

Cofabrication: A Strategy for Building Multicomponent Microsystems

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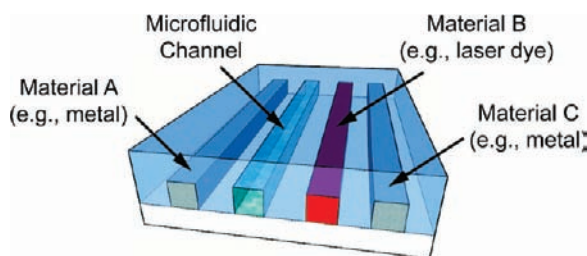
This Account describes a strategy for fabricating multicomponent microsystems in which the structures of essentially all of the components are formed in a single step of micromolding. This strategy, which we call “cofabrication”, is an alternative to multilayer microfabrication, in which multiple layers of components are sequentially aligned (“registered”) and deposited on a substrate by photolithography.

Cofabrication has several characteristics that make it an especially useful approach for building multicomponent microsystems. It rapidly and inexpensively generates correctly aligned components (for example, wires, heaters, magnetic field generators, optical waveguides, and microfluidic channels) over very large surface areas. By avoiding registration, the technique does not impose on substrates the size limitations of common registration tools, such as steppers and contact aligners. We have demonstrated multicomponent microsystems with surface areas exceeding 100 cm², but in principle, device size is only limited by the requirements of generating the original master.

In addition, cofabrication can serve as a low-cost strategy for building microsystems. The technique is amenable to a variety of laboratory settings and uses fabrication tools that are less expensive than those used for multistep microfabrication. Moreover, the process requires only small amounts of solvent and photoresist, a costly chemical required for photolithography; in cofabrication, photoresist is applied and developed only once to produce a master, which is then used to produce multiple copies of molds containing the microfluidic channels.

From a broad perspective, cofabrication represents a new processing paradigm in which the exterior (or shell) of the desired structures are produced before the interior (or core). This approach, generating the insulation or packaging structure first and injecting materials that provide function in channels in liquid phase, makes it possible to design and build microsystems with component materials that cannot be easily manipulated conventionally (such as solid materials with low melting points, liquid metals, liquid crystals, fused salts, foams, emulsions, gases, polymers, biomaterials, and fragile organics). Moreover, materials can be altered, removed, or replaced after the manufacturing stage. For example, cofabrication allows one to build devices in which a liquid flows through the device during use, or is replaced after use. Metal wires can be melted and reset by heating (in principle, repairing a break). This method leads to certain kinds of structures, such as integrated metallic wires with large cross-sectional areas or optical waveguides aligned in the same plane as microfluidic channels, that would be difficult or impossible to make with techniques such as sputter deposition or evaporation.

This Account outlines the strategy of cofabrication and describes several applications. Specifically, we highlight cofabricated systems that combine microfluidics with (i) electrical wires for microheaters, electromagnets, and organic electrodes, (ii) fluidic optical components, such as optical waveguides, lenses, and light sources, (iii) gels for biological cell cultures, and (iv) droplets for compartmentalized chemical reactions, such as protein crystallization.



I. Fabricating Microfluidic Channels

Cofabrication starts with the design and fabrication of a single layer (or plane) of microfluidic channels (Figure 1). Microchannels in PDMS can be readily produced using soft lithography.¹ By this approach, a master is first created by patterning photoresist using contact photolithography. To reduce costs for features having relatively large ($>10\ \mu\text{m}$) dimensions, a transparency with the desired pattern printed with a high-resolution printer can be used as the photomask. Subsequent casting of the PDMS prepolymer against the master and curing yields the polymer replica with a network of channels. Bringing the polymer replica in conformal contact with a flat

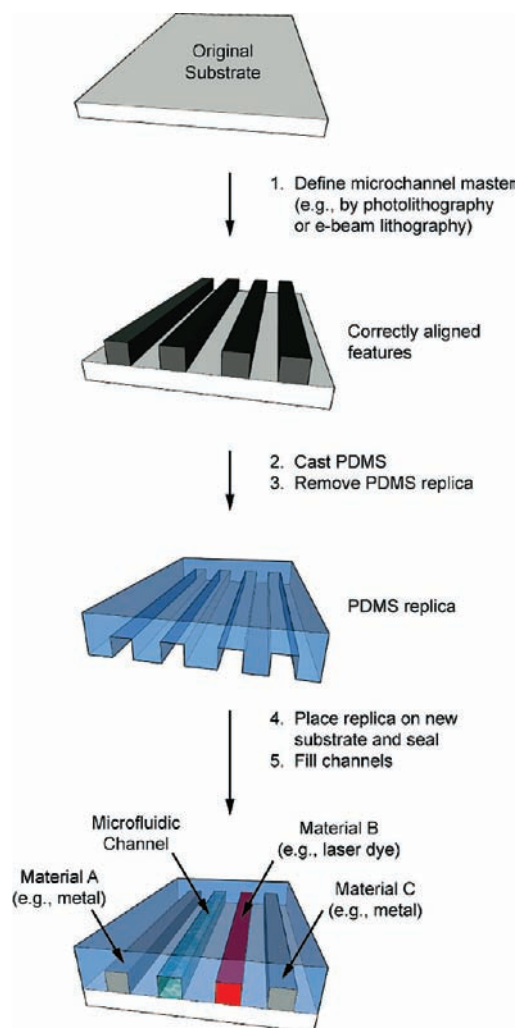


FIGURE 1. Cofabrication involves three steps: (i) designing the microsystem in such a way that a single layer of microfluidic channels provides the structural basis for all of the components and functions required in the final device, (ii) fabricating these channels by micromolding, and (iii) generating function by filling the separate subsystems of microfluidic channels with appropriate materials in fluid form. In this example, a simple step of micromolding ultimately generates two independent, aligned metal wires, a microfluidic channel, and an optical structure.

slab of PDMS (or other substrates including glass, oxidized silicon, or oxidized polystyrene) after oxidization of both surfaces irreversibly seals and completes the microchannels. The procedure for fabricating the channels takes less than 24 h from computer design to physical system.

For some applications, it is desirable to build the layer of microfluidic channels using a material other than PDMS.^{2,3} For example, bulk and surface micromachining⁴ rely on dry- or wet-etching cavities in silicon or glass substrates, followed by a bonding step with another surface. Micromolding, microimprinting or embossing, and laser ablation are commonly used to produce microfluidic channels in plastics and other polymers.^{5,6} Scanning beam lithography techniques, such as focused ion-beam, e-beam, and pulsed laser deposition, can fabricate microchannels as small as 10 nm in width.⁷ Patterning paper into hydrophobic and hydrophilic regions also provides a new approach to the fabrication of microfluidic channels.⁸

The microfluidic channels used in cofabrication have some similarities with established injection molding strategies used for manufacturing polymeric, metallic, and ceramic parts.⁹ These techniques utilize molds that are injected with a precursor (typically a powder or polymer melt) that can be processed further to form solid structures. Injection molding of miniature components (so-called "microcasting") can form microscale parts (e.g., posts, gears) with features as small as $10\ \mu\text{m}$.¹⁰ Cofabrication also involves injecting materials into molds, but unlike injection molding, the microfluidic channels are integral parts of the final structure (whereas in injection molding the mold is typically separated after injection). For academic research environments, injection molding is also limited by the cost of the equipment and the high pressures required (e.g., 3–5 MPa).¹⁰

Ultimately, the choice of material used for microfluidic channels (e.g., the *bas-relief material* of a cofabricated system) depends on the characteristics required of that material in the final application: temperature stability, elasticity, transparency, resistance to solvent, permeability, wettability, biocompatibility, scalability, index of refraction, etc. Table 1 summarizes materials often used for this purpose.

