Supplementary Material (ESI) for Lab on a Chip

Dielectrophoresis of *Caenorhabditis elegans*

Han-Sheng Chuang[a], David Raizen[b], Annesia Lamb[b], Nooreen Dabbish[b], and Haim Bau*[a]

ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Explanation of Movies

Movie S1: DEP-trapped worms of different lengths. The first part of the movie features the motion of a worm whose tail is tethered to an electrode when the worm's body length is shorter than the gap between the electrodes. The second part of the movie illustrates the trapping of a worm whose body length exceeds the gap between the electrodes.

Movie S2: DEP-trapped worm's responses to green and blue light. The worm does not mind the green light while it avoids diligently the blue light.

Movie S3: At the low electric field frequency (5 kHz) and moderate intensity (21 kV/m), the L1 stage worm is partially and reversibly paralyzed when subjected to an electric field. Once the worm leaves the range of the field, it resumes its normal swimming motion.

Preparation of the Microfluidic Chip

The microfluidic chip was made with polydimethylsiloxane (PDMS, Sylgard 184, Ellsworth Adhesives) and a glass slide (plain microscopic slide, 76.2×25.4×1 mm, Fisher Scientific). Gold electrodes were patterned on the glass slide using the lift-off technique. Briefly, positive photoresist S1813 (Shipley) was spin-coated on the glass at 2000 rpm for 25 s and then exposed to 365 nm UV light with a transparency mask for 15 s. Subsequently, the patterns were developed with developer MF-319 (Microposit) for 1 min. The developed glass slide was baked at 60 °C for 5 min on a hotplate. Then, 20 nm thick chromium (Cr) film was deposited on the glass with an e-beam evaporator to form an adhesive layer. A 150 nm thick gold (Au) film was deposited on top of the adhesive layer using the e-beam evaporator. The glass with the metal

films was then immersed in acetone in an ultrasound bath to dissolve the photoresist and obtain the electrode patterns depicted in Fig. S1. Two different electrode patterns were fabricated: spiked electrodes (Fig. S1a) separated by a distance of 167 μm and flat electrodes (Fig. S1b) separated by a distance of 330 μm.

The flow conduit was formed in PDMS. Standard photolithography was used to create a mold with negative photoresist SU8 on a 3" silicon wafer. The thickness and width of the straight conduit were, respectively, 118 μm and 300 μm. The total length of the conduit was 17 mm. PDMS mixture was prepared with prepolymer and cure agent in the ratio of 1:9 and then baked on a hot plate at 70 °C for 2 hours. The cured PDMS was peeled off from the wafer and cut into the desired size. A flat-tipped needle was employed to puncture holes at both ends of the conduit to form an inlet port and an outlet port. The PDMS slab was placed on the top of the glass slide with the patterned electrodes and aligned under a microscope so that the line connecting the spikes was parallel to the axis of the conduit and located at the midwidth of the conduit. The PDMS slab was then clamped to the glass slide. Permanent bonding of the PDMS slab to the glass was not necessary as the liquid in the system was not subject to significant pressure.

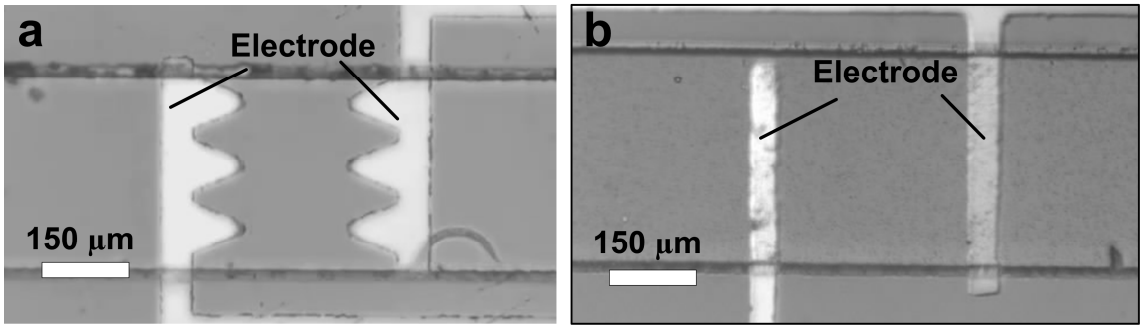

Fig. S1: Electrodes (white color) patterned on the bottom, glass surface of a straight conduit. The conduit was molded in a PDMS. (a) Spiked electrodes; (b) Flat electrodes.

