

CHAPTER 2

Basic Microfluidic and Soft Lithographic Techniques

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2-1 Introduction

Most optofluidic devices share a similar fluidic platform. The design, fabrication, and operation of the fluidic systems in these devices are based on those developed for microfluidics used in biochemical analysis. This chapter describes the basic ideas of microfluidics. We first summarize the materials most commonly used in fabricating microfluidic systems and the techniques developed for fabricating them. We then describe the characteristics of flow in these systems and illustrate the principle of operation of some important microfluidic components.

We focus our discussion on the use of poly(dimethylsiloxane) (PDMS) for fabricating microfluidic systems. PDMS has been the most widely used material in the research and development of microfluidics. PDMS is an optically transparent elastomer whose stiffness can be controlled from very soft (easily deformed by finger pressure) to much stiffer. The fabrication of systems of microchannels in PDMS is particularly straightforward. The use of PDMS as a material allows rapid prototyping of devices, and facilitates the demonstration and the testing of new concepts. The physical and chemical properties of PDMS also

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make possible the fabrication of devices with a useful range of functions, ranging from molecular analysis to frequency-tunable lasing.

2-2 Historical Background

Microfluidic systems have the properties required for applications in a wide range of areas: molecular analysis, biodefense, molecular biology, microelectronics, clinical diagnostics, and drug development [1]. There are many benefits resulting from the miniaturization of devices for use in these areas, including decreased cost in manufacture, use, and disposal; decreased time of analysis; reduced consumption of reagents and analytes; reduced production of potentially harmful by-products; increased separation efficiency; decreased weight and volume; and increased portability [1]. The growth of molecular biology has stimulated the development of systems for analysis of biomolecules, DNA, and proteins. The first microfluidic device was a miniaturized gas chromatography (GC) system developed by Terry et al. [2] at Stanford University in the 1970s. The laboratories of Manz [3–5], Harrison [6–10], Ramsey [11–15], and Mathies [16–18] were among the first to develop microfluidic systems to analyze aqueous solutions. The technology used to fabricate these early systems—photolithography and etching in silicon and glass—was derived from microelectronics, as these technologies were available and highly developed. These materials and techniques are expensive and time-consuming, however, and they require access to specialized facilities. They are therefore only marginally useful in research requiring rapid evaluation of prototypes. Their major advantage—chemical inertness—is so far required only in the still-undeveloped area of organic synthesis.

2-3 Materials for Fabricating Microfluidic Devices

Most research in microfluidic systems is now carried out in PDMS and other polymers. Fabrication in polymers is easier, more flexible, and much less expensive than in silicon or glass. It also avoids other problems of hard materials (e.g., formation of sharp shards on breakage) and enables certain components (e.g., pneumatic valves) that cannot be fabricated in rigid materials. In the following sections, we will focus on the use of PDMS for the development of microfluidic systems. PDMS has several attractive properties that make it suitable as a material for rapid prototyping of microfluidic devices capable of supporting a wide range of applications. Table 2-1 summarizes some of these properties and consequences.

2-3-1 Mechanical Properties of PDMS

PDMS is elastomeric. It has tunable Young's modulus, typically around 750 kPa [19]. It deforms easily, conforms to surfaces, and

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Property	Characteristics	Consequence
Optical	Transparent; UV cutoff, 240 nm	Optical detection from 240 to 1100 nm
Electrical	Insulating; breakdown voltage, 2×10^7 V/m	Allows embedded circuits; intentional breakdown to open connections
Mechanical	Elastomeric; tunable Young's modulus, typical value of ~ 750 kPa	Conforms to surfaces; allows actuation by reversible deformation; facilitates release from molds
Thermal	Insulating; thermal conductivity, 0.2 W/(m · K); coefficient of thermal expansion, $310 \mu\text{m}/(\text{m} \cdot ^\circ\text{C})$; stable up to $\sim 300^\circ\text{C}$	Can be used to insulate heated solutions; does not allow dissipation of resistive heating from electrophoretic separation
Interfacial	Low surface free energy ~ 20 erg/cm ²	Replicas release easily from molds; can be reversibly sealed to materials; not wetted by water unless oxidized to SiOH presenting surface
Permeability	Low permeability to liquid water; permeable to gases and nonpolar organic solvents	Contains aqueous solutions in channels; allows gas transport through the bulk material; incompatible with many organic solvents
Reactivity	Inert; can be oxidized by exposure to a plasma	Unreactive toward most reagents; surface can be etched; can be modified to be hydrophilic and also reactive toward silanes
Toxicity	Nontoxic	Can be implanted in vivo; supports mammalian cell growth

(Adapted from J. C. McDonald and G. M. Whitesides, "Poly(dimethylsiloxane) as a material for fabricating microfluidic devices," *Acc. Chem. Res.*, 35, (2002), 491–499.)

TABLE 2-1 Physical and Chemical Properties of PDMS

releases from features of a mold—including undercut features—without damaging them or itself. Microfluidic channels and other features on the micron scale can therefore be reproduced with high fidelity in PDMS by replica molding. Using composite stamps composed of two layers—a stiff layer supported by a flexible layer of PDMS, replication of features below 1 nm has been demonstrated [20, 21]. Because

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PDMS is elastomeric, it is possible to form optical components whose dimensions can be tuned mechanically. Stretching or compressing a surface-relief grating or Fresnel lens made of PDMS, for example, changes the periodicity of the grooves on the grating or the lens, and the respective diffraction pattern generated or the focal properties of the lens [22, 23].

2-3-2 Surface Chemistry of PDMS

The surface of PDMS is hydrophobic as it contains repeating units of $-\text{O}-\text{Si}(\text{CH}_3)_2-$ groups. By exposing it to oxygen or air plasma, this surface can be made hydrophilic. Exposure to plasma introduces silanol ($\text{Si}-\text{OH}$) groups, and destroys methyl groups ($\text{Si}-\text{CH}_3$). Plasma-oxidized PDMS can be wetted by aqueous, polar solvents, and eutectic gallium-indium, a liquid metal alloy. On standing, a hydrophilic, oxidized PDMS surface becomes hydrophobic, as the surface reconstructs and as non-crosslinked components of the prepolymer bloom to the surface. It is possible to keep PDMS that has been plasma-treated hydrophilic indefinitely by keeping the surfaces in contact with water or polar organic solvents.

