Dielectrophoretic Particle Characterization and Manipulation

CNF Project # 1360-05
Principal Investigator(s): Brian J. Kirby
User(s): Benjamin G. Hawkins

Affiliation(s): Department of Biomedical Engineering, Cornell University
Primary Research Funding: National Science Foundation, Department of Energy
Contact: bk88@cornell.edu, bgf6@cornell.edu
Web Site: http://www.kirbyresearch.com/index.cfm/page/rp/dep.htm

Abstract:
This report discusses two aspects of the development of a high-throughput separation technique aimed at separating cells based on phenotypic differences in membrane composition using dielectrophoresis (DEP): automated particle characterization and electrodeless DEP separations. First we discuss an automated technique to measure cellular dielectrophoretic mobility as a function of frequency. Second, separation of a three-component suspension of polystyrene particles with diameters of 0.5 µm, 1.0 µm, and 2.0 µm was achieved using electrodeless DEP. An analysis of the geometry indicates that separations can be achieved spanning orders of magnitude in DEP mobility.

Introduction:
This project involves the development of a dielectrophoresis-based, high-throughput microfluidic technique for identifying and separating cell populations with phenotypic differences in membrane composition. We focus on Mycobacterium in particular as the membrane composition of M. tuberculosis has been implicated in both pathogenicity and drug resistance of the organism. The acid-fast bacterial membrane is composed of a cell membrane, peptidoglycan, and associated extra-membrane lipids. These outer membrane lipids include covalently and non-covalently attached moieties that we postulate contribute, in a detectable way, to the cellular “electrical phenotype” [2].

We have developed electrodeless DEP particle sorting techniques capable of either high sensitivity or high dynamic range operation, depending on sample characteristics. To determine the range of sample characteristics, and thereby design effective separation techniques, we utilize an interdigitated gold electrode array on a glass substrate to measure the dielectrophoretic response of particles as a function of frequency. Using LabView to automate the experiment, we measure particle trapping on the electrode array via fluorescence intensity at various electric potential magnitudes and frequencies. In this way, we obtain data on particle frequency response, proportional to the dielectrophoretic mobility.

Separation of particles of interest, based on dielectrophoretic mobility, has been demonstrated using an electrodeless DEP device consisting of a curved constriction in channel depth set in a curved microfluidic channel. The channel is hot embossed from a silicon master into Zeonor 1020R polymer.

Constriction curvature leads to variation in the DEP force and causes a spatial separation of particle populations based on DEP mobility. Channel curvature superposes additional variation in the DEP force, leading to and increase in the dynamic range of the device proportional to the radius of curvature.

Research Summary:
Automated experiments are carried out on a gold electrode array deposited on a glass substrate. Device fabrication follows standard lift-off processing techniques using e-beam...
deposition. Final electrode dimensions are 10 µm wide on 30 µm centers. A PDMS microfluidic channel, 100 µm by 25 µm by 1 cm, is bonded to the glass substrate. Sample particle suspensions are introduced via ports cut in the PDMS channel and actuated using a Chemyx syringe pump (Figure 1). Electric potential, controlled via LabView and an Agilent 33200 function generator, applied to the electrodes generates electric field gradients and DEP forces. Particle trapping is quantified by measuring fluorescence intensity as a function of electric field frequency and magnitude (Figure 2).

Particle separation experiments utilize a fabrication technique (previously reported [1]) for hot embossing Zeonor 1020R polymeric devices from a silicon “stamp.” After the devices are embossed, a chemical bonding procedure is used to enclose the channel with a second Zeonor lid. The silicon master “stamp” was created using a two-step Bosch etch process to define microfluidic channels 50 µm deep and 2500 µm wide. Channel constrictions are 40 µm tall, leaving a channel gap of 10 µm (Figure 3). We have characterized our fabrication technique using mechanical profilometry after hot-embossing and confocal microscopy after enclosure.

After fabrication, fluorescent polystyrene microparticles are used to characterize device performance. A mixture of 0.5 µm, 1.0 µm, and 2 µm diameter spheres are suspended in DI water and introduced via reservoirs affixed to the Zeonor substrate. Particle motion was observed using a X-Cite 120 fluorescence source and a Nikon TE2000U inverted microscope. Images and movies were recorded with a Q-Imaging Retiga EXiFAST camera and Phylum software. Image analysis was carried out using MATLAB (MathWorks).

We have demonstrated successful separation of 0.5 µm, 1 µm, and 2 µm polystyrene beads using a curved constriction in channel depth placed in a curved microfluidic channel (Figure 3). Successful separation of the three microsphere populations was achieved and characterized using time-lapse images (containing approximately 200 frames of video, Figure 4). The added curvature of the microchannel leads to predictable variation in the bulk electric field and additional modulation of electric field gradients.

References:
