34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Selective *E. coli* Trapping with 3D Insulator-based Dielectrophoresis using DC-Biased, AC Electric Fields*

Phillip A. Zellner[1], Ali Sahari[2], Yahya Hosseini[1], Bahareh Behkam[2], and Masoud Agah[1], *[1]VT MEMS Lab, Virginia Tech, [2]MicroN BASE Lab, Virginia Tech*

Abstract— **We present the development of a batch trapping, insulator-based dielectrophoretic (iDEP) device with three-dimensional design. The microfluidic devices use DC-biased, AC electric fields to selectively manipulate biological particles based on their electric properties. The mold for the polymer microdevices is fabricated using an RIE-lag technique which creates microchannels with varying depths using a single etch process. The resulting three-dimensional insulating constrictions permit operation at low applied voltages. By varying both the applied frequency and the ratio of AC to DC electric fields, the iDEP device can trap and separate polystyrene beads and *E. coli* cells.**

Key Words: dielectrophoresis, particle separation, three dimensional fabrication

I. BACKGROUND

Dielectrophoresis (DEP) is a well-known electric field based technique for separating, moving, and trapping biological particles. DEP devices have been shown to separate and concentrate beads, bacteria, viruses, and mammalian cells [1]. Insulator-based dielectrophoresis (iDEP) uses insulating structures rather than electrode patterns to produce the nonuniform electric fields necessary for DEP. The lack of embedded electrodes makes the devices very inexpensive to fabricate.

iDEP devices typically use either high voltage DC fields or high voltage AC fields to trap particles. Previously DC-biased AC fields have been used to continuously separate polymer beads [2] and to affect DEP trapping at very low frequencies (<2Hz) [3]. In this study, a new 3D iDEP device is used to selectively trap and separate *E. coli* and 1μm polystyrene beads. The 1μm beads were chosen because they are similar in size to E.coli and thus difficult to separate with only DC fields. Both negative and positive DEP was observed over a frequency range of DC to 600kHz. The 3D channel design increases the DEP force allowing for device operation at 200V. To our best knowledge, this is the first use of DC-biased AC fields to selectively trap bacteria.

*Research supported by the NSF CAREER award (ECCS-0747600) and the Virginia Tech Institute for Critical Technologies and Applied Science (ICTAS).

P. A. Zellner, Y. Hossei ni, and M. Agah are with the VT MEMS Lab, Virginia Tech, VA 24060 USA (phone: 757-310-3135 ; fax: 540-231-3362; e-mail: pzellner@vt.edu).

A. Sahari and B. Behkam are with the MicroN BASE , Virginia Tech, VA 24060 USA. (e-mail:sahari@vt.edu).

II. THEORY

Dielectrophoresis is the motion of polarizable particles that are suspended in an electrolyte when subjected to a spatially nonuniform electric field [4]. The DEP force felt by a spherical particle suspended in a medium is:

$$\mathbf{F_{DEP}} = 2\pi R^3 \varepsilon_m \mathrm{Re}[f_{CM}] \nabla |\mathbf{E}|^2 \quad (1)$$

where R is the radius of the particle, ε_m is the permittivity of the medium, **E** is the local electric field. $\mathrm{Re}[f_{CM}]$ is the real part of the Clausius-Mossotti factor which is:

$$f_{CM} = (\varepsilon_p^* - \varepsilon_m^*)/(\varepsilon_p^* + 2\varepsilon_m^*) \quad (2)$$

where ε_p^* and ε_m^* are the complex permittivities of the particle and the medium, respectively. Complex permittivity is defined as:

