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Trapping and viability of swimming bacteria in an optoelectric trap

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Abstract

Non-contact manipulation methods capable of trapping and transporting swimming bacteria can significantly aid in chemotaxis studies. However, high swimming speed makes the trapping of these organisms an inherently challenging task. We demonstrate that an optoelectric technique, rapid electrokinetic patterning (REP), can effectively trap and manipulate *Enterobacter aerogenes* bacteria swimming at velocities greater than 20 $\mu\text{m/s}$. REP uses electro-orientation, laser-induced AC electrothermal flow, and particle-electrode interactions for capturing these cells. In contrast to trapping non-swimming bacteria and inert microspheres, we observe that electro-orientation is critical to the trapping of the swimming cells, since unaligned bacteria can swim faster than the radially inward electrothermal flow and escape the trap. By assessing the cell membrane integrity, we study the effect of REP trapping conditions, including optical radiation, laser-induced heating, and the electric field on cell viability. When applied individually, the optical radiation and laser-induced heating have negligible effect on cells. At the standard REP trapping conditions fewer than 2% of cells have a compromised membrane after four minutes. To our knowledge this is the first study detailing the effect of REP trapping on cell viability. The presented results provide a clear guideline on selecting suitable REP parameters for trapping living bacteria.

Graphical Abstract

We demonstrate an optoelectric technique (REP) for dynamic trapping and manipulation of swimming bacteria, and characterize the effect of trapping conditions on the viability of the trapped cells.

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Electronic Supplementary Information (ESI) available: [PDF file provides temperature rise induced by laser beams of different power, beam intensity profile in a REP trap, and introduction to finite element modeling of electrothermal flow. Movie S1 shows trapping of swimming cells. Movie S2 shows manipulation of cells. Movie S3 shows migration of cells to electrode surface after electro-orientation. Movie S4 shows an unaligned cell attempting to escape the REP trap.]. See DOI: 10.1039/x0xx00000x

Introduction

Motility is vital to many bacteria for their survival,^{1,2} dispersal,³ and pathogenicity.⁴ For example, directional migration of the Enterobacteriaceae enables their acquisition of vital resources for proliferation. Not surprisingly, the hydrodynamic^{5–7} and biochemical aspects⁸ of bacterial motility have served as a topic of constant attention for many decades. In peritrichous bacteria, propulsion is achieved by the rotary action of flagellar motor proteins:⁶ concerted rotation in the counterclockwise direction causes flagella to bundle together and propel the cell forward (run motion), but clockwise rotation of some motors causes the flagella to unbundle and the cell to vacillate (tumble motion). Both motions are influenced by chemical stimuli in the environment, or chemotaxis. In the absence of chemical stimuli, bacteria swim in a random fashion with straight runs followed by randomizing tumbles.⁵ In the presence of chemoattractants or nutrients, tumbling is reduced and a biased random walk towards the chemical source is commonly observed.

Investigations with swimming bacteria responsive to chemical stimuli can be enhanced with non-contact manipulation tools that can trap and transport cells with high spatial resolution, and monitor response by adjusting cell proximity to the stimulus source. Apart from analyzing bacterial taxis, these tools can also be used for studying cell mechanics,⁹ interaction between cells,^{10,11} and for separating individual cells or a small group of cells from the general population. However, trapping swimming bacteria is a challenging task due to their high velocities (>20 $\mu\text{m/s}$). Techniques such as acoustophoresis¹² and hydrodynamic trapping¹³ have been used previously for capturing particles and non-swimming cells but trapping and manipulation of swimming microorganisms have not been demonstrated by these methods. Only a few techniques such as optical tweezers^{11,14–21} and rotating magnetic beads²² have proven capable of trapping and manipulating swimming bacteria. However, the high-intensity radiation used in optical tweezers ($\sim 10^7 \text{ W/cm}^2$) can induce photodamage in cells during trapping,^{23–29} resulting in cell death which is also referred to as “optocution”.³⁰

Recently optoelectric methods have emerged that offer a similar level of dynamic control as optical tweezers in trapping and manipulating particles.^{31–36} Rapid electrokinetic patterning (REP)^{31,34} is such an emerging particle manipulation method. REP uses a combination of electrokinetic and hydrodynamic effects to achieve trapping.^{31,37–41} A laser-induced AC electrothermal (ET) vortex sweeps particles into the stagnation zone at the center of the vortex on the electrode surface where particles are held by particle-electrode forces³⁷. REP has been demonstrated previously with non-swimming bacteria⁴² and spherical polystyrene particles.⁴¹