The silanol groups on the surface of PDMS allow it to react with a wide range of silanes ($\text{Si}-\text{R}$) that are terminated with important functional groups (i.e., $\text{R} = \text{NH}_2, \text{COOH}, \text{SH}$). By using different functional groups, it is possible to adjust the surface of PDMS to be hydrophilic or hydrophobic, or to introduce other reactive groups. Grafting a poly(ethylene glycol)di-(triethoxy)silane onto an oxidized PDMS surface makes the surface hydrophilic permanently, and reduces non-specific adsorption of proteins. Silanizing oxidized PDMS with an amino-terminated silane (aminopropyltriethoxysilane) provides a reactive surface for a bifunctional cross-linker for protein attachment [24]. These modified polar surfaces can, however, become hydrophobic again through blooming of mobile, nonpolar siloxanes. Application of a sol-gel coating may be more protective, but has not been extensively developed [25].

Irreversible Sealing

It is simpler to seal channels made in PDMS than channels that are made in glass, silicon, or thermoplastics, as high temperatures, pressures, and voltages are not required. For example, sealing glass to glass or silicon to silicon requires high temperatures ($\sim 600^\circ\text{C}$ for glass; $>800^\circ\text{C}$ for silicon) and/or voltages (500–1500 V for anodic bonding of glass). Sealing of channels in PDMS can be performed in ambient laboratory conditions. By exposing the surface of PDMS and the surface of the substrate to an air- or oxygen-based plasma, PDMS channels can be sealed irreversibly to PDMS, glass, silicon, polystyrene, polyethylene, or silicon nitride [24]. Plasma oxidation produces silanol groups on PDMS, and $-\text{OH}$ -containing functional groups on the other materials. When the surfaces are brought into contact, the

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polar groups form covalent –O-Si-O-bonds with oxidized PDMS; the channel is therefore sealed irreversibly. It should be noted that the two surfaces must be brought into contact quickly (< 1 min) after oxidation, because the surface of the oxidized PDMS reconstructs in air. Empirical evidence shows that sealing works best when the samples and chamber are clean, the samples are dry, the surfaces are smooth (on the micron scale), and the oxidized surfaces are not mechanically stressed. Heating a weak seal at 70°C can sometimes improve the strength of the seal [19]. Another way to seal two pieces of PDMS irreversibly involves adding an excess of the monomer to one surface and an excess of the curing agent to the other. When the two surfaces are cured together, an irreversible seal that is indistinguishable from the bulk properties of PDMS forms [24].

Reversible Sealing

Another advantage of PDMS over glass, silicon, and hard plastics is that it makes reversible conformal contact (van der Waals contact) to smooth surfaces. PDMS devices can therefore be demountable, and resealing can occur multiple times without degradation in the PDMS.

Microfluidic devices that are demountable can be used to pattern surfaces with proteins, cells, and other biomolecules using fluid flow [24]. Our group [26] and others [27] have performed binding assays using a demountable device. Antibodies were first patterned on a glass substrate by flowing a solution of antibody through a set of parallel channels. The PDMS device was then peeled off from the glass substrate, rinsed, and placed perpendicular to the first set of channels. Solutions containing antigens were then introduced through the channels. Antibody-antigen complexes were subsequently detected at the crossings of stripes of antibodies and the channels.

PDMS channels can also seal reversibly to silicone (or cellophane) adhesive tapes [19]. To make a mechanically stable support, double-sided tape—with one side applied to a flat plastic or glass slab—is a valuable component. Polymeric adhesive tapes are convenient because they are mechanically flexible, and they form a stronger (but still reversible) bond than that between PDMS and other flat surfaces. They also allow nonsealing functional layers such as filter papers and membranes to be incorporated into the microfluidic system [26].

Compatibility with Solvents

PDMS is compatible with water, and most polar organic solvents (such as methanol and glycerol); it swells, however, in nonpolar organic solvents (such as pentane and chloroform) [28], and will absorb nonpolar solutes from aqueous solutions. To reduce the absorption of small molecules and the swelling by nonpolar organic solvents, one can modify PDMS with silica particles [29], or coat the surface with a glass-like layer using sol-gel chemistry [25] (Fig. 2-1).

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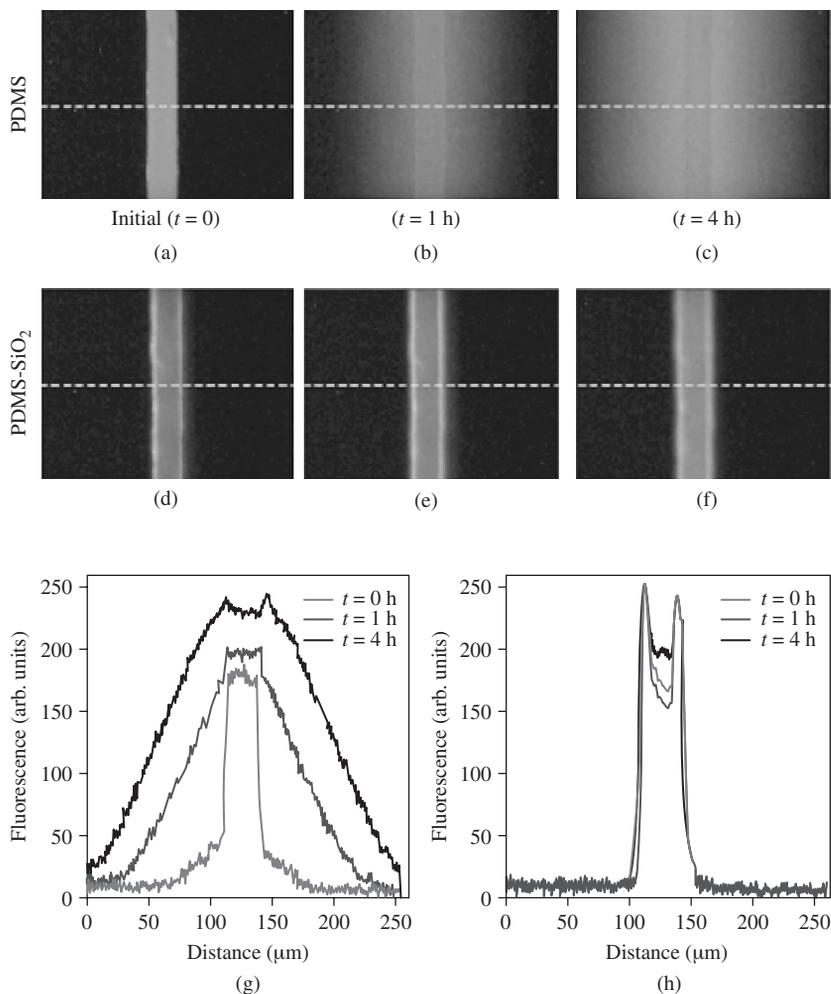


FIGURE 2-1 Images of PDMS (a–c) and PDMS-SiO₂ (d–f) devices are shown. The channels on these devices are filled with 10 μM rhodamine B in a 10 mM (pH 9.5) sodium borate solution. The images were acquired over a 4 h period. Fluorescent profiles of the PDMS and PDMS-SiO₂ channels are also shown in g and h, respectively. These profiles were taken along the white dotted line in images A–F. (Adapted from Ref. 29.)

