Micro and Nano Systems Letters

LETTER

Open Access

Patternable particle microarray utilizing controllable particle delivery

Sanghyun Lee[1†], Hojin Kim[2†], Wonhyung Lee[1] and Joonwon Kim[1*]

Abstract

In this study, we demonstrate an on-demand delivering and sequential arraying of single microparticles utilizing multiple pneumatic pressure-driven elastomer valves and a deterministic particle arraying mechanism. Two types of separate microfluidic devices are combined: (i) a particle transfer device and (ii) a particle arraying device, to construct a desired particle pattern. The elastomer valve integrated in the transfer device acts as a removable particle trap that enables the trapping and on-demand releasing of a particle depending on the application of the pneumatic pressure. The arraying device is composed of highly packed particle trapping sites to deterministically array incoming particles that are released and transferred from a transfer device. Repeating the "trapping-transfer-and-array" sequence can construct an array of different types of particles in a certain pattern. One-dimensional linear and two-dimensional planar microparticle-based patterns were demonstrated using bare and red-fluorescent microparticles.

Keywords: Microfluidics, Microparticle, Pneumatic microvalve, Controllable particle delivery, Patterning

Introduction

Recently, particle-incorporated microfluidic platforms have emerged as effective tools to conduct various analyses in biological and chemical research fields [1–5]. When compared with flat substrates or supports, microparticles can serve as a mobile substrate and provide multiple functionalities including huge analytical surface and the capability of effective mixing, sorting, and transporting of molecules of interest [6]. For most cases in these platforms, particles are trapped in an array format within microchannel networks with embedded physical barrier structures (e.g., weirs or micropillars) or external active forces (e.g., electric forces). Depending on the target applications, surfaces of particles can be functionalized (e.g., DNA or antibody conjugation). To construct the particle array, the particles (i.e., array elements) are introduced from an off-chip environment and subsequently arrayed within microfluidic devices [7–11].

Generally, different types of functionalized particles are arrayed randomly to perform analyses; thus, the encoding and decoding of individual particles must be performed for their identification and readout of the results after their reactions (i.e., mix-and-match). Although several strategies for particle encoding exist [12–14], a high-resolution imaging system is typically required to identify individual particles based on their encoding method. Meanwhile, a position-based signal readout method facilitates an easy readout of the results such as the wellplate-based enzyme-linked immunosorbent assay (ELISA). Thus, the advantages of particle-based analysis and position-based easy readout can be combined in an integrated system.

Several studies have been carried out to generate specific particle patterns using microfluidic platforms. A 1-D microfluidic bead array was constructed by depositing particles one by one using vacuum tweezers [15, 16]. This approach can form a desired particle pattern in predefined channels, but is a cumbersome process. As an alternative approach, an additional microbead loading channel was integrated to deposit the functionalized microparticles into predetermined positions [17, 18]. This requires additional components and has limitation to enable a single particle level patterning.

*Correspondence: joonwon@postech.ac.kr
†Sanghyun Lee and Hojin Kim contributed equally to this work
[1] Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 37673, South Korea
Full list of author information is available at the end of the article

Without using physical trap structures, dynamic particle patterning was demonstrated using standing surface acoustic waves (SSAWs) [19, 20]. However, complicated device fabrication and system setup are required.

In this regard, we present a method to construct a particle array in a desired pattern (i.e., patternable particle array) instead of a random pattern, to enable a position-based easy readout using different types of particles with a single-particle-level resolution. Our strategy combines two types of separate microfluidic devices that offer different functions: (i) a particle transfer device and (ii) a particle arraying device. Pneumatic microvalve-based removable trap techniques and deterministic particle arraying techniques are used in the transfer and arraying devices, respectively. A transfer device can selectively transfer the particles of interest; these can be patterned in the arraying device in a desired pattern. Many studies have demonstrated particle manipulation (e.g., trapping, releasing, and pairing) using pneumatic pressure-driven elastomer microvalve structures [21–25]. Depending on the state of the valve (i.e., either "on" or "off"), particles can be either trapped or released. An arraying device can capture the introduced particles deterministically (i.e., from the transfer device) based on flow fractionation [10, 26]. As a proof-of-concept of our method, we demonstrated particle patterns of one-dimensional (1D) linear (e.g., "dot-dash" line) and two-dimensional (2D) planar (e.g., Braille numbers) using bare and red-fluorescent polystyrene particles.