In this work, we show that REP can effectively trap swimming bacteria moving at an average speed of 23 $\mu\text{m/s}$, faster than the radially inward flow ($\approx 15 \mu\text{m/s}$) of the AC electrothermal vortex. Unlike previous REP studies with non-swimming bacteria and polymer microspheres,^{31,43} unique characteristics are observed in the trapping behavior of swimming bacteria. We find that the electro-orientation is paramount to trapping of these swimming cells, and that unaligned bacteria with sufficient energy can escape the ET vortex. We also observe that cells aligned with the electric field swim toward the electrode surface upon application of the electric field, thereby improving the ability to efficiently trap these highly motile bacteria.

In addition, we investigate the effect of REP trapping conditions on the captured cells. Parameters such as optical radiation, laser-induced heating, and electric field strength were varied to evaluate their effect on cell viability post REP trapping. The individual effects of optical radiation and laser-induced heating on cell wall integrity were observed to be negligible. Our standard trapping conditions using moderate electric field (110 kVpp/m at 20 kHz) and laser beam (20 mW) settings easily maintain cell membrane integrity for four minutes in a REP trap. To the best of our knowledge, this is the first study that discusses the viability of cells during REP capture.

Materials and Methods

The REP setup consists of an optical microscope, a pair of parallel-plate indium tin oxide (ITO) coated electrodes separated by a 100 μm spacer to form a microchannel, and a continuous-wave 1064 nm laser projecting into the microchannel through a 40 $\times$ objective lens, which was also used for darkfield imaging of bacteria cells (Fig. 1a).

REP Chip Preparation

REP chips were prepared from glass slides and coverslips coated with 700 nm ITO (SPI Supplies Inc., PA, USA), which served as the top and bottom electrodes, respectively. Two holes were drilled in the ITO glass slide to provide inlet and outlet ports. The ITO-coated substrates were then immersed for 30 minutes in acetone in an ultrasonic cleaning bath, rinsed with deionized water, soaked overnight in 10% bovine serum albumin (BSA) solution, followed by a second rinsing and drying. The BSA prevented nonspecific binding of bacteria onto the electrode surfaces. Two layers of 50- μm double-sided tape were used as spacers to form a microchannel between the electrodes. Electrical contacts were provided through two strips of conductive copper tape connected to the ITO surfaces.

Apparatus

A Telulex Model SG-100/A function generator (Berkeley Nucleonics Corp., CA, USA) was used to provide an AC electric field in the channel of the REP chip. The applied peak-to-peak AC voltage and frequency were monitored with an Agilent 54610B oscilloscope (Agilent Technologies, CA, USA). A continuous-wave, 1064-nm Nd:YVO₄ laser was integrated into an inverted epifluorescence Nikon TE2000U microscope. The bacterial cells were observed with a Nikon 40 $\times$ ELWD objective (0.6 NA) under darkfield and fluorescence microscopy. A Nikon air dark field condenser (0.80–0.95 NA) was used for darkfield

microscopy, and fluorescence excitation was conducted with a 120 W lamp (Xcite series 120PC, EXFO Life Sciences & Industrial Division, Canada). The darkfield images were recorded with a grayscale CCD camera (PCO.1600, Cooke Corporation, MI, USA), which was controlled with the manufacturer-provided Camware software. The laser beam was focused so that the diffraction limited spot was positioned 25 μm above the top electrode surface (imaging plane), resulting in a beam waist of $\approx 15 \mu\text{m}$ on the electrode surface.

Sample preparation

All culture materials and bacterial specimens were used as received from VWR and the Carolina Biological Supply Company. *E. aerogenes* (ATCC No. 13048) was grown using standard culture conditions. An overnight culture in nutrient broth (BD Diagnostics) at 37 °C was subcultured in a fresh nutrient broth at 100X dilution with continuous shaking at 230 rpm. These were grown for 4 h before being subcultured again at a 100X dilution. After 3 h a third subculture was performed at the same dilution and allowed to grow for 2 h, or until the culture reached the mid log phase with an optical density of 0.2 at 600 nm. After such time, the bacteria were collected by centrifugation at 2000 *g* over ten minutes. Dispersion in dilute phosphate-buffered saline (PBS; 1:200 dilution) was performed by gentle inversion of the centrifuge tube before using bacteria in REP studies.

