Comparative modeling and analysis of microfluidic and conventional DNA microarrays

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Abstract

A theoretical analysis was developed to predict molecular hybridization rates for microarrays where samples flow through microfluidic channels and for conventional microarrays where samples remain stationary during hybridization. The theory was validated by using a multiplexed microfluidic microarray where eight samples were hybridized simultaneously against eight probes using 60-mer DNA strands. Mass transfer coefficients ranged over three orders of magnitude where either kinetic reaction rates or molecular diffusion rates controlled overall hybridization rates. Probes were printed using microfluidic channels and also conventional spotting techniques. Consistent with the theoretical model, the microfluidic microarray demonstrated the ability to print DNA probes in less than 1 min and to detect 10-pM target concentrations with hybridization times in less than 5 min.

Keywords: Microarray; Hybridization; Biochips; DNA; Mass transport

Hybridization assays account for a substantial fraction of all biomarker detection and quantification assays in research and clinical use. In research applications, the discovery of new potential gene expression biomarkers has been greatly facilitated by the development of high-throughput testing platforms such as microarrays, which test a single sample for tens of thousands of potential targets [1]. Although hundreds of high-value potential biomarkers may be initially selected by using microarrays with small patient populations, it is necessary to screen much larger sample populations to further identify and validate the few biomarkers that may become clinically valuable [2–4]. New hybridization platforms are essential to reduce test costs per sample in validating potential biomarkers and to ultimately increase the accuracy and reliability of testing for eventual clinical applications.

Commercial platforms have become available to fulfill this need for low-cost testing of more samples against fewer targets. Contract printing companies prepare microarrays with small numbers of probes. Consumables and automation companies offer robotic printers and 96-well microtiter plates where each well bottom can be printed with hybridization probes. Bead-based hybridization systems are also available where many targets can be tested simultaneously in a single microtiter plate well. To date, these systems have not found wide use due to their complexity, the time and cost required to acquire and prepare small sets of unique selected probes, large sample volumes, long hybridization times, and variability of test results. Furthermore, most platforms require the purchase of expensive instrumentation and automation along with the maintenance of a dedicated and trained staff to use these assays effectively. Overall, these platforms remain expensive and time-consuming and are still beyond the reach of many researchers for rapid inexpensive testing of small collections of targets.
Nomenclature

<table>
<thead>
<tr>
<th>Variables</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>$C$</td>
<td>Concentration of a reactant in a fluid volume (mol cc$^{-1}$)</td>
</tr>
<tr>
<td>$C_{init}$</td>
<td>Initial concentration of mobile reactant in solution phase (mol cc$^{-1}$)</td>
</tr>
<tr>
<td>$C_{mean}$</td>
<td>Mean concentration of mobile reactant in solution phase (mol cc$^{-1}$)</td>
</tr>
<tr>
<td>$C_{wall}$</td>
<td>Mean concentration of mobile reactant at the surface of the hybridization site (mol cc$^{-1}$)</td>
</tr>
<tr>
<td>$D$</td>
<td>Mobile species diffusion coefficient (cm$^2$ s$^{-1}$)</td>
</tr>
<tr>
<td>$d_h$</td>
<td>Hydraulic diameter of a microfluidic channel (4 [flow area]/[wetted perimeter], cm)</td>
</tr>
<tr>
<td>$h_{diff}$</td>
<td>Diffusion mass transfer coefficient (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$H_{react}$</td>
<td>Reaction mass transfer coefficient (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$H_{tot}$</td>
<td>Total mass transfer coefficient (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{assoc}$</td>
<td>Hybridization association reaction rate constant (mol$^{-1}$ cm$^3$ s$^{-1}$)</td>
</tr>
<tr>
<td>$k_{diss}$</td>
<td>Hybridization disassociation reaction rate constant (s$^{-1}$)</td>
</tr>
<tr>
<td>$L$</td>
<td>Total downstream length of a hybridization site (cm)</td>
</tr>
<tr>
<td>$M_{diff}$</td>
<td>Mass per unit area of a reactant diffused to a specific hybridization site (mol cm$^{-2}$)</td>
</tr>
<tr>
<td>$M_{hyb}$</td>
<td>Mass per unit area of a reactant hybridized at a specific hybridization site (mol cm$^{-2}$)</td>
</tr>
<tr>
<td>$M_{imm}$</td>
<td>Total mass of a reactant immobilized at a specific hybridization site (mol cm$^{-2}$)</td>
</tr>
<tr>
<td>$Q$</td>
<td>Mean volumetric flow rate in a channel (cm$^3$ s$^{-1}$)</td>
</tr>
<tr>
<td>$r_o$</td>
<td>Radius of hybridization spot in a stationary system (cm)</td>
</tr>
<tr>
<td>$t_{hyb}$</td>
<td>Total hybridization time (s)</td>
</tr>
<tr>
<td>$v_x$</td>
<td>Fluid velocity (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$w$</td>
<td>Width of a hybridization site in a flowing channel (cm)</td>
</tr>
<tr>
<td>$x$</td>
<td>Length of a hybridization site in a flowing channel (cm)</td>
</tr>
</tbody>
</table>

