Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Methods for

Electrical Sorting of *Caenorhabditis elegans*

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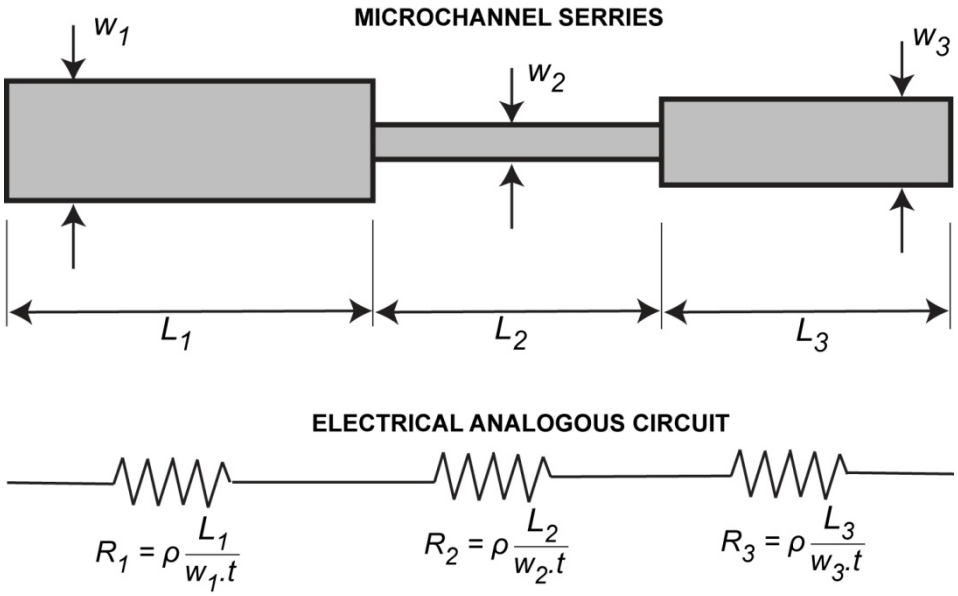
1. Electric field across microchannels

Supplementary Figure 1 illustrates a network of 3 channels arranged in series. The channels have different lengths and widths, but have the same thickness. The electric field (*EF*) across a microchannel with uniform cross sectional area is $EF = V/L$, where V is the voltage drop across that section and L is the length of the channel. The voltage can be obtained from equation (1).

$$V = RI = \frac{\rho L}{A} I = \frac{\rho L I}{wt} \qquad (1),$$

where R is the electrical resistance (Ω), ρ is the electrical resistivity (Ω.m) of the media in the channel, I is the current (A), $A(=wt)$ is the cross sectional area (m^2) of the channel, and w and t are the width and the thickness of the channel (m), respectively.



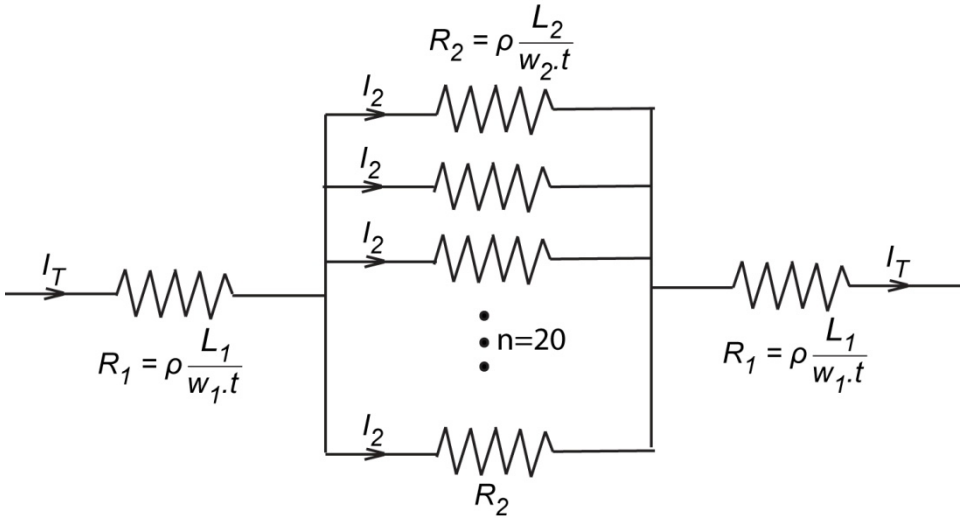
Supplementary Figure 1. A three-channel network and its electrical analog circuit

Considering equation (1), the electric field across each microchannel section (wide-narrow-wide) in series in Supplementary Figure 1 can be calculated from equation (2)

$$EF = \frac{V}{L} = \frac{\rho I}{wt} \tag{2}$$

Equation (2) demonstrates that the ratio between electric fields of two microchannels (same thickness) arranged in series (same current passing through both) and different in width is inversely proportional to their width ratio (i.e. $EF_1 / EF_2 = w_2 / w_1$).

For the case of the continuous sorting device, the electric analogy diagram is illustrated in Supplementary Figure 2. As can be seen, the loading and separation chambers were treated as resistors R_1, and each electric trap as a single resistor R_2.



