A microfluidic-based hydrodynamic trap: design and implementation†

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We report an integrated microfluidic device for fine-scale manipulation and confinement of micro- and nanoscale particles in free-solution. Using this device, single particles are trapped in a stagnation point flow at the junction of two intersecting microchannels. The hydrodynamic trap is based on active flow control at a fluid stagnation point using an integrated on-chip valve in a monolithic PDMS-based microfluidic device. In this work, we characterize device design parameters enabling precise control of stagnation point position for efficient trap performance. The microfluidic-based hydrodynamic trap facilitates particle trapping using the sole action of fluid flow and provides a viable alternative to existing confinement and manipulation techniques based on electric, optical, magnetic or acoustic force fields. Overall, the hydrodynamic trap enables non-contact confinement of fluorescent and non-fluorescent particles for extended times and provides a new platform for fundamental studies in biology, biotechnology and materials science.

Introduction

Trapping and manipulation of single particles and molecules has enabled remarkable progress in many fields of science and engineering. Over the past several years, a diverse set of tools has been developed to manipulate particles in solution by direct control of their position and velocity. Particle manipulation techniques commonly rely on application of force fields, including optical, electric, magnetic, acoustic and hydrodynamic forces in order to confine micro- and nanoscale particles. Each manipulation method has advantages and limitations as a manipulation tool for living cells and biological systems, where perturbations need to be minimized for viability of biological systems.

Particle trapping using hydrodynamic forces was first demonstrated by G. I. Taylor in 1934. Taylor developed a “four-roll mill” apparatus to study dynamics and breakup of macroscopic, millimetre-sized oil droplets in linear viscous flows. Several years later, Bentley and Leal developed a computer-controlled version of the four-roll mill to study particle and droplet dynamics in flow and reported confinement of macroscopic droplets (typically 1 mm diameter) to within 0.5–1.0 mm of the trap center for shear rates up to 5 s⁻¹. Recently, a microfluidic version of the four-roll mill was reported by Hudson et al. and Lee et al., and these studies mainly focused on the generation of linear mixed flows with a well-defined flow characteristic ranging between simple shear flow and extensional flow in a microfluidic device. Using these devices, large carbon nanotube flocs (typically ~100 μm in size) were studied in viscous (500 cP) polymer solutions.

Recent efforts have focused on combining particle confinement and manipulation methods into integrated devices in order to capitalize on microfluidic technology. Several methods employ hydrodynamic forces for capturing and/or manipulating cells or particles and can be classified into two categories: contact-based and non-contact methods. Contact-based methods use fluid flow to immobilize and physically confine particles against microfabricated obstacles or channel walls, whereas non-contact methods rely on stagnation point flows, microvortices and microeddies. Contact-based methods are efficient in trapping large numbers of particles in an array format for high-throughput studies, however the ability for fine-scale manipulation of individual particles is limited. Non-contact particle confinement methods based on stagnation point flows can provide high resolution manipulation of single particles, though previous work has largely focused on trapping macroscopic particles in aqueous solutions.

Microfluidic-based stagnation point flows have also been used to study the dynamics of single DNA molecules. DNA dynamics were characterized in a passive stagnation point flow without the active feedback control and in a stagnation point flow with the human-facilitated manual feedback control. However, these microfluidic-based approaches are not practical for precise or long-term confinement of particles in solution due to finite...
residence times in passive flows, where particles ultimately escape into the flowing solution.

Here, we report a microfluidic-based hydrodynamic trap for free-solution particle confinement and manipulation. Hydrodynamic trapping is based on the sole action of fluid flow in a microfluidic device. The hydrodynamic trap is automated and facilitates high resolution, non-contact confinement of micro- and nanoscale particles in a stagnation point flow. The underlying principle of the hydrodynamic trap is the active feedback control of the fluid flow in order to maintain particle center-of-mass position at the trap center. The hydrodynamic trap consists of a simple integrated, two-layer microfluidic device and achieves particle trapping without the need for complex device synthesis or coupling of external fields (optical, electric, or magnetic) into the device.

In this work, we characterize key microfluidic device design parameters for efficient trap performance. We systematically study hydrodynamic trap response as a function of device properties, including membrane valve thickness and stiffness, and channel dimensions. We develop theoretical models for the response of the fluid stagnation point as a function of valve cross-sectional area and applied pressure to the membrane valve, and in all cases, we validate theoretical expressions with experimental measurements. Overall, proper understanding of trap response is essential for engineering and implementing microfluidic-based hydrodynamic traps.

Hydrodynamic trapping: mechanism and control

The hydrodynamic trap is based on the active control of a stagnation point flow generated at a cross-slot junction in a PDMS-based microfluidic device (Fig. 1). Two opposing laminar streams converge at the cross-slot junction and exit through perpendicular outlet channels (Fig. 1c), thereby creating a planar extensional flow, which is a two-dimensional flow containing a fluid stagnation point (zero-velocity point). Planar extensional flows consist of purely extensional and compressional components with no rotational flow character. In the vicinity of the microchannel junction, the fluid velocity is given by:

\[ \mathbf{u} = \dot{\varepsilon}(-x, y) \]  

where \( \mathbf{u} \) is the velocity vector, \( \dot{\varepsilon} \) is the strain rate and \((x, y)\) are the coordinates along the inlet (compressional) and outlet (extensional) directions, respectively, with the origin located at the stagnation point. In a planar extensional flow, the magnitude of the fluid velocity is proportional to the distance from the stagnation point along each component direction.

The key concept behind hydrodynamic trapping is active control of the stagnation point position, thereby enabling dynamic and precise control of the hydrodynamic force exerted on a particle by the fluid. In this way, the flow field is actively controlled to confine and maintain a particle at the fluid stagnation point, which allows for trapping and manipulation of particles in free-solution.

Using the hydrodynamic trap, single micro- and nanoscale particles are trapped at a predetermined target position (trap center) in the microchannel junction. Consider a freely suspended particle entering the cross-channel geometry in the vicinity of the stagnation point. Initially, the particle follows a pathline determined by the hyperbolic fluid streamlines (Fig. 1c). Upon activating the trap, the particle is confined at the trap center by successive iteration of the following experimental
steps: (i) the centroid position of the particle is determined by image acquisition and image analysis, (ii) a feedback controller determines an updated stagnation point position in order to exert a hydrodynamic force on the particle directing it toward the trap center, and (iii) the stagnation point is re-positioned and the particle moves toward the trap center. In this manner, the stagnation point position is continuously adjusted in order to “steer” a particle towards the trap center. After the particle reaches the trap center, the particle is confined at the stagnation point. In an ideal trap, a particle positioned precisely at the stagnation point achieves zero velocity at the trap center. However, micro- and nanoscale particles are subject to thermal fluctuations and may become displaced by Brownian motion or small perturbations in the flow field, thereby requiring active feedback control.

The stagnation point represents a point of minimum (maximum) flow potential along the inlet (outlet) streams. Therefore, a particle experiences an attractive potential toward the stagnation point along the inlet channel direction and a repulsive potential along the outlet direction with respect to the stagnation point. To achieve particle trapping, it is sufficient to manipulate the stagnation point position along the outflow axis.