Devices consisting of microfluidic channels that flow samples past immobilized probes are being explored increasingly due to their low cost and potential to perform hybridization assays rapidly and accurately with reduced sample volumes. Several platforms that demonstrate the feasibility of using microfluidic channels for rapid hybridization have been reported [5–9]. Groups of parallel microfluidic channels have also attracted interest for their ability to test many samples simultaneously; these can then be read with a single imaging step, resulting in increased accuracy. A practical approach to fabricating these multiplexed microfluidic microarrays has been to first print lines of probe using parallel microfluidic channels and then to expose targets to these lines through channels arranged orthogonally to the lines. Historically, this approach has been used for membrane slot blot assays [10,11]. This format has been further explored by several groups using different detection approaches, including surface plasmon resonance [12–14], waveguides [15–17], and standard fluorescence imaging [18–20]. These studies have demonstrated the broad utility of this platform for the detection of a wide range of interactions between nucleic acids and proteins and the identification of organisms. This platform has also been used to determine the concentrations of sample targets [20,21]. However, experimental conditions used in these studies generally have led to highly saturated immobilized probes where the rate of hybridization changes rapidly with time, making determination of target concentrations and sample-to-sample comparisons difficult [22,23].

A more complete theoretical analysis is clearly needed to develop the potential of microfluidic microarrays for increased hybridization speed and accuracy and to compare results from different samples and platforms. We have developed a theoretical model, validated by experimental data, for predicting hybridization rates in both microfluidic channels and conventional microarrays, combining diffusion resistance and hybridization reaction resistance. A series of experiments were performed using an eight-channel elastomeric microfluidic microarray where parameters such as probe/target concentrations, probe printing methodologies, and sample flow rates and residence times in the microfluidic channels were varied. The results were then compared with those of conventional microarrays.

Materials and methods

Theoretical analysis

In a hybridization reaction, labeled molecular targets are transported from a bulk sample volume to a hybridization site by both diffusion and convection. At the hybridization site, they react with or hybridize to a complementary immobilized group of molecular probes. Analytical solutions for conventional microarrays, where the sample volume remains stationary, have been reported for labeled targets diffusing vertically down to a hybridization surface with a finite reaction rate and for labeled targets diffusing radially to a hybridization surface with an infinite reaction rate [24,25]. Computational solutions have also been reported for fluidic microarrays where the sample is flowing during hybridization [6,9]. There remains a need for an analytical
approach that allows prediction of overall hybridization rates in both conventional stationary and flowing hybridization systems. Exact solutions for the combined diffusion and reaction equations governing these cases are difficult to achieve. Although numerical solutions for mass transfer and chemical reaction problems are used routinely in the chemical process industry, these solutions require extensive effort and are difficult to use for device optimization.

In this article, we link exact analytical solutions for diffusion equations to the hybridization reaction equations, applying the assumption that the concentration of sample target at the hybridization site is constant. This assumption is valid where probesaturation levels and reverse hybridization rates do not significantly affect overall hybridization rates. A simple analytical expression for designing and optimizing microfluidic microarrays is derived, comparing results achieved on a microfluidic platform with those achieved with stationary microarrays. In section A below, the overall microarray mass transfer and hybridization reaction, applicable to both flowing and stationary systems, is modeled using diffusion and reaction mass transfer coefficients. Mass conservation at the hybridization site is used to link target diffusion to hybridization rate using these coefficients. To use this model, values must be obtained for the relevant mass transfer coefficients, diffusion coefficients, and hybridization reaction rate constants.

In subsequent Sections B–D, expressions for the mass transfer coefficients used in Section A are derived. Finally, in Section E, values for diffusion coefficients and reaction rate constants required to obtain values for the mass transfer coefficients are reported.

