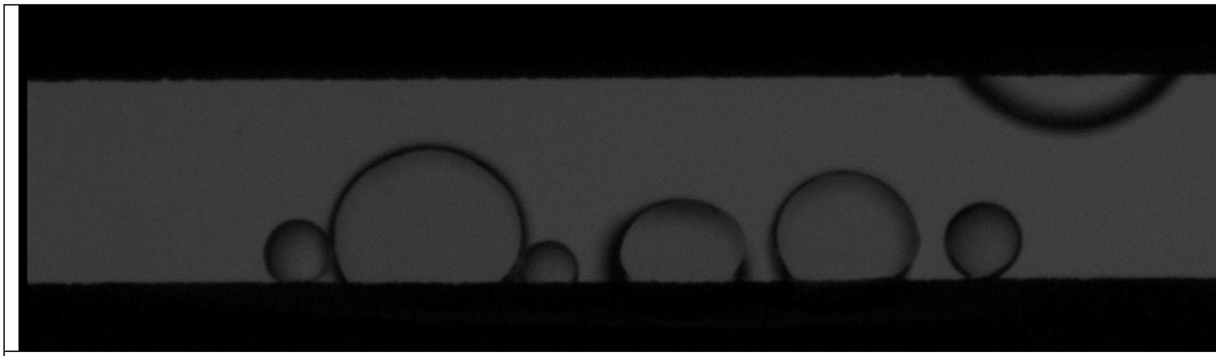

Fig S1. Bubble formation in the microchannel. A microscopic image of a pair of electrodes (black rectangles) inside the microchannel shows bubble formation on the edges of the electrodes that blocks the microchannel when the potential exceeds the redox limit.

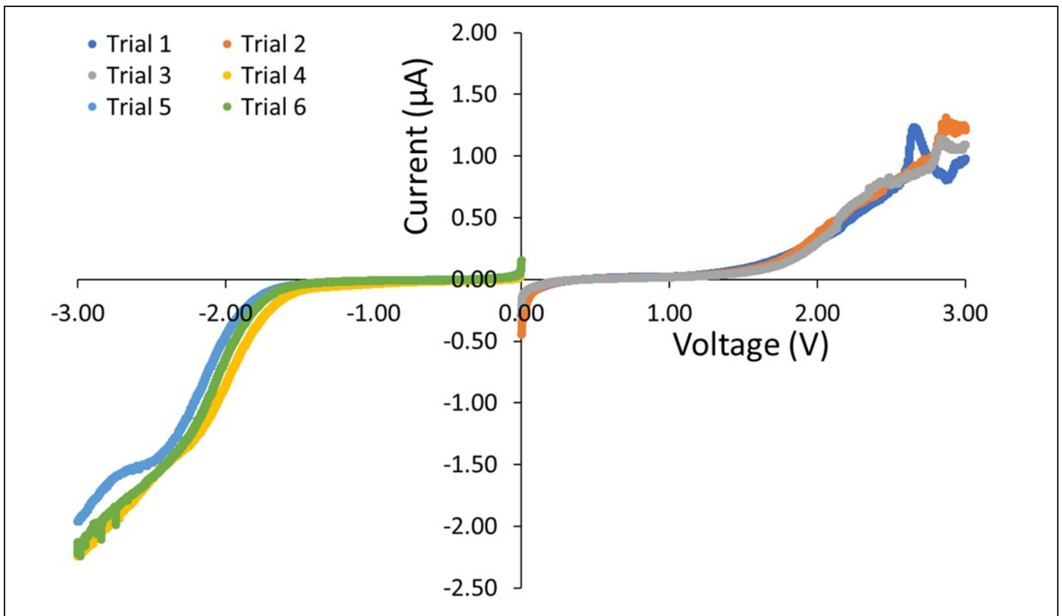

Fig S2. Voltage sweeps of the TrACE device. Voltage from 0 to ± 3V was applied with the sweep rate of 0.01 V/s while monitoring the current. The positive and negative sweeps were each repeated three times.

