Modular LEGO Brick Microfluidics

By

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B.S.E., Mechanical Engineering
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ABSTRACT

Wider use and adaptation of microfluidic systems is hindered by the infrastructure, knowledge, and time required to build prototype devices, especially when multiple fluid operations and measurements are required. As a result, rapid prototyping methods based on planar and three-dimensional printing are attracting interest; however, these techniques cannot produce structures with the resolution, smoothness, and feature size needed for standard microfluidic devices. Herein I present a new approach to rapidly construct modular microfluidic systems by modification and assembly of interlocking injection-molded blocks. I demonstrate this principle using micromilling of store-bought LEGO® bricks to create surface fluidic pathways on bricks, and develop procedures for sealing and interconnecting bricks to form modular, reconfigurable microfluidic systems. Micromilling using a desktop machine achieves channel dimensions of 50 μm in depth and 150 μm in width, or greater, etched into the sidewalls of blocks. Sealing these channels with adhesive films allows internal fluid pressure of at least 400 kPa. The intrinsic tolerances of injection molded bricks and their elastically averaged connections gives mechanical locating repeatability of 1 μm, which enables fluid to pass between bricks via an O-ring with >99.9% sealing reliability. Using the LEGO-based approach, I build systems made of assembled brick units for generating droplets, sensing light, sorting with inertial and magnetic forces, and repeatably positioning a smartphone camera, and characterize their performance. Then, I fabricate and measure LEGO-like bricks made by FDM and SLA three-dimensional printing, showing that they can integrate with injection-molded bricks to add useful function, although their surface quality, resolution, and material limit performance. In addition, I adapt these components for two educational activities for high school students: a colorimetric titration device and a modular designable boat. The standard interface among all bricks enables a wide variety of brick units to be incorporated onto a common platform, making this “lab on a brick” a new and viable platform for advancing research and education in microfluidics.

Thesis Supervisor: Anastasios John Hart
Title: Associate Professor of Mechanical Engineering
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I finally would like to recognize Helen Xu, a high school student who contributed to the titration system for educational use described in Chapter 7, and thank the user on grabcad.com, Daniel Herzberg, who provided the SolidWorks model of a LEGO brick that I have used extensively throughout this work, with slight modifications, for illustration and 3D printing.

I have enjoyed a thrilling first couple of years at MIT, and hope for several more.
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In fluid flow at miniature scales, viscous forces dominate over inertial forces, allowing an exact solution to the then-simplified Navier-Stokes equation, resulting in excellent control and understanding of the fluid motion. The field of study of this flow regime is termed microfluidics, and its related technology is used in a vast variety of fluid-based systems for analysis and research, including commercial inkjet printheads and pregnancy tests (a type of lateral flow test). Other applications include an increasing number of clinical diagnostics and point-of-care medical exams such as for diabetes and lactose intolerance, as well as tools for water testing, genetic testing, chemical synthesis, and more. Microfluidics enables unique and useful tools to perform biological analysis and chemical synthesis with rapid and precise control of concentrations and good measurement sensitivity even for high-throughput procedures. Microfluidics can also be used to understand reaction products and investigate the fundamental science of transport at sub-micron scales.\textsuperscript{1–3}

However, unlike circuit electronics and machine elements that largely use standard components and leverage digital design tools, microfluidic systems have no universally established construction paradigm. The process of designing and fabricating a microfluidic system is compounded by requirements for high precision manufacturing and practical issues (e.g., materials, tight sealing) and, as a result, traditional microfabrication
methods such as chemical etching of microchannels in silicon wafers remain the
dominant method of fabricating microfluidic devices. Indeed, the commercial viability of
many lab-on-a-chip and diagnostic tools has been limited by the high capital cost of
manufacturing such devices, especially at the micrometer-scale and considering stringent
dimensional tolerances required for reliability.\textsuperscript{4,5} Infrastructure alone costs hundreds of
thousands of dollars for cleanroom lithography, and these traditional microfluidic devices
cost $\sim$50-1500 each, in labor and materials, in part because of low production volume
and the need for mostly-manual labor (Table 1-1).

<table>
<thead>
<tr>
<th>Method</th>
<th>PDMS cast from silicon wafer, photolithography</th>
<th>PDMS cast from print\textsuperscript{6}</th>
<th>3D printing (various methods)</th>
<th>Injection molding\textsuperscript{7}</th>
<th>LEGO microfluidics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production time (low volume)</td>
<td>~1 week for master mold</td>
<td>&lt;24 hr</td>
<td>20 min/cm\textsuperscript{3}</td>
<td>Seconds to minutes</td>
<td>3 min/part</td>
</tr>
<tr>
<td>Production time (high volume)</td>
<td>≥1 hr, can be molded in parallel</td>
<td>&quot;--&quot;</td>
<td>No change</td>
<td>Seconds to minutes</td>
<td>3 min/part\textsuperscript{1}</td>
</tr>
<tr>
<td>Cost per part</td>
<td>$50-500</td>
<td>$20</td>
<td>$0.25/cm\textsuperscript{3}</td>
<td>Cents</td>
<td>$0.10</td>
</tr>
<tr>
<td>Infrastructure cost</td>
<td>$&gt;100,000</td>
<td>$&lt;2000</td>
<td>$500</td>
<td>$1k-10k/mold, $50k/machine</td>
<td>$5,000</td>
</tr>
<tr>
<td>Minimum channel size</td>
<td>&lt;1 μm</td>
<td>&lt;20 μm</td>
<td>~500 μm</td>
<td>&lt;1 μm \textsuperscript{8}</td>
<td>~10-100 μm</td>
</tr>
<tr>
<td>Surface roughness</td>
<td>&lt;10 nm</td>
<td>Same as print</td>
<td>0.4-6 μm (also depends on orientation)</td>
<td>&lt;50 nm</td>
<td>~1 μm</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Production rate could be faster at high volume with a higher-performance milling machine and automated load/unload
The adaptation of soft lithography (e.g., replica molding of PDMS) to microfluidics in 1998 catalyzed wider adoption of microfluidics in research because users could design, prototype, and replicate new microfluidic devices using replica casting of polydimethylsiloxane (PDMS) from fabricated silicon or other molds, bringing production time from ~1 week to ~3 hours once a master mold was formed, and providing new material properties via the soft and flexible elastomers. Nevertheless, functional microfluidic systems, and thus the underlying fabrication methods and materials, must still meet a number of strict performance requirements that include small feature size and smooth surfaces, thermal and chemical stability, tight seals and pressure stability, capability for in situ measurements and imaging, and more.

In most cases, microfluidic devices are designed to integrate all necessary functions on a single chip. An alternative approach to building microfluidic systems, which leverages rapid prototyping, is to divide the system into “blocks” that can assemble in many ways to form complete devices (which enables quick changes between biological assays or chemical synthesis by rearranging different unit operations) remain a major area of interest in microfluidics. Modularity offers significant benefits from their capacity to enable rapid prototyping and reconfiguration of system concepts, along with simplifying the development and sharing of new techniques as individual units that would then be integrated easily into other modular systems, relying only on a universal interconnection. These modular systems are especially desirable if they can be made by common machining and laboratory tools, so that any researcher could develop or modify units. In addition, microfluidic components developed and validated within a research team can
be made available for download worldwide.\textsuperscript{2} This would allow future researchers building on the work to use the same system instead of recreating it based on published figures and text, lowering the learning curve, reducing repetition and, as a result, hastening the discovery process.

In addition, modular pieces that perform complex but well-characterized functions, perhaps for field generation or particle sorting, may be added to a device without fully understanding the nuances of their internal processes and be used solely for their output, much as circuit elements are used today. This would enable final devices to be quite complex with incremental effort required only to design and make the new module and verify its performance (Fig 1-1 shows complex systems made from many individual pieces. Few people understand how each piece works but still we can use them for new and useful work).

\textbf{Figure 1-1} Notable modular and standardized systems enabling complex designs. a) Circuit board from a Samsung Galaxy S6 using circuit elements, (b) mechanism of a mechanical watch using standard mechanical components, (c) Google datacenter using standard hardware. Everyone has used one of these, but few people including the assembler can describe how each part fundamentally works.

\textsuperscript{2} We find inspiration from a number of sites that share 3D-printing design files. A new open-source platform for microfluidics design in particular is metafluidics.com, which has two dozen 3D printed component design files (as of Jan 2017).
More recently, researchers have sought to adapt consumer-grade printing technologies for lower-cost manufacturing methods for microfluidics. These include most notably inkjet toner printing, wax printing, other paper-coating methods, and 3D printing.\textsuperscript{11,12}

An important recent success in these areas has been the proliferation of two- and three-dimensional printed designs to produce both monolithic and modular systems,\textsuperscript{3,13–21} with notable prototypes that assemble DNA,\textsuperscript{22} create emulsions,\textsuperscript{3} apply electronics-like operations like resistance and capacitance to control fluid flow,\textsuperscript{13} integrate optical and other sensors directly into printed units,\textsuperscript{23} and generally connect in a simple, reconfigurable manner (Fig. 1-2).\textsuperscript{17,24} In addition, researchers have demonstrated functional microfluidic prototype systems based on laser printing\textsuperscript{25,26} and simple paper methods.\textsuperscript{27,28} Printed systems work well when tailored for a single application with lower resolution requirements, particularly for single-use (disposable) systems for field applications as they may cost under $1 per part.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure12.png}
\caption{Notable modular microfluidic systems, from left to right: 3D-printed pieces able to make a variety of topologies;\textsuperscript{3} plastic pieces mounted on Legos for multi-part lateral flow diagnostics in the developing world;\textsuperscript{3} and a mold for PDMS-based puzzle pieces.\textsuperscript{29}}
\end{figure}

\textsuperscript{3} From the MIT media lab, http://littledevices.org/
However, the use of 2D and 3D printing methods to make general microfluidic devices is restricted by universal limitations in the current technology in material choice and minimum feature dimensions. Polymer 3D printers use either a thermoplastic (as in fused deposition modeling), or a photopolymerizable resin (as in stereolithography), for which a handful of translucent resins are available, though their chemical makeup is proprietary (e.g., Clear resin from Formlabs; Watershed from DSM Somos). The dimensional resolution is restricted by layer height, typically 50-150μm, surfaces are rough, and printed materials may suffer from uncertain and poor long-term dimensional stability (particularly when in contact with a fluid) (Table 1-1). Modularity places additional stringent requirements on accuracy, repeatability, interchangeability — essential criteria to enable rapid construction of systems from a component library —, and for maintenance of tight sealing performance between modules, particularly if the system is reconfigurable. When these requirements are met, modularity is attractive because it enables a microfluidic device to be fabricated rapidly, benefiting prototyping iterations, research speed, and fabrication.

