Generating fixed concentration arrays in a microfluidic device

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Abstract

We have designed and built a laminar microfluidic diffusion diluter (\muDD) to obtain fixed concentration gradients inside lithographically patterned lab-on-a-chip architectures. The driving force for this investigation was the desire to minimize the amount of precious analyte consumed in high throughput measurements performed as a function of concentration. This was achieved by engineering a microfluidic system capable of delivering minute volumes of analyte by very slow pressure-driven flow. The \muDD consists of a Y-junction that allows inflow of two different streams into a main channel, which eventually splits into a linear array of independent microchannels. The arraying technique is based on convective/diffusive transport of nanoliter quantities of an analyte from one fluid stream into the other. The \muDD design allows output channels to exhibit predetermined analyte concentration values, which can be controlled by regulating the flow rate. Experiments were performed for flow rates ranging from 500 to 50 nl/min. Theoretical studies of convective/diffusive transport in the main channel have been performed as a function of the Peclet number and the normalized channel dimensions. These results were validated using fluorescence microscopy experiments as well as two- and three-dimensional numerical simulations. The computational results compared well with the experimental measurements, validating the \muDD design.

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1. Introduction

Microfluidic technology has attracted increasing interest over the last decade. The impetus behind recent advances such as microvalves [1–5], surface modification [6–11], unique geometries [12,13], serial and parallel diluters [14–16], as well as 3D lithography [17–19] has been the need to control the movement and mixing of minute quantities of fluids quickly and precisely at low Reynolds numbers. One particularly intriguing achievement was the development of the T-sensor [20–22] and similar devices [23–25]. This technique combines two flowing tributaries into a single stream and allows the contents to mix by diffusion as the liquids progress down the channel. Fluorescent tags or calorimetric changes can then be exploited to monitor analytes as a function of chemical or physical properties at any point along the diffusion gradient.

In the present work we exploit diffusional mixing inside a flowing stream as a first step in a procedure to permanently separate chemically distinct aqueous solutions into a series of parallel channels. We refer to this process as \muDD. The purpose of this technique is to isolate arrays of analytes for (1) surface patterning, (2) heterogeneous assay development, and (3) the creation of multidimensional screens for combinatorial chemistry. The general concept is illustrated schematically in Fig. 1a. Two streams of liquid are combined at a junction and allowed to diffuse into each other as they flow downstream side by side. After a fixed distance, they are partitioned into a series of smaller channels that emanate from the main flow stream. The shape of the concentration gradient formed by this process was studied as a function of flow rate using (1) experiments performed in glass microchips and (2) computational fluid dynamics simulations.

2. Theory

2.1. Analysis of convective diffusive transport in straight channels

Incompressible fluid flow and convective/diffusive transport of a passively advected scalar field are governed by the
2.2. Three dimensional velocity field and concentration distribution

CFD-ACE+ (CFD Research Corporation, Huntsville, Alabama) was used to obtain three-dimensional velocity and concentration distributions in the main channel. Simulations were performed for a volumetric flow rate of 500 nl/min and normalized concentration values of Θ = 0 and 1 across half of the channel width, respectively. The channel width is 500 µm, while its height is ~6 µm; hence, the channel aspect ratio (width to height ratio) was ~90. For such large aspect ratios, mostly two-dimensional streamwise velocity (u) distribution is observed, as shown in Fig. 2a. The streamwise velocity profile is mostly uniform in the spanwise direction (v), except very close to the side walls it decays to zero to comply with the no-slip boundary conditions. Streamwise velocity (u) variation is parabolic across the height of the channel (z-direction), typical of pressure driven flows between two parallel plates. The velocity components along the y- and z-directions are negligible.

Concentration variations obtained from three-dimensional simulations are shown in Fig. 2b. The concentration distribution on the lateral plane in the middle of the channel is shown, as well as several vertical cross sections. Slight transverse diffusive broadening is observed only very near the entry to the main channel [26,27]. Even at small distances away from entry, the concentration contours shown in Fig. 2b indicate no variations along the height of the channel. Therefore, diffusion mainly takes place along the streamwise and spanwise direction and the concentration profile can be assumed to be two-dimensional, and transverse diffusive broadening is negligible for high aspect ratio channels [26,27].

2.3. Analytical model for concentration distribution

Eqs. (1)–(3) can be simplified by assuming steady (∂u/∂t = 0 and ∂Θ/∂t = 0), fully developed flow (∂u/∂x = 0) conditions. The latter approximation is valid due to the simple geometry, where the flow development effects are confined to a very small portion of the channel inlet for small Reynolds number flows (in the present case: Re < 1.389). In addition, the main velocity component is in the streamwise direction while the spanwise and cross flow velocity components are negligible (i.e. \( \vec{u} \approx u_e \hat{x} \), where \( e_x \) is the unit vector in the streamwise direction).