Toxicity

PDMS is nontoxic to proteins and cells. It is permeable to oxygen and carbon dioxide, but only slowly permeable to water. It is therefore suitable for biological studies: for example, mammalian cells can be cultured on it directly [30].

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2-3-3 Optical Properties of PDMS

PDMS is optically transparent from 240 to 1100 nm [19], and has a refractive index around 1.41. It has negligible birefringence. It is therefore possible to enclose optofluidic components in PDMS, and couple light through PDMS, with minimal loss due to absorption. Commercially available PDMS—Silgard 184—does, unfortunately, contain nanoparticles of silica that introduce unwanted scattering of light. In the devices we and others have fabricated, the thickness of PDMS for enclosure of microfluidic components is limited (usually < 1 cm), and thus scattering due to passage of light through PDMS does not cause significant loss during the coupling of light into and out of the devices.

We have not identified a polymer that lacks these scatterers, and still possesses the other desirable qualities of PDMS. The Norland optical adhesives (photocurable polyurethanes), for example, contain no scattering particles, but they are not soft, and cannot be processed the same way as PDMS. This need for an elastomeric polymer with high optical transparency and easy sealability presents an opportunity for future research in material science. To summarize, PDMS has attractive features that make it useful for a wide range of applications in laboratory, and for prototyping in research, though it may not be the ultimate material used in large-scale manufacturing. Other polymers used for fabricating microfluidic systems include h-PDMS, photocurable perfluoropolyethers (PFPE), cyclic olefin copolymer (a thermoplastic polymer), thermoset polyester, polymethylmethacrylate, polycarbonate, and polyurethanes [31]. Each material has its own advantages and disadvantages; depending on the application, one material may be more suitable than the other. For example, PFPEs, a class of fluoropolymers that are liquids at room temperature, are chemically resistant (like Teflon). They are compatible with organic solvents such as toluene and dichloromethane (both of which swell PDMS). The fabrication process for channels in PFPE involves procedures that are more complicated than with PDMS, however. There is no simple procedure for adhesive-free contact sealing, and these polymers are much more expensive.

2-4 Fabrication of Microfluidic Systems in PDMS

Systems in PDMS are typically fabricated using techniques in soft lithography [19]. Soft lithography involves the replication of a topographically defined (typically in photoresist) structure on a master in a soft elastomer. The process can be carried out in ambient laboratory conditions. Replication can also be repeated multiple times. Soft lithography therefore enables rapid, simple, and inexpensive fabrication processes.

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The details of fabrication using soft lithography can be found elsewhere [19]. Here we provide a summary of the processes involved. The microfluidic channels are designed in a CAD program and printed onto a high-resolution transparency (~5000 dpi) (or, somewhat less conveniently and more expensively, converted into a conventional chrome mask). This transparency is used as a photomask in 1:1 contact photolithography (usually using SU-8 or PMMA as photoresist) to produce a master. This master consists of a positive bas-relief of photoresist on a silicon wafer, and serves as a mold for PDMS. Liquid PDMS prepolymer is poured over the master and cured for 1 h at 70°C. The PDMS replica is then peeled from the master and sealed (following plasma oxidation of the interfaces involved) to a flat PDMS, glass, or silicon surface to form the microfluidic channels. The overall process takes approximately 24 h. Figure 2-2 shows a schematic diagram of the procedures involved.

2-5 Characteristics of Flow in Microchannels

A basic understanding of fluid dynamics in microsystems is useful in the design and development of microfluidic devices. This section summarizes a few characteristics of flow in microchannels that are important in common microfluidic components. Comprehensive reviews on the physics of fluids in microfluidic systems can be found elsewhere [32–34].

In general, as the physical length scale of the system decreases, gravity becomes less important. Surface forces (surface tension, electrical, van der Waals, and surface roughness) become dominant [33]. Most microfluidic devices are in the micro- or nanoscale range, and the relative importance of forces typically follows this order: interfacial force \gg viscous forces $>$ gravitation \sim inertial force $>$ buoyancy [35]. Most microfluidic devices involve the use of miscible liquids only. Interfacial tension is therefore usually negligible. Viscous forces dominate, and as a result, the flow is primarily laminar without turbulence; mixing occurs by diffusion only [32]. We will describe laminar flow and diffusion in more details in the following section.

2-5-1 Laminar Flow

Flow in microchannels is commonly characterized by the Reynolds number, Re . The Reynolds number describes the tendency of fluid to develop turbulence. It represents the relative importance of inertia to viscous dissipation ($Re = vl/\mu$, where v is the average flow speed, l is the characteristic length scale of the channel, and μ is the kinematic viscosity) [32]. For Re much less than 2000, viscous forces dominate, and the flow is laminar. As Re increases above 2000, the flow becomes dominated by inertial forces, which tend to produce instability leading to turbulence.

