

Rapid isolation of cancer cells using microfluidic deterministic lateral displacement structure

Zongbin Liu,¹ Fei Huang,^{1,2} Jinghui Du,^{1,3} Weiliang Shu,¹ Hongtao Feng,¹ Xiaoping Xu,^{1,4} and Yan Chen^{1,a)} ¹Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences,

Shenzhen 518055, People's Republic of China ²College of Chemical Engineering, Sichuan University, Chengdu 610065, People's Republic of China ³Medical Department of Nanchang University Graduate School, Nanchang 330006, People's Republic of China ⁴Hong Kong University Shenzhen Hospital, Shenzhen 518053, People's Republic of China

(Received 8 August 2012; accepted 15 October 2012; published online 7 January 2013)

This work reports a microfluidic device with deterministic lateral displacement (DLD) arrays allowing rapid and label-free cancer cell separation and enrichment from diluted peripheral whole blood, by exploiting the size-dependent hydrodynamic forces. Experiment data and theoretical simulation are presented to evaluate the isolation efficiency of various types of cancer cells in the microfluidic DLD structure. We also demonstrated the use of both circular and triangular post arrays for cancer cell separation in cell solution and blood samples. The device was able to achieve high cancer cell isolation efficiency and enrichment factor with our optimized design. Therefore, this platform with DLD structure shows great potential on fundamental and clinical studies of circulating tumor cells. © *2013 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4774308]

I. INTRODUCTION

Circulating tumor cells (CTCs) are isolated tumor cells disseminated from disease site in metastatic or primary cancers and can be used as a diagnostic tool for disease severity, monitoring the effectiveness of therapy, and serving as an independent prognostic factor.^{1–3} However, CTCs concentration in peripheral blood sample is extremely low (1 in 10⁹ cells), which makes isolation and detection of CTCs technically challenging.^{4,5} Detection of these extremely rare cells within the circulation may provide important prospect for cancer prognosis and progression, assessment of personalized medicine, and optimization of targeted therapy. To date, the only technology approved by the US FDA (Food and Drug Administration) for CTCs detection is called CellSearch.^{6,7} The principle of capturing CTCs in CellSearch is based on their affinity to antibodies against an epithelial cell adhesion molecule (EpCAM). However, this technique is expensive, laborious, and time-consuming with variable efficiency.^{8,9}

Recently, microfluidic devices have shown the potential to efficiently isolate and detect rare CTCs in cancer patients. A variety of on-chip blood analysis has been demonstrated by several groups.^{8,10–18} Nagrath *et al.* developed a microfluidic device consisting of microposts coated with EpCAM antibodies.¹² The device achieved 99% cell capture yield with 47% purity at flow rate less than 2 ml/h. Stott *et al.* and Wang *et al.* each described a chaotic mixing structure to enhance the interaction between CTCs and antibody-coated chip surface for better CTCs capture.^{10,14} Combining the microfluidic and magnetic beads technologies, Lien *et al.* reported a three dimensional (3D) device to detect CTCs with detection limit of 5×10^1 cells/ml.¹⁵ These microfluidic devices have shown great capability of efficiently and selectively separating of viable CTCs from peripheral blood samples. However, one of the reasons that these devices fail to

^{a)}Author to whom correspondence should be addressed. Electronic mail: yan.chen@siat.ac.cn.

011801-2 Liu et al.

satisfy clinical requirements is the ability to process a milliliter volume of blood within a reasonable time, because the blood sample can only be processed at a low flow rate (\sim mL/h). Hence, there is a clear demand to develop a simple and more efficient platform to rapidly isolate CTCs from a large volume of whole blood.

