High-throughput inertial particle focusing in a curved microchannel: Insights into the flow-rate regulation mechanism and process model

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(Received 22 June 2013; accepted 31 July 2013; published online 8 August 2013)

In this work, we design and fabricate a miniaturized spiral-shaped microchannel device which can be used for high-throughput particle/cell ordering, enrichment, and purification. To probe into the flow rate regulation mechanism, an experimental investigation is carried out on the focusing behaviors of particles with significantly different sizes in this device. A complete picture of the focusing position shifting process is unfolded to clarify the confusing results obtained from flow regimes with different dominant forces in past research. Specifically, with the increase of the flow rate, particles are observed to first move towards the inner wall under the dominant inertial migration, then stabilize at a specific position and finally shift away from the inner wall due to the alternation of the dominant force. Novel phenomena of focusing instability, co-focusing, and focusing position interchange of differently sized particles are also observed and investigated. Based on the obtained experimental data, we develop and validate, for the first time, a five-stage model of the particle focusing process with increasing flow rate for interpreting particle behaviors in terms of the competition between inertial lift and Dean drag forces. These new experimental findings and the proposed process model provide an important supplement to the existing mechanism of inertial particle flow and enable more flexible and precise particle manipulation. Additionally, we examine the focusing behaviors of bioparticles with a polydisperse size distribution to validate the explored mechanisms and thus help realize efficient enrichment and purification of these particles.
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I. INTRODUCTION

As a passive manipulation scheme, inertial particle focusing offers significant advantages such as continuous high-throughput processing, non-invasive manipulation without special labels, and simple operation without external fields.^{1,2} It has therefore been employed as an important pretreatment strategy for wide-ranging applications such as size-based separation,^{3–6} membrane-free filtration or enrichment,^{7–9} sheathless microflow cytometry,^{10–12} efficient solution exchange,¹³ high-yield cell-in-droplet encapsulation,^{14,15} and large population mechanical phenotyping.¹⁶ With the prosperous development in novel biomedical applications of inertial focusing, the elucidation of the underlying mechanisms has attracted significant attention.

Recent progress on inertial focusing mechanism has been achieved mainly in two channel configurations. For the investigation of inertial particle migration in straight channels, experimental or numerical approaches have been applied to systematically investigate particle equilibrium position,^{17,18} particle–particle interaction,^{19,20} lift force scaling,^{21,22} and particle focusing

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process^{23,24} in inertial flows. The manipulation objects have evolved from rigid polymer particles to irregular and deformable particles/cells or droplets.^{25,26} For more complex, curved channels where flowing particles experience both inertial migration effects and influences from the Dean flow,^{6,27} the particle dynamics have been reported in several types of channel geometries, including single curves,^{27,28} symmetric or asymmetric sinusoidal lines,²⁷ and spirals.^{29–31} The spiral channels, which feature asymmetric structure and gradually varied curvature, have drawn special attention for in these channels the strong Dean flow can be maintained for an extended period of time in a compact manner. Specifically, Kuntaegowdanahalli *et al.*²⁹ reported a new scheme for high-throughput separation of multi-particles utilizing spiral channel design and characterized, in the meantime, the moving of the focusing position near the outlets at relatively high flow rates. Martel *et al.*³⁰ studied the migration behaviors of single-sized particles in spiral channels of various aspect ratios and proposed a new ratio of forces threshold for achieving high quality focusing. And in our previous work, we studied the formation process of ordered particle streams both at outlets and along the microchannel and elucidated the corresponding migration mechanisms.³¹ Although efforts have been made to understand particle focusing behaviors, fundamentals disclosing regulation mechanisms of the flow rate remain elusive. For example, previous studies have focused on the particle behaviors in single flow regime, with little attention paid to a panorama of single/hybrid particles' focusing behaviors across flow regimes with different dominant forces. In addition, a theoretical model of the inertial focusing process for interpreting detailed particle focusing behaviors with increasing flow rate is still lacking.

In this work, we design and fabricate a spiral microfluidic device with a small footprint and then experimentally investigate the focusing behaviors of differently sized particles across flow regimes with different dominant forces in this channel configuration. The effects of flow rate on particle focusing position and focusing ratio are quantitatively characterized through analyzing the obtained fluorescent stream images and intensity spectrums. More importantly, we develop and validate, for the first time, a five-stage model for further elucidating the particle focusing process with increasing flow rate. The improved understanding of flow-rate regulation mechanism would provide insights into the device design and the operation protocol improvement.

