Supporting Information for

Focusing of mammalian cells under an ultrahigh pH gradient created by unidirectional electropulsation in a confined microchamber

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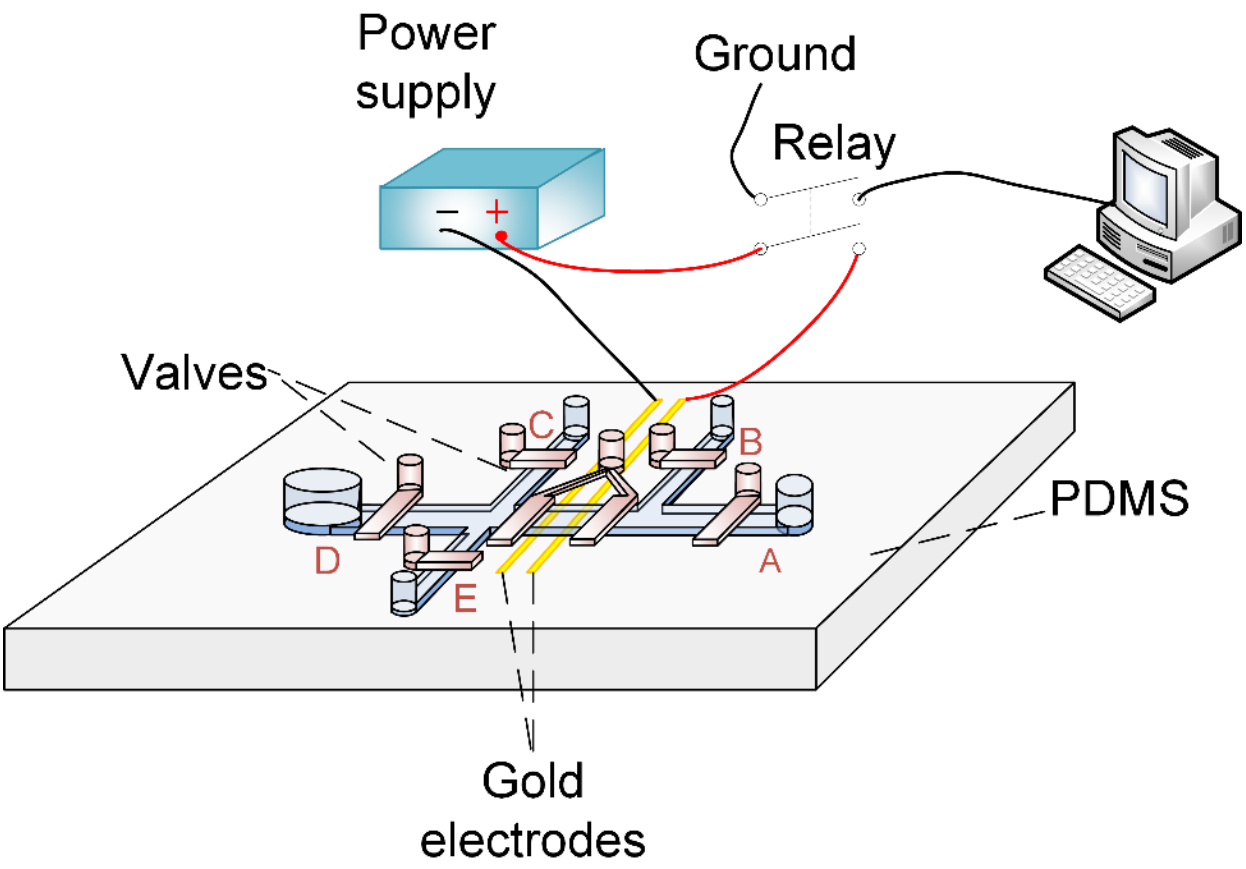

Figure S1. The design and setup of the entire microfluidic chip. The microfluidic device is made of PDMS and consists of two layers (fluidic layer in blue and control layer in pink). The fluidic layer has 3 inlets (A, B, C) and 2 outlets (D, E). All these ports can be closed by the microvalves that are adjacent to them. By selective closing of valves, the cells initially flow into the device from inlet B to outlet E. After the microfluidic chamber is populated with cells, the two valves close to the electrodes close to seal the chamber. Then buffer flows from E to C, and from A to B, to flush out the cells that are not in the electropulsation chamber. All the valves are closed during electropulsation. Two surface gold electrodes are in the electropulsation chamber, and connected to a circuit including a dc power supply and a relay. A computer is used to control the frequency and the duration of the pulses.