In this way, the stagnation point is actively re-positioned such that the particle is situated between the trap center and the stagnation point, thereby yielding a hydrodynamic restoring force exerted on the particle in the direction of the trap center. Using this method, particles are confined in two-dimensions by implementing active feedback control in a single direction, corresponding to the outlet (extensional) flow direction in the microfluidic device. The stagnation point position is actively controlled along the outflow direction via an integrated, on-chip valve by adjusting the relative flow rates in the two outlet channels. In this work, we utilize a single membrane valve located on one of the outlet channels (Fig. 1) to effectively manipulate the stagnation point position along the outlet channels with high precision. Here, the on-chip valve is not used to gate fluid flow in a binary (on/off) fashion; rather, it functions as a metering valve for fine-scale adjustment of the relative flow rates in the outlet channels. Closing this valve, for instance, would increase the flow resistance and reduce the flow rate through the outlet channel, which consequently re-positions the stagnation point position towards the same outlet channel.

In this work, we implement a linear feedback controller for particle trapping. Using this control algorithm, the stagnation point is re-positioned to a distance linearly proportional to the displacement offset between the particle and trap center (ESI†). The hydrodynamic force exerted on a point particle is linearly proportional to fluid velocity and therefore linearly proportional to the distance between the particle and the stagnation point along the extensional axis (eqn (1)), which is a consequence of viscous-dominated laminar flow in the microchannels. The linearity in hydrodynamic force with particle displacement aids in implementing a simple linear feedback control algorithm for particle trapping.

**Experimental**

**Device design and fabrication**

We built the hydrodynamic trap by designing and fabricating a hybrid poly(dimethylsiloxane) (PDMS)/glass microfluidic device using standard multilayer soft-lithography techniques (Fig. 1a and b). The hydrodynamic trap is a two-layer microfluidic device consisting of two patterned layers in PDMS. A thin PDMS layer (fluidic layer) containing the flow channels (inlet, outlet, and sample microchannels) is sandwiched between a glass substrate and a thick PDMS layer (control layer). The control layer contains an elastomeric membrane valve, which consists of a pressurized microchannel positioned above one of the outlet channels. The fluidic layer consists of four buffer inlet channels, two outlet channels and a sample inlet channel. Two buffer inlet streams on each half of the device converge to form two opposing inlet streams, which meet at the cross-slot microchannel junction. The sample inlet stream is introduced through a separate port and is focused between two adjacent inlet buffer streams, thereby delivering particles to the center of the microchannel junction (trapping region). Typical channel dimensions range between 100 and 500 μm in width and 10 and 50 μm in height.

The elastomeric membrane valve is an essential component of the microfluidic-based hydrodynamic trap. The valve consists of a microchannel positioned above one of the outflow channels downstream of the cross-slot configuration and is separated from the flow channels by a thin (20–100 μm) elastomeric (PDMS) membrane. By applying pressure to the control layer microchannel (valve), the membrane is deflected downwards onto the flow channel, thereby changing the cross-sectional area and altering the flow resistance within the outlet stream situated beneath the valve. In this manner, the monolithic membrane valve serves as an on-chip metering valve capable of adjusting the relative flow rates in the outlet channels, which enables fine-scale control of the stagnation point position and facilitates particle confinement. In addition, a constriction is fabricated in the opposite outlet channel, which requires a constant offset pressure to be applied to the membrane valve in order to maintain the stagnation point in the center of the cross-slot. In this work, we demonstrate device operation using a single membrane valve in one of the outlet channels, thereby enabling particle confinement.

The fluidic and control layers are individually patterned in PDMS as two separate layers by replica molding. The molds for the two layers were prepared by spin coating a thin layer (10–50 μm) of negative photoresist (SU-8) onto silicon wafers (3″ diameter) and patterning with UV exposure using a high-resolution transparency film as a mask. The molds are developed using propylene glycol methyl ether acetate (PGMEA) followed by surface treatment with trichlorosilane vapor under vacuum to prevent the adhesion of cured PDMS. Next, the thin fluidic layer is obtained by spin coating the fluidic mold with PDMS at 20 : 1 (w/w) base : crosslinker ratio yielding a thickness of ~70 to 110 μm. Depending on the channel height (10–50 μm), spin coating results in a ~20 to 100 μm thick membrane between the control and fluidic layers. The control layer is formed by casting a thick layer (4–6 mm) of PDMS with 5 : 1 (w/w) base : crosslinker ratio on the corresponding control layer mold. Next, each PDMS layer was partially cured by baking at 70 °C for 30 minutes. The thick PDMS replica (control layer) is then peeled from the control mold, aligned and hermetically sealed onto the thin PDMS layer (fluidic layer) by baking together overnight at 70 °C to form a monolithic device. The PDMS replica containing the two device layers is peeled off the fluidic mold and access ports for the microchannels in both layers are punched out using
a blunt needle. Finally, the PDMS slab is bonded to a coverslip by plasma oxidation to yield a functional device.

**Experimental setup and trapping algorithm**

The experimental setup consists of the microfluidic device (hydrodynamic trap) mounted on the stage of an inverted microscope (Olympus IX71) equipped with a CCD camera for image acquisition and a 10× or 40× high numerical aperture objective lens for particle detection. A syringe pump (Harvard Apparatus) is used to deliver fluid into the device, and an electronic pressure regulator (Proportion Air) is used to actuate the membrane valve. Particles are trapped using an automated feedback control mechanism. A custom LabVIEW code executes the following steps in the feedback control algorithm: (1) capturing an image of the particles in the trapping region, (2) tracking and localizing the center-of-mass position of a “target” particle in the trapping region, (3) calculating the displacement offset between the particle and trap center and determination of the pressure required for the on-chip valve using a linear feedback controller, (4) signalling the pressure transducer to apply an updated pressure to the on-chip valve, which adjusts the stagnation point position to steer the trapped particle towards the trap center. In this work, the duration of one cycle of the feedback loop is 60–140 ms (7–15 Hz feedback loop rate), which efficiently confines particles at the trap center.

**Results and discussion**

**Particle trapping**

Using the microfluidic trap, we confined micro- and nanoscale particles (100 nm–15 µm diameter) for long timescales (minutes) in free-solution. In addition, we trapped both fluorescent and non-fluorescent beads and single cells (bacterial and mammalian) using the device. Fig. 2a shows the trajectory of a trapped particle (2.2 µm diameter fluorescent polystyrene bead) confined for nearly 5 minutes in the microchannel junction. Fig. 2b and c show the histogram of bead displacement from the trap center along the inlet and outlet channel directions, respectively. The trajectory of a trapped particle may be obtained by either using the centroid position data recorded by the LabVIEW code, or by tracking and localizing the trapped particle from the recorded movie of the trapping region.