$$\varepsilon^* = \varepsilon + \sigma/(j\omega) \quad (3)$$

Thus, $\mathbf{F_{DEP}}$ will fluctuate greatly depending on the operating frequency and even change direction. At DC and very low frequency fields, DEP separation of bioparticles is mostly dominated by the size difference of the particles due to the cubed radius term and the low conductivity of celluar membranes. However, at high frequencies, the fields are able to penetrate the cell membrane into the electrically conductive cytoplasm. Thus cells can have a higher DEP frequency dependency than solid particles. If a particle is attracted to a region with a high electric field, it experiences positive DEP (pDEP). Conversely, if a particle is repelled by a region with a high electric field, it experiences negative DEP (nDEP). In order for a particle to become trapped in these devices, the DEP force must overcome the electrokinetic forces. Fluid flow in these devices is driven by electroosmotic flow. Additionally particles move with respect to the fluid due to electrophoresis. The velocity of a particle in the microchannel is:

$$\mathbf{u} = \mu_{DEP} \nabla(\mathbf{E} \bullet \mathbf{E}) + \mu_{EO}\mathbf{E} + \mu_{EP}\mathbf{E} \quad (4)$$

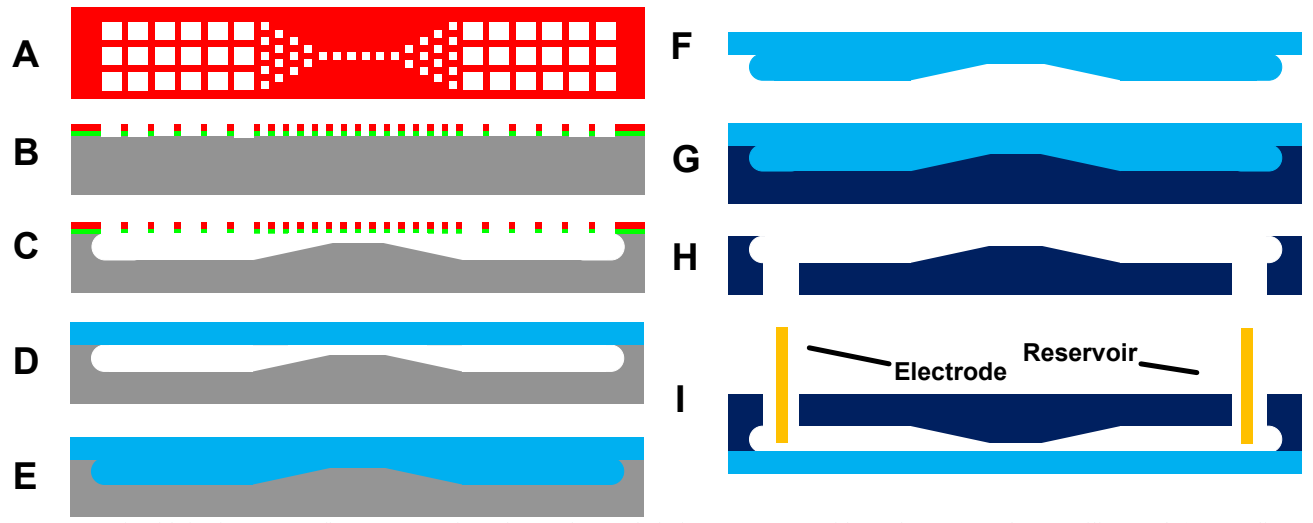

Figure 1 Device fabrication process flow. (A) Top view of DRIE lag mask design (B) Pattern oxide mask (C) DRIE lag 3D silicon etch (D) Anodic bonding of pyrex wafer (E) Melt pyrex into silicon mold (F) Etch all silicon with KOH (G) Pour PDMS over pyrex master and cure (H) Remove PDMS from glass master and punch ports (I) Plasma bond to glass slide, attach reservoirs, and insert electrodes

Where μ_{DEP}, μ_{EO}, and μ_{EP} are the DEP, electroosmotic, and eletrophoretic mobilities. At DC electric fields, electrophoresis, electroosmosis and dielectrophoresis are all significant; as the frequency is increased, the DEP term dominates the equation and only dielectrophoresis is significant [2]. In this work, we use a single AC signal with a DC offset. The DC offset provides motion of particles through the channel as well as some DEP force. The AC electric field provides only a DEP force that is tunable. The ratio of the magnitudes of the applied electric fields is α.