A. Mass conservation equation and definition of mass transfer coefficient

Solutions to both the mass transport and kinetic reaction equations can be formulated in terms of a mass transfer coefficient, $h$, which relates mass concentration gradients to mass transport rates. Using mass conservation, the amount of target immobilized at the hybridization site can be expressed in terms of the mass transport coefficients as

$$M_{\text{hyb}} = h_{\text{diff}}(C_{\text{mean}} - C_{\text{wall}})t_{\text{hyb}} = h_{\text{react}}C_{\text{wall}}t_{\text{hyb}},$$

(1a)

where $M_{\text{hyb}}$ is the mass per unit area of a reactant that has been immobilized at a specific hybridization site, $h_{\text{diff}}$ is the mass transfer coefficient resulting from diffusing and flowing target, $(C_{\text{mean}} - C_{\text{wall}})$ is the difference in mean target concentration in a sample volume and concentration of the target at the hybridization site, $h_{\text{react}}$ is the mass transfer coefficient resulting from target hybridization, $C_{\text{wall}}$ is the concentration of target at the hybridization site driving the hybridization reaction, and $t_{\text{hyb}}$ is the total hybridization time. The reciprocal of the mass transfer coefficients can be added to eliminate $C_{\text{wall}}$ from this equation to define a total mass transfer coefficient, $H_{\text{tot}}$, for a hybridization platform:

$$H_{\text{tot}} = (h_{\text{diff}}h_{\text{react}})/(h_{\text{diff}} + h_{\text{react}}).$$

(1c)

When a microarray reader measures signal intensity at a hybridization site, the value recorded is proportional to the number of labels attached to the hybridized targets per unit area of the hybridization site, $M_{\text{hyb}}$. Therefore, Eq. (1b) can be rearranged to present a relationship between signal intensity and target concentration:

$$(\text{Signal Intensity})/(H_{\text{tot}}C_{\text{mean}}h_{\text{hyb}}) = \text{Const.}$$

(1d)

Using Eq. (1d), the target concentration in a sample can be determined by measuring the hybridization site signal intensity, using a platform-specific derived value for $H_{\text{tot}}$, and working with a user-specified $t_{\text{hyb}}$.

B. Derivation of a diffusion mass transfer coefficient, $h_{\text{diff}}$, for a conventional microarray

Conventional microarrays consist of flat surfaces that contain spots of immobilized probes. These surfaces are immersed in a stationary sample solution containing labeled targets for 16–24 h to allow hybridization to take place. Mechanically spotted microarrays typically have collections of approximately 100-μm diameter hybridization spots at 250-μm center-to-center distances. To develop a useful analytical model for DNA diffusion to hybridization sites on conventional microarrays, at least two approximate approaches have been proposed. Pappaert et al. [24] modeled the process as DNA diffusing perpendicularly to the surface containing the hybridization site, whereas Gadgil et al. [25] modeled the process as DNA diffusing only radially toward and parallel to the hybridization site. Both approaches result in lower bound predictions for actual diffusion rate as they do not include diffusion from both vertical and horizontal directions. We propose modeling the process as DNA diffusing spherically to the hybridization site, which is itself modeled as a half-sphere with radius $r_o$. This model approaches an exact solution for diffusion rates where the height of the sample volume over the hybridization site is much larger than the diameter of the hybridization site, resulting in an analytical solution that is easy to evaluate mathematically. Using spherical coordinates, the following mass transport equation applies:

$$\frac{\partial C}{\partial t} = \frac{D}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial C}{\partial r} \right),$$

(2)

where $D$ is the diffusion coefficient of the labeled target and $r$ is the spherical distance from the hybridization site. Applying the appropriate boundary conditions, $C = C_{\text{init}}$ at $r = \infty$ and $C = C_{\text{wall}}$ at $r = r_o$ (valid throughout the hybridization time) to Eq. (2),

$$C = ((C_{\text{init}} - C_{\text{wall}})r_o/r) \text{erfc}(r - r_o)/(2(Dh_{\text{hyb}})^{1/2}),$$

(3a)

where erfc refers to Gauss’s error integral. Subsequent evaluation of Eq. (2) with respect to time produces the following analytical expression:
\[ M_{\text{diff}} = \frac{D}{(2r_o)(1 + 2r_o(\pi D_{\text{hyb}})^{-1/2})(C_{\text{init}} - C_{\text{wall}}) t_{\text{hyb}}}, \]  

where \( M_{\text{diff}} \) is the mass per unit area of DNA that has diffused to the semispherical hybridization site of radius \( r_o \). Eq. (3b) was arrived at by applying the condition that \( C_{\text{wall}} \) stays constant over the hybridization time. This is a valid assumption for conventional microarrays because diffusion resistance controls the overall hybridization rate, with \( C_{\text{wall}} \ll C_{\text{init}} \). Using realistic values for the DNA diffusion coefficient and hybridization site radius, the time-dependent part of Eq. (3b), and therefore the effect of modeling the hybridization site as a half-sphere rather than as a flat spot, becomes insignificant for hybridization times longer than approximately 4 h. For longer hybridization times, Eq. (3c) can be expressed in terms of the mean or average mass transfer coefficient, \( h_{\text{diff}} \), as

\[ h_{\text{diff}} = \frac{D}{(2r_o)}. \]  

