RAPID CONCENTRATION AND MANIPULATION OF COLLOIDS AND MICROORGANISMS THROUGH DOUBLE LAYER POLARIZATION ELEKTROKINETICS

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ABSTRACT
This paper reports on a novel electrokinetic technique that can concentrate, translate, and sort colloids and microorganisms. This technique, termed rapid electrokinetic patterning (REP), uses electric double layer polarization electrokinetics for particle manipulation which is fundamentally different from dielectrophoresis which uses Maxwell-Wagner interfacial polarization. Therefore REP provides a new scheme for electrokinetic sorting based on the surface characteristics of particles and their electric double layer behavior. The concentration and sorting of bacteria (Shewanella Oneidensis MR-1, Staphylococcus aureus, and Saccharomyces cerevisiae) is demonstrated herein. Also, a new non-optical REP system is introduced providing increased portability and larger colloid concentration size.

KEYWORDS: Colloids, Electrokinetics, Double-layer, Polarization, Dielectrophoresis

INTRODUCTION
Electrokinetic techniques, such as dielectrophoresis, have been integrated with lab-on-a-chip devices for a variety of functions including the transport and concentration of colloids as well as the pumping and mixing of fluids. REP is an AC electrokinetic technique that couples colloid-colloid electrokinetic interactions with electrothermal hydrodynamics in a uniform AC electric field to rapidly concentrate, pattern, and sort suspended micro- and nanoparticles. An REP-based manipulation system was introduced at MicroTAS 2008 [1] and further investigations demonstrated manipulation and sorting of polystyrene particles 49 nm to 3.0 μm [2-5]. A recent review article demonstrating this and other optically-based electrokinetic techniques is available [6]. This paper introduces two critical advancements for the future implementation of REP in life sciences and lab-on-a-chip technologies. First, the manipulation of Shewanella Oneidensis (S. Oneidensis) MR-1 bacteria and size-based separation of Staphylococcus aureus (S. aureus) and Saccharomyces cerevisiae (S. cerevisiae) bacteria is performed. This demonstrates the ability of REP as a manipulation tool for bio-particles. Second, a new non-optical REP system is introduced with predominant advantages over the original optical-based system, such as increased portability, larger concentration size, and ease of integration with existing lab-on-a-chip technologies.

THEORY
REP is a combination of a variety of electrokinetic phenomena involving colloids and hydrodynamics. The following is a brief overview; a more extensive background of REP is discussed elsewhere [2, 3]. First, a non-uniform temperature gradient is created by applying a highly-focused laser beam to a substrate which absorbs the illumination. This substrate also serves as half of a parallel-plate electrode configuration. The combination of non-uniform temperatures with a uniform electric field results in a microfluidic vortex, whose circulation center is on the substrate where the laser is applied [7]. The generated heat has an increased magnitude of less than 7 °C with temperature gradients in the range of approximately 0.05 to 0.2 °C/μm [2, 3]. This vortex carries suspended particles towards the electrode surface where low-frequency (< 100 kHz) particle-electrode electrokinetics continuously trap the colloids.

EXPERIMENTAL
Figure 1 illustrates the optically-based REP configuration. Two indium tin oxide (ITO) electrodes on glass substrates were separated by 50 μm double-sided adhesive containing microfluidic features. Inlet and outlet holes were drilled into one of the ports for fluid access. A 1.064 nm wavelength laser is delivered to the chip through a 60X (water immersion, 1.2 NA) objective lens. The applied laser power was no greater than 30 mW. S. Oneidensis MR-1, S. aureus, and S. cerevisiae were prepared and resuspended in DI water; the sample had a measured conductivity of 1.5-2.0×10⁻³ S/m. Prior to sample injection, the chamber was filled with bovine serum albumin (BSA) and left for 13 hours to prevent the adhesion of the bacteria on the ITO surfaces.

Instead of using highly-focused lasers, a non-optical REP chip with patterned thin film resistive heaters has been fabricated to generate non-uniform temperature fields. An array of thin film copper heater strips 200 nm thick, 1.0 mm long, and 25 μm wide were patterned using photolithography and lift-off on a glass substrate (1.5 mm thick). A 1.0 μm thick SiO₂ insulating layer was deposited through PECVD and windows were etched to expose the underlying heater bonding pads. Next, a thin layer of aluminum (200 nm thick) was deposited, providing one-half of the parallel plate electrode. A double sided ad-
hesive spacer was used to separate the chip from an ITO coverslip. Inlet and outlet holes were drilled in the thicker glass substrate.

RESULTS AND DISCUSSION

Over 600 MR-1 are rapidly aggregated with an AC signal of 18.7 kHz and 17.8 V_{pp} and a laser power of 20 mW (Figure 2a). Multiple aggregation sites and/or patterning of the bacteria is demonstrated in Figure 2b with two laser spots (total power 30 mW) and an AC signal of 17.3 kHz and 16.9 V_{pp}. Translation of the bacteria aggregation is shown in Figures 2c and 2d. The bacteria are originally aggregated at the location in Fig. 2c before the laser spot is translated laterally to a spot 40 μm away; the hydrodynamic vortex has been relocated to this new spot, carrying the bacteria with it. This translation was accomplished at 18.7 kHz, 13.8 V_{pp}, and a laser power of 20 mW.

Figure 3a demonstrates the initial capture of *S. aureus* and *S. cerevisiae* at 17.5 kHz, 10.07 V_{pp}, and 20 mW. The larger *S. cerevisiae* are removed at an increased AC frequency of 38.9 kHz (Fig. 3b). This result is consistent with previous findings that the relaxation frequency of similar colloids is greater for larger-diameter particles [2, 3]. However, more experiments are necessary to determine the relationship between polarization and relaxation of biological ionic double layers.

![Figure 1: Illustration of optically-based REP configuration.](image)

![Figure 2. Manipulation of Shewanella Oneidensis MR-1 bacteria using REP technique. Solid-circles in the figure represent the location of a laser illumination. (a) Aggregation, (b) patterning, and (c)-(d) translation of MR-1. The applied electrical frequency and voltage is 17.32 kHz and 16.9V_{pp}, and the laser power is 30mW.](image)

![Figure 3. Size-based separation of the smaller Staphylococcus aureus and larger Saccharomyces cerevisiae using REP technique. (a) Aggregation of both at 17.5 kHz, 10.07 V_{pp} and 20 mW. (b) Only S. aureus are captured at an increased frequency of 38.9 kHz.](image)
An illustration the non-optical REP system is shown in Figure 4a. An embedded thin-film resistive heater controlled by an independent DC signal is the source of the non-uniform heating within the AC-biased parallel-plate system, as opposed to a highly focused illumination spot (refer to Fig. 1). Figure 4b demonstrates trapping of 500 nm fluorescent polystyrene particles with a 25 μm wide and 1 mm long heater strip. The generated heat has an increased magnitude of less than 5 °C with a temperature gradient of approximately 0.1 °C/μm. Sorting of 2.0 μm particles from 500 nm particles was also accomplished (not pictured).

Figure 4. (a) Illustration of a non-optical REP system using thin-film resistive heaters. (b) 500 nm particles captured using a 25 μm wide heater at 30 kHz and 8 Vpp.

CONCLUSION

The ability for REP to manipulate biological samples lays the foundation for future applications for the sorting and concentration of microorganisms utilizing double layer electrokinetics. Colloid manipulation with a simplified, less-expensive non-optical REP chip enables more users to use this technology and integrate it with other lab-on-a-chip devices.

REFERENCES


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