

Diffusion Based Extraction in A Microfabricated Device

James P. Brody and Paul Yager

Center for Bioengineering, Box 352141, University of Washington
Seattle, WA 98195

jpbrody@u.washington.edu

yagerp@u.washington.edu

ABSTRACT

Microfabricated fluid systems allow complex chemical analyses to be performed on sub-nanoliter volumes. However, many common laboratory procedures, including filtration, have yet to be robustly implemented in micro-fluid systems. A device has been developed to separate particles and molecules based on their diffusion coefficients; the process is demonstrated using a micromachined device with fluid channels as small as 20 μm . A simple model predicts exponential dependence of the output concentration on diffusion coefficient in certain regimes. Experiments confirm the model.

Introduction

It has recently begun to be possible to fabricate intricate fluid handling systems with channel sizes as small as 1 μm using the tools developed by the semiconductor industry to miniaturize electronics. These devices can be mass produced inexpensively and some may soon be in widespread use for simple analytical tests [1,2]. There are many advantages these miniaturized devices could offer, including the promise of performing some analytical functions more rapidly and on smaller samples [3] than with macroscopic systems.

However, miniaturization of analytic instruments is not a simple matter of reducing their size. At small scales different effects become important, rendering some processes inefficient and others useless. It may also be difficult to replicate smaller versions of some devices because of material or process limitations. For these reasons it may prove necessary to develop new methods for performing common laboratory tasks on the microscale.

This report describes a fluid-integrated microfabricated device that uses diffusion to separate particles by transferring them from one fluid to another. It demonstrates that an important step, separation, needed in many analytical tests, can be done quickly and easily in a micro-fluid system. It illustrates the importance of redesigning macroscopic devices, like filters, to use microscopic effects instead of just miniaturizing conventional designs. Also, it demonstrates that the unique properties of low-Reynolds number flow, flow conditions that are ubiquitous in microscopic conditions, can be used to develop new types of devices that do not have macroscopic counterparts.

In Maxwell's famous *Gedankenexperiment* [4], a demon operates a door between two adjacent boxes of gas at the same temperature. The demon sorts the molecules, putting the faster molecules in one box and the slower in the other, thereby violating the basic laws of thermodynamics. This paradox has since been resolved in many different ways. [5]

A similar arrangement can be used to separate particles by diffusion, without violating any fundamental laws of thermodynamics. Consider two boxes; that on the left contains mixture of particles of two very different sizes, and that on the right is empty. (See Figure 1.) A demon removes the wall between the two boxes for some time, τ , and then replaces it a distance λ_b to the right of its original position. The process can be characterized by a quantity D_f where $D_f = \lambda_b^2 / \tau$. Virtually no particles with a diffusion

coefficient substantially less than D_f will be present in the box on the right, while particles with a diffusion coefficient much greater than D_f will be in the box on the right at a substantial concentration. If the time, τ , is long enough, the concentration of smaller particles in the two boxes will be the same. By choosing the ratio of volumes of the two boxes, one can choose the final concentration of the smaller particles to be arbitrarily small. This provides both a way to isolate smaller molecules from large ones, and larger molecules from small ones, although both are slightly diluted from their original concentrations.

The concept of diffusion-based separation is described in Figure 3. The process relies on the non-mixing nature of low Reynolds number flow along with the fast diffusion times for small molecules. The idea was originally implemented in a system with two glass plates separated by a shim by Giddings [9]. We present here a greatly modified version of this implemented in a microfabricated fluid system.

A simple model can be used to quantify this "extraction" process. The normalized probability density function, $y(x)$, for the one-dimensional distance, x , which a particle (with diffusion coefficient D) diffuses in a time, t , follows a Gaussian distribution.

$$y(x) = \frac{1}{\sqrt{4\pi D \tau}} \exp\left(-\frac{x^2}{4D\tau}\right) \quad (1)$$

In the simple approximation that the width of the box on the left is much less than the distance λ_b , the concentration of particles in the output will be given by

$$C(D) = C_o \int_{\lambda_b}^{\infty} y(x) dx, \quad (2)$$

which is related to the well-tabulated function $\text{erf}(x)$.

Because the function $y(x)$ falls off rapidly with increasing x , this approximation turns out to be remarkably good; the behavior of the solution is not substantially changed by solving the complete problem. The result is that the concentration of a molecule with $D=D_f/n$ is reduced in the output by a factor of e^{-n} . Since the diffusion coefficient of a molecule generally varies as its linear size, this can be a very sensitive process for discriminating among molecules.