In terms of manufacturing volume, another technique, injection molding, sits at the opposite end of the spectrum from 3D printing. With a production speed of seconds per part, it can produce very smooth surfaces (<50 nm surface roughness), employs thermally and chemically stable polymers (common thermoplastics), and provides excellent dimensional control (<50 μm tolerances) (Table 1-1). However, the high tooling cost makes injection molding prohibitive except for production of a large number of identical units.
In this thesis, I present the use of store-bought LEGO® bricks, arguably the world’s most famous and widely accessible toy, as a high-precision and low-cost platform for building modular microfluidic devices and systems. This hybrid approach leverages the materials and dimensional quality of standard injection molded units, with custom modifications enabling deployment of a library of bricks with both fluidic and active (field-applying, electronic) functions. LEGO-based microfluidic bricks can be fabricated using a desktop micromill and widely accessible laboratory tools, assembled and interchanged with ease, and interfaced with electronics and sensors for prototyping microfluidic systems. In Chapter 2, I demonstrate micron-scale repeatability and very close dimensional tolerances of LEGO bricks, machining processes to fabricate the microfluidic system, sealing and surface modification. In Chapter 3, I discuss flow and sealing performance. In Chapter 4, I demonstrate two functional applications: mixing of liquids in laminar flow and controlled generation of water-in-oil droplets, as well as integration of a brick-mounted optical sensor to monitor both functions. In Chapter 5, I compare geometry and material properties of micromachined LEGO bricks with those of 3D-printed components. In Chapter 6, I discuss initial demonstrations of systems with high school programs. And in Chapter 7, I consider the most applicable future directions and discuss benefits and current limitations of Lego microfluidics.
1.1 Concept

LEGO-based microfluidic devices were created by modifying as-purchased LEGO bricks to include surface-machined microfluidic pathways. Systems were built by assembling these modified bricks ('fluidic bricks') on a standard LEGO baseplate (Fig. 2-1). Microfluidic channels with rectangular cross-sections were created by micromilling into bricks made of ABS (opaque/colored) or polycarbonate (clear/translucent) plastic. Individual bricks were machined with arbitrary 2D pathways, with width and depth determined by the tool diameter and cutting path. By this approach and systems could be created by machining a complete device pathway in a single fluidic brick, or by assembling multiple fluidic bricks along with inlet/outlet bricks connected to tubing.

After milling, each fluidic brick was sealed by applying a strip of adhesive polyethylene film with apertures located at the desired ends of the milled channels (Fig 2-1c). The apertures were sized such that capillary pressure retained water inside when a brick was pulled from a system and apertures were exposed to air. Reversible contact-based sealing between bricks was achieved using miniature O-rings placed in micromilled grooves, which allow fluid to pass between bricks without leaking. The dimensional tolerances of LEGO bricks, and the ensuing repeatability and interchangeability of
mounting, resulted in O-rings forming reliable and reversible sealing between adjacent bricks on contact, without additional steps or hardware.

Among many candidate methods for creating channels in bricks such as laser ablation and hot embossing, micromilling was chosen because of its attractive combination of accessibility, versatility, speed, and dimensional quality (Table 2-1). Exploration of alternate fabrication methods is discussed in Appendix A. For this work, a
three-axis desktop micromill (Roland SRM-20, ~$5,000 as of Spring 2016; stepper-driven axes with XYZ resolution of 1 μm, max spindle speed of 9500 revolutions per minute, 50 W power consumption according to [rolanddga.com]) was employed along with carbide end mills (Performance Micro Tool carbide endmills, 2 flutes, diameter 0.0040"-0.0200"). Milling parameters were suggested by software (GWizard, CNCCookbook), and refined by experiments to reduce tool breakage and surface roughness. Typical cutting parameters are listed in Table 2-2, with 9500 revolutions per minute and 2-flute endmills. Climb milling was preferred to improve surface texture. Fluidic bricks took 1 to 40 minutes to fabricate, depending on geometry, number of faces milled, and channel dimensions, though the typical speed for bricks presented in this chapter was 1 to 10 minutes per brick.

**Table 2-1** Alternate methods to create channels were compared qualitatively. Micromilling was selected for its combination of speed, precision, and serial nature.

<table>
<thead>
<tr>
<th>Poor</th>
<th>Good</th>
<th>Great</th>
<th>Laser ablation</th>
<th>Hot embossing</th>
<th>Milling</th>
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<tr>
<td>Ability to customize each piece</td>
<td><img src="image" alt="Circle" /></td>
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</table>

Bricks were held in place using a LEGO fixture attached to the table of the micromill with double-sided tape; this was possible because of the low cutting forces of micromilling (<5 N). The repeatability of brick mounting made it easy to rotate and reposition blocks to mill on different faces, allowing a 2.5-dimensional structure with corners that lined up well between different faces (Fig. 2-2).
a) Milling fixture

Figure 2-2 Micromilling fixture using LEGO bricks. (a, b) A bottom layer of LEGO bricks was adhered via double-sided tape to the micromill table; a middle layer was included to meet height requirements of the spindle and reduce the necessary cantilevered length of the endmill; and a third layer held fixtured bricks to be milled (b). Each layer had three rows of 3-4 bricks for stability, and one or more large plate of LEGOs holding the entire fixture together on both the top and bottom surface. (c) For geometries that required connected features on more than one face (i.e., all of them presented in this thesis), bricks were rotated around the corner of interest, into the adjacent spot on the fixture, instead of being rotated in place, in order to reduce the effect of any angular misalignment of the fixture or mill. For example, the pictured brick would be milled sequentially in spots labeled 1, 2, and 3 to modify each side (d, e) This resulted in well-aligned corner cuts on the shown advective mixing unit.4

The programmed trajectory of the machine removed material in sequential passes with rectangular cross-sections (when using a square endmill), creating microchannels with in-plane trajectories specified by a CAD drawing (Fig. 2-3a-c,e). With this setup the mill successfully machined channels with final widths as small as \( \approx 150 \, \mu m \) and depths tunable from 50-1,000 \( \mu m \); machined corners had radii of \( \approx 5-10 \, \mu m \) and average surface

---

4 Misaligned corners should improve the mixing ability of the shown brick by introducing more intense geometrical changes, but it is desirable to reduce misalignment where it is due purely to error.
roughness of 0.90 μm as constrained by the motor on the micromill (Fig 2-3d-g; Table 2-3, on the low end for micromilling\(^3\)). In particular, the runout of the spindle (~5 μm), measured with a dial gauge, limited the smallest tool that could be used without breaking, and the spindle rotation speed limited the smallest well-made cut. To make a smooth feature, one has to move the endmill along the surface fast enough to avoid heat buildup in one area, because when heated these thermoplastics will soften and deform, leading to low-quality features. However, a high chip load (material removal rate per flute per revolution) causes greater forces on the tool, which lead to deflection and breakage. Thus, spindle rotation speed limits the cutting diameter of the endmill (Fig 2-3c, rightmost). The mill could also produce standing walls with width down to 20 μm and aspect ratio greater than 5.

**Table 2-2** Typical cutting parameters used in micromilling here, for 9500 revolutions per minute and 2 flutes: tool diameter, feed, depth of cut material removal rate (MRR), cutting velocity (Vc), and chip load (feed per cut).

<table>
<thead>
<tr>
<th>Tool diameter (in)</th>
<th>Feed (mm/min)</th>
<th>Depth (mm)</th>
<th>MRR (mm(^3)/min)</th>
<th>Vc (mm/min) (m/s)</th>
<th>Chip load (mm(^3)/rev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS</td>
<td>PC</td>
<td>ABS</td>
<td>PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0040</td>
<td>0.102</td>
<td>50</td>
<td>30</td>
<td>0.03</td>
<td>0.2</td>
</tr>
<tr>
<td>0.0070</td>
<td>0.178</td>
<td>90</td>
<td>60</td>
<td>0.04</td>
<td>0.6</td>
</tr>
<tr>
<td>0.0100</td>
<td>0.254</td>
<td>120</td>
<td>90</td>
<td>0.06</td>
<td>1.8</td>
</tr>
<tr>
<td>0.0190</td>
<td>0.483</td>
<td>270</td>
<td>190</td>
<td>0.12</td>
<td>15.6</td>
</tr>
</tbody>
</table>

A scanning electron micrograph shows that the bottom of the as-milled channel surface has circular corrugations (Fig 2-3e) and a laser-interferometry scan (Keyence VK-X) shows sidewalls in a brick imaged at an angle (Fig 2-3f). Native LEGO bricks had average roughness of 38 nm for polycarbonate bricks and 78 nm for ABS bricks on the injection-molded sidewalls, measured with a stylus profilometer (2 μm radius stylus,
I expected that roughness of the milled surfaces would be inversely proportional to feed rate, though measurements showed that bricks (milled with a 0.0200" diameter endmill) had only a slight dependence on milling parameters, increasing when feed rate was excessive (Fig 2-3g).

Figure 2-3 Micromilling of channels into the faces of as-purchased ABS LEGO bricks with (a) a micromill that cuts channels of (b) 10-1000μm in depth and (c) 150-500μm in width with a square profile. (d) A comparison of endmill size to channel size shows good agreement for endmills wider than 200 μm, with the dotted line indicating a channel width equal to the endmill diameter (at cutting parameters noted in Table 2-2); a mill with a higher spindle speed would be expected to perform better at smaller dimensions. (e,f) A scanning electron micrograph and laser-scanning image show circular corrugations left by the endmill, where in (f) the blue section is the bottom of the channel with the brick imaged at an angle. (g) Surface roughness was measured as a function of milling feed rate.
The ability to work within this dimensional range enables fabrication of planar microfluidic pathways for mixing and controlled droplet generation as well as for processes that benefit from larger dimensions and nonplanar geometries including advective mixing, filtration, and manipulation of inertial effects to generate secondary flows for particle separation. This size scale met our requirements for demonstration; however, other mills could create even smaller features with greater precision. For instance, high-performance micromilling has been shown to create features as small as 20 μm in width and below and much smaller depth with accuracy as tight as 100 nm. \(^5\) Other techniques including laser ablation and hot embossing could be used to create even smaller channels and/or complex surface textures on the brick surfaces.

The milled channels were covered with a thin film of polyethylene with acrylic adhesive (110μm thickness, ThermalSeal) underlaid with cyanoacrylate adhesive (Krazy Glue KG925) brushed around the edges to anchor the film. The film was pierced with the corner of a standard razor blade at fluid inlet points to make apertures using divots milled into the bricks to guide the razor. A laser-cutter likewise could create the apertures. This process took <1 min (Appendix C).

I chose to mill on sidewalls and connect bricks face-to-face in plane rather than vertically in a stack primarily because of the marks left by injection molding (Fig. 2-4). Specifically, (1) ejector pin marks on the bottom surface formed large indents of >300μm change in depth, which would require extra steps to bypass (Fig. 2-4c,g), (2) a draft angle of ~0.5° widened the brick 50 μm over a 9.54 mm height, and (3) bottom surfaces were both rougher than sidewalls and deflected a greater amount by attachment to other bricks.

In addition, a decoupling of the attachment region from the fluidic region was desirable to prevent one from influencing the other. Milling entirely internal features, such as through the brick, was not possible because LEGO bricks have reasonably thin walls (1.4-1.5 mm).

![Image of LEGO brick imperfections](image)

**Figure 2-4** Native imperfections in LEGO bricks due to injection molding. On an ABS-molded brick (a-c) or polycarbonate brick (d-h), one may observe (a, f) the gate on the top of a central post, where molten plastic first enters the injection mold, (b) a thin-walled cross-section with constant thickness including 10 little bars at inside contact points with the posts, and (c, g) semicircular ejector pin marks on the lower surface. In addition, (e) text identifying the manufacturer and mold ID are present on the inner top surface and (h) thin weld lines marked in black are present on the end of the brick furthest from the gate, where segments of cooling molten plastic fused during mold filling.

I explored three methods to treat the surface of milled bricks to alter their roughness, wettability, and solvent resistance. First, while milled microfluidic features are relatively smooth compared to machining processes, lithography-based methods often achieve roughness \(<10\) nm, and roughness will reduce flow efficiency and control,\(^{31}\) and so I investigated a method of solvent treatment to smooth features. ABS plastic is soluble
in acetone. I flowed a stream of acetone through a milled brick to soften and smooth the channels, and found it to be successful (Fig. 2-5), though the acetone could be diluted or flowed more rapidly to precisely smooth the walls.

