.
- Most swelling solvent is diisopropylamine, which cause linear expansion of PDMS 2.13 times.

In acids and bases PDMS generally does not swell, unless it is dissolved by concentrated acids like 18M sulfuric acid or 100% trifluoroacetic acid. But swelling can be also used to extract unbound PDMS strands from elastomeric network, after such an extraction PDMS exhibit much better surface stability when plasma oxidized (return of hydrophobicity is considerably slower)

1.5.4 Microfabrication in PDMS

Typically microstructures are formed into PDMS using replica molding technique, which is described in previous chapter (Ch 1.4). In replica molding liquid mixture of PDMS prepolymers is

poured to mold and then left to cure. Solid PDMS is be peeled off, which leaves negative relief of mold into PDMS. PDMS is excellent due to its low surface free energy that enables easy release from many surfaces. For all microfabrication purposes it is important to degas PDMS before. It means that mixture of prepolymers, which during stirring easily captures small bubbles, is evacuated in desiccator in order to remove bubbles that may cause defects otherwise. Due to the elasticity aspect ratios of structures in PDMS are limited into order of 0.2...2, otherwise they will collapse²⁶.

Spin coating

When thin PDMS layers are desirable, spin coating can be used (Spin coating technique is described in previous chapter 1.4). Spin coating model for Sylgard 184 was developed by Zhang *et al*³⁸.

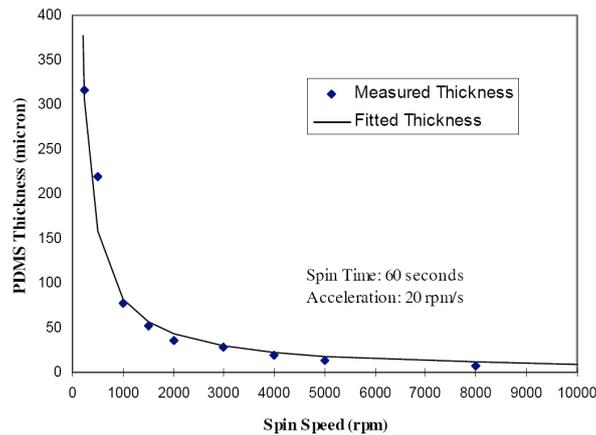


Figure 1.5-6. Thickness of spin-coated PDMS depending on spin speed (Figure by Zhang *et al*³⁸) Empirical formula describing thickness is following

$$t = \frac{KC^{\beta}\eta^{\gamma}}{\omega^{\alpha}}$$

where t is film thickness, and ω spin speed. $K=6.2661$, $C=0.90909$, $\alpha=0.9450$, $\beta=5.2707$, $\eta=4000\text{mPa}\cdot\text{s}$, $\gamma=6.2051$.

Etching

PDMS can be also chemically etched, which allows for example *in situ* modification of microfluidic channels (eg. Takayama *et al*³⁹). Best found etching solution is tetrabutylammonium fluoride (TBAF) in NMP (Also DMF and DMSO give satisfactory result). Particular solvents are suitable since they do not swell polymer, but readily dissolve etching product and etchant.

Photopatternable PDMS

Dow Corning offer also photopatternable spin-on silicone materials (WL-5000 serie)⁴⁰. These materials act as a negative photoresists and are processed respectively: spinned, soft baked, UV-exposed, post baked and developed. Material is shipped as single component mixture and is more demanding as it needs to be stored at temperatures below -15°C (then life time 6 months, while at room temperature it is only 3 days). Also photopatternable silicones are more expensive (250mL/560EUR).

1.6 Valves in microfluidic systems

Since first microvalve solution reported in 1979, variety of different designs has been proposed for controlling fluid flows in small scales⁴¹. By principles they can be mechanical membrane valves controlled by temperature, magnetic or electric methods or instead take advantage of special kind of “smart” materials, which can change their phase upon external stimuli. Since this thesis is concerning design of microvalve systems, it is also important to review already existing concepts and understand their advantages and shortcomings. Nevertheless increasing the complexity of fabrication microvalves are desirable since they allow much faster control compare to external valves which have large dead volumes. In some simpler system, where only input of the flow has to be switched, it is sufficient to use solution exchange systems like described by Bridle *et al*⁴². However their applicability is limited to certain designs, whereas internal valves are more generic and can also outperform external solution exchange system in speed.