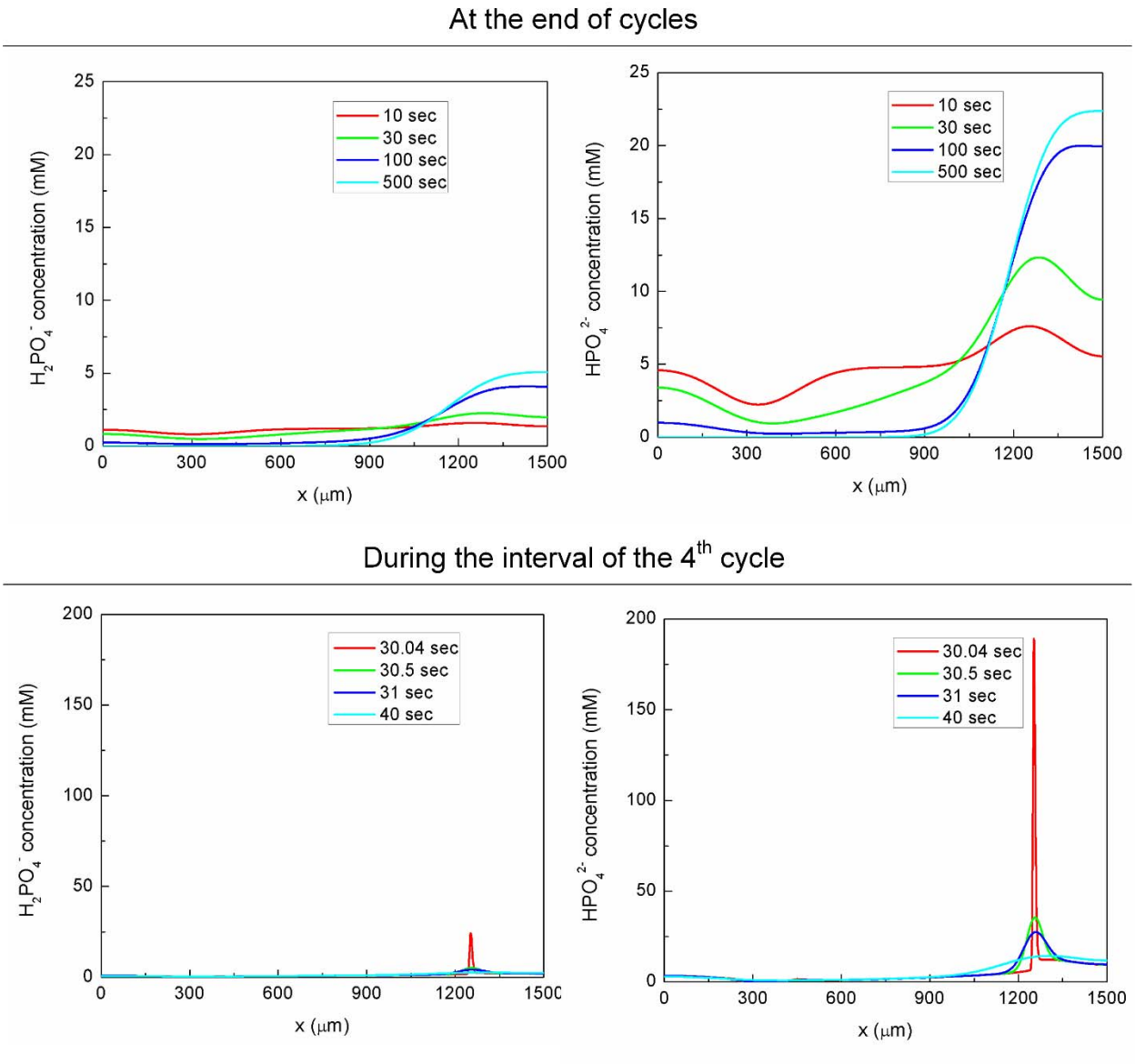

Figure S2. The modeled concentration profiles of $H_2PO_4^-$ and HPO_4^{2-} during the process of electropulsation. To simplify the modeling, we consider only electrophoresis and diffusion of the ions in the buffer (Na^+, K^+, $H_2PO_4^-$ and HPO_4^{2-}) for the solution containing 4.8 mM Na_2HPO_4, 1.2 mM KH_2PO_4 without involving the proton and hydroxide production. The pulses (1000 V/cm) are applied once every 10 s and last for the first 30 ms of each 10 s cycle. The cathode is located at x=130-250 μm, the anode is located at x=1250-1370 μm, and both electrodes are 120 μm wide. At the end of the 4th cycle, 71% of $H_2PO_4^-$ and 83% of HPO_4^{2-} are at the proximity of the anode (within a 300 μm distance along x).