In this paper, we demonstrate a microfluidic chip for the continuous high throughput (~ml/min) processing of whole blood with efficient isolation of cancer cells using deterministic lateral displacement (DLD) structure. Size-based separation,^{13,19,20} which is based on the fact that cancer cells are often larger than native leukocytes, has been developed to a leading technology for cancer cell isolation. In particular, we adopt this size-based particle sorting method because it can process a large volume of blood with fast rate while maintaining high cell viability.²¹ Particles above a critical size follow bump migration path and are separated after a certain distance.^{21–27} As the size of CTCs is on average $15-25 \,\mu\text{m}$, which is larger than the size of blood cells $(5-15 \,\mu\text{m})$, it is possible to use the microfluidic DLD array for CTCs isolation. Recently, Loutherback *et al.* has used the DLD method to isolate cancer cells from blood with an efficiency greater than 85% at 10 ml/min.²¹ DLD micropost shape and flow velocity are the two essential parameters that determine the isolation efficiency. In this work, we studied the effect of these two parameters on cancer cells isolation efficiency. To our knowledge, we are the first to thoroughly study this high-throughput structure on various types of cancer cells. We constructed a high throughput microfluidic device and optimized the design to achieve high efficiency cancer cell enrichment and recovery, and high cell viability for post-capture processing, which could lead to the development of clinical application of microfluidic technologies for cancer detection.

II. MATERIALS AND METHODS

A. Microfluidic device design

The microfluidic DLD design consisted of an inlet, three outlets, and a central flow chamber with micropost array, as shown in Fig. 1(a). The flow chamber was 35 mm long, 3.5 mm wide, and 30 μ m high. Fig. 1(b) shows the mirrored circular microposts with 25 μ m radius and 25 μ m gaps. The microarray had a tilted angle 3.2° to the fluid flow direction. According to the theory developed by Inglis *et al.*,²² the calculated critical particle size was about 5–6 μ m. In blood samples, large cells (cancer cells and part of leukocytes) flow in a bumping mode and thus are concentrated in the centre of chamber, while small cells (erythrocytes and most of leukocytes) follow the streamline direction. We optimized the design in the outlet portion to maximize the CTCs enrichment. Fig. 1(c) shows one narrow outlet channel and two broad outlet



FIG. 1. Schematic illustration of the microfluidic DLD design for cancer cell isolation from blood. (a) Overview of the device. (b) Flow chamber with circular micropost array showing the trajectory of cancer cells, leukocytes, and erythrocytes. (c) Three outlet channels showing cancer cell isolation effect: cancer cells are collected by the middle narrow channel. (d) Triangular micropost array.

011801-3 Liu et al.

channels. Cancer cells were collected in the narrow channel. Besides the circular micropost array, a triangular micropost array was also studied, as shown in Fig. 1(d). The triangular micropost had 25 μ m side length and 25 μ m gaps with a tilted angle 3.8°. Based on the theoretical principle by Loutherback *et al.*,²⁷ the calculated critical particle size was also about 5–6 μ m. The outlet portion of the device consists of a row of microposts with gradually decreasing gaps from 25 μ m to 8 μ m (Fig. 2(b)). With this design, although part of the erythrocytes and leukocytes escaped through the gaps at the outlet, the cancer cells can be fully collected once they were concentrated here.

B. Device fabrication

Microfluidic devices were fabricated using standard photolithography and soft lithography. Negative photoresist SU8-3025 (Microchem corp., Naton, MA) was used to fabricate the master on silicon wafer with a photomask. Next, the patterned silicon wafers were silanized with chlorotrimethylsilane (ABCR GmbH & CO. KG, Germany) to facilitate particle desorption mass spectrometry (PDMS) mold release. PDMS prepolymer mixed with curing agent (5:1 w/w ratio) was poured onto the silicon wafer and curried at 80 °C for 1 h. Finally, holes were punched for inlet and outlets, and the PDMS mold was bonded to glass slides after oxygen plasma treatment (PDC-M, Chengdu Mingheng Science. & Technology Co., LTD, P. R. China). The fabricated cancer cell isolation chip is shown in Fig. 2.

C. Cell culture and preparation

Five commercially available human cancer cell lines MCF7 (HTB- 22^{TM} , human breast cancer cell line, ATCC, USA), MDAMB231 (HTB- 26^{TM} , human breast cancer cell line, ATCC, USA), A549 (CCL-185TM, human lung cancer cell line, ATCC, USA), HEPG2 (HB8065TM, human liver cancer cell line, ATCC, USA), and KYSE150 (BH-HCOO4, human oesophageal squamous cancer cell line, BioHermes, China) were used to mimic CTCs isolation in the microfluidic devices. Cancer cells were cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cell culture was maintained at 37 °C with 5% (v/v) CO₂, and medium was changed every 2–3 days. Cells were harvested through incubation in 0.05% Trysin-0.53 mM EDTA at 37 °C for 5 min. The cell suspension was then diluted to the desired concentration.