**Figure 2-5** Acetone smoothing of micromilled channels in bricks. (a) A 500 μm channel was milled into an ABS LEGO brick, and then acetone, in which ABS is soluble, was flowed through the system for 10 seconds, resulting in a smooth channel (b) with a rounded profile (c) that previously had been rectangular as in Fig 2-3b. Controlled vaporization of acetone onto the surface, such as by suspending a brick over a heated flask (with appropriate safety precautions) may achieve controlled smoothing without this excessive deformation.

It is often desirable to change the wettability of a surface made for microfluidics, such as to control the behavior of emulsions, or for separations. Polycarbonate is also soluble in acetone. In this case, however, as Cui, et al. first noted, PC recrystallizes as acetone evaporates after first dissolving its surface. The submersion time in acetone can be controlled to generate micro- and nano-scale features (as in Fig. 2-4c), rendering the surface superhydrophobic after a 5-minute treatment using acetone and air. Polycarbonate LEGOs were able to undergo this change; however, large-scale cracks along the surface always formed, making them unable to create a smooth surface. It also rendered them cloudy-white, and expanded the brick widths ~100 μm per treated side. Still, this process remains an option to modify brick wettability over small regions.
To create a solvent-resistant barrier, I coated bricks with a 4 μm layer of Parylene-C (Di-chloro-di-p-xylene; Galentis S.P.A.), which is transparent and used to coat implanted medical devices that hold electronics because it forms a resistant, nonporous barrier to water and a wide range of organic solvents. This coating successfully protected bricks from a variety of organic solvents which did discolor and scar regular bricks (acetonitrile, dimethyl sulfoxide, tetrahydrofuran, toluene, dichloromethane, N,N-Diisopropylethylamine, hexanes, and dimethylformamide).

Figure 2-6 Surface structure change of polycarbonate due to acetone. (a) Initial brick surface before milling. (b) Top view of milled channel after soaking the brick in acetone, showing features that indicate some material dissolved and rounded. (c) Closer view of another segment, showing the features that change the wetting – small-scale recrystallized bodies.

Figure 2-7 Parylene-C coating on bricks (a) which provides a transparent, solvent-resistant barrier. (b) Coated bricks were unaffected by various organic solvents until the coating was locally scraped off; then, deformation and discoloration were contained in the de-coated areas (i.e., did not leak below the coating).
1.2 Repeatability and gap size

Modular microfluidic systems require the building units to have tight dimensional tolerances and mounting interfaces that impart highly repeatable positioning. This depends on how the consistency of the fabrication process, and the consistency of the attachment. For a system with mostly horizontal flow interconnection (such as this one), these values depend on the variation in width among different bricks, \( \sigma_b \), and the repeatability of mounting of any single brick, \( R_b \), which I define as one standard deviation of the position of a brick edge as a brick is repeatedly removed and reattached to a baseplate. Assuming the respective variances are independent and normally distributed, I define the total interchangeability \( I \) of a brick to be

\[
I = \sqrt{R_b^2 + \sigma_b^2}.
\]

As-received bricks and modified bricks were measured to have \( I = 7-25 \ \mu\text{m} \), depending on brick type and material (Fig. 2-10f). This is imparted by the consistent dimensions of injection-molded LEGO bricks, along with the elastically averaged interface which involves contact between regularly spaced top cylindrical posts, and a bottom rectangular web. When assembled, the mechanical interference between these features causes frictional and elastic forces that hold the bricks together (Fig 2-7a). Two bricks can attach to the same larger brick (e.g., a baseplate) without interference because the brick outer dimensions guarantee a small \((\sim 100 \ \mu\text{m})\) but uniform gap between adjacent sidewalls.\(^6\) Bricks expand elastically when mounted due to the stress exerted by posts on the baseplate, but not enough to completely fill this gap \((<50 \ \mu\text{m}, \text{Fig } 2-7b)\); in fact, the

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\(^6\) The LEGO Group could have chosen to make well-fitting bricks by using a more compliant material that would deform to reduce interference, which would result in poor repeatability on the micron scale. Lucky for us, they decided to use precision and stiffer materials instead.
use of bricks for the milling fixture self-compensates for this deformation during milling. If bricks were milled when held in a vice instead, both this small expansion and the attributes of having been injection molded (the draft angle on sidewalls or gate (bump) on one post) would result in less accurate channel geometries.

![Figure 2-7](image)

**Figure 2-7** Attachment mechanics of LEGO bricks on LEGO baseplates. (a) Bricks attach when a series of posts make contact with the inside of a second brick, exerting force on post and brick walls. (b) Finite element model of a mounted brick showing the resulting deformation (exaggerated scale running from cyan (highest) to blue (none)). Magenta arrows locate the applied force and green objects indicate fixed surfaces. The true scale of maximum deflection is <50 µm per side as measured with a micrometer. The force exerted on the outer walls of the brick by a post was estimated as 10 N using a Hertzian contact stress analysis based on the deformed area of a post attached to a transparent brick. (c) Photograph of two bricks cut in half and attached together, showing both a uniform thickness cross-section and points of contact. (d) Locations of contact points for the five most common bricks, shown on the top (light gray) and bottom (dark gray) brick surfaces, and once for each type of post attachment. Note: (d) was styled after a nicely illustrative figure in.

When multiple posts fit into multiple webs, the compliant posts each deform slightly, causing an elastic averaging of position that (in its ideal form) reduces the error in position; the theory of elastic averaging suggests the brick-brick repeatability should scale as \( c \cdot \frac{r}{\sqrt{n}} \) for a constant \( c \geq 1 \), surface roughness \( r \) and \( n \) contact points (where here bricks have multiple (3-4) contact points per post (Fig. 2-3).
roughness of <0.1 μm for injection-molded LEGO bricks, theory estimates the repeatability to be of the same order of magnitude.

Thus, to guide design of the microfluidic system and understand the dimensional limits of performance, I measured the gap spacing and repeatability of LEGO bricks on a baseplate. The size distribution of LEGO bricks was measured using a digital micrometer (Mitutoyo IP65, resolution 0.001 mm). The total brick width at mid-height was measured, and was used to determine the size distribution of the narrow gaps that exist between bricks on a baseplate by comparing the brick width to the average distance from the end of one post to the beginning of the next, or post pitch, which was calculated from measurements with each of 179 bricks (Fig 2-5a).

Figure 2-8 Gap spacing and repeatability measurement experimental setup. (a) The distance x was measured between two bricks side-by-side using optical imaging and processing for (b) a series of trials. In each trial, the brick was taken off and replaced on the baseplate. (c) The number of such trials necessary for a measurement was determined as the point when variation in repeatability was within the measurement error, typically at least 20 trials. (d) A second set of experiments was performed for the bricks with an additional top plate. (e) Values identified for gap spacing measurements.
For a baseplate pitch of $P$, assumed to be perfectly regular, and gap spacing $g$, the total width, $W$, of a brick that is $N$ posts wide in that direction must be (Fig 2-8e)

$$W = NP - g.$$ 

Measurements for all bricks were compiled to determine $P = 7.988$ mm, and then $g$ was determined for each individual brick size. The measurement was done in this way in order to decouple the gap spacing measurement from the brick repeatability. While the posts are not a perfect datum reference, post position was regular because it is one of the primary features required for bricks to function appropriately. These values were confirmed by microscope measurements of bricks placed side-by-side on a baseplate (as in Fig 2-8a), and by the success of later experiments that relied on these values.

The gap spacing between bricks averaged 177 μm with a standard deviation of 25 μm for all LEGO considered together. Distribution varied with particular brick size, with 2-post bricks having the minimum variation of 6 μm and 8-post bricks having the maximum of 25 μm (Fig 2-6a-c). There was no strong correlation with brick size, however, and so variation is more likely due to differences in parameters used by The Lego Group in molding each type of brick.

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7 This somewhat strange value is corroborated by a rumor that LEGO's original molds were not made to the correct size, as they had specified 8 mm, but because of their commitment to quality, once they discovered the error they continued to produce bricks with this pitch so that the earliest bricks would fit together with later bricks.
Figure 2-9  Gap width between mounted LEGO bricks. (a) Average gap size for various bricks and (b, c) distribution of brick-brick gap between pairs of randomly selected 1x2-post and 2x2-post bricks. Error bars show 1σ of measurements.

Repeatability of mounting LEGO bricks on a LEGO baseplate was measured by placing randomly selected pairs of same-size bricks onto a baseplate, focusing on the edges of the two bricks in the center, and measuring position of one untouched brick relative to the second one, while the second one was detached and re-attached in the same location between images (Zeiss Z1m; see Fig. 2-8). Images were analyzed (with AxioVision software) to determine the repeatability of position of the edge of the moved brick relative to the edge of the stationary brick, averaging the distance along a ~2 mm length of brick edge (Fig 2-8). The software took an image and processed it based on color to locate the gap and edges of the gap. All images were inspected visually for quality. The program then calculated the average width of the identified gap with a user-specified formula to divide the area by the micrograph's width (i.e., considering it to be a parallelogram, \( \text{area} = \text{width} \times \text{height} \); the area is found by processing, and the width is set by the image width which sets one pair of borders).
Figure 2-10  Gap spacing measurements with computer program AxioVision. A screenshot from the image shows the gap between bricks highlighted in red.

A minimum measurement repeatability, or noise level from pixel and focus resolution, of ~0.5 μm was determined from a set of control experiments in which the brick assembly was re-imaged but bricks were not disassembled. This minimum resolution depended on the contrast between the gap and the bricks. This was affected by brick color being less distinguishable from the gap for darker bricks, and is indicated by the error bar for each measurement set. All measurements were performed by the author.

Repeatability improved with increasing brick size (number of posts) as expected from elastic averaging theory. In addition, when the pairs of bricks were also constrained on the top using a third brick that spanned the gap (essentially doubling the number of engaged posts per brick; see Fig. 2-8d), repeatability dropped below 1.4 μm (Fig 2-5, 2-6a). Thus, any brick with 2 or more posts that had both top and bottom constraints, or any 2x2-post brick, was considered well-placed when designing microfluidic systems as these were roughly equivalent to the best repeatability regular bricks could achieve.

A fit of the repeatability data to the ideal model showed good agreement, with equations of \( R_d = 3.9/\sqrt{N} \) and \( 7.1/\sqrt{n} \), respectively (Fig. 2-11d,e), for \( N \) posts or \( n \) contact.
points, as before. The data and fit are quite similar. Still, there are useful insights from the comparison. Considering trends based on \( n \) rather than \( N \) distinguishes, for instance, square bricks with 2x2 posts from long bricks with 1x4 posts. Square 2x2 bricks have 3 contact points per post due to geometry and the long 1x4 bricks have 4, giving slightly superior repeatability. The direction of contacts affected the repeatability when it was measured for bricks on top of a thermoformed LEGO baseplate which was more flexible than the injection-molded plates (Fig. 2-12).

Fluidic bricks with O-rings and sealing film had repeatability of 1.6 \( \mu \)m and 1 \( \mu \)m, measured without and with a top brick constraint on the fluidic brick and connected to the stationary brick, respectively. This is improved over the unmodified bricks of the same size, suggesting that the O-ring provided a further elastically averaged constraint. The distribution of brick dimensions and repeatability for populations of different brick styles helped us to develop quantitative guidelines for selection of brick styles and to understand the dimensional requirements for robust sealing (Fig. 2-6f): to maximize consistency and enable prototyping of compact systems, I selected 1x2 and 2x2 brick sizes with the same color (2x2 – white ABS, 1x2 – transparent PC) for much of the following work.
**Figure 2-11** Mounting repeatability of LEGO bricks. (a) Micron-scale average mounting repeatability values for multiple LEGO brick sizes on a baseplate and with an additional top plate constraining position and distributions of edge position when removing and replacing one brick from the baseplate for (b) 1x2-post and (c) 2x2-post bricks. (d,e) Repeatability from (a) plotted against number of connections, or points of contact between brick and baseplate where interference occurs (n; 3-4 per post) or number of posts engaged including top and bottom (N). Dotted lines show a fitted equation for repeatability for each set. (f) Interchangeability of bricks calculated as the quadrature sum of width distribution and repeatability including a top plate.