II. Filling Microfluidic Channels with Materials

Microfluidic channels are typically filled with liquids using either capillary action or active injection by pump/vacuum. Capillarity depends on the relative free energies of the interfaces in a channel to "wick" aqueous fluids into the channel.

TABLE 1. Properties of Materials Used To Form Microfluidic Channels in Cofabricated Systems

material for forming channels	method for forming channels ^a	advantages	disadvantages
PDMS	SL	fast prototyping, biocompatible, well studied, transparent, flexible/unbreakable	gas permeable, melts at high temp, hydrophobic, absorbs small molecules, swells with solvents
polycarbonate	μ M, IM, HE, e-B, LL	non-gas-permeable structure, strong, transparent	hydrophobic, susceptible to organic acids/bases, inflexible
polymethylmethacrylate	μ M, IM, HE, e-B, LL	non-gas-permeable structure, strong, transparent, inexpensive	hydrophobic, susceptible to organic acids/bases, inflexible
cydoolefin copolymer	μ M, IM, HE, e-B	non-gas-permeable, inexpensive, resists solvents and acids/bases	hydrophobic
perfluoropolyethers	μ M, IM, HE	biocompatible, resists solvents and acids/bases, liquid at RT	gas permeable, not commercially available, requires UV to cure
polyurethanes	SL	non-gas-permeable, easy to make, hydrophilic, inexpensive, flexible/unbreakable	more complex fabrication than PDMS, difficult to bond layers or perform surface modifications
silicon	μ M, e-B, LL	well-studied, non-gas-permeable, resists solvents and most acids/bases	breaks easily, not electrically insulating, expensive, opaque
glass	μ M, e-B, LL	inexpensive, resists solvents and most acids/bases, non-gas-permeable, transparent	breaks easily, difficult to etch (requires HF) and seal to itself (requires high temp)
thiolene	SL	fast prototyping, inexpensive, resists solvents, transparent, low gas permeability, biocompatible	requires UV to cure

^a SL = soft lithography, μ M = bulk/surface micromachining, IM = injection molding, HE = hot embossing, e-B = e-beam lithography, LL = laser lithography.

It is also possible to fill channels by applying an external vacuum or pressure to force a material into the channel; this approach is especially useful for replacing fluids in previously filled channels or for applications in which the fluid must flow through the channel to provide function (e.g., optofluidics).

Microfluidic channels can be filled with solid materials by injecting the material into the channel in liquid form and then allowing the material to solidify inside. We have found it particularly useful to be able to inject molten metals and alloys (e.g., In, Sn, Ga, In/Sn, In/Ag, Ga/In) into microfluidic channels by heating the metals and channels using a hot plate or heat gun, drawing the metal into the channel using house vacuum, and allowing the metal to solidify by cooling; PDMS is able to withstand temperatures up to 400 °C for limited times.¹¹ It is also possible to inject liquid materials into microfluidic channels and to solidify these liquids into solids by curing or chemical cross-linking. These materials include sol-gels,¹² thermally curable epoxies,¹³ and photocurable prepolymers.¹⁴ Crystallization in microfluidic channels has also been demonstrated¹⁵ (Table 2).

For some materials, it is necessary to modify the interior surface of the microfluidic channels chemically to improve the wettability of the injected material. For example, prior to injecting low-melting point metal (e.g., In) or metal alloy (e.g., eutectic In/Sn) into a PDMS channel to form a long, continuous wire, it is first necessary to oxidize the surface of the channel, and then to modify it with a silane (3-mercaptopropyltrimethoxysilane) so that the metal wets it.¹¹ When injected into untreated PDMS channels, most metals in liquid form do not

wet the walls of the channels. As a result, they tend to fracture upon cooling and solidification, and to lose their electrical connectivity/conductivity.

III. Devices Produced Using Cofabrication

This section describes several devices that demonstrate the design, operation, and characterization of cofabricated microsystems.

Liquid-Filled Channels (Microfluidics). Over the past decade, the study of the flow of liquids in microfluidic channels and the applications of liquid-filled microchannels has emerged as an area of great scientific and technological interest.^{16,17} A number of groups have investigated and modeled the physics of microfluidic laminar flow in microsystems.¹⁸ Microfluidic channels filled with media are useful for the study of cells¹⁹ or whole microorganisms.²⁰ Liquid-filled channels have been applied for the development of a variety of practical “lab-on-a-chip” devices including devices for high-throughput analysis,²¹ generation of chemical gradients,²² fluidic mixing,²³ and detection and diagnosis of disease.²⁴

Metal-Filled Channels (Microsolidics). A growing interest in automated lab-on-a-chip systems has fueled the development of microfluidic systems in which metal wires provide electronic function to the system.²⁵ Using the principles of cofabrication, we have produced current-carrying wires by injecting liquid metal in microfluidic channels and allowing the metal to cool and solidify;¹¹ for very low melting tempera-

TABLE 2. Relationship between the Function of a Cofabricated Microsystem and the Material Injected into Its Microfluidic Channels

function class	function	phase	material in microfluidic channel
fluidic	control and handling of liquid chemicals	liquid	aqueous-based solvents, buffers ^{16,17,22,23}
	control and handling of biological samples for diagnostics	liquid	blood serum, saliva, other bodily fluids ^{8,24}
	supply of nutrients to cell cultures	liquid, gel	liquid-based media, agar ¹⁹
		gas	oxygen, CO ₂ , or other bioactive gases
	supply of nutrients to multicell organisms	liquid	liquid-based media ²⁰
	application of pressure for valves/pumps	gas	air or other inert gases
		gel	pH-responsive hydrogels ³⁸
	colorimetric molecular sensing	gel	polydiacetylene-based hydrogel ³⁹
	compartmentalization of reactions	emulsion	aqueous detergents suspended in fluorinated carrier fluids ⁵²
	molecular crystallization	emulsion	aqueous detergents suspended in fluorinated carrier fluids ^{15,53}
electronic	conduction of electrical current (wires, circuits, antennas)	solid	low-melting-temperature metals ¹¹
	generation of heat or control of temp	solid	low-melting-temperature metals ¹¹
	generation of electrostatic fields (dielectrophoresis, cell lysis)	solid	low-melting-temperature metals
	generation of magnetic fields	solid	low-melting-temperature metals ^{27,28}
	molecular electronics or testing of SAMs	liquid	non-Newtonian fluidic metals (EGaln) ^{26,35,36}
	electromechanical fluid actuation	sol	ferrofluids
electrochemical	storage of electrical energy (batteries)	liquid	electrolytes or conductive liquids
	electroplating or electrochemical reactions	liquid	electrolytes or conductive liquids
	electrophoretic separation	gel	polyacrylamide or agarose gels ³⁷
	electrochromatographic separation	sol–gel	porous photopolymerized sol–gel
optical	guiding and switching light (waveguides)	liquid	liquids with high or low index of refraction ^{29,30}
	focusing light (lenses)	liquid	liquids with high or low index of refraction ³¹
	generation of light (lasers, optical sources)	liquid	fluorescent or phosphorescent dyes ^{32,33}
	diffraction of light (diffraction gratings)	foam	gas–liquid foams ⁵¹
structural	micromechanical support (scaffolding)	solid	epoxies, cements, UV or heat curable polymers, or low-melting-point metals ^{11–13}
	solid support for bead packing and chromatography	solid, gel	porous polymer and gel-based monoliths ¹²
	support for plant growth (hydrostats/artificial soils)	gel	sodium/potassium/ammonium polyacrylates
	tissue engineering	gel	collagen, gels, other biocompatible solids ^{14,40–44}