FIG. 2. The fabricated cancer cell isolation chip. (a) Device overview. (b) The outlet portion of the device consists of a row of microposts with gradually decreasing gaps, as indicated in the blue circle.

011801-4 Liu et al.

D. Microfluidic CTCs isolation

To determine the isolation efficiency of our device, cancer cells were spiked into phosphate buffered saline (PBS) at 10^5 cells/ml. The inlet with cell suspension solution was connected to a syringe pump, which controlled the fluid velocity. At flow rates from 0.01 ml/min to 2 ml/min, the Reynolds number (Re = VD/v, where V is the average velocity, D is the hydrodynamic diameter, and v is the kinematic viscosity) of the flow between microposts can be calculated ranging from 0.1 to 20. Therefore, the fluid flow is laminar for all the flow rates. The cell movement in the microchannels was observed under microscope and recorded by a high speed CCD camera. The concentrated cells were collected in the middle narrow outlet, which was connected to another syringe pump controlling the output flow. By measuring the cell concentration and volume of the output, we can calculate the isolation efficiency with the following equation:

Isolation efficiency =
$$\frac{C_1 V_1}{C_1 V_1 + C_2 V_2}$$

where C_1 and V_1 are the cancer cell concentration and solution volume in the collection outlet, and C_2 and V_2 are the cancer cell concentration and solution volume in the two waste collection outlets.

E. Blood specimen processing

Blood samples were provided by Second People's Hospital of Shenzhen, China. All blood samples were collected into EDTA-containing tubes and were processed within 12 h. As blood has high viscosity, it was diluted 10 times with physiological saline. Cancer cells were first stained by Vybrant[®] DyeCycleTM Green (Life Technologies, Carlsbad, CA) to be discriminated from other blood cells by fluorescence microscope. Next, cancer cells were spiked into the diluted blood specimens at $\sim 10^4$ cells/ml. After cancer cell isolation, the cell isolation efficiency was finally calculated.

III. RESULTS

A. Cancer cell isolation by circular DLD array

To test how the flow rates affect the isolation efficiency of the circular DLD array, cell suspensions of MCF-7, KYSE150, MDAMB231, A549, and HEPG2 were injected into the device at flow rates of 30, 50, 100, 500, 1000, and 2000 μ l/min, respectively. As shown in Fig. 3(b), cells were focused and collected by the output channel at flow rate of 30 μ l/min. Fig. 3(c) shows the calculated cell isolation efficiency. The cell isolation efficiency decreased significantly with the increase of flow rate from 30 to 100 μ l/min and eventually reached a stable level. At flow rate of 30 μ l/min, MCF-7 and KYSE150 achieved 100% isolation efficiency while MDAMB231, A549, and HEPG2 achieved more than 90% isolation efficiency, as shown in Fig. 3(d). At flow rate of 100 μ l/min, the isolation efficiency for MCF-7 and KYSE150 was about 50%, while the efficiency for MDAMB231, A549, and HEPG2 was between 20%–50%. Cell sizes of MCF-7 and KYSE150 were between 15–25 μ m (Fig. 3(a)), which were larger than those of MDAMB231, A549, and HEPG2 (10–15 μ m). The result shows that the larger cells have higher isolation efficiency, since their flow profiles fit more into the displacement zone of the DLD arrays.