II. BASIC PHYSICS OF PARTICLE FOCUSING

In finite-Reynolds-number curved channel flows, the coupling of inertial migration effect and Dean flow effect leads to a transverse migration of the particles flowing in the channel.^{29,31} As illustrated in Fig. 1(a), particles that are randomly distributed near the inlet gradually form a well-ordered particle stream while migrating to the outlets. The inertial lift force (F_L) that causes the inertial migration of particles is the net force of a shear-induced lift force directed to the channel wall and a wall-induced lift force to the center,^{6,32,33}

$$F_L = \frac{\rho U_m^2 a_p^4}{D_h^2} f_L(\text{Re}_c, X_p). \quad (1)$$

Here ρ is the density of the fluid, U_m is the maximum fluid velocity, a_p is the particle diameter, f_L is the lift coefficient which is dependent on the channel Reynolds number (Re_c) and the particle position within the channel cross-section (X_p), and D_h is the hydraulic diameter, approximated as $2wh/(w+h)$ for rectangular cross-sections (w and h denoting the channel width and height, respectively).

Meanwhile, a specific Dean flow superimposed on the main flow is induced by the fluid inertia effects in curved channels and is illustrated as a pair of symmetric-counter rotating vortices located respectively in the upper and lower half of the channel. The strength of this rotational flow is quantified by a dimensionless number called the Dean number ($De = \text{Re}_c(D_h/2R)^{1/2}$, where R is the channel radius).^{1,34} The additional drag force (Dean drag, F_D) applied by this secondary flow further alters the transverse position of the flowing particles.

FIG. 1. (a) Schematic of inertial particle focusing in a spiral microchannel. Particles that are randomly dispersed in the inlet region gradually form a well-ordered particle stream at a specific lateral position while migrating towards the outlets. (b) Photograph of the finished PDMS microfluidic device (the channel of which was filled with red ink for visualization). The inset (i) shows a microscope image of the channel cross-section.

With the Dean drag force and the Dean velocity being in the same direction, the magnitude of this drag force scales as follows:³³

$$F_D \propto \rho U_m^2 a_p D_h^2 R^{-1}. \quad (2)$$

It should be noted that the ratio of particle diameter (a_p) to the characteristic length of the channel cross-section (L_c) should satisfy $a_p/L_c \geq 0.07$ to ensure that particles can be well ordered under specific operating conditions.⁷ The characteristic length of the low-aspect-ratio channel used in this work approximately equals the channel height.³¹

III. EXPERIMENTAL

A. Device design and fabrication

The channel unit employed in our miniaturized particle focusing devices was a simple five-loop Archimedean spiral structure with a low-aspect-ratio ($AR = h/w = 1:3$) cross-section. One inlet and two bifurcated outlets were arranged at the channel ends for the enrichment and sheathless focusing of bioparticles. The detailed channel structure is $150 \mu\text{m}$ wide with the spacing between two adjacent loops fixed at $500 \mu\text{m}$ and an initial radius of the spiral at 3.5 mm . The total length of the main spiral channel is calculated to be $\sim 16.6 \text{ cm}$. This device was cost-effectively prototyped in polydimethylsiloxane (PDMS) using our maskless lithography system (SF-100 Xtreme, Intelligent Micro Patterning) and micromolding techniques. More details on device fabrication and packaging methods have been provided in our recent publication.³⁵

Photograph of the finished device is shown in Fig. 1(b). The small overall size ($\sim 2\text{ cm}^2$) and the simple planar nature of the device permit its flexible integration with existing Lab-on-a-chip units.

B. Sample preparation

Two polymer microparticles (particle density 1.05 g/cm^3) with significantly different sizes ($5\text{ }\mu\text{m}$, Green fluorescence, Thermo Fisher Scientific, Inc. and $10\text{ }\mu\text{m}$, Blue fluorescence, Invitrogen) served as cell surrogates in the focusing behavior testing. The particle sample (1% solids) was diluted in 0.5 wt% Tween 20 (Sigma-Aldrich) aqueous solutions, obtaining a homogeneous suspension with the concentration of $\sim 0.008\%$. Zymosan bioparticles from *Saccharomyces cerevisiae* were covalently labeled with Texas Red[®] fluorophores (Molecular Probes, Inc.) for better observation. The particles were dispersed in 0.01 M sterile phosphate-buffered saline (PBS, Sigma) that contains 0.5 wt. % Tween 20 (Sigma-Aldrich) to reduce aggregation. The obtained particle solution was incubated at 37°C for 2 h and then vigorously vortexed into the homogeneous suspension (particle concentration $\sim 0.04\text{ mg/ml}$). The extremely low particle concentrations used in this work ensure that the interparticle interactions can be neglected in the analysis of particle dynamics.

