

High-Throughput Submicron Particle Separation Using Cascaded Inertial Microfluidics

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Abstract

Extracellular vesicles serve as promising biomarkers and therapeutic carriers; however, no isolation protocol currently achieves both high recovery rates and high specificity. Inertial microfluidics offers significantly higher throughput than alternative methods, although the focusing capability diminishes for particles smaller than approximately 1 μm . In this study, we introduce a cascaded spiral platform comprising two serially arranged devices designed to resolve a broader size range than a single device could achieve independently. Both stages were modeled using COMSOL Multiphysics[®], where the velocity field within the spiral microchannel was calculated assuming stationary laminar flow, followed by a time-dependent simulation tracing particles ranging from 10 μm to 100 nm. The first stage, featuring a cross-sectional area of $250 \times 50 \mu\text{m}$, effectively removes 10 μm particles, whereas the second stage, with an 8 μm height, separates 1 μm particles from 100 nm particles. The initial stage was fabricated accordingly, and experimental validation using fluorescent polystyrene beads confirmed the predicted separation: 10 μm polystyrene beads exited through the inner outlet, and 1 μm polystyrene beads through the outer outlet.

Keywords: inertial microfluidics; spiral microchannel; Dean flow; extracellular vesicles; particle tracing; COMSOL Multiphysics[®]

Introduction

Extracellular vesicles (EVs) are heterogeneous lipid bilayer particles secreted and internalized by nearly all types of cells [1]. These lipid vesicles are classified by size, origin, biogenesis, surface markers, and clinical significance. Small EVs (SEVs, 30-200 nm) are produced inside the endosomal system, whereas large EVs (LEVs, 200 - 1000 nm) are formed directly from the plasma membrane [2]. Due to their abundance of biomolecules, EVs have attracted interest as biomarkers for liquid biopsies [1]. Furthermore, as mediators of intercellular communication, EVs play crucial roles in many physiological and pathological processes, including intercellular signaling, tissue development, and cancer metastasis [1]. Over the past decade, EVs have been extensively studied to clarify their biological functions and to harness them as non-invasive biomarkers for the early diagnosis of cancer and other inflammatory diseases [3]. Beyond diagnostics, EVs are emerging as powerful therapeutic tools for pathological conditions, such as neurological diseases and cancers [4]. Their ability to cross biological barriers (e.g., the blood-brain barrier, the gut, and dense tumors) enables them to support natural repair processes or serve as engineered nanocarriers for therapeutic agents and

tissue-specific ligands for targeted drug delivery [5,6].

However, reliable isolation of EVs from complex bioliquid remains an unresolved obstacle. The International Society of Extracellular Vesicles has yet to identify a protocol offering both high recovery and high specificity [6]. Conventional isolation techniques, such as Ultracentrifugation (UC), can process relatively large sample volumes and are available in most medical research facilities. However, UC requires a very high acceleration ($>10^5$ g), which may rupture EVs, thereby resulting in poor EV recovery [2]. Microfluidic technologies offer several advantages, including cost-effectiveness, reduced consumption of biological samples and reagents, and portability. Passive inertial devices, of which the spiral microchannel is one type, operate at much higher flow rates and offer high throughput, and separation relies on inertial lift and Dean forces for particle sorting. However, inertial microfluidics struggles to separate submicron particles [7]. Zhou et al. 2019 presented a ca. 30 mm long wavy microchannel for EVs separation using elasto-inertial sorting by formation of Dean vortices. The authors have shown that focusing on 1 μm , 500 nm, and 300 nm polystyrene particles. However, heterogeneous samples with polydisperse particle populations were not investigated. They have also

used pre-treated samples via centrifugation to remove large cells and apoptotic bodies [8]. The proposed system combines two spiral inertial microfluidic devices in series. The first-stage spiral removes large particles and cellular debris, and the second-stage spiral separates micron-sized particles ($\sim 1 \mu\text{m}$) from submicron particles ($\sim 100 \text{ nm}$). This cascade architecture enriches nanoscale particles progressively while maintaining high throughput and label-free operation. Both devices were simulated individually in COMSOL Multiphysics® to optimize the channel geometry and flow conditions required by each stage. Further, the first-stage spiral was then fabricated and experimentally validated with fluorescent polystyrene particles and cells. The second-stage spiral was characterized by simulation alone, which also gives the fabrication tolerance it demands.