Nominal diameter (µm)	$\sigma_{\text{measured displacment}}$ (µm)	$\sigma_{\text{measurement noise}}$ (µm)	Experimental diffusion length (µm)	Theoretical diffusion length (µm)
0.53 ± 0.013	0.14 ± 0.03	0.02 ± 0.02	0.12	0.128
1.00 ± 0.016	0.11 ± 0.08	0.04 ± 0.02	0.07	0.092
1.90 ± 0.095	0.10 ± 0.06	0.07 ± 0.04	0.03	0.066

Table S1. Comparison between experimental and theoretical diffusion lengths. Experimental diffusion lengths are recorded from particle displacements in the y-dimension between frames in the suspended particles and the sum of the Brownian motion and the measurement noise. Measurement noise measures particle displacements in a control with stationary particles captured in a thin PDMS layer. Experimental diffusion lengths are the subtracted values between the measured displacement and noise. The time between each frame is 0.01s. Theoretical diffusion length is calculated using $l = \sqrt{2Dt}$, where t = 0.01 s.

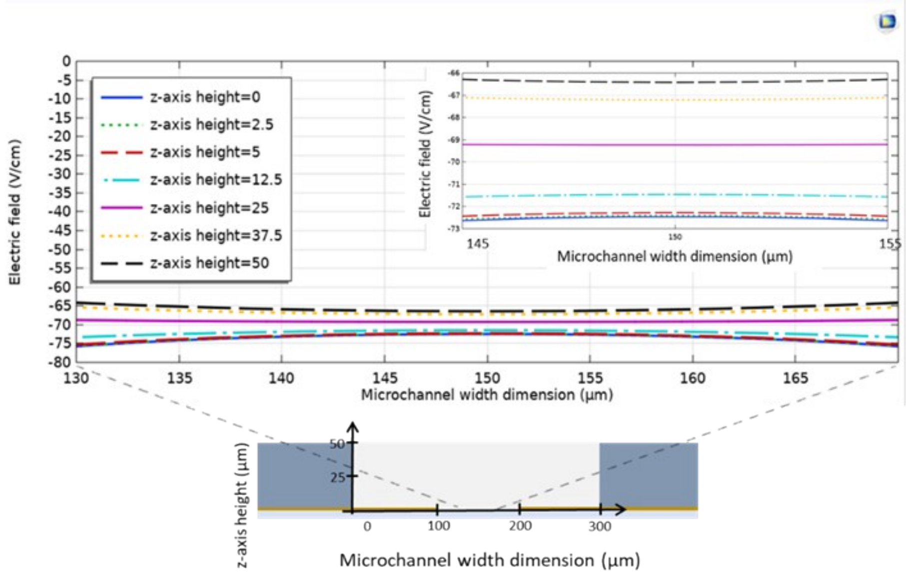

Fig S3. Electric field variation. A finite element analysis model of microchannel cross-sectional to quantify the variation in the electric field along the z-axis height. The width and height of the microchannel is 300 μm and 50 μm respectively, and the electrodes are 100 μm apart from one another on top of the glass substrate. We focus on quantifying the divergence in the electric field in the middle of the microchannel as we only analyze the particles traveling within this region experimentally. The plot is computed with the boundary conditions of one electrode at 1 V and the other at ground.