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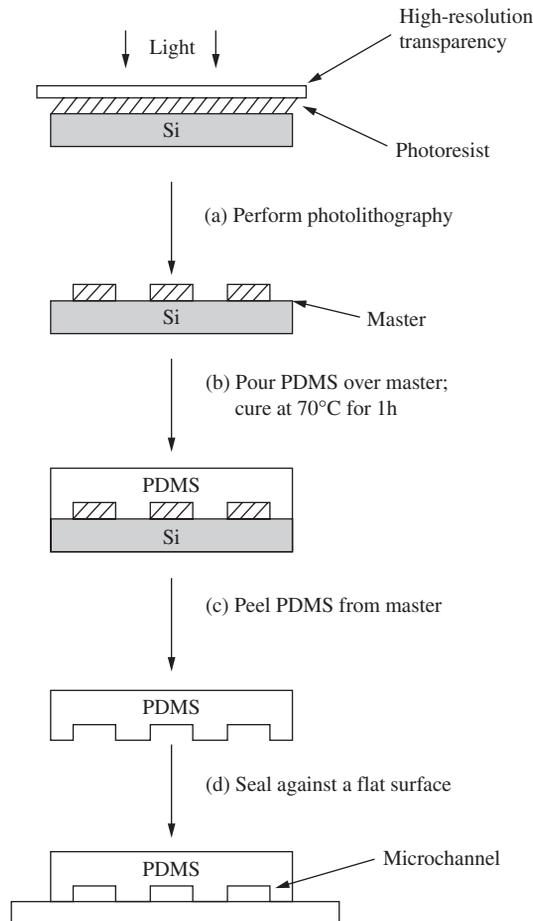


FIGURE 2-2 Scheme describing rapid prototyping of microfluidic systems. A system of channels is designed in a CAD program. A commercial printer uses the CAD file to produce a high-resolution transparency (~5000 dpi). (a) This transparency is used as a photomask in contact photolithography to produce a master. A master consists of a positive relief of photoresist on a silicon wafer and serves as a mold for PDMS. (b) Liquid PDMS prepolymer is poured over the master and cured for 1 h at 70°C. (c) The PDMS replica is peeled from the master. (d) The replica is sealed to a flat surface to enclose the channels. The overall process takes ~24 h. (Adapted from Ref. 19.)

Since the length scale of microfluidic systems is small ($< 500 \mu\text{m}$ typically), the flow of fluids in microchannels takes place in the regime where the Reynolds number is low (typical $Re < 10$). Viscous forces dominate, and the flow is laminar. The liquids can be treated as laminae (layers) of uniform thickness; their boundaries remain fixed as the liquid moves between them; the only mixing of the streams

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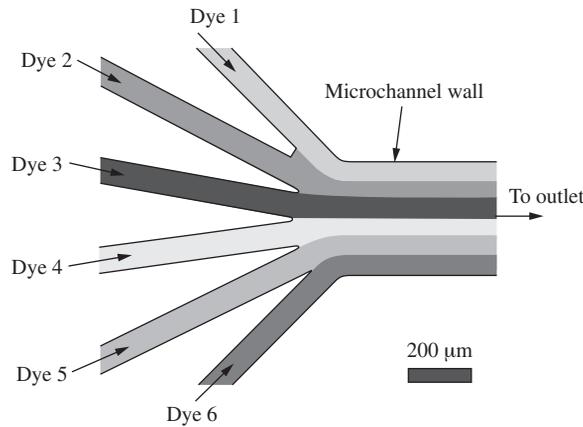


FIGURE 2-3 Optical micrograph of laminar flow of six streams of different dye solutions in a microchannel flowing from left to right. The height of the channel is 100 μm . (Adapted from Ref. 40.)

occurs by diffusion across the liquid-liquid interface [36]. Figure 2-3 shows an example of laminar flow of six different streams of solutions of dye flowing (from left to right) into a single channel. There is no turbulent mixing, and the interface between these laminar streams remains parallel and distinct. Note that this interface is at dynamic steady state: a continuous flow of liquids is necessary to maintain this interface. As a result, manipulating the conditions of flow can reconfigure the shape and position of this interface dynamically. This feature is attractive for some applications in optics, and has been exploited for constructing optical devices; more details can be found in Chap. 3.

2-5-2 Diffusion

Although there is no turbulence, mixing in laminar streams still occurs due to diffusion. There is a transverse (in the y -direction in Fig. 2-4) concentration gradient across laminar streams that contain different concentrations of solutes (or other properties, such as temperature). Transverse diffusion of solutes broadens the laminar interface, flattens the concentration gradient across the streams, and homogenizes the liquids. Figure 2-4 shows this idea. To visualize the spatial extent of transverse diffusive mixing, two nonfluorescent chemical species (carried separately by the two flowing solution streams) are used. The product of these two species is fluorescent, and can therefore be imaged with a confocal microscope.

The Péclet number ($Pe \equiv vw/D$) compares the typical time scale for diffusive transport to that for convective transport (for channel width w , velocity of fluid v , and diffusivity D) [32]. For solute ions with typical diffusivity $D \sim 2 \times 10^3 \mu\text{m}^2\text{s}^{-1}$ flowing through a channel

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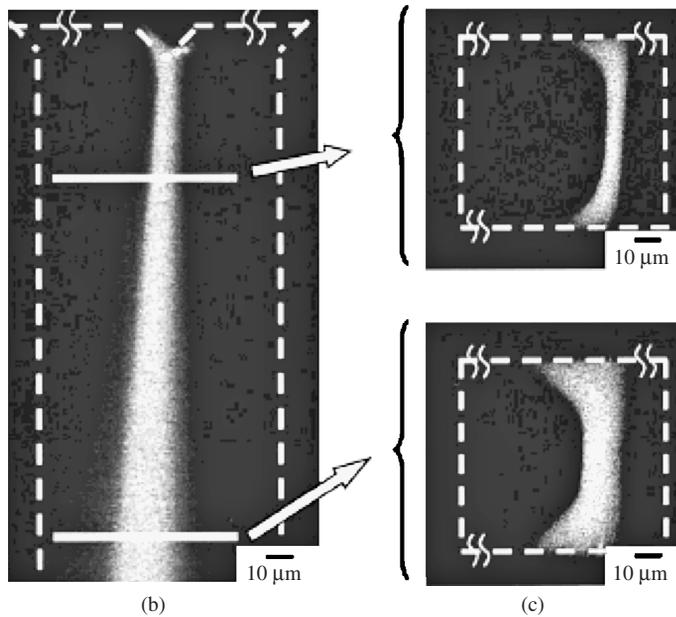
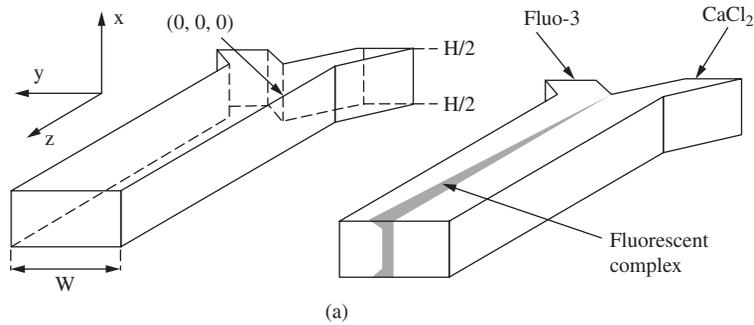