In the typical microarray hybridization time of 16 h, the distance that the diffusion front travels away from the hybridization spot can be approximated by \((D t_{\text{hyb}})^{1/2}\). This distance, on the order of 0.7 mm, is well within the typical sample volume heights used in conventional well-based microarrays. For microarrays where a coverslip is used to contain samples, typical sample volume heights are in the 70-μm range. This case places a finite constraint on the growth of the diffusion front, and Eq. (3c) can be considered an upper bound for \( h_{\text{diff}} \).

C. Derivation of a diffusion mass transfer coefficient, \( h_{\text{diff}} \), for a microfluidic microarray

Creating a model consistent with our experimental device (10 × 40 μm rectangular microchannels), the system was modeled as a rectangular duct insulated on three sides (the polydimethylsiloxane [PDMS] walls) with constant concentration at the remaining wall formed by the glass slide, based on the assumption that nonspecific DNA adhesion to the hydrophobic PDMS is insignificant compared with the adhesion to the polar surface of a commercially treated microarray slide or a complementary hybridization site. The following mass transport equation applies:

\[ \nu_x \frac{\partial C}{\partial x} = D \frac{\partial^2 C}{\partial y^2}, \]  

where \( \nu_x \) is the fluid velocity along the channel and \( y \) is the channel height. The boundary conditions are \( C = C_{\text{wall}} \) at \( y = 0 \) where DNA is being transferred to the surface. At all other walls, there are no concentration gradients.

There are distinct solutions to Eq. (4) for the printing and hybridization reactions. During the printing process, DNA is transferred to the glass slide surface for the entire length of the microchannels, whereas for the hybridization process blocking agents present in the solution result in binding only in regions where probe and target DNA are complementary. Accordingly, we modeled the printing and hybridization processes separately.

As probe DNA flows down the channel during the initial printing process, DNA is immobilized to the surface of the glass slide, causing a concentration gradient to be established in the fluid over these sites. For typical flow rates used experimentally to flow DNA probes through the microchannels and deposit them on the slide surface (2 cm s\(^{-1}\)), the mass transfer concentration profile becomes fully developed at approximately 2 mm into the microchannel. Working in the fully developed regime in the printing region, the diffusion mass transfer coefficient, \( h_{\text{diff}} \), can be approximated by

\[ h_{\text{diff}} = 3.81D/d_h, \]  

where \( d_h \) is the hydraulic channel diameter for the aforementioned geometry and boundary conditions [26]. When microfluidic manifolds are used to print probe DNA, the concentration of DNA drops significantly, immobilized to the slide in the connecting microfluidic channels before reaching the hybridization region. Therefore, \( C_x \) is position dependent, related to \( h_{\text{diff}} \) by

\[ C_x = C_i e^{-h_{\text{diff}}w Q}, \]  

where \( C_i \) is the sample concentration at the channel entrance, \( C_i \) is the mean concentration of the sample at position \( x \) along the channel, \( w \) is the channel width, and \( Q \) is the sample volumetric flow rate (Fig. 1A). The mass per unit area of probe printed (\( M_{\text{immn}} \)) can then be determined by

\[ M_{\text{immn}} = h_{\text{diff}} C_w t_{\text{print}}, \]  

where \( t_{\text{print}} \) is the total print time or the time required to pass the print sample through the microfluidic device. For the hybridization process, samples are flowed across the glass slide orthogonal to the printed probes. No binding occurs when targets flow over the glass surface or sites containing noncomplementary probes. However, target hybridization occurs at sites containing complementary probes, initiating the development of a concentration gradient. The mass transport rates for flowing probes over a hybridization site can be approximated using a parallel plate model where diffusion boundary layer heights are much smaller than the distance between the parallel plates [22,23,26]. Adapting this approximation to our microchannel model, the diffusion mass transfer coefficient can be defined as

\[ h_{\text{diff}} \frac{m}{m} = 1.47(D/d_o)^{1/3}(Q/(wL))^{1/3}, \]  

where \( h_{\text{diff}} \frac{m}{m} \) is the mean or average mass transfer coefficient over a complementary hybridization site of length \( L \) and \( d_o \) is the channel height (Fig. 1B).