In regard to species transport, mainly convective/diffusive transport was observed in the spanwise and streamwise directions. Fig. 2a shows that steady flow species transport in large aspect ratio channels is not affected by the local velocity distribution. Hence, it can be assumed that streamwise velocity (u) in the species transport Eq. (2) can be approximated by the channel averaged streamwise velocity (\( \bar{u} \)). Under these simplifications, the steady species transport
equation is reduced to:

\[
\frac{\bar{u}}{u} \frac{\partial \Theta}{\partial x} = \frac{1}{Pe} \left[ \frac{\partial^2 \Theta}{\partial x^2} + \frac{\partial^2 \Theta}{\partial y^2} \right]
\]

where \( \bar{u} \) is normalized with the reference velocity \( U \). Since the channel-averaged velocity is a constant over the entire domain, without a loss of generality, it can be stated that \( U = \bar{u} \).

A further simplification can be made using an order of magnitude analysis. Namely, the streamwise diffusion was negligible compared to the spanwise diffusion (i.e. \( \frac{\partial^2 \Theta}{\partial x^2} \approx \frac{\partial^2 \Theta}{\partial y^2} \)) [28]. Hence, Eq. (4) can be reduced to the following form:

\[
\frac{\bar{u}}{u} \frac{\partial \Theta}{\partial x} = \frac{1}{Pe} \frac{\partial^2 \Theta}{\partial y^2}
\]
An analytical solution of (5) was obtained for half of the channel using a separation of variables technique. The incoming fluid had concentration value of $\Theta_0$, while at the channel center ($y = h/2$), $\Theta_c = \Theta_0/2$. This value was fixed along the entire channel ($0 \leq x \leq L$), due to the presence of a zero-concentration equal flow rate stream on the upper half of the domain. On the wall ($y = 0$), zero Neumann conditions ($\partial \Theta / \partial y = 0$) are assumed. Based on these boundary conditions, the analytical solution of (5) becomes:

$$\frac{\Theta - \Theta_c}{\Theta_0 - \Theta_c} = \sum_{n=0}^{\infty} \frac{2(1)^n}{(n + (1/2))\pi} \exp\left(-\left(n + \frac{1}{2}\right)^2 \frac{4x}{hPe}\right) \times \cos\left(\left(n + \frac{1}{2}\right) \pi \frac{2y}{h}\right)$$

(6)

Using Eq. (6), streamwise concentration variations were identified as a function of the diffusion distance $x$, the $Pe$ number and the width of the channel ($h$), which together form a non-dimensional parameter:

$$\kappa = \frac{x}{hPe} = \frac{xa}{h^2u}$$

(7)

In Fig. 3a, a typical concentration variation in the spanwise direction across half of the main channel ($\kappa = 0.0052$) is presented. This was obtained by three- and two-dimensional solutions of Eqs. (1)–(3) using CFD-ACE+ and our spectral element algorithm [28], as well as the analytical solution given by (6). Concentration variations in the spanwise direction obtained with an increased level of simplifications are similar to the three-dimensional solution. Detailed comparisons between the three- and two-dimensional simulations and the analytical model can be found in [29].

Local values of $\kappa$ were used to determine the concentration variation at any location in the channel. Since zero Neumann conditions on the walls are specified, the wall concentration value was also determined using the analytical (or numerical) solution. Fig. 3b shows the variation of the side wall concentration ($\Theta_0/\Theta_c$) as a function of $\kappa$. This figure was obtained by a superposition of five different simulations at various $Pe$ numbers, and also by evaluating the analytical solution at $y = 0$. Due to the problem definition, the normalized right and left wall concentrations were related to each other in the following form:

$$\frac{\Theta}{\Theta_0|_{right-wall}} = 1 - \frac{\Theta}{\Theta_0|_{left-wall}}$$

In addition, a unique concentration profile exists for any given $\kappa$. Fig. 3b shows the normalized concentration distribution obtained at various $\kappa$ values. The symbols and lines show the analytical results and the two-dimensional spectral element solutions, respectively. Concentration profiles obtained in this section were used to evaluate the inlet conditions for numerical simulations of the μDD device.