B. Cancer cell isolation by triangular DLD array

It was previously reported that the isolation efficiency of triangular DLD array was better than that of circular DLD array.²⁷ The triangle posts offer more advantages for cell processing in the device because they provide less clogging and cell deformation. In our study, microfluidic device with triangular DLD array was designed and fabricated, and we thoroughly studied the isolation effect of various cell types in this structure at different flow rates. Cell suspensions of MCF-7 and MDAMB231 were introduced to the device to test the isolation efficiency. Fig. 4(a) is a video frame showing that the cancer cells were focused to the centre of chamber



FIG. 3. Cell isolation efficiency of circular DLD array. (a) Images of MCF-7, KYSE150, A549, HEPG2, and MDAMB231 cells. (b) Image of cancer cells isolated and concentrated to the centre narrow channel at flow rate of 30μ l/min. (c) Isolation efficiency for MCF-7, KYSE150, MDAMB231, A549, and HEPG2 cell lines at flow rates of 30, 50, 100, 500, 1000, and 2000 μ l/min. (d) Isolation efficiency at lower flow rates with error bars representing standard deviations (n = 3).

and collected by the output channel. Fig. 4(b) shows the relationship between isolation efficiency and flow rates. For MCF-7, the isolation efficiency was 100% at flow rate of 100 μ l/min and had little change with the increase of the flow rate. For smaller cells such as MDAMB231, the isolation efficiency was about 100% at flow rate 100 μ l/min and decreased to about 70% at flow rate of 1000 μ l/min, as illustrated in Fig. 3(c).

The difference of isolation efficiency between circular DLD array and triangular DLD array was further analyzed and summarized in Fig. 5. For both MCF-7 and MDAMB231, high isolation efficiency was accomplished at low flow rate of 50 μ l/min in circular and triangular DLD arrays. However, the isolation efficiency of circular DLD array decreased significantly at high flow rate while triangular DLD array was able to keep the high isolation efficiency. Therefore, we concluded that triangular DLD array has improved performance and is more suitable for cancer cell isolation than circular DLD array.

C. Cancer cell enrichment in blood sample

Because triangular DLD array performed better cancer cell isolation efficiency, it was utilized to study cancer cell isolation from peripheral blood samples. To model the presence of cancer cell in human peripheral blood, we spiked a small amount of MCF-7 and MDAMB231 cells into $10 \times$ diluted blood samples. The concentration of cell mixture is 10^4 cells/ml, and the initial



FIG. 4. Cell isolation efficiency of triangular DLD array. Error bars show standard deviations (n = 3). (a) Cells were isolated and focused to the centre narrow channel at flow rate of 50 μ l/min. (b) Isolation efficiency for MCF-7 and MDAMB231 cell lines at flow rates of 50, 100, 500, 1000, and 2000 μ l/min. (c) Isolation efficiency for MCF-7 and MDAMB231 cell lines at flow rates of 100 and 1000 μ l/min.

concentration ratio of cancer cells MCF-7 to blood cells was 0.01%. The results are shown in Fig. 6. The cancer cell spiked blood sample was highly concentrated after processing, Figs. 6(c) and 6(d) shows the enrichment results of MCF-7 cells. For MCF-7 cells, isolation efficiency 99% was achieved and maintained with the increase of flow rate from 50 to 2000 μ l/min. For MDAMB231 cells, the isolation efficiency was nearly 100% at flow rate less than 100 μ l/min and decreased to about 80% at flow rate 2000 μ l/min. The results of cancer cell isolation efficiency in blood sample were consistent with separation in PBS solution.

The relative concentrations of MCF-7 cells, erythrocytes, and leukocytes recovered from the collected solution was further measured by hemocytometer counting and illustrated in Table I.



FIG. 5. Comparison of cancer cell isolation efficiency between circular and triangular DLD arrays. Error bars show standard deviations (n = 3). (a) MCF-7 isolation efficiency. (b) MDAMB231 isolation efficiency.



FIG. 6. Isolation of MCF-7 and MDAMB231 from spiked diluted blood sample with triangular DLD array. (a) Image of blood sample flowing through the microfluidic device. (b) MCF-7 and MDAMB231 isolation efficiency. (c) Image of MCF-7 cells in diluted blood sample before processing. MCF-7 cells were pre-stained by Vybrant[®] DyeCycleTM Green. (d) Image of MCF-7 cells (green) in the collected solution of triangular micropost array. After flowing through the chip, the MCF-7 cells were significantly enriched.