C. Experiment set-up and operation

Polyetheretherketone (PEEK) tubing pieces ($1/32''$, Upchurch Scientific) were directly inserted into the ports of the microfluidic device (diameter $\sim 0.75\text{ mm}$) for sample introduction and collection. A syringe pump (Legato 270, KD Scientific, Inc.) was employed to precisely and stably drive the particle suspension at a specific flow rate. The spiral device was clamped on the stage of an inverted fluorescence microscope (IX71, Olympus), and the observation plane was fixed at the bottom of the microchannel. High-speed motions of single/hybrid particles labeled with different fluorescence dyes were visualized and differentiated by switching the equipped fluorescence mirror units (U-MWU2, U-MWB2). A 14-bit CCD camera (Exi Blue, Qimaging) and the IMAGE-PRO Express software (Media Cybernetics, Inc.) were used for recording experimental data into image sequence format.

D. Image processing and analyzing

A set of over 50 contiguous image frames was captured at each location under high exposure time of 600 ms or 3 s. The IMAGEJ software (Version 1.45 s, NIH) was used to stack and Z project the obtained discrete image frames, making a real composite image out of all these frames. The fluorescence intensity across the microchannel width was also measured by applying the "Plot Profile" function of this software. As an important prerequisite to ensuring measurement accuracy, the microchannel walls in a dark fluorescence spectrum were precisely determined by stacking with each image a bright-field image of the same position.

IV. RESULTS AND DISCUSSION

A. Flow rate regulation mechanism

As the sole controllable operating parameter in passive inertial manipulation, the driving flow rate is directly responsible for the regulation of particle focusing dynamics. To precisely control the transverse focusing positions of particles, it is of great importance to understand the flow rate regulation mechanism. Therefore, we separately investigated the focusing behavior of two particles with significantly different sizes ($5\text{ }\mu\text{m}$ and $10\text{ }\mu\text{m}$ diameters) at the outlet region under various flow rates ($De = 0.86 \sim 15.53$). Fig. 2(a) shows the fluorescent stream images categorized in terms of flow rate and particle size. We found that the occurrence of lateral migration was distinctly observed under all the tested conditions, and that the flow rate effects on focusing behavior are mainly embodied in the changes of the particle stream width and focusing position. Moreover, a new instability phenomenon (double focusing positions) was

FIG. 2. (a) Fluorescent stream images illustrating the focusing behaviors of two particles in the outlet region at different flow rates. (b) Fluorescence intensity distributions across the channel width at flow rates where the instability occurs. (c) Lateral focusing position as a function of De ; and gap between the main and minor focusing positions of 10 μm particles.

discovered in the focusing behaviors of large particles. The instability phenomenon reported in previous studies has either been in the form of multiple particle trains which appeared as a result of the high particle volume fraction of the suspension,²⁰ or particle defocusing which emerged due to the strong Dean mixing effect at high flow rates.^{27,33} In this work, however, we found that the instability phenomenon occurs when the applied particle concentration is extremely low and, more importantly, that the unstable particles resume single-position focusing as the flow rate further increases. We next characterized and plotted the fluorescence intensity distributions across the channel width in the flow rate range ($De = 4.31 \sim 11.21$) to illustrate the dynamics of this newly found instability phenomenon (see Fig. 2(b)). The results show that with the main focusing position moving towards the inner wall, a fixed minor focusing position emerges between the wall and the main position, and that the main and minor positions meet and merge as the flow rate increases to be higher than $De = 10.35$. By analyzing the variation of the peak intensity, we also noticed that the decrease of stream width and intensity in the main focusing position is accompanied by the increase of intensity in the minor one. These results indicate that the new focusing instability actually is a stepwise particle transferring from the main focusing position to the potentially more stable minor one.