Particles are trapped “on-demand” such that the user can continue to trap a “target” particle or simply release the trapped object by terminating the feedback controller and select a new target particle. Selection of a new “target” particle is accomplished using a manual or automated scheme based on particle properties (e.g., size and morphology). For example, particles matching a desired size and morphology criteria can be identified via custom pattern recognition and particle measurement algorithms in LabVIEW and subsequently confined at the trapping region for further observation and analysis. In addition, a trapped particle can be manipulated by translating the trap center position. In this case, the stagnation point position is adjusted to steer the particle and ultimately confine it at the new desired trap center. Using the feedback control algorithm, microscale particles are confined to within ±1 µm of the trap center along the inlet and outlet channel directions.

**Stagnation point position**

Particle confinement and manipulation is achieved through precise control of the stagnation point position, which, in turn, is adjusted using an integrated on-chip valve. Therefore, the effect of valve pressurization on the stagnation point position will determine the overall trap performance.

In order to design and engineer a robust microfluidic-based trap, we systematically analyzed the response mechanism for each step in the trapping process. The overall process flow scheme for hydrodynamic trapping is summarized by the following steps (Fig. 3).

1. On-chip membrane valve is pressurized.
2. Cross-sectional area of the valve changes.
3. Relative flow resistance in outlet channels change.
4. Stagnation point position moves to a new location.
5. Particle moves toward trap center via an updated force.

In this article, we model each step in the trapping process with regard to microfluidic device design parameters in order to quantify trap response. For each step, we develop a theoretical model and provide experimental validation of overall device performance. We analyze device parameters directly impacting control of stagnation point position, including the membrane valve design parameters, such as the width, height and the length of the constriction. In addition, we examine the structural characteristics of the membrane valve, such as the membrane thickness and stiffness via the PDMS base-to-crosslinker ratio.
The effect of membrane valve pressurization on valve cross-sectional area

Membrane deflection changes the cross-sectional area of the fluidic channel beneath the control layer, thereby adjusting the relative flow resistance in one outlet channel with respect to the other (Fig. 4a). In this manner, the control layer acts as a dynamic metering valve controlling flow rates in the fluidic layer, which enables the fine-scale control of stagnation point position. The response of the valve against applied pressure depends on the physical properties of the membrane, such as membrane thickness and stiffness. In order to fully characterize valve response, we fabricated microfluidic devices with varying membrane thickness ($d = 31, 37, 51$ and $66 \mu m$) between the control and the fluidic layer. We characterized the effect of membrane thickness on valve function by measuring the valve opening for several pressure values (0–130 kPa) applied to the membrane. Previous work reported valve response in the context of binary on/off valves.\(^9\) Here, we characterize valve response in the context of dynamic metering valves for hydrodynamic trapping, which is essential for optimal device performance. To determine valve opening at a specific pressure value, the microchannel in the fluidic layer is filled with a fluorescent dye solution, and the section of the microchannel under the membrane valve is imaged by a CCD camera at variable pressure. Valve opening is determined by calculating the ratio of the total fluorescence intensity under the membrane valve at a given pressure relative to zero applied pressure (fully open).

Valves with thinner membranes close at relatively low pressure values compared to those with thicker membranes (Fig. 4b). Valves with thicker membranes are less responsive, though they exhibit a wider range of linear response against changes in pressure. For instance, the membrane valve shows a linear response range between 0 and 20 kPa, and 0 and 90 kPa for $31$ and $66 \mu m$ membrane thicknesses, respectively. A wide range of linear valve response represents an important design advantage for valve control, as it facilitates implementation of a linear (proportional) feedback control algorithm for particle trapping. In this work, we used “push-down” valves on microchannels with rectangular cross-sections such that the membrane valves are not able to fully close the microchannels. In the context of
a hydrodynamic trap, inability to fully close valves is not a disadvantage per se because membrane valves are utilized as metering valves (typically within the linear response range) rather than binary on/off valves.

We also characterized the effect of membrane stiffness on valve performance. Here, we varied PDMS base : crosslinker ratio and studied valve function by measuring valve opening for several pressure values (0–110 kPa) applied to the membrane (Fig. 4c). A two-layer PDMS device is fabricated by bonding two PDMS device layers containing excess amounts of either base or crosslinker. We used a 5 : 1 base : crosslinker ratio for the control layer and 12 : 1, 15 : 1 and 30 : 1 ratios for the fluidic layer, which contains the membrane portion of the on-chip valve. During the curing process, the base : crosslinker ratio shifts towards a ~10 : 1 ratio at the interface of the two layers due to the diffusion of excess crosslinker across the layers. However, the desired target ratios of 12 : 1, 15 : 1 and 30 : 1 are retained within the membrane valve, because microchannels are situated immediately above and below the membrane, and the membrane does not come into conformal contact with the control layer. Therefore, the membrane valve is expected to become increasingly flexible and more responsive with increasing base : crosslinker ratio in the fluidic layer. Indeed, as the base : crosslinker ratio in the fluidic layer is increased from 12 : 1 to 30 : 1 (Fig. 4c), the membrane valve becomes more responsive, i.e. the change in valve opening per unit pressure change increases.

**Effect of valve cross-sectional area on the relative flow rates through the outlet channels**

In the hydrodynamic trap, the on-chip membrane valve is used to adjust flow resistance by forming a variable constriction within one of the outlet channels, resulting in an overall change in the relative flow rates distributed through both outlet channels.

The flow resistance within an outlet channel can be adjusted using a constriction that changes the cross-sectional area along the height or width of the channel (or both). For a microchannel with rectangular cross-section, the flow resistance is an asymmetric function of channel height and width with a stronger dependence on the former (see ESI†). Therefore, a unit change in channel height and width along the constriction would yield different overall flow resistances for the outlet channel (see Fig. 5 and discussion below). Depending on the desired response, membrane valves can be designed to induce a constriction along either the height or width of the cross-sectional area of the channel. In order to study the effect of changing the constriction width on flow resistance, we designed and built a series of model devices with varying constriction widths. In addition, in order to characterize the effect of changing the constriction height on flow resistance, we employed push-down membrane valves to induce changes in the channel height.

