Published in final edited form as:

Angew Chem Int Ed Engl. 2019 June 03; 58(23): 7606–7610. doi:10.1002/anie.201901412.

Integration of Lateral Filter Arrays with Immunoaffinity for Circulating Tumor Cell Isolation

Kangfu Chen^[a], Pablo Dopico^[a], Jose Varillas^[b], Jinling Zhang^[a], Thomas J. George^[c], and Z. Hugh Fan^{[a],[b]}

^[a]Interdisciplinary Microsystems Group (IMG), Department of Mechanical and Aerospace Engineering, University of Florida, P.O. BOX 116250, Gainesville, FL, 32611 (USA)

^[b]J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, 32611 (USA)

^[c]Department of Medicine, University of Florida, Gainesville, FL, 32610 (USA)

Abstract

Circulating tumor cells (CTCs) are considered an important biomarker for cancer prognosis and treatment monitoring. A variety of methods have been employed to isolate a small number of CTCs from billions of normal blood cells. These methods are generally based on either physical (e.g. size) or biological properties (e.g. immunoaffinity) of CTCs. However, the heterogeneity of CTCs in both physical and biological properties limits the efficiency of various approaches for CTC isolation. To address this challenge, we have developed a lateral filter array microfluidic (LFAM) device to integrate size-based separation with immunoaffinity-based CTC isolation. LFAM consists of a serpentine main channel for the majority of a sample passing by and an array of lateral filters for CTC isolation. The unique device design produces a two-dimensional flow, which reduces nonspecific geometric capture of normal cells as typically observed in vertical filters. LFAM is further functionalized by immobilizing antibodies that are specific to the target cells. The resultant LFAM devices show capture efficiency of $98.7 \pm 1.2\%$ for pancreatic cancer cells (L3.6pl) spiked in blood samples. LFAM also demonstrates better performance than a previously reported immunoaffinity-based micromixer device when both devices are compared for isolating CTCs from patients with metastatic colorectal cancer, indicating its potential for clinical applications.

Graphical Abstract

Correspondence to: Z. Hugh Fan.

Supporting information for this article is given via a link at the end of the document.

A lateral filter array microfluidic (LFAM) device integrates size-based separation with immunoaffinity-enabled isolation for detection of circulating tumor cells. A serpentine channel for main flow and lateral filter arrays for filtration, like a parking lot, prevent cells from clogging and increase CTC capture efficiency through combined effects.

Keywords

Circulating tumor cells; Microfluidics; Lateral filter array; Immunoaffinity; Simulation

Circulating tumor cells (CTCs) are cancer cells shed from a primary tumor into peripheral blood. CTCs are considered an important biomarker for detection of metastasis, treatment monitoring and disease prognosis.^[1] Compared with traditional tissue biopsy, CTC enumeration is a noninvasive ‘liquid biopsy’, being cheaper and less harmful to the patient.^[2] However, the rarity of CTCs in blood (tens of CTCs among billions of healthy blood cells per mL blood) makes their detection a technological challenge.^[3] The FDA-approved CellSearch® is hindered by its relatively low sensitivity and high cost.

The common methods for CTC enumeration are generally classified into two categories: physical-property-based separation and biological-property-based isolation. Size is one of the most prevalent physical properties used to differentiate CTCs from normal blood cells.^[4–12] Size-based CTC isolation assumes that CTCs are larger than most normal blood cells.^[12] Immunoaffinity is the primary biological property used to isolate CTCs because of the specific conjugation between surface biomarkers on CTCs (e.g., epithelial cell adhesion molecules or EpCAM) and antibodies immobilized on a solid surface.^[13–23] However, the heterogeneity of CTCs in both physical and biological properties makes such ‘single-criterion’ CTC isolation methods not universally applicable for clinical applications. For example, CTCs can have the similar size with white blood cells (WBCs),^[24] and some CTCs express little or no EpCAM (or other epithelial markers) due to epithelial-to-mesenchymal transition (EMT).^[25, 26]