1.6.1 Pneumatic valves

The largest class of microvalves is miniaturization of macro-world ones, which are based on deflection of membrane that closes flow underneath. Despite of wide variety of complicated machinery built in hard materials, probably the most elegant in its simplicity is concept proposed by Quake's group⁴³. These devices were fabricated from PDMS elastomer using a replica molding techniques and their construction took advantage of elasticity of PDMS. The device is composed of 3 major layers. First is substrate to seal flow channels (without structures), second layer contains flow channels and it is covered by third layer containing control channels. These upper layers are made of films of PDMS (middle layer is thin 40 μ m) and they are aligned and irreversibly bound to each other forming a monolithic unit. Actuation of the valve occurs, when pressure is applied to the control channel, which cause soft PDMS membrane to bend and close flow channel underneath (the opening of valve has linear dependence on the applied control pressure). The strong advantage of this solution is control speed, which is in the order of 1ms. Also the device has long lifetime (4 million open-close actuations without observable fatigue). They have shown that the number of layers and complexity of device can be easily increased by mounting more layers to each other (it was tested with 7 layers). Similar approach can be also used to fabricate peristaltic pumps for microfluidics. Besides these devices can be made multiple usable, by reversible bonding of substrate, which means that substrate can be removed, flow channels can be rinsed and device can be reused. The possible drawback compare to other solutions is the need for external pressure control unit that makes device more complicated and less portable. However it is probably one of the best approaches for precise scientific apparatus. But in order to circumvent need for external pressure control, different thermopneumatic valves has been described^{34; 44}. In these valves some volatile liquid or just a gas is captured into cavities underneath the pneumatic membrane valve. Evaporation or thermal expansion causes pressure increase and closure of flow channel. Heat can be provided on site by microfabricated heating coils, which makes whole design directly electrical-ly controllable and thus much more compact. However all of these devices have been made in hard materials and the fabrication process is rather complicated. Additional problems in case of high integration of thermopneumatic valves can be thermal crosstalk between each valve and also with controllable medium. Last of them can be minimize, by locating pressure generation and heating on different region of chip.

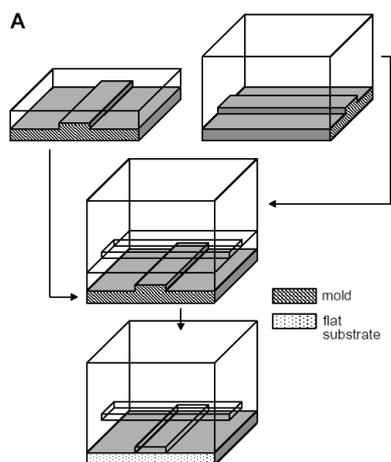


Figure 1.6-1. Pneumatic valves in PDMS (Figure by Quake *et al*)

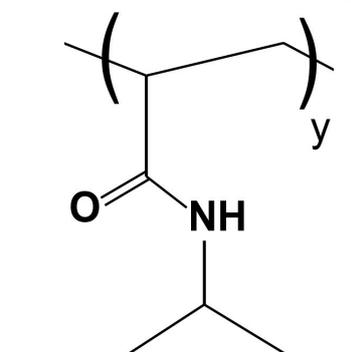
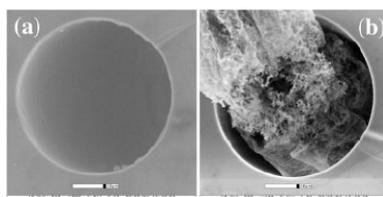


Figure 1.6-2. Temperature sensitive PNIPAAm hydrogel (Hydrophilic state left and Hydrophobic right. Figure by Fréchet *et al*)