Materials and methods

Device design and operation

To demonstrate on-demand single particle delivery and the sequential arraying of particles, two types of microfluidic devices are integrated: (i) a particle transfer device and (ii) a particle arraying device (Fig. 1a). The outlet of the transfer device and the inlet of the arraying device are connected using a tube for particle transfer. The particle transfer device is composed of pneumatic pressure inlets, particle inlets, wastes, and an outlet. A pneumatic channel is used to operate the elastomeric pneumatic microvalve. The particle arraying device is composed of an inlet, a particle arraying site, and an outlet.

In the transfer device, particles are introduced through the particle inlet and abundant particles are washed out toward waste. The particle transfer function can be enabled by operating the elastomeric pneumatic microvalve that acts as a removable particle trap depending on the applied pneumatic pressure [25]. When a positive pneumatic pressure is applied through the pneumatic channel, a thin membrane (i.e., channel wall) is deflected and as a result, a narrowed branch pocket that can trap a single particle is formed (Fig. 1c). The pillar structure can guide the particles to migrate close to the channel wall; this facilitates particle trapping [27]. Once a single particle

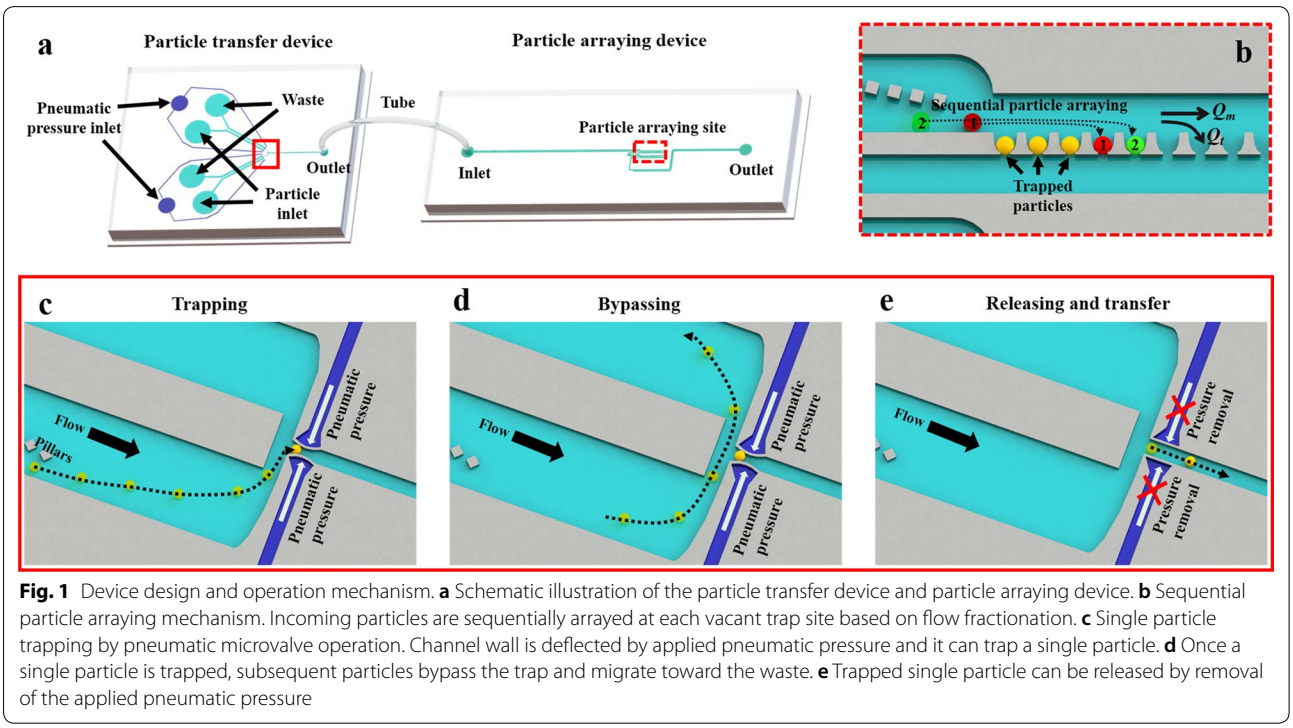

Fig. 1 Device design and operation mechanism. **a** Schematic illustration of the particle transfer device and particle arraying device. **b** Sequential particle arraying mechanism. Incoming particles are sequentially arrayed at each vacant trap site based on flow fractionation. **c** Single particle trapping by pneumatic microvalve operation. Channel wall is deflected by applied pneumatic pressure and it can trap a single particle. **d** Once a single particle is trapped, subsequent particles bypass the trap and migrate toward the waste. **e** Trapped single particle can be released by removal of the applied pneumatic pressure