FIGURE 2-4 (a) Schematic drawing of the experiment used to generate fluorescence in the microchannel, and the coordinate system employed. The dashed lines indicate the internal edges of the channel. H is the height of the channel. The origin of the coordinate system is located in the middle of the vertical edge shared by both channels in the Y junction. (b) and (c) Data obtained for the fluo-3/ Ca^{2+} system by confocal fluorescence microscopy in: (b) A $100 \times 250 \mu\text{m}^2$ yz slice obtained $12 \mu\text{m}$ from the top of the channel. (c) Corresponding $90 \times 90 \mu\text{m}^2$ cross sections in the xy plane of the channel obtained $50 \mu\text{m}$ (top) and $200 \mu\text{m}$ (bottom) from the Y junction (*Adapted from Ref. 37.*)

with width $w = 100 \mu\text{m}$ at velocity $v = 100 \mu\text{m/s}$, it would take a time of $t \sim w^2/D = 5 \text{ s}$ for the ions to diffuse across the entire width of the channel. During this time, the stripe will have moved a distance $Z \sim vw^2/D = 500 \mu\text{m}$ down the channel. The number of channel widths

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required for complete mixing would be of order $Pe \equiv Z/w = vw/D = 5$. It is possible to increase the time before complete mixing occurs [or to decrease the spatial extent of transverse diffusive broadening for a given channel length (in the z -direction)] by applying a higher rate of flow, as long as the Reynolds number is still small enough for the flow to remain laminar, or by using liquids with higher viscosities and thereby lowering diffusivity.

For larger species with lower diffusivities, pure diffusive mixing can be slow. For example, small proteins ($D \sim 40 \mu\text{m}^2\text{s}^{-1}$) flowing through a $100\text{-}\mu\text{m}$ channel at $100 \mu\text{m/s}$ would require approximately 4 min to mix completely. This time scale can be undesirably long for some biochemical applications. To enhance mixing, special channel designs have been developed. We will discuss various forms of on-chip mixers in the next section.

Note that the extents of diffusive mixing in the middle of the channel and close to the top wall (ceiling) and bottom wall (floor) of the channel are different. The cross-sectional profile (in the xy plane) of the laminar interface is not entirely vertical to the ceiling/floor of the channel (Fig. 2-4). At steady state, near the ceiling and the floor of the channel, the extent of transverse diffusive mixing across the liquid-liquid interface scales as the one-third power of the axial distance (in the z -direction) along the channel [37]. Near the middle of the channel, the extent of mixing scales is the one-half power of the axial distance, and is therefore smaller than that close to the ceiling/floor at the same position (z) down the channel. As a result, the cross-sectional profile of the laminar interface becomes curved.

2-5 Components Fabricated in PDMS

This section describes examples of microfluidic components, which are the building blocks of more complex, multifunctional microfluidic systems with applications in polymerase chain reaction (PCR), protein crystallization, lab-on-a-chip, and other micro total analytical systems (μTAS). These examples illustrate the general methods to manipulate fluids in microchannels, and the basic design rules of microfluidic devices.

2-6-1 Inlets, Outlets, and Connectors

To introduce and recover liquids from microchannels made in PDMS, polyethylene tubing can be inserted into holes bore in PDMS that are slightly too small, so the PDMS must stretch to fit. This fitting provides a waterproof seal, and prevents leaking of liquids at this PDMS-tubing interface [19]. Syringes are usually used to provide pressure or vacuum, and thus to drive the flow of fluids in the channels. The polyethylene tubing also conforms to syringe needles. This ability

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allows for syringes (and syringe pumps) to be coupled easily to microfluidic channels.

2-6-2 Valves and Pumps

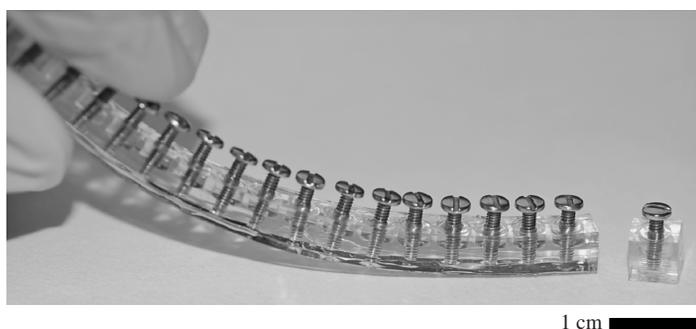
Several groups have used the elasticity of PDMS in the actuation of valves and pumps [19]. The valves operate by applying a force that pinches a fluidic channel closed at a precise location. Compression of the channels can be introduced in various ways, including: fluid pressure [38, 39], torque actuation from embedded machine screws [40] or solenoids [41], expansion of a hydrogel [42], magnetic actuation [43], or the thermal response of shape-memory alloys [44]. Takayama et al. have also used the pins of a piezoelectric Braille display as valves in microfluidic systems [45].

Quake valves are perhaps the most commonly used microfluidic valves in elastomeric devices. The Quake valve is a three-layer microfluidic structure, consisting of a flow channel in one layer separated by a thin elastomeric membrane from a (usually perpendicular) control channel in the layer above. The application of pressurized air to the control channel closes the flow channel. These valves are compatible with soft lithography, and can be used in parallel at high densities because of their small footprint. Their fabrication and operation are complicated, however, and require costly and bulky off-chip infrastructure (computer-controlled pneumatic actuators, gas distribution system, etc.). These valves are sometimes overkill for simple microfluidic applications that require only one, or a small number, of valves.