ture metals, we also used the metal directly in liquid form.²⁶ This method of fabrication, which we call *microsolidics*, ultimately generates *solid* metal structures in two or three dimensions that can be fabricated in close alignment with microfluidic channels (Figure 2). The wires can be fabricated with high aspect ratios and cross sectional areas up to $10 \times 10^6 \mu\text{m}^2$; this large cross-sectional area is difficult to achieve in a small footprint using alternative techniques such as evaporation or sputter deposition and makes it possible to use the wires for applications requiring high electrical current (e.g., for generation of magnetic fields). The general procedure consists of five steps: (i) fabrication of microfluidic channels in PDMS using soft lithography and rapid prototyping; (ii) plasma oxidation and silanization of the inside surfaces of the microchannels with 3-mercaptopropyltrimethoxysilane (0.1 M solution in acetonitrile) to make them wettable to the metals we use; (iii) introduction of molten metal into the channels by applying a vacuum to draw metal into the channels (walls of silanized channels are rapidly wet, for example, by liquid indium, eutectic indium–tin, or eutectic gallium–indium, while walls of unmodified channels are not.); (iv) cooling the channels to form solid metal microstructures; and (v) (if desired) forming the solder-filled system of channels into non-planar topologies by bending, twisting, rolling, or deforming. This last step, modifying the shape, works particularly well when the metal is encapsulated in thin (50–200 μm) layers

of PDMS. By this technique, it is possible to fabricate metallic structures with lateral dimensions as small as 50 μm , and thicknesses of 10 μm to 1 mm. Because the structures are embedded in polymer insulation, breaks or fractures in the metal can often be repaired by heating the metal above its melting point and cooling (perhaps with brief sonication).

Microheaters. Microsolidic wires can be used to build precision-integrated heaters for microfluidic channels.¹¹ Indium metal (100%) was injected in microfluidic channels and cooled to form solid, resistive metal wires in microchannels 100 $\mu\text{m} \times 100 \mu\text{m}$ in cross-sectional area. Passing electrical current through the wires increased the temperature of the metal by Joule heating, which, in turn, increased the temperature of adjacent microfluidic channels. Microsolidic heaters with cross-sectional areas of $10^4 \mu\text{m}^2$ supported currents up to 2 A ($2 \times 10^8 \text{ A} \cdot \text{m}^{-2}$) without breaking down and have been shown to heat static or flowing aqueous fluids in adjacent microfluidic channels to $>100 \text{ }^\circ\text{C}$.

In-Plane Electromagnets. Microsolidic wires have also been used to build integrated electromagnets for separations (Figure 3).^{27,28} Siegel et al. cofabricated metal wires in close proximity (distance $\sim 10 \mu\text{m}$) to liquid-filled microfluidic channels. Passing electrical current through the wires generated magnetic fields up to 2.8 mT and magnetic field gradients up to $40 \text{ T} \cdot \text{m}^{-1}$, orthogonal to the direction of current flow. These gradients captured/released and sorted superparamag-

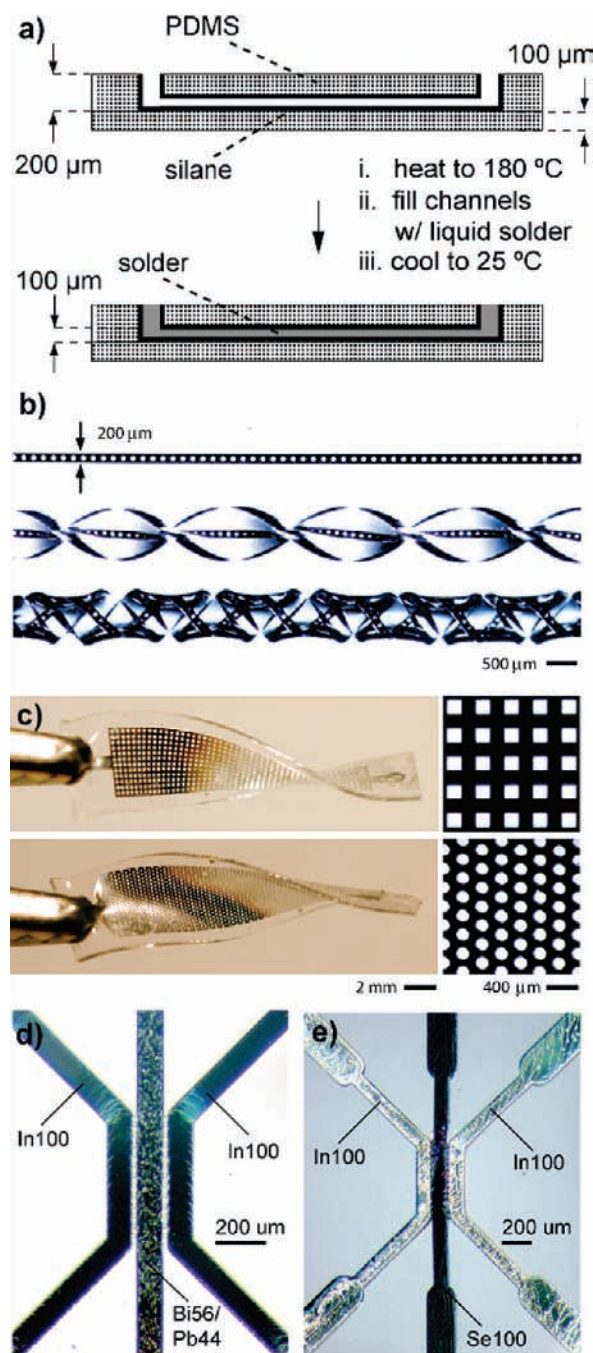


FIGURE 2. Filling microchannels with low-melting-temperature solders (microsolidics): (a) schematic diagram describing the injection of low-melting-point solders in microchannels; (b) a metallic wire (100% In) embedded in a PDMS substrate; the wire can be twisted while still maintaining electrical conductivity; (c) mesh structures formed by injecting metal in PDMS microfluidic chambers; (d, e) a system of three microfluidic channels, each filled with a different low-melting-temperature material. In panel d, the outer channels are filled with 100% In metal, and the central channel is filled with a Bi56/Pb44 solder. In panel e, the outer channels are filled with 100% In metal, and the central channel is filled with 100% Se (a semiconducting nonmetal); the three channels are connected together electrically and form a metal–semiconductor–metal junction. Panels b and c reproduced from ref 11, Copyright 2007 Wiley-VCH.

