Optimization of microfluidic microsphere-trap arrays

Xiaoxiao Xu,^{1,a)} Pinaki Sarder,^{2,a)} Zhenyu Li,^{3,a)} and Arye Nehorai^{1,b)}

¹*The Preston M. Green Department of Electrical and Systems Engineering,
Washington University in St. Louis, St. Louis, Missouri 63130, USA*

²*The Mallinckrodt Institute of Radiology, Washington University School of Medicine
in St. Louis, St. Louis, Missouri 63110, USA*

³*Department of Electrical and Computer Engineering, The George Washington University,
Washington, D.C. 20052, USA*

(Received 31 October 2012; accepted 28 December 2012; published online 27 February 2013)

Microarray devices are powerful for detecting and analyzing biological targets. However, the potential of these devices may not be fully realized due to the lack of optimization of their design and implementation. In this work, we consider a microsphere-trap array device by employing microfluidic techniques and a hydrodynamic trapping mechanism. We design a novel geometric structure of the trap array in the device, and develop a comprehensive and robust framework to optimize the values of the geometric parameters to maximize the microsphere arrays' packing density. We also simultaneously optimize multiple criteria, such as efficiently immobilizing a single microsphere in each trap, effectively eliminating fluidic errors such as channel clogging and multiple microspheres in a single trap, minimizing errors in subsequent imaging experiments, and easily recovering targets. We use finite element simulations to validate the trapping mechanism of the device, and to study the effects of the optimization geometric parameters. We further perform microsphere-trapping experiments using the optimized device and a device with randomly selected geometric parameters, which we denote as the un-optimized device. These experiments demonstrate easy control of the transportation and manipulation of the microspheres in the optimized device. They also show that the optimized device greatly outperforms the un-optimized device by increasing the packing density by a factor of two, improving the microsphere trapping efficiency from 58% to 99%, and reducing fluidic errors from 48% to a negligible level (less than 1%). The optimization framework lays the foundation for the future goal of developing a modular, reliable, efficient, and inexpensive lab-on-a-chip system. © 2013 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4793713>]

I. INTRODUCTION

With the heightened interest in developing lab-on-a-chip medical diagnostic devices,^{1–3} there has been a growing need to bridge multiple disciplines in implementing such technologies to perform rapid disease diagnosis and prognosis.⁴ Microarrays can detect different biological targets, such as DNAs, mRNAs, proteins, antibodies, and cells in a single device. They have recently been proven to be a great platform for building lab-on-a-chip systems.⁵ Figure 1 shows schematics of a microsphere array device and its target detection and quantification mechanism.^{6,7} Here, the microspheres are conjugated on the surface with molecular probes to capture targets of interest. The targets are tagged with labels (e.g., quantum dots (QDs), fluorescent dyes, etc.) with conjugated receptors. These labels radiate under fluorescence optical imaging and provide information for target detection and concentration estimation using statistical analysis tools.

^{a)}X. Xu, P. Sarder, and Z. Li contributed equally to this work.

^{b)}Author to whom correspondence should be addressed. Electronic mail: nehorai@ese.wustl.edu.

FIG. 1. Schematic diagram of the microsphere arrays and the target detection and quantification mechanism. (a) The microspheres separated by a distance d are arranged so that their centers are positioned in a plane parallel to the xy plane. They are encoded with specific receptors (not shown) to capture one side of the targets of interest. (b) To detect and quantify the targets, labels (e.g., quantum dots (QDs), fluorescent dyes, etc.) with conjugated receptors tag the other side of the targets. These labels radiate upon excitation under fluorescence optical imaging and provide information for target detection and quantity estimation.^{6,7}

To fabricate the microsphere array device, the industrial standard methods are robotic printing,⁸ photolithography patterned *in situ* synthesis (such as Affymetrix),⁹ and self-assembly of microbeads (such as Illumina).^{10,11} However, due to the limited size of their printing spots, robotic-printed microarrays suffer from inhomogeneous distribution⁸ and inefficient packing. Photolithographic patterned microarrays are costly and complicated to implement.⁹ Self-assembled microarrays need specially fabricated substrates such as etched fiber optic bundles or silicon wafers, and thus they are also relatively expensive. To eliminate these drawbacks of the existing methods, researchers recently have implemented the microsphere array system by microfluidic techniques (we denote them as microfluidic microsphere-trap arrays).^{12–14} The microfluidics microsphere-array device has the advantages of having a fast reaction rate due to active flow and providing a gentle liquid environment for biological samples. The device also can employ on-chip micromechanical valves and isolated chambers to distinguish diverse targets in its different compartments.^{15,16}

As an independent and dedicated platform, the performance of the microfluidic microsphere-trap array device depends on a careful optimization of the device architecture. Several criteria should be taken into account, including maximizing microspheres' packing density to make the device compact, efficiently immobilizing microspheres, effectively eliminating fluidic errors, minimizing errors introduced during the device's fabrication, and minimizing aberrations induced during the subsequent fluorescence imaging.⁶ However, to date (to our knowledge), no studies have been reported about simultaneous optimization of these multiple criteria.

To address the above problems, we design for the microfluidic microsphere-trap array device a novel trap array geometry (traps in inverted-trapezoid shapes) and employ a hydrodynamic trapping mechanism to immobilize the microspheres in the traps. We further develop an analytical method to optimize the values of the trap's geometric parameters to maximize the microsphere arrays' packing density. In this optimization, we simultaneously satisfy also other criteria, such as efficiently immobilizing a single microsphere in a single trap, effectively eliminating fluidic errors, and minimizing error in imaging the microspheres. We compute the optimized geometric parameters for a device capturing microspheres of radius $5\text{ }\mu\text{m}$ and use finite element simulations to validate the trapping mechanism of the device and investigate the effects of these parameters on the packing density. Microsphere-trapping experiments performed using the optimized device demonstrate the easy-control of the transportation, immobilization, and manipulation of microspheres in the trap arrays. We also fabricate another device with randomly selected values of the geometric parameters, which we denote as the un-optimized device for convenient reference. Further quantitative comparisons also show that the optimized device greatly outperforms the un-optimized device. The optimized device has a much higher packing density (1438 traps/mm^2) than that of the un-optimized one (762 traps/mm^2). Moreover, the optimized device has a higher microsphere trapping efficiency (a single microsphere in a trap) than the un-optimized one. In particular, for the former more than 99% of the traps are found to be filled with a single microsphere, whereas for the latter the percentage is 58.