Before embarking on experiments, we theoretically modellled the membrane valve as a constriction with either variable height (h_v) or variable width (w_v) located in one of the outlet channels (Fig. 5a and b, see ESI†). We derived the fluid equations describing the dependence of Q_v/Q_tot on the variable constriction width (w_v), height (h_v) and length (L_v) by calculating the flow resistances of each outlet channel, and the results are plotted in Fig. 5c. Overall, the relative flow rate (Q_v/Q_tot) exhibits a sigmoidal response as a function of normalized variable constriction width and height. Locally, and for small changes in the constriction dimension, the flow rate partitioning function Q_v/Q_tot can be modelled as a linear function of normalized variable constriction width or height, which facilitates

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**Fig. 5** Device design: the effect of valve cross-sectional area on the relative flow rates. (a) Device layout showing one fixed and one variable constriction situated on opposing outlet channels. Here, the channel width is modelled as the variable parameter for the constriction. (b) The channel height can also be modelled as the variable parameter for the constriction. (c) For device configurations illustrated in a and b, the theoretical ratio of flow rates through the outlet channel with the variable constriction (Q_v) to the total flow rate (Q_tot) as a function of normalized variable constriction width (γ_wv), height (γ_vh) and length (γ_vl) is shown. Flow rates (Q_v/Q_tot) were also experimentally measured for seven devices with different variable constriction width. For these devices, one of the outlet channels had a fixed constriction width (w_v = 100 μm) whereas the other outlet channel had a variable constriction width (w_v = 40–260 μm). Constriction widths (w_v, w_c = 0–300 μm), height (h_v = 0–50 μm) and lengths (L_v = 1 mm, L_c = 0–2 mm) are normalized to the outlet channel width (w = 300 μm), height (h = 50 μm) and length (L = 10 mm) respectively. Experimental data at seven different variable constriction width values show good agreement with the theoretical curve. The flow splits equally at γ_wv = γ_vh = 0.333, i.e. w_v = w_c = 100 μm, such that Q_v = Q_c and Q_v/Q_tot = 0.5. (Inset) The outlet channel flow resistances increase nearly linearly with the constriction length (L_v or L_c) leading to a linear decrease in the flow rate ratio.
implementing a linear proportional feedback controller for particle trapping. \( \frac{Q_v}{Q_{tot}} \) decreases nearly linearly as a function of normalized variable constriction length. In addition, results from these calculations suggest that relatively small changes in the volumetric flow rate partitioning occur for partially open or nearly fully open valves, whereas large changes occur for nearly closed valve positions (Fig. 5c).

To experimentally characterize the response of actual membrane valves, we first designed and built a series of model devices with varying constriction widths. These devices contain constrictions with varying width \( (w_v) \) located on one of the outlet channels, as shown in Fig. 5a. The opposing outlet channel contains a constriction with a fixed width \( (w_c) \) whereas the other outlet channel has a variable constriction width \( (w_v) \) (Fig. 5c). Flow rates are experimentally determined by particle tracking measurements (see ESI†). For model devices, the flow rate partitioning function \( \frac{Q_v}{Q_{tot}} \) shows good agreement between experimental data and theory when \( \frac{Q_v}{Q_{tot}} \) is plotted as a function of the variable constriction width normalized to the microchannel width \( (\gamma_{wv} = w_v/w) \). Finally, we experimentally determined the effect of changing constriction height on fluid flow by directly measuring stagnation point position, as discussed in subsequent sections (Fig. 6).

Is the action of membrane valves in actual devices better described by channel constrictions varying in width or height? For actual hydrodynamic trap devices with push-down membrane valves, our results suggest membrane valves are more accurately modelled by changes in constriction channel height, as discussed in the following section (see Fig. 6). In general, push-down membrane valves induce non-uniform changes in both channel height and width. However, valve opening exhibits a linear response over a wide range of applied pressures used in particle trapping (Fig. 4b and c), thereby suggesting that channel height changes linearly during actual device operation.

**Effect of relative flow rates in the outlet channels on stagnation point position**

Inlet fluid streams approach the cross-slot junction along the compressional flow direction and split into two opposing outlet streams (Fig. 6a and b, and S3, ESI†). For equally balanced volumetric flow rates within the inlet and outlet channels, the stagnation point is located at the center of the cross-slot junction. Varying the relative flow rates within the two outlet channels effectively moves the stagnation point along a line parallel to the outlet flow direction. At any instant in time, the stagnation point is located on the partition line at which the inlet streams split and flow into the two outlet channels (Fig. 6b). Therefore, the position of the stagnation point intrinsically determines the flow rates exiting through each outlet channel. In order to model the location of the stagnation point as a function of membrane valve position, we calculated the ratio of the flow rate exiting one outlet channel relative to the total flow rate \( (\frac{Q_v}{Q_{tot}}) \) as a function of the stagnation point position (see ESI†). The results suggest that the incoming flow splits nearly linearly with the stagnation point position (Fig. S3, ESI†). The flat (plug-like) fluid velocity profile in the inlet and outlet channels (Fig. S1f), as well as the fluid

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**Fig. 6** Device design: channel constriction width and height. Fluid stagnation point position as a function of constriction width and height was determined. (a) One constriction in each outlet channel is used to manipulate the position of the stagnation point position. One of the outlet channels has a fixed constriction \( (w_c = 100 \mu m) \) whereas the other outlet channel has a variable constriction width \( (w_v = 40–260 \mu m) \). (b) As the variable constriction size (width or height) is changed, the position of the stagnation point is effectively moved along the outlet channels. (c) Theoretical (solid line) and experimental (squares, diamonds) data for the stagnation point position change with constriction size. We built microfluidic devices with 12 different variable constriction widths and measured the normalized stagnation point position (squares). The variable constriction width \( (w_v) \) and the stagnation point position are normalized to the microchannel width \( (w = 300 \mu m) \). At \( (\gamma_{wv} = \gamma_{wc} = 0.333, i.e. w_v = w_c = 100 \mu m) \), the flow is split equally and the stagnation point is located at the center of the channel. The effect of membrane valve opening on the stagnation point position shows a response curve similar to the theoretical response curve for changes in constriction height, thereby suggesting that the membrane valve mainly induces a change in channel height along the outlet channel.
velocity profile in the vicinity of the stagnation point (eqn (1)),
result in the linear relationship between flow rate partitioning
and stagnation point position. Near the channel walls (within
10% of the channel width), the fluid velocity approaches zero,
resulting in deviations from the linear response (Fig. S3†).

Overall, precise regulation of the relative flow rates through
the outlet channels enables linear, fine-scale adjustment of the
stagnation point position at the microchannel junction.
Furthermore, the linear relationship allows for control of stаг-
nation point position using a linear feedback controller. In this
way, a flow rate increase in one of the outlet channels would
move the stagnation point away from this channel outlet in
a linear fashion.

Effect of valve cross-sectional area on stagnation point position

As a final step in modelling the microfluidic-based trapping
mechanism, we characterize the effect of valve cross-sectional
area on stagnation point position using a model system and
actual trap devices. Overall, these experiments build on the
knowledge of valve response presented in Fig. 5 by quantifying
the stagnation point response as a function of valve constriction
width and height.

First, we calculated the theoretical dependence of the stagna-
tion point position as a function of the variable constriction
width ($w_v$) and height ($h_v$) by combining the dependence of the
relative flow rates through outlet channels (Fig. 5) with the effect
of the relative flow rates on stagnation point position (Fig. S3†).
As the variable constriction width changes, the position of the
stagnation point effectively moves along the principal axis of
extension (axis parallel to the outlet channels as shown in Fig. 6b
and c). Results describing the response of the stagnation point
position to changes in the variable constriction width are pre-
sent in Fig. 6c. Overall, the fluid stagnation point position
exhibits a sigmoidal response to changes in the variable
constriction width. As expected, the stagnation point is located at
the center of the channel when the constriction widths for both
outlet channels are equal (i.e. $w_v = w_o$), thereby resulting in equal
flow partitioning.

