Methods

Casting Mold Fabrication

We fabricated the ratchet devices using the polydimethylsiloxane (PDMS) rapid prototyping technique[28].

Photolithography chrome masks (3" plates, Nanofilm) were patterned using a Heidelberg Instruments µPG 101 laser writer (10 mW, 100 % pixel pulse) and Bitmap pattern design files with a resolution of 1 µm per pixel. Masks patterned with the laser writer were developed using a solution of AZ® 400K developer (Clariant) to deionised water (DI) in a ratio 1 : 4 (1 min). Next we etched the Cr with pure CEP-200 Micro-Chrome Etchant (Microchrome Technology) solution (2 min). Finally the sample was rinsed with acetone and then DI.

Pre-cleaned silicon substrates (4" wafers, Silicon) were dehydration baked using a hotplate (200 °C, 5 min) before we applied a 25 µm thick layer of negative tone photo-patternable epoxy resin (SU-8 3025, Microchem) on the silicon wafers using a Laurell WS-400B-6NPP-LITE (3100 rpm, 30 s) spin coater and softbaked them on a hotplate (95 °C, 10 min).

We used developed masks to pattern the SU-8 photoresist in a Quintel Q-2001 CT Mask Aligner (7 s, 250 W) to create prototype masters by UV lithography. The SU-8 photoresist was then subjected to post-exposure baking on a hotplate (65 °C, 1 min then 95 °C, 5 min) before we immersed it in pure SU-8 developer (Microchem) for at least 5 min, and rinsed it with isopropyl alcohol (IPA). Resist residue left on masks was removed by immersion in Nanostripper solution or acid piranha solution (3 : 1 concentrated sulfuric acid to 34 % hydrogen peroxide volume ratio).

The finished casting mold was glued with rubber cement into an aluminum dish with a diameter of 70 mm.

Channel Fabrication

We mixed PDMS from Dow Corning (Sylgard 184) in a ratio of 3.5 g elastomer to 0.35 g curing agent, degassed it for 5 min in an desiccator, and cast it into the mold, which we then baked for 35 min at 100 °C. Afterwards, we took the hardened PDMS from the mold and cut it on a clean glass plate with a scalpel such that the channel ends were open.

Sample assembly

To assemble a complete cell, we glued a glass ring (height 1 mm) cut from a test tube onto a cover glass (diameter 22 mm) with Norland N61, a UV glue, which was then hardened within 2 h under a 365 nm UV lamp. After the glue had hardened, this glass dish along with the PDMS channel was treated with air plasma for 3 min to increase the hydrophilicity. Without this treatment, colloidal suspension would not pervade the channels later on and air bubbles would remain stuck in the channels. Afterwards, we laid the channels face-down into the glass dish, which we then filled with colloidal suspension. In contrast to this setup, we placed the closed channel used for Fig. 3 face-up since colloidal suspension would not enter the channel otherwise.

We chose silica colloids of 3.0 μm (catalog code SS05N) and 4.3 μm in diameter from Bangs Laboratories Inc. and diluted them in aqueous solution (ratio 1 : 7500) to achieve the required low density in the channels. The demonstration of particle separation (Fig. 3) was done with 3.0 μm and 4.3 μm colloids. The experiments to verify the modeled direction reversal shown in Fig. 4 were carried out with 3.0 μm colloids. To seal the cell, a second covering glass with 19 mm in diameter was treated with air plasma for 3 min, carefully slid onto the glass ring and finally glued with UV glue, as described above.