This paper is organized as follows. Section II describes the structure of our device, the hydrodynamic trapping mechanism to immobilize the microspheres in the traps, and the optimization formulation of the trap geometry. Section III shows the finite element fluidic dynamics simulation results. In Section IV, we compare the results of the microsphere trapping experiments using the optimized device and the un-optimized device. We also discuss the comparison between our device and self-assembled three-dimensional (3D) microarrays. Section V concludes the paper.

II. OPTIMIZING MICROFLUIDIC MICROSPHERE-TRAP ARRAYS

We first briefly describe the structure of our microsphere-trap arrays and their hydrodynamic trapping mechanism. We then present the geometry of a single trap and its surrounding microfluidic channels and formulate the optimization problem for this geometry. We note that *trapping* here means to immobilize the microspheres at predetermined locations in the trap arrays during the experiments, as Figure 2 shows. Embedded receptors on the trapped microspheres capture targets in subsequent experiments.^{6,7}

A. Structure of the microfluidic microsphere-trap arrays

Figure 2(a) is a schematic of the microfluidic microsphere-trap array. It presents the top view of the microfluidic channels with hydrodynamic trap arrays. The traps in the arrays are made of polydimethylsiloxane (PDMS). Each trap is made of inverted-trapezoid grooves. The microfluidic channels are connected with each other by a common inlet and outlet, as shown in Figure 2. Note that a microfluidic channel describes the path between any two consecutive traps and between any two rows of the trap array. To fill the traps, a liquid, such as phosphate buffered saline (PBS), containing the microspheres with specific receptors flows through the channels. The microspheres are immobilized by the traps during the process. To avoid cross contamination, in the intermissions of the microspheres' loading operation, the residual spheres are washed out using buffer solution.

In our design of the trap array device, each row of the traps is offset horizontally with respect to the one above it (Figure 2(a); inset *a*). This offset ensures the microspheres not trapped

FIG. 2. Schematic of the microfluidic microsphere-trap array. (a) Layout (top view): Microfluidic channels with hydrodynamic trap arrays. The channels are connected by a common inlet and a common outlet. Liquid solution carrying the microspheres flows from the inlet and through the chamber. Microspheres are trapped by the hydrodynamic trap arrays during the process. Inset *a* shows a zoomed-in view of trap arrays in a microfluidic channel, and inset *b* shows a single trap for capturing one microsphere. The white dashed square shows the area *S* of the single trap and its surroundings, whose length and width are defined by *x* and *y*. (b) Trapping mechanism: The top figure shows how an empty trap automatically captures a single microsphere, because the corresponding path *P*₁ is designed to have a lower flow resistance than path *P*₂. We denote this mechanism as *trapping*. Once the trap through path *P*₁ is filled, the flow resistance of path *P*₁ increases dramatically and is much larger than that in path *P*₂. Thus, subsequent microspheres flow through path *P*₂. We denote this mechanism as *bypassing*.

by the first row can easily be captured by the next row of traps. The separations between adjacent traps and rows are optimized to ensure minimal channel clogging (channel clogging refers to obstruction in a channel region that restricts the flow of microspheres. As a result, unwanted microspheres aggregate in that region.¹⁷). Such separations also eliminate the possibility of two microspheres arriving at a trap simultaneously and intending to fill in the same trap.

Next, we will explain the hydrodynamic trapping mechanism of microspheres in the trap arrays. We remind the readers that the device is designed for later use of detecting multiple targets, such as DNAs and antibodies, captured by receptors embedded on the surface of the microspheres.^{6,7}

B. Hydrodynamic trapping mechanism

The proposed device employs fluidic resistance engineering to perform hydrodynamic trapping of microspheres.^{13,18,19} To explain this mechanism, we schematically present the possible flow paths of a microsphere in Figure 2(b). In this figure, path P_1 (pink line) is the *trapping* path and path P_2 (green line) is the *bypassing* path. Here we define *trapping* as a microsphere flowing into the trap, and we define *bypassing* as the flow of subsequent microspheres through the channels next to the trap. This scheme for a single trap is applicable for all the traps.

In order to trap the microspheres as shown in Figure 2(b), the trap array geometry should be designed so that the *trapping* path P_1 for an empty trap has a lower flow resistance than the *bypassing* path P_2 . Then during the loading process, a microsphere in the fluid is most likely to move into an empty trap through P_1 (Figure 2(b), top). However, once the trap through P_1 is loaded by a microsphere, the flow resistance in P_1 dramatically increases and is much larger than that in P_2 , and thus subsequent microspheres divert to path P_2 and bypass the filled trap (Figure 2(b), bottom).

C. Trap geometry and optimization

Obeying the hydrodynamic trapping mechanism explained above, we have designed a modular trap geometry to immobilize the microspheres, particularly to ensure a single microsphere in each trap. We have optimized this geometry to increase the microspheres' packing density and simultaneously satisfied other design criteria, such as eliminating channel clogging,¹⁷ avoiding multiple microspheres trapping at one trap location, satisfying the trap array device's microfabrication tolerance and feasibility,²⁰ and achieving the optimal distance d_0 between microspheres obtained in the statistical design to minimize image analysis error.⁶ Image analysis error is experienced during analysis of the fluorescence images of targets captured by the microsphere array device.⁶ In the following, we first present the proposed trap geometry, then discuss the formulation of the optimization for this geometry, including the objective function and constraints.

Figure 3 shows a schematic diagram of the trap geometry and depicts the corresponding geometric parameters. We define the radius of the microsphere as r ; the height of the groove (i.e., height of the channel) as h ; the length and the upper width of the groove walls as l and t , respectively; the trapezoid angle of the trap as α ; and the upper and the bottom widths of the trap opening as u and b , respectively. We also define the width of the channel as g , the distance between two microspheres in the same row as d , and the minimal distance between a trap and a microsphere filled in a consecutive row as v . To eliminate the units of these parameters, we normalize them by dividing by the groove height h (see Figure 3). We use below the sign $\sim$ to represent the resulting parameters; e.g., $\tilde{r}$ represents the normalized r . Furthermore, we define the area of a single trap and its surroundings as S , whose length and width are x and y , respectively (see the white dashed square in Figure 2(a); inset b). Finally, we define the packing density of the arrays as ρ .