**Figure 2-12** Initial measurements of brick repeatability on a thermoformed baseplate indicated a much worse repeatability for bricks of all sizes due to the flexibility of the plate. In addition, as for each N value there are multiple plotted points, there was a significant difference in stiffness in the different orientations of the brick of the same size, perhaps locally restraining the baseplate, which changed the repeatability.
In theory, the lower limit for repeatability is the surface roughness (Ra = 40 nm for LEGO brick sidewalls, 1.2 μm for the bottom surface). To explain the deviations from this value for 2x4-post bricks (Fig 2-6a), I located the maximum stable angular error for a brick, where one side of the brick made solid contact with the baseplate and the position was first stable to inversion (Fig 2-7b). Before the stable point, the brick would attempt to push away from the baseplate (the elastic restoring force likely being greater than the frictional holding force at that point). The resulting stable position error on the side of the brick increases with brick size. The force to entirely assemble bricks together also increases with brick size at a rate of 2 Newtons per post (Fig 2-7a), so that it becomes increasingly difficult to assemble larger bricks with a firm, level attachment by hand.

Figure 2-13 Measurements of force to assemble bricks and possible abbe error affecting repeatability. (a) Vertical force required to assemble one brick on top of another was measured with a Flexiforce sensor (Tekscan) and Arduino microcontroller. Aspect ratio refers to the ratio of posts/brick in one direction versus the other. (b) Measurements of θ were performed with an optical microscope, and L was measured with a micrometer to calculate the expected error mid-height L away from the hinge point, and Error = L(1-cos θ). This may help explain why repeatability does not strictly decrease with brick size. (c) Exaggerated illustration of Abbe error stackup with many bricks.
The dimensional variation of brick size and mounting guided our design of the O-ring sealing interface between fluidic bricks (Fig 2-8). O-rings (size 001-1/2, 1/8" outer diameter, EPDM rubber, McMaster-Carr) were seated in milled circular grooves such that the O-ring protruded into the gap with sufficient margin to form a seal upon contact with the adjacent brick. Therefore, the reliability of sealing depends on the space between the surfaces that constrain the O-ring; the total distance between brick faces includes the native gap and milled hole, minus one layer of plastic film (Fig 2-8a). Using a screw-driven compression fixture (Fig 2-15) I measured the maximum fluid pressure that the O-ring seal could hold, versus the degree of compression. The O-ring sealed when compression was high enough to seal against the brick surface (10% compression, or 100 μm in a 1018 μm-thick O-ring\(^8\)), and when compression was low enough that the O-ring was not squeezed out of its seat when bricks were assembled (50% compression). As a result, I designed the groove depth to give nominal compression of 30% of the O-ring thickness and predict based on the gap statistics that >99.9% of brick assemblies (4.5σ) will seal until either the O-ring material fails or it is dislodged by fluid pressure, at a pressure of 5-25 MPa for the O-ring used here, based on data from an O-ring handbook (Fig 2-8b).\(^3\) When bricks were assembled, I never observed leakage at fluid pressures lower than 200 kPa, at which pressure the sealing film occasionally delaminated.

The sealing capacity was calculated from values provided in an O-ring handbook\(^3\) for compression force on Shore A – 70 durometer materials and material failure limits (Fig 2-14c), indicating pressure capacity of at least 5 MPa for the minimum compression of 10%. From holding force requirements, I calculated the fluid pressure that would be

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\(^8\) I opted to mill the O-ring groove with a smaller endmill to reduce the mean length of machining marks and lower the minimum compression required for sealing.\(^6\)
required to counteract that force over the area of the O-ring (using the maximum possible contact area as a conservative estimate). The handbook does not provide calculated groove dimensions for face seals for 001-1/2 (or below 004) so calculations of groove depth were done by hand and checked by experiment where possible. In this case, the gap variation in only one dimension was important, as the inner diameter of the O-ring is large enough to absorb much greater (~0.5 mm) misalignment of the bricks parallel to the wall holding the O-ring without breaking the seal.

Figure 2-14 Design of the O-ring seat in a milled brick. (a) Components of the O-ring seat that influence sealing capabilities (b) by influencing the percentage compression of the O-ring, which is controlled to increase probability of sealing at the O-ring, calculated as the area under the curve of regions beyond the lower and upper sealing thresholds, with an O-ring seat variation of $\sigma = 45 \mu m$. (c) Sealing capacity as a function of O-ring compression (only using data and equations from 37). The left axis indicates the fluid pressure required to dislodge the O-ring normal to the sealing face, with values calculated based on handbook values for the holding load required for a given compression value (and Pressure = (Holding force)/(O-ring projected area)). Red lines indicate the fluid pressure at which the O-ring seal will fail by material extrusion into the gap, which depends on material, gap size, and fluid pressure, not compression.
O-ring compression fixture to experimentally test. This device was used to vary the spacing between bricks, connected by an O-ring, to calculate sealing pressure as a function of O-ring compression. A liquid pressure dial gauge (McMaster-Carr #4026K3) was attached between the right brick and a syringe, and the left brick was closed. Leakage pressure was identified as the maximum reading on the pressure gauge, after which the pressure decreased and fluid emerged.

Alternative options to O-rings considered to seal between bricks were other sizes and materials of O-ring, short segments of Tygon tubing as used in inlet bricks, and thin layers of punctured PDMS or gasket material. The compressibility of materials was particularly important—humans can exert an average force of 36 N (8 lbf) with a pinching motion, so material had to be soft enough to deform the requisite amount at these lower forces (as a result stiff Buna-N O-rings were rejected), and sufficiently stiff to retain shape in the gap, (and thus soft Silicone O-rings were rejected). Calculations indicated that a thin layer of PDMS could feasibly fill the gap and suitably deform. However, layers of PDMS spin-coated to the desired thicknesses (200-400 μm) proved too fragile and sticky and were found to tear and delaminate (due to compression-induced shear stress) when placing bricks together. EPDM rubber was selected as it had the best rated resistance to the chosen working fluids (water and silicone oil). For greater chemical compatibility, O-ring materials including Kalrez, PTFE, and FEP would be suitable; however, the O-ring seat would need to be redesigned to accommodate the higher stiffness of these materials.

This design enables sealing between bricks by simply placing one next to another.
on a baseplate, without additional steps or hardware, so that building a system is as simple and as quick as building something with unmodified LEGO bricks. Upon attachment, the O-ring is compressed, forming the seal. The compression force is small, making assembly easy, but the area of compression is also small because the O-ring is miniature, enabling sealing to high pressures.

Permanent sealing of the bricks such as by solvent or thermal welding of a plastic cover instead of the adhesive film on the surface should give a higher pressure capacity. In solvent welding using acetone, a thin layer of ABS or polycarbonate was able to seal one surface of bricks, but tended to fracture when I attempted to seal a channel around a corner.
CHAPTER 3

FLOW CHARACTERIZATION

In order to enable modular system design, it is necessary to know how channel dimensions and other characteristics of fluidic bricks influence their flow behavior. The flow resistance, defined as $\Delta P/Q$ for a pressure drop $\Delta P$ and volumetric flow rate $Q$, provides an engineering metric to characterize the flow resistance in the brick modules. To this end, a simple system was assembled, comprising inlet and outlet bricks and a series of fluidic bricks with straight channels of the same cross-section. This allowed us to investigate the relationship between resistance and path length, and to remove/replace bricks so we could assess the repeatability and interchangeability of flow characteristics.

A resistor model was developed for the system, where the flow was initially assumed to be viscous, pressure-driven, and laminar, with fluid resistance being proportional to $R \sim \Delta P h^4 / Q L$, for a hydraulic diameter $h$, length $L$, and viscosity $\mu$. This model was used to guide the selection of dimensions of ancillary fluidic paths including tubing, inlets, and outlets so that the greatest pressure drop would occur within the fluidic bricks (Fig 3-1a).

The inlet/outlet brick was created by milling 1/8" through-holes concentric with brick posts on a 1x2 brick, and press-fitting 1/8" outer diameter elastomer tubing into the hole (Tygon, McMaster-Carr #5103K42). Cyanoacrylate adhesive (Krazy Glue KG925) was optionally used to form a permanent seal between the tubing and hole. An O-ring seal
and small through-hole were milled into the body of the brick to enable sealing with fluidic bricks. All connections between bricks occurred through these holes cut on the smooth sides of the bricks.

Pressure was measured directly upstream of the LEGO fluid system using a liquid pressure dial gauge (McMaster-Carr #4026K3), when water was pumped through the system using a syringe pump (NE-300, Pump Systems Inc.). Final leaking pressure was measured by inletting a constant flow of water, plugging the outlet, and recording the pressure at which the system first began to leak.

The pressure to drive fluid through bricks was linearly related to flow rate, consistent with Poiseuille flow in a rectangular channel (Fig. 4a), where \( \Delta P = \frac{12 \mu L}{w h^3} Q \) for an incompressible, Newtonian fluid. Fluidic bricks with a channel cross section of 500 \( \mu m \times 500 \mu m \) had a resistance of 730 Pa-s/mL, adding linearly with each brick in series (Fig 5a).

This value was consistent within resolution of the pressure gauge (63 Pa) when bricks were repositioned, and consistent within 135 Pa/(mL/s) when a brick was refurbished. For refurbishing the tape was removed from a brick, and the brick was cleaned thoroughly with isopropyl alcohol, dried, covered with new sealing film, received a new O-ring, and reinserted. By design, inlet and outlet bricks had fluid resistance of <1% of the fluidic bricks, and both the corner bend and O-ring seat contributed negligibly (<5%) to the total pressure drop (Fig 3-1a). Finally, the effects of aging of a brick was considered; the flow resistance was measured and then the brick was allowed to sit, filled with water. When remeasured after three weeks, average flow resistance had diminished by 24%, perhaps due to partial delamination and bulging of the sealing film under
pressure, as suggested by the nonlinear (decreasing) flow resistance with increasing pressure (Fig 3-3).

**Figure 3-1** Pressure drop for water flow through LEGO microfluidic brick series. (a) Resistor model of brick system depicting contributions to flow resistance from each component. (b) Pressure drop for a series of fluidic bricks increases linearly with flow rate, where “build” refers to a brick refurbishment which includes replacing the sealing film and O-ring and cleaning brick surfaces with isopropyl alcohol. It is compared with the pressure drop for two bricks in series which has a doubled value, as expected. “All builds” refers to an averaged value. Error bars indicate standard deviation in pressure drop when bricks were disassembled and reassembled without rebuilding between tests, and are within the noise of the pressure gauge. (c) A system size limit due to pressure capacity (415 kPa) is calculated based on flow rate and hydraulic diameter of the channel for a square channel cross-section, using a resistor model based on Hagen-Poiseuille flow. For example, the region using 4-8 units is highlighted in green. Arrows to the right of the 10 mL/min line indicate the allowed region (i.e., if a point on the line works, then for the same flow rate, a system with fewer bricks or larger diameter will also work).