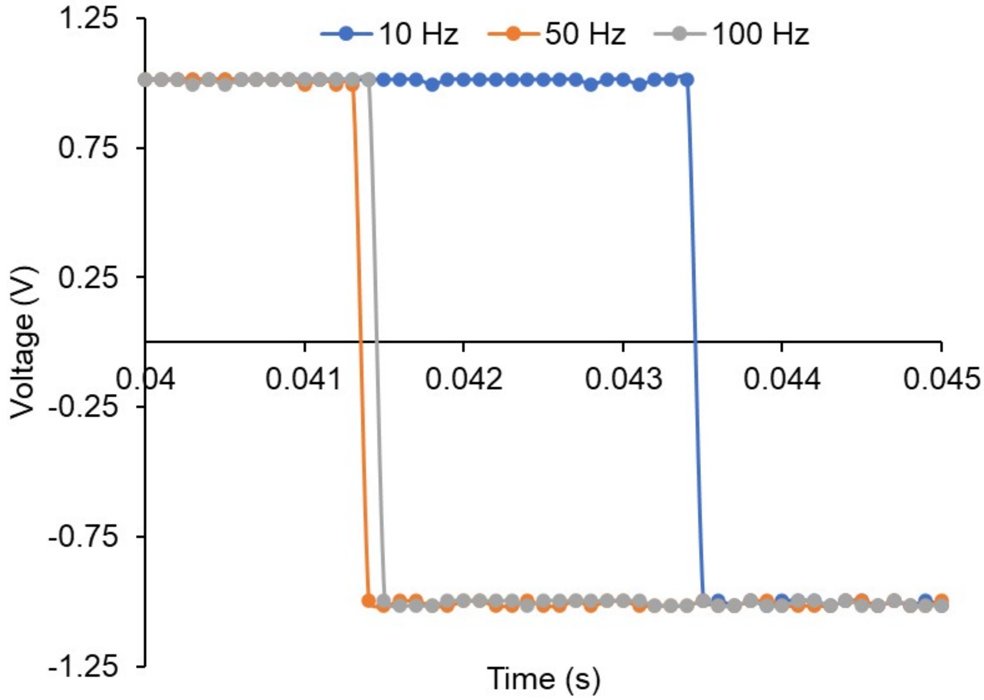

Fig S4. Characterization of applied square waves. Square waves that the function generator applies to the TrACE system are measured using an oscilloscope with a sampling interval of 100 µs (100 X faster than the 100 Hz electric square wave).

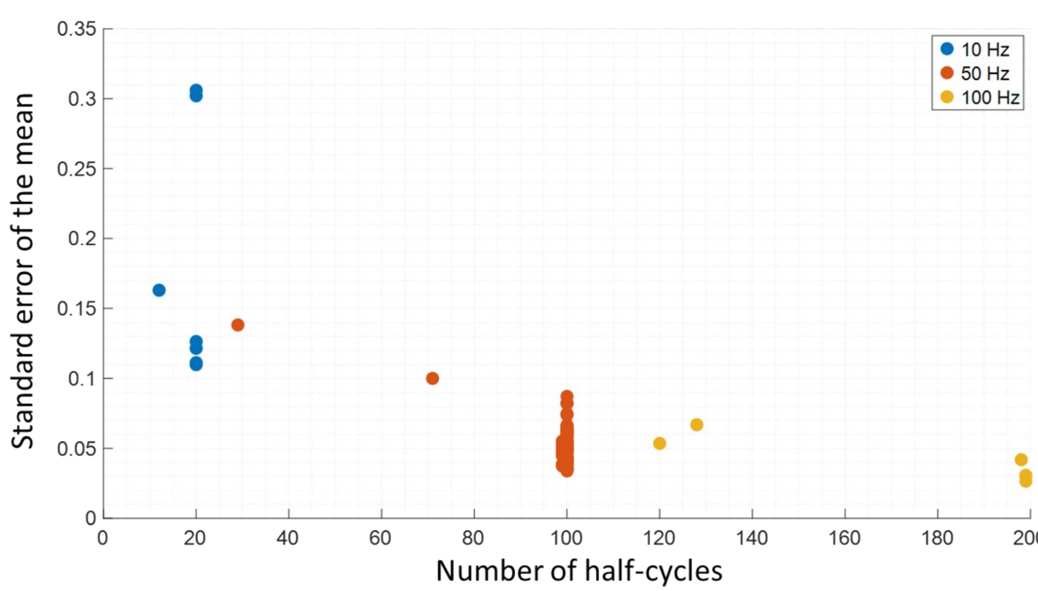

Fig S5. Relation between the standard error of the mean and the number of half-cycles. The number of half-cycles refers to the number of measurements taken on the particle. The standard error of the mean is calculated by dividing the standard deviation by the square root of the number of half-cycles.