Combining size and immunoaffinity for CTC isolation has been explored by Juncker’s group who used an antibody-functionalized membrane filter for tumor cell detection.^[27] Lee et al. enlarged the size of tumor cells by binding them with antibody-conjugated beads before filtration.^[28] However, membrane filters are limited by the pore size selected^[29] and possible cell clogging in the membrane can lower the cell purity (defined as the number of

target cells captured over the number of all types of cells isolated).^[30] The short contact time between a tumor cell and a pore as well as only one contact opportunity limits the capture efficiency (defined as the number of target cells captured over the number of cells introduced). For binding tumor cells with antibody-conjugated beads, this sample treatment can cause cell fragmentation or loss as in the FDA-approved CellSearch assay.^[31]

To address these challenges, we have developed a lateral filter array microfluidic (LFAM) device and integrated it with immunoaffinity-based CTC capture (Figure 1 and Figure S1 in Supporting Information). As shown in the inset of Figure 1A and Figure 1B, we embedded lateral filters in a serpentine main channel, producing a main flow in the serpentine channel and lateral flows through filters, in a design like a parking lot. The main channel allows a blood sample passing through quickly; the filters are designed to permit normal blood cells through while trapping CTCs. The existence of lateral flows induces two velocity components of a flowing cell, as illustrated in Figure 1C. The parking-space-like arrangement of filters helps adjust the flow pattern when a filter is “filled” by a CTC, changing the flow paths of a subsequent cell. This arrangement prevents cells from clogging, increasing the cell purity. In addition, various filter dimensions from 10 μm to 6 μm (Figure S1) are created in one device to address the size heterogeneity of CTCs.

The geometry of filters forces the direct contact between CTCs and antibodies immobilized on filters (Figure 1D) because of their comparable size. The large number of filters (13,600 filters) creates sufficiently high contact frequency between CTCs and antibody-functionalized surfaces, significantly increasing the probability of immunocapture.

LFAM devices were fabricated using soft lithography, consisting of a glass cover and a polydimethylsiloxane (PDMS) substrate. The serpentine main channel is 300 μm wide and 45 μm in height. All filters have the same height as the main channel, and they are divided into five zones. Each filter zone consists of 10 columns of lateral filters. The size of filters within each zone is identical, but the sizes in different zones vary from 10 μm to 6 μm , with a deduction of 1 μm in each subsequent zone. The 10- μm -filter zone is located near the inlet and the 6- μm -filter zone is near the outlet (Figure S1).

To ensure interactions between CTCs and filters, the device is designed to pass all cells through filters with no cells staying in the main channel only. The device designs are studied and optimized by analysing the flow paths of cells as discussed below.

A lumped element model was developed using MATLAB to simulate the streamline pattern and optimize the design of LFAM. The microflow system is analogous to a circuit network (Figure S2). The main channel and lateral filters are modelled as a series of hydrodynamic resistors.

The flow pattern in LFAM is affected by the distribution of hydrodynamic resistance (Figure S3). Within each column of a microchannel, the mainstream is defined as the flow through the channel elbow, as shown in Figure 2A. The total flow is defined as the flow through both filters and channel elbow in the same column. The mainstream ratio is defined as the ratio of the mainstream to the total flow. Figure S4 shows that the mainstream ratio determines the region of the mainstream zone, which describes the interactions between the flow and filters

in the downstream. Adjusting the mainstream ratio leads to various streamline patterns in LFAM. The theoretically predicted flow pattern was then compared with experimental observation. As shown in Figure 2A, when the mainstream ratio is smaller than 50%, the streamline pattern is divided into 3 zones: one mainstream zone through the top elbow, one mainstream zone through the bottom elbow, and one middle zone covering only filters. As the mainstream ratio increases, the middle zone is squeezed, and the two mainstream zones expand. When the mainstream ratio equals 50%, the middle zone disappears, with each mainstream zone covering one column of filters periodically (Figure 2B). When mainstream ratio exceeds 50%, a new middle zone appears due to the overlap of the neighbouring mainstream zones (Figure 2C). The overlapped zone stays in the serpentine main channel without passing any filters. Cells staying in the overlapped zone will not interact with any lateral filters, resulting in possible cell loss. As a result, the mainstream ratio should be no more than 50%. Our simulation results are summarized in Figure 2D, which shows that the mainstream ratio increases from 23.2% to 46.9% when the filter size decreases from 10 μm to 6 μm .