**Figure 3-2** (Left) Brick lifetime. Flow over time was monitored for a brick that was allowed to sit, filled with water. When remeasured after three weeks, average flow resistance had decreased by 24%, perhaps due to partial delamination and bulging of the sealing film under pressure, as suggested by the nonlinear (decreasing) flow resistance at higher pressures.
The leaking pressure was above 415 kPa, the upper limit of the pressure I could exert using available tools and sensors, while calculations suggest a sealing capacity >5 MPa for the O-ring and >10 MPa for the sealing film (Fig 2-14).\textsuperscript{39} I elected to use the highest measured pressure (415 MPa) in later calculations.

The pressure-flow behavior allows me to estimate the total pressure drop of a brick network, versus the channel hydraulic diameter and number of bricks (assuming they are all similar). For example, at a moderate flow rate of 0.1 mL/min, analysis using the resistor models predicts networks of >100 bricks in series could be combined within the measured pressure limits (Fig 3-1b). For comparison, recently reported microfluidic systems in the journal \textit{Lab on a Chip} typically have \textasciitilde4-8 procedures such as inlets and outlets, testing and incubation chambers, heaters, sensors, and cameras in a complete device (Fig 3-3; highlighted in Fig 3-1b).\textsuperscript{40-59} Therefore, I can conclude that the brick system could be scaled to accommodate this typical size, and much larger systems.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{chart.png}
\caption{(Right) Number of different functions performed by the microfluidic devices published in \textit{Lab on a Chip}, 2016 and 2012, issues 1, where in an equivalent modular system one could envision decomposing the devices into different bricks. Values greater than 28 are from multiplexed systems with an average value of \textasciitilde1000 components.}
\end{figure}
Figure 3-4 (a) Replotting pressure drop versus Reynolds number for experimental and theoretical values for laminar flow suggest effects from inertia and a transition to turbulence or another effect. (b,) Two geometries with different numbers of 90 degree corners and dissimilar lengths have indistinguishable pressure drop per length, indicating minimal effects from corners at moderate flow rates. (c,d) Bricks used in the system.

In a modular system, reconfigurability is a key property. To aid this, I designed the apertures in the bricks to contain fluid by capillary pressure. This is possible for fluids that do not wet the sealing film, polyethylene, or brick materials polycarbonate (the transparent brick material) or ABS.9 The required aperture size was calculated via a balance of capillary pressure due to surface tension and gravitational (i.e., hydrostatic) pressure so that a finished brick could be removed from a brick network and freely inverted (Fig 3-5). This outlet hole diameter (or minimum width, if noncircular) is less than the capillary length for most fluids, \( l_c = \frac{\sigma}{\sqrt{\rho g}} \) for surface tension \( \sigma \), fluid density \( \rho \), and acceleration due to gravity, \( g \).

---

9 The contact angle of water on brick surfaces was measured by dispensing a droplet of water onto the side surface and measuring using a contact angle goniometer (Rame-Hart, Model 590).
Additional system properties are summarized in Table 3-1 below including manufacturing size limits, flow and assembly attributes, and material properties.

**Table 3-1** Demonstrated specifications and properties of LEGO-based fluidic bricks.

<table>
<thead>
<tr>
<th>Category</th>
<th>Attribute</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Manufacturing</strong></td>
<td>Channel dimensions</td>
<td>50-1500μm (depth)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150+μm (width)</td>
</tr>
<tr>
<td></td>
<td>Channel geometries</td>
<td>Rounded or rectangular cross-section, any two-dimensional path on a surface</td>
</tr>
<tr>
<td></td>
<td>Surface roughness (average, Ra)</td>
<td>Native brick: 0.045μm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Machined brick: 0.6-1.2 μm</td>
</tr>
<tr>
<td></td>
<td>Time to make (average per brick)</td>
<td>10 min (milling)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 min (cleaning and sealing with film)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 sec (assembly)</td>
</tr>
<tr>
<td><strong>Fluid flow</strong></td>
<td>Pressure limit</td>
<td>&gt;400 kPa</td>
</tr>
<tr>
<td></td>
<td>Flow rates tested</td>
<td>2 μl/min to 5 mL/min</td>
</tr>
<tr>
<td></td>
<td>Lifetime</td>
<td>Depends on pressure</td>
</tr>
<tr>
<td></td>
<td>O-ring sealing success rate</td>
<td>&gt;99%</td>
</tr>
<tr>
<td><strong>Assembly</strong></td>
<td>Repeatability of positioning 1 brick</td>
<td>&lt;3 μm</td>
</tr>
<tr>
<td></td>
<td>Interchangeability</td>
<td>&lt;15 μm</td>
</tr>
<tr>
<td><strong>Material properties</strong></td>
<td>Material</td>
<td>Polycarbonate (PC) or acrylonitrile butadiene styrene (ABS)</td>
</tr>
<tr>
<td></td>
<td>Operating temperature</td>
<td>-3 to 70°C (based on material properties of all components)</td>
</tr>
</tbody>
</table>
Figure 3-5 Calculation of geometry to retain fluid inside bricks via surface tension. (Right) Demonstration of a brick that was filled to the top with green fluid when it was in a brick network, and removed to be imaged, without leaking.
4.1 LEGO-mounted Light Sensor

The brick-based approach, as well as the ability to mount bricks with precise and repeatable positioning on a common baseplate, also facilitates integration of optical imaging and sensing of the fluid path. I demonstrate this in simple modular systems for optical imaging during two fundamental microfluidic flow processes: controlled mixing of two liquids in laminar flow, and generation of water-in-oil and water-in-air emulsions, where water is darkly colored (Fig 4-1).

An exemplary brick-based sensor was constructed by adhering an infrared light sensor (940nm, LiteOn LTR-301, as used in 3) onto the back of a standard L-shaped LEGO brick, with a small through-hole milled to provide direct optical access through the brick wall. This sensor was paired with a brick with a through-hole holding the end of a 1/8"-diameter fiber optic cable attached at the other end to an emitting light source (here, the flashlight of an iPhone 4s, Apple Inc.), similar in design to many laboratory spectrophotometry light sources. These were mounted in-line with each other on opposite sides of a transparent fluidic brick with the fluid channel in-line with the light path of the sensor pair (Fig. 4-1a,c). The LEGO mounts ensured consistent alignment between the emitter and sensor bricks and to the fluidic brick when repositioned to any
point on the fluid path. The passage of fluid reduced light transmission, and light level was recorded as a voltage output by a microprocessor (Arduino Uno) that also powered the circuit and collected data. The transparent polycarbonate brick and polyethylene film together allowed 80% transmission of light, which was determined by comparing transmission with and without the empty brick between the light source and sensor.

Using a fluidic brick designed for combining two miscible solutions, a fluidic system consisting of two inlets, a flow combination brick, and an outlet was built, and the sensor was mated to the system by attachment onto the same baseplate. I constructed a curve of light transmission vs percent concentration with two different sensors with sensitivity of 2% concentration or 0.5 volt / 100% (Fig. 4-1d). Such a method can be used to observe the extent of a chemical reaction that affects the absorbance or color of a solution, such as in a precipitation or synthesis reaction, or to calculate the concentration of a substance via the Beer-Lambert law, which predicts the concentration of a solution based on absorbance. This was measured with two LEGO-based light sensors that were used in turn at the same location. They were calibrated first by measuring 95% and 5% concentration to match the values. The two sensors provided the same linear relation within 1% and individual data points within 8% (experimental limit, 0.01 V) in the linear regime of the analysis, with error up to 0.27 V when fluid is highly concentrated (Fig. 4-1b).

For a second analysis, a T-junction was milled into a transparent brick to form a droplet generator, and was placed into the same system with two inlets and one outlet, and the fluid in one inlet was changed (Fig. 4-1c-f, as in Fig. 2-1d). Ambient air was used as the continuous phase and water as the dispersed phase. Water had a contact angle between 80° and 90° for both brick materials.
Figure 4-1 Light sensor for measuring absorbance and counting droplet passage. (a) A LEGO-mounted light source and light sensor were mounted on opposite sides of a transparent fluidic brick, shown here with the sensor present and removed to reveal the droplet generating brick behind. (b) When mixing two fluids for a series of concentrations, two interchangeable sensors measured a calibration curve for different mixing ratios, which followed a linear trend, as expected by the Beer-Lambert law, giving the same linear slope within experimental error (<1%). The inset darkfield micrograph demonstrates laminar flow during mixing at 50% upstream of the sensor. Flow was balanced so the exit flow was 0.5-1.0 mL/min and no flow was less than 0.01 mL/min. (c) The sensor detected the passage of fluid as it changed color. A top-down view shows the sensor and light source aligned around the illuminated post, which is where the droplet brick would be placed. When generating droplets, (d) one sensor recorded data that was analyzed to (e) mark the passage of droplets, indicated by a spike, and (f) measure the average size and distribution of droplet size as a function of flow rate ratio, and represented here by passage time. Further data processing can measure droplet size later. Droplet generation flow rates were 0.5 mL/min for air and 0.1 mL/min for water.
The data from the light sensor (Fig. 4-1d) was processed to indicate the passage of the droplets with a vertical spike (Fig. 4-1e). Each spike indicated the passage of one droplet, and the width of the spikes was used along with input flow rates to calculate the size distribution of the droplets at two speeds of droplet generation, and their polydisperity (Fig. 4-1c,f). The reaction time of the sensor was reported to be 15 µs and the microprocessor limit was 250k bits/s, though here measurements were taken at 9600 bits/s.

### 4.2 Magnetic sorting

Another key application of microfluidics is separation and processing of colloidal materials, including cells. For example, paramagnetic beads may be functionalized with a material (such as biotin) which would bond to another marker attached to cells that a researcher would like to isolate from a population. By separating particles in the flow via magnetic forces, the cells attached to paramagnetic particles could be captured, removed from the paramagnetic beads (such as via solvent exchange) and further analyzed, for example by a molecular assay. To this end, a passive sorting device is being developed that uses a permanent neodymium magnet mounted in a brick to selectively sort suspended paramagnetic particles into one of two outlets (Fig. 4-2a).

A modular system can simplify the design and use of microfluidics for separation, because the sorted outputs can be collected into bricks. The bricks can then be removed wholesale from the device and moved to a new device for downstream analysis, or moved before the inlet in the same system and re-sorted to increase the purity or increase the capture rate of the final separated product. This method could also use a single device
with two outputs to sort particles into multiple distinct segments, such as by sequentially increasing the magnetic field (by shifting the magnet-holding brick over by one post) or by altering the flow rate and saving captured solutions from one output and re-running the other. The repositionability of the brick means that one can reliably change the strength and direction of the magnetic field by discrete steps, and reliably move it back to the original position without additional hardware.

The sorting device was made with combined with a single inlet brick, and each outlet was connected to a collector brick with a large vacant space to hold fluid, and an outlet. The magnet-holding brick was placed to the side of the sorting brick to pull paramagnetic particles to that side. A preliminary test with stationary fluid with paramagnetic particles (Fluorescent YG Superparamagnetic Microparticles, 1-3 μm diameter; Polysciences) was used to measure particle flow rate towards the magnet and this value was combined with data from the permanent magnet manufacturer (K&J Magnetics) (Fig. 4-2f) to estimate paramagnetic particle velocity towards the magnet as a function of distance (Fig. 4-2g). Good separation will occur if the fluid velocity towards the second outlet is less than the magnetic migration velocity towards the first outlet (limiting this setup to a flow rate of 0.01 mL/min under current conditions), and so a magnet closer to the flow will enable faster separation. This device merits further study.
4.3 Inertial sorting

A second common variety of passive sorting is sorting due to inertial effects in the fluid, which typically is used to separate particles by size or density. Nivedita, et al.
previously showed a spiral path for sorting blood cells that achieved ~95% sorting efficiency and throughput with rates up to $10^6$ cells per minute.$^61$ To investigate and validate the suitability of LEGO systems for this type of separation, a LEGO block was milled with this design and connected to specialized inlet and outlet bricks which then connected to regular outlets, in order to accommodate the specialized geometry with irregular aperture points (Fig 4-4a, b).