To experimentally characterize changes in the stagnation point
position as a function of constriction width, we fabricated
devices with 12 different variable constriction widths ($w_v = 40–
260 \mu m$ as shown in Fig. 6a) and experimentally measured the
fluid stagnation point position. In these model devices, one of the
outlet channels has a fixed constriction ($w_c = 100 \mu m$), whereas
the width of the variable constriction ($w_v$) on the opposite outlet
channel is changed. Experimental results are shown in Fig. 6c
and agree well with theoretical calculations. Experimental
determination of the stagnation point position is accomplished
by particle tracking as described in the ES† (Fig. S4).

Finally, we examined the actual response of the stagnation
point position against membrane valve opening in functional
hydrodynamic trap devices. Here, we varied the pressure applied
to the membrane valve and measured the stagnation point
position by particle tracking. As shown in Fig. 6c, the stagnation
point response to changes in valve opening is sigmoidal for
functional trap devices. The experimental response curve
suggests that the membrane valve acts as a constriction
decreasing in height with increasing applied pressure. For this
analysis, the applied pressure is converted to a percent valve
opening using experimental data shown in Fig. 4b and c. Results
shown in Fig. 6 suggest that the constriction formed by the
membrane valve is accurately modelled by variations in
constriction channel height rather than channel width, which
might be explained by the relatively low aspect ratios in our
microchannels ($0.1 < \alpha < 0.166$). In addition, the channel width
($w = 300 \mu m$) is significantly smaller than the constriction length
($L_c = 1 mm$), thereby resulting in more uniform changes in the
channel height with valve closure along the channel length.

Conclusion

The hydrodynamic trap facilitates particle confinement and
manipulation using a stagnation point flow generated in a cross-
slot microfluidic device. Particles are confined at a user-defined
set point (trap center) using an automated feedback control
mechanism. The feedback controller continuously tracks particle
position and adjusts stagnation point position using an inte-
grated on-chip metering valve. Using the hydrodynamic trap,
particles are confined in free-solution at a stagnation point,
whereas the vast majority of existing microfluidic methods for
particle manipulation relies on physical barriers (which necessi-
tate particle-wall contact), circulating flows or microeddies.
Overall, the microfluidic-based hydrodynamic trap offers a new
method for non-contact and free-solution confinement of arbi-
trary particles or cells for long time scales.

In this work, we investigate the hydrodynamic trap response as
a function of microfluidic device design parameters, including
membrane valve characteristics and channel dimensions. We
developed a model for stagnation point response as a function of
applied valve pressure and validated the theoretical model with
experiments. Overall, the model describing the on-chip
membrane valve enables quantitative understanding of valve
response. Furthermore, precise understanding of stagnation
point position as a function of valve response will allow for
design and implementation of robust hydrodynamic traps for
tailored applications, including confinement of small nano-
particles and manipulation of arbitrary particles in flow. Select-
ing and implementing specific valve design parameters will
enable custom engineering of the on-chip membrane valve and
facilitate rational design of the trap response. For example, one
can envision designing a hydrodynamic trap with a response
curve with a given slope and linear response region for trapping
applications specific to “target” particles or cells.

In this manuscript, we characterize key design parameters
determining the response of the stagnation point to changes in
the applied valve pressure. By combining experiments and
theory, we develop a quantitative model for the microfluidic-
based hydrodynamic trap and provide a general framework for
building and implementing an effective model-based control
algorithm. The experiments described in this paper utilize a linear
feedback control algorithm for particle trapping. However,
custom model-based feedback controllers utilizing valve
response curves for any microdevice trap design may be imple-
mented in the hydrodynamic trap. 39

Although we used a push-down (or actuate-to-close) valve that
mainly changes the microchannel height in this work, a valve that
changes the microchannel width can also be employed. 34 In this
way, a hydrodynamic trap with a tailored response can be achieved. Implementation of valves with well-characterized response curves would allow for custom trap design for specific particle trapping applications.

The microfluidic trap offers several advantages for the confinement and manipulation of micro and nanoscale particles. First, particles are trapped in free-solution, thereby allowing for non-perturbative and non-contact confinement of single particles or cells. In addition, trapping is achieved by the sole action of hydrodynamic flow, thereby eliminating the need for optical, electric, magnetic or acoustic fields. Hydrodynamic trapping is possible for any arbitrary particle with no specific requirements on material composition or chemical/physical properties (e.g. surface charge, refractive index) of the trapped object. Furthermore, single particles may be hydrodynamically trapped in a concentrated solution of particles, which enables confinement, micromanipulation and isolation of a single target particle in a crowded solution, difficult to achieve using alternative force trapping methods. In addition, hydrodynamic trapping allows for dynamic exchange of the surrounding medium (pH, temperature, ion concentration, etc.) of a trapped particle, coupled with concomitant and direct imaging for real-time characterization of single nanoparticles or cells. Finally, hydrodynamically trapped particles may be monitored using a wide variety of microscopy techniques including bright field, phase contrast and fluorescence microscopy. Overall, the microfluidic-based hydrodynamic trap offers a powerful and versatile platform for non-perturbative, fine-scale confinement and manipulation of micro and nanometre-sized particles for long-time observation without surface immobilization.

Acknowledgements

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References

SUPPLEMENTARY MATERIAL

A microfluidic-based hydrodynamic trap: Design and implementation†

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Flow in a rectangular channel

The Navier-Stokes equation and the associated boundary conditions for a pressure-driven, steady-state flow in a microchannel with a rectangular cross section is given by:

\[
\left( \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) u = -\frac{\Delta p}{\eta L}, \quad \text{for} \quad \frac{w}{2} < y < \frac{w}{2} \quad \text{and} \quad 0 < z < h
\]

\[
u = 0, \quad \text{at the boundary, i.e.} \quad y = \pm \frac{w}{2}, \quad z = 0, \quad z = h
\]

where \( u \): velocity,
\( \Delta p \): pressure difference,
\( \eta \): viscosity,
\( w, h, L \): width, height and length of the channel respectively,
\( x, y, z \) are the coordinate axes along the channel length, width and height respectively.

The solution for the velocity along the channel width (\( y \)) and height (\( z \)) is given by a Fourier series expansion:

\[
u(y, z) = \frac{4h^2\Delta p}{\pi^3\eta L} \sum_{n,odd} \frac{1}{n^3} \left[ 1 - \frac{\cosh(n\pi \frac{w}{h})}{\cosh(n\pi \frac{w}{2h})} \right] \sin(n\pi \frac{z}{h})
\]

Fig. S1 shows the velocity profile (shown in (a)) and the contours of the velocity along the channel width and height (shown in (b) and (c) respectively). The flow rate \( Q \) can be found by integrating \( \nu(y, z) \) along these axes:

\[
Q = 2 \int_{0}^{w/2} dy \int_{0}^{h} dz \nu(y, z) = \frac{h^4\Delta p}{12\eta La} \left[ 1 - \sum_{n,odd} \frac{192\alpha}{(n\pi)^2} \tanh\left(\frac{n\pi}{2a}\right) \right]
\]

where \( \alpha = h/w \) is the aspect ratio of the channel. For channels with low aspect ratio \( \alpha \to 0 \), the flow rate (\( Q \)) can be approximated by:

\[
Q \approx \frac{h^4\Delta p}{12\eta La} (1 - 0.63\alpha), \quad \text{for} \ \alpha \to 0
\]

Eq. (S3) represents a good approximation to Eq. (S2) for flow rate estimates in rectangular microchannels with low aspect ratio. For example, the error is 12.3% for \( h = w \) (square channel cross-section, \( \alpha = 1 \)), 0.15% for \( \alpha = 0.5 \) and only 0.003% for \( \alpha = 0.1 \).