is trapped, subsequent particles will migrate toward the waste without any additional trapping (Fig. 1d). The trapped particles can be released and transferred to the arraying device in an on-demand manner when the applied pneumatic pressure is removed (Fig. 1e).

The target particle can be transferred from the transfer device to the arraying device through the tube connection. Based on the flow fractionation at each vacant trap sites (i.e., flow is distributed into main flow Q_m and trapping flow Q_t), the transferred particle is trapped deterministically at each vacant trap site (sequentially from upstream to downstream) of the particle arraying device (Fig. 1b) [26]. By repeating the "trapping-transfer-and-array" sequence, we can construct the particle array in a controllable manner (i.e., patternable particle array). We can select specific particles to be transferred using different types of particle suspensions at different particle inlets. The numbers of particle inlets and pneumatic pressure inlets can be varied based on the types of particles that are arrayed.

Device fabrication

Both microfluidic devices were fabricated using polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning Inc.) by standard soft lithography [28]. Negative tone photoresist (KMPR 1025, MicroChem, Inc.) was used to prepare the master molds. It was deposited onto two four-inch silicon wafers with the same thickness (32 μm) by spin coating and soft baking (100 °C for 15 min). Ultraviolet (UV) exposure through a photomask and post-exposure baking (100 °C for 3 min) and development (SU-8 developer, MicroChem, Inc.) were proceeded to define the patterns.

Two different PDMS prepolymer mixtures (i.e., different PDMS monomer base to crosslinker ratio) were used to prepare PDMS replicas using the master molds: (i) 12:1 w/w ratio mixture for the particle transfer device and (ii) a 10:1 w/w ratio mixture for the particle arraying device. Regardless of the PDMS mixture ratio, PDMS replicas were prepared by the same procedure. The PDMS mixture was poured onto the master mold and degassed; this was then thermally cured at 100 °C for 20 min. The cured PDMS replica was peeled off from the master mold and holes were punched using a disposable biopsy punch. The holes were rinsed with isopropanol. The PDMS replica and glass substrate were irreversibly bonded by contact after air plasma treatment (CUTE-MP, FemtoScience). The prepared devices were stored at room temperature for 24 h for a reliable device operation.

Experimental setup

For system operation, separate custom-built pneumatic pressure supply systems are used: (i) positive pressure supply system to operate the elastomeric valve and infuse particles and (ii) a negative pressure supply system to facilitate particle transfer and arraying. A positive pressure application system (connected with the pneumatic/particle inlet of the transfer device) consists of a pressure pump (i.e., positive pressure generation), solenoid valves, a pressure regulator, and a pressure monitor. A negative pressure application system (connected with the outlet of the arraying device) consists of a pressure pump (i.e., negative pressure generation), pressure regulator, and pressure monitor. Microscopic images were acquired using an inverted microscope (IX 73, Olympus) with a charge-coupled device (CCD) camera (DP80, Olympus). For fluorescence detection, a SOLA light engine (SM 365, lumencor) and filter cube (U-FGWA, Olympus) were used as the light source and fluorescence filter, respectively.