$$\alpha = |E_{AC}|/|E_{DC}| \quad (5)$$

In our device, the insulating 3D posts constrict the current, creating a high electric field between the posts and a low electric field away from the posts. Due to the fluid flow, a particle experiencing pDEP will be trapped on the downstream end of the posts and particles experiencing nDEP will be trapped on the upstream end of the posts. By varying the frequency and α of the applied signal, particles can be selectively trapped based on their geometry and electrical properties. Additionally, the 3D geometry increases the gradient of the electric field by providing a larger change in cross sectional area for current to pass through. This allows for large DEP forces to be generated with low applied electric fields (200V/cm).

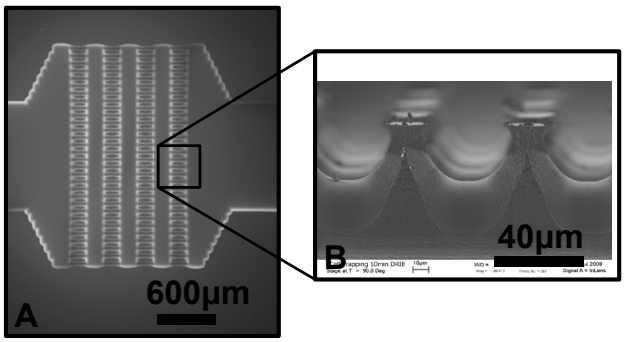

Figure 2 (A) SEM of a iDEP device comprised of 3D microposts. (B) SEM of the cross section of the posts showing the depth change of the structures

III. EXPERIMENTAL PROCEDURE

A. Device Fabrication

Fabrication (Fig 1) starts by depositing and patterning an oxide layer. Silicon is etched isotropically using SF_6 plasma. We utilize RIE lag and our published models [5, 6] to smoothly and arbitrarily vary the depth of the microfluidic channel in a single-etch-step process by changing the geometrical pattern on the mask layout. PDMS devices are fabricated according to our recently developed 3D polymer fabrication technique [7]. Silicon is bonded to a Pyrex wafer under vacuum and then heated to 700°C for 8 hours. The Pyrex substrate is melted and fills the cavities on silicon. Silicon is then completely etched with a KOH solution and the Pyrex wafer is used as a master mold for PDMS. Molded PDMS devices were then bonded to glass substrates after being treating with oxygen plasma. The resulting microchannels are 90µm deep containing microposts spaced 40µm with the depth of 40µm for the constriction region between the posts (Fig 2).

B. Experimental Setup

A function generator (4079, BK Precision) was connected to a power amplifier (EW2350, Tegam) which provided up to $200V_{p-p}$ across a frequency range of DC to 1MHz. The electrodes were placed in the fluid reservoirs, spaced 1cm apart. Experiments were observed using an inverted fluorescent microscope (Axio Observer Z1) and videos of all trapping experiments were acquired using a CCD color camera and a dual band excitation filter. Images were analyzed with ImageJ (NIH). *Escherichia coli (E. coli)* strain MG1655 was grown at 37 °C in LB medium containing 1% tryptone, 0.5% NaCl, and 0.5% yeast. Tetracycline was applied as the antibiotic at 5µg/ml concentration since the strain contained pHC60, a stably maintained plasmid that constitutively expresses GFP useful for fluorescent microscopy analyses. For all experiments, *E. coli* cells and red fluorescent 1µm and 4µm beads (Flourospheres,

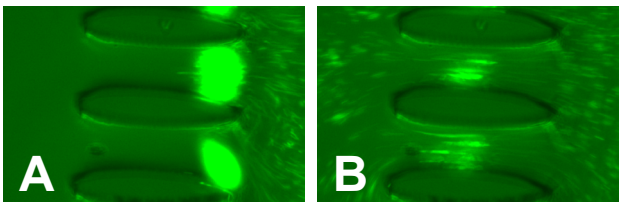

Figure 3 *E. coli* trapping at α =19 and VAC = 190 by (A) nDEP at 2kHz (B) pDEP at 400kHz. Fluid flow is from right to left