We further studied CTC capture in LFAM through force analysis. Considering a cell an elastic sphere, a compression force is required to deform the cell so that it can squeeze through a filter. The compression force was calculated using the Hertz model (Figure S5).^[32] When the hydrodynamic force a cell experiences overcomes the required compression force, the cell starts to squeeze through the filter. A computational fluid dynamic model (COMSOL Multiphysics) was employed to simulate the hydrodynamic force a CTC experiences. The hydrodynamic force under different flow rates is compared with the compression force (Figure S6). The compression force calculated for WBCs (Figure S6A) shows that WBCs can squeeze through the filters at these flow rates. CTCs with a diameter bigger than 15 μm can be trapped by the lateral filters at various flow rates. CTCs with a diameter smaller than 15 μm can be compressed into the lateral filter at a high flow rate. The capture of these types of CTCs relies on immunoaffinity. The hydrodynamic force a cell experiences in the filter is then compared with the bond force between antibodies and the cell (Figure S7). For tumor cells with high expression level of the biomarker targeted by the antibody, the bond force is more than 10 times larger than the hydrodynamic force the tumor cell experiences. The force analysis theoretically proves the feasibility of integrating lateral filter arrays with immunoaffinity for CTC isolation.

To test LFAM and verify simulation, L3.6pl cells (metastatic human pancreatic cancer cells) were spiked in Dulbecco's phosphate-buffered saline (DPBS) buffer for performance evaluation. L3.6pl cells express a high level of EpCAM. We studied devices functionalized with anti-EpCAM and compared them to devices without antibody. As shown in Figure 3A, without antibody functionalization, the capture efficiency of L3.6pl cells varies from 69.8% to 83.9% at different flow rates. The capture efficiency decreases with the flow rate because of increasing shear-induced hydrodynamic force.^[33] When LFAM is functionalized with anti-EpCAM, the capture efficiency increases 10% or more at all flow rates. The capture efficiency is $98.7 \pm 1.2\%$ at 1.8 ml/h. Even at a high flow rate of 7.2 ml/h, the capture efficiency is $87.2 \pm 5.3\%$, showing that the antibody-functionalized LFAM device maintains high capture efficiency.

The distribution of cells captured in different filter zones indicates the effect of immunoaffinity on cell capture. In a LFAM device without antibody immobilized (Figure 3B), at the flow rate of 1.8 ml/h, $80.2 \pm 7.7\%$ of captured cells are in the 10- μm filter zone. As flow rate increases, more cells are captured downstream (i.e., 9 μm to 6 μm filter zones); only $21.8 \pm 7.4\%$ of captured cells are in the 10- μm filter zone when the flow rate is 7.2 ml/h.

For comparison, in a LFAM device functionalized with anti-EpCAM (Figure 3C), the captured cell distribution patterns are almost identical for different flow rates. Over 90% of captured cells are distributed in the 10- μm filter zone for all four flow rates. This shows that the functionalization of antibody significantly increases the capability of filters to capture tumor cells.

To further demonstrate the advantages of integrating size-based separation with immunoaffinity-based isolation, MCF7 cells (human breast adenocarcinoma cells) were spiked in the buffer and tested in LFAM. Significant improvement in cell capture efficiency was also observed when LFAM was functionalized with anti-EpCAM (Figure S10). The change of distribution pattern for captured MCF7 cells after antibody functionalization in LFAM was similar to what we observed for L3.6pl cells (Figure S10).