3. Experimental section

3.1. Glass microchip fabrication

Standard 50 mm × 75 mm soda-lime microscope slides were spin coated with Microposit S1813 photoresist (Shipley, Marlborough, MA) to a thickness of ~7 μm and baked in a convection oven at 90 °C for 1 h. The photomask was produced by reducing a negative image printed from a 1200 dpi laser printer onto Kodak technical pan photographic film (which ultimately served as the contact photomask) using a Pentax K100 camera fitted with an SMC Pentax-A 1:2 50 mm lens. Samples were exposed using a Quintel 6000 mask aligner and developed in a 1:1 solution of Microposit developer concentrate (Microchem) and DI water. Slides were etched and bonded using a process adapted from Lin et al. [30]. Photopatterned slides were gently wafted by hand in a buffer oxide etchant (BOE) solution (1:6 ratio of 48% HF:200 g NH$_4$F in 300 ml DI water) for 2.5–3 min and washed in a 1 M HCl solution for 20 s. The dimensions of the etched pattern are shown in Fig. 4. Inlet holes 1 mm in diameter were drilled into the glass using a diamond coated drill bit (Wale Apparatus). A 25 mm × 37.5 mm planar soda-lime glass slide section was used as a cover for the channel system. Covers and etched
were attached on each side of the block behind the bearings to extend the overall length of the pusher block by 6.4 cm. Additional linear bearings were fitted to the rear of the new aluminum blocks to give the pusher block assembly a total of four linear bearings. Furthermore, the stock guide rails were replaced with precision-machined steel rails (PIC Design), which were exactly matched to the linear bearings. Finally, a custom adapter was machined to fit to the front of the pusher block, which had thumbscrew fittings that directly attached the syringe plunger to the block. These modifications eliminated slip–stick friction and pulsation under slow flow conditions. The modified pump was fitted with two 100 µl syringes (Hamilton, Reno, NV) and PTFE lines were attached with PEEK (1/4)-20:10-32 fingertight adapters (Upchurch, Oak Harbor, WA) and run to a homemade manifold for interfacing with the glass chips.

3.3. Fabrication of chip holder and flow rate experiments

A Plexiglas manifold was machined with two inlet ports into which Teflon lines and PEEK fingertight fittings were inserted. This manifold was fastened to an aluminum chip clamp. A screw mounted over each outlet from the manifold clamped the chip into place with the inlet holes directly over the manifold outlet. The pressure from porous Teflon pads at the end of the clamp screw pressed the chip into place with the inlet holes directly over the manifold outlet. The pressure from porous Teflon pads at the end of the clamp screw pressed the chip against Teflon orings and flattened them slightly to form a high-pressure seal. The Plexiglas/aluminum chip assembly was mounted onto the stage of an E800 microscope (Nikon). Using the modified syringe pump, the flow rate was varied and images were taken using a Micromax 1024 CCD camera (Princeton Instruments) atop the microscope. Data was collected using Metamorph software and normalized using Sigmaplot.

4. Results

4.1. Dilation experiments in glass microchips

To demonstrate the arrayer concept, the modified syringe pump was used to inject an aqueous green fluorescent dye, Alexa 488, and an aqueous red fluorescent dye, Alexa 594, by positive pressure (100 nl/min) into the two inlet ports of the device. As the pump infused the dyes, diffusional mixing occurred over a ~22 mm path length before the liquid stream was separated into a series of 23 parallel microchannels. The array was imaged by epifluorescence microscopy (Fig. 1b). As can be clearly seen, the concentration gradient present at the end of the main channel was continuously isolated and maintained in the microchannel array. The channel on the far left-hand-side contained mostly Alexa 488 while the channel on the far-right contained mostly Alexa 594. In between, the channels held a stepwise gradient of concentrations. This is shown quantitatively in Fig. 1c by the line profile of fluorescence intensities.
To investigate the effect of flow rates on the distribution of Alexa 594, the pump was run at several speeds varying from 50 to 500 nl/min. In this case an 11 channel device was employed to accommodate fluid dynamic calculations, which became increasingly time consuming as more channels were added. The line profiles for four flow rates are shown in Fig. 5. At the highest rate, the concentration was only moderately distributed across the series of channels. From right to left, the concentration of the dye molecule increased from background noise to its maximum across only seven channels. This was representative of how a high flow rate only allows for minimal diffusion. The difference between the channels with the highest and lowest dye concentrations was at least three-orders of magnitude and probably more. The ability to place an upper boundary on this value was ultimately limited by the dynamic range of our CCD camera. Slowing the flow rate to 250 nl/min spread the dye over 8 of the 11 channels and 100 nl/min covered 10 microchannels. Finally, at 50 nl/min, the Alexa 594 had migrated to cover all the channels. Fits to this data (shown...
4.2. Computational modeling

The convective/diffusive transport problem was divided into two different parts: the main channel and the microchannels. The main channel section was modeled using the analysis presented in the theory section. On the other hand, the microchannel array was simulated using a two-dimensional spectral element algorithm, which is essentially a high-order finite element method [28]. Eqs. (1)–(3) were solved on a computational domain, geometrically similar to the actual μDD. Details of the computational domain and the independent grid studies can be found elsewhere [29].