1. Optimization objective function

We aim to maximize ρ of the microsphere arrays. This is equivalent to minimizing the area S of each trap and its surroundings, as seen in Figures 2 and 3. From these figures,

FIG. 3. Schematic diagram of the proposed trap array geometry. Three adjacent traps are presented here, with the first two traps in the same row and the third trap in a subsequent row. Each trap is made of inverted-trapezoid grooves. This diagram also shows the two flow paths of a microsphere encountering the first trap: the *trapping* path (pink line) and the *bypassing* path (green line). The microsphere chooses the *trapping* path when it experiences smaller flow resistance in this path than in the *bypassing* path; otherwise it chooses the *bypassing* path. The *trapping* path consists of the sub-paths P_{11} , P_{12} , and P_{13} , and the *bypassing* path consists of the sub-paths P_{21} , P_{22} , P_{23} , P_{24} , and P_{25} ; see more details in *Constraint 1* of Subsection II C 2.

$$x = u + 2t + g, \quad y = g + l, \quad S = xy. \quad (1)$$

Therefore, the optimization objective function is $\rho = 1/S$, where S is to be minimized with respect to the trap array geometric parameters $\delta = [r, h, l, u, b, t, g, d, v]^T$. For simplicity, we keep the values of r and h fixed in δ , and denote the other parameters as the optimization parameters. To summarize, the optimization objective is

$$\rho_{\text{opt}} = 1/S_{\text{opt}}, \quad \text{with} \quad S_{\text{opt}} = h^2 \cdot \min_{\delta} (\tilde{g} + \tilde{l}) \cdot (\tilde{u} + 2\tilde{t} + \tilde{g}). \quad (2)$$

2. Optimization constraints

The optimization constraints are formulated to achieve the multiple criteria we proposed in the Introduction, i.e., the desired hydrodynamic trapping, a feasible device fabrication, a high microsphere trapping efficiency, small fluidic errors, and minimal errors in imaging the microspheres after they capture targets.

Constraint 1: We first formalize the constraint for the desired hydrodynamic trapping. According to this mechanism, we require a smaller flow resistance in path P_1 (pink line in Figure 3) than that in path P_2 (green line), for an empty trap. This in turn requires the volumetric flow rate Q_1 along the path P_1 be higher than the rate Q_2 along the path P_2 ,^{18,19} and thus the volumetric flow rate ratio $Q_1/Q_2 > 1$. Note that volumetric flow rate defines the volume of fluid that passes through a given surface per unit time.²¹ Volumetric flow rates Q_1 and Q_2 are related to the pressure drops along the paths P_1 (ΔP_1) and P_2 (ΔP_2), respectively.^{18,19} Therefore, we compute ΔP_1 and ΔP_2 first.

The general expression of the pressure drop ΔP in a rectangular microchannel is derived¹³ based on Darcy-Weisbach equation and the Hagen-Poiseuille flow problem for continuity and momentum equations.²² Here, fully established flow is assumed inside the trapping area, which

in practice can be achieved by fabricating the trapping area far enough from the liquid entrance port. The expression of ΔP is given by

$$\Delta P = \frac{f(\beta)\mu Q C^2 L}{32A^3}, \quad (3)$$

where μ is the fluid viscosity, L is the length of the channel, Q is the volumetric flow rate, and A and C are the channel's cross-sectional area and perimeter. The function $f(\beta)$ is a known polynomial of the aspect ratio β ,²¹ which is given by

$$f(\beta) = 96(1 - 1.3553\beta + 1.9467\beta^2 - 1.7012\beta^3 + 0.9564\beta^4 - 0.2537\beta^5),$$

where β is the ratio of the height and width of the rectangular channel, such that $0 \leq \beta \leq 1$.

For the trap array geometry in Figure 3, we compute ΔP_1 and ΔP_2 as explained as follows:

- ΔP_1 (pink line in Figure 3): Path P_1 consists of the sub-paths P_{11} (above the trap), P_{12} (through the trap), and P_{13} (below the trap). We have the length of P_{12} as $P_{12}^l = l$, where l has been defined as the length of the groove. The width of P_{12} is continuously changing from the top opening u to the bottom opening b , both of which are several μm long. Moreover, the widths of P_{11} and P_{13} equal the length of the whole horizontal channel, which is more than $1 \times 10^3 \mu\text{m}$ long. Therefore, the widths of P_{11} and P_{13} are much greater than that of P_{12} , the pressure drops along P_{11} and P_{13} are negligible and most of the pressure drop in P_1 occurs along P_{12} .²¹

Therefore, from Eq. (3) and Figure 3, we have

$$\Delta P_1 = \int_0^l \frac{f(\beta)\mu Q_1 C^2}{32A^3} dl'. \quad (4)$$

where $A = wh$, $C = 2(w + h)$, $\beta = w/h$, with w denoting the width of P_{12} . For the sub-path P_{12} through the trap, at any moment the microsphere is flowing in a piece-wise rectangular channel of infinitesimally small width $d w$. This infinitesimal metric changes with the length l of the triangular shape inside the trap, and we thus substitute w with l while deriving the pressure drop along P_{12} . Therefore, substituting $w = \frac{(b-u)}{l} \cdot l' + u$, $\tilde{w} = w/h$, and $\tilde{l}' = l'/h$ into Eq. (4), we obtain

$$\Delta P_1 = \int_0^{\tilde{l}} \frac{f(\tilde{w})\mu Q_1 (\tilde{w} + 1)^2}{8\tilde{w}^3 h^3} d\tilde{l}'. \quad (5)$$

- ΔP_2 (green line in Figure 3): Path P_2 has the same start and end points as path P_1 , and it consists of the sub-paths P_{21} (above the trap), P_{22} (above the separation between the traps), P_{23} (through the separation between the traps), P_{24} (below the separation between the traps), and P_{25} (below the trap). Again, the widths of P_{22} and P_{24} (equaling the length of the whole horizontal channel) are so large that we ignore the pressure drops along them. Most of the pressure drops happen along the sub-paths P_{21} , P_{23} , and P_{25} , which have the same width g . The length of P_2^l becomes $P_2^l = P_{21}^l + P_{23}^l + P_{25}^l = u + 2t + g + l$. Therefore, using $A = gh$ and $C = 2(g + h)$ in Eq. (3), we obtain

$$\Delta P_2 = \frac{f(\tilde{g})\mu Q_2 (\tilde{g} + 1)^2 \tilde{P}_2^l}{8\tilde{g}^3 h^3}, \quad (6)$$

where $\tilde{P}_2^l = P_2^l/h$, $\tilde{g} = \tilde{g}$ if $\tilde{g} \leq 1$, and $\tilde{g} = \tilde{g}^{-1}$ otherwise.