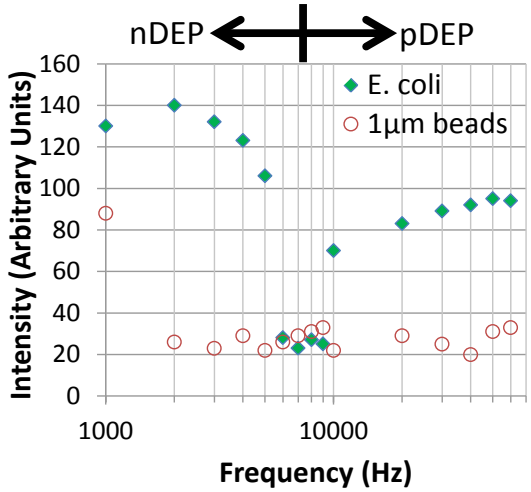

Figure 4 Trapping of *E. coli* and beads as function of frequency 190VACp-p offset by10 VDC (α =19) .

Invitrogen) were suspended in DI water with a measured conductivity of 800µS/m.

IV. RESULTS AND DISCUSSION

A. Characterization of particles

E. coli, 1µm, and 4µm polystyrene beads suspended in DI water were placed in the channel. Particles which are different in size can be separated easily due to the cubed radius term in the DEP force equation 1. For example, with this device, we observed 100% trapping of 4µm polystyrene beads with an applied DC potential of 30V. Both *E. coli* and the 1µm polystyrene beads trapped at DC potentials of 100V. Thus, while the 4µm beads may be separated from *E. coli* with DC fields, the 1µm beads require a more complex separation technique.

The frequency response of the *E. coli* was evaluated by holding α constant at 19 (10 VDC and 190VDC) and sweeping the frequency from 1kHz to 600kHz. Both pDEP and nDEP trapping of *E. coli* was observed (Fig. 3). The observed nDEP trapping was able to trap 100% of the *E. coli* that passed through constriction region; however, the pDEP trapping was much weaker and was only able to trap approximately 20% of the *E. coli*. The trapping was measured after 30 seconds of applied field by quantifying the intensity of the fluorescent in the trapping region of the device (Fig. 4). As seen that in Fig. 4, when no trapping occurred, the background fluorescent intensity observed was between 20-40AU. This resulted neither from particle

