The Concrete Mathematics of Microfluidic Mixing, Part I*

UNM TR-CS-2006-09

Jennifer Sager, Maxwell Young, and Darko Stefanovic
Department of Computer Science
University of New Mexico
Albuquerque, NM 87131

May 1, 2006

*Supplementary Material to *Characterization of Transverse Channel Concentration Profiles Obtainable With a Class of Microfluidic Networks*, published in *Langmuir*, 22(9), 4452–4455 (2006)

Abstract

We analyze mathematically a previously reported class of passive microfluidic mixing networks. The networks produce nonhomogeneous concentrations in the output channel, resulting in diverse concentration profiles. We formally prove that all profiles obtainable with this class of networks can be described as polynomials of degree no higher than the number of input channels less one. We derive explicit formulas for the calculation of resultant output concentration profiles and conversely for the calculation of input concentrations needed to obtain set output profiles.

1 Introduction

Microfluidic technology presents the opportunity for low-cost fabrication of sophisticated reaction assemblies in which chemical and biochemical reactions, including open-system reactions, can be performed with very small reactant volumes and with high volumetric accuracy. For instance, microfluidic assemblies have found uses in the design of reaction chambers for DNA computing [14, 15, 4]. Fluid flow in microfluidic channels is entirely laminar, owing to typical channel cross-sections, flow velocities, and fluid properties. Therefore, when two miscible flows are merged into a common channel, they mix only by diffusion. This means that mixing is generally slower than with turbulent flows and special care must be taken to achieve complete mixing of flows (assuming this is desired). On the other hand, the geometries of microfluidic channels and laminar flow permit the diffusion to be described accurately by relatively simple and tractable equations. Consequently, it is possible to calculate the requisite channel length such that two fluids entering the channel side by side unmixed leave the channel essentially completely mixed. (See Stroock [11] for an improvement that induces chaotic flow by means of a herringbone channel floor pattern; Hardt [5] is a recent review of such passive mixing techniques. One can also use active folding mixing in a rotary mixing chamber [1], or mixing by means of folding in oil droplets [12].)

But what if our goal is not to achieve a completely homogeneous mixture at the end of the mixing channel, but rather a deliberately nonhomogeneous one? Recently, Whitesides' group demonstrated a microfluidic network that produces a non-uniform concentration profile in the output channel, measured in the cross-section transverse to the flow. Their contribution was described in multiple publications. First, Jeon et al. [7] obtained a gradient, i.e., a roughly linear dependence of concentration on the transverse coordinate x across the output channel. Second, Dertinger et al. [2] obtained either a roughly linear dependence or a roughly quadratic dependence, depending on the particulars of the microfluidic network. Third, significant applications of non-uniform concentration profiles were described [6, 3, 8].

The microfluidic network they designed is shown schematically in Figure 1. The network consists of k stages, and has p inlets and $p + k$ outlets. Each stage splits n flows into $n + 1$ flows, for $n = p, \ldots, p + k - 1$. It is assumed that the channels are fabricated with a degree of precision that allows all channel widths at the same level, and consequently all flows at the same level, to be assumed equal. The splitting of inlet flows in a stage is simple because the flow is perfectly laminar. Each inlet flow is split into exactly two outlet flows. Each outlet flow is a combination of exactly two inlet flows except for the two extremal outlets, each of which carries the unmixed flow from its corresponding extremal inlet. After the splitting, complete mixing [7, 11] occurs in the long and narrow serpentine channels.

Dertinger et al. report that they "numerically simulated" their mixing model and "found empirically" that for a mixing network of the above design with p inlets the calculated profile agrees with a polynomial of degree $p - 1$. This is supported by their laboratory results for $p = 2$ (concentration varies roughly linearly across the channel, as

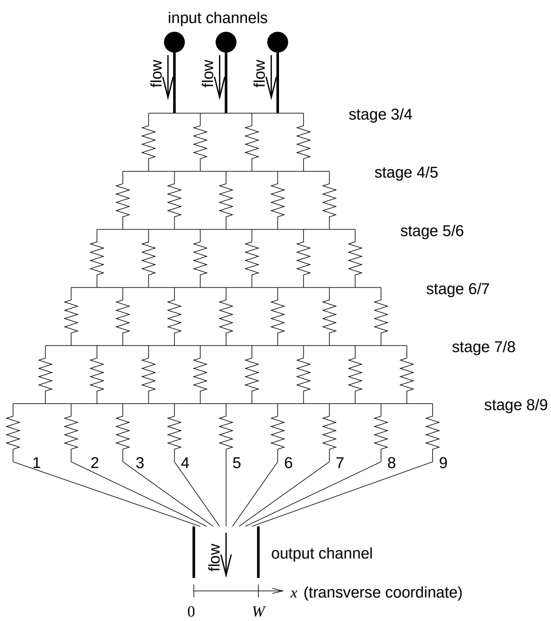

Figure 1: A microfluidic mixing network (after Jeon et al. [7]) with $p = 3$ inlets to the first stage, $k = 6$ stages, and $p + k = 9$ outlets from the final stage.

evidenced in fluorescence micrographs) and $p = 3$ (concentration varies roughly parabolically across the channel).

Each outlet of the final stage carries a homogeneous flow. Thus, the transverse profile of the concentration (the x-direction in Figure 1) is a staircase function, which can be viewed as the sampling at $p + k$ uniformly spaced points of some target function. Now, any p samples uniquely determine a polynomial of degree $p - 1$, but the network as described has k more samples, and whereas one may wish to choose all $p + k$ samples freely, it appears that they cannot be independently chosen. Formally, we shall term *Dertinger's conjecture* the statement that *all* $p + k$ samples conform to a unique polynomial of degree $p - 1$. More precisely: for a given mixing network specified by parameters p and k, the concentrations in the $p + k$ network outlets, expressed as a function of the x coordinate at $p + k$ discrete points, are all described by a polynomial of degree $p - 1$.