For comparisons of numerical and experimental results, the experimental data were normalized by using the maximum fluorescence intensity in the main channel obtained from the 500 nl/min case. The normalized fluorescence intensity distribution at the entry of the μDD (at x = 21.4 mm) is shown in Fig. 6. Comparisons of the experimental measurements with theoretical analysis required accurate predictions for the diffusion coefficient for the Alexa 594 dye. In order to determine the diffusion coefficient, the measured fluorescence intensity for the 500 nl/min case was matched with the theoretical model given by Eq. (6). The concentration distribution at $\kappa = 0.002$ matched the normalized intensity measurements quite well. Using this one-point matching at $\kappa = 0.002$, as well as the channel averaged velocity ($2.78 \text{ mm/s}$) and the channel width (500 μm), the $Pe$ was calculated to be 21,400 from Eq. (7). This value of $Pe$ requires a molecular diffusion coefficient of $x = 6.5 \times 10^{-11} \text{ m}^2/\text{s}$. In Table 1, the $\kappa$ values, $Re$ and $Pe$ numbers obtained for all the flow rates (500–50 nl/min) are shown. Agreement between the experimental measurements and the theoretical results was observed over a wide range of flow rates. This validates the consistency between the numerical results and the experimental measurements.

The theoretically obtained concentration variations were utilized as the inlet (boundary) conditions for numerical simulations of the μDD as well as for the calculated concentration profiles in the output channels (bars in Fig. 5). For all cases, experimentally measured fluorescence intensity values were normalized against the fluorescence intensity of the 500 nl/min flow rate. The final concentration profile in the 500 nl/min case did not diffuse much after entering the microchannels. This was expected, since the $Pe$ number was quite high and the process was almost entirely convection dominated. At 500 nl/min, the first three microchannels experience concentration values close to $\Theta = 0$, while the last four microchannels have values near $\Theta = 1$. The results for the 250, 100 and 50 nl/min cases are also shown in Fig. 5. As expected, the diffused zone in the middle of the micro

![Fig. 6. Main channel analysis. The gray squares represent fluorescence linescans across the width of the main channel and the lines are the predicted concentration profiles.](image-url)
channels grows with decreased flow rate. Overall, the computational results closely follow the experimental measurements. Hence, it is possible to generate a desired concentration variation in the μDD, and theoretical and numerical models can be used to accurately predict the underlying physics and μDD performance.

5. Discussion

In the experiments presented above, a right angle geometry was employed to separate the main channel into distinct microchannels. Though other geometries were tested, this particular layout proved superior because the streamwise velocity remained constant throughout the main channel and microchannels. Furthermore, the design enabled efficient packing of the device onto a relatively small rectangular chip. As mentioned above, this design has consequences for the distribution of concentrations in the microchannels. Namely, the inflection point of the concentration distribution in the microchannels does not center around the middle microchannel, even though the concentration distribution is symmetric in the main channel. In fact, the inflection point is closer to the first microchannel in all cases. This asymmetry arises from the fact that the left wall of the main channel was 3 mm (black region in Fig. 4) longer than the right side. However, this makes no practical difference in terms of assay development, since the analyte concentrations can be calculated or quantified experimentally in each channel by fluorescence measurements.

Myriad chemical and biochemical processes are concentration dependent. This fact has traditionally forced researchers to undertake the laborious task of preparing serial dilutions for step-by-step analysis of concentration dependent phenomena. Recently, several research groups have constructed various on-chip dilution devices to circumvent this problem [14–16]. The first approach by Ramsey and coworkers used electrokinetically driven methods. This device is limited to conducting solutions and may lead to sample damage by Joule heating in certain cases. Whitesides and coworkers designed a pressure driven device that employed a branching mechanism. This required an entire mixing row for every new channel added to the array. Although such devices are capable of producing linear concentration gradients, they require long mixing lengths, have comparatively large dead volumes, and consume relatively large amounts of precious analyte due to fast flow rates.

The development of μDD was motivated by the need to obtain the maximum amount of data from the minimum amount of sample. Indeed, this technique can be expanded to probe a variety chemical systems [8,32]. For example, the array-based field of proteomics could benefit from μDD because of its capability to collecting massive amounts of data from a few microliters of newly isolated protein. Specifically, glass microchips could be employed to investigate the various concentration dependent behaviors of protein/ligand interactions and, thus, rapidly obtain kinetic and thermodynamic parameters. This could be done by creating a concentration gradient of protein over a chemically modified ligand-containing surface to study binding affinity.

6. Conclusions

We have designed and characterized a microfluidic diffusion diluter for the combinatorial study of concentration dependent phenomena. In high aspect ratio systems, it is reasonable to simplify three-dimensional numerical simulations to two dimensions, since diffusion dominates spanwise transport and convection dominates streamwise transport of analyte. We have developed a non-dimensional parameter \( \kappa \), which describes this process and allows the efficient prediction of concentration values in the μDD.

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