This hypothetical separation scheme can be implemented in a microfluid system--one in which the dimensions of the channels are sufficiently small that only low-Reynolds number ($R_e \ll 1$) flow can occur. Initially two separate flow streams (a carrier stream and a dilutant stream) are brought together into a central channel in which particles can diffuse between the two non-mixing streams. (See Figure 2.) Whereas at higher

Reynolds numbers ($Re \gg 1$), mixing independent of diffusion would occur between the two fluids in the central channel, when the channels are no more than tens of micrometers in size, the two adjacent streams flow in parallel without turbulence for the length of the channel. Only diffusive mixing will occur even in low-viscosity fluids. At the end of the parallel flow channel a fraction of the carrier flow stream is split off into an output channel. The time for diffusive exchange between the two fluids, $\tau = l/v$, is controlled by the velocity, v , of fluid in the central channel and the length, l , of the channel. The barrier distance, λ_b , is determined by the precise fraction of the carrier flow stream that flows into the waste stream. The barrier distance can be controlled by changing the geometry and/or pressures of the four channels.

Experimental

Silicon micromachining was done in the Washington Technology Center Microfabrication facility. The process was modified from previous work \cite{brody95} to avoid the problem of coating deep features uniformly with photoresist. In our experience, spin coating photoresist on a wafer that had features greater than a few microns deep led to a non-uniform coating that either left undeveloped areas after exposure or areas uncoated with photoresist. These problems were avoided with this process.

This process required two mask levels. One level defined connection ports, which were etched completely through the wafer, and the second level defined the fluid transport channels on the face of the wafer, typically only 10 μm deep.

We started with 4" chrome masks made to our specifications by Photo Sciences, Inc. (Torrance, CA) and 3" silicon wafers ($\{100\}$, n-type) with 1000 nm of wet thermal SiO_2 grown on them. A primer (P-10M, MicroSi, Phoenix, AZ) was spun on at 3000 rpm. About one micron of AZ-1512 (Hoechst) photoresist was deposited by spin coating (3000 rpm), and this was followed by a soft bake (30 min at 90 C).

A contact aligner was used to align and expose wafers. Exposure time (nominally 15 seconds) was varied to yield best results. No post-exposure bake was done. Wafers were developed in AZ-351 (Hoechst) (diluted 4:1) for one minute, and rinsed in DI water. Blue tack tape (Semiconductor Equipment Corporation, Moorpark, CA) was applied to the backsides of the wafers to protect the oxide from the oxide etch.

The fluid channel mask was exposed first. After developing, the wafers were immersed in a buffered oxide etch (BOE, 10:1 HF (49%) and NH_4F (10%)) to etch away

600 nm of the unprotected oxide. The blue tack tape was removed by hand, and the photoresist was removed in an acetone rinse.

Wafers were again coated with photoresist and the fluid connection mask was aligned and exposed on the wafers. These wafers were developed and blue tack tape was once again applied to the backsides of the wafers to protect the oxide, and then immersed in the BOE to remove all the unprotected oxide. Blue tack tape and photoresist were removed as before.

Silicon etching was done in a mixture of ethylene-diamine, pyro-catechol, and water (EDP F-etch) set up in a reflux boiling flask. This etch attacks the {100} planes of silicon at a rate of about 100 μm per hour [8]. First fluid connection ports were etched through the wafer, then 400 nm of the oxide were removed in a BOE. Finally, flow channels between fluid connection ports were etched about 10 μm deep. After final processing the wafers were cleaned in a Piranha bath and rinsed in DI water.

We used anodic bonding to attach Pyrex glass to the silicon devices. We obtained 3" diameter pieces of Pyrex glass (100 μm thickness) from Esco Products Inc (Oak Ridge, New Jersey). First, the silicon and Pyrex glass were immersed in a solution of H_2O_2 , NH_4OH , and H_2O (1:4:6) heated to 50 C. This process removes any organic matter on the surfaces and also makes the surfaces hydrophilic. After 20 minutes in this solution, the silicon and Pyrex were placed in a rinser-dryer. Upon removing, the Pyrex glass was immediately placed into contact with the silicon wafer and set on a hot plate for anodic bonding. Bonding was done at 485 C with 800 V applied between the Pyrex and the silicon.

The Pyrex-silicon structures were diced into individual 1 cm by 1 cm devices by manually marking the silicon with a diamond tipped scribe and gently applying stress along the scratch. The Pyrex-silicon fractured along the silicon fault without problems.

Fluid connections were made to ports on the back side of the wafer. A glass tube (3 mm inner diameter, ~3 cm long) was epoxied around each fluid port. The flow was driven by a pressure difference between the entrance ports and the exit port. This pressure difference, equivalent to about 3 cm of H_2O , was sufficient to induce a mean flow velocity of greater than 100 $\mu\text{m}/\text{sec}$.