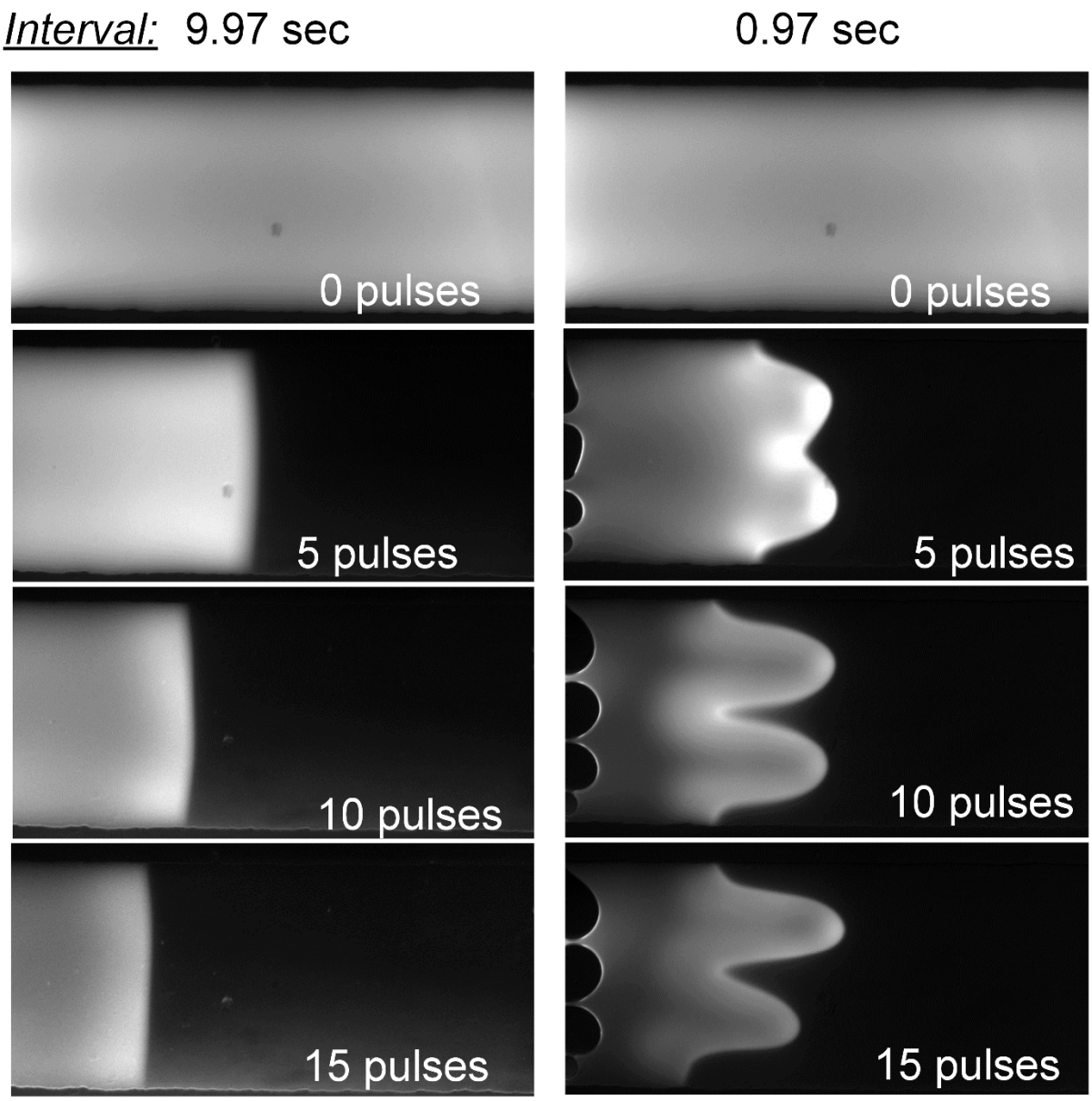

Figure S3. Time-lapse images of the buffer (4.8 mM Na_2HPO_4, 1.2 mM KH_2PO_4, and 250 mM sucrose) containing fluorescein during electropulsation (50 pulses of 30 ms duration and 1000 V/cm intensity). The left panel was taken when the interval was 9.97 s between pulses and the right panel had intervals of 0.97s.