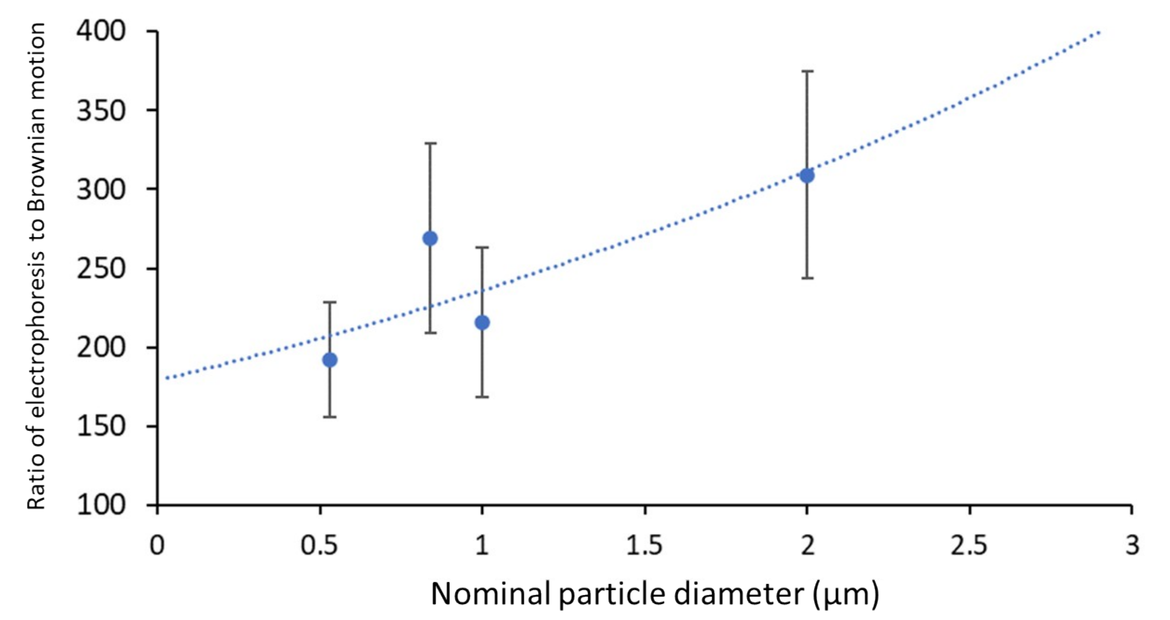

Fig S6. The ratio of electrophoresis to Brownian motion as a function of the particle size. The ratio is calculated using Eq (17) for each particle. The mean ratio for the particle population is plotted, and the standard deviation is represented as the error bars. The large error bars are most likely caused by the inherent variability of the particles in both size and electrophoretic mobility. The exponential curve is fitted to the dataset.