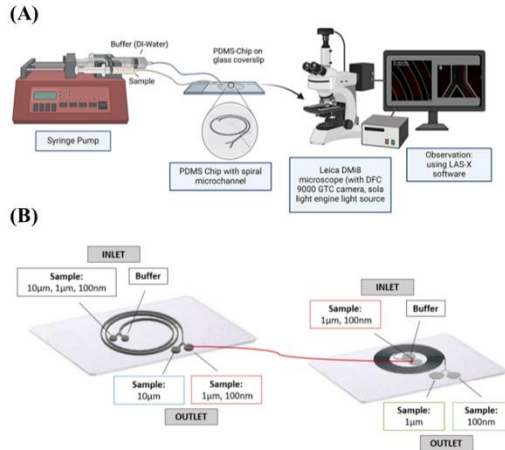


Figure 1. Cascaded spiral platform. Schematic of two spiral devices in series. The first device receives a sample of $10 \mu\text{m}$, $1 \mu\text{m}$, and 100 nm particles at one inlet and a buffer stream at the other, and divides them between an outlet carrying the $10 \mu\text{m}$ fraction and an outlet carrying the $1 \mu\text{m}$ and 100 nm fractions. The second device receives that combined submicron fraction and separates the $1 \mu\text{m}$ from the 100 nm particles at its two outlets. [Created with BioRender.com].

Theory

The Reynolds number, the ratio of inertial to viscous forces, characterizes the flow regime of a fluidic system, as shown in equation (1),

$$Re = \frac{\rho U_f D_h}{\mu} \quad (1)$$

where ρ is the fluid density, U_f the mean fluid velocity, μ the dynamic viscosity and D_h the hydraulic diameter [9]. The hydraulic diameter for a rectangular cross-section is given by equation (2),

$$D_h = \frac{2wh}{w + h} \quad (2)$$

where w is the channel width and h the channel height [9]. While most microfluidic devices run below $Re = 1$, where viscous forces dominate, and

inertia can be neglected [9], spiral inertial devices operate at far higher flow speeds, so inertial forces become significant [10]. Flow stays laminar in either case, because the transition to turbulence near $Re = 2000$ is never reached at micron-sized channel dimensions.

Solving the Navier-Stokes equations for steady, fully developed flow yields the parabolic Hagen-Poiseuille profile, where velocity is highest at the center of the channel and lowest at the walls [10]. Within a straight channel, two inertial lift forces act on a suspended particle. Shear-gradient lift follows from the parabolic profile and drives the particle toward the wall, while wall-induced lift pushes it back as the wall is approached [10,11]. Their sum is the net inertial lift, as shown in equation (3),

$$F_L = \rho G^2 C_L a_p^4 \quad (3)$$

where G is the shear rate, C_L the lift coefficient and a_p the particle diameter [12]. The fourth-power dependence makes the force strongly size-selective, and in a straight square channel, it leaves four equilibrium positions, one at the center of each wall, the corners being excluded because wall-induced lift acts there from two walls at once.

In curved channels, a third force becomes relevant. Centrifugal effects push the fluid at the channel center toward the outer wall, and because the fluid is incompressible, it returns along the top and bottom walls, establishing two counter-rotating vortices in the cross-section, as shown by the looped arrows in Figure 2 [10]. This secondary flow is called Dean flow, and its strength is quantified by the Dean number, as shown in equation (4),

$$De = Re \sqrt{\frac{D_h}{2R}} \quad (4)$$

where R is the mean radius of curvature [9,10]. Dean number rises with flow speed and channel size and falls with radius. Assuming Stokes drag and estimating the mean Dean velocity as $U_{De} = 1.84 \times 10^{-4} De^{1.63}$ [13], the force the secondary flow exerts on a particle is given by equation (5),

$$F_D = 5.4 \times 10^{-4} \pi \mu De^{1.63} a_p \quad (5)$$

which is linear in particle diameter [10,13]. The Dean force reduces the four equilibrium positions of the straight channel to one. At the outer, top and bottom walls the inertial lift and the Dean force act in the same direction, whereas at the inner wall they oppose one another, leaving a single stable position there.