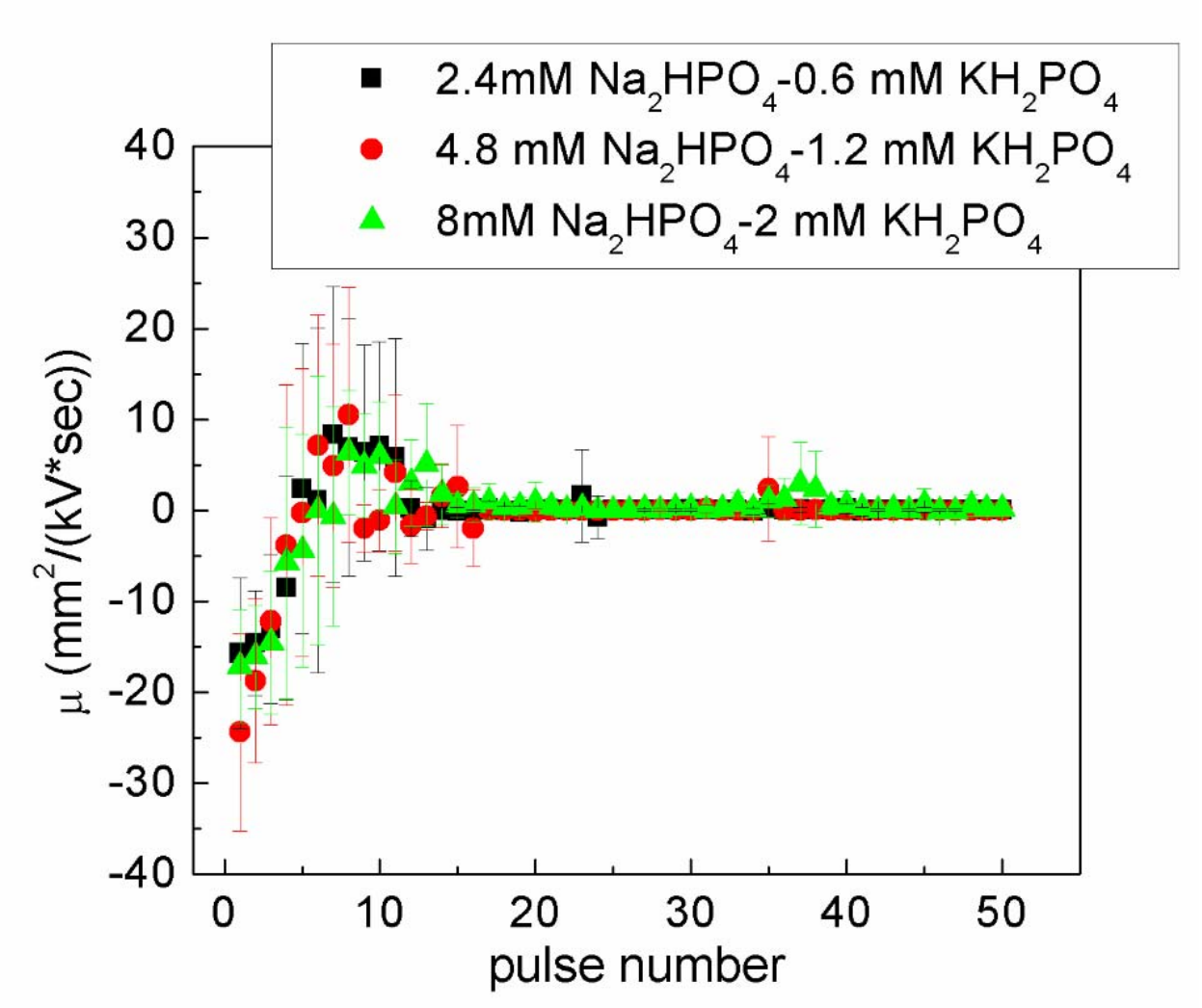

Figure S4. The average mobility of cells during each pulse for experiments performed in buffers of various ion concentrations. Three different buffers containing 2.4 mM Na_2HPO_4-0.6 mM KH_2PO_4, 4.8 mM Na_2HPO_4-1.2 mM KH_2PO_4, and 8 mM Na_2HPO_4-2 mM KH_2PO_4, supplemented with 250 mM sucrose in all cases, were tested. The electropulsation was conducted by applying 50 pulses with 1000 V/cm intensity, 30 ms duration, and 9.97 s intervals.