Labeling protocol

Loss of membrane integrity is correlated with bacterial cell death. While even porous cells may survive for a time, and some of them recover viability⁴⁴, cells with an undamaged membrane are assumed to retain full viability. Therefore, the effect of REP trapping on cell membrane integrity was tested with L13152 LIVE/DEAD BacLight™ bacteria viability kit (Life Technologies, NY, USA). The cells were stained according to the manufacturer's protocol. Briefly, a 1:1 mixture of bacteria in dilute PBS buffer and 2× stock solution of SYTO9 and propidium iodide (PI) was shaken and incubated for 15 minutes in the dark at room temperature. After incubation, 20 μL of this sample was used directly for experiments. Direct use without centrifugation to isolate bacteria ensured that PI was present to stain compromised cells during the REP trapping process. The excitation/emission peaks for SYTO9 and PI are about 480/500 nm and 490/635 nm, respectively.

Particle Tracking Velocimetry

The particle tracking experiments were conducted to measure the velocity profile of the electrothermal vortex flow near the top electrode surface as a particle moves in a REP trap. Experiments were performed with neutrally buoyant, 1- μm Fluoro-Max™ polystyrene beads (Thermo Scientific, USA). A minimum of 20 trajectories of particles were recorded with typical REP trapping conditions of 20 mW laser and a 110 $\text{kV}_{\text{pp}}/\text{m}$ electric field at 20 kHz. The particles were tracked with the Mosaic plugin in ImageJ. For calculating the average speed of motile *E. aerogenes* cells, 20 cells were randomly selected and tracked in the field of view with the Manual Tracking plugin in ImageJ. The average speed of the released cells was calculated by tracking released cells with the Manual Tracking plugin. For estimating propulsive power, cells were assumed to be swimming 0.5 μm below the top electrode surface.

Finite element modelling of electrothermal flow

COMSOL Multiphysics v4.4 was used to simulate ET flow and laser-induced temperature increase; more details can be found in the Supplementary Information.

Results

A culture of motile *Enterobacter aerogenes* (10^7 cells/mL) in dilute PBS was gently introduced between the transparent electrodes. An AC electric field of 110 kV_{pp}/m at 20 kHz was used for REP trapping. REP trapping could be achieved and imaged on either the top or bottom electrode surface, however the top electrode was used in our studies to avoid complications related to the settling of non-motile cells.

Trapping of swimming *E. aerogenes*

In the absence of the electric field or laser, bacteria were freely swimming in the channel with an average speed of 23 ± 7 $\mu\text{m/s}$, as shown in Fig. 1b. When the AC electric field was applied, motile cells became aligned with the electric field in vertical orientation (in head-up or head-down configuration) due to an effect known as electro-orientation.⁴⁵ In brief, the rod-shaped bacteria become electrically polarized and support an induced dipole, which generates a torque that orients cells along the direction of the electric field (Fig. 1c).^{45,46}

Applying the AC electric field with the laser focused onto the top electrode produces a toroidal ET vortex, which sweeps the bacteria to the stagnation zone at the center of the vortex (Fig. 1d). The entire trapping process is shown in Movie S1. The laser-induced ET vortex, which is the primary driver of the REP trapping mechanism, is created by a temperature gradient on the ITO-coated electrode surface.³⁸ The incident laser radiation is partially absorbed in the 700 nm ITO layer and dissipated as heat from the electrode. This localized heat dissipation produces a temperature gradient in the solution within a fraction of a second (~ 100 ms) of laser irradiation.⁴⁷ Since the electrical conductivity and permittivity of fluids are temperature-dependent, the temperature gradient is also responsible for spatial gradients in solution conductivity and permittivity, which couple with the applied AC electric field to produce an ET vortex.⁴⁵

The ET vortex transports bacteria to the stagnation zone at the center of the vortex, below the top electrode, where cells are held by short-range particle-electrode interactions,³⁴ which prevent trapped cells from being carried toward the channel center by the ET flow. The trapped cells were immediately released upon turning off the laser and electric field, with simultaneous loss of electro-orientation (Fig. 1e). The released cells appeared to be fully functional and migrated from their former confinement site at an average speed of 22 ± 7 $\mu\text{m/s}$ which is statistically indistinguishable (t-test, $p > 0.05$) from the speed observed prior to the REP trapping (see Movie S1). This suggests that the flagellar rotation and proton motive force across the cell membrane were unaffected by the trapping process. The trapped cells can be relocated on the electrode by moving the microscope stage while maintaining the laser in a fixed position, as depicted in Fig. 1f. The trapped cells were observed to retain their vertical orientation, and were translated at the same velocity as stage repositioning (see Movie S2).