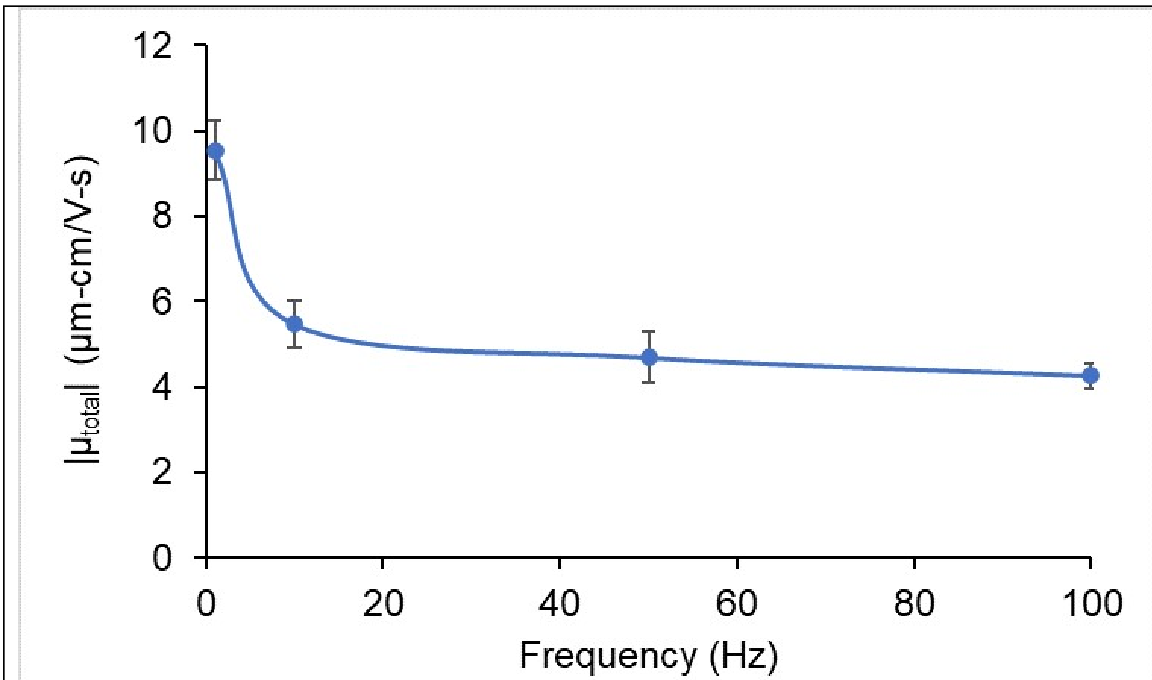

Fig S7. Total measured mobility as a function of electric wave frequency. The observed mobility is the sum of the electrophoretic and electroosmotic mobilities. The electrophoretic mobility is independent of the frequency, but the electroosmotic mobility decreases with increasing frequency. As the change in total mobility approaches zero, the remaining mobility is the electrophoretic mobility. The total mobility of 2 µm carboxyl particles is calculated from the particle's y-displacement at frequencies 1, 10, 50, and 100 Hz. Thus, the electrophoretic mobility measurements at 50 and 100-hz have a negligible contribution from EOF.

	No PVP	1% PVP	2% PVP
Mean electrophoretic mobility (µm-cm/V-s)	-5.2 ± 0.7	-4.8 ± 0.4	-4.9 ± 0.3
Sample size	12	26	21

Table S2. Mean electrophoretic mobility with and without a PVP coating layer. A particle's electrophoretic mobility is measured based on the y-velocity in three different conditions: 1) No PVP in the channel, 2) 1% PVP coating layer in the channel, and 3) 2% PVP coating layer in the channel. The sample size is equal to the number of particles analyzed. Experimental conditions are 0.1mM phosphate buffer at pH 7.4 with the specified PVP concentration. The amplitude of the applied square wave is 1 V amplitude, and the frequency is 50 Hz. The mean electrophoretic mobility is calculated by averaging all the electrophoretic mobilities in each condition. No significant difference in the mean electrophoretic mobility is observed across all three conditions.