Using Eq. (S3), the flow resistance of a rectangular microchannel is approximated by:

\[
R = \frac{\Delta p}{Q} \approx \frac{12\eta La}{h^4} \left( \frac{1}{1 - 0.63\alpha} \right), \quad \text{for} \ \alpha \to 0
\]

The effect of constriction width and length on the relative flow rates through outlet channels

Let us consider the cross-slot geometry used in hydrodynamic trapping (Fig. S2a). We would like to calculate \( (Q_c/Q_{\text{tot}}) \), the ratio of the flow rate through the outlet channel with the constriction (\( Q_c \)) to the total flow rate (\( Q_{\text{tot}} = Q_c + Q_0 \)) as a function of the constriction width (\( w_c \)) and length (\( L_c \)). We assume that the flow is pressure-driven from a common source and the outlet channels

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Fig. S1 Velocity Profile in a Rectangular Microchannel. (a) Velocity profile for laminar flow in a rectangular microchannel with aspect ratio of $\alpha = h/w = 0.166$. The axes are normalized to the channel width ($w = 300 \, \mu m$), the channel height ($h = 50 \, \mu m$) and the maximum velocity. (b) The velocity profile along the channel height is parabolic with increasing maximum velocity towards the centre of the channel along the channel height. (c) For low aspect ratios, the flow in rectangular microchannels is plug-like flow and the global maximum velocity is attained at a distance ($0.2 \times $ channel width) from the channel walls along the channel width. (d) The experimental velocity profile along the channel width is obtained by tracking and measuring the velocity of individual fluorescent beads flowing in the microchannel at half height. From the fit to the experimental data, the maximum velocity in the microchannel is obtained to calculate the flow rates in Fig. 5 in the main text.

open to atmospheric pressure. In this case, the pressure drop across both outlet channels is the same and the outlet channels are analogous to resistors connected in parallel. As a result, the flow rate through the outlet channel with the constriction is proportional to the flow resistance of the opposite channel:

$$\frac{Q_c}{Q_{tot}} = \frac{R_n}{R_{tot}} = \frac{R_n}{R_n + R_c} = \frac{1}{1 + \frac{R_c}{R_n}} \quad (S5)$$

We can obtain an expression for $(Q_c/Q_{tot})$ using the approximate flow resistance formula in Eq. (S4) where $R_n$ and $R_c$ are given by:

$$R_n \approx \frac{12\eta L}{h^4} \frac{1}{(1 - 0.63\alpha)} \quad \text{and} \quad R_c \approx \frac{12\eta L}{h^4} \left( \frac{1 - \gamma_c}{(1 - 0.63\alpha)} + \frac{\gamma_c}{\gamma_{wc} - 0.63\alpha} \right), \quad \text{for} \quad \alpha \to 0 \quad (S6)$$

where $\gamma_{wc} = w_c/w$ and $\gamma_c = L_c/L$ are the normalized constriction width and length respectively. Combining Eq. (S5) and (S6), we obtain $(Q_c/Q_{tot})$ as a function of $\gamma_{wc}$ and $\gamma_c$:

$$\frac{Q_c}{Q_{tot}} [\gamma_{wc} \gamma_c] = \frac{1}{2 + \gamma_c \left( \frac{1 - \gamma_{wc}}{\gamma_{wc} - 0.63\alpha} \right)} \quad (S7)$$

As $\gamma_{wc} \to 1$ or $\gamma_c \to 0$, the constriction vanishes and Eq. (S7) correctly predicts that the flow splits equally $(Q_c/Q_{tot} \to 1/2)$.
The flow resistance of the outlet channel with the fixed constriction in Fig. 5 is given in the main text. Now, let us consider the device layout with a cross-slot geometry and two constrictions (one on each outlet channel) as illustrated in Fig. 5a in the main text. One constriction has a fixed width while the other has a variable width. We calculated \( \frac{Q_v}{Q_{tot}} \), the ratio of the flow rate through the outlet channel with the variable constriction \( Q_v \) to the total flow rate \( Q_{tot} = Q_c + Q_v \) as a function of the variable constriction width \( w_c \) and length \( L_c \). Similar to Eq. (S5), \( \frac{Q_v}{Q_{tot}} \) is given by:

\[
\frac{Q_v}{Q_{tot}} = \frac{R_c}{R_{tot}} = \frac{R_c}{R_c + R_v} = \frac{1}{1 + \frac{R_v}{R_c}}
\]

The flow resistance of the outlet channel with the variable constriction \( R_v \) is given by:

\[
R_v = R_n(1 - \gamma_{wc}) + R_{var} \quad \text{where} \quad R_{var} = \frac{12\eta L a}{h^4} \gamma_{wc} \left( \frac{\gamma_{wc}}{\gamma_{wc}} \right) \left[ 1 - \sum_{n=0}^{\infty} \frac{192}{(n\pi)^3} \alpha \tanh \left( \frac{n\pi \gamma_{wc}}{2\alpha} \right) \right]^{-1}
\]

The first 50 terms of the power series expansion are included in the calculation of the flow resistances. The resulting \( \frac{Q_v}{Q_{tot}} \) is plotted as a function of normalized constriction width \( \gamma_{wc} \) (see Exact plot) and length \( \gamma_{wc} \) (see inset plot). As expected, this solution produces a more accurate result and predicts correctly that as \( \gamma_{wc} \to 0 \), \( \frac{Q_c}{Q_{tot}} \to 0 \). For the device design depicted in Fig. S2a, we conclude that \( \frac{Q_v}{Q_{tot}} \) exhibits a sigmoidal and a linear decay response against changes in constriction width \( w_c \) and length \( L_c \) respectively.