To study nonspecific capture of control cells in LFAM, a mixture containing 1000 L3.6pl cells and 3×10^6 WBCs (control cells) were infused to the antibody-functionalized LFAM. Figure 3D shows the non-specific capture of WBCs in LFAM at a flow rate of 3.6 ml/h and 7.2 ml/h, at a value of $(2.50 \pm 0.24) \times 10^4$ WBCs (or 0.83% of WBCs introduced) and $(1.08 \pm 0.11) \times 10^4$ WBCs (or 0.36%), respectively. The capture efficiency of the target L3.6pl cells are $93.8 \pm 1.5\%$ at 3.6 ml/h and $88.5 \pm 1.6\%$ at 7.2 ml/h.

The captured L3.6pl cells were then released from LFAM by pumping from the outlet (Supporting Info). As shown in Figure 3E, the cell release efficiency in an antibody functionalized device is $90.2 \pm 3.5\%$, which is higher than the corresponding release efficiency of $81.0 \pm 3.7\%$ in LFAM without antibody. This result seems to be counter intuitive, but it can be explained by two facts: (1) more than 90% of captured L3.6pl cells are located in the 10- μm filter zone (i.e., near the inlet) of the antibody-functionalized LFAM and they are easier to be pumped out with a reverse flow; and (2) antibody-functionalized LFAM is treated with a trypsin solution to disassociate EpCAM-antibody bond and the solution may slightly affect the cell membrane structure. Figure 3F shows that the viability of L3.6pl cells released from LFAM without antibody is $92.9 \pm 2.4\%$, which is close to the viability of the cells before infusion, $96.1 \pm 1.2\%$ (Supporting Info). The viability of cells released from the antibody-functionalized device is $83.8 \pm 5.1\%$; the decrease in viability probably results from the trypsin treatment. The released L3.6pl cells from antibody-functionalized LFAM were re-cultured and proliferated in a petri dish for two weeks (Figure S14).

Before using LFAM for clinical application, we studied the device by spiking 10–1000 of L3.6pl cells into 1 ml of 2-time diluted healthy blood samples and then infusing the sample into the anti-EpCAM-functionalized device. As shown in Figure S15, the capture efficiency is $95.4 \pm 1.1\%$ and $88.7 \pm 3.5\%$ at a flow rate of 3.6 ml/h and 7.2 ml/h, respectively. The

antibody-functionalized LFAM gives sufficiently high capture efficiency even at a high flow rate.

Finally, anti-EpCAM functionalized devices were used for CTC isolation from clinical samples. De-identified blood samples were voluntarily collected from patients with metastatic colorectal cancer after approval from the University of Florida institutional review board (IRB). A half of blood sample (4 mL) was diluted with equal volume of DPBS and then processed in the antibody-functionalized LFAM. The equal volume blood dilution was to reduce the blood viscosity and diminish the effect of viscosity variation among different patients, as practiced in commercial Ficoll-Paque process and in the literature.^[34] For comparison, the other half of the blood sample was processed under the same condition (e.g., immobilized with anti-EpCAM) using a previously reported, geometrically enhanced mixing (GEM) device containing herringbone micromixers.^[35] Captured cells were fixed with 4% paraformaldehyde (PFA) and permeabilized with Triton X-100. Three reagents, 4', 6-diamidino-2-phenylindole (DAPI), anti-cytokeratin (CK) labelled with fluorescein isothiocyanate (FITC), and anti-CD45 labelled with phycoerythrin (PE), were used to interrogate the cells captured. DAPI+/CK+/CD45- cells were considered CTCs. A total of 12 clinical samples were processed. CTCs were detected in 12/12 patients using LFAM and 11/12 using GEM. LFAM generally gives better CTC capture efficiency than GEM, as shown in Figure 4A. Their box plots are given in Figure 4B, showing an average of 3.2 CTCs/ml with a range of 1–8.25 CTCs/ml using LFAM, and an average of 1.3 CTCs/ml with a range of 0–4 CTCs/ml using GEM. The difference in the CTC number between LFAM and GEM is statistically significant ($p=0.00224$).