It is not intuitively clear why the network design of Figure 1 should yield polynomial profiles. Indeed, it turns out it is surprisingly difficult to prove that this is the case. The primary contribution of this paper is a proof of Dertinger's conjecture. Our proof is divided into three parts. First, we describe the effect of a single stage of the microfluidic mixing network using a transfer matrix and develop a closed-form solution for the aggregate transfer matrix of multiple successive stages. Second, we derive a formula for finite differences over the columns of the aggregate transfer matrix. Third, we prove that a particular-order finite difference of that matrix is everywhere zero. As we detail below, these three steps suffice to prove the conjecture.

Our proof shows that a mixing network of the Whitesides' group's design does indeed always result in a sampling of a polynomial transverse profile of concentration in the output channel (or more precisely, at the very entrance of that channel before diffusion has smeared it). The number of input channels p determines the degree of the polynomial, and the number of outlets of the final stage, $p+k$, determines the granularity of sampling.

We also explicitly develop an expression for the resultant polynomial profile, and, conversely, show how to compute the requisite input channel concentrations for a given desired polynomial output profile.

2 Proof

2.1 Transfer matrix product

The transfer matrix for a flow-splitting stage with n inlets and m outlets describes how the flows are split and mixed. If the concentrations of a particular solute in the n inlets are grouped into a column vector $\mathbf{c}^{\text{in}}$ of n values and concentrations in the m outlets are grouped into a column vector $\mathbf{c}^{\text{out}}$ of m values, then we have $\mathbf{c}^{\text{out}} = \mathbf{T}_{m,n}\mathbf{c}^{\text{in}}$, where $\mathbf{T}_{m,n}$ is the transfer matrix.

Each row of a transfer matrix gives the composition of a single outlet flow in terms of the inlet flows. Conversely, each column of a transfer matrix describes how a single inlet flow is distributed across the outlet flows.

Restating Dertinger's analysis [2] in matrix form, the transfer matrix for a single stage with p inlets and $p+1$ outlets is a $(p+1) \times p$ band matrix:

$$\mathbf{M}_{p+1,p} = \begin{bmatrix} 1 & 0 & \cdots & & & \\ \frac{1}{p} & \frac{p-1}{p} & 0 & \cdots & & \\ 0 & \frac{2}{p} & \frac{p-2}{p} & 0 & \cdots & \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ & \cdots & 0 & \frac{p-2}{p} & \frac{2}{p} & 0 \\ & & \cdots & 0 & \frac{p-1}{p} & \frac{1}{p} \\ & & & \cdots & 0 & 1 \end{bmatrix}$$

That is, the elements of $M_{p+1,p}$ are given by:

$$m_{i,j}^{p+1,p} = \frac{1}{p} \cdot \begin{cases} i-1 & \text{if } j = i-1 \\ p-i+1 & \text{if } j = i \\ 0 & \text{otherwise} \end{cases} \tag{1}$$

The transfer matrix for k stages, where the first stage has p inlets and the final stage has $p+k$ outlets, is given by the product of k single-stage matrices:

$$\mathbf{T}_{p+k,p} = \mathbf{M}_{p+k,p+k-1} \cdots \mathbf{M}_{p+1,p}$$

It is a $(p+k) \times p$ band matrix. The elements of $\mathbf{T}_{p+k,p}$ are given by:

$$t_{i,j}^{p+k,p} = \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}}$$

Example: For the network of Figure 1, we have:

$$\mathbf{M}_{4,3} = \begin{bmatrix} 1 & 0 & 0 \\ \frac{1}{3} & \frac{2}{3} & 0 \\ 0 & \frac{2}{3} & \frac{1}{3} \\ 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{M}_{5,4} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ \frac{1}{4} & \frac{3}{4} & 0 & 0 \\ 0 & \frac{2}{4} & \frac{2}{4} & 0 \\ 0 & 0 & \frac{3}{4} & \frac{1}{4} \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{M}_{6,5} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ \frac{1}{5} & \frac{4}{5} & 0 & 0 & 0 \\ 0 & \frac{2}{5} & \frac{3}{5} & 0 & 0 \\ 0 & 0 & \frac{3}{5} & \frac{2}{5} & 0 \\ 0 & 0 & 0 & \frac{4}{5} & \frac{1}{5} \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{M}_{7,6} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ \frac{1}{6} & \frac{5}{6} & 0 & 0 & 0 & 0 \\ 0 & \frac{2}{6} & \frac{4}{6} & 0 & 0 & 0 \\ 0 & 0 & \frac{3}{6} & \frac{3}{6} & 0 & 0 \\ 0 & 0 & 0 & \frac{4}{6} & \frac{2}{6} & 0 \\ 0 & 0 & 0 & 0 & \frac{5}{6} & \frac{1}{6} \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{M}_{8,7} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{1}{7} & \frac{6}{7} & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{2}{7} & \frac{5}{7} & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{3}{7} & \frac{4}{7} & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{4}{7} & \frac{3}{7} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{5}{7} & \frac{2}{7} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{6}{7} & \frac{1}{7} \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{M}_{9,8} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ \frac{1}{8} & \frac{7}{8} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \frac{2}{8} & \frac{6}{8} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{3}{8} & \frac{5}{8} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{4}{8} & \frac{4}{8} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{5}{8} & \frac{3}{8} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{6}{8} & \frac{2}{8} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \frac{7}{8} & \frac{1}{8} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

$$\mathbf{T}_{9,3} = \mathbf{M}_{9,8}\mathbf{M}_{8,7}\mathbf{M}_{7,6}\mathbf{M}_{6,5}\mathbf{M}_{5,4}\mathbf{M}_{4,3} = \begin{bmatrix} 1 & 0 & 0 \\ \frac{3}{4} & \frac{1}{4} & 0 \\ \frac{15}{28} & \frac{3}{7} & \frac{1}{28} \\ \frac{5}{14} & \frac{15}{28} & \frac{3}{28} \\ \frac{3}{14} & \frac{4}{7} & \frac{3}{14} \\ \frac{3}{28} & \frac{15}{28} & \frac{5}{14} \\ \frac{1}{28} & \frac{3}{7} & \frac{15}{28} \\ 0 & \frac{1}{4} & \frac{3}{4} \\ 0 & 0 & 1 \end{bmatrix}$$

Theorem 1. *The elements of $T_{p+k,p}$ are given by:*

$$t_{i,j}^{p+k,p} = \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}}$$

The reasoning that led us to this form is sketched below in Section 2.1.1.