A small silicon device was fabricated to demonstrate the separation scheme. A typical image allowing one to monitor the flow in such a device is shown in Figure 5. A mixture of particles suspended in a carrier fluid enters a central channel from the bottom left and a diluting fluid enters from the bottom right. (See Figure 3.) The ratio of carrier fluid to

diluting fluid in the central channel is controlled by the pressure behind each of the two entrance channels.

At the exit of the central channel, the fluid is split into two separate flow streams. Since the flow is in the low-Reynolds number regime, this splitting can be done without any gross mixing. The volume ratio of these two can be set by the geometry of the exit channels. If the volume of the diluting fluid entering the channel is greater than the volume of the sample stream and the two exit streams are identical, a fluid barrier of width λ_b , is formed.

Any particles that enter on the upper left (the sample stream) and exit on the lower right (the filtered output) must diffuse across the fluid barrier. By controlling the length of the central channel and the flow rate inside it, the time, t , during which particles may diffuse across the barrier is determined. A critical parameter, $D_f = \lambda_b^2/t$, characterizes the device.

Results and Discussion

Microfluidic devices are inherently characterized by low-Reynolds number flow. The Reynolds number, $R_e = h v/\nu$, is determined by the velocity, v , and kinematic viscosity, ν , of the fluid and the characteristic size, h , of the channel. At $R_e \ll 1$, inertial effects in the flow are negligible, and therefore mixing by fluid transport is non-existent[6]. Because of this lack of fluid mixing, one can bring two fluids into contact and completely separate them smoothly, without mixing. This is something that is not possible at Reynolds numbers much greater than one.

Diffusion is a process that can easily be neglected at large scales, but rapidly becomes important at the micro-scale. The characteristic time, $\langle t \rangle$, for a molecule to diffuse across a distance, h is $\langle t \rangle = h^2/D$, where D is the diffusion coefficient. For a protein (hemoglobin, $D = 7 \times 10^{-7}$ cm²/s, in water at room temperature), it takes about 10⁶ s (10 days!) to diffuse across a 1 cm pipe, but only about 1 s to diffuse across a 10 μ m channel. Microfluid devices, with their micron-sized dimensions, can take advantage of diffusion as rapid separation mechanism.

The parameter that characterizes the device, $D_f = \lambda_b^2 v/h$, can easily be controlled from less than 10⁻⁹ cm²/s to greater than 10⁻⁴ cm²/s by varying the geometry of the structure and the flow rate. This range of diffusion coefficients includes even the smallest molecules. For instance, protons diffuse in room temperature water at about 10⁻⁴ cm²/s, and this is

the fastest diffusing ion. Tobacco mosaic virus (TMV), a large virus 300 nm in length, has a diffusion coefficient of about 5×10^{-8} cm²/s.

This method can be scaled up to arbitrarily large flow rates. Since the volumetric flow rate, $Q = vhw = R_e v w$, depends linearly on both R_e and w , the throughput can be increased without limit by simply increasing w . However, for a fixed w , the maximum throughput is set when the low Reynolds number flow conditions begin to break down. This critical Reynolds number depends on the specific geometry of the device and flow, but is somewhere in the range of $R_e = 1-100$. The maximum throughput per unit width is achieved in a device with dimensions of $\lambda_b^2/lh = D_f/(R_e v)$ where the volumetric flow rate is given by $Q/w = R_e v$. For a device intended to extract all molecules smaller than a typical protein ($D = 10^{-6}$ cm²/s) and operating at $R_e = 1$ in water at room temperature ($v = 100$ cm²/s), the ratio, $\lambda_b^2/lh = 10^{-8}$. A device with $\lambda_b = 0.1$ μm would be 10 cm long, have a depth of 10 μm and require a pressure drop of about 100 PSI. It would provide a flow rate of about 10 $\mu\text{l/s}$ per mm of width. We are currently fabricating larger devices in a variety of geometries to test these predictions.

Conclusions

Separation by diffusion can be used to allow sensitive analytical tests to be performed on microscopic amounts of fluid. This device can provide easily tunable separation for particles ranging in size from ions to large viruses. Unlike porous filters it will not clog or foul, since there is no membrane; it is reusable, and could readily be operated in a continuous flow mode. While the initial demonstration of this device was made using silicon, there is also no reason that it could not be implemented in materials that lend themselves to making much larger devices, allowing unlimited throughput. This suggests many potential applications beyond our primary goal of separation of small molecule analytes from whole blood for optical monitoring. For example, the device could be used for continuous separation of small molecules from cells in culture, and for hemodialysis, in which process the absence of a membrane could prove to be a substantial advantage.

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Table 1: Parameters for a range of biomolecules in a device 50 μm deep and 1 mm long flowing at a velocity of 10 mm/sec. ($R_e = 0.1$) This would be driven with a pressure drop of about 1 PSI/cm and result in a volume flow rate of 2 $\mu\text{l}/\text{sec}$ for each mm of width.