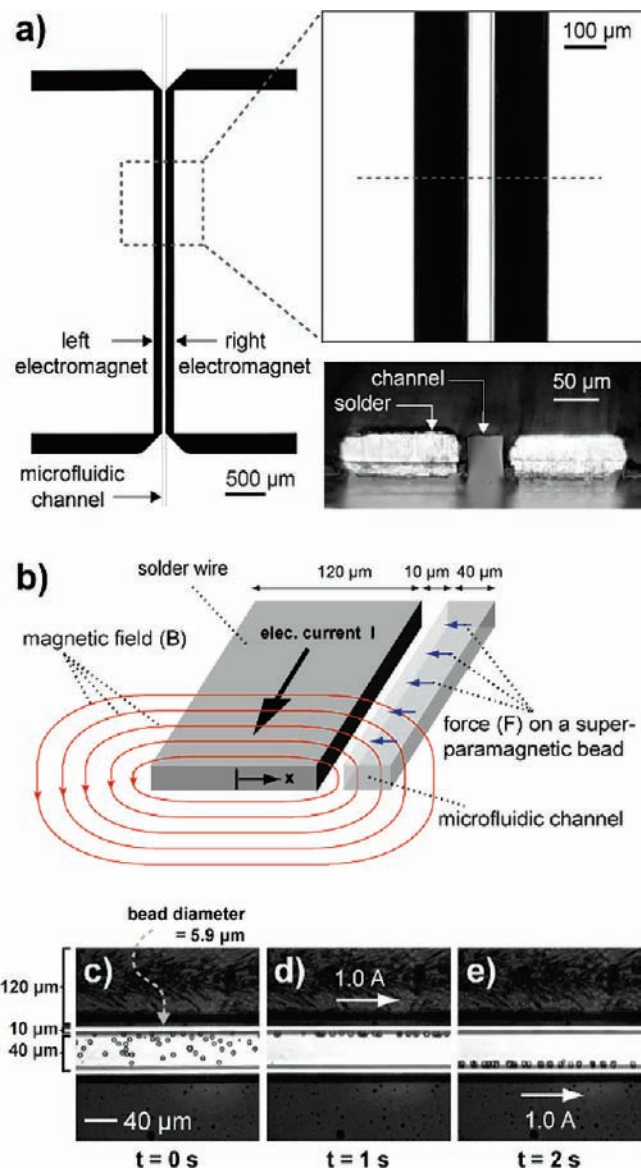


FIGURE 3. Cofabricated electromagnets: (a) Photographs of three microfluidic channels as viewed from above at low magnification (left) and high magnification (upper right), and the cross section of the channels (lower right); all channels are $40\ \mu\text{m}$ in height. The two outer channels, which form the electromagnets, are filled with solder, and the central channel is filled with solution. (b) Passing electrical current through the outer metal wires generates a magnetic field, which is subsequently used to move superparamagnetic beads suspended in solution in the central channel. (c–e) The capture and release of superparamagnetic beads in a microfluidic channel in proximity to two electromagnets (in black); the arrows represent the direction of electrical current passing through each electromagnet. The current generated a magnetic field oriented into the plane of the page with greatest magnitude at the edge of the channel closest to the electromagnet. The process was repeated over 1000 times with similar results. Panels reproduced from ref 27, Copyright 2006 Wiley-VCH.

netic beads suspended in the adjacent liquid-filled microfluidic channels with forces up to $3.3\ \text{pN}$.

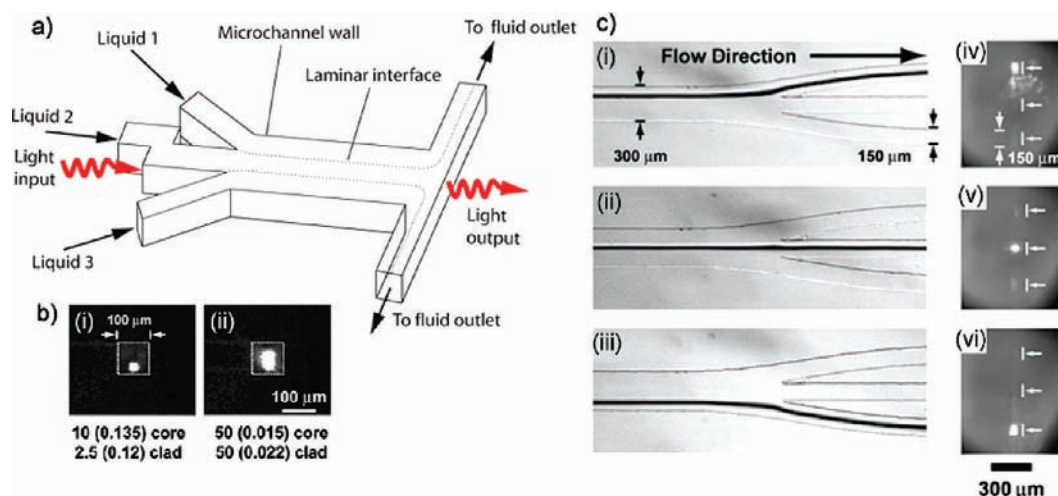


FIGURE 4. (a) Schematic representation of the typical design of a L^2 waveguide. The index of refraction of liquid 2 is higher than that of liquids 1 and 3 for the guiding of light. (b) Optical micrographs of the cross section of the outlet of the microfluidic channel (outlined by the dashed box) as viewed through the transparent window. The rates of flow ($\mu\text{L}/\text{min}$) (and the residence time in seconds) of the core and the cladding are listed. (c) Optical switch: (i–iii) optical micrographs of the top view of the microfluidic channels; (iv–vi) optical micrographs of the cross section of the end of the channel showing light exiting the L^2 waveguides. The white arrows and lines represent the location of the ends of the branches of the microfluidic channel. Panels reproduced from ref 29, Copyright 2004 National Academy of Sciences.

Liquid–Liquid Optical Systems. The principles of cofabrication can also be applied to fabricate reconfigurable optical components.^{29,30} One category of these devices, which we call liquid–liquid (L^2) systems, typically comprises two or more streams of liquid flowing in microfluidic channels, each possessing different optical properties (such as refractive indices) (Figure 4a). At low and moderate Reynolds numbers, flow of these streams is laminar;¹⁷ this property creates optically smooth liquid–liquid interfaces, which exhibit low optical losses from scattering.

Liquids can be replaced or replenished continuously in liquid–liquid systems. This capability for replacement allows injection of liquids with different properties (e.g., index of refraction, absorption, fluorescence) to tune the optical output of a microsystem in real time. The ability to replenish liquids minimizes the impact of photobleaching and related phenomena because bleached elements are replaced continuously. In addition, the liquid–liquid interface is deformable: by manipulating the flow conditions, it is possible to change the position or the shape of the liquid–liquid interface and, therefore, the path of light inside the optofluidic devices.

Optofluidic Waveguides. Optical waveguides can be formed in microfluidic channels in which the laminar streams themselves control the direction of the flow of light.²⁹ These devices consist of two streams of liquids with lower refractive index (the cladding) sandwiching a stream of liquid with higher refractive index (the core) in a single microchannel; light is supplied from an optical fiber inserted in PDMS through a fiber port fabricated at the end of the channel (Fig-

ure 4b). By controlling the relative rates of flow of the core and cladding liquids, we changed the width of the core stream and, therefore, the width of the optical waveguide. Decreasing the ratio of flow rates of the core to the cladding streams decreased the core size from >100 to <10 μm and switched the guiding from multi- to single-mode.

Optofluidic Switches and Couplers. Cofabricated liquid–liquid components can be used to build complex devices that control the direction of propagation of light in microfluidic channels.²⁹ We built an optical switch by branching a liquid–liquid waveguide into three separate outlet channels (Figure 4c). Changing the relative rates of flow of the cladding liquids changed the path of the core liquid and, therefore, the path of the light. It was also possible to fabricate an evanescent coupler comprising two liquid–liquid waveguides sharing an inner cladding stream with a width <5 μm . The efficiency of coupling of evanescent fields between the two waveguides was modified by changing the rate of flow of the liquids, which in turn, adjusted the width of the inner cladding stream.