Proof. We proceed by induction on k.

The base case, when $k = 1$, is trivially true because $T_{p+1,p} = M_{p+1,p}$. Thus for the base case, we have:

$$t_{i,j}^{p+1,p} = m_{i,j}^{p+1,p}$$

After substituting Equation 1 for the right hand side, we obtain:

$$\begin{aligned} t_{i,j}^{p+1,p} &= \frac{1}{p} \cdot \begin{cases} i-1 & \text{if } j = i-1 \\ p-i+1 & \text{if } j = i \\ 0 & \text{otherwise} \end{cases} \\ &= \frac{\binom{p-1}{j-1}\binom{1}{i-j}}{\binom{p}{i-1}} \end{aligned}$$

Now assume by the inductive hypothesis that the theorem is correct and let $1 \le h \le p+k$.

$$\begin{aligned} t_{i,j}^{p+k+1,p} &= t_{i,h}^{p+k+1,p+k} t_{h,j}^{p+k,p} \text{ from the matrix multiplication} \\ &= m_{i,h}^{p+k+1,p+k} t_{h,j}^{p+k,p} \end{aligned}$$

Since the only non-zero elements of $m_{i,h}^{p+k+1,p+k}$ are $m_{i,i-1}$ and $m_{i,i}$:

$$\begin{aligned} t_{i,j}^{p+k+1,p} &= \frac{i-1}{p+k} t_{i-1,j}^{p+k,p} + \frac{p+k-i+1}{p+k} t_{i,j}^{p+k,p} \\ &= \frac{i-1}{p+k} \cdot \frac{\binom{p-1}{j-1}\binom{k}{i-1-j}}{\binom{p+k-1}{i-2}} + \frac{p+k-i+1}{p+k} \cdot \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}} \\ &= \frac{\binom{p-1}{j-1}\binom{k+1}{i-j}}{\binom{p+k}{i-1}} \end{aligned}$$

□

2.1.1 Intuition

Here we show how we arrived at the formula $t^{p+k,p}_{i,j} = \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}}$ as a hypothesis for the closed form of transfer matrix product.*

If we write out the transfer matrix products in a very specific form, we can see a pattern emerge. In the following, parentheses are used to emphasize the pattern.

$$\mathbf{T}_{9,8} = \frac{1}{8}\begin{bmatrix} 1(8) & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1(1) & 1(7) & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1(2) & 1(6) & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1(3) & 1(5) & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1(4) & 1(4) & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1(5) & 1(3) & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1(6) & 1(2) & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1(7) & 1(1) \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1(8) \end{bmatrix}$$

$$\mathbf{T}_{9,7} = \frac{1}{8\cdot 7}\begin{bmatrix} 1(8\cdot 7) & 0 & 0 & 0 & 0 & 0 & 0 \\ 2(1)(7) & 1(7\cdot 6) & 0 & 0 & 0 & 0 & 0 \\ 1(2\cdot 1) & 2(2)(6) & 1(6\cdot 5) & 0 & 0 & 0 & 0 \\ 0 & 1(3\cdot 2) & 2(3)(5) & 1(5\cdot 4) & 0 & 0 & 0 \\ 0 & 0 & 1(4\cdot 3) & 2(4)(4) & 1(4\cdot 3) & 0 & 0 \\ 0 & 0 & 0 & 1(5\cdot 4) & 2(5)(3) & 1(3\cdot 2) & 0 \\ 0 & 0 & 0 & 0 & 1(6\cdot 5) & 2(6)(2) & 1(2\cdot 1) \\ 0 & 0 & 0 & 0 & 0 & 1(7\cdot 6) & 2(7)(1) \\ 0 & 0 & 0 & 0 & 0 & 0 & 1(8\cdot 7) \end{bmatrix}$$

$$\mathbf{T}_{9,6} = \frac{1}{8\cdot 7\cdot 6}\begin{bmatrix} 1(8\cdot 7\cdot 6) & 0 & 0 & 0 & 0 & 0 \\ 3(1)(7\cdot 6) & 1(7\cdot 6\cdot 5) & 0 & 0 & 0 & 0 \\ 3(2\cdot 1)(6) & 3(2)(6\cdot 5) & 1(6\cdot 5\cdot 4) & 0 & 0 & 0 \\ 1(3\cdot 2\cdot 1) & 3(3\cdot 2)(5) & 3(3)(5\cdot 4) & 1(5\cdot 4\cdot 3) & 0 & 0 \\ 0 & 1(4\cdot 3\cdot 2) & 3(4\cdot 3)(4) & 3(4)(4\cdot 3) & 1(4\cdot 3\cdot 2) & 0 \\ 0 & 0 & 1(5\cdot 4\cdot 3) & 3(5\cdot 4)(3) & 3(5)(3\cdot 2) & 1(3\cdot 2\cdot 1) \\ 0 & 0 & 0 & 1(6\cdot 5\cdot 4) & 3(6\cdot 5)(2) & 3(6)(2\cdot 1) \\ 0 & 0 & 0 & 0 & 1(7\cdot 6\cdot 5) & 3(7\cdot 6)(1) \\ 0 & 0 & 0 & 0 & 0 & 1(8\cdot 7\cdot 6) \end{bmatrix}$$