Role of electro-orientation in trapping of swimming bacteria

Some unique characteristics are observed in the trapping behavior of swimming cells as compared to the trapping process of non-swimming cells and polymer particles. When the electric field is applied, most cells align with the electric field. At this point, bacteria can only swim in the z -direction (the direction of the alignment). We observe that the aligned cells swim to both the top and bottom electrode surfaces from the interior of the channel (see Fig. 2). Prior to applying the AC electric field ($t = 0$ s), only a single, unaligned cell was present in the field of view; within a few seconds of applying the field ($t = 8$ s), multiple cells swam to the top electrode, all with the uniaxial alignment in z -direction (see Fig. 2 and Movie S3). The electrokinetically-induced swimming of bacteria in the z -direction, toward electrodes, inherently improves the probability of trapping by REP.

Essentially, uniaxial alignment of cells with the AC electric field is paramount to the trapping of cells. Velocity profiles of the ET flow near the top electrode surface (obtained by tracking 1- μm beads) indicated a maximum velocity of $13.2 \pm 1.0 \mu\text{m/s}$ (Fig. 3), whereas motile *E. aerogenes* could swim at an average velocity of $23 \pm 7 \mu\text{m/s}$, which is fast enough to escape the maximum trapping velocity. However, cells are vertically aligned with the electric field, which limits their lateral mobility, resulting in their facile entrapment by the radially inward flow of the ET vortex.

In our experiments, we noticed that a small percentage of cells ($\approx 5\%$) were not aligned with the electric field, and that some of these unaligned cells were able to swim through the vortex without being trapped. The fraction of unaligned cells could be reduced to less than 2% by starting with an AC frequency of 100 kHz and then lowering the frequency to 20 kHz. The aligning torque induced by the electric field depends on the dielectric properties of both cells and medium, as well as cell volume and AC frequency⁴⁵. Therefore, slight differences in dielectric properties and cell size among bacteria in a sample can introduce variations in electric torque, resulting in a fractional population of unaligned cells.⁴⁸ Though the reasons for non-alignment are still under investigation, the conditions in which unaligned cells escape the REP trap can be quantified by determining the minimum propulsive power required by cells to swim against the drag force exerted by the radially inward flow in the ET vortex. This threshold power can be estimated by using the velocity profile shown in Fig. 3. By treating bacteria as elongated (prolate) spheroids with major and minor semiaxes a and b , the drag force acting on the bacteria is expressed as

$$F_{\infty} = \frac{4\pi\eta aU}{\ln(2a/b) - 1/2} \quad (1)$$

Where η is the viscosity of the solution and U is the velocity of the flow.^{20,49} From darkfield microscopy images, the mean length and diameter of an *E. aerogenes* cell was measured to be $7.3 \pm 1.6 \mu\text{m}$ and $1.6 \pm 0.2 \mu\text{m}$, respectively. For bacteria swimming near the surface of the top electrode, the wall effects on drag force are also taken into account. Following the analysis of Happel and Brenner,⁴⁹ the modified drag force is $F = F_{\infty}/(1 - k(F_{\infty}/6\pi\eta U))$.²⁰ In this equation, l is the distance between the electrode surface and the major axis of the prolate

spheroid, and k is a dimensionless constant equal to $9/16$.⁴⁹ An unaligned bacterium can thus escape the trap if it has propulsive power $P = F \times U$ at each radial position. Therefore, a cell trying to escape from the center of the trap must generate a peak propulsive power of 12.2 ± 3.2 aW (Fig. 3). Movie S4 in the Supplementary Information shows an unaligned cell attempting to escape the trap. When swimming against the flow, the escaping cell slowed down, aligned with the electric field, and was pulled toward the trap by the inward flow.