Experimental Setup

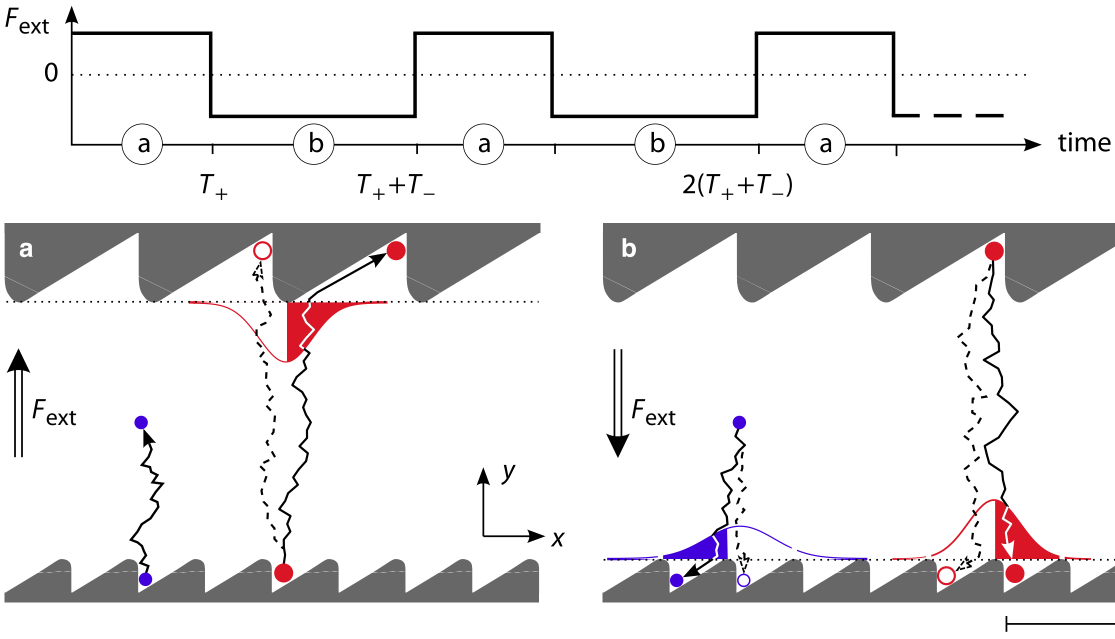
The tiltable stage consists basically of a pendulum, on which the following components are aligned: an *xy*-stage and an in-plane rotary stage (all equipped with micrometer screws) for sample alignment, LED illumination, a Marlin CCD camera from ALLIED Vision technologies on a micrometer slider for focusing, a 1x optical tube and a 10x or 5x ocular. The pendulum is suspended in its center of mass and driven by a stepper motor with a custom made controller, which can be addressed via RS232. The mechanism allows for an inclination of $\pm 90^\circ$.

Discussion

Detailed description of the separation mechanism

We consider a complete cycle $T_+ + T_-$ of the external force. Due to our choice for T_-, particles will always start the cycle in a minimum of the small sawtooth profile, i.e., in the very corner. First, we consider a single particle, which is sufficiently fast to reach a minimum of the large sawtooth profile during T_+ (red particle in Supplementary Fig. 1**a**). While crossing the channel under the influence of F_{ext}, the particle's trajectory deviates from a straight path due to Brownian motion, such that the probability to find the particle at a certain x-position on the dotted line is given by a Gaussian-like distribution $P(x)$. Note that it is the interaction of the particle with the small profile that causes deviations from an exact Gaussian distribution, because diffusion in x -direction is partially impeded.

The distribution P explains how the interaction with the profile causes a mean displacement in $+x$-direction. The parts of P between two adjacent edges of the profile give the probability p_i for a particle to reach the corresponding minimum in between. Reaching a certain minimum causes a lateral displacement to the initial x-position (at the opposite profile). For the chosen asymmetry of the large profile, the resulting mean displacement is positive. Regarding for instance the situation schematically depicted in Supplementary Fig. 1**a**, a displacement to the right is clearly larger as one to the left. However, the corresponding probabilities p_+ and p_- are approximately equal.



Supplementary Fig. 1 Top view on the channel separator, showing in (**a**) the first part of a separation cycle with particles featuring different drift velocities at their start and end points, resp. An external force F_{ext} drags the particles towards $+y$ during this part, which lasts for a time T_+. The second part of the cycle, which is characterized by F_{ext} pointing towards $-y$ for a time T_-, is depicted in (**b**), including the respective start and end points of each particle. The timeline above illustrates the periodic switching of F_{ext} between state (**a**) and (**b**). Diffusion paths and probability densities P to find a particle at a certain position along the dotted lines are schematically depicted. Channel width d, large sawtooth length L, and profile amplitudes h and $h/2$ are indicated.

When the external force changes its direction the particle approaches the small sawtooth, as depicted in Supplementary Fig. 1**b**. In full analogy to the situation at the larger profile, the mean displacement caused by the interaction with the small profile is negative, due to the inverse asymmetry. Since the large profile has twice the spatial periodicity of the small profile, the mean displacement $\langle \Delta x \rangle$ after a full up-down cycle is positive, i.e., the particle is transported to the right ($+x$).