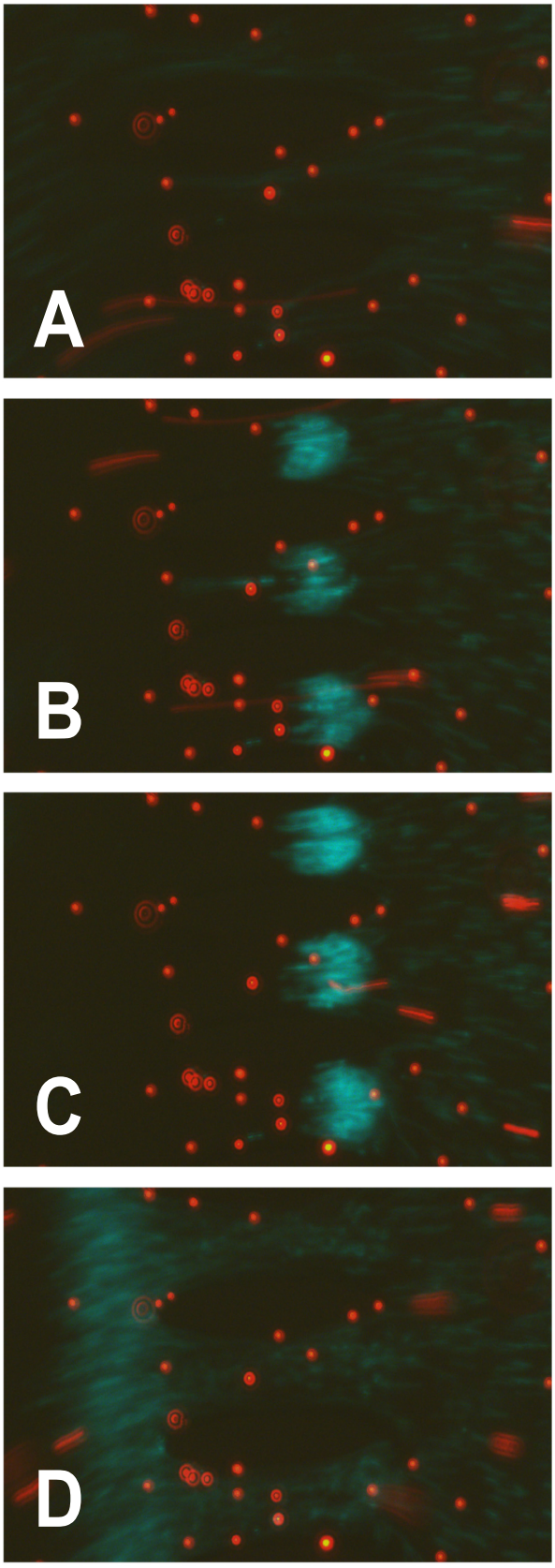

Figure 6 Selective trapping of *E. coli* (green) from red 1µm beads. Fluid flow is from right to left (A) No applied field or fluid flow. (B and C) 180Vpp at 2kHz offset by 20VDC. (D) E.coli are release by turning removing the AC signal

trapping nor fouling but from particles moving through the trapping region. In other words, only intensities above 40 AU indicate DEP trapping. Particles trapped in the upstream half of the constriction were under pDEP while those trapped in the downstream half of the constriction were under nDEP. The *E. coli* showed nDEP trapping below 5kHz and pDEP above 100kHz with no trapping between 6kHz and 90kHz, putting the DEP crossover frequency in this region. The frequency response of the polystyrene beads suspended in DI water was evaluated with the same methodology. Only nDEP of the beads was observed and this trapping occurred at 1kHz (Fig. 4). This indicates that the *E. coli* are more responsive to high α DEP in this frequency range.

B. *Separation of particles*

E. coli and 1µm beads were mixed into a single solution in DI water and placed in the channel. Separation was obtained by fixing the frequency to 2kHz for nDEP and 400kHz for pDEP to maximize *E. coli* trapping. Holding the magnitude of the sum of VDC and VAC constant at 200V, the value of α was varied. After 30 seconds, the intensity of the different colors were quantified in the trapping region. Fig 5a and Fig. 5b show the results for operation at 2kHz and 400kHz, respectively. Again, the background fluorescent intensity was observed to be between 20-40 AU. Thus only values above 40 indicate particle accumulation and trapping. The trapping of all particles increased with decreasing α due to the increased electro-osmotic fluid flow and thus a larger number of particles crossing the trapping region in the 30s timeframe. For both the pDEP and nDEP frequencies, the *E. coli* separated easily from the beads for high values of α. However, for low values of α both *E. coli* and beads were trapped as the DC component of the DEP force became more dominant. If the extreme is considered, when α = 0 and only DC fields are applied, both E. coli and 1um beads are trapped making separation very difficult. It should be noted that all observed trapping of beads was nDEP due to the DC component of the applied signal. While there was some fouling of polystyrene beads in the channel, no beads were observed trapping at α = 9 or 19 (Fig 6). Fig 6 shows the E. coli being release back into the sample, when the AC voltage is removed. Using these operating conditions, it is possible to completely separate the E. coli in a sample from the beads by running the entire sample through the device while trapping E.coli and then running a clean media through the device and releasing the bacteria. These results show the feasibility of selectively trapping bio-particles with either positive or negative DEP in the same iDEP device using DC-biased AC fields. Due to the flexibility, selectivity, economy of fabrication, and portability of these devices, they have the potential to realize critical bioparticle applications such as monitoring drinking water for harmful pathogens. Using a large array of 3D structures in parallel, E.coli and other harmful pathogens could be selectively concentrated from the water sample. The concentrated sample would then be released and detected downstream. The device could be tuned to the particular application by altering the operating frequencies and AC to DC ratio of the applied signal.