Results and discussion

1D linear patterning of particles

On-demand single particle release and delivery was demonstrated with elastomeric microvalve operation. The valve deflection state (i.e., ON or OFF) can be controlled using the applied pneumatic pressure and solenoid valve operation (Fig. 2a). When the valve state is "ON" (i.e., the wall of the valve is deflected under the application of pneumatic pressure), a single particle can be trapped at the narrow channel created by valve deflection (Fig. 2b). The average diameter of the particle used in this test was approximately 25 μm. Particle suspensions were introduced into the particle transfer chip under a pneumatic pressure of 5 kPa. To trap a single particle, we applied a pneumatic pressure of 200 kPa to deflect the wall of the microvalve. After particle trapping, subsequent particles passed the valve and migrated toward the waste channel owing to a sudden increase in hydraulic resistance of the channel through the microvalve. When the applied pneumatic pressure was removed by triggering a solenoid valve (switching time of 100 ms) in an on-demand manner, the trapped single particle was selectively released and migrated to the outlet of the transfer device (Fig. 2c). This particle was subsequently transferred to the particle arraying device under an applied vacuum pressure of −15 kPa at the outlet of the arraying device.

1D linear particle patterning was demonstrated using two types of particles: (i) bare polystyrene beads (mean diameter ≈ 25 μm; SD: ±0.21 μm, Sigma-Aldrich), (ii) red-fluorescent polystyrene beads (mean diameter ≈25 μm; SD: ±0.20 μm, excitation/emission = 530/607 nm, microParticles). Concentrations of particle suspensions were set to 10,000 particles/mL for both particle types. By repeating the "trapping-transfer-and-array" sequence (i.e., one time red-fluorescent

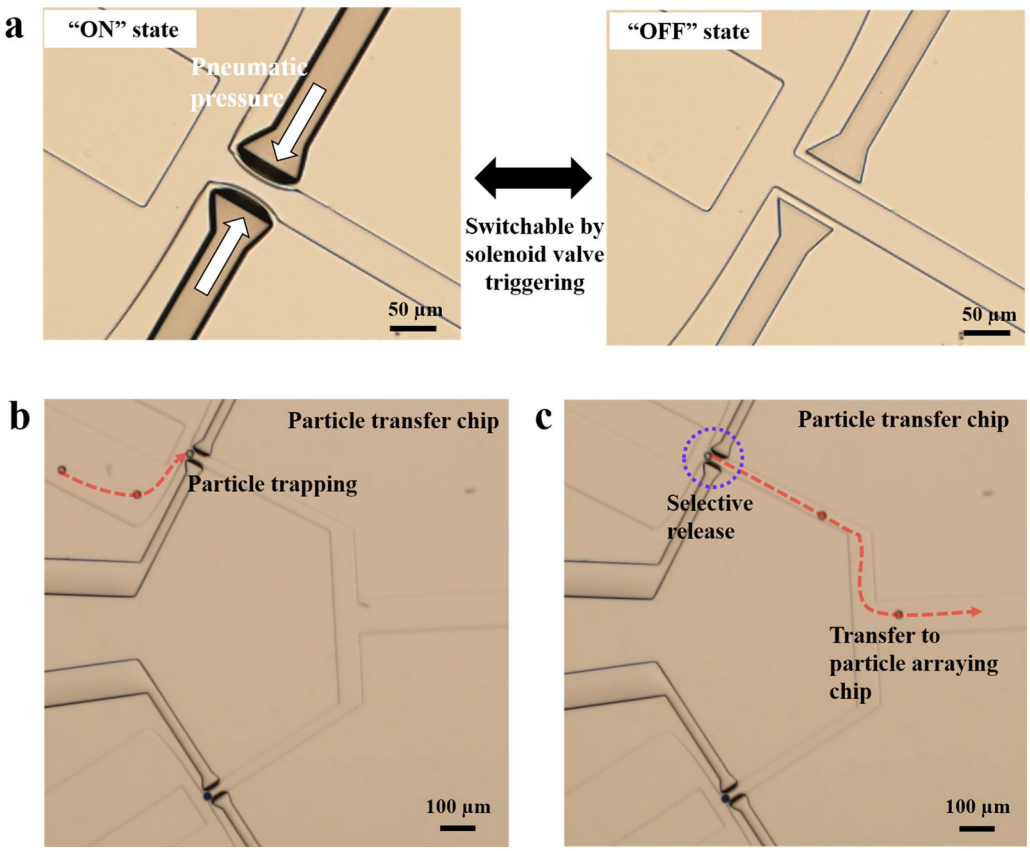

Fig. 2 On-demand particle transfer utilizing pneumatic valve operation. **a** Depending on the applied pneumatic pressure, the valve wall is either deflected ("ON" state) or not ("OFF" state). These two states are switchable by controlling the integrated solenoid valve. **b** Particle trapping. Single particle is trapped at the narrow channel created by valve deflection. **c** Particle releasing. Removing the pneumatic pressure allows the trapped particle to be released and transferred in an on-demand manner

particle and two times bare particles) using individual solenoid valve triggering, we can construct a line pattern, as shown in Fig. 3a. The fluorescence image reveals that the particle line pattern is constructed as a "dot-dash" line (Fig. 3b).