$$\mathbf{T}_{9,5} = \frac{1}{8\cdot 7\cdot 6\cdot 5}\begin{bmatrix} 1(8\cdot 7\cdot 6\cdot 5) & 0 & 0 & 0 & 0 \\ 4(1)(7\cdot 6\cdot 5) & 1(7\cdot 6\cdot 5\cdot 4) & 0 & 0 & 0 \\ 6(2\cdot 1)(6\cdot 5) & 4(2)(6\cdot 5\cdot 4) & 1(6\cdot 5\cdot 4\cdot 3) & 0 & 0 \\ 4(3\cdot 2\cdot 1)(5) & 6(3\cdot 2)(5\cdot 4) & 4(3)(5\cdot 4\cdot 3) & 1(5\cdot 4\cdot 3\cdot 2) & 0 \\ 1(4\cdot 3\cdot 2\cdot 1) & 4(4\cdot 3\cdot 2)(4) & 6(4\cdot 3)(4\cdot 3) & 4(4)(4\cdot 3\cdot 2) & 1(4\cdot 3\cdot 2\cdot 1)) \\ 0 & 1(5\cdot 4\cdot 3\cdot 2) & 4(5\cdot 4\cdot 3)(3) & 6(5\cdot 4)(3\cdot 2) & 4(5)(3\cdot 2\cdot 1) \\ 0 & 0 & 1(6\cdot 5\cdot 4\cdot 3) & 4(6\cdot 5\cdot 4)(2) & 6(6\cdot 5)(2\cdot 1) \\ 0 & 0 & 0 & 1(7\cdot 6\cdot 5\cdot 4) & 4(7\cdot 6\cdot 5)(1) \\ 0 & 0 & 0 & 0 & 1(8\cdot 7\cdot 6\cdot 5) \end{bmatrix}$$

$$\mathbf{T}_{9,4} = \frac{1}{8\cdot 7\cdot 6\cdot 5\cdot 4}\begin{bmatrix} 1(8\cdot 7\cdot 6\cdot 5\cdot 4) & 0 & 0 & 0 \\ 5(1)(7\cdot 6\cdot 5\cdot 4) & 1(7\cdot 6\cdot 5\cdot 4\cdot 3) & 0 & 0 \\ 10(2\cdot 1)(6\cdot 5\cdot 4) & 5(2)(6\cdot 5\cdot 4\cdot 3) & 1(6\cdot 5\cdot 4\cdot 3\cdot 2) & 0 \\ 10(3\cdot 2\cdot 1)(5\cdot 4) & 10(3\cdot 2)(5\cdot 4\cdot 3) & 5(3)(5\cdot 4\cdot 3\cdot 2) & 1(5\cdot 4\cdot 3\cdot 2\cdot 1) \\ 5(4\cdot 3\cdot 2\cdot 1)(4) & 10(4\cdot 3\cdot 2)(4\cdot 3) & 10(4\cdot 3)(4\cdot 3\cdot 2) & 5(4)(4\cdot 3\cdot 2\cdot 1) \\ 1(5\cdot 4\cdot 3\cdot 2\cdot 1) & 5(5\cdot 4\cdot 3\cdot 2)(3) & 10(5\cdot 4\cdot 3)(3\cdot 2) & 10(5\cdot 4)(3\cdot 2\cdot 1) \\ 0 & 1(6\cdot 5\cdot 4\cdot 3\cdot 2) & 5(6\cdot 5\cdot 4\cdot 3)(2) & 10(6\cdot 5\cdot 4)(2\cdot 1) \\ 0 & 0 & 1(7\cdot 6\cdot 5\cdot 4\cdot 3) & 5(7\cdot 6\cdot 5\cdot 4)(1) \\ 0 & 0 & 0 & 1(8\cdot 7\cdot 6\cdot 5\cdot 4) \end{bmatrix}$$

*We thank the anonymous reviewers for suggesting we should include this explanation.

$$
\mathbf{T}_{9,3} = \frac{1}{8\cdot7\cdot6\cdot5\cdot4\cdot3}\begin{bmatrix}
1(8\cdot7\cdot6\cdot5\cdot4\cdot3) & 0 & 0 \\
6(1)(7\cdot6\cdot5\cdot4\cdot3) & 1(7\cdot6\cdot5\cdot4\cdot3\cdot2) & 0 \\
15(2\cdot1)(6\cdot5\cdot4\cdot3) & 6(2)(6\cdot5\cdot4\cdot3\cdot2) & 1(6\cdot5\cdot4\cdot3\cdot2\cdot1) \\
20(3\cdot2\cdot1)(5\cdot4\cdot3) & 15(3\cdot2)(5\cdot4\cdot3\cdot2) & 6(3)(5\cdot4\cdot3\cdot2\cdot1) \\
15(4\cdot3\cdot2\cdot1)(4\cdot3) & 20(4\cdot3\cdot2)(4\cdot3\cdot2) & 15(4\cdot3)(4\cdot3\cdot2\cdot1) \\
6(5\cdot4\cdot3\cdot2\cdot1)(3) & 15(5\cdot4\cdot3\cdot2)(3\cdot2) & 20(5\cdot4\cdot3)(3\cdot2\cdot1) \\
1(6\cdot5\cdot4\cdot3\cdot2\cdot1) & 6(6\cdot5\cdot4\cdot3\cdot2)(2) & 15(6\cdot5\cdot4\cdot3)(2\cdot1) \\
0 & 1(7\cdot6\cdot5\cdot4\cdot3\cdot2) & 6(7\cdot6\cdot5\cdot4\cdot3)(1) \\
0 & 0 & 1(8\cdot7\cdot6\cdot5\cdot4\cdot3)
\end{bmatrix}
$$

From these examples, we can guess that $t_{i,j}^{p+k,p}$ can be written as a product of four terms. The first term, $\frac{(p-1)!}{(p+k-1)!}$, is the coefficient of the matrix. The second term is $\binom{k}{i-j}$. The intuition for why the second term is a binomial can be found in Pascal's triangle. Consider the set of matrices whose elements are equal to $\binom{k}{i-j}$. Each column is a cyclic permutation of the previous column. Thus, adding two adjacent columns produces a new column whose elements are:

$$
\binom{k}{i-j} + \binom{k}{i-(j+1)} = \binom{k+1}{i-j}
$$

because this action follows the same sequence as Pascal's triangle. Since there are exactly two non-zero elements in each column of $\mathbf{M}_{p+1,p}$ and they are adjacent, the products of $\mathbf{T}_{p+k,p+1}\mathbf{M}_{p+1,p}$ produce elements which can be expressed as the sum of the number of non-zero terms in the pair of corresponding horizonally adjacent elements in $\mathbf{T}_{p+k,p+1}$. Since this sequence also follows the pattern in Pascal's triangle, it can be described by a binomial. The remaining terms can be described by: $\frac{(i-1)!}{(j-1)!}$ and $\frac{(p+k-i)!}{(p-j)!}$. Thus we have:

$$
t_{i,j}^{p+k,p} = \left(\frac{(p-1)!}{(p+k-1)!}\right)\binom{k}{i-j}\left(\frac{(i-1)!}{(j-1)!}\right)\left(\frac{(p+k-i)!}{(p-j)!}\right)
$$