Optofluidic Lenses. We used the principles of cofabrication to build a liquid–liquid lens, where the curvature of the interface could be modified dynamically (Figure 5a).³¹ The lens was formed by laminar flow of three streams of fluids in a microchannel; the index of refraction of the core stream was higher than the index of the cladding streams. The streams entered an “expansion chamber”, or a region of the microchannel with increased width. For appropriate rates of flow, the shape of the interface between the core and cladding

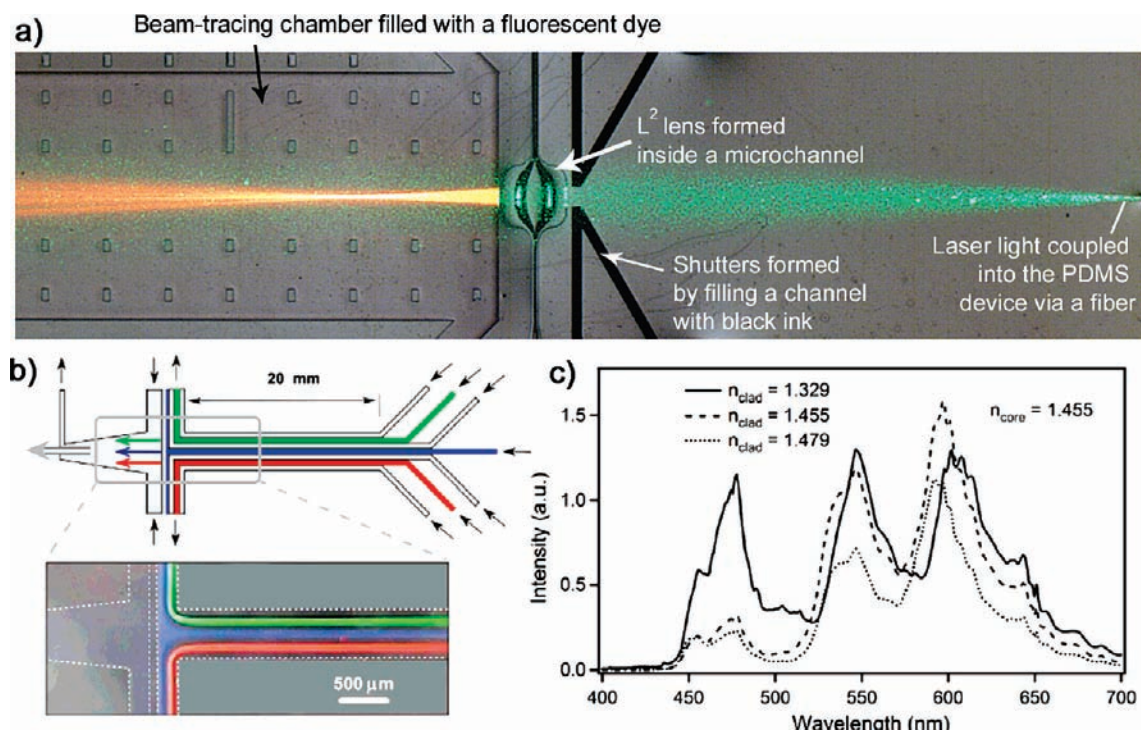


FIGURE 5. (a) Bright-field image a L^2 lens cofabricated in a single layer of lithography. (b) Top-view scheme for the array of L^2 fluorescent light sources, consisting of parallel L^2 waveguides in a single PDMS microchannel. The inset shows an optical micrograph of the T-junction. (c) Spectral output (solid line) from an array of L^2 fluorescent light sources containing 0.5 mM solutions of perylene, fluorescein, and sulforhodamine B in DMSO/EG (1:1), with various cladding liquids. Panel a reproduced from ref 31, Copyright 2008 Royal Society of Chemistry. Panels b and c reproduced from ref 32, Copyright 2005 American Chemical Society.

streams in this region was biconvex; this structure focused light propagating in the plane of the expansion chamber perpendicular to the direction of flow of the liquids. Changing the relative rates of flow of the three streams modified the curvature of the interface, and thus the focal distance of the lens, in real time.

Optofluidic Light Sources. We developed various on-chip fluidic light sources for optical detection and spectroscopic analysis in integrated microanalytical systems (μ TAS).^{32,33} In these systems, solutions containing fluorescent dye flowed through microchannels and were excited optically by incident light from an external halogen bulb or a pump laser (Figure 5b,c). Microchannel-based liquid–liquid fluorescent light sources circumvent the need to align and secure optical fibers to microfluidic channels and can be produced with a range of cross-sectional diameters.

Electronic Interfacing with Surfaces. Sometimes it is possible to gain additional function in a cofabricated system by placing the layer of microfluidic channels, roughly and with minimal precision, perpendicular to a chemically patterned surface. We cofabricated an array of seven molecular scale tunneling junctions³⁴ each comprising an electrically conductive self-assembled monolayer (SAM) connected to two metal-

lic electrodes via a microfluidic channel (Figure 6a).³⁵ We aligned a PDMS microchannel perpendicularly to Ag electrodes on a glass substrate and sealed it to the surface; the Ag electrodes were patterned with $SC_{11}Fc$ (Fc = ferrocene) SAMs. Injecting liquid metal (EGaln = eutectic Ga–In)³⁶ in the microchannel formed electrical contact between the SAM–Ag electrode pair and completed the molecular junction. An optical micrograph of the devices is shown in Figure 6b. The resulting Ag– $SC_{11}Fc$ /EGaln junctions (“–” indicates a covalent bond and “//” indicates a van der Waals contact) rectified currents with rectification ratios R ($R = |J(-V)|/|J(+V)|$), J = current density (A/cm^2), V = the voltage (V) of 102 at $V = \pm 1.0$ V. The electrodes and microfluidic channel were arranged in a 7×1 crossbar scheme (comprising seven Ag electrodes and one EGaln-filled microfluidic channel).

The cofabricated tunneling junctions gave working devices in $\sim 80\%$ yield and reliably rectified AC electronic signals.³⁵ Figure 6c shows the average current density as a function of the potential of the tunnel junctions. These results represent a first step in applying cofabrication to produce functional molecular electronic devices in microfluidic channels.

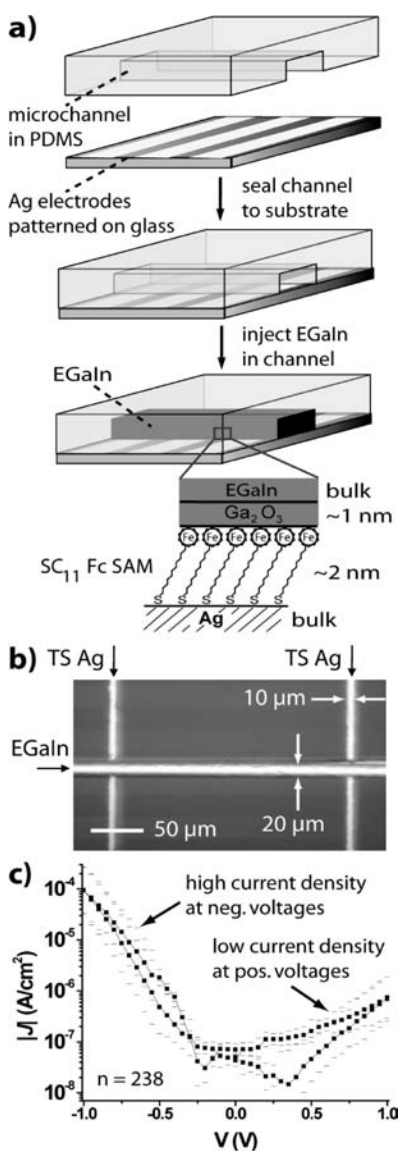


FIGURE 6. Cofabrication of molecular electronic devices in microfluidic channels: (a) schematic of the fabrication of the metal–SAM//EGaIn junctions; (b) top-down micrograph of two TS Ag electrodes (of seven total) with EGaIn in the perpendicularly aligned PDMS channel; (c) the average values of the current density (J) as a function of voltage (V) applied across the junction. The shape of the J/V curve is asymmetric; the difference in average slope of J for negative values of V and positive values of V illustrates the rectification of electronic signals through the device (adapted from ref 35).

In-Channel Gels for Electrophoresis and Cellular Growth.