Preparation of *C. elegans*

Worms were cultured and handled as previously described[1]. Worms were cultivated at 20°C on NGM agar plates and fed the streptomycin-resistant *E. coli* strain DA837[2]. *C. elegans* Bristol strain N2 was used as wild-type. In order to obtain a synchronized population of worms for an experiment, a just starved plate of worms was subjected to alkaline bleach egg preparation, and the isolated eggs were allowed to hatch for 12 hours in M9 Buffer. L1s, L3s and 1-day old

adults were obtained by picking worms 6 hours, 28 hours, and 72 hours, respectively after plating the arrested L1 worms onto food. Prior to DEP experiments, worms were kept at room temperature 21°C-24°C for up to 8 hours.

Experimental Setup and Methods

Dielectrophoretic Trapping

Experiments were carried out both with AC and DC electric fields. The AC field was generated with a function generator (CFG250, Tektronix) and a power amplifier (Power Amplifier, E&I). AC sinusoidal electrical potentials of various frequencies and magnitudes were applied across the electrode pair.

The DC electric field was generated with two electrical wires inserted in the inlet and outlet ports. DC field was not applied across the patterned electrodes to avoid corroding the electrodes. The DC potential was supplied with a DC power supply (TPS-4000, Toward Electric Instruments).

The conduit was initially filled with deionized (DI) water, which had a measured conductivity of 1×10^{-3} S/m. Worms from a synchronous culture were transferred with minimal bacteria from the culture dish and placed in the inlet of the microfluidic chip. We found that worms could stay alive and healthy in the non-buffer environment for at least 24 hours. Gentle flow (~0.4 mL/hr) was generated by a syringe pump to guide the worms towards the electrodes. The guided flow was switched off when the electric field was applied. The observations were carried out with a single worm in the vicinity of electrodes. Once a set of experiments has been concluded, the worm was released, washed away, and replaced with a new worm. Occasionally, we observed random, background flow due to the imbalanced pressure between the inlet and outlet ports. All experiments were carried out after the background flow nearly subsided.

Photophobic Behavior

An upright microscope (BX51, Olympus) equipped with a high speed CCD camera (1600, PCO) was used to record the worms' behavior in the presence of the electric field. The magnifications of the objectives were 4x and 10x. Two filter cubes, RSGFP (Exciter: HQ480/Dichroic:Q505p/Emitter:HQ535) and TRITC (Exciter: HQ545/Dichroic: Q570LP/Emitter: HQ610), were used to generate different wavelengths to prompt worms'

photophobic responses. Fig. S2 depicts the time fraction that the worm's head (n=3) stayed in the region illuminated by the green light and the blue light (see also Movie S2). The movies were analyzed frame by frame to obtain the worm's position. The measurements started after a few seconds when the worms perceived the light. Trapped worms show significant photophobic responses turning their heads away from the blue light while remaining poised under green light illumination.

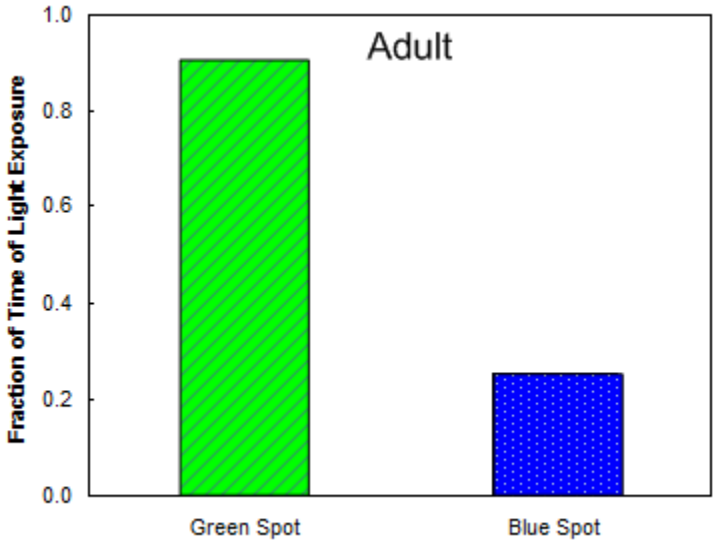

Fig. S2: The fraction of the time that the trapped worm's head (n=3) stayed exposed to green (green bar) and blue (blue bar) light.