The sorting effect is a result of secondary flow set up by the spiral motion, and is characterized by the Dean's number of the flow,$^62$ which is related to Reynolds number by

$$De = Re \frac{d}{\sqrt{2r}}$$

for a hydraulic diameter $d$ and radius of curvature of the spiral $r$. At a critical $De$ around 10,$^61$ the secondary flow will have a significant effect. Thus, a smaller $r$ will allow more effective sorting – either stronger separation capacity or a smaller spiral needed for the same separation. The minimum radius (at the center of the spiral) has been limited by the size of inlet tubing needed to input fluid to standard microfluidic devices, and the margin designed in to allow one to punch out the tubing (Fig. 4-3). Because the inlet here is formed by a simple aperture in sealing film, with an O-ring pressed to the reverse side, the center of the spiral can have an initial $r$ as small as can be milled with unmilled regions large enough to seal with sealing film, shown here as $r = 1.25$ mm for the first spiral and increasing outwards (Fig. 4-4c), which could be reduced further by using a smaller endmill or cutting subsequent spirals closer together.
Devices of this design typically have >80% sorting efficiency for very high flow rates (>1 mL/min). While increasing spiral efficiency and reducing the required planar area of the device is good, it is also advantageous to reorient the spiral sorter to be vertical, effectively reducing any area of the device to the width of the LEGO brick, and the inlets and outlets in a compact, stacked structure, even with a larger spiral.
Figure 4-4 (a) Components of a device based around a (b) spiral sorting device. Micrographs of the fluid-filled system show (c) the inlet of the spiral, (d) one bend in the curve, and (e) the separation point with stationary particles that have adhered to the sealing film. Channel width is 500 μm.
3D PRINTED BRICKS AND INTEGRATED COMPONENTS

As the performance of additive manufacturing (AM) processes continues to improve, and as AM machines (i.e., 3D printers) become more widely available, 3D printing (3DP) is being investigated for use in constructing microfluidic systems. Despite this great and growing interest, the utility of 2D and 3D printing methods to make broadly useful microfluidic devices is restricted by universal limitations in the current technology: material choice and producible dimensions. Polymer 3D printers must use either a thermoplastic (as in fused deposition modeling, FDM), of which there is none that can print with optical clarity comparable to glass or high-quality molded plastics, or a photopolymerizable resin (as in stereolithography, SLA), which may be translucent but often has a proprietary formula, and can pose chemical hazards to fluids if not fully cleaned or cured. The dimensional resolution of 3DP methods is restricted by layer height, typically 20-200 μm, which influences surface roughness, and 3DP parts may further suffer from uncertain or poor long-term dimensional stability (particularly when in contact with a fluid).

However, mating 3DP components with standard injection-molded bricks would greatly increase the versatility and functionality of the modular system presented in this thesis. Therefore, the goal of this chapter is to investigate the consistency and repeatability of 3D printed bricks and to use 3DP to demonstrate exemplary functions
including components with internal fluid paths having reduced unnecessary volume compared to modified bricks, and fixture for repeatable mounting of a smartphone camera for imaging flow in a system.

To understand how 3DP bricks compared to milled injection-molded bricks, I printed LEGO-like bricks using two methods with hobby-scale machines available: stereolithography and fused deposition modeling. 3D-printed bricks were made from a computer aided design model of a LEGO (SolidWorks) using SLA (Clear resin, Form2; Formlabs, layer height 0.25 mm, oblique orientation) and FDM (ABS plastic, Stratasys Mojo; layer height 0.170 mm, vertical orientation with posts facing down, and Zortrax M200, layer height 0.90 mm, same orientation). 10

In the stereolithography (SLA) process used here, a stage is lowered into a vat of photocurable resin. To make each layer, a scanning laser (405 nm wavelength) below the tank cures segments of the resin in a thin gap between the bottom of the tank and the stage or previous layer. The stage moves incrementally out of the tank (after each layer is scanned) thus vertically incrementing the part, and the layer height is set by that increment, typically 25-100 μm. Because the formulation of the resin is photo-cured to solidify, in principle (though not here in practice) the curing parameters can give a variety of material properties, including high-heat resistance and transparency. Resolution is limited by the layer height, and by the spot size of the laser, here 300 μm. In addition, structures are printed with a “forest” of support structure (built from the same, single material) that must be removed to give the final part, often leaving pits or bumps on the part surface (Fig 5-1b).

---

10 The Zortrax prints without support dissolvable. This upside-down orientation was found to produce bricks with no defects, compared to the other 3 possible orientations.
In fused deposition modeling (FDM), a heated nozzle extrudes a line of molten thermoplastic onto a stage to fill in a pattern, moving sequentially upwards. The Mojo printer used here has a second nozzle to extrude support material, which can later be dissolved away. The support material allows printing of features such as overhanging regions or internal cavities that would collapse without an underlying layer. The Zortrax printer extrudes coarse sections of the regular printed material to form support structures. Resolution is limited by the nozzle diameter (and in addition, the material is typically broadened upon extrusion due to die swell, and to impart good adhesion between adjacent deposited lines), and material must be a thermoplastic. Typically the extrusion temperature is comparable to the melt temperature used for injection molding.

Another common 3DP method used for microfluidics is jetting, which can achieve smaller features, though only in-plane and in line with printer axes. That technology, most prominently brought to market by Objet (now owned by Stratasys) is currently limited by material and machine cost and accessibility, and therefore is considered to be less accessible than FDM and SLA and was not studied here.
Figure 5-1 Printing of bricks by SLA and FDM. (a) The Formlabs Form2 includes a stage submerged in a resin bath, and a scanning laser that locally polymerizes the resin to create individual layers of the printed object (yellow glow). (b) Objects are printed with support structure made from the same material, which must be removed after printing, leaving scars (c, k). (d) The surface texture is rough compared with injection-molded bricks, but the oblique printing angle obscures individual layers. (e) The Stratasys Mojo prints by extrusion of material via heated nozzle onto (f) a vertically moving stage inside the printer, which holds (g) a build tray which here holds the just-printed pieces. The orange material is ABS plastic and white material is the dissolvable support. (h, i, l, m) Individual layers are distinct, as this printing mechanism favors printing orientations level with the part axes, and the layers are larger. (j-m) Side-by-side comparisons of orange injection-molded LEGO bricks with (j-k) SLA bricks and (l-m) FDM bricks.

Next, I measured the feature resolution and positioning repeatability of these bricks (using the methods of Ch. 2) to compare their interchangeability with that of injection-molded bricks. Although surfaces were rough compared to injection-molded bricks (RMS
~4 μm vs ~0.07 μm), the repeatability of these units onto injection-molded LEGO bricks was comparable at 6 to 10 μm for SLA bricks and 2 μm (!) for FDM bricks (Fig. 5-2). Wear-in was observed for both FDM and SLA bricks (Fig. 5-2b,c). In addition, the SLA-printed bricks fatigued rapidly until failure (Fig. 5-2b), and always fractured or loosened after general use.

The impressive repeatability of the 3D printed bricks is an interesting phenomenon, as the brick surfaces are quite rough. The roughness that governs repeatability may then be the roughness of the surface within each layer, or it may simply be the roughness of the smoothest surface in the attachment, i.e., the injection-molded LEGOs. This might be tested by measuring repeatability versus layer thickness, and by comparing the observed repeatabilities of Fig. 5-2a with those of 3DP bricks mounted onto other 3DP bricks, at which point the layered texture on each structure would be expected to interact, and perhaps interlock. The influence of nominal brick dimensions (i.e., amount of interference between post and cage) is also of interest, and could be studied using 3DP bricks.
Figure 5-2 Repeatability of 3D-printed bricks when mounted to a standard LEGO brick. (a) Final results show repeatability ranging from 2 to 10 µm for different sizes of the same bricks. Here, unlike before, post count refers to the number of posts orthogonal to the measured edge, instead of complete post count, to observe for effects of printing orientation. Measurements of repeated mountings of the same 3DP bricks on a standard LEGO brick shows systematic effects, plotted for one experiment each for (b) SLA and (C) FDM bricks. For SLA-printed bricks, this includes an initial widely varying initial variation in position, termed “wearing in,” a narrower range of position with a mean downward drift, and then the brick appears to experience failure after which point variation in position is quite large. Repeatability in (a) is indicated for the middle range only. (c) FDM-printed bricks exhibit the same initial change in position, but afterwards mostly converge onto a narrow and consistent distribution. (d) After experiments, I observed permanent deformation at the contact points (black arrows) and a fracture on the SLA brick.

The dimensional variability of SLA and FDM brick widths (measured as in Ch. 2) was σ = 70 and 30 µm, respectively, leading to a total interchangeability of 70 and 30 µm (compared with 7 to 25 µm for LEGO bricks, again being dominated by the difference in size between bricks). I observed that the SLA bricks stretched and/or swelled after repeated contacts and exposure to water.
As of January 2017, the material cost per brick for the Mojo (ABS) is $0.39/cm³, plus support material that may reasonably double the cost, and $0.15/mL for Clear Resin for the Form2. The material required to print bricks of different sizes is included in Table 5-1, and support material volume was calculated using the software for the Form2 (Preform 2.8.0, Formlabs).

### Table 5-1 Material volumes (mL=cm³) and cost for printed bricks, compared with ~$0.05-0.10 for commercial injection-molded LEGO bricks or <$0.03 for similar bricks from other manufacturers.

<table>
<thead>
<tr>
<th>Brick size</th>
<th>FDM (Mojo)</th>
<th>SLA (Form2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Material volume (cm³)</td>
<td>Material cost ($)</td>
</tr>
<tr>
<td>1x2</td>
<td>0.77</td>
<td>$0.30</td>
</tr>
<tr>
<td>2x2</td>
<td>1.27</td>
<td>$0.50</td>
</tr>
<tr>
<td>2x4</td>
<td>2.35</td>
<td>$0.92</td>
</tr>
</tbody>
</table>

Although micromilling on injection-molded bricks produces finished products that are less expensive, smoother, and more repeatable than 3DP bricks, 3DP does enable rapid fabrication of complementary pieces for microfluidics. Unlike any other process, 3D printing allows the fabrication of pieces that have internal, complex, and custom geometry, which in particular can create large channels going through the body of bricks. Indeed, the inlet brick used in earlier chapters was made of three stacked LEGO plates. I often found it useful to instead use a 3D printed version (made by SLA for flow visibility) with a smoothly turning cross-section, so that the inlet was easier to clean and less able to contaminate flows between experiments. This eliminated dead space and sharp changes in geometry, but was limited by the properties of the available resin, restricting
transparency, dimensional stability, and roughness (affecting interchangeability and “click” connection between bricks), and minimum resolution. Several other studies have exploited 3DP to print microfluidic devices with enclosed channels. However, this is limited by channel length through the part and printing orientation and I found prints to be unreliable for channel diameters <750 μm for channels passing through a brick.