Cofabrication of gels and other multiphase materials in a single layer of microfluidic channels produces microdevices with useful function in biology. Typically, gels are injected in the channels in liquid form and then cured by heating, cooling, or UV irradiation. A variety of groups have injected polyacrylamide gels in microfluidic channels or networks of channels for applications in 1D and 2D electro-

phoretic separation.³⁷ Other types of gels, including collagen- and acrylate-based hydrogels, have been injected in microfluidic channels for microfluidic valving and flow control,³⁸ colorimetric molecular sensing,³⁹ tissue engineering,^{40,41} and constructing cellular and biomolecular microenvironments.^{42–44}

Bubble and Droplet Generation in Microchannels.

There has been growing interest in producing emulsions such as foams in microfluidic devices.^{45,46} We and others have used cofabrication to generate monodisperse emulsions of bubbles,⁴⁷ droplets,⁴⁸ and polymer particles⁴⁹ in microchannels that exhibit remarkable regularity. Flow-focusing^{47,49} and T-junction⁴⁸ geometries allow the user to tune the size and the fraction of volume that the bubbles and droplets occupy in the outlet channel. Garstecki et al. demonstrated the self-organization of bubbles in a variety of ordered gas–liquid and liquid–liquid lattices in microfluidic channels.⁴⁷ The form and structure of these lattices was adjusted by changing the pressures applied to the two immiscible fluids. These microfluidic lattices have four practical benefits. They are (i) self-organizing, (ii) self-healing, (iii) dynamically addressable, and (iv) made from components that are constantly replenished for real-time control and modification of the lattice.

Complex Periodic Emulsions. Cofabricating multiple flow-focusing generators in a single layer of microfluidic channels makes it possible to produce complex, periodic structures of bubbles and droplets (Figure 7a).⁵⁰ The generators, when placed in close proximity to each other, allow fluctuations in the local fluidic resistance in a single channel or in a network of channels and, thus, make possible the synchronization of timing in formation of droplets and bubbles. This synchronization can produce stable composite structures comprising different emulsions (droplets or bubbles) in the same continuous phase.⁴⁷ In one application of this approach, Hashimoto et al. used the self-organization of bubbles to produce microfluidic diffraction gratings.⁵¹ Changing the external flow parameters (i.e., the rate of flow of water and the applied pressure of nitrogen) adjusted the diameter of the bubbles that were generated from the flow-focusing geometry (and thus the lattice constant of the grating structure) and made it possible to tune the angle of diffraction of light from the device *dynamically* and in real time.

Chemical Reactions in Droplets. Droplets formed by flowing immiscible fluids in cofabricated microfluidic channels can be used as vehicles for performing chemical reactions (Figure 7b).⁵² Compartmentalization in droplets allows for the rapid mixing of reagents, provides precise control of the timing of reactions, and makes it possible to synthesize

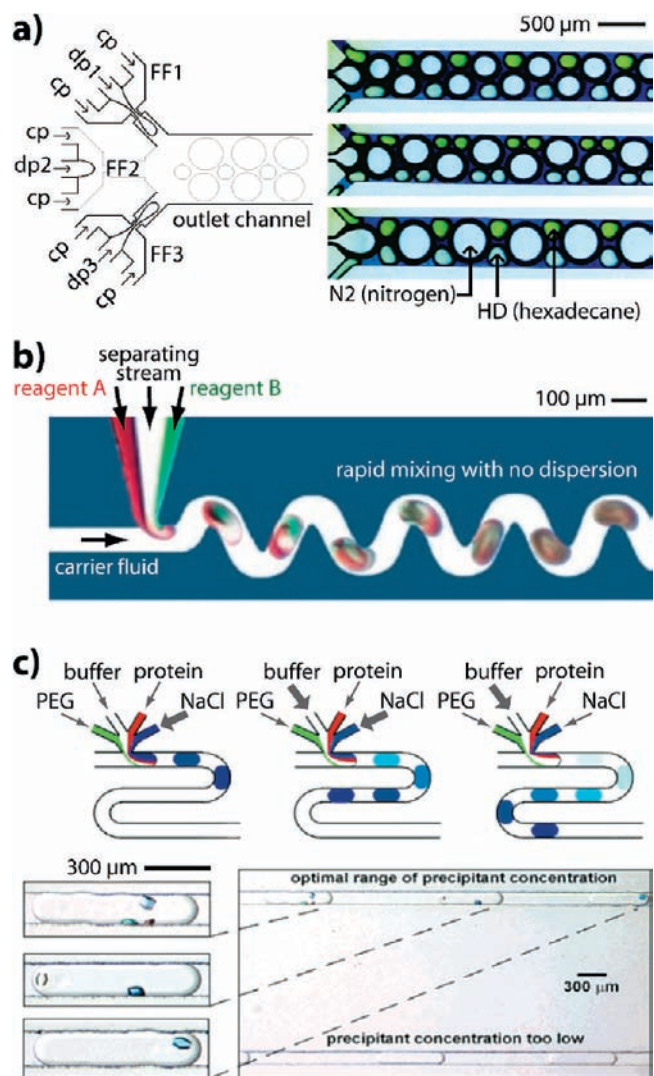


FIGURE 7. Cofabrication of emulsions in microfluidic channels. (a) (left) A schematic representation of three flow-focusing generators connected to a single outlet channel. The abbreviations “cp” and “dp” denote inlets with continuous phase and dispersed phase, respectively, and arrows indicate the direction of flow. (right) Optical micrographs of composite lattices comprising nitrogen bubbles and hexadecane droplets suspended in water (reproduced from ref 50, Copyright 2007 Wiley-VCH). (b) Using droplets as microreactors. Aqueous droplets containing two reagents were formed and encapsulated in a fluorinated carrier fluid to facilitate transport through a buffer (reproduced from ref 52, Copyright 2006 Wiley-VCH). (c) Crystallization in droplets. (top) A schematic illustration of the droplet-forming process; decreasing the flow rate of the NaCl stream and increasing the flow rate of the buffer increased the concentration of NaCl in each droplet. (bottom) A polarized microphotograph showing crystallization of lysozyme inside 7.5 nL aqueous droplets containing PEG, salt, lysozyme, and acetate buffer with variable composition (reproduced from ref 53, Copyright 2003 American Chemical Society).

and transport solid products. Ismagilov and co-workers applied this strategy to the crystallization of proteins in microfluidic channels (Figures 7c).⁵³ The crystallization trials were set up

inside 7.5 nL aqueous droplets, each containing solutions of protein, precipitants, and additives in variable ratios. This method allowed researchers to set up hundreds of crystallization trials under computer control at rates of several trials per second.

Integrated Droplet Generation and Dielectrophoretic Sorting. One advantage of cofabrication is the ability to combine multiple materials, and thereby multiple functions, in a single device. An example is fluorescence-activated droplet sorting.⁵⁴ In this approach, Baret et al. compartmentalized single *Escherichia coli* in droplets produced from a cofabricated microfluidic droplet generator and then sorted the cells dielectrophoretically into separate chambers using metallic electrodes cofabricated in the same device. This method combines the advantages of microtiter-plate screening and traditional fluorescence-activated cell sorting and demonstrates how two strategies of cofabrication (here metal electrodes and droplet generation) can be integrated in practical applications.

IV. Expanding Capabilities through Cofabrication: New Methods and Materials

Cofabrication is already a useful technique for a variety of applications in science and technology, but the approach faces several hurdles on its path to becoming a reliable technique for mass production. For example, the technique is currently limited to materials with low melting points (generally <300 °C) and with affinity for the surface of the channel walls. In addition, while materials such as aqueous fluids are carried into channels via capillary action, others must be injected manually using vacuum or pressure (typically via a syringe), making it difficult to produce devices rapidly *en masse*.

