Refinement of Insulator-based Dielectrophoresis

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The geometries were changed based on altering the shape of the base insulator, adding smaller length scale insulating features to the tops of the base insulators, altering the shapes of the small features, and altering the alignment of the insulators relative to each other (Figure S1 and S2).

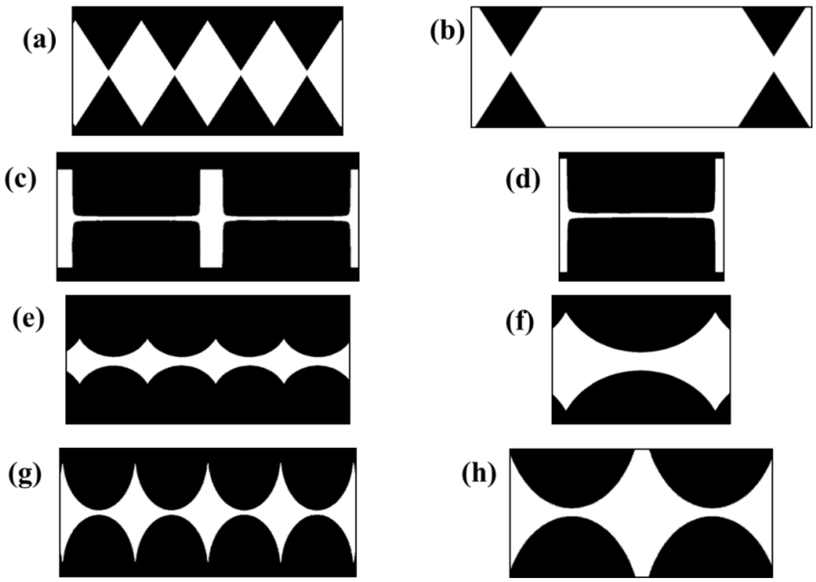

Figure S1 Examples of different base insulators modeled. (a,c,e,g) represent a view of several insulators (b,d,f,h) represent a zoom in of the points of constriction in the microchannel. (a&b) triangle/diamond (c&d) *Inverse 20× Curve* (e&f) circle (g&h) ellipse

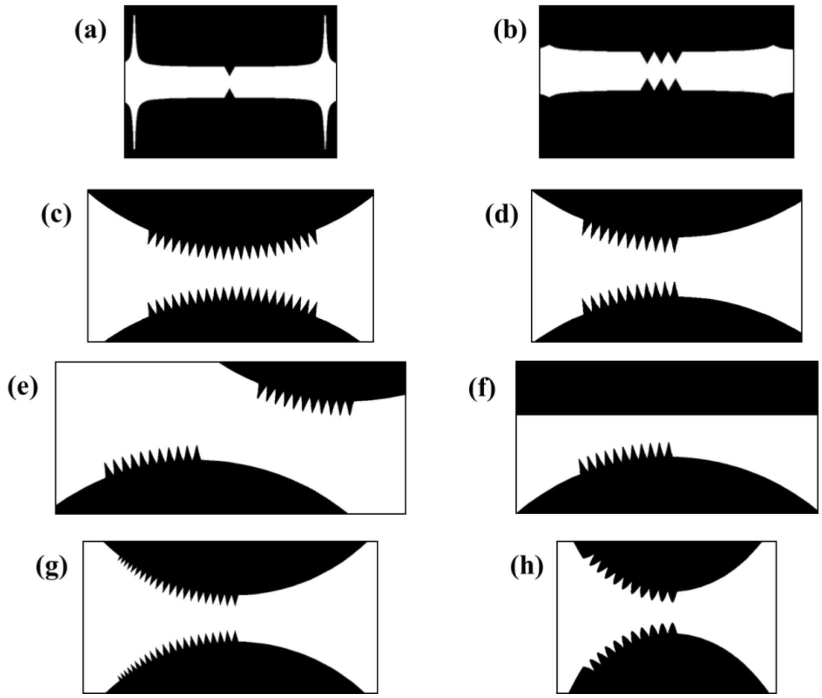

Figure S2 Examples of various insulators tested where all of the small insulators are 20 μm tall at the point of greatest constriction. (a) *Inverse 20× Curve* base insulator with 1 small triangle insulator (b) *Inverse 20× Curve* base insulator with 3 small triangular insulators (c) Circle base insulator with ellipse small insulators across the whole top of the insulator (d) Circle base insulator with ellipse small insulators across half the top, were the last small insulator is the point of greatest constriction. (e) Circle base insulator with ellipse small insulators such that the last small insulators on both side of the microchannel are offs set by 200 μm (f) Circle base insulator with ellipse small insulators where the wall of the microchannel is the other side of the constriction. (g) Circle base insulator with small ellipse insulators that diminish in size the further from the point of greatest constriction. (h) Ellipse based insulator with small elliptical insulators that are inset into the base insulator.

The mathematical derivation of the trapping equation is included below. All equations and variables are as defined in the paper.