This formula can be rearranged as:

$$
\begin{aligned}
t_{i,j}^{p+k,p} &= \left(\frac{(p-1)!}{(j-1)!(p-1-(j-1))!}\right)\binom{k}{i-j}\left(\frac{(i-1)!(p+k-1-(i-1))!}{(p+k-1)!}\right) \\
&= \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}}
\end{aligned}
$$

2.2 Finite difference matrix

Each column of the transfer matrix for k stages, $\mathbf{T}_{p+k,p}$, describes the distribution of one of the p inlet flows across the $p+k$ outlet flows. We claim that the elements of each column are samples of a polynomial and prove this by constructing a table of repeated finite differences—the p-th order repeated finite differences of a $(p-1)$-degree polynomial vanish. Finite differences are usually defined for vectors; we extend the notation to matrices, taking the finite differences column-wise.

We denote the r-th repeated finite difference operator by Δ^r. Of interest is the p-th repeated finite difference of the transfer matrix for a k-stage mixing network, $\mathbf{T}_{p+k,p}$, that is, $\Delta^p \mathbf{T}_{p+k,p}$. The elements $d_{i,j}^{p+k,p}$ of this $k \times p$ matrix are given by:

$$d_{i,j}^{p+k,p} = \sum_{s=0}^{p} (-1)^s \frac{\binom{p}{s}\binom{p-1}{j-1}\binom{k}{s+i-j}}{\binom{p+k-1}{s+i-1}} \tag{2}$$

We first introduce some notation.

Definition Let f_i denote a sequence of values. We denote the finite forward difference by $\Delta f_i = f_{i+1} - f_i$. We recursively define the m-th finite forward difference by $\Delta^m f_i = \Delta^{m-1} f_{i+1} - \Delta^{m-1} f_i$.

We employ the well known formula for the m-th finite forward difference:

$$\Delta^m f_i = \sum_{s=0}^{m} (-1)^s \binom{m}{s} f_{i+s} \tag{3}$$

In our case, $m = p$ since we are interested in the p-th finite forward difference. Our sequence of values is defined by the entries of a single column of $T_{p+k,p}$, so we have:

$$f_i = t_{i,j}^{p+k,p} = \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}} \tag{4}$$

Consequently, we obtain:

$$f_{i+s} = \frac{\binom{p-1}{j-1}\binom{k}{s+i-j}}{\binom{p+k-1}{s+i-1}} \tag{5}$$

Putting all of this together with (3) gives the desired result (2).
The menacing right-hand side of (2) sums to zero, which we now prove using hypergeometric summation techniques [10, 9].
We first introduce the necessary concepts and notation [10].

Definition A *hypergeometric series* $\sum_{s\geq 0} t_s$ is one in which $t_0 = 1$ and

$$\frac{t_{s+1}}{t_s} = \frac{(s+a_1)(s+a_2)...(s+a_m)}{(s+b_1)(s+b_2)...(s+b_n)(s+1)} c \tag{6}$$

where a's and b's are known as upper and lower parameters, respectively, and c is a constant. Furthermore, we succinctly represent $\sum_{s\geq 0} t_s$ as

$${}_mF_n = \left[\begin{matrix} a_1 & a_2 & ... & a_m \\ b_1 & b_2 & ... & b_n \end{matrix} \; ; \mathrm{c} \right]. \tag{7}$$

Given a hypergeometric series $\sum_{s\geq 0} t_s$, its ${}_mF_n$ representation can often be derived with the following algorithm [10]:

1. Shift the summation index s so that the sum begins at $s = 0$ and the first term is non-zero. Extract the term corresponding to $s = 0$ as a common factor so that the first term of the sum is now 1.

2. Obtain and simplify the ratio t_{s+1}/t_s so that is of the form illustrated in Equation 6.

3. Use the upper and lower parameters and the constant c to formulate ${}_mF_n$.

Having obtained a representation of $\sum_{s\geq 0} t_s$, one can reference a list of hypergeometric identities known, colloquially, as a "hypergeometric database"[†]. By employing one or more known identities, it is often possible to transform ${}_mF_n$ into a more useful representation. To apply this technique to our problem, we split the proof into two parts. The first assumes that $j \leq i$ and the second assumes that $i < j$; in both cases we show that $d_{i,j}^{p+k,p} = 0$.

Lemma 1. *Let $2 \leq p$, $1 \leq k$, $1 \leq i \leq k$, and $1 \leq j \leq p$. Assume that $j \leq i$. Then:*

$$d_{i,j}^{p+k,p} = \sum_{s=0}^{p} (-1)^s \frac{\binom{p}{s}\binom{p-1}{j-1}\binom{k}{s+i-j}}{\binom{p+k-1}{s+i-1}} = 0$$

Proof. Consider two consecutive terms of the sum, t_s and t_{s+1}. Then:

$$\frac{t_{s+1}}{t_s} = \frac{(s+i)(s-p)(s+i-j-k)}{(s+i-p-k)(s+i-j+1)(s+1)}.$$

Therefore, $d_{i,j}^{p+k,p}$ is a hypergeometric series. Since $j \leq i$, the first term is non-zero. By the algorithm summarized above, we extract the first term as a common factor to get:

$$d_{i,j}^{p+k,p} = \frac{\binom{p-1}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}} \left[\begin{matrix} i & -p & i-j-k \\ i-p-k & i-j+1 & \end{matrix} ; 1 \right] \tag{8}$$