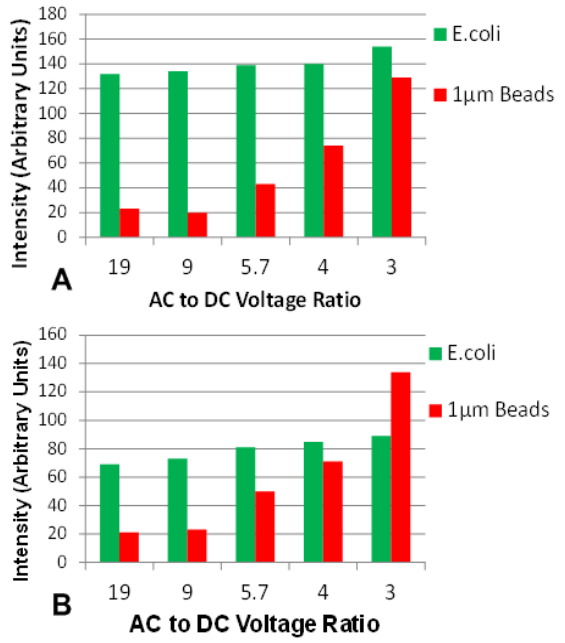

Figure 5 Quantification of selective trapping as a function of α for (A) nDEP at 2kHz (pDEP) at 400kHz

V. CONCLUSION

The presented iDEP device demonstrated the first selective trapping of bacteria using DC-biased AC electric fields. High DEP forces were obtained at low applied voltages using 3D constrictions in the microchannel. It was shown that separation can be maximized by careful selection of both the applied frequency and the ratio of the applied AC and DC fields. Separation was demonstrated with both positive and negative DEP. These results demonstrate the potential of DC-biased, AC field iDEP devices for bioparticle characterization, concentration, and separation.

REFERENCES

[1] S. K. Srivastava, A. Gencoglu, and A. R. Minerick, "DC insulator dielectrophoretic applications in microdevice technology: a review," Analytical and Bioanalytical Chemistry, vol. 399, pp. 301-321, Jan 2011.

[2] B. G. Hawkins, A. E. Smith, Y. A. Syed, and B. J. Kirby, "Continuous-flow particle separation by 3D insulative dielectrophoresis using coherently shaped, dc-biased, ac electric fields," Analytical Chemistry, vol. 79, pp. 7291-7300, Oct 1 2007.

[3] J. L. Baylon-Cardiel, N. M. Jesus-Perez, A. V. Chavez-Santoscoy, and B. H. Lapizco-Encinas, "Controlled microparticle manipulation employing low frequency alternating electric fields in an array of insulators," Lab on a Chip, vol. 10, pp. 3235-3242, 2010.

[4] H. Pohl, Appl. Phys., vol. 22, pp. 869-871, 1951.

[5] K. Gantz, L. Renaghan, and M. Agah, "Development of a comprehensive model for RIE-lag-based three-dimensional microchannel fabrication," Journal of Micromechanics and Microengineering, vol. 18, Feb 2008.

[6] P. Zellner, L. Renaghan, Z. Hasnain, and M. Agah, "A fabrication technology for three-dimensional micro total analysis systems," Journal of Micromechanics and Microengineering, vol. 20, Apr 2010.

[7] P. Z. Y. Hosseini, and M. Agah, "A Single-Mask Process for 3-D icrostructure Fabrication in Glass and Elastomers," presented at the MEMS 2012, Paris France, 2012.