- Equating ΔP_1 and ΔP_2 , we obtain the expression of Q_1/Q_2 . Recall that we require $Q_1/Q_2 > 1$ to achieve hydrodynamic trapping, *Constraint 1* is $C_1 = \{G(\delta) < 0\}$, where

$$G(\delta) = \int_0^{\tilde{l}} \frac{f(\tilde{w})(\tilde{w}+1)^2}{w^3} d\tilde{w} - \frac{f(\tilde{g})(\tilde{g}+1)^2 \tilde{P}_2^l}{\tilde{g}^3}. \quad (7)$$

Constraint 2: To ensure that a microsphere is trapped in a trap and to reduce the chance that multiple microspheres are in a trap, we require b to be smaller than the microsphere's diameter ($\tilde{b} < 2\tilde{r}$). We also require u and l to be smaller than the sum of two microspheres' diameters ($\tilde{u} < 4\tilde{r}$ and $\tilde{l} < 4\tilde{r}$). To avoid the cases when fabrication variations hinder the values of these parameters to satisfy this constraint, we use $2\mu\text{m}$ safety margins.²³ Therefore, *Constraint 2* is given by $\mathbf{C}_2 = \{\tilde{b} \leq 2\tilde{r} - 2/h, \tilde{u} \leq 4\tilde{r} - 2/h, \tilde{l} \leq 4\tilde{r} - 2/h\}$.

Constraint 3: To ensure stable trapping of the microspheres, i.e., a microsphere is retained in a trap and is not swept away due to the transient flow motion around the trap, we require the trapezoid angle $\alpha = 2\arctan(0.5(\tilde{u} - \tilde{b})/\tilde{l})$ to be greater than 5° . For α smaller than 5° , the vertical component of the trapping force would become too small to hold the microspheres in the traps, and we observed in experiments the microspheres can escape through the openings. We also require l to be larger than the radius of the microsphere ($l > r$). Therefore, *Constraint 3* is $\mathbf{C}_3 = \{-\alpha \leq -5^\circ, -\tilde{l} \leq -\tilde{r}\}$.

Constraint 4: To avoid channel clogging, we require $\tilde{g} > 2\tilde{r}$ to allow one microsphere flowing through the channel during the *bypassing* process. We also require $\tilde{g} < 4\tilde{r}$ to avoid multiple microspheres flowing simultaneously through the channel. Similar to *Constraint 2*, we use $2\mu\text{m}$ margins, considering fabrication variations. Therefore, we modify this inequality to be $2\tilde{r} + 2/h < \tilde{g} < 4\tilde{r} - 2/h$.

We also require v , the minimal distance between a trap and a microsphere filled in a consecutive row, to be greater than the microsphere's diameter, i.e., $\tilde{v} > 2\tilde{r}$, where $\tilde{v}^2 = (\tilde{g} - \sqrt{\max(0, \tilde{r}^2 - (0.5\tilde{u})^2)} - \tilde{r})^2 + (0.5\tilde{g})^2$. Allowing for fabrication variations, the requirement becomes $\tilde{v} > 2\tilde{r} + 2/h$. Therefore, *Constraint 4* is $\mathbf{C}_4 = \{\tilde{g} \leq 4\tilde{r} - 2/h, -\tilde{g} \leq -2\tilde{r} - 2/h, -\tilde{v} \leq -2\tilde{r} - 2/h\}$.

Constraint 5: For fabrication feasibility, the possible aspect ratios (the ratio of transverse dimensions to height, for example, t/h , i.e., $\tilde{t}$) of the geometric parameters in the device should be limited in the range of $[0.4, 2.5]$. Features with too small aspect ratios are difficult to fabricate using soft lithography, and channels with too large aspect ratios easily collapse. Therefore, *Constraint 5* is $\mathbf{C}_5 = \{\tilde{l}, \tilde{g}, \tilde{b}, \tilde{u}, \tilde{t} \leq 2.5, -\tilde{l}, -\tilde{g}, -\tilde{b}, -\tilde{u}, -\tilde{t} \leq -0.4\}$.

Constraint 6: To minimize the error in imaging the targets captured by the microspheres, the distance $d = u + 2t + g$ between the centers of two immobilized microspheres should be greater than the minimal distance d_0 that can be computed using the method developed in our earlier publication.⁶ Therefore, *Constraint 6* is given by $\mathbf{C}_6 = \{-\tilde{d} \leq -\frac{d_0}{h}\}$.

The optimization problem is summarized as

$$\rho_{\text{opt}} = 1/S_{\text{opt}}, \quad \text{with} \quad S_{\text{opt}} = h^2 \cdot \min_{\delta} (\tilde{g} + \tilde{l}) \cdot (\tilde{u} + 2\tilde{t} + \tilde{g}), \quad (8)$$

where $\delta \in \{\mathbf{C}_1 \cap \mathbf{C}_2 \cap \mathbf{C}_3 \cap \mathbf{C}_4 \cap \mathbf{C}_5 \cap \mathbf{C}_6\}$.

To solve Eq. (8), we used the interior-point optimization algorithm.²⁴ We further confirmed the result obtained from this method using the grid-search method²⁵ on the feasible parameter space defined by δ .

III. FINITE ELEMENT SIMULATION

In this section, by solving Eq. (8), we compute the optimal trap array geometry for trapping microspheres of radius $r = 5\mu\text{m}$. We use finite element simulation to validate the hydrodynamic trapping of the microspheres in the device. We also investigate the sensitivities of the packing density ρ to the optimization geometric parameters in δ , to evaluate the effects of these parameters.

First, we set the fixed parameter h to be $13\mu\text{m}$, for microspheres of radius $5\mu\text{m}$. For our optimization, h acts as a normalizing factor but does not affect the packing density of the

TABLE I. Fixed and optimization geometric parameters for the microfluidic microsphere-trap array.

Fixed values (μm)	r	h			
	5	13			
Optimized values (μm)	l_{opt}	u_{opt}	b_{opt}	t_{opt}	g_{opt}
Interior-point	5.210	10.001	6.915	5.205	14.546
Grid-search	5.200	10.020	6.900	5.200	14.600

device. However, h should be larger than one microsphere's diameter to avoid the microsphere flowing out of the channel. It also should be shallow enough to avoid one microsphere flowing on top of another microsphere so that the two arrive at the trap simultaneously. Based on experimental testing results, we choose $h = 2.6r$. The values of r and h are summarized in Table I. We note that the minimal distance d_0 to minimize the imaging error for microspheres of radius $5 \mu\text{m}$ is $20 \mu\text{m}$.⁶

We then obtain the optimal values of the optimization parameters in δ , following the method described in Subsection II C. As stated, the interior-point algorithm and the grid-search method are used to solve Eq. (8). The two optimization methods give almost identical results for the optimization parameters l , u , b , t , and g ; see Table I. To restate, l is the length of the groove walls, u is the upper width of the trap opening, and b is the bottom opening width. t is the upper width of the groove wall, and g is the width of the channel. Note that the parameters d and v in δ are not listed as they are functions of the other parameters. The S_{opt} computed from the interior-point method and the grid-search method are $690.61 \mu\text{m}^2$ and $686.39 \mu\text{m}^2$, respectively, with corresponding ρ_{opt} of 1448 traps/ mm^2 and 1456 traps/ mm^2 .