Viability of cells in a REP trap

Even when successfully trapped, it is important to consider how bacteria are affected by REP parameters such as laser radiation, temperature and electric field. We studied the effect of these conditions on cell viability by assessing cell membrane integrity using SYTO9/PI staining; the former dye penetrates all cells and stains them green, whereas the latter penetrates cells with compromised membrane and stains them orange or red. *E. aerogenes* were dispersed in dilute PBS buffer solution which contained both stains. The cells were then exposed to optical radiation, laser-induced heating, and electric field separately to study their individual effect on cells. Finally, the combined effect of all these conditions in a REP trap was investigated by observing trapped cells for a trapping period of five minutes. Each experiment was repeated 10 times, and error bars report the standard deviation of the data.

Effect of optical radiation—The effects of high-intensity radiation on cells has been widely studied in optical tweezer experiments.^{23–29} Results show that the photodamage depends upon the wavelength and intensity of the laser beam, and is caused by photochemical generation of toxic chemical species.^{23,27} Neuman *et al.* demonstrated that a damage threshold does not exist for the high intensity radiation in optical tweezers; photodamage begins from the time that cells are trapped.²³ The laser intensities used in REP trapping are three orders of magnitude lower than that of optical tweezers (see Fig. S1), so photodamage to trapped bacteria is expected to be negligible, which is confirmed by stain testing. *E. aerogenes* cells exposed for five minutes to laser radiation between 15 to 40 mW in a glass channel exhibited no differences in viability relative to unirradiated controls.

Effect of laser-induced heating—Temperature rise is another important factor to consider during REP trapping. Finite-element modeling of ET flow, driven by a 20-mW laser, shows a maximum temperature rise of 17.6 °C above room temperature at the center of the trap (see Fig. 4 and Supplementary Information for simulation details). The simulation suggests that fluid temperature decays in both the radial and axial directions, such that cells within 20 μm of the trap center ($r = 0$, $z = 0$) experience temperatures in the range of 27.0 to 37.6 °C, below the threshold of thermal shock. As expected, the temperature rise decreases with a reduction in laser power: For cells trapped in the ET vortex created by a 15-mW laser beam, the temperatures range from 25.2 to 33.2 °C (see Fig. S2), well within the range of viability. The effect of laser-induced heating on membrane integrity was studied by exposing bacteria to the laser beam for five minutes on the ITO surface in the absence of electric field. As shown in Fig. 4b, even a laser beam as high as 40 mW in power was found to have a minimal effect. Only 1.2% of the cells exhibited a loss in cell wall integrity during the experiment.

Effect of AC electric field—Another aspect to be considered is the strength of the applied AC electric field.^{44,50} An electric field above a threshold value can cause reversible electroporation of cells, which is often used to introduce exogenous molecules into cells while retaining cell viability.⁴⁴ The effect of an electric field on the membrane integrity of cells was studied by exposing bacteria to fields of varying strength for five minutes in a REP chip. As evident in Fig. 5, an electric field of 110 kV_{pp}/m was found to have only a minor effect on cell membrane integrity, since fewer than 2% of all bacteria were orange or red after five minutes. However, as the field strength was increased above 150 kV_{pp}/m, the percentage of bacteria with compromised cell walls increased. At 200 kV_{pp}/m, approximately 21% of all cells showed signs of electroporation after five minutes.

Effect of REP trapping conditions—So far we have characterized the individual effects of optical radiation, laser-induced heating, and AC electric field on cell viability. During REP trapping, all three of these factors are present along with shear forces from the ET flow. The overall effect of REP trapping was studied by quantifying the population of red and green stained bacteria in the trap over time at different electric field strengths and laser powers (see Movie S5 and Fig. S3). Under standard trapping conditions (20 mW laser, 110 kV_{pp}/m electric field), more than 98% of the cells had intact membranes after four minutes (see Fig. 6). However, REP conditions operating at higher field strengths had a negative impact on the membrane integrity of trapped cells: at 150 and 200 kV_{pp}/m, 58% and 98% of trapped cells lost their membrane integrity after five minutes (see Fig. 6). This contrasts with only about 5% and 21% loss of membrane integrity at these same field strengths when only electric field was applied. The higher rate of electroporation can be explained by the presence of shear forces and higher local temperature in the REP trap, which can cause faster electroporation.⁵¹ This hypothesis is further validated by studying the effect of an increase in laser power on membrane integrity (see Fig. 7). With increase in power, the temperature of the solution increases (see Fig. S4) causing an increase in poration of the cells. At 150 kV_{pp}/m, an increase in laser power from 20 to 30 mW caused a 15% increase in cells with compromised membranes. In the future, further studies to understand the effect of REP trapping on swimming bacteria can be performed by using a rotating bacterial cell assay²³ or by monitoring changes in the pH gradient across cell membranes.^{24,25}