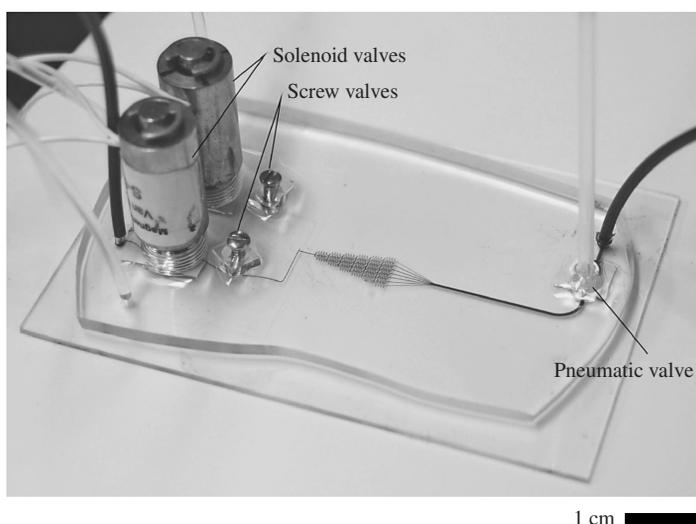
TWIST and solenoid valves developed by our group are simpler to construct and operate, and are suitable for situations that require only small number of valves [40, 41]. To construct a TWIST valve, a small machine screw is introduced directly above a microfluidic channel in a PDMS device. Rotation of the screw results in downward motion of the screw and compression of the underlying channel, and therefore the closing of the channel. To construct a solenoid valve, a cylindrical, push-type solenoid is placed directly on top of a channel. To focus the force from the solenoid onto a small area, a small bead is inserted between the armature of the solenoid and the top of the channel. Applying a voltage to the solenoid actuates the valve.

Recently Hulme et al. showed that it is possible to fabricate these valves [pneumatic (Quake-like), screw (TWIST-like), and solenoid valves] en masse, ahead of time, and then positioned and embedded in microfluidic devices as needed [41] (Fig. 2-5). These valves are suitable for systems in which they are needed only in small numbers, and in which fabrication of an integrated system is not required. Since the valves are prefabricated using a standardized procedure, uniform operation of the valves is possible. The disadvantage of this type of valves is the need for component-level assembly and a relatively large footprint for each valve.

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(a)



(b)

FIGURE 2-5 (a) A photograph of a strip of prefabricated screw valves. A single valve has been separated from the strip using a razor blade. (b) A photograph of a microfluidic gradient generator containing two embedded solenoid valves, two embedded screw valves, and one embedded pneumatic valve. (*Adapted from Ref. 41.*)

2-6-3 Mixers

Mixing of fluids in microchannels is important for many biological and chemical applications. Mixing in simple microchannels can be slow, as discussed in the previous section. Mixers are therefore essential in enhancing mixing efficiency and in homogenizing reagents rapidly. All mixing ultimately occurs due to molecular diffusion. The basic idea behind mixers is reducing the distance over which mixing must occur [32].

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A wide variety of mixers have been developed. They can be broadly classified as active (involving input of external energy) or passive (making use of the fluid dynamics in specific geometry of the channel in the absence of external forces). Passive mixers are usually easier to fabricate than active mixers, and are more suitable for applications involving sensitive species as they do not impose electrical, mechanical, or thermal agitation [46].

One of the passive mixers developed involves a staggered herringbone structure to generate chaotic advection in a microchannel [47] (Fig. 2-6). This mixer uses asymmetric grooves on the floor of the channel (the “staggered herringbone” design) to generate a transverse component to the flow when an axial pressure gradient is applied. Because of this transverse component, the fluid elements are stretched and folded into one another; this process increases the contact area between the flowing streams and facilitates mixing by diffusion. Channels with the staggered herringbone design thus have a higher efficiency of mixing laminar streams of fluid than channels with smooth walls.

Another type of passive mixer involves the use of serpentine channels [42, 46]. Fluids flowing through curved channels experience both inertial forces and centrifugal forces. Under suitable conditions, these

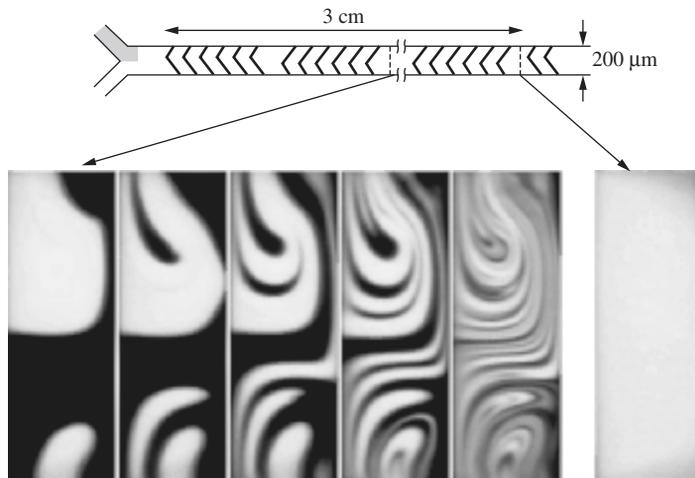


FIGURE 2-6 Continuous-flow staggered herringbone mixer, in which grooved channel walls drive alternating, asymmetric helical secondary flows that chaotically stir the fluid. Each cycle cuts the distance between stripes in half, so that the distance between stripes decreases exponentially with the number of cycles. Diffusive mixing occurs when the tracer can diffuse from one stripe to the next before another cycle has occurred, giving a mixing time that depends logarithmically on Pe . Thus the channel cross section is rapidly mixed. (Adapted from Ref. 47.)

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effects establish a radial pressure gradient whose magnitude can become sufficient to generate a transverse flow (“Dean flow”) [32] across the streams. This transverse flow increases the contact area between the streams, and enables more efficient mixing of the liquids.

Active mixers have also been developed for enhancing mixing: rotary mixers, where solutions to be mixed are actively pumped peristaltically in a circulating loop [48]; mixers based on electrowetting [49], nonlinear electrokinetic effects [50, 51], and acoustic streaming [52]. These systems are usually complicated to fabricate, however. Recently, a simple, portable, hand-powered mixer has been developed that exploits the introduction and movement of bubbles in microchannels to mix the continuous fluids [53].

2-6-4 Diluters for Generating Concentration Gradients in Microchannels

Gradients in the concentration of solutions are important in many biological and chemical processes, such as chemotaxis and nerve growth cone guidance. Various forms of diffusion-based dilution microfluidic devices have been developed to generate concentration gradients. The general design consists of two inlets, one for the reagent to be diluted, and the other for the diluting agent or buffer, leading into a network of multistep fluid-dividers [54] (Fig. 2-7). Mixers are usually incorporated to ensure the complete mixing of the reagent and the buffer. The ratio of fluidic resistance in the branches determines the ratio of volumetric flow of the reagent and the buffer in each branch, which in turn determines the output concentration. The fluidic resistance can be increased by increasing the length of the channel, or by decreasing the cross-sectional area of the channel. Different schemes have been developed to generate linear and logarithmic gradients [54–62].