To validate the hydrodynamic trapping mechanism for immobilizing the microspheres in our device, we perform finite element simulation of the transient motion of the microspheres flowing with the fluid into the device, by use of COMSOL MULTIPHYSICS 3.5;²⁶ simulation details are described in the supplementary material (finite element simulation).²⁷ Due to the high computational demand in 3D fluidic dynamics simulations, the simulations are done in 2D. Figures 4 and 5 present the

FIG. 4. Finite element simulation of one microsphere (denoted as 1) trapping process ((a)-(d)). Fluid flows into the inlet with fully developed laminar characteristics with a parabolic velocity profile. The boundary condition for the outlet is 0 pa pressure with no viscous stress.

FIG. 5. Finite element simulation of one microsphere (denoted as 2) bypassing process ((a)-(d)), when the trap is already filled by a microsphere (denoted as 1). Fluid flows into the inlet with fully developed laminar characteristics with a parabolic velocity profile. The boundary condition for the outlet is 0 pa pressure with no viscous stress.

positions of the microspheres, as well as the fluid velocity surface plot and streamline plot, at several time points. Particularly, Figure 4 demonstrates that when the trap is empty, the microsphere directly flows into the trap and is immobilized (the *trapping* process). Figure 5 shows that when the trap is filled with a microsphere, the subsequent microsphere passes by the trap (the *bypassing* process). The hydrodynamic interactions among the microspheres and also between the microspheres and the traps are shown using pressure field plots in Figures S1–S3 in the supplementary material.²⁷ These finite element simulation results clearly verify the flow resistance based design parameters given above.

To study the effects of the optimization geometric parameters and compare the different sensitivities of ρ in response to their changes, in Figure 6 we plot ρ as individual functions of l , u , b , t , and g . In each sub-plot of a specific parameter, the range of the x -axis is this parameter's feasible range as determined by the optimization constraints (Eq. (8)), and the other four parameters are all set at their optimal values obtained from the grid-search method. For example, in Figure 6(a), l is feasible in the range $[5.2 \mu\text{m}, 18 \mu\text{m}]$, $u = u_{\text{opt}} (10.02 \mu\text{m})$, $b = b_{\text{opt}} (6.9 \mu\text{m})$, $t = t_{\text{opt}} (5.2 \mu\text{m})$, and $g = g_{\text{opt}} (14.6 \mu\text{m})$. Among the five parameters, g appears to exert the most dramatic effect on ρ (Figure 6(e)). Explicitly, a slight increase of g away from the optimal value $g_{\text{opt}} = 14.6 \mu\text{m}$ induces a large decrease of ρ , as indicated by the largest first derivative of ρ with respect to g . In contrast, l , u , and t are less influential on ρ since ρ is less sensitive to their changes (Figures 6(a), 6(c), and 6(d), respectively). ρ is independent of b (Figure 6(b)). Figure 6 also implies that the feasible ranges of the five parameters are large enough to tolerate fabrication errors. The analysis of various geometric parameters provides insight into their relative significance, which guides us in controlling the precision of these parameters when fabricating the trap arrays.

The simulated optimal values of the geometric parameters here are used in the fabrication of the optimized microfluidic microsphere-trap array device. More details are given in Section IV.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

To evaluate the optimization results, we fabricated ten devices with the optimized geometric parameters obtained from the simulation. For performance comparison with the optimized devices, we also fabricated another ten devices. The geometric parameters of these ten devices were randomly selected, which satisfy only the flow resistance constraint to ensure hydrodynamic trapping (*Constraint 1*). We denote these ten devices as un-optimized devices for

FIG. 6. Effects of the optimization geometric parameters of (a) l , (b) u , (c) b , (d) t , and (e) g , on the packing density ρ of the microfluidic microsphere-trap arrays. These parameters are plotted in their feasible ranges with respect to the optimization constraints. The first derivatives of ρ with respect to l , u , b , t , and g are computed at these parameters' optimal values obtained from the grid-search method.

convenient reference; though the values of their parameters may not satisfy the other proposed constraints. The geometric parameters of the optimized and un-optimized devices are listed in Table II. Considering the fabrication feasibility, we constrained the parameter precision to $0.1\ \mu\text{m}$. A number of microsphere-trapping experiments were performed for each set. In these experiments, both devices were tested under the same operation conditions, including driving pressure, microsphere concentration, and microsphere solution viscosity, etc. Details are given below.

A. Device fabrication

Microfluidic trap array devices were fabricated by using standard soft lithography techniques.^{16,28} The devices were made of PDMS, a widely used material in microfluidics and micro-optics. Briefly, we first fabricated a patterned photoresist SU8 mold on a silicon wafer using photolithography. Then PDMS prepolymer (RTV615, 1:10 ratio) was poured onto the mold and degassed in a vacuum chamber. The prepolymer was partially cured in a 60°C oven for 45 min. The 45 min curing time was found to be optimal as: shorter curing time led to collapsed structures in the final device, and longer curing time made the release of PDMS from the mold difficult. The partially cured PDMS was peeled off from the mold, and the liquid inlet and outlet ports were punched through the whole layer, using a biopsy punch. The PDMS layer was permanently bonded to a standard glass slide by oxygen plasma treatment. The master SU8 molds could be reused many times, thus reducing the fabrication cost and time.

B. Device operation

The PDMS microfluidic device was mounted on an inverted microscope (Olympus IX71, San Jose, CA) equipped with an iXon+ EMCCD camera (Andor, South Windsor, CT). A solution of $10\ \mu\text{m}$ polystyrene microspheres (Bangs Lab, Fishers, IN) was prepared in 1X PBS buffer with 0.05% Tween-20 (Sigma-Aldrich, St. Louis, MO) at a concentration of $10^5/\text{ml}$. The microsphere solution was loaded into a 22 gauge Tygon tubing (Cole Parmer, Vernon Hills, IL). One end of the tubing was connected to the device input port via a stainless steel tube and the other end was connected to a pressure source controlled by a pressure regulator with a resolution of 0.4 psi. The microsphere solution was pushed into the device by applying 1-2 psi pressure to the Tygon tubing. Snapshots and videos of the microsphere trapping process were captured by the EMCCD camera. The schematic diagram of the experimental setup is shown in Figure 7.