The MCF-7 enrichment factor can be calculated by dividing MCF-7 relative concentration with blood cell relative concentration. The calculated results showed that MCF-7 cells had $\sim 115 \times$ enrichment over erythrocytes and $\sim 40 \times$ enrichment over leukocytes. Similar to other sized based cell separation methods, such as inertial microfluidic,¹³ our approach is difficult to completely isolate cancer cells that cover leukocyte size scale. As shown in Table I, there was still a small fraction of erythrocytes and leukocytes in the collected solution. Fig. 6 and Table I indicated that the triangular DLD array enabled the continuous separation of cancer cells with high isolation efficiency and a high throughput of 2 ml/min. Although the high flow rate induced the deformation of cancer cells, the triangular DLD array was able to achieve enhanced separation efficiency and cancer cell enrichment factor.

IV. DISCUSSION

Microfluidic DLD arrays have shown great potential for size based particle separation. Given the array parameters, such as shape, gap, and tilt angle, the critical particle size can be calculated according to the theory developed by Inglis and Loutherback.^{22,27} This theory is used for hard spherical particles separation, while biological fluids usually consist of soft and non-spherical particles such as cells. It has been reported that red blood cells can change the shape and deform under shear stress in blood.^{28–30} Since the goal of our study is to fast process large volumes of blood samples, the high fluid velocities in our devices may subject the cells to high shear stress, resulting in the deformation of cells. The cell deformation can influence the cell isolation efficiency of DLD device. However, there is little research on the effect of fluid flow on cancer cells deformation. Therefore, the relationship between flow rate, DLD array shape, and cell deformation is further explored.

The flow velocity distribution of the channel with circular and triangle posts obstacles were analyzed using a laminar flow law of Newton fluid based on N-S equations in FLUID MECHANICS

011801-8 Liu et al.

	Concentration (%)		
	Erythrocytes	Leukocytes	MCF-7
Sample	100	100	100
Collected solution	0.86 ± 0.09	2.41 ± 0.15	98.7 ± 0.7

TABLE I. Relative concentrations of erythrocytes, leukocytes, and MCF-7 recovered from the collected solution of triangular micropost array at flow rate 2000μ l/min.

software for computational fluid dynamics (CFD) simulation in order to estimate the shear stress in the flow channels. First, a 3D model of the microfluidic system was established by GAMBIT software. Then, the mesh file was introduced into ANSYS FLUID software by setting proper compute parameter under acceptable grid density and local grid refine. The laminar flow module was selected for this CFD-simulation. The flow module enabled the motion type present in the system to be determined and the velocity profile to be analyzed through the Navier-Stokes equations for an isothermal incompressible fluid. It was also assumed that the viscosity and density of the buffer solution are almost identical to that of water.

Fig. 7(a) shows the simulation results of cancer cells moving through the circular post gap. Cells were constrained to lie in the plane of post. Due to the difference of flow velocities between the gap and post edge, cells were subjected to shear stress. According to the theory by



Flow rate 1000 µL/min

FIG. 7. Simulation of cell movement and experimental observation in DLD arrays. (a) and (b) Schematic illustration of cell movement and deformation in circular and triangular DLD arrays. Cells were added after simulation. (c) Cell deformation at flow rate $1000 \,\mu$ /min in circular and triangular DLD arrays. Cells had significant deformation in circular DLD structure while there was no cell deformation in triangular DLD structure.

Tegenfeldt *et al.*, the cell deformation degree is proportional to the shear stress.²⁸ When the cell moved in line with two posts, it experienced the largest deformation. Cell deformation was demonstrated by images acquired by a high-speed camera, as shown in Fig. 7(c). Cell deformation resulted in the decrease of cell effective radius. If the effective radius is less than the critical particle size of the DLD array, cells cannot be separated by the device. Cell deformation explains why the cell isolation efficiency of circular DLD array decreased with the increase of flow rates in our study (Fig. 3).

For triangular post array, cells had the largest shear stress at vertex of the triangular post, as illustrated in Fig. 7(b). As the contact area between a cell and a post is a point rather than a plane, it is difficult for cells to deform. We observed that cells rotated rapidly around the vertex without deformation, as shown in Fig. 7(c). Therefore, the fluid flow velocity had no effect on cancer cell isolation efficiency in triangular DLD array. However, the vertex of the triangular post was practically not a point but a rounded corner due to the limitation of fabrication conditions. So the high flow velocities also affected cancer cell isolation efficiency of triangular DLD array in our devices.