\[ \text{Abbreviations used:} \ PDMS, \ polydimethylsiloxane; \ BSA, \ bovine \ serum \ albumin; \ SSC, \ sodium \ saline \ citrate; \ SDS, \ sodium \ dodecyl \ sulfate. \]
D. Derivation of a reaction mass transfer coefficient, $h_{\text{react}}$, for stationary and microfluidic microarrays

Hybridization kinetics follows second-order reaction dynamics dependent on both the concentrations of the target and the probe. The conventional model of reaction rate kinetics, modified for one analyte attached to a surface, can be expressed as

$$\frac{dM_{\text{hyb}}}{dt} = k_{\text{assoc}} M_{\text{imm}} C_{\text{wall}} - M_{\text{hyb}} (k_{\text{assoc}} C_{\text{wall}} + k_{\text{diss}}),$$  \hspace{1cm} (6a)

where $M_{\text{imm}}$ is moles of immobilized probe per unit area of hybridization surface available for hybridization and $k_{\text{assoc}}$ and $k_{\text{diss}}$ are the forward and reverse hybridization reaction rate constants, respectively. If the value of $M_{\text{hyb}} (k_{\text{assoc}} C_{\text{wall}} + k_{\text{diss}})$ remains small (probe saturation levels and reverse hybridization rates are small), the hybridization kinetics can be modeled as first-order, $h_{\text{react}}$ remains constant over the course of a hybridization protocol, and can be defined as

$$h_{\text{react}} = k_{\text{assoc}} M_{\text{imm}}.$$  \hspace{1cm} (6b)

However, if the value of $M_{\text{hyb}} (k_{\text{assoc}} C_{\text{wall}} + k_{\text{diss}})$ becomes significant over the course of a hybridization reaction, the value of $h_{\text{react}}$ will not remain a constant but rather will decrease with time until it becomes rate limiting for hybridization and $C_{\text{wall}}$ will approach $C_{\text{mean}}$. This will result in an overprediction for the value of $h_{\text{react}}$ and thus in overall hybridization rates.

E. DNA diffusion coefficients and kinetic hybridization reaction constants

DNA diffusion coefficients are proportional to absolute temperature, are inversely proportional to the viscosity of the solvent, and are also influenced by the shape and dimensions of the molecules [27,28]. The DNA targets diffusing in a hybridization device are single-stranded and can range from tens to thousands of bases long. These targets carry labels such as biotin and a fluorophore that can generate steric hindrance effects and retard DNA diffusion rates. Stellwagen et al. [28] used Eq. (7a), originally proposed by Liu and Giddings [29], to predict reported single-strand DNA diffusion coefficients from approximately 10–10^4 bases at 20 °C in water.

$$D = 7.38 \times 10^{-6} L^2 / B^{0.539},$$  \hspace{1cm} (7a)

where $D$ is the DNA single-strand diffusion coefficient and $B$ is the number of bases in the DNA strand. Tinland et al. [30] used both pKS–fluorescein and pKS–YOYO at 21 °C, and Nkodo et al. [31] used Rox at 30 °C to measure the diffusion coefficients of labeled single-strand DNA. Their reported diffusion coefficients are predicted by Eq. (7b):

$$D_{\text{label}} = 4 \times 10^{-6} L^2 / B^{0.539}. $$  \hspace{1cm} (7b)

There have been numerous reports pertaining to the measurement of DNA hybridization reaction rate constants on solid substrates [32]. Sensitive rate measurements have been performed using techniques such as quartz microbalances [33], resonant mirrors [34], and BIAcore instruments.
[35,36] examining hybridization kinetics as a function of probed length, salt concentration, and temperature. Previously reported experimentally derived rate constants are on the order of $10^3$–$10^5$ for the association constant, $k_{\text{assoc}}$, and $10^{-5}$–$10^{-2}$ for the dissociation constant, $k_{\text{diss}}$. Larger values of $k_{\text{assoc}}$ were observed for longer chain lengths, higher ionic strength, and higher temperatures, whereas $k_{\text{diss}}$ increased with temperature but decreased with ionic strength and chain length [33]. Steric hindrance issues, related to a specific solid substrate, probe linker, and degree and position of homology between target and probe, would also affect the value of the hybridization reaction rate, $k_{\text{assoc}}$. Because the microfluidic flow environment used in our experimental platform is unique, lacking previously published rate constant data, the bracketed values of $k_{\text{assoc}}$ and $k_{\text{diss}}$ were used as starting reference points from which estimated values were obtained for a best fit of our experimental data.