C. Results

We present the results of the microsphere-trapping experiments of the optimized and un-optimized devices. The optimization is to maximize the packing density ρ of the trap arrays, favors a single microsphere in each trap, and avoids multiple trapping and channel clogging. To compare the performances of the optimized and un-optimized devices, in addition to ρ , we define four experimental measurements as follows:

- *single*, the fraction of traps that immobilizes a single microsphere;
- *multiple*, the fraction of traps that immobilizes more than one microsphere;
- *empty*, the fraction of traps without immobilized microspheres;
- *clogged*, the fraction of channels clogged by the microspheres.

TABLE II. Geometric parameters of the optimized and un-optimized microfluidic microsphere-trap arrays.

Values (μm)	h	l	u	b	t	g
Optimized device	13	5.2	10.1	6.9	5.2	14.6
Un-optimized device	13	14.6	27.5	5.0	17.5	12.5

FIG. 7. Schematic diagram of the experimental setup.

Illustrative examples of the above measurements are highlighted in Figure 8(c). We expect that an optimized device should have large values for ρ and *single*, but small values for *multiple*, *empty*, and *clogged*.

From Table II, we compute the areas of each trap and its surroundings for the optimized device and the un-optimized device as $694.98 \mu\text{m}^2$ and $1312.5 \mu\text{m}^2$. Therefore, the packing densities ρ of the two devices are 1438 traps/ mm^2 and 762 traps/ mm^2 , respectively. Compared with the un-optimized device, the optimized one improves the packing density by a factor of two.

For a qualitative comparison of the trapping effectiveness of both devices, we present snapshots of one microsphere-trapping experiment at three critical time points: the start (Figure 8(a)), middle (Figure 8(b)), and end (Figure 8(c)). We observe that the optimized device is remarkably more compact and neat in the layout of the trapped microspheres (larger *single*; smaller *multiple*, *empty*, and *clogged*) than the un-optimized one. Though the optimized device requires a slightly longer time (18.67 min) to completely fill up the traps than the un-optimized one does (16 min), it traps many more microspheres, virtually all of them *single*. Snapshots of the time-resolved progress of the entire trapping experiment of the two devices are available in Figures S4 and S5

FIG. 8. Time-lapse high-speed camera snapshots of one microsphere-trapping experiment of an optimized device (left) and an un-optimized device (right), at (a) the start time point, (b) the middle time point, and (c) the end time point. The packing densities for the optimized and the un-optimized devices are 1390 traps/ mm^2 and 762 traps/ mm^2 , respectively. Illustrative examples of trapping results: *single* (white circle), *multiple* (yellow circle), *empty* (blue circle), and *clogged* (red circle) are highlighted in (c). Note that due to their negligible fractions, *clogged* is not found in the snapshot of the optimized device, neither is *empty* in the snapshot of the un-optimized device.

FIG. 9. Time-lapse plots of the *single* values of the optimized device and the un-optimized device, with five replicate trapping experiments on each. Error bars indicate the standard deviations. The average experiment times taken to fill all the traps for the optimized device and the un-optimized device are 18.67 min and 16.0 min, respectively.

in the supplementary material.²⁷ Illustrative videos showing how the microspheres being trapped are in Video S1 and Video S2 (descriptions of the two videos are in Ref. 27).

To further compare the microsphere trapping performances of the optimized and un-optimized devices, we performed five replicate experiments on each device and plotted the values of *single* as a function of time in Figure 9. The *single* value of the optimized device experiences a sharp linear increase until 14 min, when over 90% of the traps are occupied correctly with a single microsphere. After this time point, the increase of *single* slows down because the still-available traps may be relatively less accessible. At the end time point, *single* of the optimized device achieves more than 99% (see Figure 10 for more details). The *single* value of the un-optimized device, however, experiences a slow and concave increase almost from the beginning and reaches the limit of around 58% in the end. This figure shows that the optimized device is more efficient and accurate in trapping a single microsphere in each trap.

As an evaluation of the final outcomes of the optimized and un-optimized devices, we compute the *single*, *multiple*, *empty*, and *clogged* of ten optimized and ten un-optimized devices, at the conclusions of the experiments (such as shown in Figure 8(c)). These values are

FIG. 10. Trapping results for the optimized devices and un-optimized devices at the conclusions of the experiments. The reported values are averaged results obtained on ten devices. Error bars indicate the standard deviations of the results on ten devices.

presented in Figure 10 and Table S1 (see supplementary material).²⁷ The small standard deviations of these measurements for both devices suggest the trapping processes are highly reproducible and the results are statistically representative. The values of *empty* are close to 0% for both devices, indicating that almost no traps remain empty in the end. As long as there exist paths for the microspheres to reach the empty traps, these traps will be eventually filled as the experiment proceeds. However, filling the empty traps runs the risk of getting more microspheres trapped at a single trap or clogging the channels. As we have observed from Figures 8(c) and 10, the optimized device effectively avoids such risk. In other words, most of the influent microspheres in the optimized device, if not immobilized in the still-vacant traps, will pass by the channels directly. Therefore, in the optimized device, *single* is dominant (99.29%) and the undesired *multiple* and *clogged* are negligible (0.38% and 0%, respectively). On the contrary, in the un-optimized device, the risk of multiple-trapping and channel clogging is obviously dramatic (Figure 8(c)). That is, the influent microspheres in the un-optimized device are more likely to aggregate in the already occupied traps or channels, rather than pass through. Therefore, compared to the optimized device, *single* of the un-optimized device is much lower (58.57%), and its *multiple* and *clogged* are much higher (41.43% and 6.93%, respectively). Overall, Figure 10 confirms the effectiveness of the optimization with highly reproducible experimental results.

The microsphere-trapping experiments, with highly reproducible results, successfully demonstrate the advantages of the optimized device over the device with randomly selected geometric parameters (the un-optimized device). The optimized device remarkably improves the packing density and the efficiency in trapping a single microsphere at each trap. It also effectively reduces the undesirable behaviors (multiple trapping and channel clogging) in the trapping process.

The systematic optimization framework for building the optimal structure of the microfluidic microsphere-trap arrays is comprehensive and efficient. The hydrodynamic trapping mechanism employed in the optimization is accurate and effective in immobilizing the microspheres. The framework is highly robust to incorporate the specific sizes of the microspheres into the optimization problem (Eq. (8)). The other parameters in Eq. (8) are also readily to modify with respect to varying requirements of device fabrication and applications. This optimization problem is simple to solve and takes less than 5 s to yield results.