Saalschütz's identity [9, 10] is commonly contained within a hypergeometric database. It states that when c is a negative integer and $d + e = a + b + c + 1$, then:

$${}_3F_2 \left[\begin{matrix} a & b & c \\ d & e & \end{matrix} ; 1 \right] = \frac{(d-a)_{|c|}(d-b)_{|c|}}{d_{|c|}(d-a-b)_{|c|}} \tag{9}$$

[†]There is really no single standard hypergeometric database. Rather, it is simply a collection of useful identities that one may obtain from many different sources. The included identities may change from source to source.

where $(a)_n$ denotes the rising factorial[‡]. Note that $c = i - j - k \leq -1$ and so $|c| = j + k - i \geq 1$. Therefore, this identity allows us to transform Equation 8 into:

$$\frac{\binom{p}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}} \frac{(-p-k)_{j+k-i}(i-k)_{j+k-i}}{(i-p-k)_{j+k-i}(-k)_{j+k-i}} \tag{10}$$

We can rewrite (10):

$$\frac{\binom{p}{j-1}\binom{k}{i-j}}{\binom{p+k-1}{i-1}} \frac{(-p-k)(-p-k+1)...(-p+j-i-1)\cdot(i-k)(i-k+1)...(j-1)}{(i-p-k)(i-p-k+1)...(-p+j-1)\cdot(-k)(-k+1)...(j-i-1)} \tag{11}$$

The multiplicative terms of $(-p-k)_{j+k-i}$, $(i-p-k)_{j+k-i}$, and $(-k)_{j+k-i}$ start negative and stay negative. However, exactly one of the multiplicative terms in the numerator, $(i-k)_{j+k-i} = (i-k)(i-k+1)\cdots(i-k+j+k-i-1)$, equals zero since the terms start negative and end with a non-negative term. Therefore, (11) equals zero. $\square$

Lemma 2. *Let $2 \leq p$, $1 \leq k$, $1 \leq i \leq k$, and $1 \leq j \leq p$. Assume that $i < j$. Then:*

$$d_{i,j}^{p+k,p} = \sum_{s=0}^{p} (-1)^s \frac{\binom{p}{s}\binom{p-1}{j-1}\binom{k}{s+i-j}}{\binom{p+k-1}{s+i-1}} = 0$$

Proof. This closely follows the proof for Lemma 1. However, now that $i < j$, the terms of $d_{i,j}^{p+k,p}$ will be zero until $s = j - i$. Therefore, in order to use the algorithm summarized earlier, we must rewrite our sum. Certainly, we can start our sum with $s = j - i$, however, this violates the constraint that our summation must start at index zero. We can rewrite the sum and abide by the constraints of the algorithm to get:

$$d_{i,j}^{p+k,p} = \sum_{s'=0}^{p+i-j} (-1)^{(s'+j-i)} \frac{\binom{p}{s'+j-i}\binom{p-1}{j-1}\binom{k}{s'}}{\binom{p+k-1}{s'+j-1}} \tag{12}$$

As we should expect, this is still hypergeometric since:

$$\frac{t_{s'+1}}{t_{s'}} = \frac{(s'+j)(s'-k)(s'+j-i-p)}{(s'-p-k+j)(s'+j+1-i)(s'+1)} \tag{13}$$

By the same method as before, we can express Equation 12 as:

$$\frac{\binom{p}{j-i}\binom{p-1}{j-1}}{\binom{p+k-1}{j-1}} \begin{bmatrix} j & -k & j-i-p & \\ -p-k+j & j+1-i & & ;1 \end{bmatrix} \tag{14}$$

[‡]Also known as the Pochhammer symbol. This is defined for non-negative n as: $(a)_n = (a)(a+1)(a+2)...(a+n-1)$ if $n \geq 1$, otherwise $(a)_0 = 1$.

Since $c = j - i - p \leq -1$ and, therefore, $|c| = p + i - j \geq 1$, we can again use Saalschütz's identity which gives us:

$$\frac{\binom{p}{j-i}\binom{p-1}{j-1}}{\binom{p+k-1}{j-1}} \frac{(-p-k)_{p+i-j}(j-p)_{p+i-j}}{(j-p-k)_{p+i-j}(-p)_{p+i-j}} \tag{15}$$

Finally, note that $(j-p)_{p+i-j} = (j-p)(j-p+1)...(i-1)$ is the only rising factorial term that contains a multiplicative term of zero; therefore, (15) equals zero. $\square$

Theorem 2. *Let* $2 \leq p$, $1 \leq k$, $1 \leq i \leq k$, *and* $1 \leq j \leq p$. *Then:*

$$d_{i,j}^{p+k,p} = \sum_{s=0}^{p} (-1)^s \frac{\binom{p}{s}\binom{p-1}{j-1}\binom{k}{s+i-j}}{\binom{p+k-1}{s+i-1}} = 0$$

Proof. This follows directly from Lemma 1 and Lemma 2. $\square$

In the example shown previously, $\Delta^0 \mathbf{T}_{9,3} = \mathbf{T}_{9,3}$ and we have:

$$\Delta^1 \mathbf{T}_{9,3} = \begin{bmatrix} \frac{-1}{4} & \frac{1}{4} & 0 \\ \frac{-3}{14} & \frac{5}{28} & \frac{1}{28} \\ \frac{-5}{28} & \frac{3}{28} & \frac{1}{14} \\ \frac{-1}{7} & \frac{1}{28} & \frac{3}{28} \\ \frac{-3}{28} & \frac{-1}{28} & \frac{1}{7} \\ \frac{-1}{14} & \frac{-3}{28} & \frac{5}{28} \\ \frac{-1}{28} & \frac{-5}{28} & \frac{3}{14} \\ 0 & \frac{-1}{4} & \frac{1}{4} \end{bmatrix}$$

$$\Delta^2 \mathbf{T}_{9,3} = \begin{bmatrix} \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \\ \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \\ \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \\ \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \\ \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \\ \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \\ \frac{1}{28} & \frac{-1}{14} & \frac{1}{28} \end{bmatrix}$$

$$\Delta^3 \mathbf{T}_{9,3} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

2.3 Conclusion of proof

A fundamental algebraic result is that if the p-th finite differences of a sequence are zero, the sequence represents the (equispaced) values of a polynomial of degree $p - 1$ [13]. Applying this reasoning to the transfer matrix $T_{p+k,p}$ column-wise, we conclude that each of the p inlet flows generates a polynomial of degree $p - 1$ across the $p + k$ outlet flows. Finally, the concentration profile in the output channel is the sum of the concentrations generated by all the inlet flows, and is therefore also a polynomial of degree $p - 1$.