Conclusions

In this work, we have shown that REP is an efficient method for trapping and manipulating swimming bacteria. Compared to the previously studied trapping of polymer microspheres and non-swimming bacteria, we have noticed unique characteristics in the trapping behavior of swimming cells. We observe that the alignment of cells along the electric field is critical to their successful trapping, since unaligned swimming cells are powerful enough to overcome the trapping velocity of the radially inward flow of the electrothermal vortex. Therefore, if bacteria are non-aligned with the electric field and possess sufficient energy, they can swim against the inward flow and escape the trap. In our experiments, most of the cells (98%) are aligned with the electric field and successfully trapped. Though reasons for non-alignment of some of the cells are still under investigation, we have quantified the minimum propulsive power required by cells for escaping the trap.

We have also investigated how REP trapping conditions affect cell viability by assessing cell membrane integrity. The laser-induced temperature rise by itself was found to have minimal effect on bacteria. However, the use of high electric field strength (200 kV_{pp}/m) resulted in electroporation of more than 21% of the bacteria within five minutes. The combined effect of laser-induced temperature rise, electric field, and electrothermal flow in a REP trap was found to accelerate the poration of the cells as compared to when only electric field or laser was applied. At the standard REP trapping conditions, the trapped cells were barely affected for the first four minutes in the trap. These membrane integrity results provide a clear guideline for the trapping duration, electric field strength, and laser power that can be used during REP trapping of living bacteria. In the future, we expect REP to be used in studying chemotaxis, cell migration, and in applications involving cell sorting or compartmentalization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

(a) A schematic diagram of the experimental setup. An inverted Nikon microscope was used for microscopy and trapping. A Rapid Electrokinetic Patterning (REP) chip consists of two parallel-plate Indium Tin Oxide (ITO) coated transparent electrodes across which an AC electric field is applied. A suspension of bacteria was introduced between the electrodes. During the trapping and manipulation experiments, an AC electric field of 110 kVpp/m at 20 kHz was used. The REP trapping was initiated by projecting a 20 mW 1064 nm laser beam onto the top electrode (see Movie S1). (b, c) Freely motile *E. aerogenes* experienced electro-orientation to a net vertical alignment within 0.5 s of applying an AC electric field. (d) Projection of a 20-mW laser beam onto the electrode surface resulted in REP trapping of bacteria within 20s. (e) The trapped cells were released upon switching off the laser and electric field. (f) Schematic diagram illustrating the manipulation of the trapped cells by moving the microscope stage with respect to the laser (see Movie S2).

Fig. 2.

Bacteria before and after applying AC electric field. Initially, a single unaligned cell was present in the field of view ($t = 0\ s$); after applying the field six additional cells swam to the top surface ($t = 8\ s$; see Movie S3).

Fig. 3.

The velocity profile measured with 1-μm particles entering a REP trap. Starting from a distant radial position, particles accelerate before coming to rest in the trap. This velocity profile was used to calculate the minimum propulsive power required by an unaligned bacterium for escaping the vortex.

Fig. 4.

(a) The temperature rise induced by a 20 mW laser, at different axial positions, as a function of radial position from the vortex center. (b) Effect of the temperature rise induced by a 40 mW laser on the membrane integrity of the exposed bacteria in a REP chip in the absence of the electric field.

Fig. 5. Effect of the AC electric field on the membrane integrity of the bacteria as a function of time. These experiments were performed in a REP chip in the absence of the laser beam.

Fig. 6.

Effect of the REP trapping conditions on the membrane integrity of the captured bacteria as a function of time. Laser power was kept at 20 mW while electric field strength was varied from 110 kV_{pp}/m to 200 kV_{pp}/m (see Fig. S6 for longer testing).

Fig. 7.
Effect of the laser power on the membrane integrity of the captured bacteria in a REP trap after five minutes.