Device fabrication

PDMS-based microfluidic devices used for the printing and hybridization of DNA samples were fabricated using established soft lithography methods where microchannels were formed by casting the elastomer against a positive photoresist substrate [37]. Master molds for the microfluidic channels were made by spin-coating 10-μm thick layers of positive photoresist (AZ 4620, Shipley) onto 3-inch silicon wafers treated with hexamethyldisilizane and patterning them with high-resolution (3500 dpi) transparency masks. After exposure, the wafers were developed to expose the latent photoresist pattern, washed with deionized water, and dried under nitrogen. Microfluidic device fabrication was carried out by pouring PDMS (Sylgard 184, Dow Corning) on the patterned wafer and curing the pre-polymer for 20 min at 80 °C. After curing, the devices were lifted off the silicon wafer and trimmed to size (1 × 1 inch), and interconnect holes connecting the microchannels on the bottom of the devices with the top surface were made by punching the device with a 20G luer stub (BD Biosciences). As a final processing step, devices were washed with isopropyl alcohol to remove debris and were stored in Petri dishes until use. Before use, the devices were exposed to either bovine serum albumin (BSA) or DNA to inhibit nonspecific DNA adsorption onto the channel walls.

DNA printing and hybridization

Experimental data were obtained using 60-mer triple repeats of (Cy3) Mag1 (ATTCGATCAGGAGATGTCGTG) and (Cy5) Apn1 (CAGATTCCATAACACGGGAC) DNA bar code sequences from the Saccharomyces gene deletion project [38]. To print probe DNA onto a microarray slide, an eight-channel microfluidic device (Fig. 2), with channel dimensions of 40 × 10 μm, was placed channel-side down on the surface of a treated glass slide (Gaps II, Corning). Then 500-nl aliquots of DNA probes (800 pM to 800 nM), suspended in 3× sodium saline citrate (SSC) buffer, were pipetted over the sample entry ports. Parallel probe loading into the microchannels was subsequently achieved by application of gentle vacuum pressure (53–76 mmHg) to the individual microfluidic output ports, simultaneously drawing the probe samples from the top of the chip through the microchannels attached to the glass slide at 22 °C. Total print times were varied from 0.5 to 4 min, resulting in different printed probe concentrations as calculated from Eqs. (5a) through (5c). As the samples flowed through the microchannels, the DNA interacted with the silane groups on the slide surface, resulting in rapid deposition of the DNA onto the glass slide.

![Fig. 2. Schematic and micrograph of an eight-channel PDMS microfluidic array device.](image)
surface, with the eight probes printed as parallel 40-μm wide lines with a 100-μm center-to-center spacing. A series of microarrays was also printed using conventional quill spotting pins. The printed spots were arranged in lines on the microarray slide so that they could be subsequently aligned with the microfluidic channels contained in the microfluidic device used for hybridization. The microfluidic devices were then removed from the glass slide and UV-crosslinked (65 mJ/cm²) to enhance probe immobilization. After crosslinking, the arrays were air-dried and stored in the dark.

To expose targets to the microfluidically printed microarray for hybridization, an identical microfluidic device rotated 90° relative to the first device was placed on the surface of the probe-printed microarray where the channels were orthogonal to the printed parallel lines of DNA on the slide. To expose targets to the conventionally printed microarray, the microfluidic device was positioned on the surface of the microarray so that the channels lined up with and covered the spotted probes. Then 300-nl to 3-μl aliquots of labeled target DNA samples (10 pM to 10 nM in 5 × SSC/25% [v/v] formamide/0.1% sodium dodecyl sulfate [SDS]/6 μg/ml sheared salmon sperm [DNA]) were introduced into each of the sample entry ports and drawn along the channels using gentle vacuum pressure (53–76 mmHg) at 22°C from 12 s to 8 min to achieve different hybridization signal intensities. Hybridized microarray slides were subsequently scanned with the ArrayWoRx e Biochip Reader (Applied Precision). Slides were scanned for Cy3 and Cy5 fluorescence at a resolution of 9.756 μm. Peak minus mean background signal intensity was used to determine hybridization signal intensity. Data points were considered valid for signal/background > 1.25 and predicted signal intensities within the linear range of the Biochip reader (< 50,000 RFU). The viscosities of the experimental print and hybridization solutions were measured using a control stress rheometer (AR 1000-N, TA Instruments) with deionized water as a reference fluid.

Results and discussion

Qualitative results

Qualitative experiments were performed to show how effectively microfluidic microarrays can be used to rapidly detect specific DNA targets in the presence of competing probes and targets. Fig. 3 shows the results of a hybridization experiment for two DNA bar code targets, Mag1 (Cy3) and Apn1 (Cy5), hybridized against their printed complement (20 pmol cm⁻²). Cy3 and Cy5 targets were detected down to 100 and 10 pM, respectively, with low cross-hybridization background observed between nonspecific pairs. All printing was performed in less than 1 min, and all hybridizations were performed in less than 5 min.