3 Concentration Profile

As a practical matter, it is important to know not just that the resulting profile is a polynomial, but also what that polynomial is. Given the linearity of the system, the resulting profile, as a function of the transverse coordinate x, is

$$r(x) = \sum_{j=1}^{p} \mathbf{c}_j^{\text{in}} h_j^{p+k,p}(x),$$

where each $h_j^{p+k,p}(x)$ is the "impulse response" to a unit concentration in input channel j. The impulse response $h_j^{p+k,p}(x)$, a polynomial of degree $p-1$, can be reconstructed by well-known techniques [13] from the column j of the transfer matrix $\mathbf{T}_{p+k,p}$ and its finite differences, computed above:

$$h_j^{p+k,p}(x) = \sum_{m=0}^{p-1} \lambda_m \prod_{q=0}^{m-1} (x - \alpha_q)$$

where

$\alpha_q = \alpha_0 + qw$ for $0 \leq q \leq p-1$

and

$$\lambda_m = \frac{1}{m! w^m} \Delta^m t_{1,j}^{p+k,p}$$

Here the α_q are the points at which the polynomial is tabulated, and w is the distance between each two. From the geometry of the problem, if the output channel width is W, it is formed from $p+k$ final mixing network outlets, so $w = \frac{W}{p+k}$. We can take evaluation points to be in the middle of each mixing network outlet, so $\alpha_0 = \frac{w}{2}$. Thus, for any given mixing network structure, and for given channel widths and input concentrations, the formula above explicitly gives the resultant concentration profile in the channel.

The impulse response polynomials for the running example are:

$$\begin{aligned} h_1^{9,3}(x) &= 1 - \frac{9}{4}(x - \frac{1}{18}) + \frac{81}{56}(x - \frac{1}{18})(x - \frac{3}{18}) \\ &= \frac{255}{224} - \frac{18}{7}x + \frac{81}{56}x^2 \end{aligned}$$

$$\begin{aligned} h_2^{9,3}(x) &= \frac{9}{4}(x - \frac{1}{18}) - \frac{81}{28}(x - \frac{1}{18})(x - \frac{3}{18}) \\ &= -\frac{17}{112} + \frac{81}{28}x - \frac{81}{28}x^2 \end{aligned}$$

$$
\begin{aligned}
h_3^{9,3}(x) &= \frac{81}{56}(x - \frac{1}{18})(x - \frac{3}{18}) \\
&= \frac{3}{224} - \frac{9}{28}x + \frac{81}{56}x^2
\end{aligned}
$$

Substituting $x = \frac{1}{18}, \frac{3}{18}, ..., \frac{17}{18}$ into $h_j^{9,3}(x)$ produces the entries of the column j of $\mathbf{T}_{9,3}$. A plot of the three impulse response polynomials is provided in Figure 2.

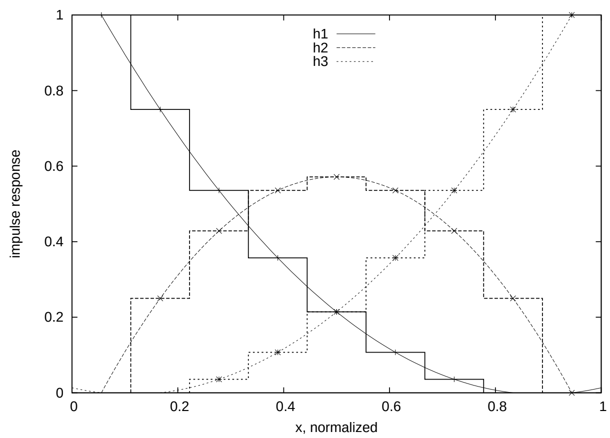

Figure 2: The impulse response polynomials $h_1^{9,3}(x)$, $h_2^{9,3}(x)$, and $h_3^{9,3}(x)$ and their staircase realizations.

4 The Inverse Problem

One interesting question is whether *all* polynomials of a given degree can be obtained using the described network. Another important consideration is how to determine input concentrations that will yield a desired concentration profile. More precisely, given a target concentration profile as the $(p+k) \times 1$ vector $\mathbf{c}^{\text{out}}$, we need a way of obtaining the $p \times 1$ vector $\mathbf{c}^{\text{in}}$.

Denote the top $p \times p$ submatrix of $\mathbf{T}_{p+k,p}$ by $\mathbf{S}_{p,p}$. Recall that the entries of $\mathbf{T}_{p+k,p}$ are given by the formula for $t_{i,j}^{p+k,p}$ and, therefore, by construction, $\mathbf{S}_{p,p}$ is a lower triangular matrix. Consequently, the columns of $\mathbf{S}_{p,p}$ constitute a basis spanning $\mathbb{R}^p$. Any polynomial of degree $p-1$ can then be obtained by specifying an input vector that employs an appropriate linear combination of these columns. The entries of the $p \times 1$ output vector will define the coefficients of the polynomial. Therefore, it is possible, in principle, to obtain any polynomial of degree $p-1$ as the output concentration profile.

Now assume that a specific concentration profile $\mathbf{c}^{\text{out}}$ is desired. The entries of $\mathbf{c}^{\text{out}}$ define a sampling of $p+k$ points from a polynomial of degree $p-1$. However, p points are sufficient to define a unique polynomial of degree $p-1$. Therefore, we may assume that the desired concentration profile is specified by a $p \times 1$ vector $\mathbf{c}_p^{\text{out}}$. The inverse $\mathbf{S}_{p,p}^{-1}$ is guaranteed to exist and is also lower triangular because $\mathbf{S}_{p,p}$ is lower triangular. The required input concentrations are then obtained as $\mathbf{c}^{\text{in}} = \mathbf{S}_{p,p}^{-1}\, \mathbf{c}_p^{\text{out}}$.