For on-chip blood analysis, it is essential to process milliliters of blood samples within a reasonable time. The throughput of our device was up to 2 ml/min with 99% MCF-7 isolation and 80% MDAMB231 isolation. If a microfluidic device is designed with five parallel isolation chambers, the total throughput will be 10 ml/min, which is faster than most of the current technologies. Microfluidic antibody based immuno-binding methods are widely used to study cancer cell isolation. In order to achieve high cell capture efficiency, blood samples must be processed at a low flow rate less than 2 ml/h.^{10,12} Therefore, this microfluidic DLD structure provides an ideal scheme for the further development of complete cancer cell capture and analysis system for clinical blood samples.

V. CONCLUSIONS

In summary, a microfluidic chip with DLD array for rapid isolation of CTCs is successfully demonstrated in this study. Circular and triangular DLD arrays were employed to achieve size-based cell separation. Triangular DLD array has better performance than circular DLD array due to less cell deformation. As an application of this device, cancer cell isolation from spiked blood sample with high isolation efficiency (99% for MCF-7 and 80% for MDAMB231) and 2 ml/min throughput was achieved. Compared to current technologies for CTC enrichment, such as microfluidic filtration,²⁰ our device has significantly higher throughput. Meanwhile, our platform has distinct advantages, such as label-free and clogging-free, and maintains high cell viability after processing. More importantly, this high throughput microfluidic device has the potential to be combined with other techniques, such as antibody based immuno-binding methods for post-capture processing. Therefore, the microfluidic DLD array device may provide a promising platform for rapid isolation and detection of CTCs in tumor research and clinical diagnostics.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant 61106128), the Knowledge Innovation Project of the Chinese Academy of Science (Grant KGCX2-YW-904), and the Guangdong Innovation Research Team Fund for Low-cost Healthcare Technologies (GIRTF-LCHT).

¹J. S. Ross and E. A. Stodkowska, Am. J. Clin. Pathol. 132(2), 237 (2009).

²M. Toner and D. Irimia, Annu. Rev. Biomed. Eng. 7, 77 (2005).

³U. Dharmasiri, M. A. Witek, A. A. Adams, and S. A. Soper, Annu. Rev. Anal. Chem. **3**, 409 (2010).

⁴K. Pantel and R. H. Brakenhoff, Nat. Rev. Cancer 4(6), 448 (2004).

⁵V. Zieglschmid, C. Hollmann, and O. Bocher, Crit. Rev. Clin. Lab. Sci. 42(2), 155 (2005).

⁶M. Cristofanilli, G. T. Budd, M. J. Ellis, A. Stopeck, J. Matera, M. C. Miller, J. M. Reuben, G. V. Doyle, W. J. Allard, L. Terstappen, and D. F. Hayes, N. Engl. J. Med. **351**(8), 781 (2004).

⁷J.-M. Hou, A. Greystoke, L. Lancashire, J. Cummings, T. Ward, R. Board, E. Amir, S. Hughes, M. Krebs, A. Hughes, M. Ranson, P. Lorigan, C. Dive, and F. H. Blackhall, Am. J. Pathol. **175**(2), 808 (2009).

011801-10 Liu et al.

⁸H. Lin, M. Balic, S. Y. Zheng, R. Datar, and R. J. Cote, Crit. Rev. Oncol. Hematol. 77(1), 2 (2011).