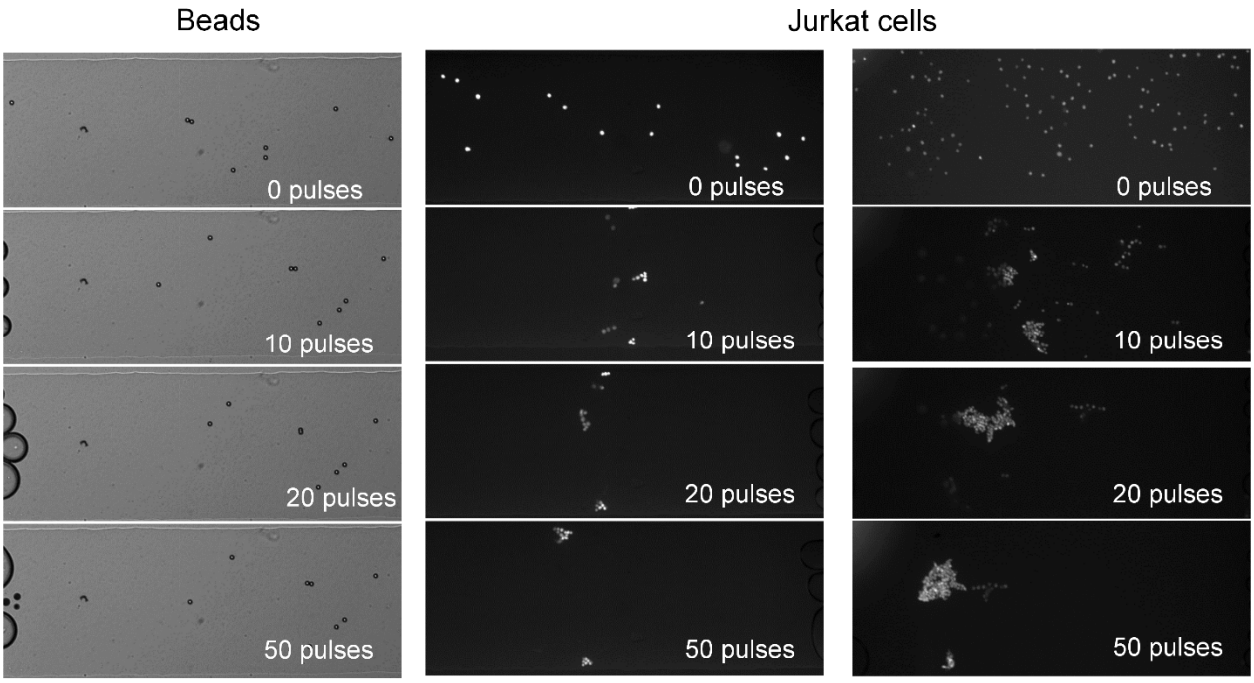

Figure S5. Time-lapse images during the application of 50 pulses of 1000 V/cm, 30 ms duration and 9.97 s intervals of polymeric beads (d~10 µm, left panel) and Jurkat cells. Jurkat cells of two different concentrations (middle and right panels) were tested. The cells and the beads were suspended in a phosphate buffer (4.8 mM Na_2HPO_4, 1.2 mM KH_2PO_4, and 250 mM sucrose). The polymeric beads did not focus under these conditions.

Video S1. CHO cell focusing observed by phase contrast imaging. The cells were suspended in a phosphate buffer (4.8 mM Na_2HPO_4, 1.2 mM KH_2PO_4, and 250 mM sucrose), and experienced 50 pulses (1000 V/cm, 30 ms in duration and 9.97 s in interval). The cells were static during the intervals between pulses. For each pulse, we took 7 images (with a span of ~ 4 s) that covered moments before and after the pulse. The video was composed by putting all 350 images together in a sequence.

Video S2. The tracked trajectories of CHO cells (labeled by Hoechst 33342) during the focusing. The cell were suspended in a phosphate buffer (4.8 mM Na_2HPO_4, 1.2 mM KH_2PO_4, and 250 mM sucrose) and were exposed to 50 pulses (1000 V/cm, 30 ms in duration and 9.97 s in interval). We took one image after each pulse and the video was composed by putting the 51 images (including 1 image before the first pulse) together in a sequence.