	MW (AMU)	D ($\mu\text{m}^2/\text{sec}$)	λ_b (μm)
TMV	$4 \cdot 10^7$	5	0.7
Myosin	$4 \cdot 10^5$	10	1
Hemoglobin	$6 \cdot 10^4$	70	2.6
Glycine	75	1000	10
O ₂	32	2000	14
Na ⁺	23	1000	10
H ⁺	1	9000	30

FIGURES

Figure 1. (a) A box containing a mixture of large and small molecules is placed adjacent to an empty box at $t < 0$. (b) When the wall between the two boxes is removed, the molecules begin to diffuse into the empty box, $0 < t < \tau$. (c) After some time, $t = \tau$, the wall is replaced between the two boxes a distance λ_b to the right of the original position. Particles that have a diffusion coefficient substantially less than λ_b^2/τ will be present at vastly reduced concentrations in the box on the right; however, particles with diffusion coefficients substantially larger than λ_b^2/τ will be in equal concentrations in both boxes.

Figure 2. (a) In low Reynolds number flow there is no mixing or secondary flows at the confluence of two channels. (b) The two flow streams move down the common channel without gross mixing. The smaller particles diffuse more quickly, and therefore can diffuse across the channel (a distance less than h) while the larger particles diffuse more slowly.

Figure 3. A fluid barrier is created in the central channel. The thickness of this barrier, λ_b , can be controlled by varying the ratio of the pressures driving the sample input and the dilutant input. Only particles that diffuse across this barrier before reaching the sample output channel will end up in the filtered output.

Figure 4. A schematic diagram of the silicon processing. (a) A 1 μm thick wet thermal oxide is grown on a 3" silicon wafer. (b) This oxide is photolithographically patterned with the flow channels and etched to a depth of 600 nm. (c) The wafer is recoated with photoresist and patterned with the through-hole connections. This oxide is completely removed from this pattern. (d) EDP etching is done to etch completely through the wafer (approximately 400 μm). (e) An oxide etch is performed to uniformly remove 400 nm of oxide from the wafer. (f) The flow channels are etched into the silicon approximately 10 μm deep. (g) The wafer is anodically bonded to a 3" disk of Pyrex glass.

Figure 5. A scanning electron micrograph of the silicon portion of the final microstructure. Silicon wafers ((100) crystal orientation) were patterned using standard photolithography techniques and etched in ethylene-diamine, pyrocatechol, and water (EDP). Fluid connections were made to ports on the back side of the wafer. A glass tube (3 mm inner diameter, ~3 cm long) was epoxied around each fluid port. The flow was driven by a pressure difference between the entrance ports and the exit port. This pressure difference, equivalent to about 3 cm of H_2O , was sufficient to induce a mean flow velocity of $>100 \mu\text{m}/\text{sec}$.

Figure 6. Fluorescence optical micrographs of operating microfabricated separation devices. Observations were made with a Zeiss ICM-405 inverted microscope and recorded with a Dage 66 silicon intensified target video camera. The dry device was wetted with isopropyl alcohol and any trapped air bubbles were removed by applying approximately 70 kPa of pressure. Then a mixture of water, the small fluorescent dye carboxyfluorescein (CF), and 0.5 μm diameter fluorescent spheres (Duke Scientific) was introduced into one of the fluid entrance ports. Pure water was introduced at the other entrance port. (a) A fluorescence image of a flowing system in which 0.5 μm spheres and free CF in water are flowing from the bottom left and pure water from the bottom right into the central channel. The CF diffuses throughout the central channel and some flows to the channel on the top right. All the 0.5 μm spheres flow to the top left. (b) An experiment similar to that shown in (a), but in which free CF was omitted. Note that all the spheres stay on the left half of the channel and travel up the left arm. In this device the sample output was recombined with the filtered output to insure that both the sample output and filtered output arms had the same geometry and pressures. Note that reflections from the tilted walls of the channels make it appear that some fluorescent material is on the fluorescence-free side of the channel near the entrance.

Figure 7. The calculated response and measured results of diffusion based separation. The curve represents equation (2) and the five points were measured in different experiments. Points 1 and 5 represent 0.5 μm spheres and CF molecules, respectively, with the parameters $\lambda_b=1 \mu\text{m}$ and $\tau=0.1 \text{ sec}$. Points 2, 3, and 4, were measured in a different device. The parameters were as follows (2) 0.5 μm spheres, $\lambda_b=6 \mu\text{m}$, $\tau=10 \text{ sec}$; (3) 0.026 μm spheres, $\lambda_b=$

15 μm , $\tau = 10$ sec, (4) fluorescein, $\lambda_b = 30$ μm , $\tau = 10$ sec. All distance measurements were ± 1 μm .

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