Quantitative results

Quantitative experiments were performed to explore the ability of the theoretical model to predict the overall mass transfer coefficient (Htot) for the microfluidic microarray over a range of target concentrations (Cmean from 10 pM to 10 nM) and printed probe concentrations (Mtot from 10⁻¹² to 10⁻¹⁷). Both are similar to values used with conventional microarrays. Viscosity-corrected diffusion coefficients, derived from Eqs. (7a) and (7b), and the range of reported hybridization association reaction rate constants [32–36] were used as starting reference points from which estimated values were obtained through a best fit of our experimental data. The measured viscosity ratios of print and hybridization solutions to water were 1.04 and 1.46, respectively. The unlabeled DNA probe diffusion coefficient (Dp) (Eq. 7a), corrected for viscosity, was 7.81 × 10⁻⁹, whereas the corrected coefficient for the labeled DNA target (Dt) (Eq. 7b) was 3 × 10⁻⁷. Best fit of experimental data was obtained with Dp = 3 × 10⁻⁷, Dt = 1.8 × 10⁻⁷, and kassoc = 1.2 × 10⁶.

Experimental hybridization results from five different microarray slides are shown in Fig. 4. Measured values for [Signal Intensity/(Cmean/λ)] were plotted against predicted values for overall mass transfer coefficients, Htot. Individual data points with similar values of Htot were generated by different microfluidic channels distributed across the five slides. Individual microfluidic channels spanned
several different hybridization sites, generating data points across the range of \( H_{tot} \). A linear relationship is expected, according to Eq. (1c), and a linear fit \((R^2=0.93)\) is observed, supporting the theoretical model. Low calculated probe saturation levels (<5%) and hybridized target concentrations (<10^{-12}) suggest that hybridization kinetics was first order, also supporting the model and the use of Eq. (6b) to determine values for \( h_{react} \). Overall slide-to-slide signal intensities were normalized to eliminate variations due to effects such as microarray reader amplification, different slide surface characteristics, and probe labeling efficiencies. The values of \( H_{tot} \) were determined primarily by the concentrations of the printed probes given that diffusion accounted for less than 10% of overall hybridization resistance. The value used for \( C_{mean} \) must reflect any reduction in target concentration during the hybridization process by being set equal to the average of the target concentration over the total hybridization time. Reduction in \( C_{mean} \) may be caused by interaction with serial probes or by significant loss through nonspecific adsorption. In these experiments, each target sample passed over eight lanes of probe, where less than 0.1% of the total target was lost at each probe site, resulting in an insignificant change in \( C_{mean} \). There were no observed differences between data from microfluidically printed slides and data from conventionally spotted slides. The data points for values of \( H_{tot} < 10^{-14} \) display a higher deviation due to lower hybridization rates and thus lower signal/background ratios \((1.25-3.00)\). Shear rates experienced by samples during hybridization \((10 \text{ s}^{-1})\) did not appear to reduce or increase hybridization levels for any of the target or probe concentrations tested. However, in separate hybridization stringency washing tests, shear rates 100 times higher resulted in loss of hybridization signal intensity, presumably from physical stripping of hybridized target from probe. It is not expected that shear forces caused by flow rates in our experiments would improve or reduce the ability of a probe to discriminate between closely related sequences. Different hybridization rates between these sequences should depend only on the differences in the values of \( k_{assoc} \) and \( k_{diss} \) between the related sequences. Flow-based studies to determine these values do not report any flow-related effects on hybridization rates [33–36].

Effect of presence of noncomplement on hybridization rates

Noncomplementary hybridization can affect the accuracy of microarrays. Values for \( k_{assoc} \) for noncomplementary binding have been measured, are much lower than values for complementary binding, and depend on the length of the DNA strand and on the number and position of base mismatches [35]. However, for high concentrations of similarly labeled noncomplementary targets, an increase in hybridization signal intensity may be recorded due to noncomplementary hybridization for both conventional and microfluidic microarrays. The magnitude will be dependent on the concentration and the values of \( k_{assoc} \) and \( k_{diss} \) for the noncomplement. The presence of noncomplementary targets may also lower the complement hybridization rate by decreasing the concentration of unoccupied hybridization sites, as can be seen from Eqs. (6a) and (6b). The theoretical model can still be used when significant noncomplementary hybridization exists if the following adjustments are made. Signal resulting from noncomplementary hybridization must be subtracted from overall measured hybridization signal, and the concentration of immobilized probe \( (M_{imm}) \) in Eq. (6b) must be adjusted to reflect the loss of hybridization sites to noncomplementary hybridization.