Quantification of Worm's Activity

The worm's undulatory motion was quantified by computing the variance of the worm's undulations. The worm's motion was imaged for a time interval of length, τ. In each frame, we connected the tip of the worm's head (**H**) and tail (**T**) with a straight line (**HT**) and denoted the distance between the line **HT** and the center of the worm's body as $b(s,t)$, where s is a coordinate along the line **HT**. The video frames were averaged to obtain the average position of the worm's body $\bar{b}(s) = \frac{1}{\tau}\int_0^{\tau} b(s,t)dt$. The variance of the worm's undulations is estimated as

$$Var(b)=\frac{1}{\tau L^3}\int_0^{\tau}\int_0^{\lambda}\left(b(s,t)-\bar{b}(s)\right)^2 dsdt\ . \qquad \text{(S1)}$$

In the above, L is the worm's body length, λ is the distance between the head and tail and we carried out the calculations only for the cases of relatively small undulations ($\underset{s}{Max}\left(\frac{b(s,t)}{\lambda}\right)<<1$). When the worm is completely paralyzed or dead, $Var(b)\approx 0$.

A Simple Estimate of the Polarization Forces Acting on the Worm

To obtain a rough estimate of the electric forces that act on the worm, we approximate the worm as a dielectric, homogeneous ellipsoid encapsulated with shells (Fig. 1c and Fig. S3). Similar models have been used to estimate the polarization forces acting on biological cells,[3, 4] The three layer model is the simplest model capable qualitatively of predicting the behavior observed in the experiments and it is consistent with prior studies of the [5] of the worm's anatomy.[5] We use the subscripts, *c*, *m*, *w*, and *l*, to represent, respectively, the permittivity of the worm's inner cavity (which models the pseudocoelomic space.), the worm's inner membrane (which models the hypodermal cell membranes), the worm's cuticle, and the suspending medium. The Clausius-Mossotti factor associated with the long axis of the multi-layered ellipsoid is[6-8]:

$$K(\omega)=\frac{\left(\varepsilon_w^*-\varepsilon_l^*\right)+X\rho_2\left[\varepsilon_w^*+A_3\left(\varepsilon_l^*-\varepsilon_w^*\right)\right]}{\left[\varepsilon_l^*+A_3\left(\varepsilon_w^*-\varepsilon_l^*\right)\right]+X\rho_2 A_3\left(1-A_3\right)\left(\varepsilon_w^*-\varepsilon_l^*\right)}. \qquad \text{(S2)}$$

In the above, the X for a live worm is

$$X_{alive}(\omega)=\frac{\left(\varepsilon_w^*-\varepsilon_l^*\right)+Z\rho_1\left[\varepsilon_w^*+A_2\left(\varepsilon_l^*-\varepsilon_w^*\right)\right]}{\left[\varepsilon_l^*+A_2\left(\varepsilon_w^*-\varepsilon_l^*\right)\right]+Z\rho_1 A_2\left(1-A_2\right)\left(\varepsilon_w^*-\varepsilon_l^*\right)} \quad ; \quad Z(\omega)=\frac{\varepsilon_c^*-\varepsilon_m^*}{\varepsilon_m^*+A_1\left(\varepsilon_c^*-\varepsilon_m^*\right)} \quad ;$$

$\varepsilon_k^*=\varepsilon_k-i\sigma_k/\omega$ is the complex permittivity, where k =*c*, *m*, *w*, and *l*.

The geometric factors, A (depolarizing factor) and e (eccentricity), are expressed as

$$A_1=\frac{1-e_1^2}{2e_1^3}\left[\ln\left(\frac{1+e_1}{1-e_1}\right)-2e_1\right] \quad ; \quad e_1=\sqrt{1-\left(\frac{b_c}{a_c}\right)^2} \quad ; \quad A_2=\frac{1-e_2^2}{2e_2^3}\left[\ln\left(\frac{1+e_2}{1-e_2}\right)-2e_2\right] \quad ;$$

$$e_2=\sqrt{1-\left(\frac{b_m}{a_m}\right)^2} \quad ; \quad \rho_1=\frac{a_c b_c^2}{a_m b_m^2} \quad ; \quad A_3=\frac{1-e_3^2}{2e_3^3}\left[\ln\left(\frac{1+e_3}{1-e_3}\right)-2e_3\right] \quad , \quad e_3=\sqrt{1-\left(\frac{b_w}{a_w}\right)^2} \quad , \quad \text{and}$$

$\rho_2 = \frac{a_m b_m^2}{a_w b_w^2}$, where a and b are, respectively, the lengths of the long and short semi-axes of the various ellipsoids (Fig. S3);

The dead worm's membrane is assumed to lose its capacitance due to irreversible permeation. Thus, the expression for X in the case of the dead worm is replaced with

$$X_{dead}(\omega) = \frac{\varepsilon_c^* - \varepsilon_w^*}{\varepsilon_w^* + A_2\left(\varepsilon_c^* - \varepsilon_w^*\right)}.$$

The final DEP force acting on the ellipsoid along its long axis is

$$F_{dep} = \varepsilon_l V \,\mathrm{Re}\left[K(\omega)\right]\nabla\left|\vec{E}\right|^2, \qquad \text{(S3)}$$

where $V = \frac{4\pi a_w b_w^2}{3}$ is the volume of the ellipsoid. To obtain the dielectrophoretic forces in other directions, one would need to use alternative expressions for the Clausius-Mossotti factor, which we do not produce here. AC electroosmotic flow was found to be insignificant in the trapping phase due to high frequencies[9, 10].