In contrast, we now consider a slow particle, which does not reach the large saw teeth during T_+ (blue particle in Supplementary Fig. 1). Since this particle only interacts with the small saw teeth, its mean displacement after a full cycle is negative, i.e., the particle is transported to the left ($-x$).

For point-like particles and very flat profiles ($h/L \ll 1$), the process can be approximated by a flashing three-state ratchet, where both profiles are represented by sawtooth potentials. A detailed theoretical description of such a ratchet system is given in Refs. 24, 30, including approximate expressions for the mean displacement.

Rescaling the experimental data

In Fig. 4., the time T_+ was rescaled using $t_{\mathrm{cross}} = d/v_{\mathrm{drift}} = 60\,\mathrm{s}$ as a time scale. We measured the drift velocity $v_{\mathrm{drift}} = 2.5\,\mu\mathrm{m/s}$ for the maximal inclination angle of 88°. Note that it takes about 70 s for the stepper motor to switch the maximum inclination from +88° to −88°. As a consequence, we performed observations for $T_+ < 50\,\mathrm{s}$ without reaching the maximum inclination. Hence, the drift velocity was smaller for those T_+. Despite that, $t_{\mathrm{cross}} = 60\,\mathrm{s}$ was used as a timescale for all values of T_+, resulting in small deviations between experimental and numerical results for $T_+/t_{\mathrm{cross}} < 1$. An inclination angle of 45° was reached for the smallest value of T_+.

Dimensionless parameters of the system

The Langevin equation describes the two-dimensional trajectory $r(t) = (x(t), y(t))$ of a particle in the investigated system. It can be written in dimensionless form using the time scale L^2/D, the length scale L and the Peclet number Pe:

$$\dot{\bar{r}} = \mathrm{Pe} f(\bar{r}, \bar{t}) + \bar{\xi}(\bar{t}),$$
$$\langle \bar{\xi}(\bar{t}), \bar{\xi}(\bar{t}')\rangle = 2\bar{\delta}(\bar{t} - \bar{t}'),$$

with D being the diffusion constant of the particle. For a spherical particle, the latter can be derived from the Stokes-Einstein relation $D = k_B T/(6\pi\eta R)$, with η being the viscosity of the fluid and R being the particle radius.

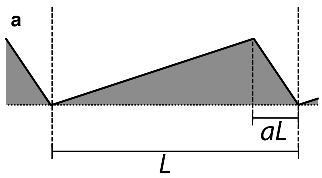
The Peclet number $\mathrm{Pe} = F_{\mathrm{ext}}/(k_B T/L)$ enters the dimensionless description of the system as the ratio between deterministic and dissipative forces. This parameter mainly determines how fast particles reach the minima of the profiles and to what extent they diffuse around the exact position of the minimum. The actual direction and time-dependence of the involved forces, including the external force F_{ext} and the interaction with the walls, is governed by $f(\bar{r}, \bar{t})$. Further, the Brownian motion is caused by a rescaled random force term $\bar{\xi} = \xi(t)L/k_B T$. The latter is unbiased, $\langle\bar{\xi}\rangle = 0$, and the corresponding auto-correlation function is proportional to the rescaled delta-function $\bar{\delta}(\bar{t}) = \delta(t)L^2/D$.

The diffusivity of the particle is given by the dimensionless ratio $\bar{t}_{\mathrm{free}} = t_{\mathrm{free}}/(L^2/D)$, where t_{free} denotes the time during which a particle travels within the channel rather than being in contact with the walls. For particles reaching both sides of the channel, $t_{\mathrm{free}} = t_{\mathrm{cross}}$, otherwise $t_{\mathrm{free}} \approx 2T_+$. Note that $\bar{t}_{\mathrm{free}}$ determines the width of the distribution $P(x)$.

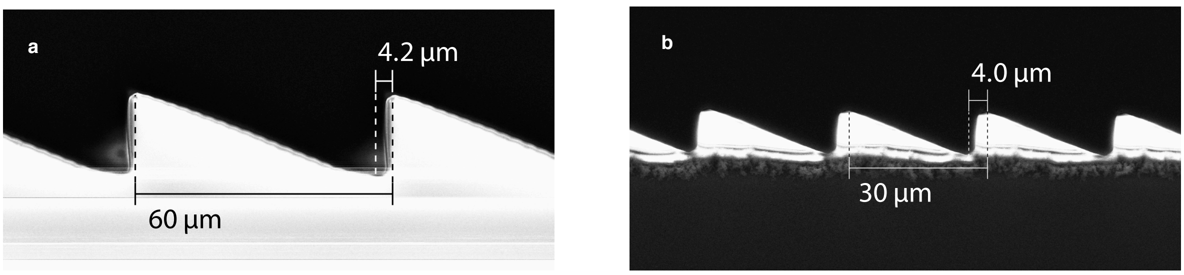
For 3.0 µm particles and the maximal inclination angle of 88°, the investigated system in Fig. 4 features $\bar{t}_{\mathrm{free}} = 0.0024$ and $\mathrm{Pe} \approx 2000$ at room temperature. For the calculation, a viscosity of $\eta = 10^{-3}\,\mathrm{Pa\,s}$ has been used.

