

Characterization of Transverse Channel Concentration Profiles Obtainable with a Class of Microfluidic Networks

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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.^{1–3} 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's manuscript⁴ for an improvement that induces chaotic flow by means of a herringbone channel floor pattern; Hardt⁵ wrote a recent review of such passive mixing techniques. One can also use active folding mixing in a rotary mixing chamber⁶ or mixing by means of folding in oil droplets.⁷)

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 nonuniform 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.⁸ obtained a gradient, i.e., a roughly linear dependence of concentration on the transverse coordinate

x across the output channel. Second, Dertinger et al.⁹ obtained either a roughly linear dependence or a roughly quadratic dependence, depending on the particulars of the microfluidic network. Third, significant applications of nonuniform concentration profiles were described.^{10–12}

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 $n = p, \dots, p + k - 1$. 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 to be 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^{4,8} 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 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

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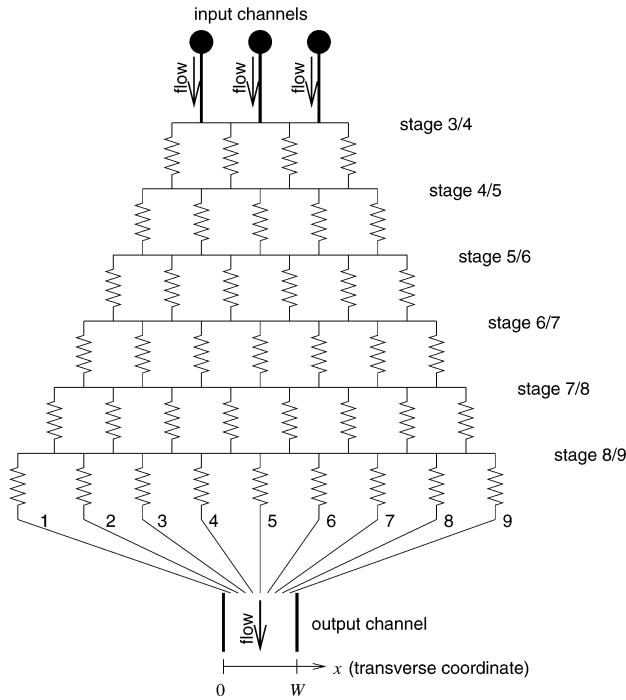


Figure 1. Microfluidic mixing network (after Jeon et al.⁸) with $p = 3$ inlets to the first stage, $k = 6$ stages, and $p + k = 9$ outlets from the final stage.

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

Here we provide the proof of Dertinger’s conjecture at a high level and with numerical examples; detailed proofs are found in the Appendix (in Supporting Information).

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}^{in} of n values and concentrations in the m outlets are grouped into a column vector \mathbf{c}^{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 analysis⁹ 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:

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

That is, the elements of $\mathbf{M}_{p+1,p}$ are given by

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

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 $\mathbf{T}_{p+k,p}$, as shown in Appendix A, are given by

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

Example: For the network of Figure 1, we have

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

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

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

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

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

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

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

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, $\Delta^p \mathbf{T}_{p+k,p}$. As shown in Appendix B, the elements $d_{i,j}^{p+k,p}$ of this $k \times p$ matrix are given by

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

The menacing right-hand side sums to zero, as we show in Appendix C using hypergeometric summation techniques.^{13,14}

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

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

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

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

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 $p - 1$.¹⁵ Applying this reasoning to the transfer matrix $\mathbf{T}_{p+k,p}$ 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 techniques¹⁵ 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

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

and

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

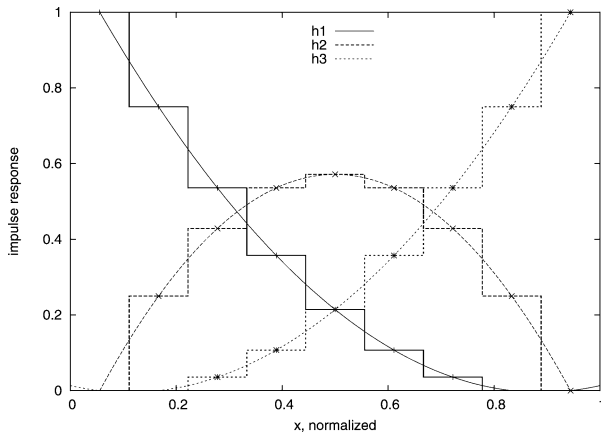


Figure 2. Impulse response polynomials $h_1^{9,3}(x)$, $h_2^{9,3}(x)$, and $h_3^{9,3}(x)$ and their staircase realizations.

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 $w = W/(p + k)$. We can take evaluation points to be in the middle of each mixing network outlet, so $\alpha_0 = w/2$. 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

$$\begin{aligned} h_1^{9,3}(x) &= 1 - \frac{9}{4}\left(x - \frac{1}{18}\right) + \frac{81}{56}\left(x - \frac{1}{18}\right)\left(x - \frac{3}{18}\right) \\ &= \frac{255}{224} - \frac{18}{7}x + \frac{81}{56}x^2 \\ h_2^{9,3}(x) &= \frac{9}{4}\left(x - \frac{1}{18}\right) - \frac{81}{28}\left(x - \frac{1}{18}\right)\left(x - \frac{3}{18}\right) \\ &= -\frac{17}{112} + \frac{81}{28}x - \frac{81}{28}x^2 \\ h_3^{9,3}(x) &= \frac{81}{56}\left(x - \frac{1}{18}\right)\left(x - \frac{3}{18}\right) \\ &= \frac{3}{224} - \frac{9}{28}x + \frac{81}{56}x^2 \end{aligned}$$

Substituting $x = 1/18, 3/18, \dots, 17/18$ 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.

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}^{out} , we need a way of obtaining the $p \times 1$ vector \mathbf{c}^{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 $t_{ij}^{p+k,p}$, 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}^{out} is desired. The entries of \mathbf{c}^{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}^{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.^{4,8}

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 multilayer 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,¹⁰ periodic profiles are of particular interest, and a better (more parsimonious) way of achieving them than the parallel repetition of networks⁹ is desirable, and also remains as a topic for future work.

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