In practice, however, concentrations are physical quantities restricted to a certain range—they cannot be negative and they cannot be above saturation. Without loss of generality, with a suitable choice of units, this range may be assumed to be $[0, 1]$. Therefore, one can only obtain output concentration profiles that lie within the image of the unit hypercube under the linear transform described by $\mathbf{S}_{p,p}$, i.e., $\mathbf{c}_p^{\text{out}} \in \mathbf{S}_{p,p}([0, 1]^p)$.

5 Discussion

All transverse concentration profiles obtained using microfluidic networks as in the Whitesides' group's design are described by polynomials of degree one less than the number of input channels. For instance, with three input channels one can obtain profiles shaped as parabolas and straight lines. The value p specifies the degree of the polynomials $h_j^{p+k,p}(x)$, and thus governs the flexibility of achievable shapes. The value k controls the granularity of the fit of the $\mathbf{c}^{\text{out}}$, i.e., the staircase actual profile, to the ideal polynomial shape $h_j^{p+k,p}(x)$.

The assumption of complete mixing in each stage of the network is crucial to this analysis; without it, discrete methods must give in to solving diffusion equations for the network as a whole, which is not likely to give useful analytical results. Fortunately, complete diffusive or chaotic mixing in each stage has been experimentally demonstrated. [7, 11]

It is interesting to consider what profiles might be obtained using more general mixing networks. For instance, mixing network stages need not use consecutive integral numbers of channels. Or, the channels might be of uneven width within a stage. Or, with current multi-layer fabrication techniques, the network topology might be more complicated than the planar network of Figure 1. The question of optimality, i.e., of obtaining a desired concentration profile using the simplest network, therefore remains open. While polynomial profiles may already be quite useful in applications [6], periodic profiles are of particular interest, and a better (more parsimonious) way of achieving them than the parallel repetition of networks [2] is desirable, and also remains as a topic for future work.

6 Acknowledgments

We are grateful to C. Moore, L. Williams, V. Kalapala, and J. Farfel (University of New Mexico) and M. N. Stojanovic (Columbia University) for helpful advice and encouragement. This material is based upon work supported by the National Science Foundation (grants CCR-0219587, CCR-0085792, EIA-0218262, EIA-0238027, and EIA-0324845), Sandia National Laboratories, Microsoft Research, and Hewlett-Packard (gift 88425.1). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the sponsors.

References

[1] CHOU, H.-P., UNGER, M. A., AND QUAKE, S. R. A microfabricated rotary pump. *Biomedical Microdevices 3*, 4 (2001), 323–330.

[2] DERTINGER, S. K. W., CHIU, D. T., JEON, N. L., AND WHITESIDES, G. M. Generation of gradients having complex shapes using microfluidic networks. *Analytical Chemistry 73* (2001), 1240–1246.

[3] DERTINGER, S. K. W., JIANG, X., LI, Z., MURTHY, V. N., AND WHITESIDES, G. M. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proceedings of the National Academy of Sciences of the USA (PNAS) 99*, 20 (2002), 12542–12547.

[4] FARFEL, J., AND STEFANOVIC, D. Towards practical biomolecular computers using microfluidic deoxyribozyme logic gate networks. In *DNA Computing: 11th International Meeting on DNA-Based Computers* (2005).

[5] HARDT, S., DRESE, K. S., HESSEL, V., AND SCHÖNFELD, F. Pasive micromixers for applications in the microreactor and μTAS fields. *Microfluidics and Nanofluidics 1*, 2 (May 2005), 108–118.

[6] JEON, N. L., BAKARAN, H., DERTINGER, S. K. W., WHITESIDES, G. M., VAN DE WATER, L., AND TONER, M. Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nature Biotechnology 20* (2002), 826–830.

[7] JEON, N. L., DERTINGER, S. K. W., CHIU, D. T., CHOI, I. S., STROOCK, A. D., AND WHITESIDES, G. M. Generation of solution and surface gradients using microfluidic systems. *Langmuir 16* (2000), 8311–8316.

[8] JIANG, X., XU, Q., DERTINGER, S. K. W., STROOCK, A. D., FU, T.-M., AND WHITESIDES, G. M. A general method for patterning gradients of biomolecules on surfaces using microfluidic networks. *Analytical Chemistry 77*, 8 (2005), 2338–2347.

[9] KOEPF, W. *Hypergeometric Summation: An Algorithmic Approach to Summation and Special Function Identities*. American Mathematical Society, Braunschweig, Germany, 1998.

[10] PETKOVŠEK, M., WILF, H. S., AND ZEILBERGER, D. *A=B*. A.K. Peters, Ltd., Wellesley, MA, 1996.

[11] STROOCK, A. D., DERTINGER, S. K. W., AJDARI, A., MEZIĆ, I., STONE, H. A., AND WHITESIDES, G. M. Chaotic mixer for microchannels. *Science 295*, 5555 (Jan. 2002), 647–651.

[12] TICE, J. D., SONG, H., LYON, A. D., AND ISMAGILOV, R. F. Formation of droplets and mixing in multiphase microfluidics at low values of the Reynolds and the capillary numbers. *Langmuir 19* (2003), 9127–9133.

[13] VAN DER WAERDEN, B. L. *Algebra*, 7th ed. Springer-Verlag, Berlin, 1966. (pp. 86–91 of English translation, Springer-Verlag, New York, 1991).

[14] VAN NOORT, D., GAST, F.-U., AND MCCASKILL, J. S. DNA computing in microreactors. In *DNA Computing: 7th International Meeting on DNA-Based Computers* (2001).

[15] VAN NOORT, D., AND MCCASKILL, J. S. Flows in micro fluidic networks: From theory to experiment. *Natural Computing 3*, 4 (2004), 395–410.