Comparison of theoretical hybridization times for microfluidic versus conventional microarrays

The reduction in hybridization time achieved with a microfluidic microarray in comparison with a conventional microarray can be assessed by evaluating the inverse ratio of their respective total mass transfer coefficients \( (H_{tot}) \). Using the same probe and target concentrations for the microfluidic and conventional microarrays, the ratio of \( H_{tot} \) will reflect the reduction in time to reach a specific signal intensity. The \( h_{diff} \) portion is evaluated using Eq. (5d) for a microfluidic microarray and Eq. (3c) for a conventional microarray. For both, the \( h_{react} \) portion is calculated using Eq. (6b). The conventional hybridization radius was set at 100 \( \mu \text{m} \), and the value of \( h_{react} \) was set at \( 1 \times 10^{-9} \), which represents the highest concentration of printed probe realized in our experiments. These results are presented in Fig. 5 as a function of the length of the diffusing DNA targets, comparing a conventional microarray with the microfluidic microarray used in the experiments and an optimized microfluidic microarray with \( 20 \times 5-\mu \text{m} \) microchannels. The increased advantage of the microfluidic microarray with longer target lengths is caused by the reduction in diffusion coefficient with increased target length, as shown by Eq. (7b). This affects hybridization rates for the diffusion-limited conventional microarray.
but not for the reaction rate-limited microfluidic microarray. The optimized design used the lowest practical channel height that avoided frequent blocking by debris and a channel width equivalent to twice the resolution of a standard microarray reader. Using 16 h as the conventional microarray hybridization time, it can be seen that, for the DNA targets used in these experiments, the current microfluidic microarray is predicted to detect the same target concentration level in 9 min. For the optimized microarray, the time is reduced to 4.8 min. In comparison, it can be seen that reducing the microchannel dimensions beyond the current prototype does not translate to a significant performance gain, only decreasing the hybridization time by approximately two times in the optimized microarray. These dimensions may represent a performance upper bound. Smaller channel dimensions may increasingly fail due to impurities in the samples more frequently blocking the microfluidic channels. Where conventional microarrays are placed in rotisserie hybridization chambers, some level of internal mixing will take place, although the amount will be restricted by the small hybridization chamber dimensions (~50 μm) where viscous effects are dominant. This mixing will result in a higher value for $h_{out}$ than that calculated from Eq. (3c), although a 16-h hybridization time is still recommended by commercial manufacturers.

The microfluidic microarray appears to meet and potentially exceed the 10-pM sensitivity limit of conventional microarrays. The targets used in the microfluidic experiments had only one fluorophore attached to a 60-mer DNA strand and were detected at a concentration of 10 pM. cDNA targets generated in a gene expression protocol are approximately 600 bases long and may have up to one fluorophore incorporated for every 20 bases, resulting in 30 times higher fluorescence per mole of target. This may result in 30 times higher sensitivity, or the ability of a microfluidic microarray to detect 333-fM targets.

### Conclusions

Faster measurement of sample target concentrations can be achieved by use of microfluidic microarrays. The ability to detect 10 pM of target concentration in 5 min using as low as 0.3 μl of sample volume was demonstrated. This represents a 200-fold decrease in hybridization time over conventional DNA microarrays. The practical limit for a decrease in hybridization time is approximately 300-fold using $20 \times 5$-μm microchannels. Further decreases are limited by how small microchannels can be fabricated in a practical manner while still allowing samples to pass through them consistently.

The theoretical model accurately predicts the overall mass transfer coefficient for a microfluidic device in the range of target concentrations used with conventional microarrays. The model demonstrates the ability to incorporate the effects of target diffusion and hybridization reaction kinetics into the predicted value of $H_{tot}$ and demonstrates that this value is independent of sample target concentration. The model can also be used to predict the effects on $H_{tot}$ of changing parameters such as microfluidic physical dimensions, flow characteristics, and physical constants. The model is valid where probe saturation levels remain less than approximately 5%, and reverse hybridization rates do not significantly affect overall hybridization rates. At higher probe saturation values, the first-order assumption for hybridization kinetics becomes invalid and the model overpredicts hybridization rates. For experimental conditions similar to conventional microarrays, our theoretical model remained linear. Microarrays (conventional or microfluidic) are generally run with probe saturation values $<<5\%$, satisfying the constraints of the model. We anticipate that the model will serve as a valuable tool for general microfluidic microarray design and optimization.

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### References