Measuring the asymmetry

The asymmetry of the sawtooth profile is defined by the parameter a being the ratio of the base length of the short side to the overall length L, as indicated in Supplementary Fig. 2**a**. Possible values for a range from 0 to 1. Our saw teeth feature rounded tips due to the fabrication process, which involves optical lithography. The intended asymmetry $a = 0$ is thereby changed, as schematically depicted in Supplementary Fig. 2**b**. Using electron microscopy, the effective asymmetry parameter was measured as $a_{\mathrm{large}} = 0.070 \pm 0.007$ for the large profile and $a_{\mathrm{small}} = 0.133 \pm 0.011$ for the small one, as shown in Supplementary Fig. 3. Note that the asymmetry parameter is dimensionless by definition.



Supplementary Fig. 2 (**a**) Definition of the asymmetry parameter a according to the simulated system[24,29]. (**b**) Although designed as $a = 0$, an effective asymmetry parameter evolves due to the smearing out of the tips and minima by the photolithographic process.



Supplementary Fig. 3 SEM images of the large (**a**) and small (**b**) sawtooth profile, lengths measured in µm.

Simulations

We performed two-dimensional Brownian dynamics simulations[30] of a single particle in the described channel device based on the dimensionless Langevin equation. The interaction between the particle and the walls was modeled with a short-ranged repulsive potential V_{wall}. For a reduced particle-wall distance $\bar{r}$ in units of L, the potential is given by $V_{\mathrm{wall}}(\bar{r})/k_B T = (\bar{r}/b)^{-12}$, with $b = 1.25 \times 10^{-3}$. The rescaled geometry parameters of the channel were set to $\bar{h} = 0.7$ and $\bar{d} = 2.5$, according to the experimental values. The asymmetry parameters for the large and small profile were set to $a_{\mathrm{large}} = 0.070$ and $a_{\mathrm{small}} = 0.133$, respectively, and the Peclet number was set to Pe $= 2000$. The simulation has been performed for a range of values for $\bar{T}_+ = T_+/(L^2/D)$, being the rescaled time for the external force to point in $+y$-direction. For the calculation of the

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particles' mean displacement $\langle \Delta x \rangle$, 100.000 up-down cycles have been simulated. For the representation of the simulation results in Fig. 4 the time intervals where rescaled using the equality $T_+/t_{\text{cross}} = \bar{T}_+ \text{Pe}/\bar{d}$.

Downscaling the device

The dimensionless form of the Langevin equation allows us to estimate the duration of one cycle for a downscaled system with identical $\langle \Delta x \rangle$. For that purpose we reduce the spatial extension of the system by a factor of ten, such that $L = 6\,\mu\text{m}$ and $R = 150\,\text{nm}$. Downscaling requires to keep the Peclet number constant in order to achieve equivalent transport properties. In line of this example, the external force needs to be increased by a factor of 10, as $\text{Pe} = LF_{\text{ext}}/k_B T$. One can roughly estimate that the rescaled duration of a full up-down-cycle is $T/(L^2/D) \approx 2\bar{t}_{\text{free}}$ (ignoring the time during which the particle interacts with the profiles). Keeping the dimensionless parameter constant with $\bar{t}_{\text{free}} = 0.0024$, the downscaled length leads to a duration of $T = 0.1$ s for a full cycle.

Legend for Movie 1

Top view on the separation process of silica colloids with 3.0 µm and 4.3 µm diameter in a channel made of PDMS on a glass substrate. The separation is driven by gravitation, hence the large particles move faster than the small ones. The width of a large sawtooth is 60 µm.

The movie has a duration of 4:48 min, which is 8x faster than real time (QuickTime h264-encoded, 6.4 MB).