Since the dielectric properties of the worm are not available, as rough estimates, we adapted, with some modifications, values reported in the literature for yeast[3] and *E-coli*[11]. The magnitudes of the permittivities and conductivities used in our calculations and the ones reported in the literature for yeast cells and bacteria are documented in Table S1. Using the values documented in table 1 and assuming the maximum $\nabla \mathbf{E} \bullet \mathbf{E}$ in our experimental set-up, we estimate that the order of magnitude of the force acting on an adult worm is around hundred nano Newtons. The above is likely an overestimate of the actual DEP force since it assumes polarization along the long axis of a rigid worm.

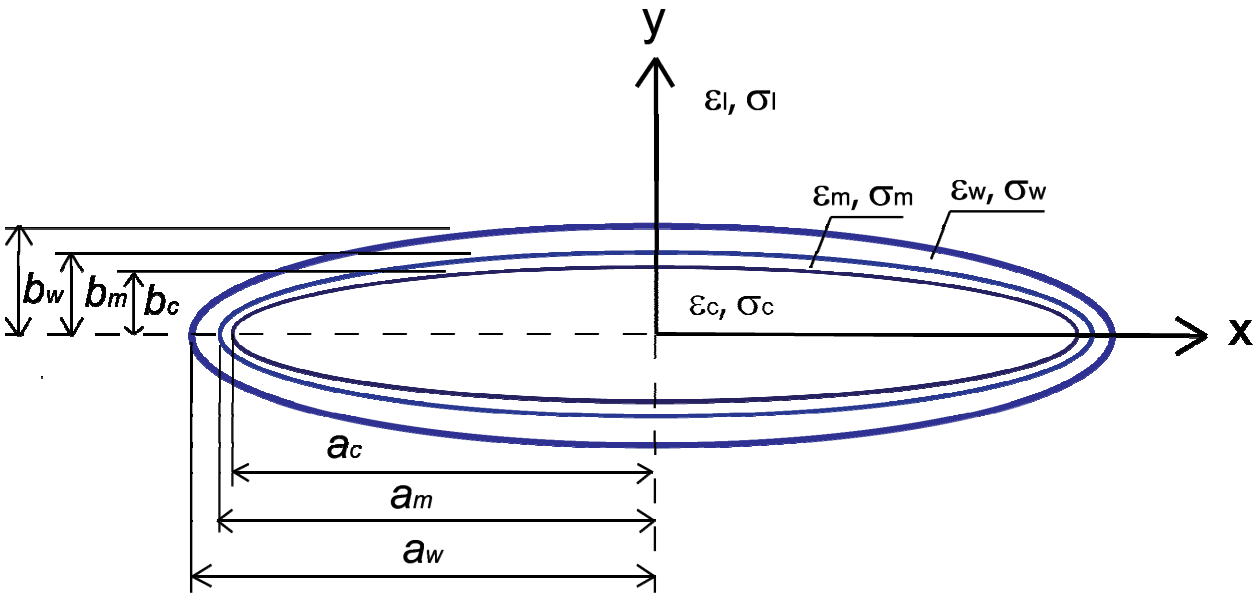

Fig. S3: DEP Model of the worm - an ellipsoid surrounded with multiple shells

Table S1: The electrical properties used in our DEP model of the worm and corresponding values reported in the literature for yeast cells[3] and *E-coli* bacteria[11].

Variable	σ_c (S/m)	σ_m (S/m)	σ_w (S/m)	σ_l (S/m)	$\varepsilon_c/\varepsilon_0$	$\varepsilon_w/\varepsilon_0$	$\varepsilon_l/\varepsilon_0$	$\varepsilon_m/\varepsilon_0$
Ours	0.5	1×10^{-6}	0.08	0.001	50	65	80	10
Jones[3, *]	0.5	n/a	0. 1	0.001	60	65	78	n/a
Mietchen et al.[11, **]	0.5	1×10^{-6}	0.007	0.001	50	77	80	8

* Yeast cell. ** *E coli* bacterium.

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