In attempts to probe into the effects of flow rate on focusing position, we measured the peak position of the intensity profile at different flow rates and plotted the acquired position values as a function of De (see Fig. 2(c)). From this figure, a complete picture of the focusing position shifting process is unfolded for clarifying the ambiguous and incomplete experimental results obtained from different flow rate ranges (i.e., flow regimes with different dominant forces) in past research.^{29,31} Specifically, we found that with increasing De , the focusing position firstly moves towards the inner wall and then remains predominantly constant (for 10 μm particles) or shifts away from the wall (for 5 μm particles). In addition, the focusing position interchange of two differently sized particles was discovered for the first time. We found that large particles are focused closer to the inner wall than small ones at high flow rates, whereas the focusing position of small particles is much closer to the wall at low flow rates (see Fig. 2(c)). This finding argues against the common view that larger particles equilibrate closer to the inner wall than smaller particles.^{29,36,37} Therefore, it is suggested that the flow rate effect on the relative positions of different particles be considered for the design of outlet systems and following Lab-on-a-chip units in spiral inertial separators.

We next duplicated this experiment using hybrid particles with the same concentration to validate and further investigate this interesting position interchange process. The captured

FIG. 3. (a) Fluorescent stream images captured at various flow rates and the corresponding fluorescence intensities across the channel width, illustrating the interchange process of focusing positions of the two particles. Red solid arrow indicates the focusing position of 5 μm particles (the fluorescent streams of these particles are artificially pseudo-colored in red for enhanced contrast); green solid arrow indicates the main focusing position of 10 μm particles; and green hollow arrow indicates the minor position of 10 μm particles. (b) Focusing ratio as a function of De . The inset (i) shows the Gaussian fitting of the fluorescence intensity distribution of 10 μm particle stream at $De = 4.31$.

fluorescent stream images and corresponding fluorescence intensities across the channel width are shown in Fig. 3(a). This figure clearly presents the detailed interchange process, in which the discussed instability phenomenon of large particles also appears. We found the main focusing position of large particles is almost coincident with that of small particles at $De = 6.04 \sim 7.76$, which means most of the two particles coexist in the same focused stream. Thus, it can be concluded that once instability is avoided in this flow rate range, particles with different sizes will be well focused at the same position. This finding serves as an important supplement to the existing mechanism that stresses the strong size dependence of inertial focusing^{33,38,39} and provides a potential scheme for sheathless ordering of particle specimens with a broad size distribution. Spacing between the two particle streams is another important parameter in inertial focusing based particle separation. Larger spacing improves the purity of the collected samples and permits convenient design of outlet collecting systems. As shown in Figs. 2(c) and 3(a), high flow rate provides much ampler spacing and higher throughput for efficient particle separation.

To investigate the effects of flow rate on focusing quality, we calculated the full width at half maximum (FWHM) by conducting Gaussian fitting of the raw intensity distribution and then defined a dimensionless parameter named focusing ratio obtained by dividing the FWHM by particle diameter. Smaller focusing ratio indicates better focusing quality. Detailed focusing ratios of the two particles at different flow rates were plotted as a function of De in Fig. 3(b). A comparison of the tendencies of the two curves demonstrates that except for the defocusing caused by the instability, the large particles generally show better focusing quality, agreeing with the experimental observations in past research.²⁹ Due to its capability of significantly weakening the focusing performance, focusing instability should be avoided in applications where measurement accuracy is largely determined by focusing quality (e.g., microflow cytometry and particle encapsulation). We also compared these curves with the position curves illustrated in Fig. 2(c), finding that flow rate has a greater impact on focusing position than on focusing ratio. Therefore, after the required particle stream width is achieved, flow rate can be used to control particle position for successful separation.

