SIMPLE, STRONG, SIZE-SELECTIVE DIELECTROPHORETIC TRAPS FOR SINGLE-CELL PATTERNING

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Abstract
We have developed a novel nDEP trap designed to pattern single cells. We tested the strength of our traps using polystyrene beads and the measurements were found to be in excellent agreement with our modeling predictions. Our DEP traps display a tunable peak size-selectivity behavior, with these traps being optimized to trap particles of ~12 μm. Finally, we have demonstrated use with cells by trapping single mouse fibroblast cells in an array.

keywords: cell patterning, dielectrophoresis, nDEP, size-selective, single-cell manipulation

Introduction
Dielectrophoretic (DEP) traps are an increasingly important method for patterning cells alongside optical tweezers [1], microcontact stamping [2], and electrophoresis [3]. DEP traps for cell patterning can use either negative dielectrophoresis (nDEP)—pushing cells away from the electrodes—or positive dielectrophoresis (pDEP)—pulling cells toward the electrodes. Prior single-cell DEP traps include nDEP octopoles [4], nDEP posts [5], and the pDEP points-and-lid geometry [6]. All of these designs are strong but are either not appropriate for patterning cells [4, 5], would not allow unobstructed cell division [5], are difficult to package [4] or fabricate [5], or cannot be used with normal cell-culture media [6]. The challenge is fabricating a strong trap that allows cell patterning, is planar, and uses nDEP. To meet this challenge, we have designed a planar nDEP trap that is simple, scalable, strong, well-suited for cells, inexpensive to fabricate, and displays size-selective trapping.

Fabrication and Packaging
The DEP traps are formed by patterning gold onto a glass slide. The minimum feature size of the traps is ≥ 10 μm, which allows the use of inexpensive transparency masks for photolithography. An overview of the experimental setup is shown in Fig. 1. The parallel-plate flow chamber was sealed using 4 binder clips for easy assembling and disassembling.

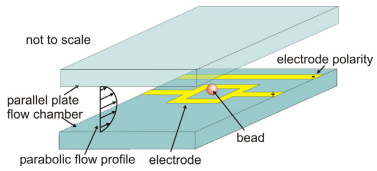

Figure 1: Overview of single-bead nDEP trapping experiments.

Results
To demonstrate the strength of our traps, we used polystyrene beads as model particles. We measured the maximum flow rate that the trapped beads could withstand before getting pushed out of the trap and compared this to predictions generated by our previously described modeling program [5]. We note that the predictions include no fitting parameters. The deviation between the predictions and experiments was ≤14% in all cases (Fig. 2). The strength of these traps is comparable to previous work [4, 5], but with significantly simpler packaging and fabrication.

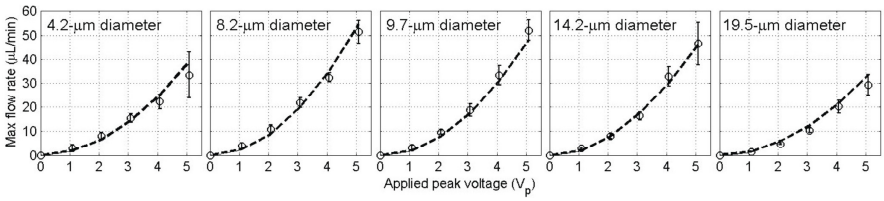

Figure 2: Experimental and simulated results for 5 bead sizes at peak voltage 1-5 V_p. Shown are the predicted values (--), mean (o), and standard deviation ($n \geq 3$ at each voltage).