2D planar patterning of particles

The multiple row patterning of 1D particle lines can yield 2D planar particle patterns. Selective and on-demand particle transfer and sequential arraying of particles were used similar to the 1D linear particle patterning method described above. Inspired by Braille patterns (Fig. 4a), which are used by the visually impaired, we constructed 2D particle patterns to display Braille numbers using controllable transfer and arraying of bare and red-fluorescent particles (Fig. 4b). Fluorescence detection revealed that several Braille number patterns (1, 2, 3, 4, and 5) were displayed by the proper combination of particles (Fig. 4c). Schematic of generating Braille-inspired particle patterns in the particle arraying device are described in Additional file 1: S1.

Conclusion

In this study, we demonstrated a patternable particle microarray by trapping, releasing, transferring, and sequentially arraying two types of particles. A particle transfer device and a particle arraying device were combined to construct desired particle patterns. In the particle trapping device, the integration of a pneumatic pressure-driven elastomer microvalve enabled single-particle trapping and selective, on-demand releasing of particles of interest. The released particle was transferred to the arraying device and subsequently trapped at vacant sites sequentially from the upstream direction. 1D linear (e.g., "dot-dash" line) and 2D planar (e.g., Braille numbers) particle patterns were constructed in a controllable manner by repeating the "trapping-transfer-and-array" sequence. Even though we demonstrated particle patterns using two

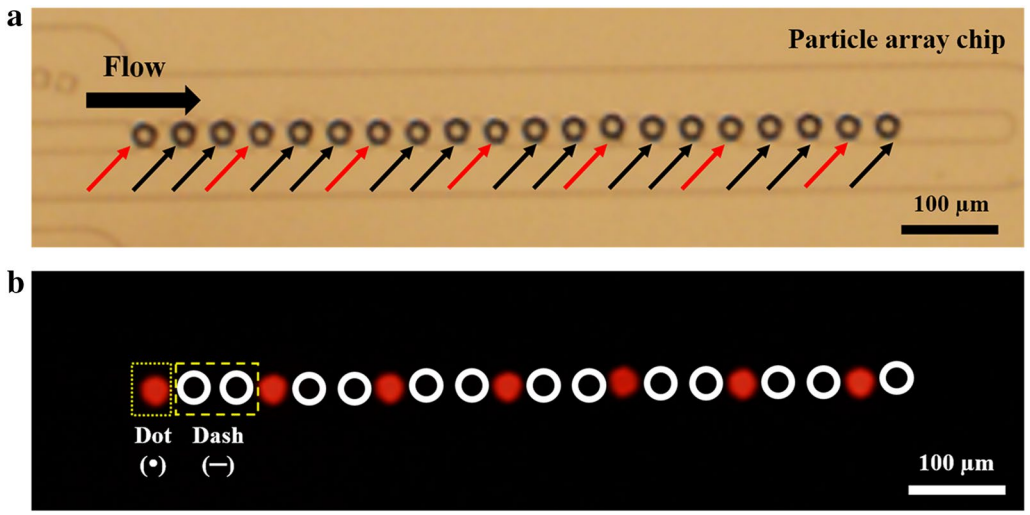

Fig. 3 1D linear pattern using two types of particles by repeating the "trapping-transfer-and-array" sequence. **a** Bright-field microscopic image of arrayed particles. Bare (indicated with black arrows) and red-fluorescent particles (indicated with red arrows) are deterministically trapped in an arraying device. **b** Fluorescent image revealing the linear pattern of the "dot-dash" line