Nonspecific capture of WBCs in LFAM was also studied. We observed about 12,100 to 64,300 WBCs per device, corresponding to 0.078% to 0.41% capture efficiency. All captured cells were then released from LFAM by using a reverse flow (Supporting Info). CTCs were released 100% and WBCs were released between 55.6% to 79.1%.

In summary, integration of size-based separation with immunoaffinity-enabled isolation has produced high CTC capture efficiency. The flow pattern in LFAM was simulated and the device design was then optimized. Our comparison studies suggest that the antibody-functionalized LFAM has better performance than the device based on tumor cell's size only (i.e. without antibody). In addition, the antibody-functionalized LFAM shows high cell purity and viability, with a greater potential for clinical applications than those devices based on immunoaffinity only. Future studies will focus on in-depth analysis of CTCs isolated using next-generation sequencing and modern single-cell technologies, as well as on clinical utilities such as anticancer treatment monitoring.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank McJunkin Family Charitable Foundation, University of Florida (UF) Division of Sponsored Research (Preparation Grant), UF Health Cancer Center, and National Cancer Institute (K25CA149080) for their support.

Pablo Dopico is supported in part by the NIH TL1 Grant under the UF Clinical & Translational Science Awards. The cancer patients who donated blood samples are gratefully acknowledged.

References

- [1]. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF, *N Engl J Med* 2004, 351.
- [2]. Pantel K, Alix-Panabières C, *Cancer Res* 2013, 73.
- [3]. Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, Lilja H, Schwartz L, Larson S, Fleisher M, Scher HI, *Clinical Cancer Research* 2007, 13, 7053–7058. [PubMed: 18056182]
- [4]. Lin HK, Zheng S, Williams AJ, Balic M, Groshen S, Scher HI, Fleisher M, Stadler W, Datar RH, Tai YC, Cote RJ, *Clin Cancer Res* 2010, 16, 5011–5018. [PubMed: 20876796]
- [5]. Moon H-S, Kwon K, Kim S-I, Han H, Sohn J, Lee S, Jung H-I, *Lab on a chip* 2011, 11, 1118–1125. [PubMed: 21298159]
- [6]. Bhagat AAS, Hou HW, Li LD, Lim CT, Han J, *Lab on a chip* 2011, 11, 1870–1878. [PubMed: 21505682]
- [7]. Sollier E, Go DE, Che J, Gossett DR, O’Byrne S, Weaver WM, Kummer N, Rettig M, Goldman J, Nickols N, McCloskey S, Kulkarni RP, Di Carlo D, *Lab on a chip* 2014, 14, 63–77. [PubMed: 24061411]
- [8]. Miller MC, Robinson PS, Wagner C, O’Shannessy DJ, *Cytometry Part A* 2018, 93, 1234–1239.
- [9]. Gogoi P, Sepehri S, Zhou Y, Gorin MA, Paolillo C, Capoluongo E, Gleason K, Payne A, Boniface B, Cristofanilli M, Morgan TM, Fortina P, Pienta KJ, Handique K, Wang Y, *PLoS One* 2016, 11, e0147400. [PubMed: 26808060]
- [10]. Adams DL, Alpaugh RK, Martin SS, Charpentier M, Chumsri S, Cristofanilli M, Adams DK, Makarova OV, Zhu P, Li S, Tang CM, Stefansson S, *RSC Adv* 2016, 6, 6405–6414. [PubMed: 29093811]
- [11]. Park ES, Jin C, Guo Q, Ang RR, Duffy SP, Matthews K, Azad A, Abdi H, Todenhofer T, Bazov J, Chi KN, Black PC, Ma H, Small (Weinheim an der Bergstrasse, Germany) 2016, 12, 1909–1919.
- [12]. Vona G, Sabile A, Louha M, Sitruk V, Romana S, Schütze K, Capron F, Franco D, Pazzagli M, Vekemans M, Lacour B, Bréchet C, Paterlini-Bréchet P, *The American Journal of Pathology* 2000, 156, 57–63. [PubMed: 10623654]
- [13]. Stott SL, Hsu C-H, Tsukrov DI, Yu M, Miyamoto DT, Waltman BA, Rothenberg SM, Shah AM, Smas ME, Korir GK, Floyd FP, Gilman AJ, Lord JB, Winokur D, Springer S, Irimia D, Nagrath S, Sequist LV, Lee RJ, Isselbacher KJ, Maheswaran S, Haber DA, Toner M, *Proceedings of the National Academy of Sciences* 2010, 107, 18392–18397.
- [14]. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Smith MR, Kwak EL, Digumarthy S, Muzikansky A, Ryan P, Balis UJ, Tompkins RG, Haber DA, Toner M, *Nature* 2007, 450, 1235–1239. [PubMed: 18097410]
- [15]. Wang S, Liu K, Liu J, Yu ZT, Xu X, Zhao L, Lee T, Lee EK, Reiss J, Lee YK, Chung LW, Huang J, Rettig M, Seligson D, Duraiswamy KN, Shen CK, Tseng HR, *Angew Chem Int Ed Engl* 2011, 50, 3084–3088. [PubMed: 21374764]
- [16]. Ahmed MG, Abate MF, Song Y, Zhu Z, Yan F, Xu Y, Wang X, Li Q, Yang C, *Angew Chem Int Edit* 2017, 56, 10681–10685.
- [17]. Smith JP, Lannin TB, Syed YA, Santana SM, Kirby BJ, *Biomedical microdevices* 2014, 16, 143–151. [PubMed: 24078270]
- [18]. Song YL, Shi YZ, Huang MJ, Wang W, Wang Y, Cheng J, Lei ZC, Zhu Z, Yang CY, *Angew Chem Int Edit* 2019, 58, 2236–2240.
- [19]. Wan Y, Zhou YG, Poudineh M, Safaei TS, Mohamadi RM, Sargent EH, Kelley SO, *Angewandte Chemie* 2014, 126, 13361–13365.
- [20]. Talasaz AH, Powell AA, Huber DE, Berbee JG, Roh KH, Yu W, Xiao W, Davis MM, Pease RF, Mindrinos MN, Jeffrey SS, Davis RW, *Proc Natl Acad Sci U S A* 2009, 106.