2-6-5 Local Heaters and Electromagnets

Incorporation of metals into microfluidic systems for applications such as on-chip heating and magnetic sorting usually require more complicated procedures as the materials and the fabrication processes are different from those of microfluidic channels, which are polymer-based. A simple method—*microsolidics*—has been developed to fabricate complex metallic structures by injecting liquid solder into microfluidic channels, and allowing the solder to cool and solidify [63, 64]. The general procedure consists of five steps (Fig. 2-8a):

1. Fabrication of microfluidic channels in PDMS.
2. Plasma oxidation and silanization of the inside surfaces of the microchannels with 3-mercaptopropyltrimethoxysilane (0.1 M solution in acetonitrile). This reduces the surface free energy of the channel surface, and allows the solders (such as In100, or 100% Indium) to wet the channel wall.

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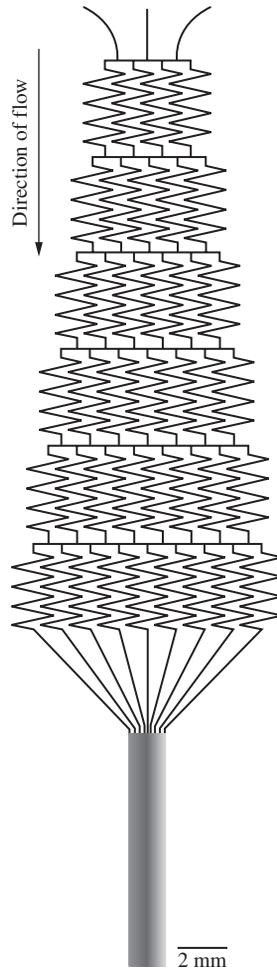


FIGURE 2-7 Photograph showing a microfluidic device we used for generating gradients of different dyes in solution. The three incoming channels (top part of the photograph) were connected to syringes via tubings (not visible). After combining the streams into a single, wide channel (bottom of the photograph), a gradient was formed across the channel, perpendicular to the direction of flow. (Adapted from Ref. 58.)

3. Injection of molten solder into the channels by applying a vacuum to draw metal into the channels.
4. Cooling the channels to form solid metal microstructures.
5. Deforming the solder-filled system of channels into nonplanar structures (if desired).

Next, we will describe two components fabricated using this method.

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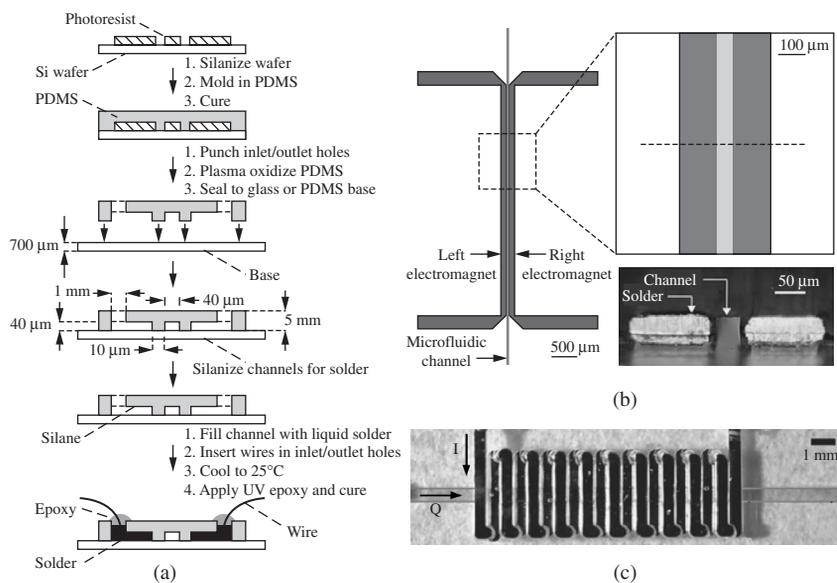


FIGURE 2-8 (a) A schematic diagram depicting the fabrication of electromagnets in PDMS. The completed device has three microfluidic channels: two outer channels filled with solder (length = 1.5 cm, width = 120 μm, height = 40 μm), and a central channel for fluids (length = 1.5 cm, width = 40 μm, height = 40 μm). (b) Electromagnet: photographs of the three channels as viewed from above at low magnification (left), high magnification (upper right) and the cross-section of the three channels (lower right). The photograph of the cross-section was obtained by sectioning the channels with a razor blade (shown as the dashed line in the upper right image); the dark line in the left electromagnet is the result of imperfect sectioning; the light areas at the bottom of the image are reflections of the metal. In the photograph at low magnification, lines were drawn to outline the location of the microfluidic channel. (c) Microheater: photograph of a solder coil positioned axisymmetrically around a central microfluidic channel; the channel was filled with dyed deionized water to make it easier to see; the exterior walls of the central microfluidic channel were highlighted for clarity. (Adapted from Ref. 63, 64.)

Electromagnet [64]

The channels for electromagnets were fabricated in close proximity (~10-μm separation), and in the same plane as the channels used to transport fluid (Fig. 2-8b). By passing electrical current through the wires, magnetic fields up to 2.8 mT and field gradients up to 40 Tm⁻¹ have been generated inside microfluidic channels. This electromagnet has been demonstrated to be capable of sorting super-paramagnetic beads.

Microheater [63]

Figure 2-8c shows a photograph of a coil microheater fabricated by the injection of solder into a PDMS channel. The device consists of a solder coil (In100, height = 80 μm, width = 800 μm, length = 12 cm) wrapped around a central microfluidic channel (height = 80 μm, width = 800 μm, length = 3 cm). This device was fabricated using a procedure similar to

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that used to fabricate a “basket-weave” microstructure: three layers of PDMS containing microfluidic channels were aligned, bonded together, and mounted to a glass slide to form a multilayer network of microfluidic channels. The network was composed of two channels: a central microfluidic channel and a “coil channel” that passed through all three microfluidic layers to surround the central channel. Solder was injected into the coil channel and cooled to form the microheater.

To characterize the microheater, electrical currents ($I = 0 - 600$ mA, at 100 mA intervals) were applied through the wire while deionized water flowed through the central channel (flow rate, $Q = 100$ $\mu\text{L}/\text{min}$). As the current passing through the solder coil increased, the temperature of the fluid passing through the microfluidic channel increased up to 40°C as a result of Joule heating.