Defined knowns:

$$\vec{v}_{EK} = \mu_{EK}\vec{E}$$

$$\vec{v}_{DEP} = -\mu_{DEP}\nabla\left|\vec{E}\right|^2$$

$$\vec{j} \approx C(\vec{v}_{EK} + \vec{v}_{DEP})$$

$$\vec{j} \cdot \vec{E} = 0$$

Therefore combining these equations the following can be stated as a trapping condition:

$$C[\mu_{EK}\vec{E} - \mu_{DEP}\nabla\left|\vec{E}\right|^2] \cdot \vec{E} \leq 0$$

$$\frac{\mu_{DEP}\ \nabla\left|\vec{E}\right|^2}{\mu_{EK}\ E^2} \cdot \vec{E} \geq 1$$

$$\frac{\nabla\left|\vec{E}\right|^2}{E^2} \cdot \vec{E} \geq \frac{\mu_{EK}}{\mu_{DEP}}$$

Design	$\nabla\lvert\vec{E}\rvert^2$ (V^2/m^3) [1]	Streaming [2]	Partial Trapping [3]	Lateral Heterogeneity [4]
Rectangle	6.3×10^{14}	Low/Medium	Medium	Low/Medium
Inverse 20× Curve	4.5×10^{14}	High	Lowest	Low
Circle	8.8×10^{14}	Medium	Low/Medium	Low/Medium
Ellipse	1.9×10^{15}	Medium	Low/Medium	Low/Medium
Triangle	3.2×10^{16}	Low	High	High
Multi-length Scale	1.7×10^{15}	High	Low	Low

Disadvantageous – Advantageous

Table S1 The designs compared in this table were limited to those presented in Figure 3 of the text. The effect of various traits is denoted as advantageous (dark maroon) to disadvantageous (white/pink). A global applied voltage of 500 V was used for all comparisons. **1** The value of $\nabla\lvert\vec{E}\rvert^2$ on the centerline at the first 34.10 µm gate was used. The highest gradient allows for the greatest dielectrophoretic force with the lowest applied global potential and/or the trapping of particles with lesser dielectrophoretic mobilities. **2** Streaming of particles towards the centerline results in a more homogeneous lateral trapping environment in the main trapping zone. This process is imperative to ensure all like-particles experience similar forces regardless of the specific pathline travelled. Designs rated 'high' indicates that particles are forced into a limited lateral zone close to the centerline. 'Medium' rated designs either allowed for limited streaming or indicated a relatively large area of interaction at the trapping area. Designs rated 'low' had limited or no streaming for some pathlines and a large area of interaction in the trapping zone. **3** Images of field lines and $\nabla\lvert\vec{E}\rvert^2$ values were inspected for lines crossing the gradient at acute angles, consistent with trapping. Advantageous designs rated 'high' had electric field lines no matter where in device that the streamlined particles are present they will either all pass a given zone or will experience their trapping ratio and be isolated. Those rated 'medium' indicate designs where the majority will experience trapping zones simultaneously, however small regions exist where analytes present close to the walls of the microchannel will experience trapping before those along the centerline. For the designs rated 'low' particles will experience larger regions close to the walls where a trapping ratio will occur, however analytes along the centerline will not experience this same phenomenon. Variations in the applied potential, constriction size, and trapping ratio will change the presence/amount of partial trapping at a given constriction making analytical comparison impractical. **4** Within the zone of capture, there will be an unavoidable range of values for the capture ratios. For each design, an assessment of the variation of the $\nabla\lvert\vec{E}\rvert^2$ values in the vertical dimension, as show in Figure 3, was performed based on the accessible area to the analytes. The most advantageous designs are rated 'low' and have limited variance across this zone. 'Medium' and 'high" ratings indicated higher variability across this dimension. The increased variability generally resulted from the particles being able to access areas near the insulators where the gradients are necessarily highest.

Video 1 – Streaming Behavior Silica Particles

Streaming Behavior of 2.7 µm silica particles with an applied potential of -600 V. The video has been sharpened in ImageJ and sped up to 3X's the original frame rate. All gates in the video are 20.5 µm, part of the Larger microchannel, and the concentration of particles was 442.0 µg/mL.

Video 2 – Streaming Behavior Polystyrene Particles

Streaming behavior of 2.0 µm yellow-green fluorescent polystyrene spheres with an applied potential of -100 V. The video was speed up from 24 fps to 30 fps, and the color scale was changed from 0-255 to 0-125 so the insulator can be seen. The gate size is 18.7 µm, part of the Analyte microchannel, and the concentration of particles is 4.6×10^7 particles/mL.

Video 3 – Capture Behavior Polystyrene Particles

Capture behavior of 2.0 µm yellow-green fluorescent polystyrene spheres with an applied potential of -400 V. The video was speed up from 24 fps to 30 fps, and the color scale was changed from 0-255 to 0-125 so the insulator can be seen. The gate size is 18.7 µm, part of the Analyte microchannel, and the concentration of particles is 4.6×10^7 particles/mL.