Using the 5 V_p data of each bead diameter from Fig. 2, we can plot the maximum flow rate as a function of bead diameter (Fig. 3*A*). We note that the bead diameter increases until 12 μm, and then decreases for larger diameters, creating a peak-size selectivity behavior that is optimized for ~12 μm particles. The trapped bead experiences *x*, *y*, and *z*-directed electric fields and ends up getting pushed out of the trap when its center of mass sees the upward *z*-directed electric fields, pushing it up into higher shear flows and out of the trap. The bead remains trapped when the bead center of mass experiences the downward *z*-directed electric fields. The line defining the transition from upwards to downwards *z*-directed electric fields—the stability transition line—was determined from the modeling software and found to have the shape in Fig. 3*B*. Although the trap has strong enough *x*-directed electric fields to resist flow rates ~200 μL/min, it is the upward *z*-directed fields that push the beads out of the trap at lower flow rates. Therefore, this stability transition line is the critical determinant of the peak size-selectivity behavior.

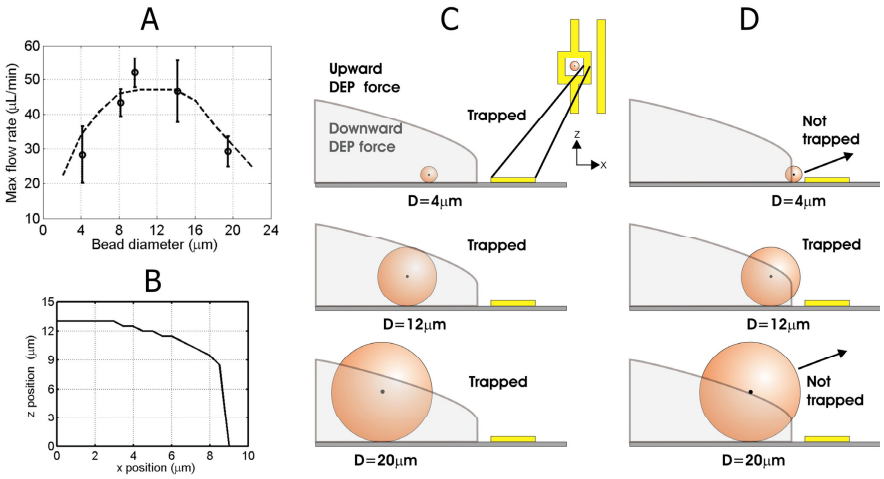

Figure 3: Peak size-selectivity behavior.

The peak size-selectivity behavior occurs because of two effects. First, at low flow rates the electric field geometry pushes larger beads further away from the electrodes (data not shown), allowing them to travel a greater distance before they get to the upward *z*-directed electric fields near the electrodes (Fig. 3*C*). Second, at higher flow rates the beads are pushed to the right, towards the upward *z*-directed fields near the square electrode. The smaller-diameter beads started out closer to the electrodes, so with flow they get pushed near the electrodes and experience the upward *z*-directed fields there (Fig. 3*D, top*). The larger-diameter beads have centers of mass that are high enough to experience the upward *z*-directed fields further away from the electrode (Fig. 3*D, bottom*). The medium-sized beads do not experience the upward *z*-directed fields until higher flow rates, making the trap optimized for them (Fig. 3*D, middle*).

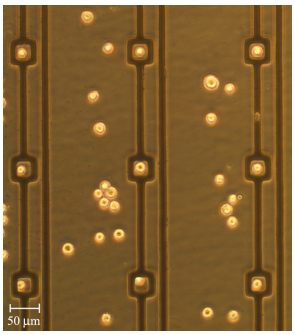

Figure 4: Mouse 3T3 fibroblast cells trapped in an array using the nDEP traps.

Finally, to demonstrate use with cells, traps were used to array single mouse fibroblasts (Fig. 4). Fibroblasts outside the traps have not been washed away and the applied signal was $2V_p$ at 5 MHz.

Conclusion

We have presented a novel DEP trap, offering a unique combination of being simple, planar, nDEP, strong, scalable, and size-selective. Our devices are easy and inexpensive to fabricate and package. Thus, we have provided the foundations for an enabling technology for patterning single cells in a wide range of configurations—allowing us to do novel cell biology experiments that were previously not possible.

Acknowledgments

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