Now, let us consider the device layout with a cross-slot geometry and two constrictions (one on each outlet channel) as illustrated in Fig. 5a in the main text. One constriction has a fixed width while the other has a variable width. We calculated \( \frac{Q_v}{Q_{tot}} \), the ratio of the flow rate through the outlet channel with the variable constriction \( Q_v \) to the total flow rate \( Q_{tot} = Q_c + Q_v \) as a function of the variable constriction width \( w_c \) and length \( L_c \). Similar to Eq. (S5), \( \frac{Q_v}{Q_{tot}} \) is given by:

\[
\frac{Q_v}{Q_{tot}} = \frac{R_c}{R_{tot}} = \frac{R_c}{R_c + R_v} = \frac{1}{1 + \frac{R_v}{R_c}}
\]

The flow resistance of the outlet channel with the variable constriction \( R_v \) is given by:

\[
R_v = R_n(1 - \gamma_{wc}) + R_{var} \quad \text{where} \quad R_{var} = \frac{12\eta L a}{h^4} \gamma_{wc} \left( \frac{\gamma_{wc}}{\gamma_{wc}} \right) \left[ 1 - \sum_{n=0}^{\infty} \frac{192}{(n\pi)^3} \alpha \tanh \left( \frac{n\pi \gamma_{wc}}{2\alpha} \right) \right]^{-1}
\]
where \( \gamma_{wv} = \frac{w_v}{w} \) and \( \gamma_{lv} = \frac{L_v}{L} \) are the normalized width and length of the variable constriction respectively. \( R_{var} \) is the flow resistance of the variable constriction. In Fig. 5c, \( (Q_v/Q_{tot}) \) is plotted as a function of the normalized width of the variable constriction \( (\gamma_{wv}) \) (main plot, solid line) and length \( (\gamma_{lv}) \) (inset). Once more, we conclude that \( (Q_v/Q_{tot}) \) is sigmoidal for changes in the width of the variable constriction \( (w_v) \) and decreases almost linearly with changes in the length of the variable constriction \( (L_v) \).

**The effect of constriction height on the relative flow rates through outlet channels**

In the previous section, we assumed that the variable parameter for the constriction is its width. However, in our microfluidic trap, the membrane valve constricts the microchannel along the width and the height. Therefore, it would be useful to calculate the effect of constriction height on the flow resistance of the microchannel and on the relative flow rates through the outlet channels.

We again consider the device design in Fig. 5a with one fixed and one variable constriction. This time, for the variable constriction, we assume that the variable parameter is the height of the constriction rather than its width \( (L_v) \) (Fig. 5b). We calculated \( (Q_v/Q_{tot}) \), the ratio of the flow rate through the outlet channel with the variable constriction \( (Q_v) \) to the total flow rate \( (Q_{tot} = Q_c + Q_v) \) as a function of the variable constriction height \( (h_v) \). Once more, \( (Q_v/Q_{tot}) \) and the flow resistance of the outlet channel with the fixed constriction \( (R_c) \) is given by Eq. (S11) and Eqs. (S8-S10) respectively. However, the flow resistance of the outlet channel with the variable constriction \( (R_v) \) is given by:

\[
R_v = R_n(1 - \gamma_{hv}) + R_{var} \quad \text{where} \quad R_{var} = \frac{12\eta L}{h^4} \frac{\gamma_{hv}}{Y_{hv}} \left[ 1 - \sum_{n=odd}^{\infty} \frac{192\pi^4 \gamma_{hv}}{(n\pi)^5} \tanh \left( \frac{n\pi}{2\alpha \gamma_{hv}} \right) \right]^{-1}
\]  

(S13)

where \( \gamma_{hv} = h_v/h \) is the normalized height of the variable constriction. Fig. 5c depicts \( (Q_v/Q_{tot}) \) plotted as a function of the normalized height of the variable constriction \( (\gamma_{hv}) \). The response of the relative flow rate \( (Q_v/Q_{tot}) \) against changes in the height of the variable constriction is also sigmoidal, however the shape of the response curve is different than the response curve for changing the constriction width. The relative flow rate response against changes in the constriction width and height are linear and sensitive between \( 0 < \gamma_{wv} < 0.2 \) and \( 0.1 < \gamma_{hv} < 0.6 \) respectively.

**Experimental determination of the relative flow rates through outlet channels**

For a pressure-driven, steady-state flow in a microchannel with a rectangular cross section, the fluid velocity averaged over the channel height is given by:

\[
\bar{u}(y) = \frac{1}{h} \int_0^h dz \ u(y, z) = \frac{8h^2\Delta p}{\pi^4\eta L} \sum_{n=odd}^{\infty} \frac{1}{n^4} \left[ 1 - \frac{\cosh \left( \frac{n\pi}{h} \right)}{\cosh \left( \frac{n\pi}{2h} \right)} \right]
\]  

(S14)

At the middle of the channel \( (y = 0) \) and at the channel wall \( (y = w/2) \), the averaged fluid velocity yields:

\[
\bar{u}(0) = \frac{h^2\Delta p}{12\eta L} \left[ 1 - \delta \right] \quad \text{where} \quad \delta = \sum_{n=odd}^{\infty} \frac{96}{(n\pi)^4 \cosh \left( \frac{n\pi}{2h} \right)} \quad \text{and} \quad \bar{u}(w/2) = 0
\]  

(S15)

The maximum fluid velocity in the channel occurs at the middle of the channel height and width:

\[
u_{max} = u(0, h/2) = \frac{h^2\Delta p}{8\eta L} \left[ 1 - \beta \right] \quad \text{where} \quad \beta = \sum_{n=odd}^{\infty} \frac{48}{(n\pi)^3 \cosh \left( \frac{n\pi}{2h} \right)}
\]  

(S16)

Now, let’s consider a pressure-driven, steady-state flow between two infinite parallel plates separated by the channel height \( (h) \). The averaged and maximum fluid velocity for this flow type are given by:

\[
\bar{u}(0) = \frac{h^2\Delta p}{12\eta L} \quad \text{and} \quad \nu_{max} = \frac{3}{2} \bar{u}(0) = \frac{h^2\Delta p}{8\eta L}
\]  

(S17)

Comparing Eqs. (S15) and (S16) with Eq. (S17), it is obvious that \( \delta \) and \( \beta \) are correction terms specifying the deviation of the averaged and maximum fluid velocity in the rectangular microchannel from the fluid velocities between two infinite parallel plates. The correction terms for five different aspect ratios are provided in Table S1.

Table S1 reveals that as the aspect ratio approaches to zero, the correction terms also approach to zero. Therefore, as the aspect ratio tends to zero, the flow in rectangular microchannels is increasingly plug-like and can be approximated by pressure driven flow between two infinite parallel plates. As a result, for a rectangular microchannel with low aspect ratio \( (\alpha \leq 0.166) \), the averaged and
Table S1 Correction terms for the averaged and maximum fluid velocity in a rectangular microchannel.

We calculated the correction factors (δ) and (β) for five different aspect ratios.