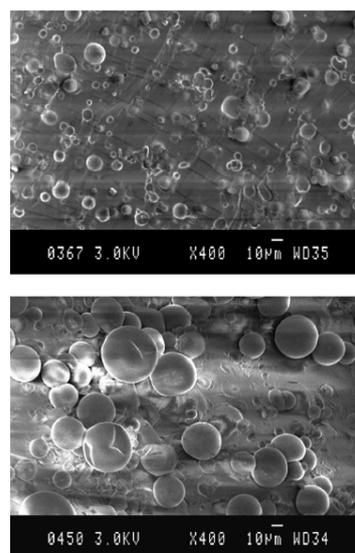


Figure 1.6-3. Expancel® microspheres before and after expansion (Figure by Samel *et al*)

1.6.2 “Smart” material valves

“Smart” hydrogel materials are responding to external stimuli such as change in pH level, concentration of chemical substances, electric field or temperature by phase change, which can cause more than order of magnitude large reversible volume change. Advantage of these materials is possibility to photo polymerize them *in situ*, which means that ready fabricated microchannels are filled by solution of monomers and photoinitiator and exposed to UV light. The exact location of polymer will be defined by photomask and rest of the monomer will be washed away. To bring an example, PNIPAAm is a standard materials for thermo responsive hydrogels^{45; 46}. Each of the valves is formed by a monolithic polymer plug inside the channel. The plug is made of cross-linked poly(N-isopropylacrylamide) (PNIPAAm), which exhibits a very rapid change of solubility (phase transition) at 32°C. Below this temperature polymer chains are hydrophilic and hydrated, which mean gel is swollen, when temperature exceeds the LCST (lower critical solution temperature) the chains become hydrophobic, gel expels the water and contract. Due to the shrinkage the plug becomes porous and penetrable to liquids. These valves have characteristics, like opening and closing times in the range 1-5 seconds and where opening is generally faster. In closed state the valve can withstand high pressure without leakage (1.38MPa) and at the opened state permit high flow rates. The opening and closing are reversible and valves survive easily large number of actuations. Since the transition temperature is relatively low, closing may need active cooling. But Fréchet *et al* also describe way how to adjust the polymer actuation temperature between 35-74°C, by varying the composition (vary ratio of monomers N-isopropylacrylamide and N-ethylacrylamide). It is combining simplicity of manufacturing, reversible actuation, and easy control, since temperature can be regulated by build-in heaters and no external pressure unit is needed. But the phase change can be also used to mechanically deflect membrane, which allows separating flow and controlling medium.

Phase change is an actuation basis also for paraffin valves^{41; 47}. Large volume expansion on melting (10-30%) can be exploited to actuate membrane valves or paraffin can be used as a meltable plug,

which can be pushed and pulled out of flow channel closing and opening of flow. Membrane valve resisting pressure 160kPa can be controlled with time 15ms using only 40mW power. Plug concept is generally slower but may withstand even higher pressures (more than 1.5MPa)

1.6.3 Expandable beads

Stemme *et al* have been using Expancel ® microspheres for designing valves and pumps for cheap and disposable microfluidic devices^{48; 49; 50}. Expancel ® microspheres are expandable on heating. They have thermoplastic shell and hydrocarbon filled interior. When the boiling point of hydrocarbons is exceeded, the forming gas expands microspheres significantly (~ 40 times of the initial volume). However this expansion is irreversible and microspheres do not contract on cooling. The principle of valves relies on the higher volume occupancy after expansion, which close flow channel. Initially their idea involved precise localization of spheres in the device. Later this concept was changed and microspheres were mixed into PDMS polymer layer and deposited as homogeneous substrate. Precise spot of expansion was defined by localized heating, which is much easier to achieve. This concept has great potential for one-time disposable consumer devices like medical diagnostics, drug delivery etc. The advantages are easy fabrication and control (no external actuators or pneumatics), also cheapness. Disadvantages are one-shot irreversible actuation, and impossibility to do valve that can be opened by stimuli. The time needed for closing is 1.5s (with 1W heating coils). However after longer times of heating the valve can withstand higher pressure (8.5s 1W heated valve withstood 140kPa counter pressure)

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