Microsolidics simplifies the incorporation of metals into microfluidic channels, but it also has several limitations. This method can only be used with metals and alloys with a low melting point (generally $< 300^\circ\text{C}$) and affinity for the surface of the channel wall. These low-melting-point solders are usually more expensive than commonly used solders, and some (those containing Pb or Cd) are not biocompatible. In addition, the wire must be fabricated as a loop; this method cannot be used to fill “dead-end” channels. Lastly, it is currently difficult to use this process to fabricate wires with cross-sectional dimensions less than 10 μm .

2-6-6 Bubble and Droplet Generator

We have focused primarily on miscible systems so far. The use of immiscible fluids for the formation of emulsions and foams in microfluidic systems is also interesting, and has undergone rapid development in recent years. The controlled formation of microscale, individual fluid segments allow compartmentalized biochemical reactions and analyses using small volumes of reagents. It has also been shown that droplet and bubble-based microfluidics can perform simple Boolean logic functions [65, 66].

There are several ways to generate droplets and bubbles in microfluidic systems; details are reviewed elsewhere [67]. Here we describe two common methods that depend on the geometry of the channel to control the generation of droplets and bubbles: the flow-focusing device and the T-junction.

Flow-Focusing Device

Figure 2-9a and 2-9b illustrates the flow-focusing device [68–70]. Gas and liquid meet upstream from the orifice at the junction of the three inlet channels. The pressure drop along the axis of the device forces the tip of the gas stream into the orifice. Here the thread breaks and periodically releases bubbles into the outlet channel. Over a wide range of parameters, this system produces almost ideally monodisperse bubbles. The frequency of bubble formation can be greater than 10 kHz.

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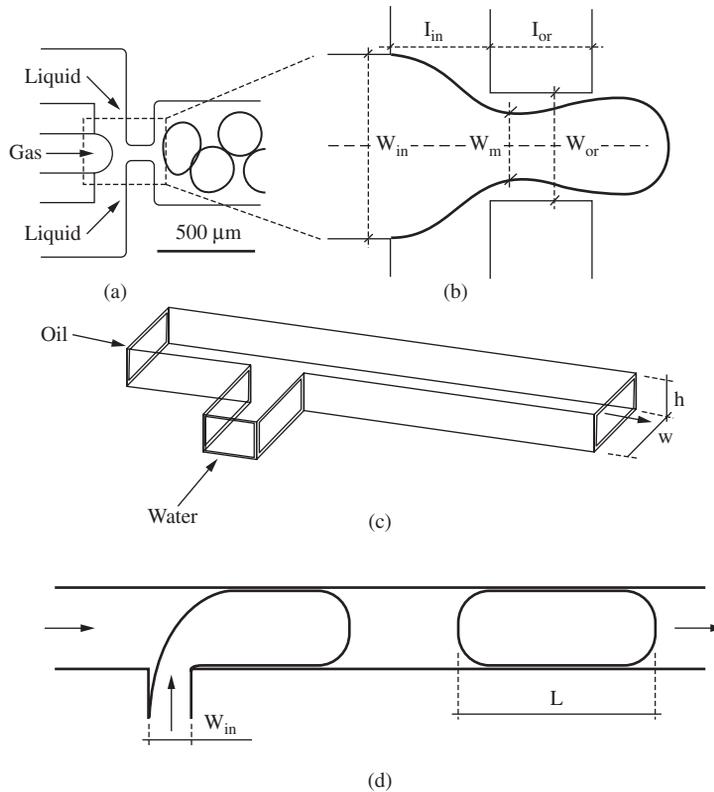


FIGURE 2-9. (a) Optical micrograph of the flow-focusing device (top view). The channels are fabricated in PDMS and have a uniform height h . (b) Schematics of the orifice region and the gas-liquid interface. The shaded areas correspond to PDMS walls and the long dashed line marks the center line and axis of mirror symmetry of the channel. (c) A schematic illustration of the microfluidic T-junction composed of rectangular channels. The channels are planar and have uniform height h . (d) A top view of the same schematic in a two dimensional representation. Flow along the main channel proceeds from left to right. The continuous fluid flows along the main channel of width w , and the fluid that will be dispersed flows via the orthogonal inlet of width w_{in} . (Adapted from Ref. 69, 71.)

The volume of the bubbles and the volume fraction of the dispersed phase can be varied independently by adjusting the pressure applied to the gas stream, and the rate of flow of the liquid. The same device can be used to generate liquid droplets in another immiscible liquid.

T-junction

Figure 2-9c and 2-9d illustrates the geometry of a T-junction [71, 72]. Two channels merge at a right angle. The main channel carries the continuous fluid (oil here) and the orthogonal channel supplies the fluid that will be dispersed (water here). As the dispersed phase

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penetrates into the main channel, shear forces generated by the continuous phase and the subsequent pressure gradient cause the tip of the dispersed phase to elongate into the main channel until the neck connecting the inlet channel with the droplet breaks. The disconnected liquid plug flows downstream in the main channel, while the tip of the stream of the dispersed phase retracts to the end of the inlet and the process repeats. The viscosity of the fluids, the interfacial tension, volumetric rates of flow of the two phases, and the geometry of junction determine the size of the droplets or gas bubbles formed.

2-6-7 Optical Components

Because PDMS is soft and deformable, it is possible to form optical components whose physical dimensions can be tuned mechanically or thermally. These components can be prepared by molding PDMS elastomers into the desired shapes. Tunable lenses and mirrors, diffraction gratings, interferometric sensors, and distributed feedback lasers have been fabricated out of PDMS [22, 23, 73–76]. Some of these devices will be described in detail in later chapters.

2-7 Conclusions

We have illustrated the basic design and construction of some important microfluidic components. Methods for the manipulation of fluids in these microfluidic systems can be used to incorporate multiple functions on the same chip, and to develop more complex optofluidic systems.

The fabrication of microfluidic components in PDMS is easier and more flexible than in silicon or glass. The use of PDMS as a material reduces the time, complexity, and cost of prototyping. Its influence on costs of manufactured systems remains to be established, but polymers are, in general, less expensive than ceramics as materials.

Some of the properties of PDMS may be disadvantageous in certain situations. For example, PDMS is incompatible with many organic solvents; it has therefore been applied primarily to aqueous solutions. When working with biological samples, nonspecific adsorption may occur. The presence of nanoparticles of silica in commercial PDMS causes undesired scattering of light. Methods to control the surface chemistry of PDMS are being actively developed to overcome these problems, however, and to expand the range of properties of PDMS-based systems.

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