B. Five-stage process model

To deepen the understanding of the particle focusing process with increasing flow rate, we estimated the competition effects between inertial lift force and Dean drag force and then developed a novel five-stage process model based on the synthesis of our experimental findings (see Fig. 4(a)). The developed model can also be generalized to other curved channel

FIG. 4. (a) Schematic of the developed five-stage process model. (b) Detailed stage distribution of the two particles.

geometries. The detailed description and experimental validation of each stage are provided as follows:

Stage One: Formation of the focused stream under the dominant inertial lift force. In this stage, where Dean flow effect is almost negligible due to low flow rates,^{33,40} flowing particles gradually migrate to form a tightly focused particle stream under the dominant inertial lift force. The increase of flow rate results in a distinct decline of the focusing ratio and rapid inward-moving of the lateral position. In our work, we found that 5 μm particles are focused into a stream with a focusing ratio of ~ 2 at $De = 3.45$, while 10 μm particles achieve a focusing ratio of ~ 1 at $De = 1.73$. The large particle focuses into a stable stream at much lower flow rates than the small one due to the fact that inertial lift force is in proportion to a_p^4 . More details on how particles gradually move towards the focusing positions with the increase in flow rate and the detailed mechanism were reported in our previous work.³¹

Stage Two: Inward moving of the focusing position under the dominant inertial lift force. In this stage, the focusing position of the stably focused particles keeps moving towards the inner wall as the flow rate increases. This conforms well to the shifting law deduced from studies of particle focusing behaviors in straight channels,^{18,41,42} indicating that inertial lift force is the dominant force that causes this inward moving. Results of our experiment show that the particle dynamics in the flow rate range of $De = 3.45 \sim 5.18$ (for small particles) or $De = 1.73 \sim 10.35$ (for large particles) reflect the characteristics of particle behavior in this stage. In addition, since Dean drag force strengthens much faster than inertial lift force as the flow rate further increases,^{29,31} the magnitude of the Dean drag force will get closer to that of the inertial lift force. In that case, the focusing position of large particles will be modified into a more stable one due to the alternation of the dominant force (the occurrence of focusing instability, see Fig. 2(a)). However, the transfer process of small particles is not prominent since they experience much weaker forces.

Stage Three: Transition process under the competition of inertial lift force and Dean drag force. In this stage, where the effects of the two forces on particle lateral migration happen to be almost identical, the focusing position stabilizes at the position extremely near the inner wall. In this work, the large particle is at this stage when the flow rate is in the range of $De = 10.35 \sim 15.53$; and the small particle has a transition stage of a much narrower range ($De = 5.18 \sim 6.04$). The difference in the flow rate range indicates that the Dean drag force acting on the small particle increases much faster than that on the large particle.

Stage Four: Outward moving of the focusing position under the dominant Dean drag force. As the flow rate further increases, the Dean drag force surpasses the inertial lift force and entrains particles away from the inner wall. This stage is validated only in our experimental observation of small particles but not in that of large particles, which implies the strong dominance of the inertial lift force at all flow rates tested in the case of large particles. One of the explanations suggested in previous studies for this difference is that inertial lift force is strongly dependent on particle diameter ($F_L \propto d_p^4$ vs. $F_D \propto a_p$)²⁹ so that the lift force dominant regime of large particles is much wider than that of small particles.

Stage Five: Defocusing induced by the Dean mixing effect. In this stage, the Dean drag force becomes more dominant with further increasing flow rate and thus causes particles to move along the Dean vortices.³³ This is the so-called particle defocusing effect. Commonly used in efficient sample mixing applications,^{40,43} this effect should, however, be avoided for precise particle manipulation. In our work, due to the leakage problem of PDMS devices at high pressure, no obvious particle defocusing was observed. Nevertheless, a slight increase in the focusing ratio of small particles was found at the end of stage Four (see Fig. 3(b)). This defocusing effect may be enhanced by increasing the flow rate.

After elucidating our novel process model, we then plotted the detailed stage distribution of the two particles used in this work (see Fig. 4(b)). From this figure, we found that particles with different sizes can be in different stages at the same flow rate. This may lead to interesting findings in the studies concerning the manipulation of multi-particles (e.g., the focusing position interchange and co-focusing of different particles found in this work).

V. VALIDATION AND APPLICATION

In order to validate the explored mechanisms and evaluate the functionality of the fabricated device, we examined the focusing behaviors and enrichment performance of zymosan bioparticles using our device. Zymosan is a polysaccharide obtained from the cell wall of *Saccharomyces cerevisiae* that has been regarded as an important microbial model for studying immune responses. Preparation of zymosan particle samples with homogeneous sizes and controllable concentrations is especially critical for improving the reliability of immunology studies. In addition, the irregular shape, polydisperse size distribution (average diameter at $3 \sim 4 \mu\text{m}$, containing submicron debris) and deformable feature of such particles make them the ideal object choice for investigating particle dynamics.