![Figure 5-3 3D printing of inlet bricks. From left to right, an entirely LEGO-based inlet made from 3 stacked thin pieces, with fluid moving in the internal rectangular cavities, and (middle) a 3D-printed substitute, which maintained a smooth profile through the brick, increasing efficiency and cleanliness. (Right) Two such inlets together were able to seal to form a fully functional modular device.](image)

The repeatability of 3DP bricks on LEGO bricks is encouraging, as it enables integration of larger and/or more complex components with the microfluidic platform, by incorporating mating attachment features. To demonstrate and leverage this functionality, I designed a 3DP mount to position a smartphone camera in line with a fluidic network; this achieved imaging repeatability of <1 mm for a focus 80 mm from the camera, as expected by the base repeatability¹¹ (Fig. 5-4). The two-piece mount holds the phone against three tall beams, and a stiff flag-like structure is pressed by the phone to deform its flagpost, causing the structure to behave like a torsional spring and press

¹¹ If the entire repeatability of the printed stand contributes to angular error, then \( \tan(\theta) = 10 \mu m/10 \text{ mm} \). The maximum focusing error due to this would be \( (\text{distance}) \times \tan(\theta) = (70 \text{ mm}) \times 0.001 = 0.07 \text{ mm error of 1 } \sigma \).
the phone into place, designed here for static loads with a factor of safety of 10 to prevent fracture (Fig. 5-4). The spacing between the two units can change to accommodate different phones. Error could be reduced further by making it a single-piece mount, strengthening the flagpost, or printing the stand by FDM if it can be redesigned to not fracture along layers when the flag is twisted.

**Figure 5-4** 3D-printed smartphone mount and characterization. (a, b) Rendering of the two components of smartphone mount, designed to hold a phone horizontally by contacting three points on the pillars, and holding the phone in place by slightly deforming the flag-like structure, which resists to keep the phone in place. (c, d) Using this mount, images were taken with a Samsung Galaxy S6 and processed in ImageJ to determine the standard deviation for each pixel over a series of 20 images when (e) the camera was left in place and the fluidic system was detached and re-attached between images, and (f) when the camera mount was detached and re-attached between images. Regions of color represent areas of variation in that color and brightness between successive images. All lines showing standard deviation are <1 mm wide. Resolution is 5312x2988 pixels, and one pixel is 17 μm on the image.
Other useful accessories for configuring peripheral hardware can be made out of Legos entirely, such as the compliant beaker-holder in Fig. 5-6, which used two rigid Lego walls and two compliant and rotating wheel assemblies to place a waste beaker next to a fluid system.

Figure 5-5 LEGO-based waste collection holder. The beaker is in contact with two rigid LEGO bricks and pressed against two relatively compliant wheels.

Finally, as an alternative to LEGO-brand blocks, I examined two other injection-molded, interlocking toy blocks: Wonderbricks, which are nearly identical to LEGO bricks (Fig. 5-6) and Nanoblocks, which are half the linear size of LEGO bricks. Both were found to have variance in dimension at the same scale as LEGOs, or 10 to 20 μm, making them viable alternative bases for block-based microfluidics. However, as noted in Fig. 5-6, the Wonderbricks are more warped than LEGO bricks, which will cause milled channels to vary in depth along the length of the brick.
In summary, using the desktop-scale prototyping tool mentioned above, micromilling injection-molded bricks can create 10x smaller features, 10x-100x smoother surfaces and 10x more consistent dimensions than 3D printing, as well as a much higher proportion of pieces that meet tolerances to snap together, more transparent color (vs. the chosen "clear" SLA resin), and more well-studied ("safe") plastic material (vs. stereolithography resins) that are known to have greater long-term stability. This suggests that micromilling on LEGOs can create a superior microfluidic system, also with customized geometry.
Figure 5-7  Comparison of properties relative to milling (normalized by the value for milling). Cost, production time, variation in width, repeatability, interchangeability, minimum enclosed channel width, and surface roughness of bricks is compared between milling on 3D-printed LEGOs or Wonderbricks, SLA, and FDM. Data is only shown where it was available. Error bars indicate range of values.

We can look forward to future advances in the performance of accessible 3DP methods, and those best suited to microfluidics. Also, advances in materials suited for microfluidics will improve the capability to integrate 3DP in microfluidic fabrication workflows, yet it will remain difficult to reach the dimensional accuracy and surface roughness of high-quality injection molded components.
CHAPTER 6

MODULAR SYSTEMS FOR EDUCATION AND OUTREACH

The current qualities of commercial LEGO bricks are sufficient for certain research-grade experiments and have the potential to bridge the gap between research and education. In addition, the familiarity and accessibility of LEGO bricks is attractive for educational use. Therefore, the LEGO-based platform provides an opportunity to design educational activities that, for example, teach principles of prototyping (milling, 3D printing), precision assembly (elastic averaging, metrology, repeatability), fluid mechanics (pressure drop, flow rates, hydrodynamics), and chemistry (reaction rates, scaling). To explore this, I am creating a pH meter for use in a chemistry class, and I developed two activities tested with high school students to learn about pH and about hydrodynamics.

6.1 High School Chemistry Titration Kit

If you think of the labs from your high school chemistry class, you might remember solutions changing color, an explosion or two, and a lot of glassware and ringstands. In the research world, microfluidics is used extensively as one tool to understand chemical synthesis, including reaction times and behavior, and product purity.

With the assistance of Helen Xu, who worked in my lab at MIT as a member of the Research Science Institute in the summer of 2016, we are envisioning a “LEGOfluidic” kit for use in high school chemistry laboratories, for example as an alternative to titration labs.
that teach the relationship between concentration of reagents in a solution and its acidity, and color-change indicators. This would also allow the laboratory to teach additional fundamentals. If the classroom has the available resources, they could design some of their own bricks to add to the system, increasing the potential for hands-on learning in design and manufacturing. This system would also reduce glassware and reagent needs (and therefore cost), and use of LEGOs would tend to increase student interest. If they do design additional components, knowing the size of the gaps between bricks is important to successfully sealing a device (see Ch. 2.2). For this purpose, I developed an entirely LEGO-based protractor system that can measure the gap between any two bricks with a resolution of tens of microns without the need for a microscope (Appendix B).

The standard goal of the chemistry lab is to drip one fluid of known concentration into another, to plot a concentration-vs-pH curve and locate the equivalence point to determine the unknown concentration. The system we have designed (Fig. 6-1) accepts a combination of reagents and diluents that are combined and passed through a sensor block that includes a standard pH sensor electrode sealed inside a brick, providing a digital readout of pH. The relative flow rates may be controlled by an external syringe pump, if available, or by altering the sizes of the “control” bricks shown (Fig 6-1) and pushing flow from a single pressure source. The system has not yet been tested in full functional form.

In addition to the pH electrode, we have developed and calibrated a second sensor: an Arduino-based colorimeter which would measure the pH of the solution by color and intensity if an appropriate pH indicator is pre-mixed with one of the inputted fluids.
Figure 6-1 System prototype to control titrations for high school chemistry laboratory. (Top) Completed system made out of (bottom) 12 bricks including an inlet for a base, acid, and water or dilutent, each having a flow control brick, two flowstream combination bricks and two mixing bricks with variable depth to generate fast advective mixing, and a sensor electrode, with the final outlet not pictured. Each brick is 15.8 mm wide.

6.2 LEGOfluidics at MicroTAS 2016: Colorimetric pH Sensor

In Ireland, between Junior and Senior level in high school, students make a decision on what science to take, if any, at the senior level – including biology, chemistry, and physics. I attended Micro Total Analysis Systems 2016 Conference in October 2016, in Dublin, Ireland, where I presented a research poster on selected work from this thesis,
and volunteered to design and deliver an outreach activity. Two hundred high schoolers at this decision point were invited to a microfluidics conference called MicroTAS in Dublin, Ireland. Scientists put together various research demonstrations to encourage the students to continue studying some aspect of science.

For this event, I simplified the design of the titration system described above to five total bricks: two inlets, two "mystery" control bricks, one mixer, and a fluid collector. This was required to be a "no gloves" activity, so chemicals were all edible: lemon juice as the acid (pH ~ 2; certain soda drinks would also work) and water (pH ~ 7). The water was pre-mixed with powdered cabbage juice (Educational Innovations IND-100), a color-changing pH indicator, and after a quick lesson on microfluidics, 10 groups of 5 students were challenged to find the correct mystery bricks to fill the cartridge with the color corresponding to the right pH (Figure 6-3). Mystery bricks differed in channel diameter, labeled on the brick, so students would determine by a mix of trial, error, and thought, the correct ratio to reach the desired pH. Most components were available in both milled and 3D-printed versions so I could see if there was a clear difference in preference or usability between the two.

In this event, I noticed students occasionally scraped off parts of the sealing film when eagerly jamming bricks together, so for subsequent development of my device designs I added dabs of cyanoacrylate adhesive onto the sealing film to prevent loss of this film.
The concept: brick by brick

(1) A microfluidic system with many interchangeable 'brick' pieces allows it to be assembled and changed easily. Its function is determined by the bricks that are chosen and the way they are arranged.

How the bricks work

Off-the-shelf Lego bricks were modified to direct the flow of fluid. An O-ring seals between bricks when they are placed down.

A cutting tool removes material from a brick surface. Here are bricks for flow focusing, mixing, and joining two fluids.

Small O-rings are compressed to reversibly seal between bricks, forming a closed system.

Designing a fluid mixing system

(2) Students build their own system to mix fluids, and observe what happens when they choose different bricks for a variable "?" position.

(3) Different choices of bricks will change the geometry of the fluid path, and with a single pressure source this results in a different mixing ratio of two fluids and a different end colour that reveals the pH of the final solution.

Inlet: Fluid 1
Mixing brick
Product
Fluid 1: water with a pH indicator (cabbage juice)
Fluid 2: lemon juice, a source of citric acid

pH color scale, indicated by cabbage juice concentrate:

Lemon juice   2  4  5  6  7  Water

Why do we do this? Connection to research.

- Fluids can be combined in a specific ratio to make a certain product.
- For example, we can use this method to add a treatment chemical to water at the right level to make it safe to drink.

How?

- Students get into groups and build a microfluidic network with different bricks.
- Students change the system by swapping bricks, and this makes different reaction products. What colours can they make? How do the bricks influence the final colour?

Learning goals

Every microfluidic system begins with a design. This activity introduces students to the general process of designing a microfluidic system and the concept of microfluidic functions, and allows them to build and test ideas in a playful setting.
A microfluidic system with many interchangeable 'brick' pieces allows it to be assembled easily. Its function is determined by the bricks that are chosen.

**WHY?**
- Fluids can be combined in a specific ratio to make a certain product.
- We can use this method to add a treatment chemical to water at the right level to make it safe to drink.

**HOW?**
- Students get into groups and build a microfluidic network with different bricks.
- Change the bricks to make different reaction products. What colors can you get?

**Figure 6-2** This poster (blue, previous page) and handout (green) were prepared for use at MicroTAS to describe the pH-measuring device and color-change of a pH indicator. Fluid was combined in two inlets and choice of mystery bricks in the second slot changed the ratio of fluid passing through, resulting in different final colors, with the color scale indicated here.
6.3 Outreach at MIT Splash: Battleboats

Third, I introduced an activity I titled “Battleboats” in a two-day outreach event for high school students called “Splash,” which is run by MIT’s Educational Studies Program. The event consisted of hundreds of single-session classes led by volunteer MIT students. I ran three sessions of a 50-minute class. Almost all components were 3D-printed because of the required geometries, and the main components were a boat chassis and pieces with angled flat edges (Fig. 6-4).
After a 30 minute introduction to hydrodynamics, including drag and thrust, students had 30 minutes to design a LEGO-fluidic boat and enter it into competition. The competition was simple: build the fastest boat. The supplies included an "engine block" which had a series of basic LEGO nubs for attachment with a nozzle opening to a balloon, so that the deflating balloon would force air into the chassis and out the bottom, providing the pressure for propulsion. This, along with other LEGO pieces, store-bought and custom-printed, allowed students to design their boats with creative freedom for shape and center of gravity. To enter the competition, though, students had to first calculate the...
drag force on their boat within a factor of 10 (by experiments to test the boat’s speed). Only two teams were able to do this.