It is noteworthy to mention that this work does not consider the inclusion of on-chip micro-mechanical valves^{15,16,29} for simultaneously detecting targets of diverse types. However, it lays the foundation for future work in integrating statistical optimization, physical device fabrication, lab-on-a-chip instrumentation, optical imaging, and statistical analysis of data to develop the microchip device. The resulting system should simplify image analysis, enable error-free target identification, and will be highly reliable, sensitive, efficient, and inexpensive. Expanded versions of the highly miniaturized arrays will be capable of processing many microarray experiments economically and are promising for the large-scale clinical applications.

D. Comparison with self-assembled 3D microarrays

Compared with the contemporary industrial 3D microarray standards, e.g., Illumina's BeadArray systems,^{10,11} our proposed microsphere arrays have several advantages but also limitations. First, the microspheres in Illumina's devices are randomly ordered and require several complex steps of hybridization and dehybridization to identify their types. Our device is capable of combining micromechanical valves and isolated microfluidic chambers to trap different types of microspheres at predetermined locations (position encoding) and use the locations to identify the types.^{6,7} This position encoding feature achieves simple and error-free identification. Second, Illumina's devices can identify thousands of different microspheres and thus can be applied to genotyping and gene expression profiling. However, due to the requirement of chambers, our device applies only when the number of microspheres types (i.e., target types) is small or moderate. Finally, the microspheres in Illumina's devices are permanently immobilized and thus the captured targets cannot be recovered. In our device, the microspheres are not

permanently immobilized, which makes it possible to recover minute and precious captured targets after imaging, for subsequent studies or assays.

E. Comparison with other hydrodynamic mechanisms

We compare here our hydrodynamic mechanism for trapping polystyrene microspheres in the proposed trap-array geometry with other mechanisms that have been recently published in the literature. In our work, we analytically optimize the trap-array to efficiently capture the microspheres in the traps, in order to use the device for sensing bio-targets. This optimization controls the differential flow resistance in and out of the traps to efficiently capture the microspheres in them using a laminar flow field. The concept of such analytical optimization could also be applied to other mechanisms involving various other hydrodynamic forces to separate microspheres without an externally applied field other than the flow field. The recent literature is rich in investigating such various other forces briefly highlighted below. Some of the hydrodynamic mechanisms considered in this vast literature could be used in conjunction with our device in conducting efficient bio-assays, and some could be used as alternatives of our mechanism for capturing bio-targets without using any traps. In the following, we briefly summarize the relevant results in the literature.

Hou *et al.*³⁰ reported a high-throughput and label-free microfluidic approach by exploiting particle deformation for intrinsic and non-specific removal of both microbes and inflammatory cellular components from whole blood. As blood flows through a narrow microchannel, deformable red blood cells migrate axially to the channel center, resulting in margination of other cells (bacteria, platelets, and leukocytes) towards the channel sides. These other cells are removed using smaller side channels. Whereas this study involves separating micron size species, it confines itself in filtering impurities from blood and thus cannot be employed for our purpose.

Tsai *et al.*³¹ proposed a high-performance microfluidic rectifier incorporating a sudden expansion channel in a microchannel. Here a block structure embedded in the expansion channel is used to induce two vortex structures at the end of the microchannel under reverse flow conditions. The vortices reduce the hydraulic diameter of the microchannel, and thus, increase the flow resistance. This way the device achieves flow rectification by exploiting viscoelastic flow effects without the need of any other moving parts. However, we note that incorporation of this mechanism would contribute additional complexity in our trapping mechanism, even though this method also allows control microfluidic flow resistance as we aim at.

Hou *et al.*³² presented a chip-scale rapid bacteria concentration technique combined with surface-enhanced Raman scattering (SERS) to enhance the detection of low bacteria count samples. This concentration technique exploits inertial effects due to vortical flow separation and the inertia of the associated particles. Whereas this method focuses on concentrating bacterial species, it may not allow capturing many targets simultaneously as we envision performing using our device. In contrast, we can exploit SERS for detecting target proteins in a liquid environment containing silver nanoparticles. This will allow perform label-free imaging of the captured bio-targets using our device with a higher sensitivity as that can be achieved using the sensing mechanism described here and in our earlier work.^{6,7}

Wang *et al.*³³ investigated the inertial effects due to vortical flow separation and due to the particles in such flow and found that oscillating microbubbles driven by ultrasound can initiate a steady streaming flow around the bubbles. This flow affects the microspheres' movement to exhibit size-dependent behaviors. Adjusting the relative strengths of the streaming flow and a superimposed Poiseuille flow allows for controlling the spheres' flow behavior, separating the trajectories of spheres with a size resolution on the order of 1 μm . We believe that the flow mechanism described in their study has a potential to be conjugated with our device to obtain position encoding without using any microfluidic chamber.

In a study using similar hydrodynamic mechanism, Yang *et al.*³⁴ proposed a novel micro-flow cytometer in which the particles are focused in the horizontal and vertical directions by means of the Saffman shear lift force generated within a microweir microchannel. Their study shows that the microweir structures can confine a microsphere stream to the center of the microchannel without the need for a shear flow. Similar to the previous mechanism, this

mechanism can also be conjugated with our proposed system to automatically sort microspheres after they capture targets. We must note that this is possible in case microspheres of different sizes are used for capturing distinct targets.

In a similar intriguing study, Kurup *et al.*³⁵ demonstrated a passive, field-free, and gravitationally driven approach to perform particle concentration inside microfluidic plugs. The method only requires changing the flow velocity for efficient performance. Their work can serve as an alternative approach to ours for detecting and identifying multiple targets in a liquid sample using functionalized microspheres without employing any microfluidic trapping mechanism.

To summarize, we believe that our proposed analytical optimization method applies to a state-of-the-art hydrodynamic mechanism based on laminar flow in a microsphere trap-array geometry. It complements very well with the recently investigated hydrodynamic mechanisms studied using cutting-edge microfluidic techniques. These two directions could be combined in a future research, for efficiently sorting, detecting, and identifying micron size species in a liquid sample.