We believe that further research in cofabrication will help overcome many of these limitations. Advances in automation, such as robotic-based fluid injection, will help facilitate production in high volume. In addition, because cofabrication does not depend on the precise alignment of layers, it may be possible to produce the *bas-relief* for the channels using techniques not commonly used for microfabrication, such as plastic injection molding or laser ablation (which can be outsourced at low cost using a commercial vendor). We believe many of the practical advancements of cofabrication will be motivated by efficiency and cost control and may emerge as the technique is adapted to commercial environments.

We also believe that new applications will evolve when new materials (and combinations of materials) are injected into microchannels. Table 2 suggests several possibilities,

some of which have been demonstrated and some of which have not. In cofabrication, structural and functional elements are added to channels postformation, and hence, the only factor limiting the choice of materials is the ability to fill channels with them. In the future, we envision systems comprising high-melting-temperature materials such as glasses, structural metals, or temperature-resistant polymers, and systems based on novel materials that are formed by combining two or more substances in a single microchannel (e.g., combination of PDMS and Fe₃O₄ to produce functional ferromagnetic ceramics).

V. Conclusions

Cofabrication represents a new model for designing and building multicomponent microsystems. The approach is especially applicable to technologies that require the integration and registration of multiple different materials in one or more layers with micrometer-precision but where simplicity and economy of fabrication are key characteristics of the design process. We anticipate that advances in cofabrication will lead to new practical applications in energy storage (e.g., batteries), lab-on-a-chip systems, flexible electronics (e.g., for radio frequency identification, RFID), systems biology, thermal management, biochemical sensors and diagnostics, self-standing 3-D structures (in which the surrounding insulation is removed after fabrication), and compound multilayer microsystems produced by layering several cofabricated structures in a single device.

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George M. Whitesides was born August 3, 1939, in Louisville, KY. He received an A.B. degree from Harvard University in 1960 and a Ph.D. from the California Institute of Technology (with J. D. Roberts) in 1964. He was a member of the faculty of the Massachusetts Institute of Technology from 1963 to 1982 and joined the Department of Chemistry of Harvard University in 1982, serving as department chairman from 1986 to 1989. He is now the Woodford L. and Ann A. Flowers University Professor.

FOOTNOTES

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REFERENCES

- McDonald, J. C.; Whitesides, G. M. Poly(dimethylsiloxane) as a Material for Fabricating Microfluidic Devices. *Acc. Chem. Res.* **2002**, *35*, 491–499.
- Mukhopadhyay, R. When PDMS Isn't the Best. *Anal. Chem.* **2007**, *79*, 3248–3253.
- Hung, L. H.; Lina, R.; Lee, A. P. Rapid Microfabrication of Solvent-Resistant Biocompatible Microfluidic Devices. *Lab Chip* **2008**, *8*, 983–987.
- Bustillo, J. M.; Howe, R. T.; Muller, R. S. Surface Micromachining for Microelectromechanical Systems. *Proc. IEEE* **1998**, *86*, 1552–1574.
- Qin, D.; Xia, Y.; Rogers, J. A.; Jackman, R. J.; Zhao, X. M.; Whitesides, G. M. Microfabrication, Microstructures and Microsystems. *Top. Curr. Chem.* **1998**, *194*, 1–20.
- Becker, H.; Gartner, C. Polymer Microfabrication Methods for Microfluidic Analytical Applications. *Electrophoresis* **2000**, *21*, 12–26.
- Mijatovic, D.; Eijkel, J. C. T.; van den Berg, A. Technologies for Nanofluidic Systems: Top-Down vs. Bottom-Up — A Review. *Lab Chip* **2005**, *5*, 492–500.
- Martinez, A. W.; Phillips, S. T.; Butte, M.; Whitesides, G. M. Patterned Paper as a Platform for Inexpensive, Low Volume, and Portable Bioassays. *Angew. Chem., Int. Ed.* **2007**, *46*, 1318–1320.
- Oswald, T. A.; Turng, L. S.; Gramann, P. *Injection Molding Handbook*, 2nd ed.; Hanser Gardner Publications: Cincinnati, OH, 2007.
- Plotter, V.; Benzler, T.; Gietzelt, T.; Ruprecht, R.; Hausselt, J. *Adv. Eng. Mat.* **2000**, *2*, 639.
- Siegel, A. C.; Bruzewicz, D. A.; Weibel, D. B.; Whitesides, G. M. Microsolidics: Fabrication of Three-Dimensional Metallic Structures using Microfluidics. *Adv. Mater.* **2007**, *19*, 727–733.