- [21]. Balasubramanian P, Lang JC, Jatana KR, Miller B, Ozer E, Old M, Schuller DE, Agrawal A, Teknos TN, Summers TA Jr., Lustberg MB, Zborowski M, Chalmers JJ, PLoS One 2012, 7, e42048. [PubMed: 22844540]
- [22]. Schiro PG, Zhao M, Kuo JS, Koehler KM, Sabath DE, Chiu DT, Angewandte Chemie International Edition 2012, 51, 4618–4622. [PubMed: 22359315]
- [23]. Witek MA, Aufforth RD, Wang H, Kamande JW, Jackson JM, Pullagurla SR, Hupert ML, Usary J, Wysham WZ, Hilliard D, Montgomery S, Bae-Jump V, Carey LA, Gehrig PA, Milowsky MI, Perou CM, Soper JT, Whang YE, Yeh JJ, Martin G, Soper SA, npj Precision Oncology 2017, 1, 24. [PubMed: 29657983]
- [24]. Marrinucci D, Bethel K, Kolatkar A, Luttgren MS, Malchiodi M, Baehring F, Voigt K, Lazar D, Nieva J, Bazhenova L, Ko AH, Korn WM, Schram E, Coward M, Yang X, Metzner T, Lamy R, Honnatti M, Yoshioka C, Kunken J, Petrova Y, Sok D, Nelson D, Kuhn P, Phys Biol 2012, 9, 016003. [PubMed: 22306768]
- [25]. Lang JM, Casavant BP, Beebe DJ, Science translational medicine 2012, 4, 141ps113–141ps113.
- [26]. Mohamadi RM, Besant JD, Mephram A, Green B, Mahmoudian L, Gibbs T, Ivanov I, Malvea A, Stojcic J, Allan AL, Lowes LE, Sargent EH, Nam RK, Kelley SO, Angew Chem Int Ed Engl 2015, 54, 139–143. [PubMed: 25377874]
- [27]. Meunier A, Hernández-Castro JA, Turner K, Li K, Veres T, Juncker D, Analytical chemistry 2016, 88, 8510–8517. [PubMed: 27442305]
- [28]. Lee HJ, Oh JH, Oh JM, Park JM, Lee JG, Kim MS, Kim YJ, Kang HJ, Jeong J, Kim SI, Lee SS, Choi J-W, Huh N, Angewandte Chemie International Edition 2013, 52, 8337–8340. [PubMed: 23825032]
- [29]. Williams A, Balic M, Datar R, Cote R, in Minimal residual disease and circulating tumor cells in breast cancer, Springer, 2012, pp. 87–95.
- [30]. McFaul SM, Lin BK, Ma H, Lab on a chip 2012, 12, 2369–2376. [PubMed: 22517056]
- [31]. Terstappen LWMM, Coumans FAW, Doggen CJM, Attard G, de Bono JS, Annals of Oncology 2010, 21, 1851–1857. [PubMed: 20147742]
- [32]. Zhang W, Kai K, Choi DS, Iwamoto T, Nguyen YH, Wong H, Landis MD, Ueno NT, Chang J, Qin L, Proceedings of the National Academy of Sciences 2012, 201209893.
- [33]. Kuo JS, Zhao Y, Schiro PG, Ng L, Lim DS, Shelby JP, Chiu DT, Lab on a chip 2010, 10, 837–842. [PubMed: 20379567]
- [34]. Xu L, Mao X, Imrali A, Syed F, Mutsvangwa K, Berney D, Cathcart P, Hines J, Shamash J, Lu YJ, PLoS One 2015, 10, e0138032. [PubMed: 26397728]
- [35]. Sheng W, Ogunwobi OO, Chen T, Zhang J, George TJ, Liu C, Fan ZH, Lab on a chip 2014, 14, 89–98. [PubMed: 24220648]