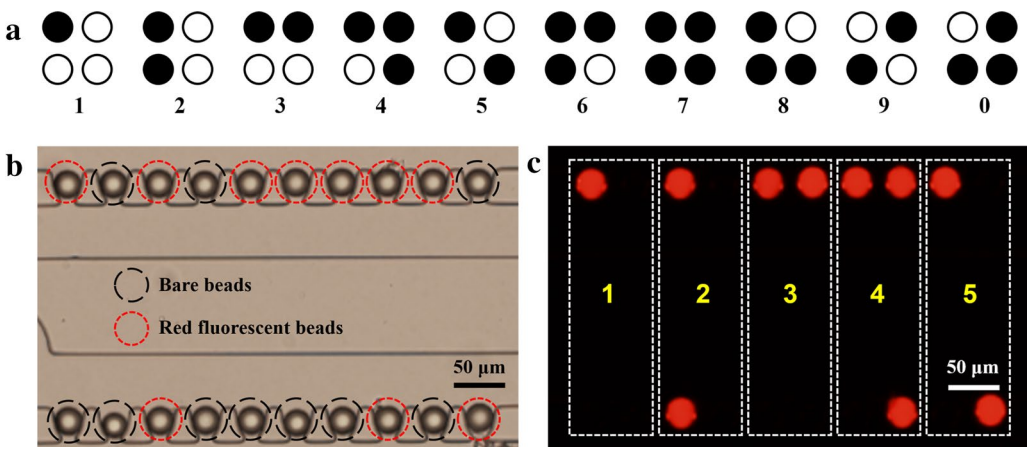

Fig. 4 2D planar pattern using two types of particles. **a** Braille representation of numbers from 0 to 9. **b** Bright-field microscopic image of arrayed particles composed of the combination of bare and red-fluorescent particles. **c** Fluorescent image revealing the planar pattern that displays the Braille numbers 1, 2, 3, 4, and 5

types of particles (i.e., bare and red-fluorescent particles), more complex particle patterns can be achieved with different types (e.g., > three types) of particles by operating additional particle inlets. The programmable operation of multiple pneumatic valves can facilitate the construction of desired particle patterns composed of different types of particles. We believe that the particle patterning technique presented herein has potential for various applications such as microarrays, drug screening, and cellular studies. Furthermore, proper integration of functionalized particles (e.g., DNA or protein-conjugated particles) can facilitate a pattern-based easy-readout multiplex immunoassay platform.

Additional file

Additional file 1. Additional information is available about schematics of generating Braille-inspired patterns.

Acknowledgements
Not applicable.

Authors' contributions
SL and HK performed the experiments, analyzed the data and wrote the manuscript. WL carried out device fabrication. JK supervised the research and reviewed the manuscript. All authors read and approved the final manuscript.

Funding
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (No. NRF-2017R1A2B4003328).

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its additional file.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 37673, South Korea. [2] BioMEMS Lab, RadianQbio, 53 Gasan digital 2-ro, Geumcheon-gu, Seoul 08588, South Korea.