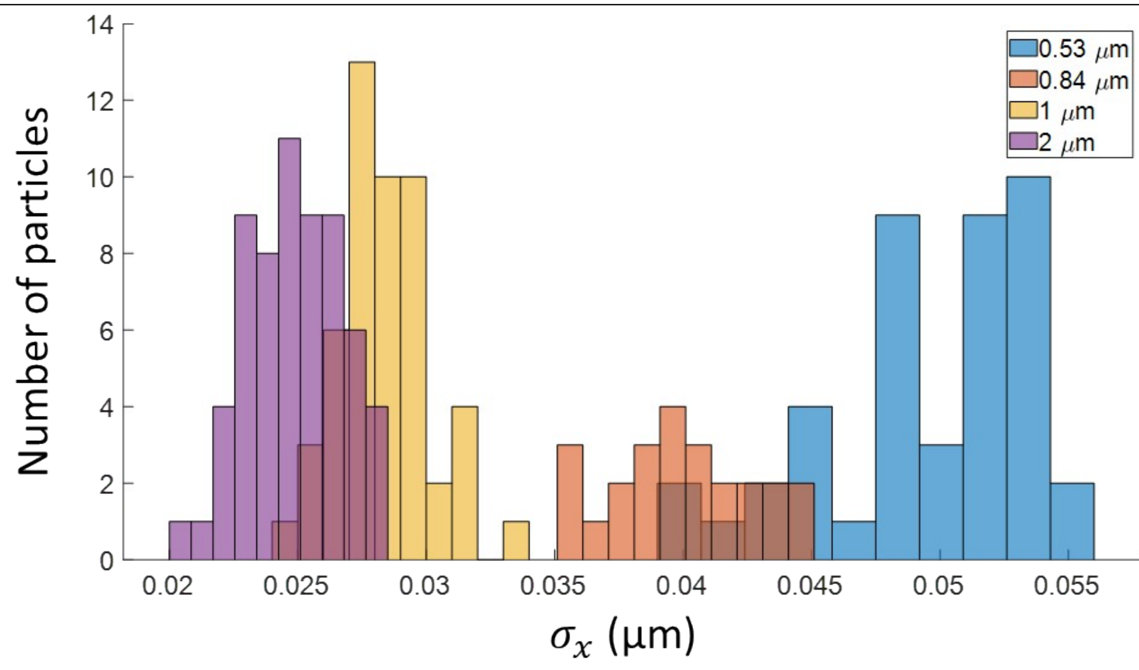

Fig S8. Measured spatial fluctuations (σ_x) as a function of particle size. Spatial fluctuations in the x-dimension are measured for each particle in 0.53, 0.84, 1, and 2 µm particle populations. The histogram shows four distinct normal distributions with peaks at different σ_x.

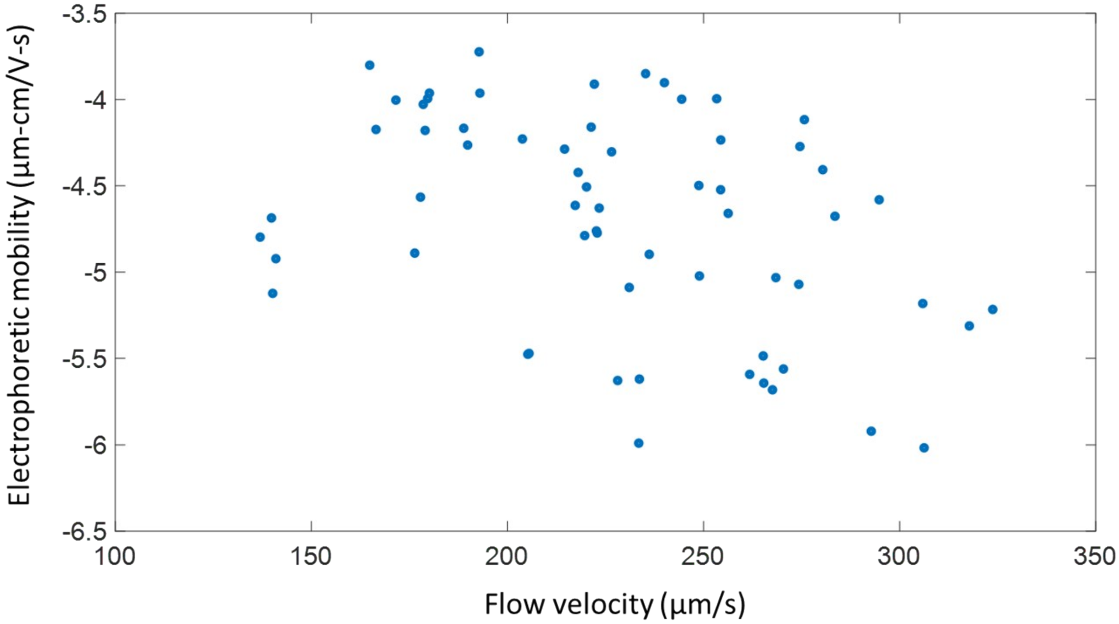

Fig S9. Electrophoretic mobility as a function of flow velocity. Particles' flow velocities are the particles' velocities in the x-direction or along the channel length. Particles' electrophoretic mobilities are calculated from the measured electrophoretic velocities in the y-direction or across the channel width. No relationship between the flow velocity and the electrophoretic mobility is observed in the system. Thus, in TrACE, particle's electrophoretic mobility can be measured independently from the particle's flow velocity.