- ⁹M. Yu, S. Stott, M. Toner, S. Maheswaran, and D. A. Haber, J. Cell Biol. **192**(3), 373 (2011).
- ¹⁰S. Wang, K. Liu, J. Liu, Z. T. F. Yu, X. Xu, L. Zhao, T. Lee, E. K. Lee, J. Reiss, Y.-K. Lee, L. W. K. Chung, J. Huang,
- M. Rettig, D. Seligson, K. N. Duraiswamy, C. K. F. Shen, and H.-R. Tseng, Angew. Chem. Int. Ed. **50**(13), 3084 (2011). ¹¹U. Dharmasiri, S. K. Njoroge, M. A. Witek, M. G. Adebiyi, J. W. Kamande, M. L. Hupert, F. Barany, and S. A. Soper, Anal Chem **83**(6), 2301 (2011).
- Anal. Chem. 83(6), 2301 (2011).
 ¹²S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U. J. Balis, R. G. Tompkins, D. A. Haber, and M. Toner, Nature 450(7173), 1235 (2007).
- ¹³A. A. S. Bhagat, H. W. Hou, L. D. Li, C. T. Lim, and J. Y. Han, Lab Chip **11**(11), 1870 (2011).
- ¹⁴S. L. Stott, C. H. Hsu, D. I. Tsukrov, M. Yu, D. T. Miyamoto, B. A. Waltman, S. M. Rothenberg, A. M. Shah, M. E. Smas, G. K. Korir, F. P. Floyd, A. J. Gilman, J. B. Lord, D. Winokur, S. Springer, D. Irimia, S. Nagrath, L. V. Sequist, R. J. Lee, K. J. Isselbacher, S. Maheswaran, D. A. Haber, and M. Toner, Proc. Natl. Acad. Sci. USA **107**(43), 18392 (2010).
- ¹⁵K. Y. Lien, Y. H. Chuang, L. Y. Hung, K. F. Hsu, W. W. Lai, C. L. Ho, C. Y. Chou, and G. B. Lee, Lab Chip 10(21), 2875 (2010).
- ¹⁶A. A. S. Bhagat, H. Bow, H. W. Hou, S. J. Tan, J. Han, and C. T. Lim, Med. Biol. Eng. Comput. **48**(10), 999 (2010).
- ¹⁷E. D. Pratt, C. Huang, B. G. Hawkins, J. P. Gleghorn, and B. J. Kirby, Chem. Eng. Sci. **66**(7), 1508 (2011).
- ¹⁸J. P. Gleghorn, E. D. Pratt, D. Denning, H. Liu, N. H. Bander, S. T. Tagawa, D. M. Nanus, P. A. Giannakakou, and B. J. Kirby, Lab Chip 10(1), 27 (2010).
- ¹⁹A. J. Mach, J. H. Kim, A. Arshi, S. C. Hur, and D. Di Carlo, Lab Chip 11(17), 2827 (2011).
- ²⁰S. Zheng, H. K. Lin, B. Lu, A. Williams, R. Datar, R. J. Cote, and Y.-C. Tai, Biomed. Microdevices 13(1), 203 (2011).
- ²¹K. Loutherback, J. D'Silva, L. Liu, A. Wu, R. H. Austin, and J. C. Sturm, AIP Adv. 2(4), 42107 (2012).
- ²²D. W. Inglis, J. A. Davis, R. H. Austin, and J. C. Sturm, Lab Chip 6(5), 655 (2006).
- ²³K. J. Morton, K. Loutherback, D. W. Inglis, O. K. Tsui, J. C. Sturm, S. Y. Chou, and R. H. Austin, Proc. Natl. Acad. Sci. USA 105(21), 7434 (2008).
- ²⁴T. A. Crowley and V. Pizziconi, Lab Chip **5**(9), 922 (2005).
- ²⁵S. H. Holm, J. P. Beech, M. P. Barrett, and J. O. Tegenfeldt, Lab Chip 11(7), 1326 (2011).
- ²⁶D. W. Inglis, Appl. Phys. Lett. **94**(1), 013510 (2009).
- ²⁷K. Loutherback, K. S. Chou, J. Newman, J. Puchalla, R. H. Austin, and J. C. Sturm, Microfluid. Nanofluid. 9(6), 1143 (2010).
- ²⁸J. P. Beech, S. H. Holm, K. Adolfsson, and J. O. Tegenfeldt, Lab Chip **12**(6), 1048 (2012).
- ²⁹Y. Park, C. A. Best, K. Badizadegan, R. R. Dasari, M. S. Feld, T. Kuriabova, M. L. Henle, A. J. Levine, and G. Popescu, Proc. Natl. Acad. Sci. USA 107(15), 6731 (2010).
- ³⁰M. Al-Fandi, M. Al-Rousan, M. A. K. Jaradat, and L. Al-Ebbini, Rob. Comput.-Integr. Manufact. 27(2), 237 (2011).