Because inertial lift scales with the fourth power of particle diameter and the Dean force only with the first, large and small particles behave differently in the same flow field. Whether a particle ever reaches the equilibrium position depends on its size relative to the channel. Focusing requires $a_p/D_h \geq 0.07$ [14, 15]. Figure 2 follows both populations along a spiral.

At the inlet (A) the sample enters at the outer wall, so large and small particles start together. Partway along (B) the vortices have carried both toward the inner wall. By the outlet (C) the large particles have collected at the inner wall and hold it, while the small ones, having no equilibrium to reach, have been carried back across the cross-section. That return is what separation exploits, and it is transient. The small particles will cross again if the channel continues, so the outlet must fall after they have completed one Dean cycle, from the outer wall to the inner and back. Because D_h alone sets the ratio, the channel cross-section decides which particles focus and which circulate.

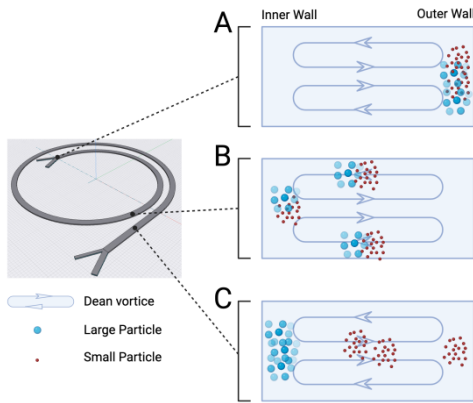


Figure 2. Particle positions across the channel cross-section at three points along a spiral, marked on the device at left. In each panel the inner wall is at the left and the outer wall at the right. Blue circles denote large particles, dark red dots small particles, and the looped arrows the counter-rotating Dean vortices. (A) At the inlet. (B) Partway along the spiral. (C) At the outlet. [Created with BioRender.com].

Governing Equations and Numerical Model

All simulations were carried out in COMSOL Multiphysics®, version 6.0 for the first-stage device and version 6.3 for the second, with the Particle Tracing Module alongside the core package. Channel geometries were drawn in Shapr3D and imported as STEP files. Two physics interfaces were used, Laminar Flow (spf) and Particle Tracing for Fluid Flow (fpt), both computed in three dimensions. Each design was solved in two steps. A stationary study of spf produced the velocity field, and a time-dependent study of fpt then tracked particles through that field.

The fluid is treated as incompressible, with constant density and viscosity. Laminar Flow solves the stationary Navier-Stokes and continuity equations, as shown in equations (6) and (7),

$$\rho(\mathbf{u} \cdot \nabla)\mathbf{u} = \nabla \cdot [-p\mathbf{I} + \mathbf{K}] + \mathbf{F} \quad (6)$$

$$\rho \nabla \cdot \mathbf{u} = 0 \quad (7)$$

where $\mathbf{u}$ is the velocity field, p the pressure, $\mathbf{I}$ the identity tensor, $\mathbf{K}$ the viscous stress tensor and $\mathbf{F}$ a volume force. Particles are advanced in a Newtonian formulation, as shown in equation (8),

$$\frac{d}{dt} \left(m_p \frac{d\mathbf{q}}{dt} \right) = \mathbf{F}_t \quad (8)$$

where m_p is the particle mass, $\mathbf{q}$ its position and $\mathbf{F}_t$ the sum of the forces acting on it. Two contributions to $\mathbf{F}_t$ were enabled. Drag follows Stokes' law, evaluated against the computed velocity field rather than the analytical estimate of equation (5). Lift uses the wall-induced formulation, which is modulated by two wall-distance functions and scales with the fourth power of the particle radius; equation (3) expresses the same dependence in terms of diameter. Water at 20 °C was used throughout, with a density of 1000 kg/m³ and a dynamic viscosity of 0.001 Pa·s. Both the sample and the sheath inlet were assigned a fully developed flow condition constrained by volumetric flow rate, and the outlet was held at a constant pressure. A no-slip condition was applied to every wall, and velocity and pressure were discretized with second-order (P2) elements. A physics-controlled mesh was used, with the Laminar Flow and Particle Tracing for Fluid Flow interfaces as contributors, generating tetrahedral elements with boundary-layer prisms along the channel walls and corner refinement. The element size was set to Coarser for the first-stage device and Extra coarse for the second-stage designs due to computational constraints.