<table>
<thead>
<tr>
<th>Aspect ratio</th>
<th>Correction term (δ) for averaged velocity</th>
<th>Correction term (β) for maximum velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.97E-07</td>
<td>4.67E-07</td>
</tr>
<tr>
<td>0.166</td>
<td>0.00016</td>
<td>0.00025</td>
</tr>
<tr>
<td>0.333</td>
<td>0.0177</td>
<td>0.0278</td>
</tr>
<tr>
<td>0.5</td>
<td>0.085</td>
<td>0.134</td>
</tr>
<tr>
<td>1</td>
<td>0.393</td>
<td>0.616</td>
</tr>
</tbody>
</table>

Maximum fluid velocities are estimated by Eq. (S17) with < 0.03 % accuracy. In this case, following Eq. (S3), the volumetric flow rate (Q) in the microchannel is given by:

\[ Q \approx \frac{h^3w \Delta \rho}{12\eta L} (1 - 0.63a) = \bar{u}A(1 - 0.63a) = \frac{2}{3} u_{\text{max}} A(1 - 0.63a) \quad \text{for } a \to 0 \]  

(S18)

where A = hw is the cross-sectional area of the microchannel. Eq. (S18) indicates that the flow rate is proportional to the maximum velocity in the microchannel. Hence, to calculate the relative flow rates through the outlet channels, the maximum velocity in each outlet channel can be used as a measure of the flow rate. For instance, \(\frac{Q_v}{Q_{\text{tot}}}\) for the device in Fig. 5a (main text) yields:

\[ \frac{Q_v}{Q_{\text{tot}}} = \frac{(u_{\text{max}})_v}{(u_{\text{max}})_c + (u_{\text{max}})_v} \]  

(S19)

where \((u_{\text{max}})_c\) and \((u_{\text{max}})_v\) are the maximum fluid velocities in the outlet channels with a fixed and variable constriction size respectively. To determine the maximum fluid velocities in the outlet channels, a dilute fluorescent bead solution is introduced to each microfluidic trapping device and individual beads are tracked while flowing through the outlet channels. For each outlet channel, the experimental velocity profile across the channel width is plotted by measuring the position and velocity of individual beads flowing through at half channel height (Fig. S1d). The maximum fluid velocity in each outlet channel is obtained by fitting the experimental velocity profile to the theoretical velocity profile at the middle of the channel height, \(u(y, h/2)\):

\[ u \left( \frac{h}{2} \right) = \frac{4h^2 \Delta \rho}{\pi^3 \eta L} \sum_{n,\text{odd}} \frac{1}{n^3} \left[ 1 - \frac{\cosh \left( \frac{\pi n y}{h} \right)}{\cosh \left( \frac{\pi n h}{2} \right)} \right] \sin \left( \frac{\pi n y}{2h} \right) \]  

(S20)

The first term in the power series expansion is used as the functional form to perform the fit yielding only 3.2% error. The relative flow rates through the outlet channels are calculated by Eq. (S19) using the maximum fluid velocity obtained from the fit for each outlet channel.

Table S2 Experimental values of relative flow rates through the outlet channels (Q_v/Q_tot) from Figure 5c.

The flow partitioning function \((Q_v/Q_{\text{tot}})\) is experimentally measured for seven different variable constriction widths to determine the effect of valve cross-sectional area on the relative flow rates through the outlet channels.

<table>
<thead>
<tr>
<th>Normalized variable constriction width</th>
<th>Relative flow rates (Q_v/Q_{tot})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.133</td>
<td>0.232 ± 0.010</td>
</tr>
<tr>
<td>0.2</td>
<td>0.388 ± 0.017</td>
</tr>
<tr>
<td>0.333</td>
<td>0.484 ± 0.019</td>
</tr>
<tr>
<td>0.466</td>
<td>0.508 ± 0.020</td>
</tr>
<tr>
<td>0.6</td>
<td>0.529 ± 0.022</td>
</tr>
<tr>
<td>0.666</td>
<td>0.532 ± 0.021</td>
</tr>
<tr>
<td>0.866</td>
<td>0.562 ± 0.022</td>
</tr>
</tbody>
</table>
**The effect of the stagnation point position on the relative flow rates through outlet channels**

Let us consider the effect of the stagnation point position on the ratio of the flow rate through one of the outlet channels ($Q_1$) to the total flow rate ($Q_{tot} = Q_1 + Q_2$) (Fig. S3). The incoming flow splits into two (shown by two different colors) along the axis passing through the stagnation point and parallel to the inlet channels. The flow rates through the two outlet channels can be given by:

$$Q_1 = \int_{-w/2}^{w/2} dy \int_0^h dz u(y,z) \quad \text{and} \quad Q_2 = \int_{y_0}^{w/2} dy \int_0^h dz u(y,z)$$  \hspace{1cm} (S21)

Combining Eq. (S1) and (S21), we get:

$$Q_1 / Q_{tot} [\gamma_{y_0}] = \frac{1}{2} \left( 1 - \sum_{n=odd}^\infty \frac{192\alpha}{(n\pi)^5} \left[ \sinh \left( \frac{n\pi \gamma_{y_0}}{\alpha} \right) \cosh \left( \frac{n\pi \alpha}{2\alpha} \right) + \tanh \left( \frac{n\pi \alpha}{2\alpha} \right) \right] \right)$$  \hspace{1cm} (S22)

where $\gamma_{y_0} = y_0/w$ is the normalized stagnation point position. If ($\gamma_{y_0} \rightarrow -1/2$), there is no flow going through outlet 1 and ($Q_1/Q_{tot} \rightarrow 0$). When the stagnation point is at the center ($\gamma_{y_0} = 0$), the flow splits equally and ($Q_1/Q_{tot} = 1/2$). As ($\gamma_{y_0} \rightarrow 1/2$), there is no flow going through outlet 2 and therefore ($Q_1/Q_{tot} \rightarrow 1$). Fig. S3 shows the plot for ($Q_1/Q_{tot}$) as a function of the normalized stagnation point position ($0 < \gamma_{y_0} < 1$) using Eq. (S22). The coordinate axis for the stagnation point position ($\gamma_{y_0}$) is translated by half a channel width ($-w/2 < y_0 < w/2$) and normalized to the channel width ($w$). As can be seen from Fig. S3, the incoming flow splits linearly with the stagnation point position which has a major implication on the design of the hydrodynamic trap as described in the main text.

**Experimental determination of the stagnation point position**

The stagnation point position data for various variable constriction widths illustrated in Fig. 6 (main text) are determined experimentally by fluorescent bead tracking. The position of the stagnation point is revealed by tracking the fluorescent beads passing through the trapping region. For this purpose, a sample stream containing 2.2 µm diameter fluorescent beads (nile red, 0.01 % w/v) is introduced to the stagnation point flow generated at the trapping region. Since the sample stream is flow-focused, the beads mainly pass through the vicinity of the stagnation point which is located nearby the center of the microchannel junction. 600 consecutive images of the trapping region are captured and recorded by a fluorescence microscope and a CCD camera. The successive images are then overlaid to reveal the stagnation point (Fig. S4). The position of the stagnation point is determined visually with one pixel accuracy which corresponds to 1 µm with our current microscopy setup (10x magnification and a CCD camera with 9.9 µm pixel size). The stagnation point position is then normalized to the channel width determined experimentally for each device used in collecting data for Fig. 6.

**Fig. S4 Determining the Stagnation Point Position.** The stagnation point position is obtained via the images of fluorescent beads flowing through the trapping region (microchannel junction). 600 successive images are overlaid to reveal the stagnation point and determine its position with one pixel accuracy corresponding to 1 µm. The stagnation point position is normalized to the microchannel width.
References