We obtained fluorescent stream images near the bifurcated outlets by injecting suspensions into the channel at various flow rates and measured the corresponding fluorescence intensities across the channel. Fig. 5(a) shows a particle-free region near the inner wall is generated while intensity distribution across the stream keeps uniform at the starting flow rate of $De = 0.86$. With increasing flow rate the focused particles (above the critical cutoff size, i.e., $\sim 3.5 \mu\text{m}$) form a stream and then gradually migrate towards the inner wall; while the smaller unfocused particles located in the outer region move to form a particle-free region, agreeing with the phenomenon reported in previous research.³¹ As the flow rate further increases to $De \geq 6.90$, particles behave in the opposite manner. Mechanism accounting for this result is that the dominant Dean drag force induced by high flow rates drags the focused particles away from the inner wall and, at the same time, enhances the mixing efficiency of the unfocused particles.³¹ Behaviors of the focused particles are entirely consistent with the above-explored mechanisms (e.g., the shifts in focusing position). According to the established five-stage process model, we found the critical flow rate for the alternation in the dominant force acting on these bioparticles is $\sim 200 \mu\text{l/min}$ ($De = 3.45$). And the best focusing quality is obtained at the flow rate range of $De = 3.45 \sim 6.90$.

Finally, we performed an enrichment operation at the flow rate of $De = 12.08$ to achieve high throughput at the $\sim \text{ml/min}$ scale. The throughput can be further improved by appropriately increasing the particle concentration or flow rate. Concentrations of the specimens collected from the inner and outer outlets were compared with that of an unprocessed specimen (dark-field microscope images of these specimens are shown in Fig. 5(b)). Quantitative enrichment

FIG. 5. (a) Schematic of inertial migration of zymosan bioparticles, fluorescent stream images captured near the bifurcated outlets and curves illustrating fluorescence intensity distribution across the channel width. The red line in the stream image of $De = 0.86$ indicates the specific position for measuring fluorescence intensity. (b) Dark-field microscope images of specimens sampled from the initial suspension and of collected samples after the enrichment operation at the flow rate of $De = 12.08$. The inset in sample three is an enlarged image showing the debris collected from the outer outlet. 2 μm polystyrene particles were artificially added into the initial suspension for size reference.

results from different sampling paradigms demonstrate that over $\sim 99\%$ of the focused particles are exported from the inner outlet while most of the small debris is collected by the outer outlet. Overall, target particles are enriched by a factor of over 2 in this single-stage system. Higher purity and concentration can be achieved if multi-stage cascaded or multilayer stacked systems are applied.

VI. CONCLUSIONS

In this work, we design and fabricate a five-loop spiral channel device with a small footprint and then perform a quantitative characterization of the inertial focusing dynamics in this spiral channel. We investigate the focusing behaviors of particles with two significantly different sizes near the outlets at various flow rates. A novel instability phenomenon with large particles is observed when the fluorescent stream images of the two particles are compared, and its occurrence process is characterized. The effects of flow rate on particle focusing position and ratio are quantified. A complete picture of the focusing position shifting process is unfolded for clarifying the ambiguous and incomplete reported results obtained from different flow regimes with different dominant forces. Focusing position interchange and co-focusing of different particles in the same stream are, for the first time, observed and validated, which offers an important supplement to the existing mechanisms. Based on the synthesis of our new findings, we establish a novel five-stage model to better understand the particle focusing process with increasing flow rate and the competition mechanism between inertial lift force and Dean drag force. The detailed stage distribution of the two particles is presented. The understanding of the flow rate regulation mechanism enables precise control of the transverse focusing positions of particles. Finally, we investigate the focusing behaviors and enrichment performances of zymosan particles which are characterized by irregular shapes, polydisperse size distribution, and

deformability. The explored focusing mechanisms of inertial particle flow and functionality of the fabricated spiral device are validated. The results show that this spiral inertial microfluidic device is applicable to high-throughput particle ordering, enrichment, and purification.

ACKNOWLEDGMENTS

This research work was supported by the Major Program of the National Natural Science Foundation of China (91023024), the National Basic Research Program of China (2011CB707601), the Specialized Research Fund for the Doctoral Program of Higher Education (20110092110003), the Natural Science Foundation of Jiangsu Province (BK2011336), and the Jiangsu Graduate Innovative Research Program (CXZZ_0138).

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