V. CONCLUSIONS

In this paper, we provided a novel geometric structure of a microfluidic microsphere-trap array device and employed fluidic resistance to hydrodynamically trap the microspheres. We built a comprehensive, robust, and simple framework to optimize the geometry of the trap arrays to maximize the packing density, while simultaneously satisfying other criteria. These criteria include efficiently immobilizing the microspheres (i.e., trapping a single microsphere in each trap stably and avoiding multiple trapping and channel clogging), and minimizing the error in imaging the target captured microspheres in subsequent studies. Microsphere-trapping experiments confirmed that the performance of the optimized device was significantly improved with respect to the optimization goal and criteria, compared with the un-optimized device.

In future work, we will combine the optimized device with statistically designed position-encoded microsphere arrays.^{6,7,36} We will use on-chip micromechanical valves and isolated microfluidic chambers for simultaneously trapping the microspheres at predetermined positions and detecting targets of diverse types, and thus achieve a multifunctional platform. We plan to conduct biomedical experiments on this platform. Specifically, epidermal growth factor receptor (EGFR) centric targeting strategies will be incorporated into the system, which will include targeting the protein receptor complex and upstream nucleic acid markers such as mRNA and DNA. A modular approach to detect the expression pattern of EGFR biomarkers across several tumor types will be included. The study will provide an integrated insight into the molecular basis of tumor proliferation in different patients.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation Grant CIF:IHCS-0963742.

¹R. E. Oosterbroek and A. van den Berg, *Lab-on-a-chip: Miniaturized Systems for (bio)Chemical Analysis and Synthesis* (Elsevier B.V., Amsterdam, 2003).

²V. Srinivasan, V. K. Pamula, and R. B. Fair, *Lab Chip* **4**, 310 (2004).

³I.-F. Cheng, H.-C. Chang, D. Hou, and H.-C. Chang, *Biomicrofluidics* **1**, 021503 (2007).

⁴S. Senapati, A. R. Mahon, J. Gordon, C. Nowak, S. Sengupta, T. H. Powell, J. Feder, D. M. Lodge, and H.-C. Chang, *Biomicrofluidics* **3**, 022407 (2009).

⁵N. Bontoux, L. Dauphinot, T. Vitalis, V. Studer, Y. Chen, J. Rossier, and M.-C. Potier, *Lab Chip* **8**, 443 (2008).

⁶P. Sarder and A. Nehorai, *IEEE Trans. Nanobiosci.* **10**, 16 (2011).

⁷X. Xu, P. Sarder, and A. Nehorai, in *Proceedings of the 45th Asilomar Conference Signals, Systems and Computers*, Pacific Grove, CA (2011), p. 1694.

⁸H. Lyng, A. Badiie, E. Hovig, O. Myklebost, and T. Stokke, *BMC Genomics* **3**, 5 (2004).

⁹S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, and S. Dennis, *Science* **251**, 767 (1991).

¹⁰A. Oliphant, D. L. Barker, J. R. Stuelpnagel, and M. S. Chee, *Biotechniques* **32**, 56 (2002).

¹¹K. L. Gunderson, S. Kruglyak, M. S. Graige, E. Wickham, J. Bierle, D. Doucet, M. Milewski, R. Yang, C. Siegmund, J. Haas, L. Zhou, and M. S. Chee, *Genome Res.* **14**, 870 (2004).

¹²C. Situma, M. Hashimoto, and S. A. Soper, *Biomol. Eng.* **23**, 213 (2006).

¹³W. H. Tan and S. Takeuchi, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1146 (2007).

¹⁴W. Xu, K. Sur, H. Zeng, A. Feinerman, D. Kelso, and J. B. Ketterson, *J. Micromech. Microeng.* **18**, 075027 (2008).

- ¹⁵M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer, and S. R. Quake, *Science* **288**, 113 (2000).
- ¹⁶Z. Li and P. Demetri, *IEEE J. Sel. Top. Quant. Electron.* **13**, 185 (2007).
- ¹⁷H. M. Wyss, D. L. Blair, J. F. Morris, H. A. Stone, and D. A. Weitz, *Phys. Rev. E* **74**, 061402 (2006).
- ¹⁸B. J. Kirby, *Micro- and Nanoscale Fluid Mechanics: Transport in Microfluidic Devices* (Cambridge University Press, Cambridge, 2010), Chap. 3.
- ¹⁹H. Bruus, *Theoretical Microfluidics* (Oxford University Press, Oxford, 2008), Chap. 3.
- ²⁰J. C. McDonald and G. M. Whitesides, *Acc. Chem. Res.* **35**, 491 (2002).
- ²¹F. M. White, *Fluid Mechanics* (McGraw-Hill, New York, 2002).
- ²²F. S. Manning and R. E. Thompson, *Natural Gas* (PennWell Books, Tulsa, 1991).
- ²³S. A. Campbell, *The Science and Engineering of Microelectronic Fabrication* (Oxford University Press, Oxford, 2001), Chap. 7.
- ²⁴G. B. Dantzig and M. N. Thapa, *Linear Programming 2: Theory and Extensions* (Springer-Verlag, New York, 2003).
- ²⁵T. G. Kolda, R. M. Lewis, and T. Virginia, *SIAM Rev.* **45**, 385 (2003).
- ²⁶See <http://www.comsol.com/> for the software description and application.
- ²⁷See supplementary material at <http://dx.doi.org/10.1063/1.4793713> for more information.
- ²⁸Y. Xia and G. M. Whitesides, *Angew. Chem.* **37**, 550 (1998).
- ²⁹T. Thorsen, S. J. Maerkl, and S. R. Quake, *Science* **298**, 580 (2002).
- ³⁰H. W. Hou, H. Y. Gan, A. A. S. Bhagat, L. D. Li, C. T. Lim *et al.*, *Biomicrofluidics* **6**, 024115 (2012).
- ³¹C. H. Tsai, C. H. Lin, L. M. Fu, and H. C. Chen, *Biomicrofluidics* **6**, 024108 (2012).
- ³²D. Hou, S. Maheshwari, and H. C. Chang, *Biomicrofluidics* **1**, 014106 (2007).
- ³³C. Wang, S. V. Jalikop, and S. Hilgenfeldt, *Biomicrofluidics* **6**, 012801 (2012).
- ³⁴R. J. Yang, H. H. Hou, Y. N. Wang, C. H. Lin, and L. M. Fu, *Biomicrofluidics* **6**, 034110 (2012).
- ³⁵G. K. Kurup and A. S. Basu, *Biomicrofluidics* **6**, 022008 (2012).
- ³⁶X. Xu, P. Sarder, N. Kotagiri, S. Achilefu, and A. Nehorai, "Performance analysis and design of position-encoded microsphere arrays using the Ziv-Zakai bound," *IEEE Trans. Nanobiosci.* (to be published).