Figure 1.

(A) Picture of a LFAM device consisting of four serpentine main channels. In each main channel, a filter array is embedded as shown in the inset. The scale bar is 200 μm . (B) The serpentine main channel creates a main flow while filters produce numerous branch flows in the lateral direction. A cell flowing through the channel has velocity components in both main channel direction and lateral direction. (C) The parking-space-like arrangement of filters helps adjust the flow paths of subsequent cells when one CTC fills a filter. The velocity component in the main channel direction of a cell prevents it from clogging. (D) The filter geometry forces the direct interaction between a cell and antibodies immobilized on the filter surfaces, increasing the probability of cell capture.

Figure 2.

(A) (top) The simulated streamline pattern in LFAM when mainstream ratio is <50%. (bottom) The observed flow pattern for 9- μm filters that have a mainstream ratio of 27.0%. (B) (top) The streamline pattern in LFAM when mainstream ratio=50%. (bottom) The observed flow pattern for 6- μm filters that have a mainstream ratio of 46.9%. (C) The streamline pattern in LFAM when mainstream ratio is >50%. (D) The mainstream ratios for different filter sizes in LFAM.

Figure 3.

(A) Comparison in capture efficiency of L3.6pl cells between a LFAM device with antibody and a device without antibody. (B) Cell distribution pattern in LFAM without antibody. Y-axis indicates the percentage of cells captured in each filter zone. (C) The cell distribution pattern in LFAM with antibody. (D) Nonspecific capture of WBCs in LFAM. (E) Release efficiency of L3.6pl cells from LFAM with or without antibody. (F) Viability of the released L3.6pl cells.

Figure 4. (A) CTCs isolated from clinical samples using LFAM or GEM. (B) The average number of CTCs per ml of blood samples detected using LFAM or GEM.