Particles were released at the outer inlet from random positions across the boundary, set to bounce on contact with a wall and freeze upon reaching an outlet, so that their final positions could be counted. The first-stage was simulated with 10 μm, 1 μm, and 100 nm particles at a density of 1050 kg/m³, the second-stage device was optimized with 1 μm and 100 nm particles at 1000 kg/m³. Trajectories were stored at 0.1 ms intervals over 1 s for the first stage and at 1 μs intervals over 5 ms for the second.

Results and Discussion

The first-stage has to remove large particles and debris while passing everything smaller particles. The hydraulic diameter and the number of turns are essential parameters for optimizing the design, as they influence particle focusing and particle trajectories, respectively. Three cross-sections were compared (Table 1). At 500 × 100 μm ($D_h = 167$ μm), both particle populations left together through the outer outlet, whereas narrowing the channel to 250 × 50 μm ($D_h = 83.3$ μm) separated 10 μm from the populations 1 μm, and 100 nm. Figure 3A shows the trajectories, with the 10 μm particles focused at the inner wall and the 1 μm and 100 nm particles carried by the Dean vortices to the outer wall.

Table 1: First-stage channel geometries tested. All simulations used a sheath flow rate of 1000 $\mu\text{L}/\text{min}$ and a sample flow rate of 100 $\mu\text{L}/\text{min}$.

$w \times h$ (μm)	D_h (μm)	a_p/D_h , 10 μm	a_p/D_h , 1 μm	a_p/D_h , 100 nm	Outcome
500×100	167	0.060	0.0059	$5.9\text{e-}4$	All populations to the outer outlet
250×50	83.3	0.12	0.012	0.0012	Separation of 10 μm from 1 μm and 100 nm, in simulation and experiment (tested with 10 μm and 1 μm)
100×50	66.7	0.15	0.015	0.0015	The fabricated device could not sustain the required flow speeds

The ratio a_p/D_h accounts for the difference, since at a hydraulic diameter of 167 μm , a 10 μm particle reaches only 0.060, below the threshold of 0.07. None of any population finds a stable equilibrium, and all circulate with the Dean vortices. At 83.3 μm the 10 μm particle reaches 0.12 and focuses at the inner wall, while the 1 μm and 100 nm particles reaches at 0.012 and 0.0012. One flow field then separates the 10 μm from the 1 μm and 100 nm. Channel length was varied next. In the $250 \times 50 \mu\text{m}$ channel, a separation of 10 μm from 1 μm and 100 nm was reached after 1.5 turns, as shown in Figure 3A. Two geometries differing by a single quarter turn showed how narrow that window is: in one, the 1 μm and 100 nm particles exit through the outer outlet; in the other, they do not. The Dean cycle accounts for this: sub-threshold particles never settle, so their position depends on how far they have traveled, and the outlet must fall where the small particles sit on the outer side of the cycle.

The simulations predict separation, and the next question was whether a physical device behaves the same way. A spiral channel with dimensions of $250 \times 50 \mu\text{m}$ was fabricated using standard soft lithography techniques and imaged at the outlet with 10 μm and 1 μm fluorescent polystyrene beads (device was not tested with 100 nm due to unavailability at that time). As Figure 3B shows, the 10 μm polystyrene beads exit through the inner outlet and the 1 μm polystyrene beads through the outer outlet, so the separation predicted by the model

also occurs in experimental data. The narrower $100 \times 50 \mu\text{m}$ channel was fabricated as well, but the soft PDMS deformed at the flow rates required for inertial focusing, altering the geometry and causing the focusing to fail.

The two do not agree in every respect. Separation was achieved only after 1.5 turns, a full turn later than the model predicted. The discrepancy is attributed to fabrication tolerances, as the measured channel height was nearly 10 μm above the 50 μm targets; the same applies to the other channel parameters and the flow speed. Since a quarter turn is enough to change the outcome, deviations of this size are sufficient to explain the shift.

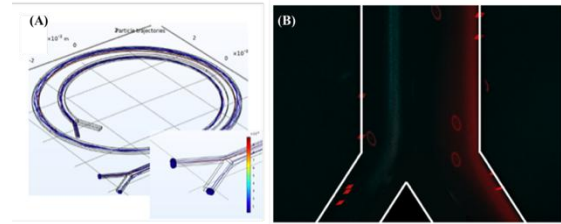


Figure 3. First-stage device, $250 \times 50 \mu\text{m}$ cross-section. (A) Simulated particle trajectories, with 10 μm particles in red and 1 μm particles in blue; the inset enlarges the outlet junction. (B) Fluorescence micrograph of the outlet of the fabricated device, channel walls outlined in white, showing cyan 10 μm polystyrene beads and red 1 μm polystyrene beads.