It proved challenging to teach to a roomful of high school students with varied backgrounds, especially in such a short time. Each team made something, and had fun, but there were new challenges for each group. The LEGO pieces had not yet arrived for the Saturday class, so they wrapped tinfoil and duct tape around standard LEGOs to make the boats and powered them using a backup fuel: baking soda and vinegar. The resulting boats often sputtered along, or foamed up, or spun in circles, unless the nozzle of the fuel tank remained oriented in the correct direction, and it was difficult to secure without a very stable (i.e., not duct-tape-and-foil) frame. Students also were hesitant to use enough fuel. Five boats were built then, each by a team of five, and three of them floated and propelled forwards. After this class, I accelerated the content and changed the design so that boats were powered by the deflation of a blown balloon.

In the second class, four teams of three people formed. We had the LEGO pieces but the students had no familiarity with drag. This was the best-performing class; four out of four boats both floated and moved forward with the balloon propulsion.

The third class, in the last session slot of the program, had four teams of two people. At this point, the LEGO pieces (printed with Form2 Clear resin) had taken on water, and no longer floated so the two boats that tied for first were the ones that floated by the end of the design period.

I had set a prerequisite grade level, 9th, and class set, physics. Still, it would be easier to teach the same students different things than to teach different students the same thing, because previous experience and knowledge of different students was both
variable and uncertain. They all had a little bit of something, some kind of knowledge about drag forces or CAD or Free Body Diagrams, but walking in I never knew what the background was, and what the needs would be. That made teaching an act of adaption.

Even so, I tried my best to teach, reach, motivate and inspire the people who showed up, who typically differed from the ones I expected and changed between class sections.

**E10637: Battleboats** Full

Difficulty: ***

Teachers: Crystal Owens

Why are cars shaped like they are? How does a jetski work? Is a hovercraft possible? Come learn the basics of hydrodynamics and jet propulsion, and then build your knowledge in a hands-on activity to design, build, and race a small Lego boat.

**Prerequisites**
A basic class in physics.

**Meeting Times**

Section 1: Sun 5:05pm–5:55pm
Section 2: Sun 5:05pm–5:55pm
Section 3: Sun 12:05pm–12:55pm

**Grades**
9 - 12

**Enrollment**

Section 1: Full! (max 15)
Section 2: Full! (max 15)
Section 3: Full! (max 15)

**Figure 6-5** Class syllabus and image of students working in teams to design their boats.

**Figure 6-6** Three resulting boats with successful designs, made by students in the second class. (a) A pontoon-style boat used additional balloons for buoyancy. (b) A LEGO-heavy design added flat surfaces (white) to the structure, and creatively incorporated their own nozzle mechanism (red straw) to aim exhaust further underwater. (c) Boat in action using two straws for buoyancy.

After the class, the boat was redesigned to be more buoyant even after long times.

The large segment before the exhaust was hollowed out. Each of the pillars that help form
the elastic fits on the bottom of the boat were mostly sealed, with an opening \( r < l_c \) on either end so that fluid would not tend to pass through the opening (in or out) on its own, but could be added with applied pressure. This would allow students to modify the center of gravity by adding water inside different pillars. Then, this activity could be expanded beyond teaching concepts of drag and thrust to discuss requirements for stability and center of gravity. That new function would make this activity more adaptable, so if students are more familiar with concepts I can advance the lesson into related topics.

The design was further modified by reducing the diameter of the ring for the balloons, and the exhaust was made into a separate brick so that students could select from a variety of pieces to determine the size and direction of the air jet for propulsion.

If the class had multiple sessions with the same students, I would add a design portion where students would develop their own ideas for boat structures after playing with examples. If they are familiar with a CAD program, they could produce printable designs. If they are learning CAD, then we would introduce some of the software and assist them in making it. Alternatively, they would suggest boat designs that we would sketch. We would produce these new designs by 3D printing during a break between classes, for instance for a multi-day class, as the printing would take \( \sim 1 \) to 2 hours per boat.
I have presented a new approach to rapidly construct modular microfluidic systems by modification and assembly of interlocking injection-molded blocks. I demonstrated this principle using micromilling of store-bought LEGO® bricks to create surface fluidic pathways on bricks, and developed procedures for sealing and interconnecting bricks to form modular, reconfigurable, and self-aligning microfluidic systems.

Micromilling using a desktop machine achieved channel dimensions of 50 μm in depth and 150 μm in width, or greater, on the sidewalls of blocks. Sealing these channels with adhesive films allowed internal fluid pressure of at least 400 kPa. The intrinsic tolerances of injection molded bricks and their elastically averaged connections gave a mechanical locating repeatability of 1 μm, which enabled fluid to pass between bricks through an O-ring with >99.9% sealing reliability and maintain repeatable flow behavior even after replacement of the sealing film and O-ring. Using the LEGO-based approach, I showed systems for variously generating droplets, sensing fluid passage by light absorbance, and repeatably positioning a smartphone camera, as well as presented designs for sorting with inertial and magnetic forces, all of which could be assembled in minutes. Then, I fabricated and measured LEGO-like bricks made by three-dimensional printing (3DP) by fused deposition modeling and stereolithography, showing that 3DP bricks can integrate with injection-molded bricks to add useful function, although their
surface quality, resolution, and material limit performance. Finally, I adapted these components for two educational activities for high school students: a colorimetric titration device and modular designable boat.

Although the methods shown for modification of LEGO bricks cannot match the resolution and fine scale limits of lithography, it is much more accessible for laboratory use, and may be a better bridge to mass-manufacturing microfluidics using molding and embossing methods. It is more practical and manufacturable at scale, and therefore useful in a way that more laborious techniques are not. Compared to extant rapid prototyping methods, LEGO microfluidics enables direct optical access for imaging and sensing, easy network construction using standard blocks, and sealing to moderate pressures without permanent bonding. The repeatable assembly by elastic averaging suggests that LEGO-like principles can be used to design new brick systems as well, using other polymers for thermal and/or chemical resistance, and to enable additional network topologies.

To my knowledge, this is the first demonstration of a fully reconfigurable modular microfluidic system that does not require a permanent sealing step, and which can have reliable channel sizes 500 μm and below. I envision this approach will facilitate improved rapid prototyping of microfluidic systems to perform, for example, droplet generation, cell counting, and biochemical analyses, and to sequence unit operations into networks.

One can conceive of many other function bricks than the ones mentioned here; the most useful of these will enable further sensing of fluid, such as temperature and impedance sensors, and processing fluid, such as to sort, dilute, concentrate, filter, and heat fluid. Further capabilities envisioned for future work may additionally require
electronic integration to control and regulate flow, and heat may be necessary to drive reaction. For analytical research, devising a system for automatically inserting various samples and reconfiguring the modules will be needed.

Demonstrations in this thesis were limited in channel size, as the current micromill could not create features below ~150 μm without loss of quality; however, high-performance micromilling has been shown to create features as small as 20 μm in width and below and much smaller depth with accuracy as tight as 100 nm. Other techniques including laser ablation and hot embossing could be used to create even smaller channels and/or complex surface textures on the brick surfaces.

Materials used in the Lego brick system allow temperatures up to 70 C, limited first by the brick materials, which restricts use for high-temperature applications like chemical synthesis. However, a capacity for moderate heating and use of biocompatible materials makes the system potentially useful for biological analysis, which may include heating, separation, filtration, and more. Sample preparation before a sophisticated stream of function is also a possible use, as LEGOs can hold relatively large volumes, allowing fast processing, and their low costs means components such as filters can be disposable.

The chemical compatibility may be improved by coating the LEGO bricks with a thin layer of a resistant material, such as Parylene-C, or by micromachining entire bricks out of a material like PTFE or PE. If the machining tolerances are tight enough, fabricated bricks with the same mechanical structure should demonstrate interchangeability similar to LEGO bricks, and with only a moderate increase in material cost.

The pressure and flow rate capabilities make applications such as water testing and biological work (cell culture, cell and other assays, DNA assembly) accessible using
LEGO microfluidics. While the surface roughness of our system is high (0.9 μm) compared to typical Silicon and glass systems (~1-10 nm), which increases the risk of trapping debris on the surface, one can take additional steps to control the surface, such as chemical smoothing, fire polishing, or adding a coating, and/or taking care to design systems with little dead space. In addition, the modular nature of legos, and 3D printed components with lego-like interfaces, enables similar interconnects to be made with any existing system, such as to insert a PDMS, glass, or Silicon chip into a mostly-brick system when particularly small or smooth features are required for a system subsection. The standard interface among all bricks enables a wide variety of brick units to be incorporated to a common platform, making this "lab on a brick" a new and viable platform for advancing research and education in microfluidics. Approaches to rationally designing ‘kits’ of brick components for specific application domains are also of interest for future work.
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Bricks were alternatively modified by maskless printing on bricks using the system described in 63. This process used a digital light processing projector (DLP) to solidify patterns in a polymer and create raised 2D and 3D features with dimensions arbitrarily either large or as small as 1-10 um that held fluid in between a pair of bricks. To fill gaps between adjacent bricks, I patterned channels on the flat side face of the brick, preserving the low surface roughness of LEGO bricks while allowing us to modify the surface for improved adhesion and sealing. The printed channel then pressed to seal mechanically and reversibly against an unmodified transparent polycarbonate brick through which one can image the flow. I was unable to find a material that could adhere to the brick surfaces and be cured by light of any wavelength, and so this branch was abandoned.
In another design scheme, systems were created with patterned inserts that were placed between bricks and folded around corners. The inserts consisted of
transparency film (TruOffice) with shallow channels made by several layers of ink deposited by a standard laser inkjet printer, and covering with an adhesive layer, similar to the process used in 26 (Fig A1-2). Holes were punched in sections of the inserts to align the film to the posts on top of LEGO bricks and on baseplates. A second style of inserts was made by 3D printing thin pieces that conformed to the brick shapes.

**Figure A1-2** A laser ink printed sheet folds around bricks that align it to drive flow.
Figure B1-1 (Left) A Lego-based protractor measures the gap between by measuring the possible angular rotation. (Right) A printable protractor if one is not available. The end should be placed on the center of the long LEGO forming the moving arm.

The presence of a gap between bricks means that when two bricks are mounted side-by-side on a single post each, so they can rotate, they can be rotated to a certain angle at which they will come into flat face-to-face contact and will not move further. Here, one long LEGO (white) sits on top of one of the two bricks being tested, to amplify the rotation to a more readable amount. Measuring that angle can give the gap width, \( g \), precise to \( \sim 10-100 \text{ um} \) using the formula

\[
g = w \left(1 - \frac{1}{\cos \theta}\right)
\]
for the brick half-width \( w \), where \( 2(g+w)=7.998 \) mm. It is more precise for smaller gaps.

![Image](image.png)

**Figure B1-2** A Lego liquid holder. Tall, transparent LEGO bricks may hold a liquid volume. The inner dimensions were measured and the volume per height was calculated. Due to the draft angle from injection molding, this is not linear, so the label is included true-to-size here.
Figure C1-1  An illustrated production scheme for adding sealing film to a LEGO brick with expected rates. If cyanoacrylate adhesive is included, it will be added right before step (3) on the outer corners of the bricks.