Supplementary Figure 2. Electric analogous diagram of the continuous sorting device. R_1 and R_2 represent the chambers and individual traps electrical resistances respectively

According to equation (2), the electric field for each chamber (EF_1) and each trap (EF_2) is:

$$EF_1 = \frac{\rho I_T}{w_{chamber}.t}$$

$$EF_2 = \frac{\rho \frac{I_T}{n}}{w_{trap}.t}$$

Therefore, for their ratio we have:

$$\frac{EF_2}{EF_1} = \frac{w_{chamber}}{nw_{trap}} = \frac{8mm}{20 \times 0.1mm} = 4$$

This is in agreement with our presented simulation results in the paper.

2. Sample Loading Methodology

To load individual worms of various stages (n=10) in the single electric trap channel (Article Fig. 1(a)), a syringe was connected to the outlet of the channel and the inlet was dipped into the worm suspension. A suction force was applied to introduce flow into the tubes and the channel. As soon as a worm was visualized in the channel (through the microscope zoomed at the entrance region), the syringe was disconnected and the tubes were leveled to kill the flow. The worm was positioned in the side wide sections.

For the continuous sorting device (Article Figure 1(b)), two sets of loading methods were used (one for individual worm behavior studies and one for separation) since the conditions in this device could be highly different from the single channel device due to the two-dimensional movement possibility provided in the chambers. In order to study individual worm's behavior in the chamber, synchronized stage samples were prepared and diluted in a similar manner described before. A few worms were picked up from the sample suspension using a pipette and loaded into the inlet tube of the device. The outlet tube of the loading chamber was opened to atmospheric pressure and the inlet and outlet tubes of the separation chamber were both closed to prevent flows from the loading chamber to the separation one. Manual pressure heads (by raising the tube for a few centimeters) in the inlet was used to load the worms from the tube into the loading chamber. As soon as a few worms were observed in the chamber (n=5-10), the inlet tube was descended at the same height of the other tubes and the flow was eliminated. The number of loaded worms was counted and a constant electric field (~1-7 V/cm in the chambers) was then applied from the loading chamber electrode towards the parallel electric traps and the separation chamber electrode as illustrated in Article Fig. 1(b). A video was recorded under the microscope and used later to analyze the worms' behavior. The parameter studied here was the number of worms attempting (by inserting 1/3rd of their body into the narrowing section of the electric traps) to move towards the separation chamber through the electric trap.

To characterize the sorting capability of the device, mixed populations (L3, L4, OA, muscle mutant, and neuronal mutant each mixed with YA in a 1:1 ratio) of worms (highly concentrated) were introduced into the loading chamber of the device. In order to achieve the populated loading, the inlet and outlet tubes of the loading chamber were both elevated at the same height in a vertical situation. Both interconnects to the separation chamber were closed. This prevented any hydraulic flow from happening. A fixed volume (60 μl) of the mixed sample was then transferred to the loading chamber inlet tube by using a pipette. The setup was then left for approximately 5 min so the worms were gravitationally forced to descend in the tube and enter the reservoir region of the loading chamber. After this time period, a pressure head between the inlet and outlet tubes of the loading chamber was used to bring the highly populated worm sample from the reservoir into the loading chamber. When the chamber was filled with worms, the inlet tube was lowered to the same height as all other tubes and any additional flow was eliminated. Proper electric fields (based on the results from the device in Article Figure 1(a)) were applied across the device from the loading chamber towards the separation chamber and videos of the sorting were recorded again. In this assay, we counted the number of worms attempting to pass (described before) and the portion of them capable of passing through the electric traps for each loaded worm stage at each applied electric field. After a few minutes that the sorting process was saturated (reduction in rate of passing), additional worms were loaded into the chamber similarly as described above while washing off the sorted animals to the outlet tubes. This was continued until all the loaded worms were experimented and exposed in the device. After sorting, different worms were recognized from their size differences in the age-based sorting experiments. For the case of mutated/wild type sorting, the wild type animals were GFP activated (VH17 strain) before mixing with the mutants. The separated population was collected from the separation chamber, transferred to a plate, and the number of GFP and non-GFP animals was counted under a microscope.

3. Effect of worms' proximity to the positive electrode

One of the main problems encountered in this device was due to the proximity of the Pt electrodes to the electric traps inside the chambers. Even though the worms did not demonstrate viability issues on the range of electric fields tested (0-7 V/cm in the chambers), we observed that at areas neighboring

the Pt wire electrodes, some worms lose their control of motion on higher electric fields (specially above ~5 V/cm) and start being attracted towards the positive electrode. We hypothesis that since the electric field streamlines are converging towards the electrodes at this region, a non-uniform electric field is generated near the electrodes causing dielectrophoresis forces to be exerted on the worms body [1]. This phenomenon could have caused reduction in the attempt rate especially for younger worms (since higher fields are required for their electrotaxis) which has to be considered for more efficient responses and hence better sorting efficiency in the future designs. However, to achieve a satisfactory rate of sorting (enough number of worms entering the chamber and hence enough number of attempts), further studies were conducted using a more populated sample loaded into the device as described in the experimental section. These assays showed promising results in sorting using electric fields in a 0-7 V/cm range as discussed later.

1 Chuang, H. S., Raizen, D. M., Lamb, A., Dabbish, N. & Bau, H. H. Dielectrophoresis of Caenorhabditis elegans. *Lab on a Chip* **11**, 599-604, doi:Doi 10.1039/C0lc00532k (2011).