The first-stage separates cells and debris from everything smaller, but the submicron population leaves through the outer outlet unsorted. To achieve a resolution difference of 1 μm from 100 nm, a hydraulic diameter of approximately 14 μm is needed. This would involve a channel width of several tens of micrometers and a height of about 8 μm , which is too small for a 10 μm cell to pass through. The second stage, therefore, has to be a separate device downstream of the first (Figure 1). Five designs that met that requirement were simulated, with hydraulic diameters ranging from 11.42 to 13.79 μm . That range places the 1 μm particles between 0.072 and 0.087, just above the threshold, while the 100 nm particles sit near 0.008. Two of the five are shown in Figure 4.

Figure 4A shows the trajectories for the $50 \times 8 \mu\text{m}$ device, which runs 2.5 loops. The 1 μm particles hold the inner wall, while the 100 nm particles travel in the center and toward the outer wall. The $30 \times 8 \mu\text{m}$ device of Figure 4B completes the same task in a single loop, at a higher Dean number. Its 1 μm particles keep a tight trajectory along the inner wall, and the 100 nm particles form clearly defined streamlines at the outer wall. All five designs separated the two populations in the simulation.

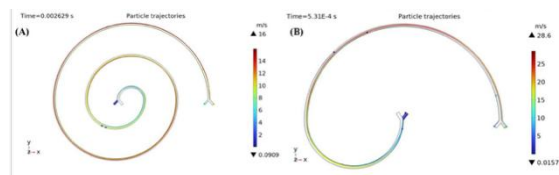


Figure 4. Simulated particle trajectories in two second-stage designs, viewed from above and colored by flow velocity in m/s. (A) $50 \times 8 \mu\text{m}$ channel over 2.5 loops. (B) $30 \times 8 \mu\text{m}$ channel over one loop. Both at a sheath flow rate of $200 \mu\text{L}/\text{min}$ and a sample flow rate of $20 \mu\text{L}/\text{min}$.

Since fabricating an $8 \mu\text{m}$ -high channel to exact specifications is challenging, the sensitivity of the focus to that dimension was directly assessed. Each design was re-simulated with the height varied between 7.5 and $9 \mu\text{m}$, while all other parameters were held constant. The dimensionless parameters barely move over that range, with a_p/D_h shifting by about 0.01 and the Dean numbers by a few percent, but the trajectories do not follow. Reducing the channel height by half a micrometer is enough to loosen the 100 nm streamlines, which tend to spread rather than follow them precisely to the outer outlet. As a result, the separation performance deteriorates gradually rather than failing suddenly. This means that the channel height must be maintained more tightly than what the dimensionless parameters alone would indicate.

Conclusions

In this work, we showed that two spiral devices in series can extend inertial separation from the micron range into the submicron, with the same criterion governing both stages. Because $a_p/D_h \geq 0.07$ ties the cut-off to the hydraulic diameter, a $250 \times 50 \mu\text{m}$ channel separates $10 \mu\text{m}$ particles from everything smaller, while a channel a few tens of micrometers wide and $8 \mu\text{m}$ high separates $1 \mu\text{m}$ particles from 100 nm ones. Simulations define the geometry and flow conditions for both stages. In the first-stage, they were tested experimentally using fluorescent polystyrene beads, and stained cells that followed the simulated paths, although separation occurred one turn later than predicted. A height sweep on the second-stage showed that its submicron streamlines loosen once the channel height falls by half a micrometer below target, which defines the tolerance the lithography must meet. Experimental verification of the second-stage is the next step, first with polystyrene beads at $1 \mu\text{m}$ and 100 nm and then with a biological sample. Coupling the two stages is the other open task, since they have so far been modeled independently. Matching the outer outlet of the first to the sample inlet of the second, and simulating the pair as a single system, would complete the platform.

Reliable submicron separation would ease the isolation bottleneck that extracellular vesicle diagnostics and therapeutics both start from. A passive cascade provides a higher throughput than active microfluidic methods, all while avoiding the forces and sample volumes required by ultracentrifugation.

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