- 12 Peterson, D. S. Solid Supports for Micro Analytical Systems. *Lab Chip* **2005**, *5*, 132–139.
- 13 Kim, E.; Xia, Y.; Whitesides, G. M. Polymer Microstructures Formed by Molding in Capillaries. *Nature* **1995**, *376*, 581–584.
- 14 Cheung, Y. K.; Gillette, B. M.; Zhong, M.; Ramcharan, S.; Sia, S. K. Direct Patterning of Composite Biocompatible Microstructures Using Microfluidics. *Lab Chip* **2007**, *7*, 574–579.
- 15 Leng, J.; Salmon, J. B. Microfluidic Crystallization. *Lab Chip* **2009**, *9*, 24–34.
- 16 Whitesides, G. M.; Stroock, A. D. Flexible Methods for Microfluidics. *Phys. Today* **2001**, *54*, 42–48.
- 17 Squires, T. M.; Quake, S. R. Microfluidics: Fluid Physics at the Nanoliter Scale. *Rev. Mod. Phys.* **2005**, *77*, 977–1026.
- 18 Atencia, J.; Beebe, D. J. Controlled Microfluidic Interfaces. *Nature* **2005**, *437*, 648–655.
- 19 Whitesides, G. M.; Ostuni, E. S.; Takayama, S.; Jiang, X.; Ingber, D. E. Soft Lithography in Biology and Biochemistry. *Ann. Rev. Biomed. Eng.* **2001**, *3*, 335–373.
- 20 Hulme, S. E.; Shevkopyas, S. S.; Samuel, A. Microfluidics: Streamlining Discovery in Worm Biology. *Nat. Methods* **2008**, *5*, 589–590.
- 21 Thorsen, T.; Maerkl, S. J.; Quake, S. R. Microfluidic Large-Scale Integration. *Science* **2002**, *298*, 580–584.
- 22 Jeon, N. L.; Dertinger, S. K.; Chiu, D. T.; Choi, I. S.; Stroock, A. D.; Whitesides, G. M. Generation of Solution and Surface Gradients Using Microfluidic Systems. *Langmuir* **2000**, *16*, 8311–8316.
- 23 Stone, H. A.; Stroock, A. D.; Ajdari, A. Engineering Flows in Small Devices. *Annu. Rev. Fluid Mech.* **2004**, *36*, 381–411.
- 24 Chin, C. D.; Linder, V.; Sia, S. K. Lab-on-a-Chip Devices for Global Health. *Lab Chip* **2007**, *7*, 41–57.
- 25 Verpoorte, E.; De Rooij, N. F. Microfluidics Meets MEMS. *Proc. IEEE* **2003**, *91*, 930–953.
- 26 Dickey, M. D.; Chiechi, R. C.; Larsen, R. J.; Weiss, E. A.; Weitz, D. A.; Whitesides, G. M. Eutectic Gallium-Indium (EGaln): A Liquid Metal Alloy for the Formation of Stable Structures in Microchannels at Room Temperature. *Adv. Funct. Mater.* **2008**, *18*, 1097–1104.
- 27 Siegel, A. C.; Shevkopyas, S. S.; Weibel, D. B.; Bruzewicz, D. A.; Martinez, A. W.; Whitesides, G. M. Co-Fabrication of Electromagnets and Microfluidic Systems in Poly(dimethylsiloxane). *Angew. Chem., Int. Ed.* **2006**, *45*, 6877–6882.
- 28 Shevkopyas, S. S.; Siegel, A. C.; Westervelt, R. M.; Prentiss, M. G.; Whitesides, G. M. The Force Acting on a Superparamagnetic Bead Due to an Applied Magnetic Field. *Lab Chip* **2007**, *7*, 1294–1302.
- 29 Wolfe, D. B.; Conroy, R. S.; Garstecki, P.; Mayers, B. T.; Fischbach, M. A.; Paul, K. E.; Prentiss, M.; Whitesides, G. M. Dynamic Control of Liquid-Core/Liquid-Cladding Optical Waveguides. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12434–12438.
- 30 Psaltis, D.; Quake, S. R.; Yang, C. H. Developing Optofluidic Technology Through the Fusion of Microfluidics and Optics. *Nature* **2006**, *442*, 381–386.
- 31 Tang, S. K. Y.; Stan, C. A.; Whitesides, G. M. Dynamically Reconfigurable Liquid-Core Liquid-Cladding Lens in a Microfluidic Channel. *Lab Chip* **2008**, *8*, 395–401.
- 32 Mayers, B. T.; Vezenov, D. V.; Vullev, V. I.; Whitesides, G. M. Arrays and Cascades of Fluorescent Liquid-Liquid Waveguides: Broadband Light Sources for Spectroscopy in Microchannels. *Anal. Chem.* **2005**, *77*, 1310–1316.
- 33 Tang, S. K. Y.; Li, Z.; Abate, A. R.; Agresti, J. J.; Weitz, D. A.; Psaltis, D.; Whitesides, G. M. A Multi-color Fast-Switching Microfluidic Droplet Dye Laser. *Lab Chip* **2009**, *9*, 2767–2771.
- 34 Akkerman, H. B.; De Boer, B. Electrical Conduction through Single Molecules and Self-Assembled Monolayers. *J. Phys.: Condens. Matter* **2008**, *20*, 013001.
- 35 Nijhuis, C. A.; Dickey, M.; Whitesides, G. M. Manuscript in preparation.
- 36 Chiechi, R. C.; Weiss, E. A.; Dickey, M. D.; Whitesides, G. M. Eutectic Gallium-Indium (EGaln): A Moldable Liquid Metal for the Electrical Characterization of Self-Assembled Monolayers (SAMs). *Angew. Chem., Int. Ed.* **2007**, *47*, 142–144.
- 37 Wu, D.; Qin, J.; Lin, B. Electrophoretic Separations on Microfluidic Chips. *J. Chromatogr. A* **2008**, *1184*, 542–559.
- 38 Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Liu, R. H.; Devadoss, C.; Jo, B. H. Functional Hydrogel Structures for Autonomous Flow Control Inside Microfluidic Channels. *Nature* **2000**, *404*, 588–590.
- 39 Lee, N. Y.; Jung, Y. K.; Park, H. G. On-chip Colorimetric Biosensor Based On Polydiacetylene (PDA) Embedded in Photopolymerized Poly(ethylene glycol) Diacrylate (PEG-DA) Hydrogel. *Biochem. Eng. J.* **2006**, *29*, 103–108.
- 40 Gillette, B. M.; Jensen, J. A.; Tang, B.; Yang, G. J.; Bazargan-Lari, A.; Zhong, M.; Sia, S. K. In Situ Collagen Assembly for Integrating Microfabricated Three-Dimensional Cell-Seeded Matrices. *Nat. Mater.* **2008**, *7*, 636–640.
- 41 Choi, N. W.; Cabodi, M.; Held, B.; Gleghorn, J. P.; Bonassar, L. J.; Stroock, A. D. Microfluidic Scaffolds for Tissue Engineering. *Nat. Mater.* **2007**, *6*, 908–915.
- 42 Wong, A. P.; Perez-Castillejos, R.; Love, J. C.; Whitesides, G. M. Partitioning Microfluidic Channels with Hydrogel to Construct Tunable 3-D Cellular Microenvironments. *Biomaterials* **2008**, *29*, 1853–1861.
- 43 Ling, Y.; Rubin, J.; Deng, Y.; Huang, C.; Demirci, U.; Karp, J.; Khademhosseini, A. A Cell-Laden Microfluidic Hydrogel. *Lab Chip* **2007**, *7*, 756–762.
- 44 Bruzewicz, D. A.; McGuigan, A. P.; Whitesides, G. M. Fabrication of a Modular Tissue Construct in a Microfluidic Chip. *Lab Chip* **2008**, *8*, 663–671.
- 45 Shaha, R. K.; Shuma, H. C.; Rowata, A. C.; Leea, D.; Agrestia, J. J.; Utada, A. S.; Chua, L. Y.; Kima, J. W.; Fernandez-Nievesa, A.; Martineza, C. J.; Weitz, D. A. Designer Emulsions Using Microfluidics. *Mater. Today* **2008**, *11*, 18–27.
- 46 Garstecki, P.; Ganan-Calvo, A. M.; Whitesides, G. M. Formation of Bubbles and Droplets in Microfluidic Systems. *Bull. Pol. Acad. Sci.: Tech. Sci.* **2005**, *53*, 361–372.
- 47 Garstecki, P.; Gitlin, I.; DiLuzio, W.; Whitesides, G. M.; Kumacheva, E.; Stone, H. A. Formation of Monodisperse Bubbles in a Microfluidic Flow-Focusing Device. *Appl. Phys. Lett.* **2004**, *85*, 2649–2651.
- 48 Thorsen, T.; Roberts, R. W.; Arnold, F. H.; Quake, S. R. Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device. *Phys. Rev. Lett.* **2001**, *86*, 4163–4166.
- 49 Xu, S. Q.; Nie, Z. H.; Seo, M.; Lewis, P.; Kumacheva, E.; Stone, H. A.; Garstecki, P.; Weibel, D. B.; Gitlin, I.; Whitesides, G. M. Generation of Monodisperse Particles by Using Microfluidics: Control over Size, Shape, and Composition. *Angew. Chem., Int. Ed.* **2005**, *44*, 724–728.
- 50 Hashimoto, M.; Garstecki, P.; Whitesides, G. M. Synthesis of Composite Emulsions and Complex Foams with the Use of Microfluidic Flow-Focusing Devices. *Small* **2007**, *3*, 1792–1802.
- 51 Hashimoto, M.; Mayers, B.; Garstecki, P.; Whitesides, G. M. Flowing Lattices of Bubbles as Tunable, Self-Assembled Diffraction Gratings. *Small* **2006**, *2*, 1292–1298.
- 52 Song, H.; Chen, D. L.; Ismagilov, R. F. Reactions in Droplets in Microfluidic Channels. *Angew. Chem., Int. Ed.* **2006**, *45*, 7336–7356.
- 53 Zheng, B.; Roach, L. S.; Ismagilov, R. F. Screening of Protein Crystallization Conditions on a Microfluidic Chip Using Nanoliter-Size Droplets. *J. Am. Chem. Soc.* **2003**, *125*, 11170–11171.
- 54 Baret, J. C.; Miller, O. J.; Taly, V.; Ryckelynck, M.; El-Harrak, A.; Frenz, L.; Rick, C.; Samuels, M. L.; Hutchison, J. B.; Agresti, J. J.; Link, D. R.; Weitz, D. A.; Griffiths, A. D. Fluorescence-Activated Droplet Sorting (FADS): Efficient Microfluidic Cell Sorting Based on Enzymatic Activity. *Lab Chip* **2009**, *9*, 1850–1858.