Received: 20 May 2019 Accepted: 18 July 2019
Published online: 23 July 2019

References

1. Birtwell S, Morgan H (2009) Microparticle encoding technologies for high-throughput multiplexed suspension assays. Integr Biol 1:345–362
2. Liu L, Wu S, Jing F, Zhou H, Jia C, Li G, Cong H, Jin Q, Zhao J (2016) Bead-based microarray immunoassay for lung cancer biomarkers using quantum dots as labels. Biosens Bioelectron 80:300–306
3. Kim H, Lee S, Lee W, Kim J (2017) High-density microfluidic particle-cluster-array device for parallel and dynamic study of interaction between engineered particles. Adv Mater 29:1701351
4. Han SW, Jang E, Koh W-G (2015) Microfluidic-based multiplex immunoassay system integrated with an array of QD-encoded microbeads. Sens Actuators B Chem 209:242–251
5. Hung L-Y, Huang T-B, Tsai Y-C, Yeh C-S, Lei H-Y, Lee G-B (2013) A microfluidic immunomagnetic bead-based system for the rapid detection of influenza infections: from purified virus particles to clinical specimens. Biomed Microdevice 15:539–551
6. Derveaux S, Stubbe B, Braeckmans K, Roelant C, Sato K, Demeester J, De Smedt S (2008) Synergism between particle-based multiplexing and microfluidics technologies may bring diagnostics closer to the patient. Anal Bioanal Chem 391:2453
7. Carlo DD, Wu LY, Lee LP (2006) Dynamic single cell culture array. Lab Chip 6:1445–1449
8. Jung Y, Hyun J-C, Choi J, Atajanov A, Yang S (2017) Manipulation of cells' position across a microfluidic channel using a series of continuously varying herringbone structures. Micro Nano Syst Lett 5:6
9. Tan W-H, Takeuchi S (2007) A trap-and-release integrated microfluidic system for dynamic microarray applications. Proc Natl Acad Sci 104:1146–1151
10. Chung K, Rivet CA, Kemp ML, Lu H (2011) Imaging single-cell signaling dynamics with a deterministic high-density single-cell trap array. Anal Chem 83:7044–7052
11. Teshima T, Ishihara H, Iwai K, Adachi A, Takeuchi S (2010) A dynamic microarray device for paired bead-based analysis. Lab Chip 10:2443–2448
12. Burger R, Reith P, Kijanka G, Akujobi V, Abgrall P, Ducrée J (2012) Array-based capture, distribution, counting and multiplexed assaying of beads on a centrifugal microfluidic platform. Lab on a Chip 12:1289–1295
13. Lee H, Kim J, Kim H, Kim J, Kwon S (2010) Colour-barcoded magnetic microparticles for multiplexed bioassays. Nat Mater 9:745
14. Appleyard DC, Chapin SC, Srinivas RL, Doyle PS (2011) Bar-coded hydrogel microparticles for protein detection: synthesis, assay and scanning. Nat Protoc 6:1761
15. Zhang H, Yang X, Wang K, Tan W, Li H, Zuo X, Wen J (2008) On-chip oligonucleotide ligation assay using one-dimensional microfluidic beads array for the detection of low-abundant DNA point mutations. Biosens Bioelectron 23:945–951
16. Zhou L et al (2006) Quantitative intracellular molecular profiling using a one-dimensional flow system. Anal Chem 78:6246–6251
17. Zhang H, Fu X, Liu L, Zhu Z, Yang K (2012) Microfluidic bead-based enzymatic primer extension for single-nucleotide discrimination using quantum dots as labels. Anal Biochem 426:30–39
18. Zhang H, Liu L, Fu X, Zhu Z (2013) Microfluidic beads-based immunosensor for sensitive detection of cancer biomarker proteins using multi-enzyme-nanoparticle amplification and quantum dotslabels. Biosens Bioelectron 42:23–30
19. Shi J, Ahmed D, Mao X, Lin S-CS, Lawit A, Huang TJ (2009) Acoustic tweezers: patterning cells and microparticles using standing surface acoustic waves (SSAW). Lab on a Chip 9:2890–2895
20. Ding X, Shi J, Lin S-CS, Yazdi S, Kiraly B, Huang TJ (2012) Tunable patterning of microparticles and cells using standing surface acoustic waves. Lab Chip 12:2491–2497
21. Tonooka T, Teshima T, Takeuchi S (2013) Clustering triple microbeads in a dynamic microarray for timing-controllable bead-based reactions. Microfluidics Nanofluidics 14:1039–1048
22. Ma C et al (2011) A clinical microchip for evaluation of single immune cells reveals high functional heterogeneity in phenotypically similar T cells. Nat Med 17:738
23. Kim HS, Devarenne TP, Han A (2015) A high-throughput microfluidic single-cell screening platform capable of selective cell extraction. Lab Chip 15:2467–2475
24. Kim H, Kim J (2014) A microfluidic-based dynamic microarray system with single-layer pneumatic valves for immobilization and selective retrieval of single microbeads. Microfluidics Nanofluidics 16:623–633
25. Kim H, Lee S, Kim J (2012) Hydrodynamic trap-and-release of single particles using dual-function elastomeric valves: design, fabrication, and characterization. Microfluidics Nanofluidics 13:835–844
26. Sochol RD, Dueck ME, Li S, Lee LP, Lin L (2012) Hydrodynamic resettability for a microfluidic particulate-based arraying system. Lab Chip 12:5051–5056
27. Iwai K, Tan W-H, Ishihara H, Takeuchi S (2011) A resettable dynamic microarray device. Biomed Microdev 13:1089–1094
28. Xia Y, Whitesides GM (1998) Soft lithography. Angewandte Chem Int Edn 37:550–575

Publisher's Note